AN INVESTIGATION INTO THE NATURE OF THE SYMPATHETIC RECEPTORS IN THE UTERUS

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ABSTRACT

An investigation has been carried out into the nature of the adrenergic receptors in the uterus of the rat, rabbit and human, using a series of sympathomimetic amines and a number of adrenergic blocking agents. Both *in vivo* and *in vitro* experiments have been performed using adult non-pregnant animals, and studies have been made on isolated uteri from immature animals. Some *in vivo* experiments have included stimulation of the hypogastric nerve and a recording of the arterial blood pressure. Experiments using human uteri have been limited to *in vitro* studies.

The presence of α-excitatory and β-inhibitory adrenergic receptors has been demonstrated in the uteri of the three species, both in adult and in immature animals. However, the actions of the sympathomimetic amines on the uteri from immature rats and rabbits and on the uterus from the human foetus differed qualitatively from their actions on the uteri from adult animals. Further, the actions of the amines on the uteri of adult rats varied according to the stage of the oestrous cycle.

A study of the influence of ovarian hormones on the uterine responses to the sympathomimetic amines was performed. Immature and ovariectomised adult rats and rabbits were treated with oestradiol and progesterone. After treatment with

oestradiol the excitatory activities of the amines were enhanced so that the responses of the uteri from animals which had received this treatment resembled those of uteri from adult animals. Administration of progesterone with the oestradiol did not modify the effects of the latter hormone. Treatment with progesterone alone did not alter qualitatively the actions of the amines. Responses of the blood pressure to the amines were not influenced appreciably by the ovarian hormones. These results suggest the possibility that a high level of oestrogen increases the number and/or the activity of the α-excitatory adrenergic receptors in the uterus.

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G E N E R A L H I S T O R I C A L

1. ADRENERGIC RECEPTORS

The earliest work relating to the idea of specialised receptors in the sympathetic system was discussed by Elliot in 1904. He found that, in the cat, following complete denervation of the iris, the dilator pupillae muscle was more sensitive to adrenaline than the muscle with its nerve supply intact. This led him to suggest that the site of action of adrenaline might be "a sort of mechanism developed out of the muscle cell in response to its union with the synapsing sympathetic fibre, the function of which is to receive and transform the nervous impulse". He concluded that adrenaline may be liberated by the nervous impulse at the nerve ending. In the following year Elliot (1905) showed the close relationship between responses elicited by adrenaline and by sympathetic nerve stimulation in many tissues. He suggested that the nature of the receptive substance determined whether the responses were either motor or inhibitor. Langley (1905) agreed with Elliot concerning the presence of receptive substances but thought them to be cell constituents, rather than that they were developed in consequence of the union between sympathetic nerves and smooth muscle. He considered that "a cell might make motor or inhibitor receptive substances or both, and that the effect of a nervous impulse depends upon the proportion of the two kinds of receptive

substance which is affected by the impulse". Dale (1906) produced definite evidence for the existence of "myotrophic structures or substances" subserving either stimulation or inhibition in the sympathetic system when he selectively antagonised only the excitatory responses of adrenaline and of nerve stimulation with ergot. Inhibitory responses were either unaffected, enhanced, or sometimes revealed after antagonism of the motor response. Dale would not speculate on the ideas of Elliot or Langley in regard to the location of these excitatory and inhibitory elements because his experiments gave "no indication as to the level at which this functional differentiation occurs".

Most of the work in the next four decades was concerned with elucidating the nature of the transmitter substance in sympathetic nerves, rather than being directed primarily at how the transmitter effected a response in the muscle. Because of the inability of any one proposed transmitter to mimic exactly the responses which occurred to sympathetic nerve stimulation, Cannon and Rosenblueth (1933) suggested that the transmitter (M) was given its characteristic type of activity by combining with either an excitatory (E) or an inhibitory (I) "receptive substance" inside the reacting cells. When these substances escaped from the affected cells into the blood stream they would induce excitatory, inhibitory or, when present together, both effects on distant organs.

The theory which is accepted at the present time as describing the nature of adrenergic receptors was proposed by Ahlquist in 1948. He classified adrenergic receptors in different effector organs largely on the basis of the order of potency of a series of five sympathomimetic amines - adrenaline, noradrenaline, isoprenaline, α-methyladrenaline and α-methylnoradrenaline. He found that only two orders of potency were revealed in all the organ systems studied. In one adrenaline was most potent and isoprenaline least potent. In the other isoprenaline was most potent and noradrenaline least potent. This suggested that only two receptor types were involved. Ahlquist called those mediating responses in the former group alpha (α) receptors, and those in the latter group beta (β) receptors. Further, all the responses mediated via α-receptors could be antagonised by ergotoxine, dibenamine and tolazoline, except that of intestinal relaxation which was not convincingly affected. The responses elicited through β-receptors were not influenced by these blocking agents. The great difference between this theory and the earlier ones was that although the older ideas described two receptor structures, these subserved either stimulation or inhibition. Ahlquist also postulated two groups of receptors but he did not classify them by the nature of the response they mediated, but by their sensitivity to a series of catecholamines in

eliciting sympathomimetic responses. In fact he found that both α- and β-receptors were involved in excitatory and inhibitory responses in the various tissues investigated.

Lands (1952) disagreed with certain aspects of Ahlquist's classification. He preferred, as did earlier workers, to classify adrenergic receptors as either excitatory or inhibitory. He found that with sympathomimetic amines like adrenaline, the 3-(or m-)hydroxyl group on the benzene ring was most important for stimulation of excitatory (Ac) receptors, while the alcoholic hydroxyl on the side chain was most important for stimulation of inhibitory (Ar) receptors. Thus receptors mediating intestinal relaxation were classified as Ar. This was in contrast to the results of Ahlquist which indicated that adrenergic receptors in the intestine were similar to those receptors in other tissues except the heart which mediated excitatory effects. The receptors in the heart were stimulated by sympathomimetic amines with a strong affinity for either Ac or Ar receptors and therefore Lands suggested that they be classified as a separate group (Acr).

Supporting evidence for Ahlquist's theory and indeed evidence which has caused its widespread acceptance came in 1958, when an antagonist of the responses elicited as a consequence of β-receptor stimulation was discovered. This antagonist, dichloroisoprenaline, was found to block both the inhibitory effects on smooth muscle as well as the excitatory

effect of catecholamines on the heart (Moran and Perkins, 1958). However it failed to influence the intestinal relaxation produced by catecholamines (Furchgott, 1959). Therefore Furchgott considered that the intestinal adrenergic receptors should be classified separately as delta receptors. In the same year however Ahlquist and Levy found that, when both α- and β-blocking agents were present, intestinal adrenergic receptors could be blocked. Thus they concluded that the intestine contains α- and β-inhibitory receptors. Furchgott confirmed these results soon afterwards (1960).

Furchgott (1959) proposed a separate class of adrenergic receptors mediating glycogenolysis which he called gamma receptors, but in the following year (Furchgott, 1960) he conceded that adrenergic receptors in the heart for glycogenolysis were of the β-type. This followed the work of Mayer and Moran (1960) who found that dichloroisoprenaline was an effective antagonist at this site. It is not yet resolved whether all adrenergic metabolic receptors may be classified as β-receptors, but glycogenolysis in the heart, skeletal muscle and liver, and adipose tissue lipolysis can be antagonised by β-blocking agents (Mayer, Williams and Smith, 1967). However α-blocking agents also antagonise hepatic glycogenolysis (Mayer, Moran and Fain, 1961).

Although the adrenergic responses in smooth muscle may be blocked by α- and β-blocking agents, either singly or in

combination, so that it appears that only two types of adrenergic receptors are present, a number of factors have emerged recently which have led to the conclusion that the β-receptors in the various tissues may not be uniformly similar. In a series of papers Levy (1964, 1966a,b, 1967b) showed that certain derivatives of methoxamine blocked β-receptors in the rat uterus, but did not block the positive chronotropic and ionotropic β-effects on the heart or on intestinal relaxation, or changes in blood flow in the hind limb of the dog, induced by isoprenaline. In support of these observations, Dunlop and Shanks (1968) found that a new blocking agent, I.C.I. 50172, antagonised the positive chronotropic and ionotropic actions of isoprenaline on the heart of the dog, but had little action on other effects mediated by β-receptors, including inhibition of the uterus of the rat. They discussed the possibility that β-receptors may not be identical in the various tissues of any one species. Dunlop and Shanks suggested, on the basis of their results and those of Levy, that β-receptors be classified into excitatory and inhibitory subgroups. Similar observations on the selectivity of action of α-methyldichloroisoprenaline at certain β-receptors were made by Van Deripe and Moran (1965), and the implications behind such a subdivision of β-receptors has been discussed by Braunwald (1966) and Moran (1966, 1967). Lands, Arnold, McAuliff, Luduena and

Brown (1967) suggested that β-receptors be divided into two subgroups, namely "B_I" receptors which include cardiac stimulatory receptors and those involved in lipolysis and "B_{II}" receptors which include receptors for bronchiolar dilatation and for glycogenolysis. Their conclusions were based on the relative potencies of agonists in effecting these responses.

In regard to subdivision in the α-group of adrenergic receptors, van Rossum (1965) found that noradrenaline possessed a greater affinity for α-receptors in certain tissues where dopamine possessed only a weak affinity. For example, α-receptors in the rabbit intestine were occupied more easily by noradrenaline than those in the rat vas deferens, whereas the reverse was true for dopamine. van Rossum speculated upon the existence of a series of α-receptors "at one end of which are receptors best fitted by noradrenaline, and at the other end receptors which are best fitted by dopamine". However, using the rather different technique of measuring dissociation constants (K_B) for receptor-antagonist interactions, Furchgott (1967) concluded that α-receptors in the aorta, stomach and duodenum of the rabbit were all of one type. This conclusion was based upon the assumption that the K_B values should be the same for any one antagonist reacting with receptors which are of the same type.

This work has thus invited speculation as to exactly

where these newer adrenergic blocking agents exert their effect in the chain of reactions leading to the observed response in the tissue. Moran (1966, 1967) has pointed out that α- or β-receptor activation is only one step in a whole sequence of events occurring between initiation and observation of the response. The possibility must not be ruled out that different events occur in different organs after receptor activation and that the newer β-blocking agents may antagonise the response through an action at such a site, thus exerting a more selective action than the earlier antagonists. It would seem therefore that a certain degree of caution is warranted in the classification of adrenergic receptors solely on the basis of susceptibility to blockade by antagonists.

In order to solve the problems regarding uniformity of type within receptor groups and the relationship between α- and β-groups, it is necessary to discover the molecular configuration of the receptors. To date the only information about the molecular nature of adrenergic receptors is based upon the chemistry of agonists and antagonists. Some workers have suggested particular enzymes or other substances which they consider may be the receptor. Others, preferring not to assign any formulation to the "whole" receptor, have pointed out a number of chemical groupings which would seem to be necessary structures on the receptor surface for its interaction with agonists or antagonists. Ariëns and his

co-workers (Ariëns, van Rossum and Simonis, 1957; Ariëns, 1967) pointed out the difference between the "affinity" of a substance for a receptor and its "intrinsic activity" in promoting a response. Agonists possess both properties while ideal antagonists have a good affinity for the receptor without initiating a response. Antagonists usually possess a greater affinity for receptors than agonists, but it is possible that both may be "multipotent", i.e. act on more than one type of receptor. It is also possible that an antagonist does not act on the same receptor as the agonist, but instead may interfere with steps in the sequence of events triggered by the action of the agonist on the receptor (Ariëns et al., 1957; Ariëns and Simonis, 1964). Large antagonist molecules may also interact with structures adjacent to the "true" receptor and thus mask its activity. All these points if not realised, may lead to erroneous conclusions regarding the structural nature of receptors as deduced from drug-receptor interactions.

In regard to the structural properties of the α-receptor, the earliest studies were made by Nickerson, Nomaguchi and Goodman (1946) and Nickerson and Gump (1949), who likened the action of dibenamine to that of the alkylating action of nitrogen mustards. They showed that the β-chloroethyl side chain of dibenamine cyclised to form an ethylene-imine ring structure which then reacted with sulphydryl groups on the receptor surface (Harvey and Nickerson, 1954). Other workers

confirmed that the action of this group of α-blocking agents was due to the formation of the ethyleneiminium ion, but a controversy arose as to the nature of the anionic site on the receptor surface with which this ion reacts. Adenosine triphosphate (ATP) appeared to be involved in the activation of α-receptors (Belleau, 1959, 1963). Those who considered that ATP was a part of the α-receptor (Belleau, 1959, 1963; Bloom and Goldman, 1966) proposed that the anionic site was a phosphate grouping of ATP which reacted with β-haloalkylamines. In contrast, other investigations indicated that the α-receptor was characterised as a lipoprotein (Dikstein and Sulman, 1965; Yong, Parrulekar, Wright and Marks, 1966). Conclusions reached by Graham and Al-Katib (1966) also favoured a protein-like structure, for they found that trypsin antagonised the alkylating mechanism of β-haloalkylamines, indicating the presence of lysine or arginine or both amino acids in the receptor structure. Further, Hokin, Sastry, Galsworthy and Yoda (1965) showed that the first stage in the breakdown of ATP by ATPase involved the transfer of the terminal ATP phosphate group to a carboxylate anion on the protein surface of the enzyme. Kimelberg and Triggle (1965), Triggle (1965) and Graham and Al-Katib (1966) suggested that a carboxylate group was the reactive site on the α-receptor for β-haloalkylamines. This led Belleau (1967) to modify his original theory (Belleau, 1959,

1960) and to propose that although ATP was involved in α-receptor activation it was not the active site for β-haloalkylamines. Instead, he proposed that the protein structure of the α-receptor was ATPase and that the reactive site for the α-blocking agents was a carboxylate group on this surface. The site of action for catecholamines however, is to date still considered to be on the terminal phosphate group of ATP (Bloom and Goldman, 1966; Belleau, 1967). It appears therefore that α-receptor agonists and α-receptor antagonists of the β-haloalkylamine type may not act at precisely the same sites.

In regard to reactions which mediate responses at the adrenergic β-receptor, Belleau (1960) and Sutherland and Rall (1960) proposed that β-receptors were involved in regulating the formation of adenosine 3'5' phosphate (cyclic AMP) from ATP. They considered that the actions of catecholamines in eliciting responses at β-receptors were related to their ability to increase the activity of adenylcyclase, the enzyme responsible for the conversion of ATP to cyclic AMP. In 1960 Belleau considered that a complex was formed between catecholamines and ATP, but although retaining this basic concept, a revision of the mechanism of combination between the reactants was necessary (Belleau, 1967) in the light of experiments conducted by Bloom and Goldman (1966). Further speculation by Robinson, Butcher and Sutherland (1967) has led them to suggest that adenylcyclase itself may be the β-receptor,

because stimulation of adenylcyclase resembles most closely those effects which have been described as being mediated by β-receptors. Other workers (Vane, 1962; Ariëns, 1963) also have suggested that β-effects may be mediated by cyclic AMP formation, without going as far as to postulate the structure of the β-receptor.

The involvement of ATP at both α- and β-receptor sites has led to some controversy as to the separate existence and function of the two receptor systems. Bloom and Goldman (1966) considered that the receptors should be regarded as enzyme-substrate complexes and emphasised their separate identities by describing the α-receptor as an ATPase + ATP complex and the β-receptor as an adenylcyclase + ATP complex. Further, it was found that ^{14}C-labelled phenoxybenzamine (Masuoka, Hansson and Appelgren, 1967) and ^{14}C-labelled propranolol (Masuoka and Hansson, 1967) were not bound to the same sites when their distribution was examined autoradiographically. On the other hand, it has been considered that adenylcyclase may be involved in both α- and β-receptor reactions (Robinson, Butcher and Sutherland, 1967) and that both receptors are concerned in the regulation by catecholamines of cyclic AMP levels in the isolated pancreatic cells from the rat (Turtle and Kipnis, 1967).

In connection with the actual location of adrenergic receptors, it has not yet been established whether they lie

within the cell, in the cell membrane or outside it. Graham and Al-Katib (1966) have found that trypsin antagonised the α-receptor blockade caused by β-haloalkylamines. They have cited evidence (Weiss, 1963) which showed that trypsin cannot penetrate living cells, and therefore they have concluded that the α-receptor site is part of the cell membrane. Paton (1967) has emphasised the importance of determining the location of receptors, for if the substance first has to penetrate the cell membrane, this process will involve structural factors in its own right. This may modify considerably the importance of structure-activity relationships in determining the nature of adrenergic receptors. Thus at the present time the structure, type and site of adrenergic receptors is still far from being elucidated.

2. ADRENERGIC RECEPTOR BLOCKING AGENTS

The use of relatively specific substances to oppose the actions of agonists is one of the most important techniques in the elucidation of the nature of receptors. In the application of Ahlquist's dual receptor theory for adrenergic receptors it is often necessary to antagonise the predominant action of sympathomimetic amines at one type of receptor, in order to expose their weaker action at the second site. Indeed, Furchgott (1967) has proposed that in a pharmacological assessment of adrenergic receptors, the true nature of a particular type of receptor can be assessed only when the other adrenergic receptors in the system are blocked. Thus, when α-receptors are being studied, β-receptor activation must be prevented, and vice versa.

A) α-RECEPTOR BLOCKING AGENTS

The first demonstrations of specific adrenergic blockade were given by Dale (1905, 1906) and Sollmann and Brown (1905). Dale found that ergotoxine and ergotamine blocked or reversed most excitatory responses to adrenaline and to sympathetic nerve stimulation. The excitatory action of adrenaline on the heart however, was not antagonised and neither were the inhibitory actions of adrenaline. These early results have been observed many times in the succeeding years, using a wide range of

substances. The following are some of the most important groups:-

(i) Ergot alkaloids and derivatives

Reduction of the alkaloids ergotamine and ergotoxine to their dihydro derivatives was found to increase their adrenergic blocking activity and to decrease their smooth muscle stimulating capacity (Stoll and Hofmann, 1943; Brügger, 1945; Rothlin and Brügger, 1945; Rothlin, 1947).

(ii) Benzodioxanes

The excitatory responses to adrenaline on the circulation, nictitating membrane, iris and uterus of certain species were shown to be blocked by piperoxan (933F) (Fourneau and Bovet, 1933). More recently, a more potent derivative, Dibozane has been described by O'Leary (1953).

(iii) Imidazoline derivatives

The adrenergic blocking action of tolazoline (Priscol) was first described by Schnetz and Fluch (1942) and more fully by Ahlquist, Huggins and Woodbury (1947) and by Gowdey (1948). Meier, Yonkman, Craver and Gross (1949) and Gross, Tripod and Meier (1951) showed that another imidazoline derivative phentolamine (Rogitine) blocked the action of adrenaline and noradrenaline on the intestine and uterus of the rabbit but had little effect on the catecholamine activity on the uterus of the guinea pig or on the cat and frog heart.

(iv) β-Haloalkylamines

The pharmacology of dibenamine was first reported by Nickerson and Goodman (1945). A more detailed report soon followed describing the actions not only of dibenamine but also of some of its analogues (Nickerson et al., 1946). However, in general, only quantitative differences between the various active members of the series were found. Another important member of this group, phenoxybenzamine (Dibenzyline) was described by McLean, Fendrick, Macko and Fellows (1951) who found it to be more potent and faster acting than dibenamine.

The prolonged action of the β-haloalkylamines, in contrast to the relatively short action of the other groups of α-blocking agents, has led to a detailed study of their mechanism of action. It appears that the blockade is produced in two stages. Initially there is a competitive type of antagonism between the catecholamine and the β-haloalkylamine for the same population of receptors (Nickerson and Gump, 1949; Furchgott, 1954; Innes, 1962a; Hansch and Lien, 1968). Later, a fully developed blockade occurs which is non-competitive, and where a stable chemical bonding between the antagonist and the receptor site is formed (Nickerson and Gump, 1949; Furchgott, 1954). This involves the formation of an ethyleneiminium intermediate (Nickerson and Nomaguchi, 1951) which then reacts with active groups on the α-receptor surface (Harvey and Nickerson, 1954; Belleau and Triggle, 1962;

Graham, 1962). The study of these interactions has greatly assisted in the definition of a molecular structure of the α-receptor (p. 16).

Throughout the first half of this century the development of adrenergic blocking agents produced only compounds which could antagonise most of the excitatory effects of catecholamines and of sympathetic nerve stimulation. The excitatory effect of adrenaline on the heart and the numerous inhibitory effects on other tissues could not be antagonised effectively, although some inconclusive reports were presented concerning the antagonism of certain of these effects (p. 29).

B) β-RECEPTOR BLOCKING AGENTS

In 1958 a new type of adrenergic blockade was described. Investigation of the compound dichloroisoprenaline (p. 280) revealed that it antagonised specifically the inhibitory components of adrenergic activity described above which could not be antagonised by any of the previously described blocking agents (Powell and Slater, 1958). Further investigations by Moran and Perkins (1958) revealed that dichloroisoprenaline also specifically blocked the positive chronotropic and ionotropic cardiac effects of adrenaline. This result substantiated the proposal by Ahlquist (1948) that catecholamine-induced responses were effected through two types of receptor (p. 10). The β-receptors were blocked by

dichloroisoprenaline, while α-receptors were blocked by the previously described blocking agents. This led Moran and Perkins (1958) to propose that adrenergic antagonists should be termed α- and β-blocking agents according to the type of receptor at which they effected a blockade. Dichloroisoprenaline did not antagonise catecholamine activity at α-receptors. Indeed, it enhanced the activity of substances which acted at these sites (Powell and Slater, 1958; Levy, 1959). In contrast to the α-blocking agents, which do not structurally resemble agonists at α-receptors, dichloroisoprenaline is very similar to isoprenaline, a potent β-receptor stimulant. Accordingly β-receptor blocking agents have been developed by various modifications of the isoprenaline structure. Because of its very close resemblance to isoprenaline, dichloroisoprenaline was found to possess quite marked sympathomimetic effects on the heart (Moran and Perkins, 1958) and particularly on the uterus of the rat where its β-sympathomimetic activity prevented it from being used as a β-blocking agent (Powell and Slater, 1958; Jensen and Sund, 1960). Consequently compounds were synthesised which possessed less intrinsic β-sympathomimetic activity but which retained their β-receptor blocking properties.

In 1962 Black and Stevenson described the adrenergic blocking properties of pronethalol (p. 281). It blocked adrenergic responses of the guinea-pig heart and tracheal

chain, and of the cat heart and fowl caecum. Relaxation of the rabbit duodenum was partially antagonised. Pronethalol did not influence the catecholamine-induced contraction of the rabbit uterus and the vasoconstrictor action of catecholamines on the rabbit ear. It was not entirely devoid of sympathomimetic activity (Lish, Weikel and Dungan, 1965) on the heart of the dog (Donald, Kvale and Shepherd, 1964) and uterus of the rat (Vanov, 1963; Vanov and Vogt, 1963), but it was withdrawn from clinical usage when it was found to produce tumours in mice (Paget, 1963). With the addition of a methoxy group into the aliphatic side chain of pronethalol to produce propranolol (p. 281) (Black, Crowther, Shanks, Smith and Dornhorst, 1964), a β-antagonist ten times more potent than pronethalol and with no sympathomimetic activity or carcinogenic potential was produced. A number of compounds have now been introduced with qualitatively similar β-blocking properties. They have the basic structure either of pronethalol, e.g. INPEA (Teotino, Polo-Friz, Steis and Della, 1963); H.13/57 (Corrodi, Persson, Carlsson and Roberts, 1963); MJ 1999 (Lish et al., 1965), or of propranolol, e.g. Kö 592 (Engelhardt, 1965); I.C.I. 45763 (Shanks, Wood, Dornhorst and Clark, 1966) and H.56/28 (Åblad, Brogard and Ek, 1967). The chemical formulae of these compounds are tabulated on p. 280.

While the β-adrenergic blocking agents described above antagonise all the β-effects of catecholamines and of

sympathetic nerve stimulation to a greater or lesser extent, a number of compounds have emerged recently which appear to block some, but not all β-receptors. Compounds related to N-isopropyl methoxamine (p. 281) were reported by Burns, Colville, Lindsay and Salvador (1964) to block adrenaline-induced release of free fatty acids from the rat epididymal fat body, but were without effect on the isoprenaline-initiated positive chronotropic and ionotropic cardiac effects. In the same year Levy found that N-isopropyl methoxamine would not block circulatory β-effects of catecholamines in the anaesthetised dog, but strongly antagonised their activity at β-receptors in the uterus of the rat. In a further study Levy (1966b) attributed these selective actions to an α-methyl group in the aliphatic side chain which is not present in other β-blocking agents. Levy (1966a) found that a related compound, butoxamine, produced a selective blockade of β-receptors in the uterus of the rat and the intestine and the femoral vascular bed of the dog, but had no influence on cardiac β-responses. Similarly α-methyl analogues of dichloroisoprenaline were found to antagonise vasodilatation but to exert only a weak blocking activity on cardiac responses to catecholamines (Van Deripe, Åblad and Moran, 1964; Van Deripe and Moran, 1965). On the basis of this differential blockade Moran (1966) suggested that β-adrenergic receptors be divided into several subclasses.

Further evidence for this was produced by Dunlop and Shanks (1968) using a new compound I.C.I. 50172 (p. 281). It caused blockade of cardiac β-responses to catecholamines, but it did not affect catecholamine-induced vasodilatation or increased blood flow in the hind limb preparation of the dog. It possessed only very weak blocking activity at β-receptors in the trachea of the guinea-pig, having only a 150th of the potency of propranolol at this site. These workers suggested from their results and from the work of Levy (1966a,b) that β-receptors should be divided into two subgroups - excitatory and inhibitory - since blocking agents have now been synthesised which selectively antagonise only cardiac excitatory, or certain inhibitory responses.

Thus it is evident that the use of adrenergic blocking agents is of value in the pharmacological definition of adrenergic receptors. Nevertheless misinterpretation of results could occur and indeed has occurred when non-specific compounds or blocking agents with numerous side effects have been employed.

Some of the most confusing results have been obtained with the use of ergot alkaloids and their derivatives (Nickerson, 1949). The potent activity of this group in stimulating smooth muscle directly is well known. That these substances will cause blockade of adrenergic excitatory responses has been reported in this section (p. 22), but many

investigators have indicated that ergot alkaloids block inhibitory responses as well. These alkaloids were found to alter the depressor responses to N-ethylnoradrenaline and isoprenaline to pressor effects (Raymond-Hamet, 1936; Hazard, Beauvallet and Guidicelli, 1947; King, 1947). These pressor responses were definitely sympathomimetic because they were reversed by benzodioxanes and by yohimbine and were enhanced by cocaine (Hazard, Beauvallet and Guidicelli, 1948). However, Lands, Luduena, Grant, Ananenko and Tainter (1950) showed that this depressor reversal was due to an increased cardiac output in the presence of a peripheral vascular bed already constricted by the alkaloid, and not a true pressor effect of isoprenaline. More recently it has been shown that some of the stimulatory effects produced by the alkaloids themselves are elicited through α-receptors (Konzett, 1960; Innes, 1962a). Ahlquist and Levy (1961) and Levy and Ahlquist (1961) revealed that dihydroergotamine possessed both α- and β-receptor blocking properties. Thus, although the activity of dihydrogergotamine on adrenergic receptors is not specific, nevertheless it has been used recently as an adrenergic blocking agent. Rudzik and Miller (1962a) stated that it blocked the inhibitory action of adrenaline on the uterus of the rat, while Clegg (1963), using it as an α-blocking agent, found that it antagonised the stimulatory effect of noradrenaline on the uterus of the guinea-pig.

However, Deis and Pickford (1964) observed that dihydroergotamine itself initiated uterine activity in the rat in dioestrus.

Similarly, the examination of the specificity of action of other adrenergic blocking agents must be considered. Attention has been focussed recently on these aspects by Furchgott (1966) and Nickerson (1967) who emphasised that the use of "receptor protection" experiments as employed by Furchgott (1954) and more recently by Innes (1962a,b, 1963), using a number of agonists, can provide direct evidence about the different sites of action of antagonists.

Marked side effects of α- and β-blocking agents on other adrenergic mechanisms will undoubtedly influence the response observed to sympathomimetic substances, especially in the whole animal. In this respect members of both groups of blocking agents inhibit the uptake of noradrenaline in sympathetic nerves. Dichloroisoprenaline and phenoxybenzamine are particularly strong inhibitors of uptake (Axelrod, Hertting and Potter, 1962). The activity of other blocking agents in this respect has been reviewed recently by Iversen (1967). Foo, Jowett and Stafford (1968) have concluded that the potency of β-receptor blocking agents in reducing noradrenaline uptake was unrelated to their potency in blocking β-receptors. All of the substances tested, including dichloroisoprenaline, pronethalol and propranolol, blocked β-receptors in

concentrations lower than those required to block the uptake of noradrenaline.

Much attention has been paid to the circulatory and cardiac responses to β-blocking agents in relation to their clinical usage. Dichloroisoprenaline (Lucchesi and Hardman, 1961) and pronethalol (Vaughan-Williams and Sekiya, 1963) exerted a non-specific antagonism to cardiac arrhythmias induced by a range of adrenergic and non-adrenergic cardiac stimulants. This action was thought to be related to the potent local anaesthetic properties of these agents (Morales-Aguilerá and Vaughan-Williams, 1965). A dose-dependent peripheral vasodilatation caused by propranolol was thought to be related also to its local anaesthetic activity (Shanks, 1967).

Other workers (Sharma, 1966; Olivares, Smith and Aronow, 1967) have indicated that propranolol may interfere with the production of α-receptor blockade by phenoxybenzamine. They argued that a pressor response produced by noradrenaline in the presence of both blocking agents was not due to a potentiation of the α-activity of noradrenaline after β-receptor blockade. It was concluded that, in some undefined way, propranolol displaced phenoxybenzamine from the α-receptors. Propranolol produced a similar effect upon the α-receptor blockade caused by tolazoline and by phentolamine (Olivares et al., 1967). More recently however, Yamamura and Horita (1968) have considered that propranolol does not act by

displacing phenoxybenzamine from α-receptors in the vasculature of the cat since the actions of methoxamine and oxymetazoline, which act only at α-receptors, were not restored after propranolol. It was shown that the actions of adrenaline and noradrenaline, which possess activity at both α- and β-receptors, were partially restored by propranolol. They proposed that this occurred as a result of an enhanced action of adrenaline and noradrenaline, after blockade of β-receptors, at residual α-receptors previously not blocked by phenoxybenzamine.

It is important therefore, to take into account the specificity of action of adrenergic blocking agents when evaluating results. This is particularly important when performing <u>in vivo</u> experiments.

E X P E R I M E N T A L

SECTIONS I - III

The experimental work is divided into three sections. Each section is complete within itself, and includes an historical, a discussion and a summary, followed by the tables and figures. A general discussion and an appendix follow Section III.

THE RESPONSES OF THE UTERUS OF THE RAT TO SYMPATHOMIMETIC AMINES AND THEIR MODIFICATION BY OVARIAN HORMONES

SECTION I

HISTORICAL

RESPONSES TO SYMPATHOMIMETIC AMINES

In 1914 Gunn and Gunn reported that adrenaline inhibited the isolated uterus of the rat. Cow (1919) confirmed this and showed that adrenaline caused a similar effect *in vivo*. Knaus and Clark (1926) found that adrenaline inhibited the uterus of the rat at all stages of the oestrous cycle. Sasaki (1938) confirmed the inhibitory action of adrenaline *in vitro* but stated that the uterus was more sensitive in dioestrus than at other stages of the oestrous cycle. Greer, Pinkston, Baxter and Brannon (1938) reported that noradrenaline produced an inhibitory action on the isolated uterus of the rat but that it was much less potent than adrenaline.

Quantitative studies of the actions of adrenaline and noradrenaline on various isolated uterine preparations enabled West (1947) to demonstrate the great difference in inhibitory potency between the two amines. He reported that *l*-adrenaline was approximately 100 times more potent than *dl*-noradrenaline on the isolated uterus of the rat. Subsequently this preparation has been employed widely in the bioassay of catecholamines, using the method of de Jalon, Bayo and de Jalon (1945), and numerous observations of the ratio of the equiactive doses of noradrenaline to adrenaline have been reported. Gaddum, Peart and Vogt (1949) quoted a

range of 75 to 300 for the ratio of l-noradrenaline to l-adrenaline; while Luduena, Ananenko, Siegmund and Miller (1949) obtained a value of 30 for the laevo isomers. Mann (1950) found that the ratio of the dose of dl-noradrenaline to the equiactive dose of l-adrenaline to cause inhibition was approximately 100 on the uterus of rats in dioestrus, in both in vivo and in vitro experiments. She obtained similar results on isolated uterine preparations from ovariectomised and from immature animals.

Observations on excitatory actions of adrenaline and noradrenaline have not been reported so widely. In both in vivo and in vitro studies Mann (1949, 1950) found that both adrenaline and noradrenaline caused excitatory responses on the uterus of the rat in the oestrous state only, either in natural oestrus or in an oestrous condition induced by the injection of oestradiol. However, the adrenaline response was biphasic, consisting of an excitatory phase followed by a more prolonged inhibition. Noradrenaline was approximately ten times more potent than adrenaline in initiating an excitatory response. The production of this excitatory response was not dependent upon the prevailing muscular tone of the uterus, for a contraction could be elicited in both quiescent and spontaneously contracting preparations. No excitatory activity to either amine was observed on the uteri from rats in

dioestrus, or from immature and ovariectomised animals. Bonney and Ferguson (1950) also showed the biphasic nature of the adrenaline response but found that a concentration of the order of 10^{-8} adrenaline excited the uterus from a rat in oestrus only when the tissue was quiescent, and that the more usually observed inhibitory action occurred when the preparation was contracting spontaneously. This excitatory action was not dependent entirely upon the tone of the uterus however, because they found that adrenaline produced solely an inhibitory action on the uterus in the dioestrous phase, regardless of whether the preparation was contracting spontaneously or was quiescent. Miller and Murray (1959) noted that, in preparations where the tone was low, adrenaline sometimes contracted the uterus, but they did not refer to any particular stage of the oestrous cycle. Draskoci, Feldberg and Haranath (1960) found that the excitatory response to adrenaline on the isolated uterus from a rat in oestrus was dependent upon the dose applied. A concentration of the order of 10^{-9} produced an inhibitory response, but on increasing the concentration 100 times an excitatory effect occurred which decreased with repeated dosage.

In a study designed to test the postulates of Ahlquist (1948, 1962) that the rat uterus contains both excitatory and inhibitory adrenergic receptors, Levy and Tozzi (1963) were unable to demonstrate any contractile effects with adrenaline

in vitro. This was true for uteri taken from rats at all stages of the oestrous cycle and from animals treated with diethylstilboestrol.

Thus it appears that the production of excitatory effects by adrenaline and noradrenaline may depend upon either the dose given, the muscular tone prevailing during the experiment, the sexual state of the animal, or perhaps upon all of these factors.

Studies on the responses of the uterus of the rat to other sympathomimetic amines have not been so numerous but the great sensitivity of the isolated preparation to *dl*-isoprenaline has been noted. Gaddum *et al*. (1949) found that it was approximately equipotent with *l*-adrenaline in producing uterine inhibition. Similar observations were recorded by Holzbauer and Vogt (1955), Rudzik and Miller (1962a), Levy and Tozzi (1963), Diamond and Brody (1966a) and Miller (1967). Other workers however, reported that it was significantly more potent than adrenaline. The ratio of potency of *dl*-isoprenaline to *l*-adrenaline was reported to be 6 (Muscholl, 1959); 20 (Brookes, Schaeppi and Pincus, 1965); 40 (Lish *et al*., 1965); and greater than 10 (Levy, 1966b). Schild (1967) has found that in the rat uterus depolarised by potassium chloride, the mean activity ratios for *l*-noradrenaline:*l*-adrenaline: *dl*-isoprenaline are 1:8:32. This inhibitory action of

isoprenaline was found to occur at all stages of the oestrous cycle, both in vitro (Levy and Tozzi, 1963) and in vivo (Lish et al., 1965).

The action of N-ethylnoradrenaline on the isolated uterus of the rat was noted by Gaddum et al. (1949). They reported its inhibitory potency to be approximately equal to that of adrenaline. The addition of an ethyl group to the α-carbon atom in the side chain of noradrenaline to form α-ethylnoradrenaline (butanephrine) did little to alter the inhibitory potency. Levy and Tozzi (1963) found that α-ethylnoradrenaline was much less potent than adrenaline, i.e. was also of a similar potency to noradrenaline.

Phenylephrine has been shown to cause inhibition of the isolated uterus of the rat (Rudzik and Miller, 1962a; Levy, 1966a) at all stages of the oestrous cycle (Levy and Tozzi, 1963). It was approximately equipotent with noradrenaline in this action (Spratto and Miller, 1967). In contrast, under the experimental conditions described by Tothill in 1967 (p. 46), phenylephrine caused a contraction of isolated uterine preparations from oestrogen-treated rats. It possessed one tenth the potency of adrenaline and noradrenaline in eliciting this action. On uteri from untreated animals only inhibitory responses to the three amines were obtained.

THE EFFECT OF HYPOGASTRIC NERVE STIMULATION

Few reports have been made on the nature of the adrenergic responses produced in the rat by stimulation of the hypogastric nerve. In a study of three rats Labate (1941) found that both adrenaline and nerve stimulation caused inhibition of the spontaneous movements of the intact uterus. Cocaine potentiated the responses to both adrenaline and nerve stimulation, thus suggesting that the fibres were adrenergic in function.

In 1950 Mann compared the effect of hypogastric nerve stimulation with the responses to adrenaline and to noadrenaline throughout the oestrous cycle. She found that excitatory responses of the uterus to nerve stimulation and to noradrenaline were produced when the animals were in natural oestrus, or in an oestrous condition produced by giving oestrogen to ovariectomised animals. In rats in dioestrus adrenaline, noradrenaline and stimulation of the hypogastric nerve caused inhibition of uterine activity. In all instances the response to nerve stimulation was similar to the noradrenaline effect, but not always similar to the adrenaline action. Thus she suggested that, in the rat, the transmitter might be noradrenaline.

THE EFFECT OF ADRENERGIC BLOCKING AGENTS

An early report of adrenergic blocking action by Mendez

(1928) indicated that ergotamine did not abolish the inhibitory action of adrenaline on the rat uterus, although it antagonised the motor effects of adrenaline on the uteri of other species. Ahlquist, Huggins and Woodbury (1947) showed similar properties for the more specific agent tolazoline. Mann (1950) showed that the motor effects produced by noradrenaline, adrenaline and hypogastric nerve stimulation on the intact uterus of the rat in oestrus were antagonised or reversed by dibenamine and by ergotoxine. The inhibitory actions of catecholamines on isolated uterine preparations were blocked by dichloroisoprenaline (Jensen and Vennerød, 1961a; Levy and Tozzi, 1963; Levy, 1964), and by pronethalol (Levy and Tozzi, 1963; Daniel, 1964; Levy, 1964; Diamond and Brody, 1966a). However, because these two substances possess considerable sympathomimetic activity on the rat uterus, other workers have reported on their inability to antagonise effectively the inhibitory actions of catecholamines (Powell and Slater, 1958; Jensen and Sund, 1960; Vanov and Vogt, 1963). The sympathomimetic actions of these blocking agents have themselves been antagonised by other more specific β-blocking agents (Lish et al., 1965; Levy, 1966b). A more effective blockade of uterine inhibitory responses to the catecholamines has been produced with propranolol (Diamond and Brody, 1966a,b and 1967; Levy, 1967a,b; Singh and Mahawar, 1968) and with MJ 1999 (Brookes et al., 1965; Lish et al., 1965; Spratto and

Miller, 1967).

Recently a group of new compounds has been synthesised which appears to block selectively some, but not all the responses which are mediated via β-receptors. These substances, N-isopropylmethoxamine (Levy, 1964), N-t-butylmethoxamine (Levy, 1966a, 1967b) and dimethylisopropylmethoxamine (Levy, 1966b), effectively antagonised the inhibitory responses to catecholamines of the isolated uterus of the rat. These findings have been interpreted to mean that β-receptors may not be identical in the various tissues of any one species (p. 13).

The inhibitory actions <u>in vitro</u> of various sympathomimetic agents have been blocked also by substances classed as α-receptor antagonists. Phentolamine was observed to block the inhibitory effect of adrenaline (Wiqvist, 1959; Rudzik and Miller, 1962a) and Jensen and Vennerød (1961b) found tolazoline effective in this respect. Dihydroergotamine was found to antagonise the inhibitory action of adrenaline (Holzbauer and Vogt, 1955; Wiqvist, 1959; Rudzik and Miller, 1962a; Levy and Tozzi, 1963; Tothill, 1967b) in concentrations which did not affect the inhibition produced by papaverine (Wiqvist, 1959) and by relaxin (Rudzik and Miller, 1962a). This led the latter workers to postulate the existence of α-inhibitory adrenergic receptors in the uterus of the rat. In contrast to this work tolazoline (Nishijama and Chuma, 1955), phenoxybenzamine (Levy and Tozzi, 1963; Farrant, Harvey and

Pennefather, 1964; Spratto and Miller, 1967) and phentolamine (Levy and Tozzi, 1963; Diamond and Brody, 1966a) were reported as having no antagonistic effects upon the inhibitory actions of catecholamines. Further, dibenamine and phenoxybenzamine (Holzbauer and Vogt, 1955), phentolamine (Brookes et al., 1965; Tothill, 1967b) and phenoxybenzamine and dihydroergotamine (Tothill, 1967b) were found to increase the inhibitory actions of adrenaline and noradrenaline. This potentiation of β-actions by α-blocking agents led Brookes et al. (1965) to suggest that α-excitatory receptors might be present in the uterus.

Stronger evidence for the presence of α-excitatory receptors has been shown by the production of an excitatory response to catecholamines in the presence of a β-blocking agent, and the subsequent antagonism of this effect by an α-blocking agent. This was reported by Jensen and Vennerød (1961a) when adrenaline inhibition (1×10^{-9}) of the uterus of a rat in oestrus was blocked by dichloroisoprenaline. A concentration of 1×10^{-7} adrenaline then produced an excitatory action which was blocked by phenoxybenzamine. Similar observations on the response to adrenaline were made by Diamond and Brody (1966a,b), also on the uterus of an oestrogen-treated rat, using propranolol and phentolamine as β- and α-blocking agents respectively.

Direct evidence for the presence of α-excitatory receptors

in the isolated uterus of the rat has been given by Diamond and Brody (1966b, 1967). They found that, in uteri from ovariectomised rats treated with oestrogen, noradrenaline produced an excitatory action which was antagonised by phentolamine. These results confirmed the work of Mann (1950) some sixteen years earlier, on studies with both intact and isolated uteri. Tothill (1967b) found, in uteri from rats treated with stilboestrol and with a low muscular tone induced by treatment with isoprenaline, that adrenaline, noradrenaline and phenylephrine all produced motor effects which were blocked by phentolamine and by phenoxybenzamine, but not by pronethalol. Since these motor effects were antagonised by the 5-hydroxytryptamine antagonist bromolysergic acid, Tothill proposed that in uteri from rats treated with oestrogens, a motor receptor designated as the "E-receptor" was present. This receptor was stimulated by catecholamines and by 5-hydroxytryptamine. However, Diamond and Brody (1966a) found that methysergide prevented responses to 5-hydroxytryptamine but not to noradrenaline on the isolated uterus of the rat. Further, Paton (1968) has shown that while the excitatory actions of both noradrenaline and 5-hydroxytryptamine may be antagonised by phenoxybenzamine, the action of 5-hydroxytryptamine is blocked selectively by dimethylaminopropylthiocinnamanilide.

Thus it is apparent that a controversy still exists as to

the nature of the adrenergic receptors in the uterus of the rat.

THE INFLUENCE OF OVARIAN HORMONES ON UTERINE ADRENERGIC RESPONSES

The observations on the activity of several sympathomimetic amines on the uterus, as reported on the preceding pages, indicate that their effects vary according to the sexual state of the animal. It has been considered that these variations may be induced through an action of the ovarian hormones.

In in vitro studies on the uterus from the rat, Balassa (1941) found that after treatment of rats with oestrogen, or oestrogen and progesterone, no alteration in the uterine response to adrenaline occurred. Mann (1949, 1950) stated that noradrenaline caused a contraction of the uterus from oestrogen-treated ovariectomised rats, i.e. a reversal of its action in untreated ovariectomised animals. The response to adrenaline also was reversed but a secondary inhibitory component followed the oestrogen-induced motor action.

Other workers (Rudzik and Miller, 1962a; Levy and Tozzi, 1963) did not record excitatory actions with sympathomimetic amines on isolated uteri from rats which had received diethylstilboestrol, and concluded that the uterus of this species contained no excitatory adrenergic receptors.

Recently however, some of the work of Mann (1950) has been confirmed and further investigated by Diamond and Brody

(1966b). They found that adrenaline and noradrenaline relaxed isolated uteri from ovariectomised rats and that pretreatment of these animals with progesterone or oestrogen and progesterone did not reverse the responses to the amines. However, in rats treated only with oestrogen the uteri were contracted by noradrenaline, although adrenaline still produced an inhibitory action. In the presence of β-receptor blockade induced by propranolol, adrenaline as well as noradrenaline was able to cause a motor action, but again this was so only if the rats had been oestrogen-treated. These workers concluded that oestrogen sensitises rat uterine α-receptors rather than that progesterone desensitises β-receptors, because in the progesterone-dominated uterus noradrenaline was less active in initiating an inhibitory response than in the oestrogen-dominated preparation.

Tothill (1967a,b) showed that adrenaline, noradrenaline and phenylephrine were able to exert motor actions on the isolated uteri of rats which had received stilboestrol. These amines produced only inhibitory actions on uteri from untreated animals in all stages of the oestrous cycle, or in ovariectomised animals which had received both oestrogen and progesterone. It was possible to produce the excitatory effects on the uterus of the oestrogen-treated animals only if the uterus was pretreated with 1×10^{-7} isoprenaline. This was reported by her to produce an effective blockade of the

β-adrenergic receptors in the uterus; a technique first described by Butterworth (1963a,b) for inducing blockade of the β-receptors in the cardiovascular system of the cat. Tothill concluded that the presence of a high level of oestrogen induced the formation of a motor receptor in the uterine tissue. More recently, Paton (1968) has confirmed that noradrenaline contracts the uterus from rats which have received diethylstilboestrol.

Thus the findings of these recent reports indicate that oestrogen treatment of rats enhances the excitatory activity of adrenaline and of noradrenaline on the uterus. To date however, no definite conclusions have been reached as to how this action is effected.

INTRODUCTION

The work reported on the preceding pages has shown that the nature of the adrenergic receptors in the uterus of the rat is still far from being elucidated. The presence of both α- and β-inhibitory receptors, as well as α-excitatory receptors has been reported, although it is not resolved whether these receptors exist in the uterus during all the sexual states of the animal.

In view of these differing results it was thought that it would be useful to re-examine the problem using a series of catecholamines which included isoprenaline, now that large doses of this substance have been reported to stimulate α-excitatory receptors (Butterworth, 1963a, b). Propranolol has been used as the β-receptor blocking agent of choice because of its lack of sympathomimetic activity.

The influence of the sexual state of the animal and of the ovarian hormones on the adrenergic responses of the uterus has been investigated. While this work was in progress other reports have been presented (Diamond and Brody, 1966b; Tothill, 1967b) concerning the influence of these factors upon the activity of sympathomimetic amines. The effects of hypogastric nerve stimulation have been studied throughout the oestrous cycle.

The present work does not include an investigation of the adrenergic responses in the pregnant animal.

METHODS

Both *in vivo* and *in vitro* studies have been made. In all experiments rats of the Wistar albino strain were used. All animals received Oxoid Modified 41B diet and tap water *ad libitum*. Four groups of experimental animals were employed:

1. ADULT RATS

Adult virgin female rats in a weight range of 170 - 380 g were used.

The animals exhibited a four-day oestrous cycle. The stage of the cycle was determined by the vaginal smear method (Long and Evans, 1922). Cells from the vaginal tract were taken up in saline introduced into the vagina from a small bulb pipette. The following stages were identified by microscopical examination of the smear:

Dioestrus - predominance of leucocytes with a few nucleated epithelial cells and absence of cornified cells.

Proestrus - predominance of round nucleated epithelial cells, with a few leucocytes and squamous cells.

Oestrus - predominance of cornified epithelial cells, with a few nucleated epithelial cells and absence of leucocytes.

Metoestrus - predominance of leucocytes with a few squamous cells.

2. IMMATURE RATS

Animals were used in which the vaginal orifice was closed. The weight range was 44 - 90 g. The uteri of animals below 40 g were generally too small and lacking in smooth muscle to be used.

3. OVARIECTOMISED RATS

Adult virgin female rats in a weight range of 200 - 360 g were used.

The ovaries were removed by a dorsal approach. The animals were anaesthetised with ether. Hair was removed from a region on either side of the back, away from the spinal column and caudal to the lowest rib. An incision was made in this region on one side and after locating the ovary it was removed carefully by paring away fat and severing the vascular and uterine connections. Ligation of the ovarian blood vessels was unnecessary as no appreciable blood loss occurred. The abdominal wall was closed with cotton thread and the body wall with Michel clips. This procedure was repeated on the opposite side for the removal of the other ovary. The animals were not used in experiments for at least fourteen days, during which time the absence of an oestrous cycle was confirmed by the daily examination of vaginal smears.

4. HORMONE-TREATED RATS

Immature and ovariectomised animals were given either oestradiol or progesterone, singly or together, in order to simulate the conditions in the oestrous cycle. All doses were given subcutaneously in arachis oil.

Oestradiol only:

(i) 50 µg oestradiol benzoate per rat per day for three days or

(ii) a single injection of 500 µg oestradiol benzoate. These animals were employed three days after the day of injection.

Progesterone only:

500 µg progesterone per day for three days.

Oestradiol and Progesterone:

50 µg oestradiol benzoate per day for three days followed by 500 µg progesterone + 10 µg oestradiol benzoate per day for three days.

Unless stated otherwise the animals were employed in experiments on the day following the last injection. The nature of the cellular components in the vaginal smear was determined before the animal was used.

IN VIVO EXPERIMENTS

Adult rats in various stages of the oestrous cycle and hormone-treated ovariectomised animals were employed. Immature and untreated ovariectomised rats could not be used owing to the undeveloped state of the uterus.

The animals were anaesthetised with a 10% solution of urethane injected intraperitoneally in a dose of 15 ml/kg body weight. The trachea was exposed but not cannulated and a cannula was tied into a femoral vein. Injections of sympathomimetic amines were made intra-arterially via a polythene catheter inserted in a retrograde manner along a femoral artery. Adrenergic blocking agents were given intravenously. In some experiments the blood pressure was recorded from a carotid artery, using a Condon capillary manometer.

An in vivo method of recording uterine movements has been developed by a modification of the technique of Bell and Robson (1937). A cannula was tied into the cervical end of one uterine horn and a small incision was made in the ovarian end. Warm 0.9% sodium chloride solution was introduced into the uterine horn so that air in the lumen was displaced through the incision. When the horn was filled with saline a ligature was tied round the uterus at the ovarian end in such a way that the saline was prevented from escaping through the incision, but the uterine blood flow was not obstructed.

Intrauterine pressure changes were recorded by connecting the cannula to a Condon capillary manometer. The changes in the height of the mercury level were magnified with a Palmer "Fixit" lever and recorded on a smoked drum (Diagram, p. 282). Contractions of the longitudinal muscle of the uterus were recorded by downward movements of the lever. Inhibitory responses were recorded as a decrease in size of the spontaneous contractions. If spontaneous movements were absent doses of acetylcholine were injected intra-arterially at regular intervals and any uterine inhibition caused by the amines was determined by the decrease in size of the subsequent acetylcholine responses. This technique afforded a good comparison with the <u>in vitro</u> method of estimating uterine inhibition.

In a number of experiments the hypogastric nerve was stimulated. The nerve was located in the mesentery near the colon, close to the abdominal aorta, below the origin of the ovarian arteries. It was tied, cut centrally and stimulated at a point distal to the inferior mesenteric ganglion using a Palmer square wave stimulator. Suitable electrical stimuli ranged from 1 - 3 V, with 0.2 - 5.0 msec pulse width at a rate of 20 - 50 per sec for a duration of 30 sec.

IN VITRO EXPERIMENTS

The sexual state of the rat was determined by taking a vaginal smear before it was used in an experiment. The animal was stunned and killed by cutting the neck. Either one or both uterine horns were removed and suspended in a physiological solution in an isolated tissue bath of 25 ml. capacity. Uterine movements were recorded on a smoked drum. In the majority of experiments using adult rats and in all experiments using immature and ovariectomised rats the bathing medium was Tyrode solution at 37 - 38°C aerated with 95% oxygen and 5% carbon dioxide. Uterine movements were recorded with an isotonic frontal writing lever.

However, in order to achieve a more accurate comparison with the numerous experimental procedures adopted by others who have used this tissue, a number of different experimental conditions were used in some of the experiments with uteri from adult rats:

(i) Either Krebs, Tyrode or de Jalon solutions were used. The composition of these solutions is given on p. 283.

(ii) Bath temperatures over a range of 29 - 38°C were employed.

(iii) The bathing solution was aerated with either air, 100% oxygen, or 95% oxygen and 5% carbon dioxide.

(iv) Simple isotonic or isometric recording levers were used.

(v) In the assessment of inhibitory activity of the sympathomimetic amines either the spontaneous rhythm of the uterus, or the acetylcholine-induced contractions of a quiescent uterus were used as indicators of inhibitory potency.

In order to obtain a better comparison between the *in vivo* and the *in vitro* work, some of the *in vitro* experiments were performed using a uterine horn from a rat in which the other horn had been used in an *in vivo* experiment. Details of the materials used in the experimental work are given in the Appendix, p. 276.

RESULTS

RATS IN DIOESTRUS

in vivo experiments

Uteri from animals in dioestrus usually showed spontaneous movements which caused pressure changes of up to 7 mm Hg and occasionally even larger contractions were observed.

In the eight experiments the five catecholamines employed, namely noradrenaline, adrenaline, α-ethylnoradrenaline, N-ethylnoradrenaline and isoprenaline, produced solely inhibitory actions (Fig. 1, 2, pp. 119, 120). These were observed either as a change in base-line tone or as a reduction of spontaneous or acetylcholine-induced contractions. The approximate ratios of equiactive doses to cause inhibition were, in terms of noradrenaline = 1 (at a dose of 6.4 μg); α-ethylnoradrenaline = 1.6; adrenaline = 0.04; N-ethylnoradrenaline = 0.006; isoprenaline = 0.0004 (Table 1, p. 107). Hypogastric nerve stimulation also caused either relaxation or inhibition of spontaneous activity (Fig. 2, p. 120).

In no instance was any reversal of response from inhibition to excitation observed to any of the catecholamines, even when comparatively large doses were used, e.g. 20 μg noradrenaline; 25 μg adrenaline; 50 μg α-ethylnoradrenaline; 500 μg N-ethylnoradrenaline or 5 mg isoprenaline. This was so

even when reversal of the blood pressure responses occurred to doses of N-ethylnoradrenaline (50 - 500 µg) and isoprenaline (1 - 10 mg). This reversal was achieved by inducing β-receptor blockade with large doses of isoprenaline. The blood pressure response to α-ethylnoradrenaline was pressor initially but subsequent similar or larger doses always produced depressor effects. In the presence of β-blockade induced by isoprenaline, α-ethylnoradrenaline always caused a pressor effect.

After propranolol (0.1 - 0.8 mg i.v.) the inhibitory action of noradrenaline was reversed to produce a contraction of the uterus (Fig. 2, p. 120). This effect however often was masked to some extent by the increased uterine motility produced by propranolol itself (Fig. 2, 3, pp. 120, 121). The inhibitory action of hypogastric nerve stimulation was influenced similarly by propranolol so that after β-receptor blockade nerve stimulation produced an excitatory effect. The inhibitory actions of adrenaline and isoprenaline were antagonised but no reversal of effect occurred after propranolol. The excitatory action of noradrenaline after propranolol was blocked by an intravenous injection of 2 mg phentolamine. Tolazoline (1.0 - 2.0 mg i.v.) and dihydroergotamine (0.1 - 1.0 mg i.v.) were without effect on the inhibitory responses produced by the catecholamines.

In nearly all the experiments doses of propranolol which

were required to produce an effective blockade of β-receptors initiated uterine contractions. This was noticeable especially when the muscular tone was low or when the spontaneous contractions were small and infrequent (Fig. 3, p. 121).

RATS IN DIOESTRUS

in vitro experiments

The degree of spontaneous activity of the preparations varied according to the nature of the different experimental conditions used and it appeared to be dependent more upon the bath temperature than upon the composition of the physiological solution employed. For example, at 29 - 32°C very little uterine activity was recorded in either Tyrode, Krebs or de Jalon solutions. At 37 - 38°C fairly regular spontaneous contractions occurred using Tyrode or Krebs solutions, but activity was still weak when de Jalon solution was employed. Aeration of Tyrode solution with either 100% oxygen, 95% oxygen/5% carbon dioxide or air did not produce significantly different results. Results recorded using de Jalon solution aerated with either 100% oxygen or air also did not differ to any great extent.

The choice of bathing media made no apparent difference to the actions of the catecholamines for in all experiments, except for one out of a total of seventeen conducted at this stage of the oestrous cycle, the five amines produced solely inhibitory actions (Fig. 4, p. 122). Only in one experiment did noradrenaline (4×10^{-6}) cause a contraction but this was followed by a prolonged inhibitory action. Subsequent similar concentrations produced only inhibition of uterine activity.

It was found that variation in the relative inhibitory potencies of the amines was considerable, but was no greater between experiments set up under the same conditions than between experiments employing different bathing media. The order of the amines, when arranged according to their relative potencies, was always the same. The values obtained for the relative inhibitory potencies, by estimation of the reduction in height of the spontaneous uterine contractions, did not differ significantly from the values obtained using acetylcholine-induced contractions. The approximate ratios of equiactive concentrations to cause inhibition were, in terms of noradrenaline = 1 (at a concentration of 12.9×10^{-8}); α-ethylnoradrenaline = 0.8; adrenaline = 0.01; N-ethylnoradrenaline = 0.0005; isoprenaline = 0.00001 (Table 2, p. 108).

Except in the one instance reported above, concentrations of up to 4×10^{-4} of each amine failed to produce excitatory actions on the uterus when the animal was in dioestrus.

The inhibitory actions of the catecholamines were not affected appreciably by tolazoline (10^{-6} - 10^{-5}) or by similar concentrations of dihydroergotamine or of phentolamine. Concentrations of phentolamine greater than 10^{-5} antagonised the inhibitory actions of the amines but also depressed spontaneous and acetylcholine-induced uterine contractions to a similar extent.

The inhibitory actions of adrenaline, noradrenaline and isoprenaline were antagonised by 4×10^{-6} pronethalol, although the β-blocking agent itself exerted inhibitory actions on the uterus in concentrations which were required for adrenergic blockade. However, 1×10^{-7} propranolol was found to antagonise the inhibitory actions of all the amines, without exerting any appreciable inhibitory action itself either on natural or on acetylcholine-induced uterine contractions. After treatment with propranolol noradrenaline, adrenaline, N-ethylnoradrenaline and α-ethylnoradrenaline produced excitatory actions, but isoprenaline failed to produce such an effect even with concentrations of up to 8×10^{-4} (Fig. 5, p. 123). The excitatory actions produced after treatment with propranolol were blocked by 4×10^{-6} phentolamine. The approximate ratios of equiactive concentrations to cause excitation after propranolol were, in terms of noradrenaline = 1 (at a concentration of 1×10^{-6}); α-ethylnoradrenaline = 12; adrenaline = 1.9; N-ethylnoradrenaline = 50 (Table 10, p. 117). These ratio values were similar to those obtained from the uteri of rats in oestrus without prior treatment with propranolol.

RATS IN PROESTRUS

in vivo experiments

Uteri of animals at this stage of the oestrous cycle usually exhibited small but regular spontaneous contractions, causing pressure changes of up to 4 mm Hg.

After initial investigations with adrenaline, noradrenaline and isoprenaline in three animals, further experiments on the intact uterus of rats in proestrus were not performed as the results were not appreciably different from those obtained when the animal was in dioestrus (compare Tables 1, 2, 3, pp. 107, 108, 109). Only inhibitory responses to the three amines and to hypogastric nerve stimulation were recorded. The approximate ratios of equiactive doses to cause inhibition were, in terms of noradrenaline = 1 (at a dose of 7.5 μg); adrenaline = 0.023; isoprenaline = 0.0001 (Table 3, p. 109).

The responses to adrenaline and to isoprenaline were antagonised and those to noradrenaline were reversed by 250 μg propranolol. A similar dose of phentolamine had no effect upon the inhibitory actions of the amines but antagonised the excitatory action of noradrenaline which occurred after the injection of propranolol.

It was observed that, as in the dioestrous and oestrous states, propranolol initiated or enhanced uterine contractions especially when the muscular tone was low. This effect was caused by doses of propranolol which were required to produce effective β-receptor blockade.

RATS IN PROESTRUS

in vitro experiments

The preparations showed little spontaneous activity so that the inhibitory effects of the catecholamines were estimated by their activities in reducing acetylcholine-induced contractions.

The actions of the catecholamines on uteri taken from rats in proestrus did not differ appreciably from their actions on uteri from rats in dioestrus, as was observed in the in vivo studies. In nineteen experiments adrenaline, noradrenaline and isoprenaline always caused inhibition of the uterine muscle, except in one experiment in which noradrenaline contracted a quiescent preparation. The approximate ratios of equiactive concentrations to cause inhibition were, in terms of noradrenaline = 1 (at a concentration of 7.9×10^{-8}); adrenaline = 0.013; isoprenaline = 0.000005 (Table 3, p. 109). Tolazoline (1×10^{-5}) did not modify the actions of the catecholamines.

During the investigations with β-blocking agents it was found that dichloroisoprenaline and pronethalol caused inhibitory actions on the uterus in concentrations which were lower than those necessary to produce β-receptor blockade. Thus it was impossible to determine to what extent β-blockade had been achieved with these agents because they depressed the acetylcholine-induced uterine contractions. In relation to the inhibitory action of noradrenaline, dichloroisoprenaline

was approximately one-tenth as active while pronethalol possessed about 1/200th the potency of noradrenaline (Fig. 6, p. 124). After the inhibitory action produced by 4×10^{-7} dichloroisoprenaline a concentration of 1×10^{-5} pronethalol failed to produce an inhibitory effect. Subsequently further concentrations of dichloroisoprenaline were without effect. The inhibitory action of adrenaline also was antagonised by these concentrations (Fig. 6, p. 124).

Propranolol caused a depression of acetylcholine-induced uterine contractions but in concentrations more than 50 times greater than those necessary to antagonise isoprenaline-induced uterine inhibition (Table 11, p. 118).

In the presence of propranolol the actions of noradrenaline and adrenaline were reversed to produce contractions of the uterus, but isoprenaline failed to exhibit excitatory actions in concentrations ranging from 10^{-10} - 10^{-4}. These actions were similar to those recorded on isolated preparations from rats in dioestrus (Fig. 5, p. 123).

RATS IN OESTRUS

in vivo experiments

Uteri from animals in oestrus, in contrast to the uteri from rats in dioestrus and proestrus were, in the majority of experiments, devoid of spontaneous activity. However muscular tone was similar to that in the other stages of the cycle.

In animals in oestrus, in contrast to those in dioestrus and proestrus, noradrenaline always produced a motor response with doses ranging from 0.1 - 20 µg (Fig. 7 - 11, pp. 125 - 129). This effect was recorded in each of a total of eleven experiments. Stimulation of the hypogastric nerve (four experiments) also resulted in uterine contraction (Fig. 8, p. 126), in contrast to the inhibitory action of nerve stimulation in dioestrus. Adrenaline however, produced an inhibitory action (eight experiments) when the animal was in oestrus, although in two instances in separate experiments a dose of 20 µg or 30 µg produced a small contraction followed by a prolonged inhibition (Fig. 11, 8, pp. 129, 126). The two substituted noradrenalines were able to induce contractions of the uterus of the animal in oestrus if large doses were injected, namely 50 µg N-ethylnoradrenaline (Fig. 7, p.125) and 20 µg α-ethylnoradrenaline (Fig. 9, p. 127), but a more powerful inhibition followed as with adrenaline. In no instance was the inhibitory action of isoprenaline reversed to

produce a contraction. This was observed even if a blood pressure reversal response, i.e. a pressor response, was produced by a large dose of isoprenaline (Fig. 12, p. 130). The approximate ratios of equiactive doses to cause excitation for the other four catecholamines were, in terms of noradrenaline = 1 (at a dose of 1.9 μg); α-ethylnoradrenaline = 10; adrenaline = 26; N-ethylnoradrenaline = 63 (Table 4, p. 110).

Although the predominant action of noradrenaline on the uteri of rats in oestrus was excitatory, an inhibitory action of the amine occurred as a secondary component of a biphasic response in four out of eleven experiments. The inhibitory response was observed as an inhibition of a following dose of either noradrenaline (Fig. 10, p. 128) or of acetylcholine (Fig. 9, p. 127). In contrast, a solely inhibitory action was the major effect produced by adrenaline, α-ethylnoradrenaline and N-ethylnoradrenaline and was the only effect produced by isoprenaline (Fig. 7, p. 125). The approximate ratios of equiactive doses to cause inhibition were, in terms of noradrenaline = 1 (at a dose of 14.3 μg); α-ethylnoradrenaline = 2.1; adrenaline = 0.13; N-ethylnoradrenaline = 0.05; isoprenaline = 0.0009 (Table 4, p. 110).

A comparison of the activities of the amines on the uteri of rats in dioestrus and in oestrus showed that adrenaline was

approximately seven times less active in causing uterine inhibition in the oestrous state (compare Tables 1 and 4, pp. 107, 110). This result could not be confirmed by in vitro studies owing to the wider variation in amine activities within any one stage of the cycle.

The motor effect of noradrenaline was antagonised and sometimes reversed by 0.2 - 2.0 mg tolazoline, 0.2 - 1.0 mg phentolamine (Fig. 11, p. 129) and 0.1 - 1.0 mg dihydroergotamine. The effect of stimulating the hypogastric nerve was antagonised to a smaller extent by these agents and could never be abolished or reversed. Propranolol (0.2 - 0.25 mg) had no effect upon the solely excitatory action of noradrenaline but it enhanced the excitatory component of a biphasic response and antagonised the inhibitory phase (Fig. 10, p. 128). The inhibitory action of adrenaline was reversed by propranolol. The subsequent excitatory effect and the excitatory effect of noradrenaline were blocked by 250 µg dihydroergotamine. The inhibitory action of isoprenaline although antagonised was never reversed by propranolol.

As in the other stages of the oestrous cycle propranolol initiated or enhanced uterine motility in doses which were required to produce β-blockade, thus making any accurate quantitative evaluation of results difficult.

In regard to the effects of the amines on the blood pressure (both i.v. and i.a. routes) the response to N-ethylnoradrenaline was, like that to adrenaline, biphasic, causing a depressor response with low doses and a pressor effect with 50 μg or more. The first response to α-ethylnoradrenaline (5 - 10 μg) was pressor, but subsequent similar or larger doses were always depressor. Reversal of the isoprenaline depressor response occurred with repeated doses of the order of 1 mg and a pressor dose-response curve was obtained with doses from 1 - 10 mg (Fig. 12, p. 130). After the isoprenaline depressor reversal, subsequent doses of the other catecholamines always produced a pressor response and changes in both the order and the ratios of potency occurred. The pressor activities of the catecholamines were:

(i) before isoprenaline depressor reversal:
noradrenaline > adrenaline > α-ethylnoradrenaline > N-ethylnoradrenaline > isoprenaline;

(ii) after isoprenaline depressor reversal:
noradrenaline = adrenaline > N-ethylnoradrenaline > α-ethylnoradrenaline > isoprenaline.

RATS IN OESTRUS

in vitro experiments

The majority of uteri were devoid of spontaneous rhythm except for an infrequent period of powerful contractions or a single maximal contraction. The activity of the smooth muscle was not influenced appreciably by changes in bath temperature from 29 - 38°C or by using either Krebs or Tyrode solutions. No spontaneous activity was recorded however, in preparations set up in de Jalon solution.

As observed in the in vivo studies, the great difference between the actions of the sympathomimetic amines at this stage and that of dioestrus was that noradrenaline elicited a motor response. This effect was observed in each of a total of 23 experiments, and such responses were recorded with both isometric and isotonic levers (Fig. 14, 15, pp. 132, 133). Temperature changes from 29 - 38°C did not alter appreciably the excitatory response to noradrenaline and contractions were obtained from preparations bathed in de Jalon, Krebs or Tyrode solutions. The contractile effect was produced with concentrations ranging from 2×10^{-8} - 4×10^{-6} in the different experiments. This wide range of effective concentrations appeared to be due to differences in the activities of the preparations and could not be attributed to the use of different bathing media or recording levers. Although a contractile response was observed more readily on quiescent preparations

(Fig. 15, p. 133), it was not dependent solely upon the tone of the musculature, for a contraction was produced in preparations to which regular concentrations of acetylcholine were applied (Fig. 13, p. 131) or in those showing spontaneous activity (Fig. 14, p. 132). In some preparations, concentrations of noradrenaline below those required to produce a uterine contraction caused an increased response to acetylcholine (Fig. 13, p. 131). This action occurred only in experiments in which noradrenaline caused solely an excitatory response. The potentiation of acetylcholine persisted for at least three contractions following the addition of noradrenaline to the bathing solution. The potentiation could be prolonged however, if noradrenaline was administered at regular intervals. A similar potentiation of spontaneous uterine contractions occurred in other experiments. In approximately half of the experiments in which noradrenaline was observed to contract the uterus, the response was followed by an inhibitory action (Fig. 17, p. 135). In these preparations a concentration of noradrenaline smaller than that which produced this biphasic action caused solely an inhibitory effect.

Although the predominant responses to adrenaline, phenylephrine, α-ethylnoradrenaline and N-ethylnoradrenaline were inhibitory, in higher concentrations these amines contracted the uterus, but these contractions were

followed always by an inhibitory phase. The greater the inhibitory activity of the amine the greater was the inhibitory component of the response in relation to the initial excitatory effect (Table 9a, p. 115). These biphasic actions were observed only in those preparations where noradrenaline caused solely an excitatory response (Fig. 15, p. 133) and not in those where its action was biphasic. Isoprenaline in concentrations up to 4×10^{-4} failed to exhibit any excitatory activity. Even after the application of high concentrations of the amine, only a pronounced inhibitory action was observed. The approximate ratios of equiactive concentrations to cause excitation were, in terms of noradrenaline = 1 (at a concentration of 114×10^{-8}); α-ethylnoradrenaline = 1.0; adrenaline = 8.9; phenylephrine = 23; N-ethylnoradrenaline = 110 (Table 5, p. 111).

All the amines were able to exert inhibitory effects either as solely inhibitory actions or as the secondary component of a biphasic response. As in the other stages of the oestrous cycle, considerable variation occurred between the concentrations required to produce similar effects in different experiments. However, when the amines were arranged according to their relative potencies, the order was the same as that found for the in vivo experiments. The approximate ratios of equiactive concentrations to cause inhibition were, in terms of

noradrenaline = 1 (at a concentration of 70 x 10^{-8}); α-ethylnoradrenaline = 1.0; adrenaline = 0.01; phenylephrine = 0.9; N-ethylnoradrenaline = 0.001; isoprenaline = 0.0000005 (Table 5, p. 111).

The excitatory actions of the amines were antagonised by 2 x 10^{-6} tolazoline (Fig. 16, p. 134) and by 4 x 10^{-7} phentolamine, and subsequently the amines produced inhibitory effects. The order of the amines, when arranged according to their relative inhibitory potencies, was identical to the order obtained for inhibitory effects on the uteri of rats in dioestrus and proestrus. In those oestrous preparations where noradrenaline caused solely an excitatory action, tolazoline exposed the inhibitory action of noradrenaline and enhanced the inhibitory activity of all the other amines except isoprenaline. In those preparations where the response to noradrenaline was biphasic, tolazoline did not potentiate the inhibitory action either of noradrenaline or of the other amines (Fig. 16, p. 134).

Propranolol (4 x 10^{-8} - 4 x 10^{-7}) blocked the inhibitory actions of all the amines. These included (1) the solely inhibitory responses, e.g. to isoprenaline; (2) the inhibitory component of a biphasic response, e.g. to adrenaline and (3) the inhibitory actions produced after treatment with tolazoline or phentolamine. The excitatory component of the

responses to the amines was enhanced after propranolol (Fig. 17, p. 135). The following approximate ratios of equiactive concentrations to cause excitation after propranolol were, in terms of noradrenaline = 1 (at a concentration of 0.6×10^{-6}); adrenaline = 2.9; phenylephrine = 12; N-ethylnoradrenaline = 56 (Table 10, p. 117). The ratios of noradrenaline to adrenaline, phenylephrine and to N-ethylnoradrenaline were smaller than those recorded in the absence of propranolol (Table 10, p. 117). Isoprenaline did not exert any excitatory effect after the addition of propranolol to the bathing solution. Even the repeated application of concentrations of the order of 10^{-4}, which did not produce uterine inhibition, failed to exert any excitatory effect (Fig. 17, p. 135).

In the concentrations in which they were employed to produce receptor blockade, the adrenergic blocking agents used in these <u>in vitro</u> experiments did not influence greatly the uterine activity, especially in regard to the modification of the acetylcholine-induced contractions which were often used as a measure of the sympathomimetic inhibitory activity. Phentolamine however, in larger concentrations (10^{-5}) than those effective for α-receptor blockade (10^{-7} - 10^{-6}), caused a non-specific depression of uterine activity.

IMMATURE RATS

in vivo experiments

Owing to the small size of the uteri of immature rats, experiments recording uterine movements in vivo were not attempted.

IMMATURE, UNTREATED RATS

in vitro experiments

In seven experiments the preparations showed very little spontaneous activity so that the inhibitory actions of the amines were estimated by their activities in reducing acetylcholine-induced contractions.

All five catecholamines produced solely inhibitory actions (Fig. 18, p. 136), comparable to their effects on the uteri from adult animals in dioestrus and proestrus. Similar concentrations were necessary to produce these effects in uteri from both immature and adult rats. The approximate ratios of equiactive concentrations to cause inhibition were, in terms of noradrenaline = 1 (at a concentration of 380×10^{-9}); α-ethylnoradrenaline = 0.7; adrenaline = 0.01; N-ethylnoradrenaline = 0.003; isoprenaline = 0.000007 (Table 6, p. 112).

Concentrations up to 4×10^{-5} of each amine failed to contract the uteri of immature rats (Fig. 18, p. 136).

After β-receptor blockade with 4×10^{-7} propranolol the inhibitory actions of the amines were antagonised. In some experiments the actions of noradrenaline, adrenaline and phenylephrine were reversed to produce motor effects, but these responses were weak and achieved only with concentrations of the order of 10^{-5}. The approximate ratios of equiactive concentrations to cause excitation after propranolol were, in terms of noradrenaline = 1 (at a concentration of 4.0×10^{-6}); adrenaline = 2.0; phenylephrine = 10 (Table 10, p. 117).

IMMATURE, OESTROGEN-TREATED RATS

in vitro experiments

Treatment of immature rats with oestradiol, given either as a single large dose (one experiment) or as repeated small doses (two experiments) (p. 51), caused a large increase in uterine weight. Uteri from the untreated immature animals always weighed less than 50 mg (wet weight). Treatment with oestradiol caused a four to eleven-fold increase in the uterine weight (excluding the weight of fluid present in the lumen produced by the hormone treatment). These preparations were devoid of spontaneous activity as were the uteri from the untreated animals.

Adrenaline, noradrenaline and isoprenaline caused inhibition of acetylcholine-induced uterine contractions, and these preparations were considerably more sensitive to the catecholamines than uteri from the untreated immature or the adult animals. However, the ratio values were similar in both untreated and oestrogen-treated animals (Table 6, p. 112). The approximate ratios of equiactive concentrations to cause inhibition were, in terms of noradrenaline = 1 (at a concentration of 4.1×10^{-9}); adrenaline = 0.004; isoprenaline = 0.00002 (Table 6, p. 112).

In all three experiments when the concentration of noradrenaline was increased to approximately 100 times that necessary to produce inhibition, a contraction of the uterus

occurred, followed by a longer period of inhibition. In one experiment a similar effect was recorded with adrenaline, which possessed about one fifth of the potency of noradrenaline in eliciting a uterine contraction (Table 6, p. 112). After 4×10^{-7} propranolol, the inhibitory component of these responses to adrenaline and to noradrenaline was antagonised without altering appreciably the motor response to noradrenaline. The excitatory effect of adrenaline, however, was enhanced, so that it became approximately equipotent with noradrenaline (Table 10, p. 117). In the two experiments in which adrenaline failed to contract the uterus, the amine produced a contraction after propranolol. The inhibitory response to isoprenaline was blocked but not reversed by propranolol.

IMMATURE, PROGESTERONE-TREATED RATS

in vitro experiments

In two animals progesterone treatment (p. 51) caused increases in the weight of the uteri but these were smaller than those of the uteri from oestradiol-treated rats. The preparations exhibited a greater degree of spontaneous activity than the uteri from untreated rats, but the contractions were relatively weak when compared with those of uterine preparations from adult animals.

Only with noradrenaline was it possible to obtain a motor response, but concentrations of the order of 10^{-5} were required to produce this effect. These concentrations were at least ten times greater than those necessary to produce such a response in uteri from animals which had received oestradiol. This effect was associated always with a following inhibitory component.

Adrenaline, noradrenaline and isoprenaline caused inhibitory actions in concentrations similar to those which were effective on uteri from rats which had received oestradiol. The approximate ratios of equiactive concentrations to cause inhibition were, in terms of noradrenaline = 1 (at a concentration of 12×10^{-9}); adrenaline = 0.01; isoprenaline = 0.00005 (Table 6, p. 112).

IMMATURE, OESTROGEN + PROGESTERONE-TREATED RATS

in vitro experiments

In three experiments the administration of oestrogen and progesterone (p. 51) caused increases in uterine weight similar to those caused by oestradiol alone. These preparations were devoid of spontaneous activity except for an infrequent, large contraction or a short period of contractile activity.

The responses to adrenaline, noradrenaline and isoprenaline did not differ qualitatively from those of uteri from rats treated with oestradiol alone. Quantitatively however, these preparations were less sensitive to the inhibitory actions of the three amines than the latter preparations (Table 6, p. 112). In one experiment noradrenaline caused solely an excitatory effect in a concentration of 2×10^{-7}. Lower concentrations caused solely inhibitory effects, while higher concentrations caused biphasic effects of excitation followed by inhibition. In this experiment adrenaline in concentrations below 1×10^{-7} produced solely inhibition, while concentrations greater than 2×10^{-7} produced biphasic effects. The ratio of the concentration of adrenaline to the equiactive concentration of noradrenaline to cause uterine excitation was 20 (Fig. 19, p. 137). In the other two experiments noradrenaline caused inhibitory effects in low concentrations and biphasic responses in higher concentrations, while adrenaline produced inhibitory

effects at all concentrations used. Isoprenaline always produced solely inhibitory effects in all three experiments. The approximate ratios of equiactive concentrations to cause inhibition were, in terms of noradrenaline = 1 (at a concentration of 413×10^{-9}); adrenaline = 0.08; isoprenaline = 0.000006 (Table 6, p. 112).

The excitatory actions of adrenaline and noradrenaline were antagonised by 2×10^{-6} tolazoline.

OVARIECTOMISED, UNTREATED RATS

in vivo experiments

Owing to the small size of the uteri of ovariectomised untreated animals, attempts at recording uterine movements in vivo were not successful.

in vitro experiments

The uteri from ovariectomised rats were smaller but otherwise of similar appearance to those from rats in dioestrus. The four preparations showed some degree of spontaneous activity which was sufficient to use in an estimation of inhibitory actions of the sympathomimetic amines.

The five amines produced only inhibitory actions as in the dioestrous and proestrous states (Fig. 20, p. 138). The approximate ratios of equiactive concentrations to cause inhibition were, in terms of noradrenaline = 1 (at a concentration of 0.47×10^{-8}); α-ethylnoradrenaline = 1.5; adrenaline = 0.014; N-ethylnoradrenaline = 0.016; isoprenaline = 0.00001 (Table 8, p. 114). These preparations were about twenty times more sensitive to the amines than those from rats in dioestrus and proestrus and, in more than one instance, inhibitory responses to concentrations of the order of 10^{-15} isoprenaline were obtained. The small disturbance caused by the addition of the solution to the bathing medium was not in itself sufficient to cause a response because a

comparable volume of saline or bathing solution produced no effect.

Treatment with propranolol antagonised the responses to isoprenaline, N-ethylnoradrenaline and α-ethylnoradrenaline. In all experiments the action of noradrenaline was reversed to produce an excitatory effect in the presence of propranolol. The responses to adrenaline were blocked but in two out of three experiments, when the concentration of adrenaline was increased to 1×10^{-6}, excitatory actions were produced. The ratio of the concentration of adrenaline to the equiactive concentration of noradrenaline to produce uterine excitation was approximately 10 in the presence of propranolol (Table 10, p. 117).

OVARIECTOMISED, OESTROGEN-TREATED RATS

in vivo experiments

The uteri of two animals which had received a single injection of 500 µg oestradiol (p. 51) were much larger and more vascular than the uteri from ovariectomised, untreated rats, and therefore, it was possible to record uterine activity. In neither experiment did the preparations exhibit any spontaneous contractions.

Doses of 2 - 10 µg noradrenaline caused excitatory actions, but in both experiments a following inhibition of uterine activity occurred. Adrenaline in doses up to 20 µg produced no excitatory action.

Both amines caused inhibition of acetylcholine-induced uterine contractions, the inhibitory action of noradrenaline occurring with doses lower than those necessary to produce excitatory effects. The ratio of the dose of adrenaline to the equiactive dose of noradrenaline to cause inhibition was 0.04 (Table 7, p. 113).

in vitro experiments

Treatment with oestradiol given either as a single large dose (two experiments) or as repeated small doses (one experiment) (p. 51), caused increases in weights (approximately 250%) of the uteri of ovariectomised rats. These preparations were usually quiescent except for an occasional large

contraction.

As with the uteri from immature animals which had received oestrogen, noradrenaline caused, in two out of three experiments, solely excitatory effects with concentrations from 10^{-7} - 10^{-6}. Higher concentrations produced biphasic effects of contraction followed by inhibition. In one experiment the amine produced only inhibitory actions in concentrations from 4×10^{-9} - 4×10^{-5}. This different response did not appear to be related to the dose of oestrogen given. Adrenaline, in one experiment only (Fig. 21, p. 139), produced a biphasic action when the concentration applied was of the order of 10,000 times that necessary to produce solely an inhibitory response.

All the five amines produced uterine inhibition and the approximate ratios of equiactive concentrations to cause inhibition were, in terms of noradrenaline = 1 (at a concentration of 38×10^{-8}); α-ethylnoradrenaline = 1.0; adrenaline = 0.004; N-ethylnoradrenaline = 0.0004; isoprenaline = 0.0000015 (Table 8, p. 114).

Treatment with propranolol antagonised the inhibitory responses to all the amines. In the experiment in which adrenaline caused a motor action propranolol enhanced this effect, but the concentration of noradrenaline required to produce a contraction was not reduced appreciably. Thus, after propranolol, the ratio of the concentration of adrenaline

to the equiactive concentration of noradrenaline was decreased from 25 to 1.3 (Table 10, p. 117).

In one experiment using the uterus from an ovariectomised rat which had not received any hormone treatment, the effect of adding a water-soluble oestrogenic material, 17β-oestradiol hemisulphate sodium, to the bathing medium was investigated. In a concentration of 1×10^{-6} this substance was allowed to remain in the bath for 30 minutes. This treatment had no effect on the response of the uterus to noradrenaline.

OVARIECTOMISED, PROGESTERONE-TREATED RATS

in vivo experiments

Treatment of ovariectomised rats with progesterone (p. 51) did not produce any noticeable degree of uterine proliferation. Owing to the small size of the uteri of these animals attempts at recording uterine movements in vivo were not successful.

in vitro experiments

In contrast to uteri from immature rats, preparations from three ovariectomised animals showed no appreciable weight gain after progesterone treatment (p. 51).

Only inhibitory responses to all the catecholamines were obtained (Fig. 22, p. 140), and although these preparations were as sensitive to the amines as uteri from normal adult animals, they were considerably less sensitive than similar preparations from ovariectomised rats which had not received progesterone treatment. The approximate ratios of equiactive concentrations to cause inhibition were, in terms of noradrenaline = 1 (at a concentration of 48×10^{-8}); α-ethylnoradrenaline = 0.27; adrenaline = 0.008; N-ethylnoradrenaline = 0.0015; isoprenaline = 0.000002 (Table 8, p. 114).

After 4×10^{-7} propranolol the inhibitory responses to all the amines were antagonised but only noradrenaline produced a motor response, i.e. a reversal of its action before propranolol (Fig. 22, p. 140).

OVARIECTOMISED, OESTROGEN + PROGESTERONE-TREATED RATS

in vivo experiments

The uteri of two animals which had received oestrogen and progesterone (p. 51) were much larger and more vascular than the uteri from ovariectomised, untreated rats or from those which had received progesterone only. In neither experiment did the preparation exhibit any spontaneous contractions.

In one experiment a dose of 0.2 µg noradrenaline produced solely an excitatory response while a tenfold increase in dose caused, as well as the motor action, a secondary inhibitory effect. This biphasic response was recorded in a subsequent experiment, when 4 µg α-ethylnoradrenaline also produced a very weak excitatory action followed by a more pronounced inhibitory effect (Fig. 23, p. 141). Adrenaline, N-ethylnoradrenaline and isoprenaline were devoid of excitatory activity.

Adrenaline caused an inhibition of acetylcholine-induced contractions in doses as low as 0.01 µg and the other catecholamines employed also caused similar effects (Fig. 23, p. 141). The following approximate ratios of equiactive doses to cause inhibition were, in terms of noradrenaline = 1 (at a dose of 3.0 µg); α-ethylnoradrenaline = 0.03; adrenaline = 0.02; N-ethylnoradrenaline = 0.005; isoprenaline = 0.001 (Table 7, p. 113).

After propranolol, not only noradrenaline, but adrenaline

and α-ethylnoradrenaline exerted solely excitatory actions. A dose of 20 μg N-ethylnoradrenaline caused a biphasic response, but a dose of 2 μg isoprenaline surmounted the propranolol blockade to produce uterine inhibition. The approximate ratios of equiactive doses to cause excitation after propranolol were, in terms of noradrenaline = 1 (at a dose of 1.0 μg); α-ethylnoradrenaline = 3.5; adrenaline = 1.5; N-ethylnoradrenaline = 450. A further increase in the dose of isoprenaline up to 5 mg failed to cause a contraction of the uterus in the presence of propranolol.

Doses of propranolol which were required to produce an effective blockade of β-receptors initiated strong uterine contractions. This effect was noticed in other in vivo experiments in normal adult rats at all stages of the oestrous cycle.

in vitro experiments

The administration of oestrogen and progesterone (p. 51) caused similar increases in uterine weight to those produced by oestradiol alone (p. 83). The preparations were devoid of spontaneous activity except for an infrequent, maximal contraction.

Excitatory responses to noradrenaline and adrenaline were obtained more frequently than in preparations from rats which had received oestradiol alone (Table 8, p. 114). In two

of the four experiments noradrenaline, in concentrations from 1×10^{-8} - 1×10^{-7}, caused solely excitatory effects, while higher concentrations produced biphasic responses. In the two other experiments noradrenaline failed to produce solely excitatory responses but caused biphasic effects in concentrations of the order of 10^{-7}. Adrenaline also produced biphasic actions but only in the two experiments in which the action of noradrenaline was solely excitatory. The ratio of the concentration of adrenaline to the equiactive concentration of noradrenaline to cause excitation was 30 (Table 8, p. 114).

α-Ethylnoradrenaline, N-ethylnoradrenaline and isoprenaline produced only inhibitory actions. Adrenaline also produced inhibition in all four experiments, but in concentrations lower than those which produced the biphasic responses. Noradrenaline caused inhibitory actions only in the two experiments in which it failed to produce solely excitatory effects and, as with adrenaline, these inhibitory effects were produced with concentrations lower than those which produced biphasic responses. The approximate ratios of equiactive concentrations to cause inhibition were, in terms of noradrenaline = 1 (at a concentration of 58×10^{-8}); α-ethylnoradrenaline = 0.25; adrenaline = 0.015; N-ethylnoradrenaline = 0.013; isoprenaline = 0.00001 (Table 8, p. 114).

In one experiment using the uterus from an ovariectomised rat which had received progesterone treatment, the effect of adding the water-soluble 17β-oestradiol hemisulphate sodium to the bathing medium was investigated. In a concentration of 1×10^{-5} this substance was allowed to remain in the bath for 30 minutes. It produced no observable differences in the responses of the uterus to noradrenaline, adrenaline and isoprenaline. Conversely, addition of 1×10^{-5} of a water soluble progestational substance, Primolut-aqueous, to the solution bathing a uterine preparation from an oestrogen-treated, ovariectomised animal, had no effect on the inhibitory response to noradrenaline.

Summaries of the results of the experiments performed on the isolated uterus of the rat are given in Tables 9a (p. 115) and 9b (p. 116). Table 9a shows the transition from the solely inhibitory actions of the sympathomimetic amines on the uteri from rats in dioestrus to their excitatory or biphasic effects on the uteri from animals in oestrus. Table 9b indicates the ratios of equiactive concentrations of the amines to produce their respective excitatory and/or inhibitory actions on the uteri from rats in different sexual states.

DISCUSSION

The employment of a series of sympathomimetic amines together with antagonists at α- and β-adrenergic receptors has confirmed the existence of α-excitatory and β-inhibitory receptors in the uterus of the rat. Although both α- and β-receptors have been shown to be present at all stages of the oestrous cycle, the actions of the amines varied according to the sexual state of the rat. In dioestrus and proestrus all the amines produced solely inhibition of the uterus. When the animal was in oestrus however, contractile responses were produced to some of the amines. Noradrenaline was the only amine to produce solely an excitatory response. Adrenaline, phenylephrine and the α-ethyl- and N-ethyl-substituted noradrenalines also initiated uterine contractions, although to a lesser extent than noradrenaline, but these effects were followed by an inhibitory phase. The greater the inhibitory activity of the amine in dioestrus, the greater was the inhibitory component of the biphasic response in relation to the initial excitatory effect in oestrus. It was important when investigating the possible motor activity of an amine on the isolated uterus from a rat in oestrus to examine the effects of the widest concentration range practicable for, except with noradrenaline, the amines always exerted inhibitory actions in concentrations lower than those necessary to exert

the biphasic actions. Further, repeated application of the amines in concentrations which produced the biphasic actions caused a decline in the excitatory component of the responses. This was because the more prolonged inhibitory phase suppressed the excitatory component on further administration of the amine. Only with noradrenaline was it possible to produce quantitatively similar responses with repeated application of the amine. Other factors which also influenced the nature of the biphasic responses are discussed on p. 266. The responses to hypogastric nerve stimulation closely paralleled those to noradrenaline. In dioestrus and proestrus nerve stimulation resulted in uterine inhibition, but in oestrus the response was reversed to a contractile effect.

Throughout this work the results obtained in the in vitro experiments correlated very well with those of the in vivo studies. This indicates the usefulness of the in vitro studies as a guide to the determination of in vivo effects when these could not be recorded, as in the immature rat. Further, it suggests that the effects of the amines in vivo on tissues other than the uterus, do not modify greatly the effects of the amines on the uterus itself.

The response of a tissue to a particular sympathomimetic amine depends to a greater or lesser extent upon the relative number and/or activity of the adrenergic α-receptors to the number and/or activity of β-receptors present in that tissue.

Thus any alteration in this relationship, such as is produced by the use of adrenergic blocking agents, will alter the response of the tissue to the amine. Since such an alteration of the responses of the uterus of the rat to the amines occurs during the oestrous cycle, it is possible that in some way the relative number and/or activity of α-receptors to β-receptors changes as the oestrous cycle progresses. The results of this work suggest that during the oestrous phase of the cycle the number and/or activity of uterine α-receptors is increased. This is suggested because: (i) the responses of the uterus from a rat in oestrus were similar to the responses of the uterus from a rat in dioestrus after treatment with propranolol; (ii) the responses of the uterus of a rat in oestrus after treatment with tolazoline or phentolamine resembled the responses of the uterus from a rat in dioestrus; (iii) larger doses of adrenaline were necessary to produce inhibition of uteri from rats in oestrus than from rats in dioestrus. An alternative interpretation of these results is that in oestrus the number and/or activity of β-receptors is decreased, but it is considered that this is unlikely because the potency of isoprenaline in causing uterine inhibition was not appreciably different in the various stages of the oestrous cycle.

In regard to inhibitory actions isoprenaline proved to be the most potent in the series of sympathomimetic amines.

Reduction in the size of the grouping attached to the nitrogen atom from $-CH(CH_3)_2$ to $-C_2H_5$, $-CH_3$ and -H, caused a progressive decrease in inhibitory potency. The addition of a $-C_2H_5$ group to the α-carbon atom in the side chain of noradrenaline did not influence appreciably the inhibitory activity while the removal of a catechol -OH group from adrenaline greatly reduced the activity. These results are in agreement with the work of recent authors (Lish et al., 1965; Schild, 1967), although in the earlier paper of Gaddum et al. (1949) it was found that an increase in the size of the groups on the nitrogen atom did little to alter the inhibitory potency of the catecholamines. This conclusion may have been reached because the uteri were suspended in de Jalon solution at 29°C. These conditions greatly reduced the sensitivity of the preparation to isoprenaline (Lish et al., 1965).

The fact that propranolol and pronethalol, and to a lesser extent dichloroisoprenaline, antagonised these inhibitory responses to the amines indicated that they were mediated via β-receptors. This is supported by the observation that tolazoline enhanced the inhibitory action of all the amines except isoprenaline on preparations from rats in oestrus, although it produced no significant effect on the uteri from animals in dioestrus. These results confirm and extend those reported recently (Levy and Tozzi, 1963; Brookes et al., 1965; Diamond and Brody, 1966a, b; Tothill, 1967b). The inhibitory

responses of uteri from rats in dioestrus and proestrus to hypogastric nerve stimulation were reversed by propranolol, thus indicating that the responses were mediated via β-receptors.

It has been reported that the α-blocking agents used in this work antagonise the inhibitory responses to the sympathomimetic amines. Wiqvist (1959) and Rudzik and Miller (1962a) found phentolamine effective in this respect, but these workers used a dose of 2.5 mg in a 50 ml. organ bath. In the work reported here it was found that doses of this order, which were at least ten times greater than those necessary for α-receptor blockade in this preparation, caused a non-specific depression of the uterus and of its response to acetylcholine. Thus the depression of the inhibitory responses to the sympathomimetic amines was non-selective. Phentolamine also depressed the activity of the amines on isolated preparations of rabbit (p. 162) and of human (p. 249) uterine tissue when large doses were employed. Similarly, Jensen and Vennerød (1961b) found that tolazoline blocked adrenaline-induced inhibition of the uterus of the rat but noted also that it depressed uterine activity and the response to histamine.

An analysis of the contractile responses, either of uteri from rats in oestrus or of uteri treated with propranolol from rats at all stages of the oestrous cycle, shows that the order of potency for eliciting motor responses was the reverse of

that for eliciting inhibitory effects on β-receptors, i.e. noradrenaline > adrenaline > phenylephrine ≫ N-ethylnoradrenaline. A comparison between noradrenaline and α-ethylnoradrenaline shows that substitution on the α-carbon atom reduces the activity in eliciting contractile responses. A greater loss of activity occurs when the substitution is on the nitrogen atom, as is shown by a comparison of the activities of the α-ethyl- and N-ethyl-substituted noradrenalines. Removal of a catechol -OH group from adrenaline to form phenylephrine also reduces the activity at the excitatory receptor. A study of these structure-activity relationships shows that it is more important for both catechol -OH groups to be present for optimum inhibitory activity than for excitatory activity, while substitution on the α-carbon atom reduces excitatory rather than inhibitory activity.

Phentolamine and tolazoline in concentrations of the order of 10^{-6} antagonised all the motor effects of the amines on the uterus. Where the response was biphasic, the inhibitory component persisted or even was enhanced, indicating that its production was not dependent upon a prior motor effect. Propranolol was effective in antagonising this secondary inhibition. The motor response to hypogastric nerve stimulation was antagonised by phentolamine, tolazoline and dihydroergotamine. These results confirm and extend those

of Mann (1950) and Diamond and Brody (1966b, 1967) and indicate that the motor effects of sympathomimetic amines and of hypogastric nerve stimulation are exerted via α-receptors. The employment of a β-blocking agent in both *in vivo* and *in vitro* preparations markedly enhanced the ability of the amines to exert a motor effect on the uteri from rats in oestrus. Moreover, it revealed a motor action of the amines on the uteri from rats in dioestrus and in proestrus. This exposure of a motor effect has been reported by Jensen and Vennerød (1961a) using dichloroisoprenaline, by Brookes *et al.* (1965) using MJ-1999, and by Diamond and Brody (1966a, b) using propranolol; all these studies being performed on *in vitro* preparations from rats in the oestrous phase only. Tothill (1967a, b) used isoprenaline to induce β-receptor blockade and reported that motor responses to adrenaline, noradrenaline and phenylephrine could be obtained but only when the animal was in an oestrous state.

In *in vitro* preparations from rats in oestrus, the concentration of noradrenaline required to produce a motor action was approximately six times that necessary to produce an inhibitory response in dioestrous preparations. For phenylephrine the increase was approximately 100 and for adrenaline 2,000. Also, higher concentrations were necessary to produce motor responses in dioestrus and proestrus after β-receptor blockade. Jensen and Vennerød (1961a) indicated

that after dichloroisoprenaline a motor effect could be obtained to adrenaline after a 100-fold increase in the concentration required to produce an inhibitory action prior to the β-receptor blockade. This failure to use higher concentrations may explain why motor effects were not observed on uteri from rats in oestrus (Rudzik and Miller, 1962a; Brookes et al., 1965; Tothill, 1967a, b), even after β-receptor blockade (Levy and Tozzi, 1963). In this latter work however, β-blockade was attempted with pronethalol, which itself has been shown by Vanov and Vogt (1963) and by Lish et al. (1965), and also reported in this work, to possess considerable inhibitory sympathomimetic activity on the uterus of the rat in concentrations lower than those required for effective β-receptor blockade. Moreover, phenylephrine was used by Levy and Tozzi (1963) as the amine most likely to stimulate α-receptors, whereas this work has indicated that noradrenaline is the most potent in stimulating α-receptors in the uterus of the rat.

In an attempt to determine how the alterations of the uterine responses to the amines are brought about it was shown in this work, and has been reported in earlier studies (Mann, 1950; Diamond and Brody, 1966b), that the production of contractile responses to noradrenaline and to adrenaline could be produced in uteri from ovariectomised adult rats which had received injections of oestradiol. The selection of the

doses of oestradiol was based upon the amount required to produce myometrial and endometrial changes comparable to those which occur naturally during the different sexual states as a result of ovarian secretions. Small doses of oestrogen (10 µg/kg) have been found to induce a vaginal smear pattern identical to that of natural oestrus and much larger single doses (e.g. 1 mg/kg) produce similar effects with little reduction in the time taken to produce endometrial proliferation (Reynolds, 1949; Parkes, 1966). Small, repeated doses and single large doses have been used in this work and it was found that both injection regimes resulted in the production of a contractile response to noradrenaline. The important factor however, is the delay involved between the injection and the observation of an endometrial change in the animal, for it is only when the vaginal smear consists predominantly of cornified cells that a reversal of the response to the amines occurs. A dose of oestrogen takes 44 - 72 hours to produce endometrial and myometrial proliferation in the rat (Allen, 1932).

A motor response of the uterus of the rat to sympathomimetic amines has been observed after either a single large injection of oestrogen (0.5 mg oestradiol dipropionate, Mann, 1950; 2.5 mg/kg diethylstilboestrol, Tothill, 1967a), or a number of small doses (50 - 75 µg diethylstilboestrol daily for 6 - 12 days, Paton, 1968), or a single small dose (50 µg

oestradiol benzoate, Diamond and Brody, 1966a). All these experiments were performed at least 40 hours after the injection. Levy and Tozzi (1963) found that sympathomimetic amines were unable to produce motor responses on the uteri of rats pretreated with 100 µg/kg diethylstilboestrol. However, it may be pertinent that their experiments were performed only 24 hours after the injection of oestrogen when the hormone was unlikely to have produced its full physiological effect.

It has been considered that the role of the ovarian hormones in altering the uterine response to sympathomimetic amines may be further implicated if an action of the hormones can be demonstrated on the isolated uterus. Graham and Gurd (1960) found, using the isolated uterus from the non-pregnant cat, that the introduction of progesterone in oil into the bathing solution was without effect on the action of catecholamines. It is possible that this may have been caused to some extent by the low solubility of the progesterone in the aqueous medium. It has been shown that water-soluble ovarian hormones, when added to the bathing solution, alter the responses of the isolated uterus of the rat to oxytocin, 5-hydroxytryptamine and acetylcholine (Neumann and Hempel, 1965). However, in this work it was found that the addition of a water-soluble oestrogen to the bathing solution failed to reverse the response to noradrenaline of a preparation from an ovariectomised rat.

During the course of *in vivo* experiments in which propranolol was employed, it was found that the blocking agent in doses necessary to cause β-blockade, initiated or enhanced the motility of the uterus. That this action did not occur *in vitro* indicates that the effect was unlikely to be produced as a consequence of a direct action of the blocking agent on the myometrium. It is possible that the effect was caused by the blockade of a sympathetic inhibitory control of the myometrium via the hypogastric nerve. This action occurred however, in animals in oestrus, when hypogastric nerve stimulation caused uterine excitation. This could be explained if it is assumed that some of the transmitter would normally excite uterine β-receptors as well as α-receptors, and therefore after β-receptor blockade more transmitter would activate α-receptors and thus increase the excitatory effect of nerve stimulation. Also, propranolol has been shown to reduce transmitter uptake into sympathetic neuronal stores (Foo *et al.*, 1968) and this action would increase the level of transmitter available to act at α-receptors. The use of an α-blocking agent however, did not show conclusively whether the increased uterine activity produced by propranolol was caused by increased α-receptor stimulation. In two experiments α-blockade did reduce the action of propranolol, but in three others no modification of its effect was seen. This may have been due to an interference with the establishment of α-blockade by

propranolol, an action which has been described by Sharma (1966), Olivares et al. (1967) and by Yamamura and Horita (1968). A further possible mechanism of action may be through a local anaesthetic effect on the uterine nerves. The potent local anaesthetic property of propranolol has been described by Lucchesi et al. (1967) and by Day, Owen and Warren (1968). Alternatively, the action of propranolol may be caused by its conversion in vivo to a substance possessing uterine excitatory properties. The development of more specific β-blocking agents will help to resolve how this action of propranolol is effected.

SUMMARY

1. Only α-excitatory and β-inhibitory adrenergic receptors have been detected in the uteri of adult rats at all stages of the oestrous cycle, in both *in vivo* and *in vitro* experiments.

2. The responses of the uterus to the sympathomimetic amines differed qualitatively in the different stages of the oestrous cycle.

Noradrenaline caused solely inhibitory effects in dioestrus and proestrus. In oestrus this effect was reversed either to a solely excitatory action or to a biphasic effect of excitation followed by inhibition.

Adrenaline caused solely inhibitory effects in dioestrus and proestrus. In oestrus, where noradrenaline produced solely an excitatory effect, adrenaline caused a contraction followed by a longer period of inhibition. α-Ethylnoradrenaline, N-ethylnoradrenaline and phenylephrine produced effects which were similar to those produced by adrenaline. The stronger the inhibitory activity of the amine the weaker was the motor component of the biphasic response which occurred in oestrus.

Isoprenaline caused solely inhibitory effects in dioestrus, proestrus and oestrus. The amine produced no α-excitatory effects at any stage of the oestrous cycle.

However it exerted a pressor effect via α-receptors in the arterial vasculature. This was elicited with doses approximately 10,000 times those necessary for a maximal depressor response.

3. Uterine inhibition produced by the sympathomimetic amines was antagonised by propranolol and subsequently all the amines except isoprenaline were able to exert a motor effect at all stages of the oestrous cycle. These motor actions and the motor actions produced in oestrus were antagonised by α-receptor blocking agents. Propranolol, in doses necessary to produce β-receptor blockade, initiated or enhanced uterine contractions in vivo at all stages of the oestrous cycle. Such an effect was not produced using isolated preparations.

4. The reponses to hypogastric nerve stimulation were very similar to the responses produced by noradrenaline. In dioestrus and proestrus nerve stimulation caused solely inhibition but this effect was reversed when the animal was in oestrus. The inhibitory responses to nerve stimulation were reversed by propranolol. The subsequent motor actions and the motor action produced in oestrus were antagonised by α-receptor blocking agents.

5. Treatment of immature and of ovariectomised adult rats with oestradiol or with oestradiol and progesterone caused the uterine responses to be converted into responses similar to those observed using rats in oestrus. Treatment with progesterone alone did not alter qualitatively the responses to the amines but lowered the sensitivity of the uteri to their effects.

6. The responses of uteri from rats in oestrus were similar to the responses from rats in dioestrus and proestrus after treatment with propranolol. The responses of uteri from oestrogen-treated immature and from oestrogen-treated ovariectomised rats were similar to the responses from untreated animals after the addition of propranolol to the bathing media. This suggests the possibility that oestrogen increases the number and/or the activity of the adrenergic α-receptors in the uterus.

TABLES 1 - 11.

FIGURES 1 - 23.

Where the abbreviations and symbols used are not explained at the foot of the Table or Figure, an explanation is given in the Appendix on page 278.

In Tables 1 - 8 each line shows the results from a single experiment. Mean values are included at the foot of each set of figures.

TABLE 1

RATS IN DIOESTRUS

Ratio of the dose of sympathomimetic amine to the equiactive dose of l-noradrenaline.

in vivo experiments

Inhibitory response

*Dose (μg) l-Nor	Ratio sympathomimetic amine/l-Nor			
	l-α-EtNor	l-Adr	l-N-EtNor	dl-Iso
5.0		0.05		0.0002
0.2				0.0005
2.0		0.10		0.0005
5.0				0.001
5.0		0.01		0.00001
20.0		0.01		
10.0	1.6	0.02		
4.0		0.03	0.006	
6.4 = 1	1.6	0.04	0.006	0.0004

*Dose by retrograde injection into the femoral artery to cause 50% inhibition of spontaneous or acetylcholine-induced uterine contractions.

TABLE 2

RATS IN DIOESTRUS

Ratio of the concentration of sympathomimetic amine to the equiactive concentration of l-noradrenaline.

in vitro experiments

Inhibitory response

*Conc^{n}. l-Nor x 10^{-8}	Ratio sympathomimetic amine/l-Nor			
	l-α-EtNor	l-Adr	l-N-EtNor	dl-Iso
10.0	0.5	0.004	0.00004	0.00000002
4.0	1.0	0.01	0.001	0.0000001
48.0	1.0	0.005	0.0005	0.00000005
24.0		0.02		0.00002
8.0		0.01		0.00005
4.0		0.02		0.000001
4.0		0.01		
8.0		0.02		
4.0		0.005		
20.0		0.004		
8.0		0.003		
12.9 = 1	0.8	0.01	0.0005	0.00001

*Concentration to cause a 50% reduction in height of spontaneous or acetylcholine-induced uterine contractions.

TABLE 3

RATS IN PROESTRUS

Ratio of the dose or concentration of sympathomimetic amine to the equiactive dose of l-noradrenaline.

Inhibitory response

a) in vivo experiments

*Dose (µg) l-Nor	Ratio sympathomimetic amine/l-Nor	
	l-Adr	dl-Iso
10	0.025	0.0001
5	0.02	
7.5 = 1	0.023	0.0001

b) in vitro experiments

*Conc.^{n} l-Nor x 10^{-8}	Ratio sympathomimetic amine/l-Nor	
	l-Adr	dl-Iso
0.8	0.01	0.0000001
0.2	0.002	0.000002
8.0	0.005	0.000001
4.0	0.04	0.00002
2.0	0.002	0.000002
20.0	0.02	0.000002
20.0	0.01	
7.9 = 1	0.013	0.000005

*Dose or concentration to cause a 50% reduction in height of spontaneous or acetylcholine-induced uterine contractions.

TABLE 4

RATS IN OESTRUS

Ratio of the dose of sympathomimetic amine to the equiactive dose of l-noradrenaline.

in vivo experiments

Dose (μg) l-Nor		Ratio sympathomimetic amine/l-Nor							
		l-α-EtNor		l-Adr		l-N-EtNor		dl-Iso	
*E	*I	E	I	E	I	E	I	E	I
2.0	10	-	0.25	-	0.1	63	0.0005	-	0.0002
2.0	5	10	4.0	-	0.25	-	0.1	-	0.002
2.5	2.5			-	0.04			-	0.0004
0.2	20			40	0.13				
2.0	-			12	1.0μg				
5.0	-			-	1.0μg				
0.25	-			-	2.0μg				
2.0	-			-	1.0μg				
0.25	-								
0.2	-								
5.0	-								
1.9=1		10		26		63		-	
	14.3=1		2.1		0.13		0.05		0.0009

Doses by retrograde injection into the femoral artery to cause:

*E = a just submaximal contraction or

*I = a 50% reduction in height of spontaneous or acetylcholine-induced uterine contractions.

- = amine used but no action observed.

TABLE 5

RATS IN OESTRUS

Ratio of the concentration of sympathomimetic amine to the equiactive concentration of l-noradrenaline.

in vitro experiments

Conc^{n} l-Nor x 10^{-8}		Ratio sympathomimetic amine/l-Nor									
		l-α-EtNor		l-Adr		l-Phe		l-N-EtNor		dl-Iso	
*E	*I	E	I	E	I	E	I	E	I	E	I
20	4		0.8	5	0.01			200	0.001	-	0.000001
80	40			10	0.01			-	0.001	-	0.0000005
160	4			5	0.01			50		-	0.0000002
2	0.4			20	0.01			80		-	0.000001
400	8	1.0		2	0.005					-	0.00000005
2	4			10	0.02					-	0.00000001
100	-			4	$4x10^{-8}$			-	$2x10^{-8}$		
80	400	-	1.2	5	0.01	25	1.0				
200	100			4	0.02	20	0.8				
16	-			25	$1x10^{-9}$						
400	-			5	$8x10^{-9}$						
60	-			7	$2x10^{-8}$						
32	-			12	$4x10^{-10}$						
40	-			10	$4x10^{-10}$						
114 = 1		1.0		8.9		23		110		-	
	70 = 1		1.0		0.01		0.9		0.001		0.0000005

Concentration to cause:

*E = a just submaximal contraction or

*I = a 50% reduction in height of spontaneous or acetylcholine-induced uterine contractions.

- = amine used but no action observed.

TABLE 6

IMMATURE RATS + HORMONE TREATMENT

Ratio of the concentration of sympathomimetic amine to the equiactive concentration of l-noradrenaline.

in vitro experiments

TREATMENT		Conc^{n} l-Nor x 10^{-9}		Ratio sympathomimetic amine/l-Nor							
				l-α-EtNor		l-Adr		l-N-EtNor		dl-Iso	
		*E	*I	E	I	E	I	E	I	E	I
U		-	40		1.0	-	0.02		0.002		
		-	600		0.3	-	0.007		0.004		
		-	800			-	0.005				0.00000005
		-	400			-	0.01				0.00001
		-	400			-	0.005				0.0000001
		-	400			-	0.01				0.000025
		-	20			-	0.01				0.0000001
			380 = 1		0.7		0.01		0.003		0.000007
O	i	800	8			-	0.01			-	0.00005
	ii	2,000	4			-	0.002			-	0.0000005
	i	800	0.4			5	0.001			-	0.0000001
			4.1 = 1				0.004				0.00002
P		20,000	4			-	0.02			-	0.0001
		20,000	20			-	0.002			-	0.0000002
			12 = 1				0.01				0.00005
O + P		1,000	1,000			-	0.01			-	0.00001
		800	200			-	0.005			-	
		200	40			20	0.01			-	0.000001
			413 = 1				0.08				0.000006

Concentration to cause:

*E = a just submaximal contraction or
*I = a 50% reduction in height of spontaneous or acetylcholine-induced uterine contractions.

- = amine used but no action observed

i and ii refers to oestrogen administration regime on p. 51.

TABLE 7

OVARIECTOMISED RATS + HORMONE TREATMENT

Ratio of the dose of sympathomimetic amine to the equiactive dose of l-noradrenaline.

in vivo experiments

Hormone treatment		Dose (µg) l-Nor		Ratio sympathomimetic amine/l-Nor							
				l-α-EtNor		l-Adr		l-N-EtNor		dl-Iso	
		*E	*I	E	I	E	I	E	I	E	I
Oestrogen	ii	10.0	5.0			-	0.05				
	ii	5.0	5.0			-	0.02				
			5.0 = 1				0.04				
Oestrogen + Progesterone		0.5	1.0	8	0.03	-	0.025	-	0.005	-	0.001
		10.0	5.0			-	0.02				
			3.0 = 1		0.03		0.02		0.005		0.001

Dose by retrograde injection into the femoral artery to cause:

*E = a just submaximal contraction or

*I = a 50% reduction in height of spontaneous or acetylcholine-induced uterine contractions.

- = amine used but no action observed.

ii refers to oestrogen administration regime on p. 51.

TABLE 8

OVARIECTOMISED RATS + HORMONE TREATMENT

Ratio of the concentration of sympathomimetic amine to the equiactive concentration of l-noradrenaline.

in vitro experiments

TREATMENT		Conc^{n} l-Nor x 10^{-8}		Ratio sympathomimetic amine/l-Nor							
				l-α-EtNor		l-Adr		l-N-EtNor		dl-Iso	
		*E	*I	E	I	E	I	E	I	E	I
U		- - -	0.03 0.04 0.8 1.0	-	2.5 0.6	- - -	0.025 0.01 0.002 0.02	- -	0.03 0.001	- - - -	0.00001 0.00004 0.0000002 0.0000004
			0.47 = 1		1.5		0.014		0.016		0.00001
O	i	-	100			-	0.002			-	0.00000002
	i	140	12			-	0.007			-	0.000003
	ii	8	10	-	1.0	25	0.004	-	0.0004		
			38 = 1		1.0		0.004		0.0004		0.0000015
P		- - -	4.0 100 40	- -	0.13 0.4	- - -	0.005 0.01 0.01	- -	0.001 0.002	- -	0.000003 0.0000004
			48 = 1		0.27		0.008		0.0015		0.000002
O + P		4 40 20 40	10 160 20 40	-	0.25	50 10 - -	0.02 0.013 0.02 0.005	-	0.013	- -	0.00002 0.000006
		26 =1	58 = 1	-	0.25	30	0.015	-	0.013	-	0.00001

Concentration to cause:

*E = a just submaximal contraction or
*I = a 50% reduction in height of spontaneous or acetylcholine-induced uterine contractions.

- = amine used but no action observed.

i and ii refer to oestrogen administration regime on p. 51.

TABLE 9a

CORRELATION OF THE SEXUAL STATE OF THE RAT WITH THE RESPONSE OF THE UTERUS TO SYMPATHOMIMETIC AMINES

in vitro experiments

Sexual state	Response: E = excitation; I = inhibition				
	l-Nor	l-Adr	l-α-EtNor	l-N-EtNor	dl-Iso
Dioestrus	14* I	14 I	3 I	3 I	9 I
Proestrus	10 I	12 I			8 I
Immature untreated	6 I	7 I	2 I	2 I	5 I
Ovariectomised untreated	4 I	4 I	2 I	2 I	4 I
Immature + progesterone	2 I	2 I			2 I
Ovariectomised + progesterone	3 I	3 I	2 I	2 I	2 I
Immature + oestrogen	3 E or EI	3 eI or I			3 I
Ovariectomised + oestrogen	3 E or EI	3 eI or I	1 I	1 I	2 I
Immature + oestrogen + progesterone	3 E or EI	3 eI or I			3 I
Ovariectomised + oestrogen + progesterone	4 E or EI	4 eI or I	1 I	1 I	2 I
Oestrus	25 E or Ei	17 EI or I	2 eI or I	7 eI or I	11 I

EI; Ei; eI - letter size indicates relative magnitude of components of biphasic responses

* = number of experiments performed.

TABLE 9b

RATIO OF EQUIACTIVE CONCENTRATIONS OF SYMPATHOMIMETIC AMINES ON THE UTERUS OF THE RAT IN DIFFERENT SEXUAL STATES

in vitro experiments

Sexual state	*Conc^{n}. l-Nor $\times 10^{-8}$	Ratio sympathomimetic amine/l-Nor				** E or I
		l-α-EtNor	l-Adr	l-N-EtNor	dl-Iso	
Dioestrus	12.9	0.8	0.01	0.0005	0.00001	I
Proestrus	7.9		0.013		0.000005	I
Immature untreated	38	0.7	0.01	0.003	0.000007	I
Ovariectomised untreated	0.47	1.5	0.014	0.016	0.00001	I
Immature + progesterone	1.2		0.01		0.00005	I
Ovariectomised + progesterone	48	0.27	0.008	0.0015	0.000002	I
Immature + oestrogen	120		5.0		-	E
	0.41		0.004		0.00002	I
Ovariectomised + oestrogen	24		25		-	E
	38	1.0	0.004	0.0004	0.0000015	I
Immature + O + P	67		20		-	E
	41		0.08		0.000006	I
Ovariectomised + O + P	26	-	30	-	-	E
	58	0.25	0.015	0.013	0.00001	I
Oestrus	114	1.0	8.9	110	-	E
	70	1.0	0.01	0.001	0.000005	I

* Concentration to cause a 50% reduction in height of spontaneous or acetylcholine-induced uterine contractions (inhibitory response), or a just submaximal uterine contraction (excitatory response).

**E = excitatory ratio; I = inhibitory ratio.

- = amine used but no action observed.

TABLE 10

EFFECT OF PROPRANOLOL ON THE EXCITATORY ACTIVITY OF SYMPATHOMIMETIC AMINES ON THE UTERUS OF THE RAT IN DIFFERENT SEXUAL STATES

in vitro experiments

Propranolol 4×10^{-7}	Sexual state	*Conc^{n}. l-Nor $\times 10^{-6}$	Ratio of the conc^{n}. of sympathomimetic to the equiactive conc^{n}. of l-Nor			
			l-α-EtNor	l-Adr	l-Phe	l-N-EtNor
Before	Dioestrus	I	I	I		I
	Oestrus	1.0	3	6	29	106
	Immature untreated	I	I	I		I
	Immature + oestrogen	0.8		5		
	Ovariectomised untreated	I	I	I		I
	Ovariectomised + oestrogen	0.08	I	25		I
After	Dioestrus	0.6	12	1.9		50
	Oestrus	0.6	I	2.9	12	56
	Immature untreated	4.0	I	2.0	10	I
	Immature + oestrogen	0.8		1.0		
	Ovariectomised untreated	0.1		10		
	Ovariectomised + oestrogen	0.8		1.3		

*Concentration to cause a just submaximal contraction.

I = inhibitory action only.

TABLE 11

RELATIVE ACTIVITY OF β-RECEPTOR BLOCKING AGENTS AS AGONISTS AND ANTAGONISTS ON THE β-RECEPTORS IN THE UTERUS OF THE RAT

in vitro experiments

β-Blocking agent	*Agonist activity	**Antagonist activity
Dichloroisoprenaline	$>1 \times 10^{-7}$	$>4 \times 10^{-6}$
Pronethalol	$>4 \times 10^{-7}$	$>4 \times 10^{-6}$
Propranolol	$>2 \times 10^{-6}$	$>4 \times 10^{-8}$

* = Concentration to cause a 50% reduction in height of acetylcholine-induced uterine contractions.

** = Concentration to cause blockade of isoprenaline-induced uterine inhibition.

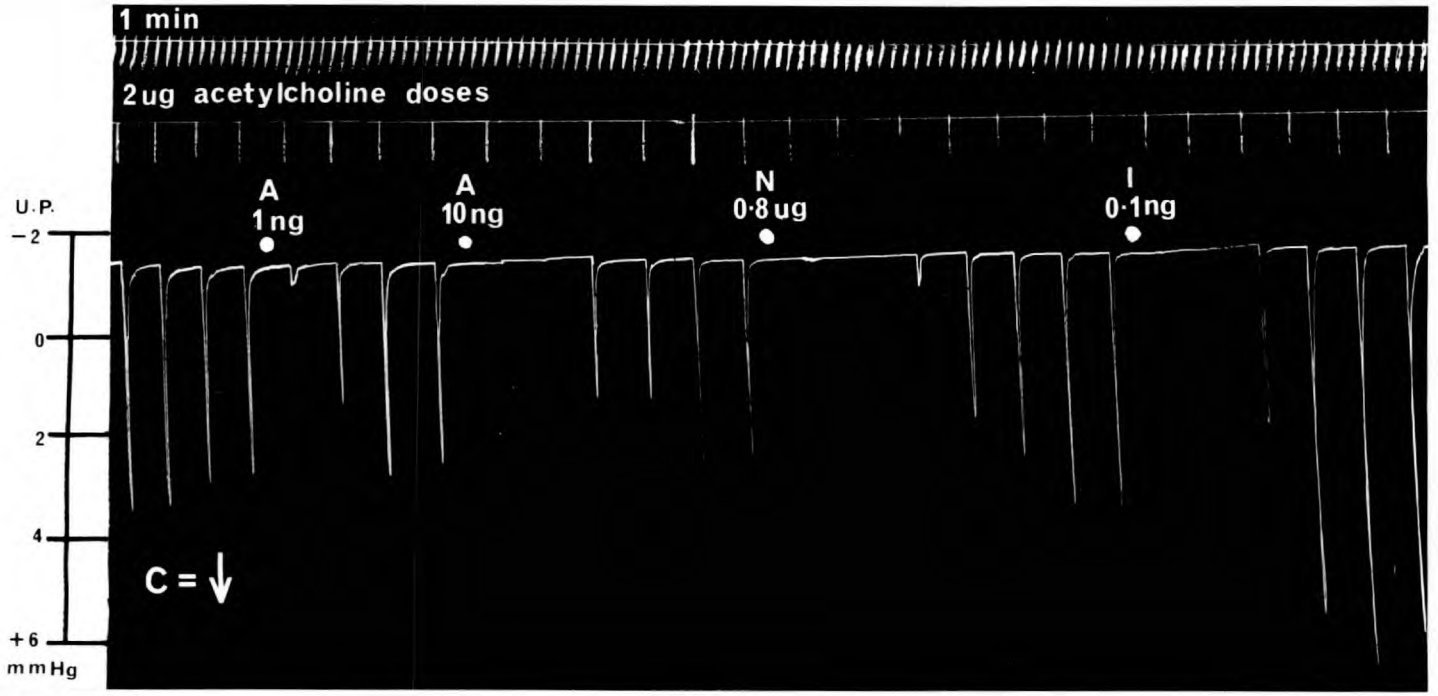


FIGURE 1. Inhibition by i.a. doses of adrenaline, noradrenaline and isoprenaline of the responses of the uterus to acetylcholine.

Rat in dioestrus, 330g.
in vivo

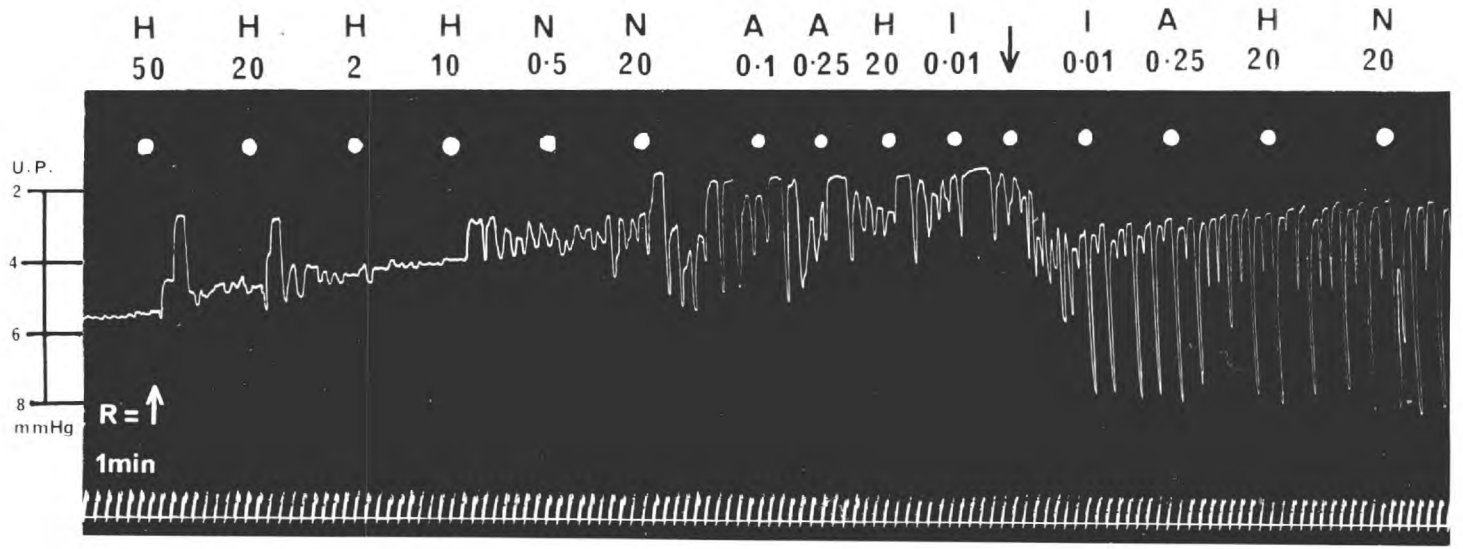


FIGURE 2. Blockade by propranolol of the responses of the uterus to μg doses (i.a.) of adrenaline, noradrenaline and isoprenaline, and to stimulation of the hypogastric nerve.

Rat in dioestrus, 340 g.
in vivo

↓ = intravenous injection of 200 μg propranolol.
H = hypogastric nerve stimulation for 30 sec at 1 V with 2 msec pulse width at e.g. 50 pulses/sec.

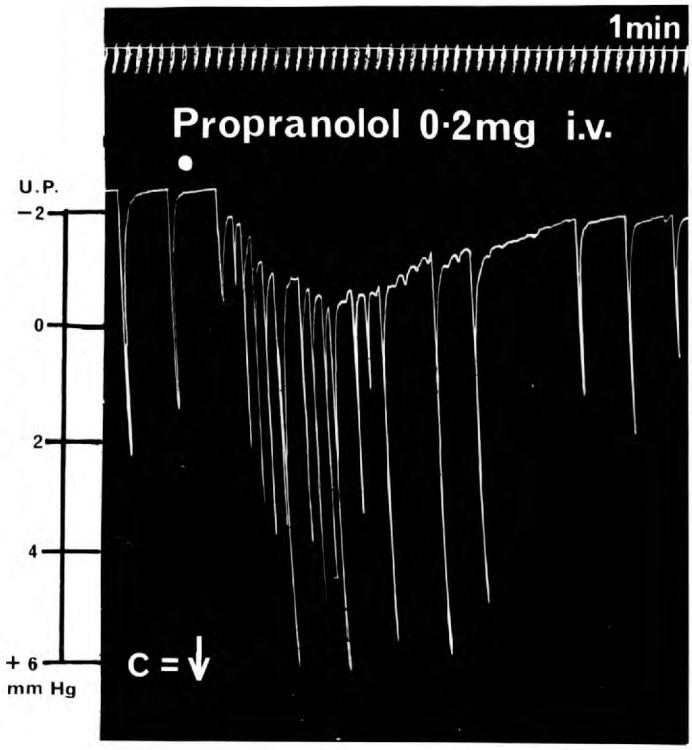


FIGURE 3. Enhancement by propranolol of the spontaneous activity of the uterus.

Rat in dioestrus, 300 g.
in vivo

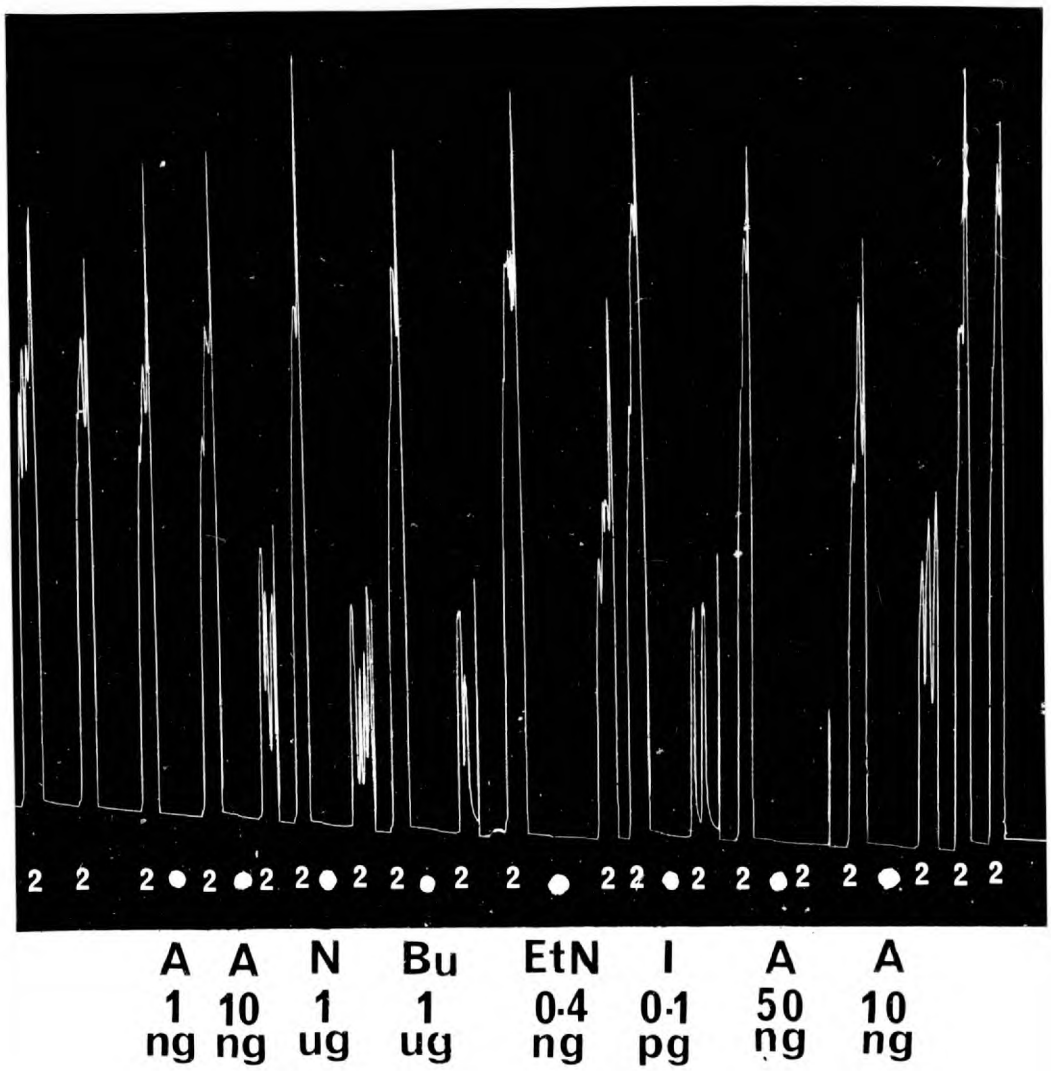


FIGURE 4. Inhibition by catecholamines of the responses of the uterus to acetylcholine.

Rat in dioestrus, 225 g.
in vitro; Tyrode solution; 37.5°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

2 = 2 µg acetylcholine.

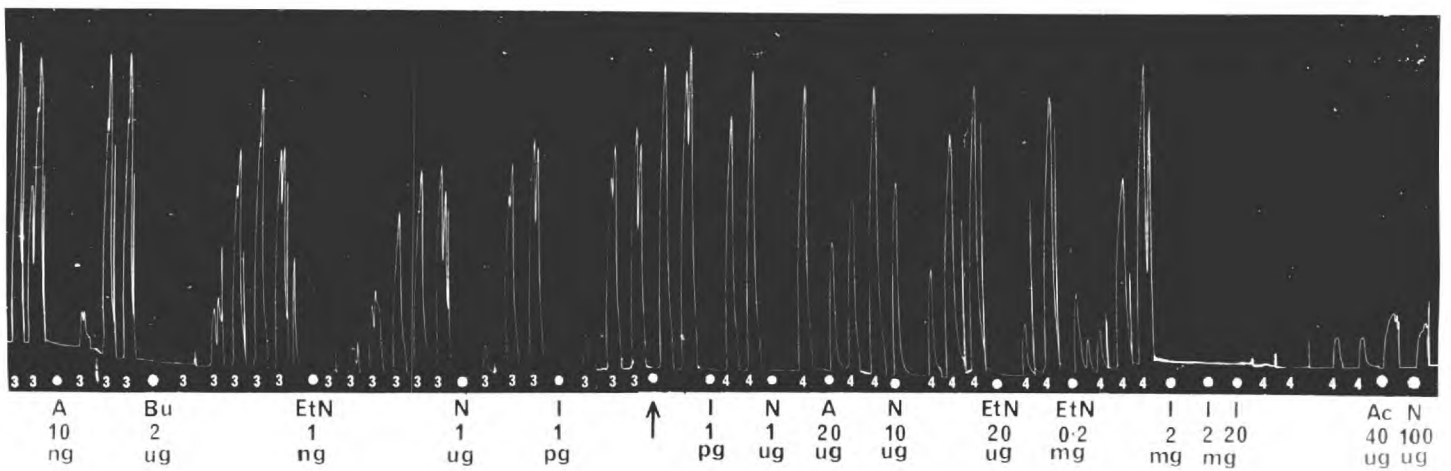


FIGURE 5. Inhibitory effects of catecholamines on acetylcholine-induced contractions of the uterus, and the subsequent blockade by propranolol of the inhibitory action of isoprenaline and the reversal by propranolol of the inhibitory actions of adrenaline, noradrenaline and N-ethylnoradrenaline.

Rat in dioestrus, 238 g.
in vitro; Tyrode solution; 37.5°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

↑ = 4×10^{-8} propranolol added to reservoir Tyrode solution.
3, 4 = 3, 4 μg acetylcholine.

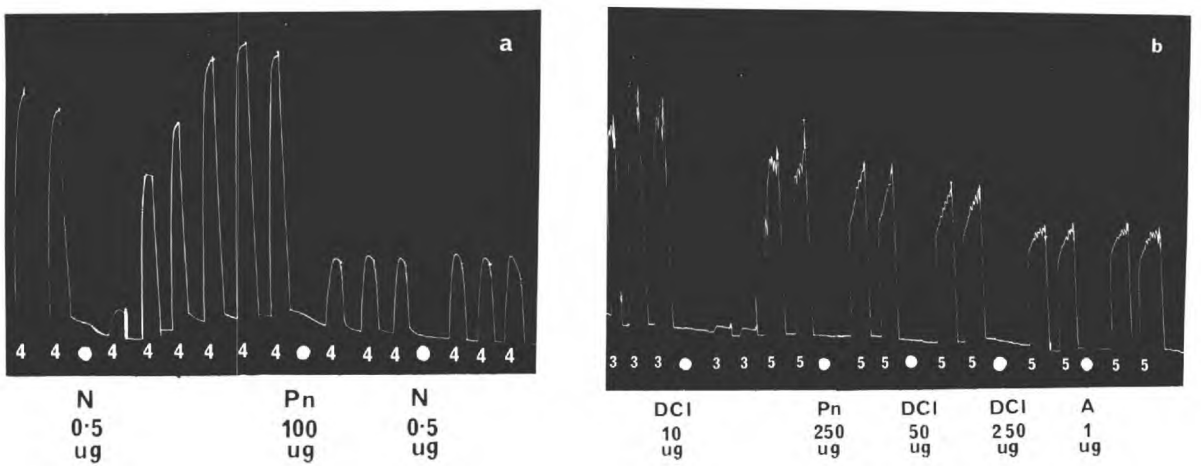


FIGURE 6. Agonist and antagonist activities of β-blocking agents on acetylcholine-induced contractions of the uterus, showing a) the effects of pronethalol and b) the effects of dichloroisoprenaline.

Rat in proestrus, 180 g.
in vitro; Tyrode solution; 37.0°C; 100% oxygen; isotonic lever.

3, 4, 5 = 3, 4, 5 μg acetylcholine.

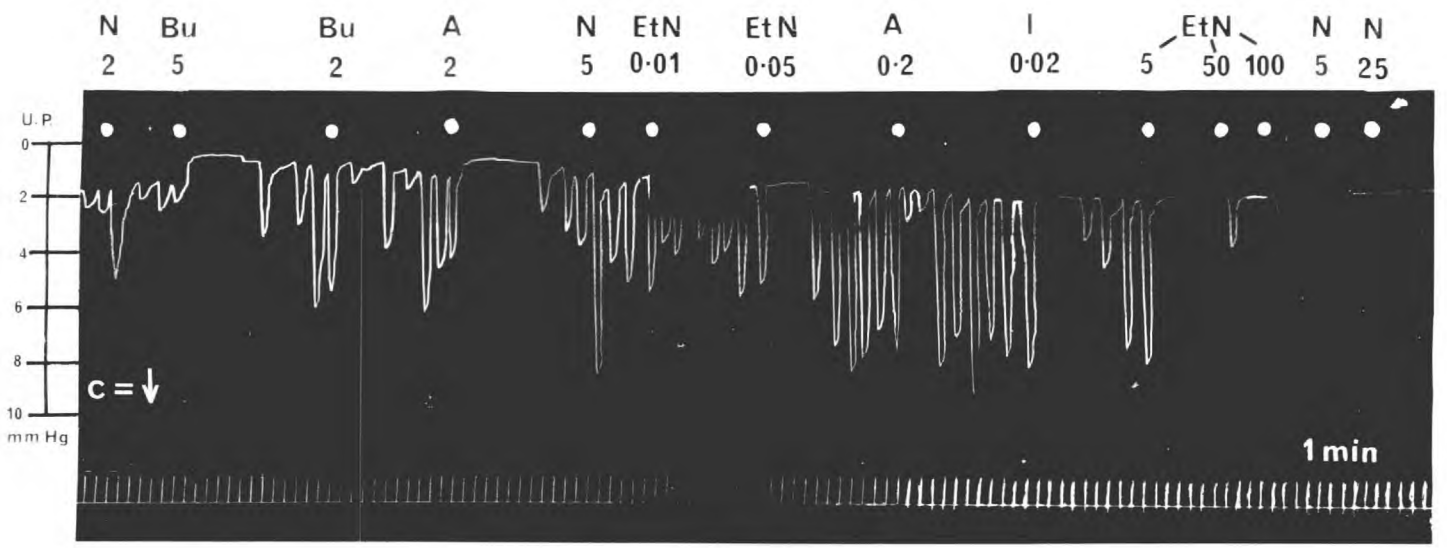


FIGURE 7. Excitatory actions of µg doses (i.a.) of noradrenaline; biphasic and inhibitory actions of N-ethylnoradrenaline; inhibitory actions of adrenaline, α-ethylnoradrenaline and isoprenaline on the spontaneous activity of the uterus.

Rat in oestrus, 265 g.
in vivo

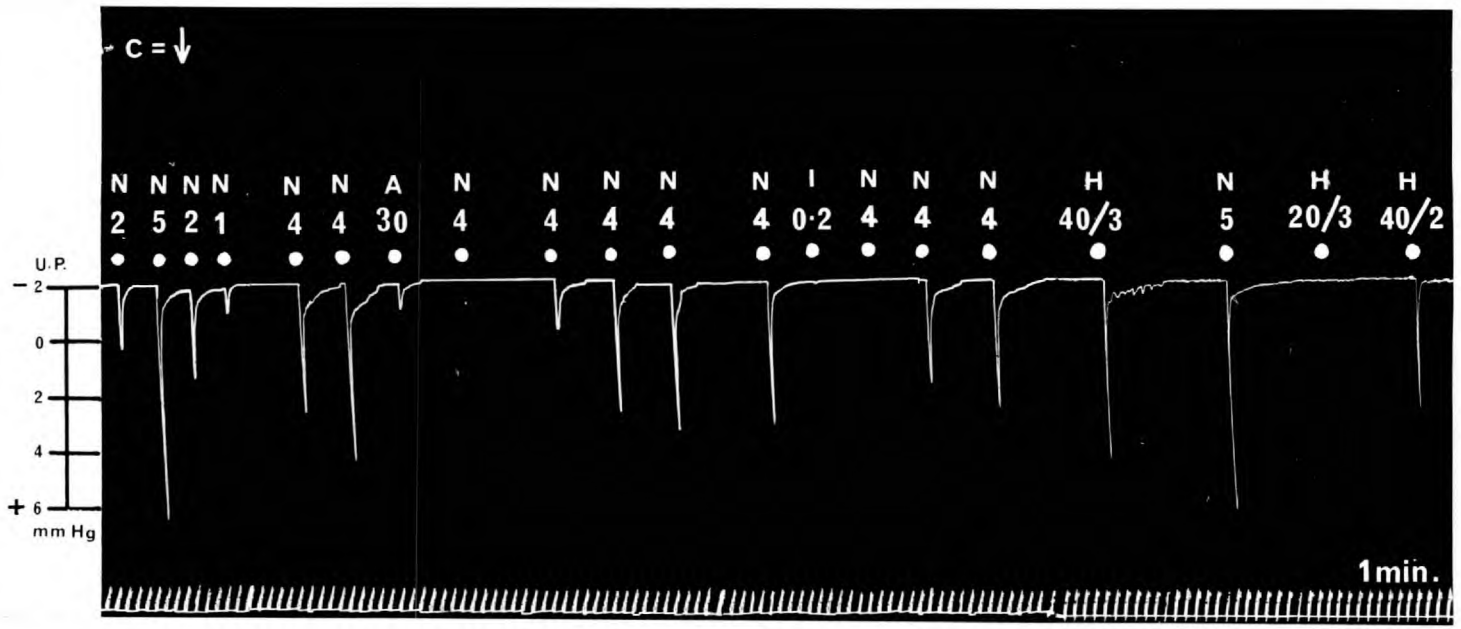


FIGURE 8. Excitatory effects of μg doses (i.a.) of noradrenaline and of hypogastric nerve stimulation; inhibitory effects of isoprenaline and biphasic effects of adrenaline on the quiescent uterus.

Rat in oestrus, 315 g.
in vivo

H = hypogastric nerve stimulation for 30 sec at e.g. 40/3 = 40 pulses per sec at 3 V. Pulse width = 2 msec.

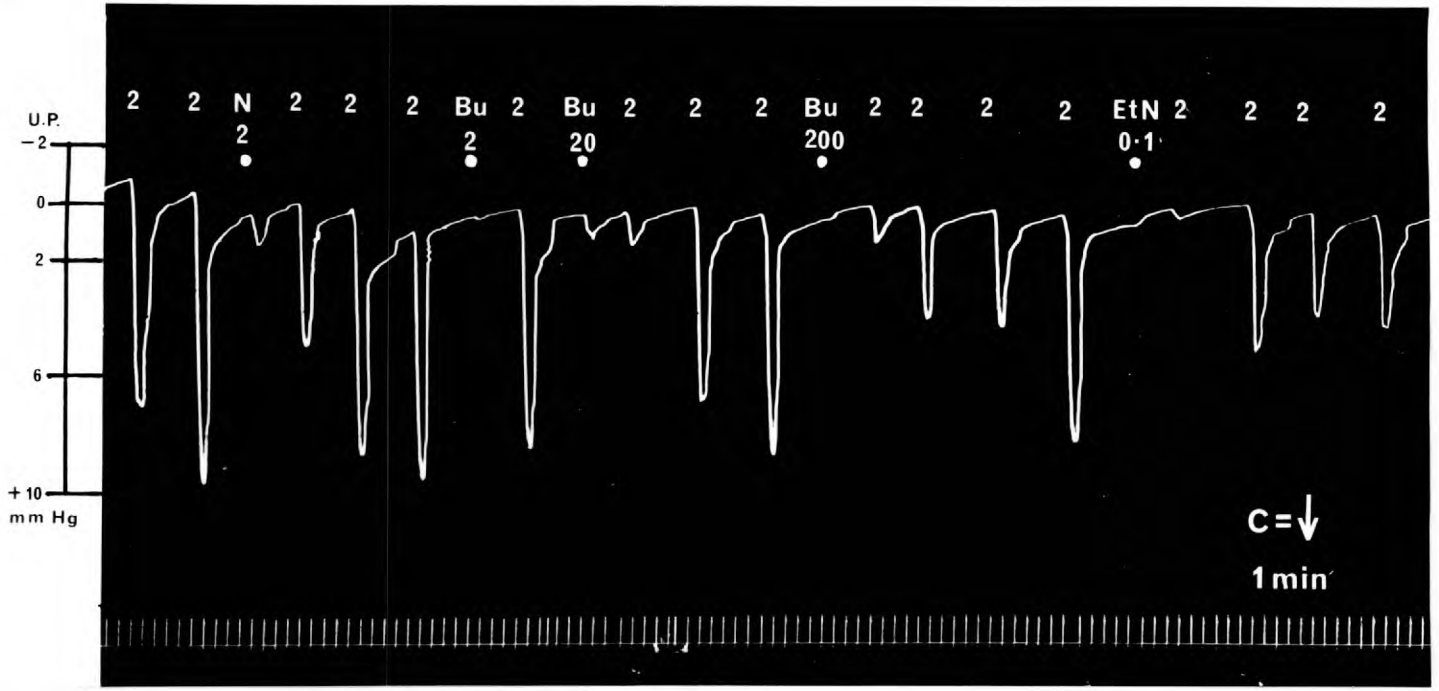


FIGURE 9. Biphasic effects of µg doses (i.a.) of noradrenaline and of α-ethylnoradrenaline, and inhibitory effect of N-ethylnoradrenaline on acetylcholine-induced contractions of the uterus.

Rat in oestrus, 310 g.
in vivo

2 = 2 µg acetylcholine, i.a.

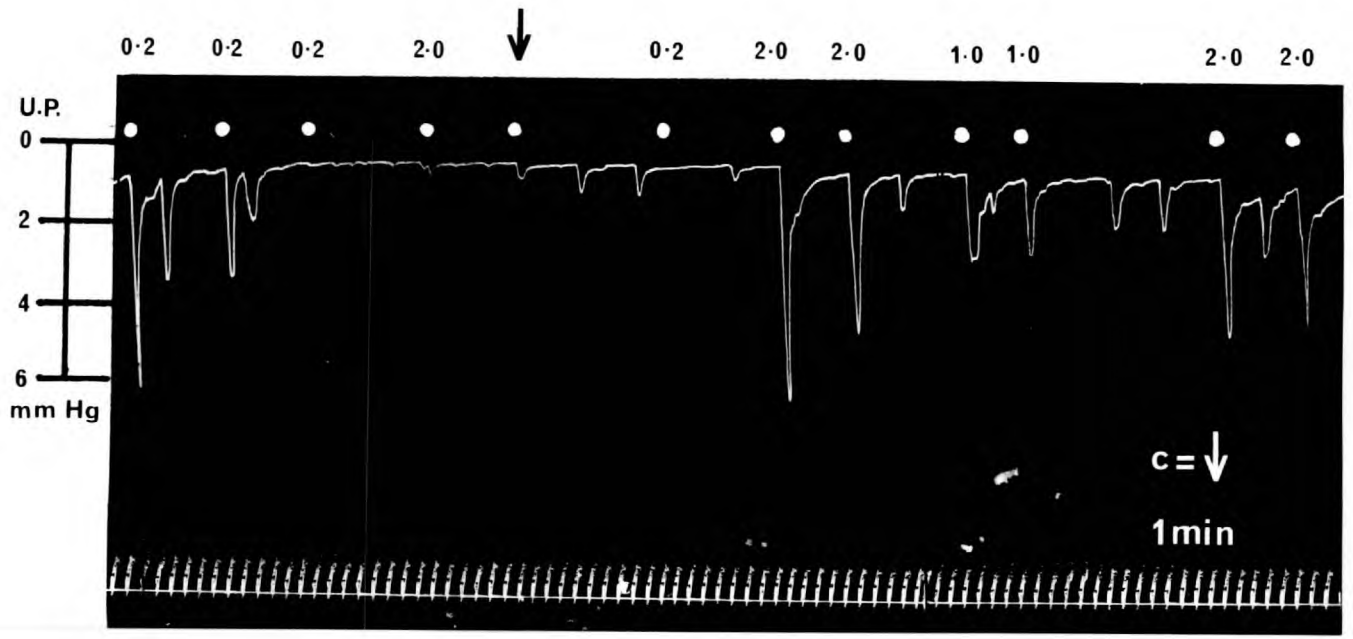


FIGURE 10. Blockade by propranolol of the inhibitory component of the biphasic effect of μg doses (i.a.) of noradrenaline on the uterus.

Rat in oestrus, 310 g.
in vivo

↓ = intravenous injection of 250 μg propranolol.

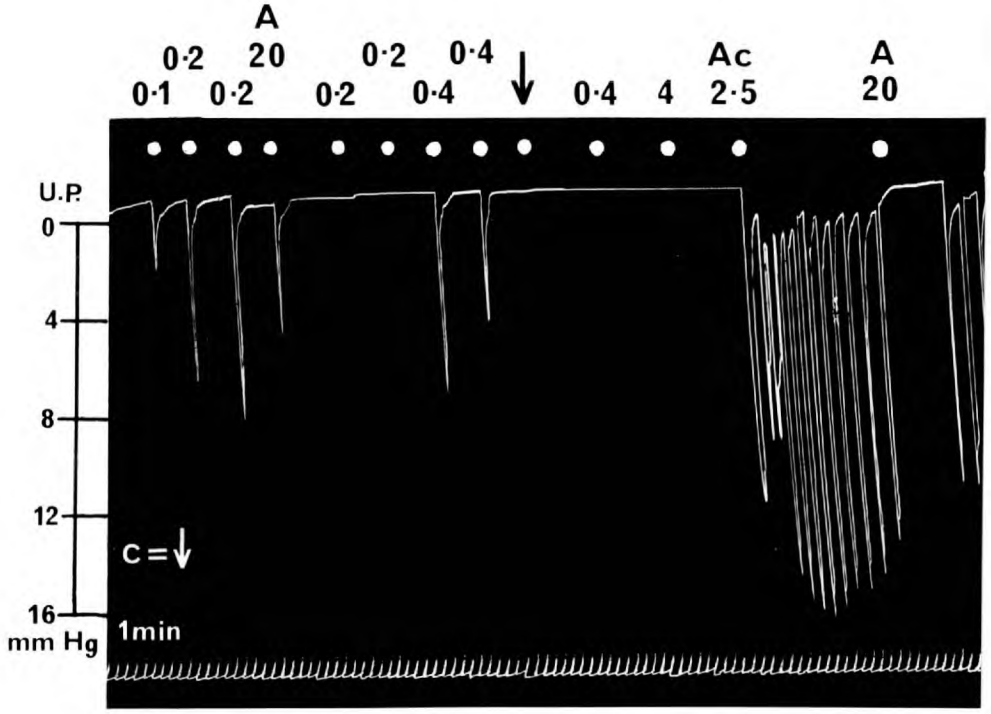


FIGURE 11. Blockade by phentolamine of the motor effects of noradrenaline and of the motor component of the biphasic effect of adrenaline on the uterus.

Rat in oestrus, 347 g.
in vivo

↓ = i.v. injection of 250 µg phentolamine. Other doses given i.a.
Unmarked doses = noradrenaline.
All doses in µg.

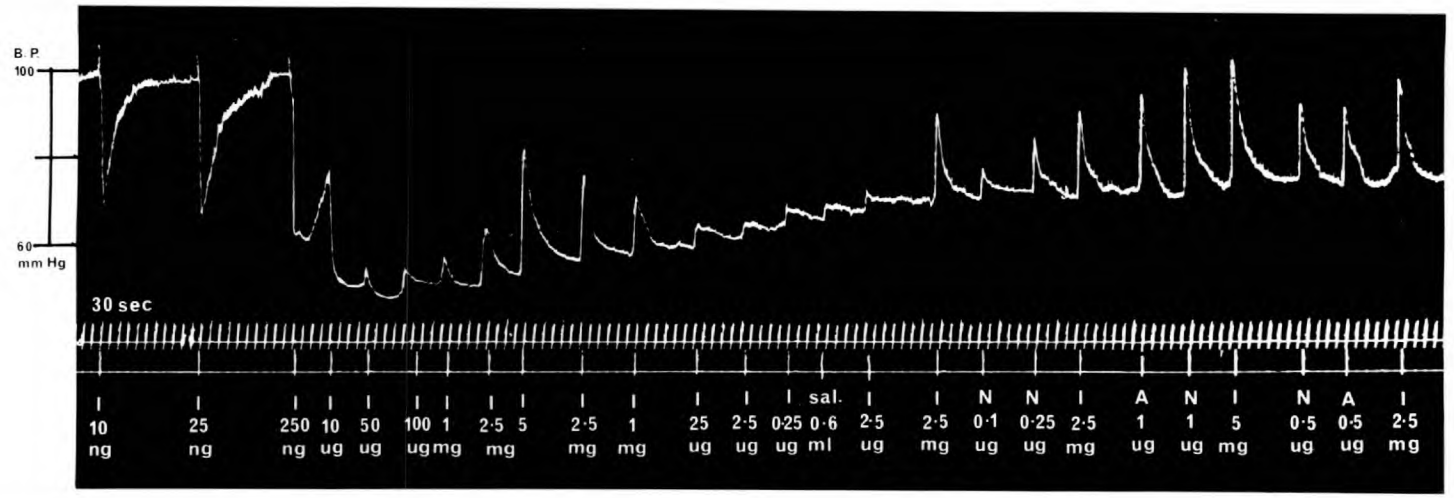


FIGURE 12. Reversal of the depressor effect of low doses of isoprenaline (i.v.) to a pressor effect with high doses, and the subsequent blockade of the depressor response.

Rat in oestrus, 320 g.
in vivo

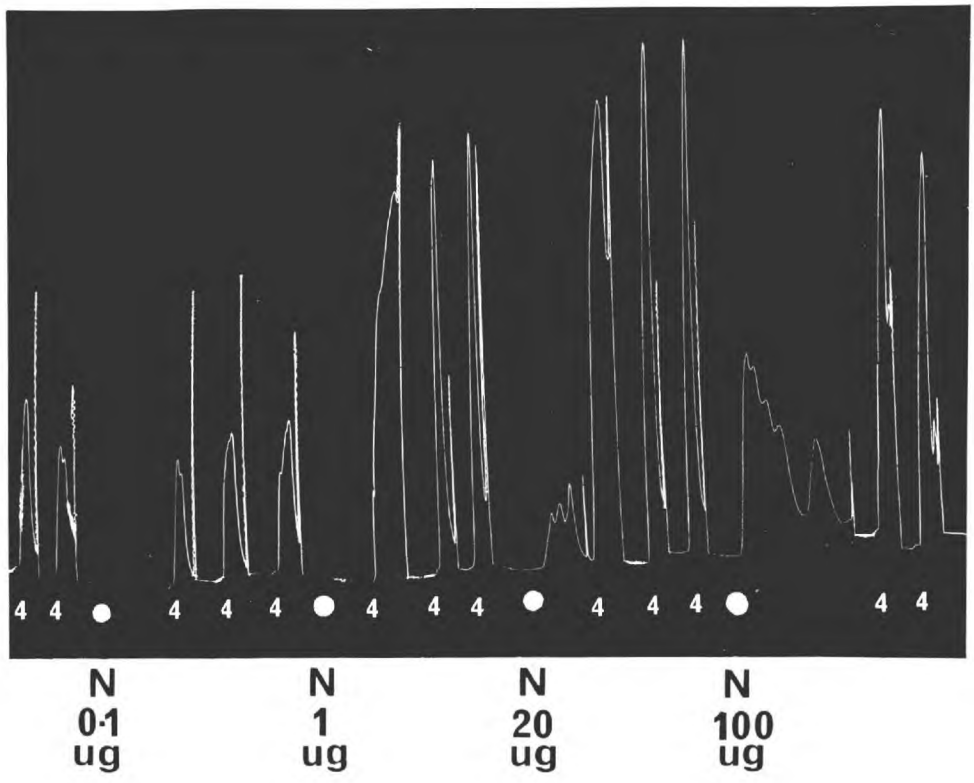


FIGURE 13. Motor actions of noradrenaline on a uterine preparation contracted by acetylcholine.

Rat in oestrus, 280 g.
in vitro; Tyrode solution; 37.5°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

4 = 4 µg acetylcholine.

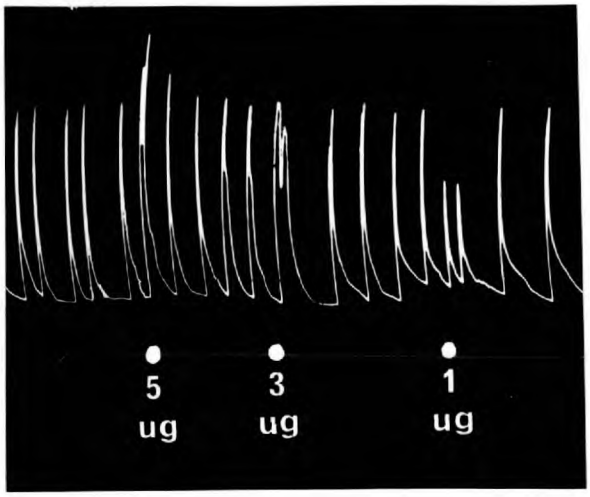


FIGURE 14. Motor actions of noradrenaline on a uterine preparation exhibiting spontaneous contractions.

Rat in oestrus, 235 g.
in vitro; Tyrode solution; 37.5°C; 95% oxygen + 5% carbon dioxide; isometric lever.

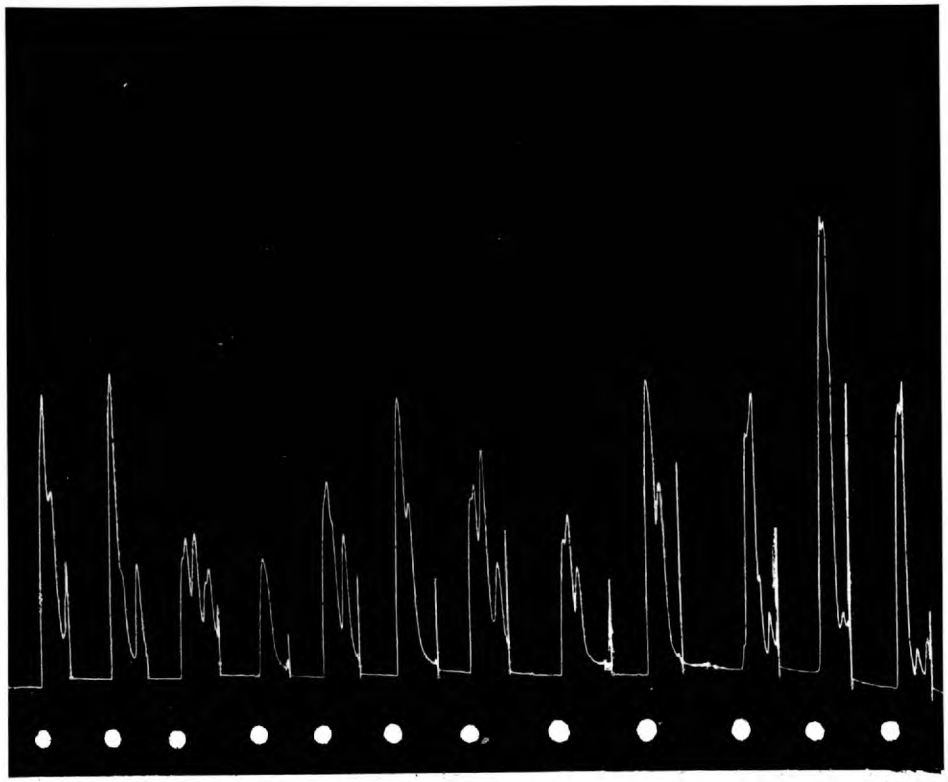


FIGURE 15. Motor actions of noradrenaline and biphasic actions of adrenaline and of phenylephrine on a quiescent uterine preparation.

Rat in oestrus, 234 g.
in vitro; Tyrode solution; 37.5°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

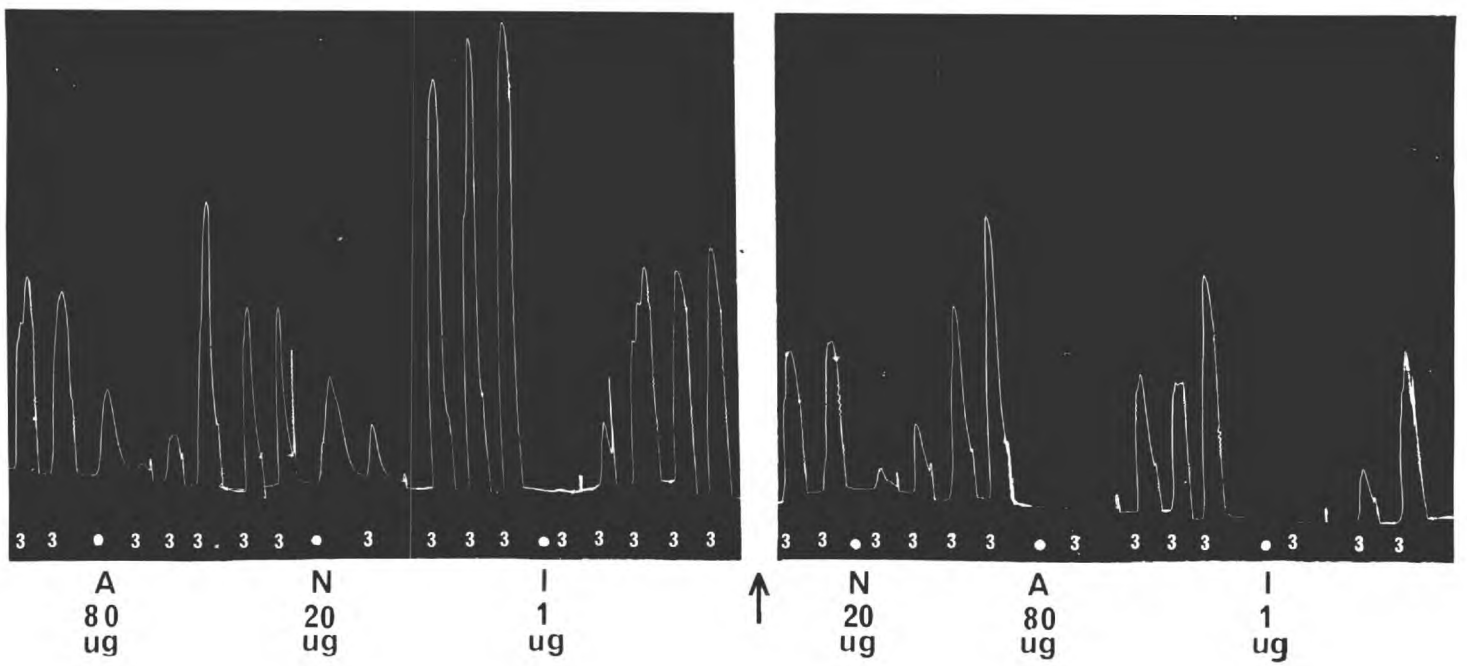


FIGURE 16. Blockade by tolazoline of the motor effects of adrenaline and noradrenaline, and the persistence of the inhibitory effect of isoprenaline on acetylcholine-induced contractions of the uterus.

Rat in oestrus, 285 g.
in vitro; Tyrode solution; 37°C; 100% oxygen; isotonic lever.

↑ = 2×10^{-6} tolazoline added to bathing solution.
3 = 3 µg acetylcholine.

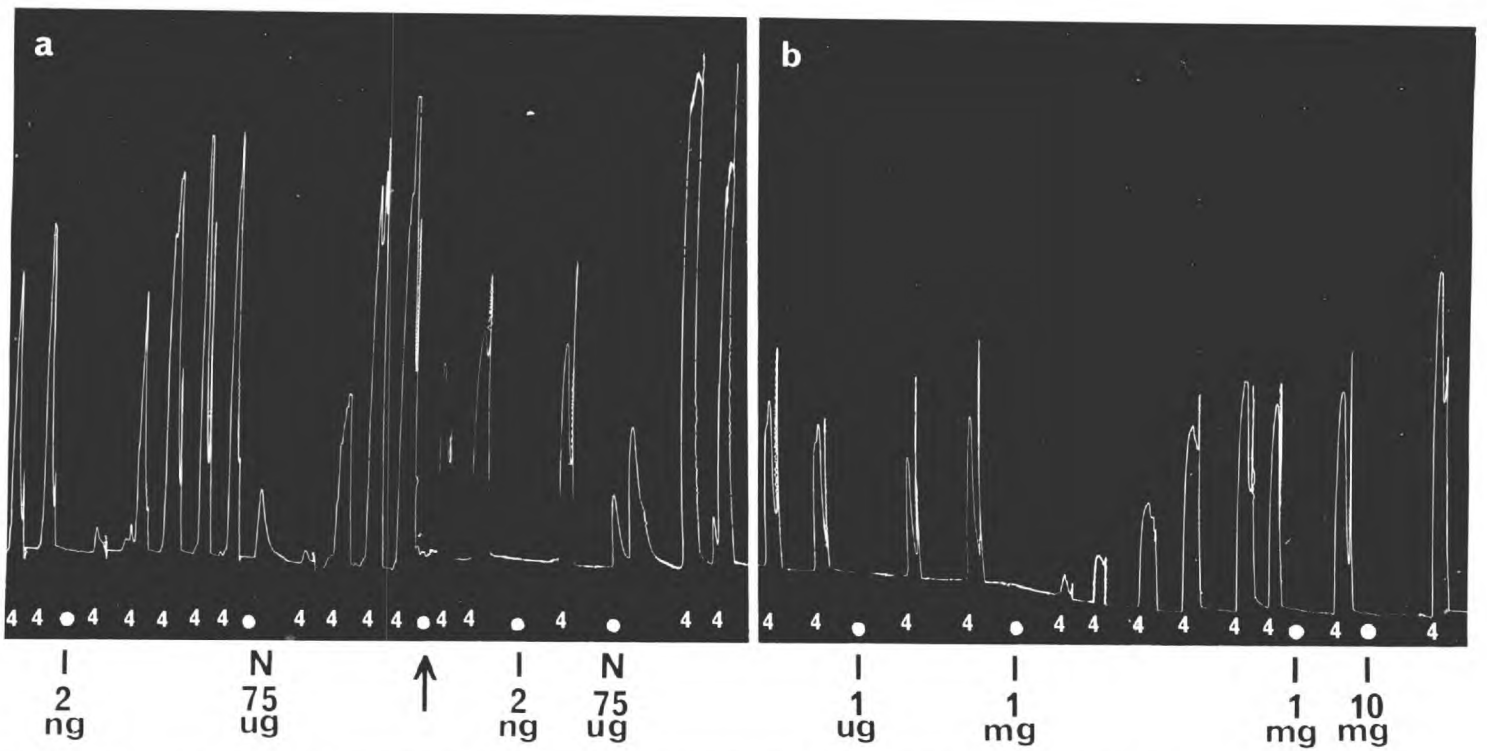


FIGURE 17. a) Potentiation by propranolol of the motor effect of noradrenaline and blockade of the inhibitory effect of isoprenaline on acetylcholine-induced contractions of the uterus.
b) Inability of high concentrations of isoprenaline to produce a motor effect in the presence of propranolol.

Rat in oestrus, 215 g.
in vitro; Tyrode solution; 37°C; 100% oxygen; isotonic lever.

↑ = 4×10^{-8} propranolol added to reservoir bathing solution.
4 = 4 µg acetylcholine.

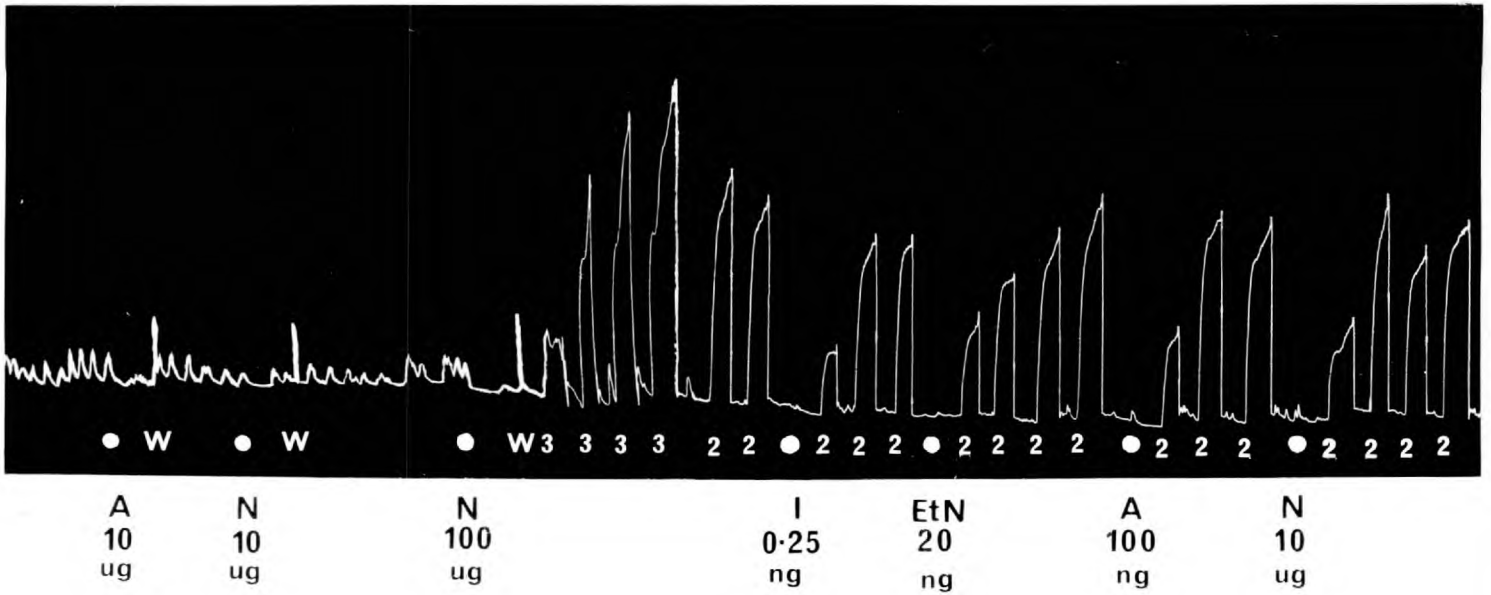


FIGURE 18. Failure of adrenaline and noradrenaline to produce excitatory effects on the uterus and the inhibitory effects of catecholamines on acetylcholine-induced contractions.

Immature, untreated rat, 60 g.
in vitro; Tyrode solution; 37.0°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

2, 3 = 2, 3 µg acetylcholine.
W = amine washed out.

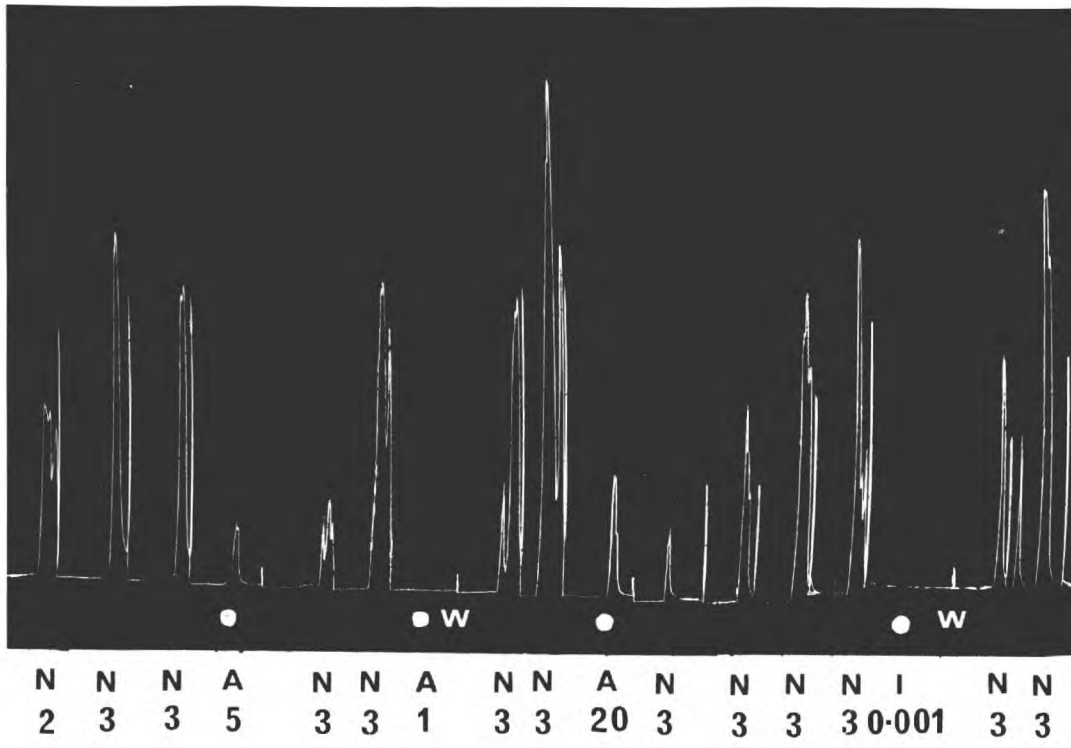


FIGURE 19. Excitatory actions of ug doses of noradrenaline; biphasic actions of adrenaline; and inhibitory action of isoprenaline on the quiescent uterus.

Immature, oestrogen + progesterone-treated rat, 86 g. in vitro; Tyrode solution; 37°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

W = amine washed out after no action observed.

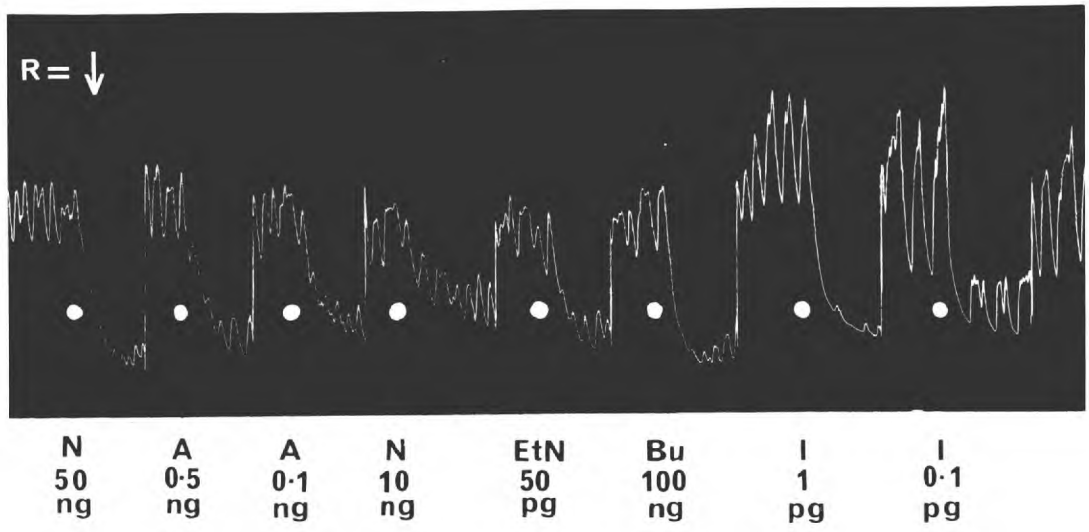


FIGURE 20. Inhibitory actions of catecholamines on the spontaneous activity of the uterus.

Ovariectomised, untreated rat, 225 g.
in vitro; Tyrode solution; 37.5°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

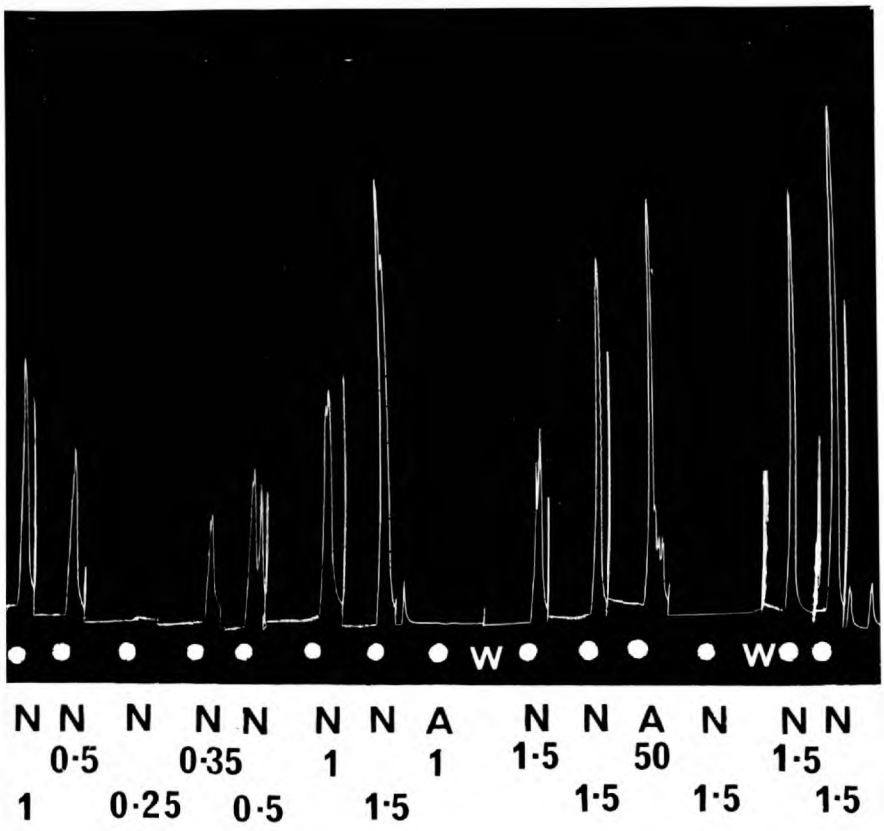


FIGURE 21. Excitatory actions of µg doses of noradrenaline; inhibitory and biphasic actions of adrenaline on the quiescent uterus.

Ovariectomised, oestrogen-treated rat, 236 g.
in vitro; Tyrode solution; 37°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

W = amine washed out after no action observed.

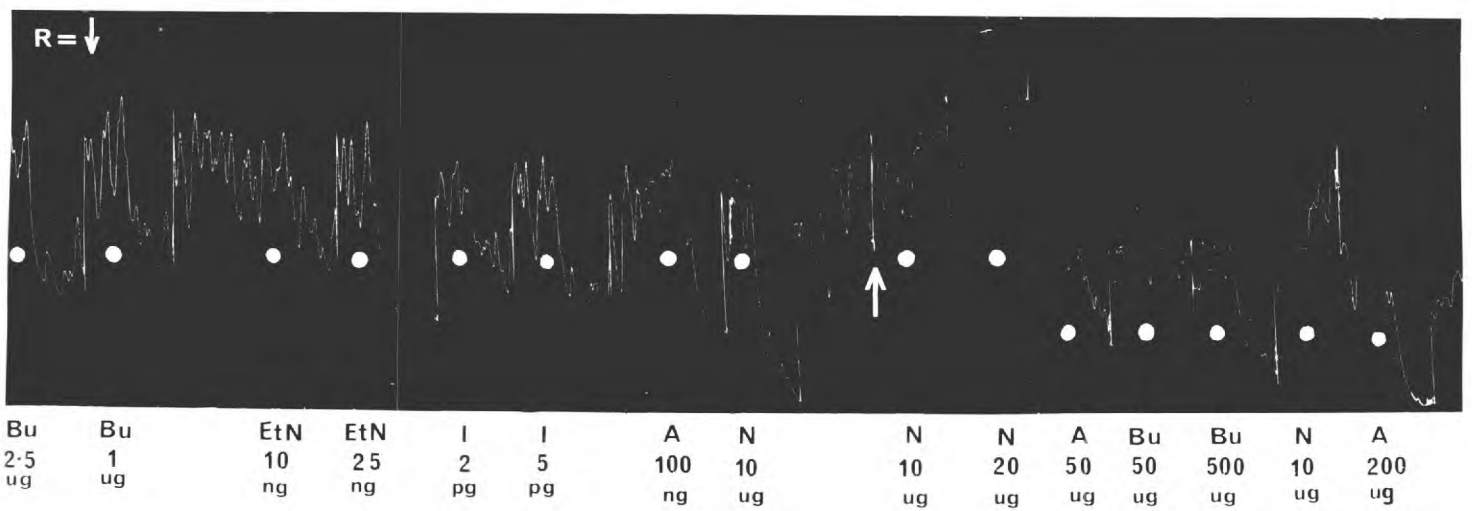


FIGURE 22. Inhibitory effects of catecholamines on the spontaneous activity of the uterus and the subsequent reversal by propranolol of the inhibitory effect of noradrenaline and the blockade by propranolol of the inhibitory effects of adrenaline and α-ethylnoradrenaline.

Ovariectomised, progesterone-treated rat, 359 g.
in vitro; Tyrode solution; 37°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

↑ = 4×10^{-8} propranolol added to reservoir Tyrode solution.

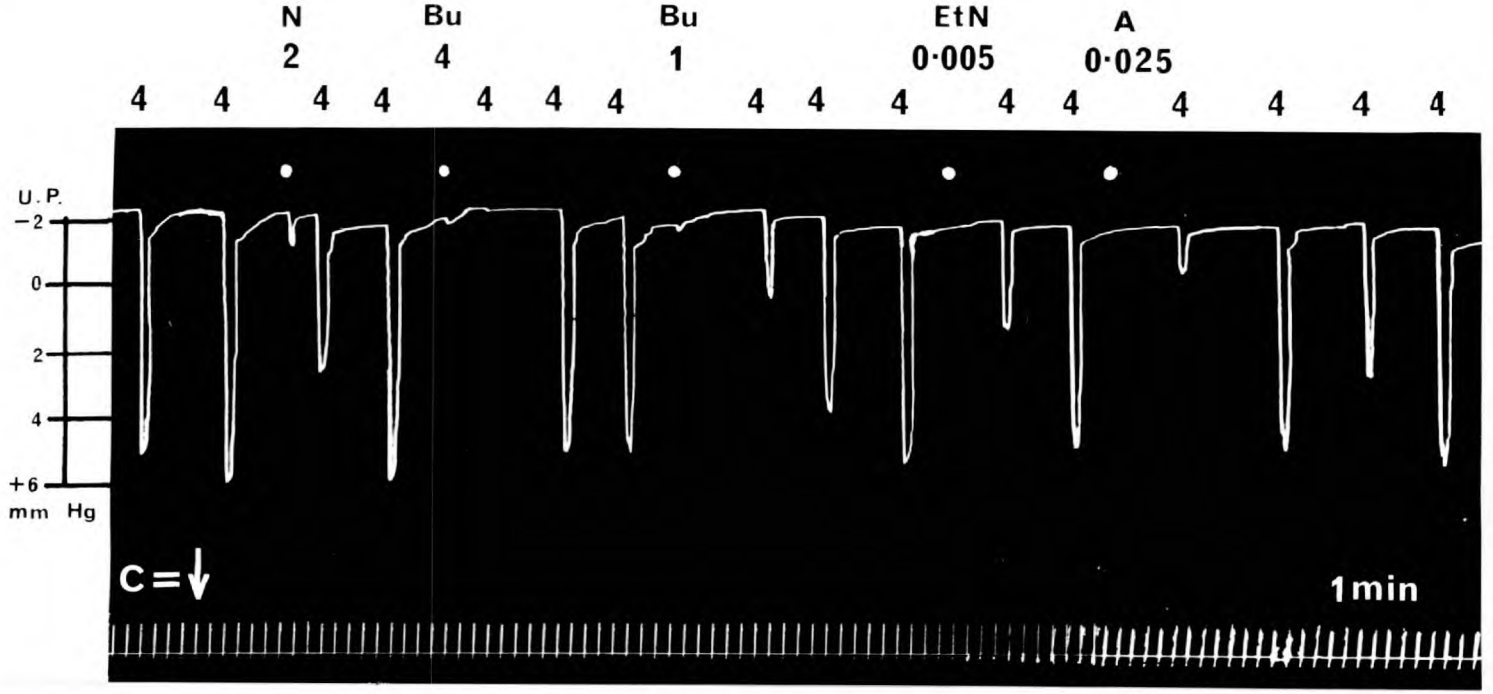


FIGURE 23. Biphasic effects of μg doses (i.a.) of noradrenaline and α-ethylnoradrenaline; inhibitory effects of adrenaline and N-ethylnoradrenaline on acetylcholine-induced contractions of the uterus.

Ovariectomised, oestrogen + progesterone-treated rat, 337 g.
in vivo

4 = 4 μg acetylcholine.

THE RESPONSES OF THE UTERUS OF THE RABBIT TO SYMPATHOMIMETIC AMINES AND THEIR MODIFICATION BY OVARIAN HORMONES

SECTION II

HISTORICAL

RESPONSES TO SYMPATHOMIMETIC AMINES

An early observation on the activity of sympathomimetic amines on the uterus of the rabbit was made by Langley in 1901 who described a motor effect of an extract of adrenal glands on the intact uterus. Elliot (1905) commented upon the similarity between the motor action of adrenaline and the response evoked by sympathetic nerve stimulation. Studying their modification by ergot alkaloids, Dale (1906) noted that *in vivo* ergot caused a reversal to inhibition of the responses both to adrenaline and to hypogastric nerve stimulation. He found that ergot alkaloids effectively antagonised only the excitatory and not the inhibitory actions of adrenaline on the uterus of various species, including the rabbit. In a study of the possible relationship between the actions of adrenaline and of the pituitary hormones, McSwiney and Brown (1926) found that the motor effect of adrenaline on the rabbit uterus could be reversed in the presence of a high tone produced by Pituitrin or histamine and concluded that the nature of the adrenaline response depended solely upon the prevailing muscular tone. Balassa (1940) stated that although adrenaline caused a contraction of the uterus of the adult rabbit, doses as great as 10 - 50 μg/kg produced no action on the uterus of the immature animal. Mann (1950) showed that similar excitatory

effects were produced by noradrenaline in the adult and in ovariectomised and immature rabbits.

Thus in most instances both adrenaline and noradrenaline cause only excitatory effects, but some workers have reported that these amines can produce inhibitory actions. Thiennes (1928) noted that whereas some uterine strips were contracted by adrenaline others were relaxed, but he forwarded no explanation for this. Rothlin and Brügger (1945) stated that low concentrations of adrenaline caused inhibition while high concentrations produced biphasic effects on some isolated preparations. Ahlquist (1948) noted that although adrenaline and noradrenaline caused solely excitatory responses on isolated uterine preparations, these amines produced biphasic effects *in vivo*. Biphasic effects were recorded also by Cross (1958) both to adrenaline and to noradrenaline in an *in vivo* study using ovariectomised oestrogen-treated rabbits. The inhibitory potency of adrenaline was approximately twice that of noradrenaline. He found that the amines inhibited the excitatory action of a following dose of Pitocin. However, Fuchs (1964) could not confirm this result, perhaps because she administered the amine and Pitocin simultaneously.

In regard to the relative potency of the two amines in eliciting motor responses, West (1947) gave the ratio of the dose of *dl*-noradrenaline to the equiactive dose of *l*-adrenaline as five and this was confirmed by Mann (1950). Luduena *et al*.

(1949) reported the ratio of l-noradrenaline to l-adrenaline as one and this was confirmed by Lands (1952). Ahlquist (1948) and Lewis (1954) found both l- and dl-adrenaline more active than dl-noradrenaline, the former being the most potent.

Studies of the responses of the uterus of the rabbit to other amines have not been so numerous, but Konzett (1940) found that 2×10^{-4} isoprenaline produced an inhibitory action on the isolated uterus when 1×10^{-6} adrenaline caused a motor effect. This inhibitory action of isoprenaline has been confirmed by Lands, Nash, McCarthy, Granger and Dertinger (1947) using a concentration of 2×10^{-7}. Ahlquist (1948) showed that this inhibitory action also occurred *in vivo*. The possibility that isoprenaline might produce a motor effect on the uterus of the rabbit has received little attention. Lewis (1954) detected a very weak action of the *laevo* isomer on *in vitro* preparations where it possessed approximately a 10,000th of the excitatory activity of l-adrenaline. A similar result was recorded by Furchgott (1959), also using *in vitro* preparations. Lish, Hillyard and Dungan (1960) found that a concentration of 8×10^{-6} l-isoprenaline contracted the uterus from a rabbit which had been injected with oestradiol. However, Luduena (1962) found that concentrations of the order of 10^{-5} were necessary to excite the uteri from untreated animals while

weaker concentrations (10^{-9} - 10^{-6}) caused only relaxation.

The action of N-ethylnoradrenaline on the isolated uterus was noted by Konzett (1940). A concentration of 1×10^{-5} produced a motor effect, indicating a potency of about one tenth that of adrenaline. This result was confirmed by Lands, Nash, Dertinger, Granger and McCarthy (1948) and by McDougal and West (1954). The latter workers reported the ratio of the concentration of dl-N-ethylnoradrenaline to the equiactive concentration of l-adrenaline as fifteen. dl-α-Ethylnoradrenaline produced a weak motor action on the isolated preparation, having only a 500th of the potency of l-adrenaline (McDougal and West, 1954).

Lewis (1954) reported that the ratio of activity of l-adrenaline to l-phenylephrine was 0.6 in producing a motor response on the isolated uterus.

THE EFFECT OF HYPOGASTRIC NERVE STIMULATION

In 1895 and 1896 Langley and Anderson made a detailed study of the anatomy of the hypogastric nerve in the rabbit. They found that stimulation of the post-ganglionic fibres from the inferior mesenteric ganglion caused a strong contraction of the uterine muscle and constriction of the uterine arterioles. Elliot (1905) noted the similarity of this action to that of adrenaline on the intact uterus. Dale (1906) and

Cushny (1906) noticed that these responses could be reversed by ergot alkaloids, which indicated that the hypogastric nerve in this species contained both excitatory and inhibitory fibres. Sauer, Jett-Jackson and Reynolds (1935) confirmed that nerve stimulation produced uterine contraction in the adult rabbit. An excitatory response to nerve stimulation was obtained by Mann (1950) in immature and ovariectomised animals as well as in the adult. In 1952 Schofield observed that a secondary inhibitory effect on the uterine musculature followed the excitatory response to nerve stimulation but considered that this was due to the intense vasoconstriction which occurred at the same time, rather than to any specific action of the transmitter on the uterine muscle.

THE EFFECT OF ADRENERGIC BLOCKING AGENTS

Many of the early reports indicated that the excitatory effects of sympathomimetic amines and of hypogastric nerve stimulation could be reversed by ergot alkaloids to produce inhibition of uterine spontaneity and a decrease in muscular tone (Dale, 1906; Cushny, 1906; Fardon, 1908; McSwiney and Brown, 1926 and Mendez, 1928). These results were recorded using both <u>in vivo</u> and <u>in vitro</u> uterine preparations.

With the introduction of more specific blocking agents, Rothlin and Brügger (1945) reported that dihydroergotamine antagonised but did not reverse the excitation of isolated

uterine muscle by adrenaline. Similar observations were made by Ahlquist *et al*. (1947) with tolazoline. They reported that the reversal of the response to adrenaline, rather than its antagonism, occurred only when the amine was applied during a contraction produced by the blocking agent. Nickerson and Goodman (1947) found that dibenamine reversed the motor effect of adrenaline both on the isolated uterus and on the uterus *in situ*. Ahlquist (1948) found, using isolated preparations, that the excitatory responses to a series of catecholamines were reversed by tolazoline. Biphasic responses were produced by the amines *in vivo*, the excitatory component of which was antagonised by tolazoline. He concluded from these results that the rabbit uterus contained α-excitatory and β-inhibitory receptors. Mann (1950) found that the excitatory responses to adrenaline and to noradrenaline could be antagonised or reversed by dibenamine in both *in vivo* and *in vitro* preparations. The action of noradrenaline was more easily antagonised, and its inhibitory activity was more easily exposed, than that of adrenaline. Gross *et al*. (1951) showed that phentolamine antagonised the actions of adrenaline and noradrenaline on α-excitatory receptors in the uterus and exposed the action of the amines on the inhibitory receptors. Luduena (1962) concluded that the excitatory action of *l*-isoprenaline on the isolated uterus was exerted via α-receptors since it was antagonised by piperoxan. Black and

Stevenson (1962) demonstrated that the excitatory action of adrenaline was not mediated via β-receptors because the response was unaltered by pronethalol.

Direct evidence for the existence of β-receptors was produced by Willems, Bernard, Delaunois and Schaepdryver (1965) and by Willems and Schaepdryver (1966) who showed that propranolol blocked the relaxation of the uterus produced by isoprenaline. Indirect evidence was presented also, in that the motor effects of adrenaline and noradrenaline were enhanced by propranolol. Lambotte, Lambert and Cession-Fossion (1967) found that the isoprenaline-induced relaxation of isolated uterine strips was antagonised by dichloroisoprenaline and that the contractions caused by adrenaline and by noradrenaline were blocked by a benzodioxane derivative, Quiloflex. This indicates the presence of both α- and β-adrenergic receptors in the uterus of the rabbit.

In regard to the action of adrenergic blocking agents on the response to hypogastric nerve stimulation, Mann (1950) found that dibenamine blocked or reversed this response and that to noradrenaline, while the effect of adrenaline was less readily antagonised. This indicated that the transmitter in the hypogastric nerve might be noradrenaline. Blockade of the excitatory effect of hypogastric nerve stimulation by α-receptor blocking agents was confirmed by Varagić (1956) using tolazoline on an isolated hypogastric nerve-uterus

preparation and by Setekleiv (1964) using dihydroergotamine and phentolamine in in vivo experiments. In their paper concerning the influence of the sexual state of the rabbit on the response of the uterus to nerve stimulation, Miller and Marshall (1965) showed that the excitatory response to nerve stimulation in oestradiol-treated animals was abolished by phentolamine, while an inhibitory effect which occurred after progesterone treatment was blocked by propranolol. Thus it was concluded that the excitatory responses to hypogastric nerve stimulation were mediated via α-receptors and that the inhibitory responses were mediated via β-receptors.

THE INFLUENCE OF OVARIAN HORMONES ON UTERINE ADRENERGIC RESPONSES

Sauer et al. (1935) reported that although adrenaline caused uterine contraction in pregnant and non-pregnant rabbits, the response to hypogastric nerve stimulation was reversed from excitation in non-pregnant animals to inhibition in pregnant animals. In an attempt to correlate this change with alterations in ovarian hormone levels they showed that nerve stimulation caused contraction of the uteri of ovariectomised animals, both before and after treatment with oestrogen. In pseudopregnant animals or in animals which had undergone prolonged luteal stimulation either no effect was recorded or uterine relaxation occurred. This work has been confirmed

recently by Miller and Marshall (1965) using an isolated hypogastric nerve-uterus preparation. Nerve stimulation caused contraction of the uteri from untreated rabbits and from rabbits previously given oestradiol, but if progesterone treatment followed the oestrogen injections, nerve stimulation then caused the uterus to relax. The latter response was blocked by propranolol and the former by phentolamine. These responses to nerve stimulation were not influenced by ganglion blocking or parasympathetic blocking agents. The hormone treatment did not alter the catecholamine content of the uterine tissue although reserpine decreased it. Reserpine also caused a decrease in the response to nerve stimulation.

Willems et al. (1965) and Willems and Schaepdryver (1966) found that in vivo the uteri of ovariectomised, oestrogen-treated rabbits were contracted by adrenaline and by noradrenaline and that these responses were reversed by phenoxybenzamine. However, the uteri of rabbits which had received Gestanon (17α-allyl-β-hydroxy-estr-4-ene), a strongly progestational agent, were relaxed by adrenaline and occasionally by noradrenaline. These responses were reversed by propranolol. This suggested that a predominance of β-receptors exists in the uteri from animals treated with Gestanon (Willems and Schaepdryver, 1966). The action of isoprenaline was not altered by the hormone treatments and the amine always caused uterine relaxation.

These results have led to the conclusion that the ovarian hormones must exert their influence at the effector cell, namely that oestrogen treatment increases the proportion of α-excitatory receptors while in progesterone-treated uterine muscle β-receptors are predominant.

INTRODUCTION

The predominant response of the uterus of the rabbit to adrenaline and to noradrenaline is a contraction of the myometrium caused by α-receptor stimulation. In this work the excitatory activity of isoprenaline also has been investigated. Recordings of the blood pressure have been made at the same time as the in vivo uterine recordings in order to determine whether a particular dose of an amine which would produce a qualitative change in the uterine response also would promote a changed response in the arterial vasculature.

The responses of the uterus of the adult rabbit to stimulation of the hypogastric nerve appear to be influenced by the ovarian hormones while the action of adrenaline is not affected (p. 150). However, since ovarian hormones have been found to influence catecholamine activity on the uterus of other mammalian species, it was considered worthwhile to reinvestigate the problem using, as well as adult rabbits, ovariectomised and immature animals, some of which were treated with ovarian hormones.

The N-ethyl analogue of adrenaline has been used in this work, for although it causes a contraction of the uterus of the adult rabbit, it possesses a greater inhibitory activity at β-receptors than adrenaline or noradrenaline, and therefore would be more suitable for the detection of any alteration in the predominance of one type of response over the other.

METHODS

As in the experiments on the uterus of the rat (Section I p. 49) both *in vivo* and *in vitro* studies have been made on the uterus of the rabbit.

In the early work New Zealand White rabbits were used but later Dutch rabbits were found to be more satisfactory and thus were used in the majority of the experiments. All animals received Oxoid S.G.1 diet and tap water *ad libitum*.

Four types of experimental animal were employed:

1. ADULT RABBITS

Adult, virgin, female New Zealand White rabbits in a weight range of 3.8 - 5.1 kg, and Dutch rabbits in a weight range of 2.2 - 3.4 kg were employed. The adult female does not exhibit an oestrous cycle but exists in an oestrous condition until mated (Parkes, 1956).

2. IMMATURE RABBITS

The smaller strains of rabbits mature more quickly and hence Dutch rabbits were used at an earlier age than the New Zealand White strain. The following weight ranges were employed:

New Zealand White 620 - 1,850 g, i.e. aged 7 - 11 weeks.

Dutch 570 - 1,235 g, i.e. aged 4 - 8 weeks.

In animals younger than these the uteri were not developed sufficiently to permit investigations to be made.

3. OVARIECTOMISED RABBITS

Adult, virgin, female Dutch rabbits in a weight range of 1.8 - 2.6 kg were employed. In the earlier experiments the ovaries were removed via a single mid-line incision in the abdominal wall. It was found more satisfactory however, to carry out the ovariectomy from two lateral incisions in the dorsal surface of the body wall. Anaesthesia was accomplished with sodium pentobarbitone, 30 mg/kg intravenously, followed by smaller doses until surgical anaesthesia was achieved. Fur was removed from a region on each side of the back, away from the spinal column and caudal to the lowest rib. An incision was made in this region in the skin and the body wall muscle layers were pared apart. The ovarian artery and vein were located and were tied off close to the ovary. A cotton thread was tied round the Fallopian tube and the ovary was removed. The body wall and skin were closed with sutures. This procedure was repeated on the opposite side for the removal of the other ovary. The animals were not used for at least 28 days.

4. HORMONE-TREATED RABBITS

Ovariectomised and immature animals were given either oestradiol or progesterone, singly or together, to simulate conditions in the adult animal.

The following treatments were used:

(i) Immature animals

Oestradiol only: 50 µg oestradiol benzoate per rabbit per day for three days.

Progesterone only: 1 mg per rabbit per day for four days.

Oestradiol and progesterone: each rabbit received 50 µg oestradiol benzoate per day for two days followed by 100 µg oestradiol benzoate + 1 mg progesterone per day, four injections on alternate days.

(ii) Ovariectomised animals

Oestradiol only: 500 µg oestradiol benzoate per rabbit per day for five days.

Progesterone only: 2.5 mg per rabbit per day, four injections on alternate days.

Oestradiol and progesterone: each rabbit received 100 µg oestradiol benzoate per day for two days followed by 100 µg oestradiol benzoate + 2.5 mg progesterone per day, four injections on alternate days.

All doses were given subcutaneously in arachis oil. The animals were used three days after the last injection. The size and vascularity of the uterus were noted as a measure of the effect of the hormones.

IN VIVO EXPERIMENTS

Immature and untreated ovariectomised rabbits could not be used owing to the relatively undeveloped state of the uterus but otherwise, as in the in vitro experiments, adult animals and hormone treated ovariectomised animals were employed.

The rabbits were anaesthetised either with a slow intravenous injection of 7 - 9 ml/kg body weight of a 25% solution of urethane, or with sodium pentobarbitone, 30 mg/kg intravenously followed by smaller doses until surgical anaesthesia was achieved. Anaesthesia with urethane was more easily carried out but it sometimes resulted in a low blood pressure.

The methods and the apparatus for setting up blood pressure and uterine recordings were identical to those described for the rat (p. 52, diagram, p. 282) except for small changes in the recording apparatus to allow for the much larger size of the rabbit uterus.

In a number of experiments the hypogastric nerve was stimulated. The location of the nerve in this species was described accurately by Langley and Anderson (1896) who stated that it followed closely the route of the abdominal aorta below the origin of the ovarian arteries. The nerve was tied, cut centrally and stimulated using a Palmer square wave stimulator at a point distal to the inferior mesenteric

ganglion, before it subdivided into many fibres in the region of the aortic bifurcation. Suitable electrical stimuli ranged from 1 - 5 V, with 0.2 - 5.0 msec pulse width at 20 - 50 per sec for a duration of 30 sec.

IN VITRO EXPERIMENTS

Similar procedures and conditions as described in Section I (p. 54) for the rat were used to study the responses of the isolated uterus. The rabbit was stunned and then killed by cutting the neck and the whole uterus was removed. Either the complete uterus, one horn, or a part of a horn was set up according to the overall size of the uterus. In some experiments pieces of tissue were stored either in Krebs or in Tyrode solution (p. 283) at 1 - 5°C for periods of up to five days before being used. In order to obtain a better comparison between the *in vivo* and the *in vitro* work some of the *in vitro* experiments were performed using a uterine horn from a rabbit in which the other horn had been used in an *in vivo* experiment.

Details of the materials used in the experimental work are given in the Appendix, p. 276.

RESULTS

ADULT RABBITS

in vivo experiments

The spontaneous activity of the uterus varied widely. In five out of fifteen experiments no activity was observed. In the other ten experiments the spontaneity varied from small contractions to large regular movements occurring every 20 - 60 seconds, sometimes producing pressure changes of up to 20 mm Hg. These variations could not be attributed to the different strains of rabbits or to the anaesthetic used. Neither did they bear any obvious relationship to the general condition of the animal during the course of the experiment, especially in regard to changes in the arterial blood pressure.

The actions of the amines on the uterus could be obtained both by intravenous and by retrograde intra-arterial injections into the femoral vessels without producing qualitatively different results. By using the latter route however, much larger doses could be given without causing pronounced actions elsewhere in the animal.

Adrenaline (0.5 - 10 µg) and noradrenaline (1.0 - 10 µg) caused contractions of the uterus, while isoprenaline in similar doses produced solely an inhibitory action. Doses of adrenaline and noradrenaline which produced strong motor effects caused, in seven out of ten experiments, secondary inhibitory responses. This was observed as a reduction in the

height of the spontaneous uterine contractions (Fig. 24, p. 210). A similar action was observed with N-ethylnoradrenaline. In no instance was an inhibitory response obtainable to any amine except isoprenaline without an excitatory effect first preceding it. Solely excitatory effects were recorded however with smaller doses of the amines than those which produced the biphasic actions, again with the exception of isoprenaline (Fig. 25, p. 211). It was possible to obtain a reversal of the isoprenaline inhibitory action when doses of the order of 1 mg were injected, i.e. approximately 1000 times the dose required for an inhibitory effect (Fig. 25, p. 211). This action was not associated with any secondary inhibitory component and smaller doses of isoprenaline, which previously had caused inhibition, did not produce such an action after the large doses had been employed. In one experiment α-ethylnoradrenaline produced excitatory effects and it appeared to be approximately equipotent with noradrenaline. The approximate ratios of equiactive doses to cause excitation were, in terms of noradrenaline = 1.0 (at a dose of 4.8 μg); adrenaline = 0.8; α-ethylnoradrenaline = 0.5; N-ethylnoradrenaline = 5; isoprenaline = 767 (Table 12, p. 202).

In some experiments the hypogastric nerve was stimulated postganglionically (Fig. 24, 25, 27; pp. 210, 211, 213). This resulted in a contraction of the uterine muscle. Where

the uterus exhibited spontaneous activity this was usually inhibited after the initial excitatory effect of nerve stimulation. However, as with adrenaline and noradrenaline, no inhibitory response was obtained without a preceding excitatory effect, but stimuli weaker than those which produced these biphasic effects produced solely excitatory responses.

In regard to the action of the amines on the blood pressure, it was noted that the pressor activity of intravenously administered adrenaline was approximately twice that of noradrenaline. Isoprenaline produced a pressor response when doses greater than 1 mg were administered (Fig. 26, p. 212). This reversal from the more commonly observed depressor response occurred in parallel with its reversal of action to a motor effect on the uterus, both reversals occurring when doses of the order of 1 mg were administered. The sequence of results recorded when administering isoprenaline in increasing doses from 0.1 - 8 mg is shown in Figures 25 and 26 (pp. 211, 212). Doses from 0.1 - 10 µg caused depressor and uterine inhibitory actions which were relatively short-lived. Larger doses produced similar but more prolonged effects, especially on the uterus, and doses of 50 µg usually caused very prolonged uterine inhibition. Subsequent administration of 1 mg isoprenaline at first produced no effect on the already inhibited musculature

of the blood vessels and of the uterus but a further dose of the order of 1 mg resulted in a pressor action and a contraction of the uterus. Doses of 1 - 8 mg produced similar but graded responses. These doses did not produce any inhibitory effects and neither did smaller doses of the order of 10 μg which previously had caused depressor and uterine inhibitory responses.

The excitatory actions on the uterus produced by noradrenaline, adrenaline and N-ethylnoradrenaline were antagonised by phentolamine (0.2 - 0.5 mg i.v.). These doses of the blocking agent were ineffective in antagonising the response to hypogastric nerve stimulation and even repetition of these doses of phentolamine failed to block completely the response to nerve stimulation (Fig. 27, p. 213). Where the responses to N-ethylnoradrenaline and noradrenaline were biphasic (excitation followed by inhibition) the inhibitory action persisted or was enhanced after phentolamine. The inhibitory action of adrenaline however, was not enhanced and in those instances in which adrenaline produced only excitation, phentolamine did not reveal any inhibitory action (Fig. 28, p. 214) unless much higher doses of adrenaline were employed (p. 168). Phentolamine, in the doses required to produce adrenergic blockade, itself caused changes in uterine activity which made any quantitative assessment of sympathomimetic amine activity unreliable. The order of the

amines however, when arranged according to their relative inhibitory potencies was isoprenaline > N-ethylnoradrenaline > noradrenaline > adrenaline.

The inhibitory action of isoprenaline was antagonised by 0.4 - 1.0 mg i.v. propranolol (Fig. 29, p. 215) and subsequent doses of isoprenaline of the order of 100 μg were able to cause excitatory effects. This was in contrast to the much larger doses (1.0 - 10.0 mg) which were necessary to cause excitation before propranolol. The excitatory action of N-ethylnoradrenaline was enhanced after propranolol, while that of adrenaline was not affected appreciably by the blocking agent. In four out of five experiments the uterine contraction caused by noradrenaline also was unaffected by β-blockade, but in one experiment the motor response to noradrenaline was enhanced. In three experiments in which the isoprenaline-induced inhibition was antagonised by propranolol, the excitatory response to hypogastric nerve stimulation was not influenced. Propranolol, in doses effective for β-receptor blockade, increased uterine motility in three out of nine experiments.

The elucidation of the nature of the activity of the sympathomimetic amines on the uterus was of prime importance in this work, but a number of observations on the actions of the amines on the blood pressure after adrenergic blockade was noted:

(i) After β-receptor blockade induced by large doses of isoprenaline, adrenaline and noradrenaline were equiactive in causing pressor effects. This was in contrast to their activities before isoprenaline, when adrenaline possessed approximately twice the potency of noradrenaline (Fig. 26, p. 212).

(ii) After β-receptor blockade induced by either propranolol or by large doses of isoprenaline the pressor actions of intravenously administered adrenaline, noradrenaline and isoprenaline were antagonised by tolazoline (10 mg) or by phentolamine (0.2 mg).

(iii) The depressor actions of small doses (1 - 4 μg) of intra-arterially administered adrenaline, noradrenaline and isoprenaline were antagonised, and in the former two instances reversed, by propranolol (0.6 mg).

(iv) The pressor actions of intravenously administered adrenaline, noradrenaline and N-ethylnoradrenaline were reversed by phentolamine (0.5 mg). Noradrenaline exhibited a greater depressor potency than adrenaline.

ADULT RABBITS

in vitro experiments

The majority of the fourteen experiments were performed using an isotonic lever, although in a few instances an isometric lever was used. This latter recording system usually reduced the spontaneity and sensitivity of the tissue, but no qualitative differences were noted in the results obtained using the two systems. The spontaneous activity of the preparations was not influenced appreciably by the different bathing media employed and the majority of preparations exhibited rhythmic contractions, although these were not apparent at first in tissues which had been stored.

Concentrations of adrenaline greater than 1×10^{-9} or of noradrenaline greater than 1×10^{-8} caused contractions of the uterus (Fig. 30a, 31; pp. 216, 217). Similar concentrations of isoprenaline inhibited the spontaneous activity (Fig. 31, 32; pp. 217, 218). In contrast to the results obtained in vivo, where in many experiments adrenaline and noradrenaline caused an inhibition of spontaneous activity after the initial excitatory effect, these amines produced solely excitatory actions at all concentrations in the in vitro preparations.

It was found that if the concentration of isoprenaline administered was approximately 1000 times greater than the minimal effective concentration to cause inhibition it would, on repeated application, produce an excitatory effect. The

first high concentration, e.g. 4×10^{-5}, produced only inhibition of the uterus. A following identical concentration usually had little effect and then subsequent concentrations caused contractions of the uterus. Concentrations of 2×10^{-5} - 4×10^{-4} were effective in producing excitatory actions and dose-response curves could be obtained (Fig. 32, p. 218). Occasionally, if the first concentration was as great as 2×10^{-4}, then a motor effect could be observed immediately, without the need for the repeated application of isoprenaline (Fig. 30b, 35; pp. 216, 221). The lower concentrations of isoprenaline, which previously had inhibited the uterus, did not produce any effects after the employment of the higher concentrations of this amine. The N-ethyl- and α-ethyl-substituted noradrenalines produced excitatory effects (Fig. 30a, p. 216), but both amines were less potent than noradrenaline. The following approximate ratios of equiactive concentrations to cause excitation were, in terms of noradrenaline = 1.0 (at a concentration of 1.9×10^{-7}); adrenaline = 0.15; N-ethylnoradrenaline = 9; α-ethylnoradrenaline = 205; isoprenaline = 1,820 (Table 13, p. 203). The excitatory actions of noradrenaline, N-ethylnoradrenaline, α-ethylnoradrenaline and isoprenaline were potentiated after the employment of concentrations of isoprenaline necessary to exert motor effects (Fig. 30b, p. 216) The following ratios of equiactive concentrations to cause

excitation after isoprenaline were, in terms of adrenaline = 1.0 (at a concentration of 2 x 10^{-8}); noradrenaline = 2.5; N-ethylnoradrenaline = 12.0; α-ethylnoradrenaline = 850; isoprenaline = 5,250.

Although isoprenaline (10^{-8} - 10^{-5}) was able to induce an inhibitory effect on the uterus (Fig. 32, p. 218), the other sympathomimetic amines studied were observed rarely to exert such an action. In one experiment (Fig. 30a, p. 216) a concentration of 8 x 10^{-7} N-ethylnoradrenaline caused a biphasic effect of excitation followed by inhibition of noradrenaline-induced contractions of the uterus, while a concentration of 1.6 x 10^{-6} or greater produced an excitatory effect. Similarly 1 x 10^{-5} α-ethylnoradrenaline caused an inhibitory effect, while 2 x 10^{-5} or a higher concentration produced a contraction. Adrenaline and noradrenaline did not inhibit either the spontaneous or the acetylcholine-induced uterine contractions. Concentrations below the lowest effective excitatory concentration produced no observable change (Fig. 31, p. 217).

The excitatory actions of adrenaline, noradrenaline, isoprenaline, α-ethyl and N-ethyl-substituted noradrenalines were blocked by 4 x 10^{-5} tolazoline (Fig. 35, p. 221), but a more complete blockade was achieved with 1 x 10^{-6} phentolamine (Fig. 34, p. 220), since the former blocking agent exhibited an excitatory effect itself in concentrations required for

α-receptor blockade. Phentolamine did not influence the inhibitory action of isoprenaline (Fig. 34, p. 220) or the excitatory effect of acetylcholine. The exposure of an inhibitory action of the amines after α-receptor blockade was most marked with N-ethylnoradrenaline. An inhibitory response was obtained also to noradrenaline after phentolamine. The inhibitory action of adrenaline in this respect was very weak, causing a small inhibitory effect in only two out of five experiments (Fig. 34, p. 220). These actions of the amines after phentolamine were very similar to those obtained <u>in vivo</u> where, although N-ethylnoradrenaline and noradrenaline readily caused inhibitory responses, adrenaline caused only a weak inhibitory effect with concentrations much higher than those necessary to produce a contractile effect before phentolamine. The approximate ratios of equiactive concentrations of the amines to cause inhibition after phentolamine were, in terms of noradrenaline = 1 (at a concentration of 8×10^{-7}); adrenaline = 2.0; N-ethylnoradrenaline = 0.25; isoprenaline = 0.05 (Table 19, p. 209). Propranolol, in concentrations of the order of 10^{-8}, antagonised the inhibitory action of low concentrations (10^{-7}) of isoprenaline. It did not alter appreciably the ability of adrenaline to exert contractile effects, but the motor actions of noradrenaline, N-ethylnoradrenaline and isoprenaline were enhanced after propranolol (Fig. 33, p. 219). The approximate ratios of

equiactive concentrations to cause excitation after propranolol were, in terms of noradrenaline = 1.0 (at a concentration of 8×10^{-8}); adrenaline = 0.25; N-ethylnoradrenaline = 3.8; isoprenaline = 250.

It was found that storage of the excised uterine tissue in Krebs or Tyrode solution at 1 - 5°C for periods of up to five days increased the sensitivity of the tissue to adrenaline, noradrenaline and isoprenaline. The number of results obtained did not allow an accurate quantitative evaluation of the activity, but it was noticeable that the increased sensitivity occurred both to the contractile effects of adrenaline and noradrenaline as well as to the relaxant effect of isoprenaline. It was not evident that the motor action of adrenaline was potentiated more than that of noradrenaline or vice versa. The maximum effect was reached after storage for two days and subsequently there was a slow loss of sensitivity to the three amines. In contrast, there was a gradual decline in sensitivity to acetylcholine throughout the period of storage of the tissue.

IMMATURE RABBITS

in vivo experiments

Owing to the small size of the uterus of immature rabbits, it was not possible to record uterine responses in vivo.

IMMATURE, UNTREATED RABBITS

in vitro experiments

The early experiments were carried out with the New Zealand White strain of rabbit. Uteri from animals less than six weeks old were devoid of spontaneous activity and did not respond to the sympathomimetic amines or to acetylcholine. In uteri from older animals responses to adrenaline and to noradrenaline were obtained. In some experiments these were excitatory but more often, in contrast to the responses of uteri from adult animals, inhibitory responses were produced. In an attempt to explain these different results in apparently similar preparations the precise ages and weights of the animals, and the weights of the uteri were recorded (Table 18, p. 208). It was found that in six experiments using uteri from rabbits aged 7 - 11 weeks both adrenaline and noradrenaline produced solely inhibitory actions (Fig. 36, p. 222), or biphasic effects. These biphasic actions consisted of a weak excitatory effect followed by a greater inhibitory component. The excitatory component of such a biphasic response to adrenaline was always larger than the

excitatory component of a response to noradrenaline which produced a comparable inhibitory effect. In contrast, in older but still immature animals the responses were qualitatively similar to those obtained in adult animals, although the uteri were generally less sensitive to adrenaline and to noradrenaline.

Similar results were obtained using Dutch rabbits (Table 18, p. 208) except that, as this strain matures earlier than the larger New Zealand White rabbit, it was possible to record responses to the sympathomimetic amines when the animals were five weeks old. In two experiments using Dutch rabbits which were four weeks old the uteri were insensitive both to the amines and to acetylcholine. In six preparations from Dutch rabbits which were 5 - 8 weeks old adrenaline and noradrenaline produced predominantly inhibitory responses. In one experiment with a uterus from a seven week old animal adrenaline produced excitatory effects while noradrenaline caused inhibition with concentrations of the order of 10^{-7} and excitation with concentrations of the order of 10^{-6} (Fig. 37, p. 223). In animals older than nine weeks the responses were similar to those obtained in the adult animal, but the preparations were less sensitive to the amines.

Isoprenaline produced an inhibitory effect in all the preparations from immature rabbits (Fig. 36, p. 222). The uteri from immature rabbits were much more sensitive to

isoprenaline than the uteri from adult animals. Effective inhibitory concentrations of isoprenaline were of the order of 10^{-10} on preparations from immature animals, while concentrations of the order of 10^{-8} produced similar effects on the uteri from adult rabbits. In no instance was it possible to elicit a contraction of the uterus from the immature rabbit with isoprenaline using concentrations as great as 10^{-4}, although similar concentrations readily caused contractions of the uteri from adult animals.

N-Ethylnoradrenaline produced solely inhibitory responses (Fig. 36, p. 222), except in one experiment with an eight week old rabbit in which a biphasic response was recorded. This contrasted with its motor action in the adult animal, except on one occasion where a biphasic response also occurred in the adult (Fig. 30a, p. 216).

The approximate ratios of equiactive concentrations to cause inhibition (using New Zealand White rabbits less than eleven weeks old, and Dutch rabbits less than eight weeks old) were, in terms of noradrenaline = 1.0 (at a concentration of 7.5×10^{-7}); adrenaline = 6.9; N-ethylnoradrenaline = 0.5; isoprenaline = 0.03 (Table 14, p. 204).

After treatment with 4×10^{-6} phentolamine, consistent inhibitory responses were obtained with concentrations of adrenaline which previously had induced only excitation. However, the α-blocking agent did not influence greatly the

inhibitory activities of adrenaline or of isoprenaline, but there were slight increases in the inhibitory activities of noradrenaline and N-ethylnoradrenaline (Table 19, p. 209). In two experiments after propranolol (4×10^{-8}) had been added to the bathing solution, the inhibitory actions of adrenaline and noradrenaline were antagonised, exposing excitatory effects (Fig. 36, p. 222). Adrenaline was approximately twice as potent as noradrenaline in initiating these excitatory effects. The inhibitory action of isoprenaline was antagonised but not reversed by propranolol. In one experiment using propranolol an N-ethylnoradrenaline-initiated inhibition was antagonised but no subsequent excitatory action was observed, even with a 50-fold increase in the concentration of N-ethylnoradrenaline (Fig. 36, p. 222).

In a Dutch rabbit which was twelve weeks old adrenaline, noradrenaline and N-ethylnoradrenaline produced motor effects which were reversed by 4×10^{-6} phentolamine, and the subsequent inhibitory responses were antagonised by 4×10^{-7} propranolol.

IMMATURE, OESTROGEN-TREATED RABBITS

in vitro experiments

Treatment with oestradiol (p. 156) caused a six- to nine-fold increase in the uterine weights when compared with the weights of uteri from untreated animals (Table 18, p. 208). Spontaneous activity varied considerably in magnitude and frequency in the four preparations studied.

In contrast to the results obtained on uteri from untreated immature animals adrenaline, noradrenaline and N-ethylnoradrenaline caused strong motor effects (Fig. 38, p. 224). Excitatory effects were observed also with concentrations of the order of 10^{-5} isoprenaline (Fig. 39, p. 225). Phenylephrine (4×10^{-8}) induced a motor response but it was less potent than adrenaline. Not only qualitatively but also quantitatively the responses to the amines were similar to those recorded from the uterus of the adult animal (Fig. 30, 32, pp. 216, 218). The approximate ratios of equiactive concentrations to cause excitation were, in terms of noradrenaline = 1.0 (at a concentration of 0.8×10^{-7}); adrenaline = 0.38; N-ethylnoradrenaline = 8; α-ethylnoradrenaline = 1,000; isoprenaline = 8,000 (Table 14, p. 204).

In regard to any inhibitory activity only isoprenaline (10^{-9} - 10^{-6}) was able to exert such an action (Fig. 39, p. 225).

IMMATURE, PROGESTERONE-TREATED RABBITS

in vitro experiments

Two Dutch rabbits which were seven weeks old were treated with progesterone (p. 156) which caused a five-fold increase in the uterine weights when compared with the weights of uteri from untreated animals of the same age (Table 18, p. 208).

The excitatory responses to adrenaline, noradrenaline and N-ethylnoradrenaline were not different qualitatively from the responses of uteri from immature animals treated with oestradiol alone. Quantitatively however, the preparations were approximately ten times less sensitive to the three amines. The approximate ratios of equiactive concentrations to cause excitation were, in terms of noradrenaline = 1.0 (at a concentration of 14×10^{-7}); adrenaline = 0.6; N-ethylnoradrenaline = 6.5 (Table 14, p. 204). α-Ethylnoradrenaline and isoprenaline, in concentrations up to 4×10^{-4}, failed to produce excitatory effects.

Inhibitory actions were produced by isoprenaline, N-ethylnoradrenaline and α-ethylnoradrenaline, but adrenaline and noradrenaline were devoid of any inhibitory activity. The sensitivity of the preparations to isoprenaline in producing inhibition was much lower than the sensitivity of uteri from untreated animals.

IMMATURE, OESTROGEN + PROGESTERONE-TREATED RABBITS

in vitro experiments

A six week old Dutch rabbit was treated with oestradiol and progesterone (p. 156) which resulted in a twelve-fold increase in the uterine weight when compared with the uterus from an untreated animal of the same age (Table 18, p. 208). In a second experiment similar treatment with oestradiol and progesterone caused a five-fold increase in the weight of the uterus of a seven week old rabbit.

Excitatory actions were produced in both experiments by adrenaline, noradrenaline, α-ethylnoradrenaline, N-ethylnoradrenaline and isoprenaline, and the approximate ratios of equiactive concentrations to cause excitation were, in terms of noradrenaline = 1.0 (at a concentration of 3.1×10^{-7}); adrenaline = 0.13; N-ethylnoradrenaline = 2.3; α-ethylnoradrenaline = 100; isoprenaline = 724 (Table 14, p. 204).

Adrenaline did not cause any inhibitory effects, but in one experiment noradrenaline, in a concentration of 4×10^{-8}, produced solely an inhibitory effect and concentrations of 8×10^{-8} - 8×10^{-7} produced biphasic responses of stimulation followed by inhibition. Higher concentrations produced solely excitatory actions. Isoprenaline produced uterine inhibition in concentrations ranging from 10^{-9} - 10^{-6}.

After treatment with 4×10^{-7} propranolol, the inhibitory component of the response to noradrenaline was abolished and the activity of the amine in eliciting a motor effect was enhanced. The motor activity of isoprenaline also was enhanced so that the following approximate ratios of equiactive concentrations to cause excitation after propranolol were, in terms of noradrenaline = 1.0 (at a concentration of 5×10^{-8}); adrenaline = 0.8; phenylephrine = 1.0; isoprenaline = 640.

OVARIECTOMISED, UNTREATED RABBITS

in vivo experiments

Four attempts were made to record uterine activity in untreated ovariectomised animals. In each experiment the uterus was considerably atrophied and avascular, and in two experiments no spontaneous activity was recorded.

These preparations were relatively insensitive to the catecholamines, and in one experiment stimulation of the hypogastric nerve had no observable influence on uterine activity. Of the five catecholamines employed only adrenaline and noradrenaline produced excitatory effects (Table 15, p. 205) and, as in the normal adult animal (Fig. 24, p. 210), these effects were usually followed by a secondary inhibitory component (Fig. 40, p. 226).

In regard to inhibitory activity not associated with a previous excitatory component, it was found that noradrenaline and N-ethylnoradrenaline in doses of the order of 1 μg caused such an effect. However, adrenaline caused in most instances a biphasic effect of stimulation followed by inhibition, as in the normal adult rabbit. In contrast to the normal adult however, if a dose of adrenaline was chosen just below that required for excitation, it was possible to produce solely an inhibitory response, but this was demonstrated only in one experiment (Fig. 40, p. 226). The approximate ratios of equiactive doses to cause inhibition were, in terms of

noradrenaline = 1.0 (at a dose of 2 μg); adrenaline = 1.0; N-ethylnoradrenaline = 0.25; isoprenaline = 0.1 (Table 15, p. 205).

Doses of adrenaline, noradrenaline and N-ethylnoradrenaline, which previously had initiated uterine inhibition, caused contractile effects after an intravenous injection of 1 mg propranolol. The approximate ratios of equiactive doses to cause excitation after propranolol were, in terms of noradrenaline = 1.0 (at a dose of 2 μg); adrenaline = 0.5; N-ethylnoradrenaline = 2.5. The action of 1 μg isoprenaline was blocked by propranolol but no excitatory activity was revealed.

Blood pressure responses recorded at the same time as the uterine movements did not appear to be appreciably different from those obtained in normal adult animals.

in vitro experiments

In four experiments one uterine horn was set up which had been stored overnight after removal from an animal which had been used in an in vivo experiment. On two occasions both uterine horns were removed from freshly killed rabbits and the second horn was used after storage overnight. Similar results were obtained from the stored tissue to that used immediately following removal from the animal. The preparations were usually quiescent although stored preparations sometimes showed

a small degree of spontaneous activity.

Excitatory responses were obtained to adrenaline, noradrenaline, N-ethyl- and α-ethylnoradrenaline. The preparations were approximately eight times less sensitive to these amines when compared with their activities in the adult animal. No contractions could be obtained with high concentrations (10^{-4}) of isoprenaline (Fig. 42, p. 228). The approximate ratios of equiactive concentrations to cause excitation were, in terms of noradrenaline = 1.0 (at a concentration of 14×10^{-7}); adrenaline = 0.19; N-ethylnoradrenaline = 14; α-ethylnoradrenaline = 200 (Table 16, p. 206).

In regard to inhibitory activity, in only two out of seven experiments was it possible to demonstrate an inhibitory action of adrenaline, and in these instances it was very weak, a small increase in concentration resulting in a contraction. All the other amines exerted inhibitory actions in concentrations considerably lower than those required to contract the tissue (Fig. 41, p. 227), e.g. for noradrenaline an inhibitory response was obtained with a concentration of the order of 10^{-9}, whereas a concentration of 1.2×10^{-6} was required for an excitatory effect.

In one experiment 8×10^{-8} noradrenaline relaxed a preparation contracted by acetylcholine, while a similar concentration of adrenaline caused a contraction which summated

with the acetylcholine contraction (Fig. 42, p. 228). As the concentration of noradrenaline was increased the response changed first to a biphasic effect of excitation followed by inhibition and then to solely an excitatory effect. The excitatory activity of noradrenaline was however, much lower, and the ratio of the concentration of noradrenaline to the equiactive concentration of adrenaline was much greater than in the normal adult rabbit. Isoprenaline was at least 100 times more potent in causing uterine inhibition in these preparations than in the adult rabbit (compare Fig. 31, 41; pp. 217, 227). The approximate ratios of equiactive concentrations to cause inhibition were, in terms of noradrenaline = 1.0 (at a concentration of 0.47×10^{-7}); adrenaline = 7.5; N-ethylnoradrenaline = 0.04; α-ethylnoradrenaline = 2.8; isoprenaline = 0.02 (Table 16, p. 206).

After treatment with 4×10^{-6} phentolamine, consistent inhibitory responses were obtained with concentrations of adrenaline which previously had induced only excitation. The α-blocking agent did not influence greatly the inhibitory potencies (Fig. 41, p. 227) of adrenaline and noradrenaline, although those of N-ethylnoradrenaline and isoprenaline were increased (Table 19, p. 209).

OVARIECTOMISED, OESTROGEN-TREATED RABBITS

in vivo experiments

In one rabbit given oestradiol (p. 156) following ovariectomy the uterus was of a similar appearance to that of the normal adult animal. The uterus was fully relaxed and devoid of spontaneity.

Adrenaline, noradrenaline and N-ethylnoradrenaline produced solely excitatory actions (Fig. 43, p. 229). The approximate ratios of equiactive doses to cause excitation were, in terms of noradrenaline = 1.0 (at a dose of 4.0 μg); adrenaline = 0.13; N-ethylnoradrenaline = 10 (Table 15, p. 205).

Although inhibitory responses would not have been directly observable, that they were not apparent was indicated by the absence of any decrease in the excitatory activity of repeated doses of the amines. α-Ethylnoradrenaline in doses of up to 0.5 mg failed to exert either excitatory or inhibitory actions.

The activity of N-ethylnoradrenaline on the blood pressure also was demonstrated. An initial intravenous dose of 2.5 μg produced a 10 mm Hg depressor response. Subsequent 2 - 5 μg doses had little action, but graded pressor responses were caused by 25 and 50 μg (Fig. 43, p. 229). The amine possessed 1/30th of the pressor activity of noradrenaline. α-Ethylnoradrenaline produced no appreciable pressor activity in doses of up to 0.5 mg, but doses of 0.05 - 0.5 mg caused small

depressor effects.

in vitro experiments

In two experiments strong spontaneous contractions were recorded and it was found beneficial to use an isometric lever.

All five catecholamines caused excitatory effects and the responses obtained were similar to those observed in the untreated adult animal. The approximate ratios of equiactive concentrations to cause excitation were, in terms of noradrenaline = 1.0 (at a concentration of 2.1×10^{-7}); adrenaline = 0.3; N-ethylnoradrenaline = 3; α-ethylnoradrenaline = 175; isoprenaline = 2,800 (Table 16, p. 206).

No inhibitory responses occurred to adrenaline, noradrenaline and N-ethylnoradrenaline. Isoprenaline produced inhibitory effects with concentrations from 10^{-9} - 10^{-6}. This indicated that the oestrogen-treated preparation was at least 100 times less sensitive to the inhibitory action of isoprenaline than was the uterus from the untreated, ovariectomised animal. In one experiment concentrations of the order of 10^{-6} α-ethylnoradrenaline inhibited the spontaneous activity after causing an initial weak motor effect.

OVARIECTOMISED, PROGESTERONE-TREATED RABBITS

in vivo experiments

In one experiment progesterone administration (p. 156) did not change the appearance of the uterus from its atrophied state caused by ovariectomy and the preparation showed no spontaneous activity.

Adrenaline, noradrenaline and N-ethylnoradrenaline produced excitatory effects, but isoprenaline was devoid of excitatory activity in doses of up to 4 mg, even though these doses produced pressor effects. The approximate ratios of equiactive doses to cause excitation were, in terms of noradrenaline = 1.0 (at a dose of 3 μg); noradrenaline = 0.67; N-ethylnoradrenaline = 2.7; α-ethylnoradrenaline = 6.7 (Table 15, p. 205).

Isoprenaline exerted inhibitory actions with doses from 1 - 10 μg. The excitatory action of N-ethylnoradrenaline was followed always by an inhibitory phase, but adrenaline and noradrenaline produced no inhibitory effects.

in vitro experiments

In contrast to the activity of the uteri from animals treated with oestradiol, these preparations from progesterone-treated rabbits exhibited only weak spontaneous contractions in the two experiments performed.

The main difference between the responses of these preparations and those from untreated, or from oestrogen-treated, ovariectomised animals was their relative insensitivity to the catecholamines. Concentrations of adrenaline, noradrenaline and N-ethylnoradrenaline necessary to produce excitatory effects were 20 - 30 times higher than those required to produce similar effects on the uteri from oestrogen-treated rabbits. This insensitivity was manifested also by the absence of any excitatory responses both to α-ethylnoradrenaline and to isoprenaline. The approximate ratios of equiactive concentrations to cause excitation were, in terms of noradrenaline = 1.0 (at a concentration of 24×10^{-7}); adrenaline = 0.5; N-ethylnoradrenaline = 2.7 (Table 16, p. 206).

Isoprenaline exerted solely inhibitory effects, but a relatively high concentration (6×10^{-8}) was necessary for a minimal response. Similarly, relatively high concentrations of N-ethylnoradrenaline (4×10^{-7}) and α-ethylnoradrenaline (4×10^{-5}) were required to produce inhibitory responses. These were at least 100 times greater than those which produced comparable effects in untreated, ovariectomised rabbits (Table 16, p. 206). Adrenaline and noradrenaline failed to exert inhibitory actions in these progesterone-treated preparations, in contrast to their effects on uteri from untreated animals.

OVARIECTOMISED, OESTROGEN + PROGESTERONE-TREATED RABBITS

in vivo experiments

In one rabbit given oestradiol and progesterone (p. 156) following ovariectomy the uterus was of a similar appearance to that of the normal adult animal. The uterus exhibited irregular spontaneous activity, the contractions varying both in intensity and in frequency.

Adrenaline and noradrenaline produced contractions of the uterus without appreciably altering the appearance of the spontaneous activity. N-ethylnoradrenaline caused a biphasic effect of contraction followed by inhibition of the uterine spontaneity. The inhibitory component of the response was more pronounced than the preceding excitatory effect. Isoprenaline in doses of up to 4 mg failed to produce excitatory actions on the uterus even though doses of this order produced pressor effects. The approximate ratios of equiactive doses to cause excitation were, in terms of noradrenaline = 1.0 (at a dose of 4 μg); adrenaline = 0.5; N-ethylnoradrenaline = 25 (Table 15, p. 205).

Isoprenaline exerted solely inhibitory actions with doses from 0.5 μg - 4.0 mg. N-ethylnoradrenaline exerted inhibitory actions with doses from 10 - 100 μg, but these effects were preceded always by a contraction of the uterus.

in vitro experiments

In three experiments results were obtained which were not greatly different from those recorded from the uteri of animals treated with oestradiol alone. However, the spontaneous activity was more pronounced than in the latter preparations.

All the five amines produced excitatory actions which were not associated with any following inhibitory phase (Fig. 44, p. 230). The approximate ratios of equiactive concentrations to cause excitation were, in terms of noradrenaline = 1.0 (at a concentration of 0.72×10^{-7}); adrenaline = 0.56; N-ethylnoradrenaline = 10.8; α-ethylnoradrenaline = 306; isoprenaline = 2,222 (Table 16, p. 206).

Inhibitory effects were produced by 10^{-9} - 10^{-7} isoprenaline and 4×10^{-7} α-ethylnoradrenaline (Fig. 44, p. 230), but neither adrenaline, noradrenaline nor N-ethylnoradrenaline exerted inhibitory actions.

In two out of three experiments β-receptor blockade was achieved with (i) propranolol (Fig. 45, p. 231), and (ii) repeated large concentrations of isoprenaline. This resulted in a lowering of the effective concentration of the sympathomimetic amine necessary to produce an excitatory effect. The approximate ratios of equiactive concentrations to cause excitation after β-receptor blockade with propranolol were, in terms of noradrenaline = 1.0 (at a concentration of 2×10^{-8});

adrenaline = 0.5; N-ethylnoradrenaline = 2.5; α-ethylnoradrenaline = 125; isoprenaline = 500.

A summary of the results of the experiments performed on the isolated uterus of the rabbit is given in Table 17 (p. 207). The table indicates the transition in the nature of the responses of the uteri to the sympathomimetic amines through the different sexual states of the animals. It also contrasts the concentrations effective for excitation and for inhibition in each sexual state.

DISCUSSION

The existence of α-excitatory and β-inhibitory adrenergic receptors in the uterine smooth muscle of the adult rabbit was demonstrated by Ahlquist (1948). His work has been extended, *in vivo* and *in vitro*, by using both the α- and β-adrenergic blocking agents currently available as well as a series of sympathomimetic amines. α-Ethylnoradrenaline has been used because of its value as a detector of adrenergic receptor types (Levy, 1959; Levy and Ahlquist, 1960). N-ethylnoradrenaline has been employed because of its intermediate structure between adrenaline and isoprenaline.

In contrast to the responses of the uterus of the rat, the predominant responses to sympathomimetic amines of the uterus of the adult rabbit were found to be excitatory. Only isoprenaline exerted solely an inhibitory action. Even with N-ethylnoradrenaline, which possesses a greater inhibitory activity than adrenaline or noradrenaline, an excitatory action was obtained. This confirms previous reports of its action *in vitro* on this tissue (Konzett, 1940; Lands *et al.*, 1948; McDougal and West, 1954). In the majority of *in vivo* experiments in which the uterus exhibited spontaneous activity, a secondary inhibitory response to adrenaline, noradrenaline and N-ethylnoradrenaline occurred, in contrast to the solely motor action of these amines *in vitro*. This was noted by Ahlquist (1948) who indicated that the effects of the amines on

the uterine vasculature were most likely to be responsible for these differences between *in vivo* and *in vitro* myometrial responses. The fact that, after administration of phentolamine, both the motor and the secondary inhibitory actions of adrenaline on the uterus were antagonised, suggests that the secondary inhibitory component occurred indirectly as a result of stimulation of α-receptors. Thus a uterine vasoconstriction could have been caused either by direct stimulation of α-receptors in the vasculature or by pressure exerted externally upon the blood vessels by the contracting uterine muscle. The resulting decrease in blood flow in the uterus may have caused a temporary ischaemia and thus produced the secondary inhibitory action observed after the injection of adrenaline. This was not so for noradrenaline and N-ethylnoradrenaline, where the secondary inhibitory component persisted after α-receptor blockade and was blocked subsequently by propranolol. This indicates the greater ability of noradrenaline and N-ethylnoradrenaline to stimulate myometrial β-inhibitory receptors as compared with adrenaline.

Isoprenaline caused an excitatory effect on the isolated uterus, an effect noted by Luduena (1962). The concentration required to produce this effect was approximately 10,000 times that necessary to produce a submaximal inhibitory action. In the work reported here, it was shown that the repeated injection of doses of this order, i.e. 1 - 10 mg, also caused

excitatory effects on the uterus in vivo. This reversal, from the usually observed inhibitory action with low doses, occurred in parallel with a reversal of its depressor action on the arterial blood pressure. The pressor response to repeated large doses of isoprenaline was reported by Butterworth (1963a, b) using the cat. The uterine excitatory responses and pressor effects caused by all the amines, including isoprenaline, were antagonised by tolazoline and phentolamine, indicating that they were mediated by α-receptors. This was supported also by the order of potency of the amines in producing a contraction of the uterus in the presence of propranolol, i.e. adrenaline > noradrenaline > N-ethylnoradrenaline > α-ethylnoradrenaline > isoprenaline.

The effects on the uterus of hypogastric nerve stimulation resembled closely the effects of adrenaline and noradrenaline in vivo, causing biphasic responses of excitation followed by inhibition. The secondary inhibitory component of this response to nerve stimulation was not observed after treatment with α-blocking agents, indicating that it was mediated indirectly by α-receptors rather than directly by β-receptors as was found with adrenaline (p. 190). The excitatory response to nerve stimulation was reduced but could never be abolished by the doses of α-blocking agents employed. Therefore it was not possible to determine whether a reversal to a solely β-inhibitory effect could be achieved as with noradrenaline.

Other workers found that, after a complete blockade of α-receptors, nerve stimulation resulted in uterine inhibition (Mann, 1950; Miller and Marshall, 1965), a response which was elicited via β-receptors (Miller and Marshall, 1965).

Although isoprenaline was the only amine which inhibited the uterus of the adult rabbit, the other amines were able to induce such an effect after treatment with phentolamine and the following order of inhibitory potency was produced: isoprenaline > N-ethylnoradrenaline > α-ethylnoradrenaline > noradrenaline > adrenaline. This order, together with the observation that the inhibitory responses were antagonised by propranolol, indicated the presence of β-receptors.

The excitatory potency of adrenaline on the uterus of the rabbit was greater than that of noradrenaline. This effect was not confined to the uterus because adrenaline also produced a greater pressor action than noradrenaline in rabbits, as indicated by Ahlquist (1948). Further, noradrenaline possessed the greater depressor action after α-blockade by phentolamine. It is also pertinent that noradrenaline was found to be the more potent in activating the uterine β-receptors, as indicated by its greater inhibitory potency on uteri of immature animals, or on uteri of adult animals after α-receptor blockade. This explains the observations that, while hypogastric nerve stimulation (Sauer et al., 1935) and noradrenaline (Euler, 1946) sometimes caused relaxation

of the uterus from a pregnant animal, adrenaline failed to produce such an effect. It also accounts for the observation that after the intravenous administration of dibenamine to the adult rabbit, the effects of noradrenaline and of hypogastric nerve stimulation were reversed to produce uterine relaxation, while the response to adrenaline was still a contraction (Mann, 1950).

The responses of the uterus of the rabbit to the sympathomimetic amines were found to vary according to the physiological state of the uterus. In the adult rabbit no appreciable variation in responses to the amines occurred between different animals. The actions of amines on the uterus of the immature rabbit however, differed from those of the adult. The predominant actions of adrenaline and noradrenaline were inhibitory and although in some experiments the two amines were able to exert motor effects these were weak and followed always by an inhibitory phase. The inhibitory action of noradrenaline was greater than that of adrenaline and the inhibitory component of a biphasic response to noradrenaline was always greater than the inhibitory component of a response to a concentration of adrenaline which produced a comparable excitatory effect. The reduced excitatory activity of the amines was evident also in that isoprenaline was unable, even in high concentrations, to exert excitatory effects. This change in activity was most clearly evident with

N-ethylnoradrenaline which exerted solely motor effects on the uterus of the adult rabbit, while on the immature uterus similar concentrations produced solely inhibition. The order of the amines, when arranged in a series according to their relative inhibitory potencies, was opposite to the order obtained for excitatory activity via α-receptors in the uterus of the adult animal. This, together with the fact that propranolol antagonised the inhibitory actions, indicated that the catecholamines acted predominantly at β-receptors in the uterus of the immature rabbit in contrast to their predominant action at α-excitatory receptors in the adult. The treatment with propranolol caused the inhibitory or biphasic actions of the amines to be converted into motor effects and the order of excitatory activity of the amines was then similar to the order obtained using the uteri of the adult. Further, the inhibitory actions of the amines on the uterus of the adult after treatment with phentolamine closely resembled the results obtained using the untreated immature uterus. In both instances noradrenaline possessed a greater β-inhibitory action than adrenaline. These results are in contrast to those of Mann (1950) who found that the responses of the immature uterus to adrenaline and to noradrenaline in vitro were not different from those recorded in adult animals. Balassa (1940) however, reported that, while adrenaline contracted the uteri of mature rabbits in vivo, it produced no effect on the uteri of

immature animals with doses of 10 - 50 μg/kg. It is most likely that these discrepancies have arisen over the term "immature" rabbit. The animals used by Balassa were 800 - 1,000 g in weight, and therefore were probably under six weeks old even if they were of a small variety (Hammond and Marshall, 1925), i.e. similar to the animals used in this work. Those used by Mann were "under six months old". The smaller strains of rabbits are capable of mating at four months (Hammond and Marshall, 1925), when presumably the hormonal status governing the uterus is comparable to that of the fully grown animal. It was found in this work that the uteri from "older immature" rabbits responded similarly to uteri from adult animals.

That the hormonal status of the animal is important in determining the nature of the responses to the catecholamines in this species was indicated by the fact that the responses of uteri from immature rabbits closely resembled those from ovariectomised adult animals. The excitatory activities of the amines were reduced using these preparations when compared with their actions on uteri from normal adult animals. Further, treatment of the immature and the ovariectomised, adult animals with oestradiol caused the uteri from these groups to respond similarly to the uteri from normal adult rabbits.

Treatment of uteri from immature or ovariectomised, adult rabbits with propranolol during the course of the experiment

caused an effect similar to that produced by oestrogen administration, and treatment of uteri from adult animals with phentolamine caused responses similar to those recorded in immature and in ovariectomised animals. The administration of oestrogen followed by progesterone did not produce appreciably different results from treatment with oestrogen alone. However, injection of progesterone alone depressed the activity of the catecholamines in exerting either excitatory or inhibitory effects; it did not qualitatively alter the nature of these effects. Miller and Marshall (1965), using an isolated hypogastric nerve-uterus preparation, reached similar conclusions concerning the influence of oestrogen on the responses of the uterus of the immature rabbit to catecholamines. Also these workers indicated that progesterone, instead of having merely a depressant effect on the uterine responses, actually had the opposite effect to oestradiol in that it enhanced activity at the β-receptors and thus accentuated or revealed the inhibitory responses to catecholamines. Similarly, Willems and Schaepdryver (1966) found that the motor effect of adrenaline *in vivo* on the uterus of the oestrogen-treated rabbit was reversed to inhibition on the uteri of rabbits which had received prior treatment with Gestanon (17α-allyl,β-hydroxy-estr-4-ene), a strongly progestational agent. It is pertinent to this work that Lish *et al.* (1960) reported that isoprenaline was able to

induce contractions of isolated, oestrogen-dominated uterine preparations with concentrations of the order of 10^{-5}. These were considerably lower than the concentrations reported by others (Furchgott, 1959; Luduena, 1962) on preparations from rabbits which had not received oestradiol.

Thus the results obtained in this study indicate that oestradiol increases the excitatory activity of sympathomimetic amines on the uterus of the rabbit. The mechanisms through which this enhancement of activity is brought about have not been elucidated, but the similarity between the effects of the amines after oestradiol treatment and after blockade with propranolol suggests that the oestrogen may exert its effect as a consequence of a changed activity and/or availability of the α- and β-adrenergic receptors in the uterus.

SUMMARY

1. The presence of α-excitatory and β-inhibitory adrenergic receptors has been demonstrated in the uterus of the adult rabbit and the ovariectomised adult rabbit, both *in vivo* and *in vitro*, and in the uterus of the immature rabbit *in vitro*.

2. Adrenaline, noradrenaline and N-ethylnoradrenaline produced qualitatively similar effects on the uterus of the adult animal. Noradrenaline possessed a stronger potency than adrenaline at uterine β-receptors and a weaker potency at α-receptors. Of the three amines, N-ethylnoradrenaline possessed the greatest activity at β-receptors and the least activity at α-receptors.

3. Isoprenaline was able to excite α-receptors in the uterus of the adult rabbit in both *in vivo* and *in vitro* preparations. This action was enhanced by blockade of β-receptors with either propranolol or large doses of isoprenaline. Doses which produced uterine contractions also were able to induce stimulation of the α-receptors in the arterial vasculature, thus initiating pressor responses.

4. Stimulation of the hypogastric nerve *in vivo* in the adult animal produced a biphasic response of excitation followed by inhibition, an effect which paralleled the actions of adrenaline and noradrenaline. Both components of these biphasic responses to the amines were blocked by phentolamine, but the effect produced by nerve stimulation was much less readily antagonised.

5. The responses of the uteri to the catecholamines varied according to the sexual states of the rabbits. Isoprenaline did not produce motor actions *in vitro* on the uteri from untreated immature rabbits or ovariectomised adult animals. Adrenaline, noradrenaline and N-ethylnoradrenaline produced inhibitory effects in these preparations, although adrenaline sometimes produced an initial contraction. These effects were similar to those produced by catecholamines on the uteri from normal adult rabbits after a partial blockade of adrenergic α-receptors.

6. Treatment of immature and of ovariectomised adult rabbits with oestradiol caused the responses of the uteri from these animals to be converted into responses similar to those observed using the uteri from intact adult rabbits. These responses to the catecholamines after oestradiol treatment were similar to the responses observed

after a partial blockade of adrenergic β-receptors in the immature and ovariectomised groups. Treatment with both oestradiol and progesterone did not produce significantly different results. Progesterone treatment alone did not qualitatively alter the responses to the catecholamines but lowered the sensitivity of the uteri to their effects.

TABLES 12 - 19

FIGURES 24 - 45

Where the abbreviations and symbols used are not explained at the foot of the Table or Figure, an explanation is given in the Appendix on page 278.

In Tables 12 - 16 each line shows the results from a single experiment.

Mean values are included at the foot of each set of figures.

TABLE 12

ADULT RABBITS

Ratio of the dose of sympathomimetic amine to the equiactive dose of l-noradrenaline.

in vivo experiments

Excitatory response

*Dose (µg) l-Nor	Ratio sympathomimetic amine/l-Nor			
	l-Adr	l-α-EtNor	l-N-EtNor	dl-Iso
5	1.0			500
5	1.0			800
2	0.5			1,000
1	0.5		6	
4	0.5		4	
10	0.25	0.5		
2	0.5			
2	2.0			
10	1.0			
10	1.0			
5	1.0			
2	1.0			
5	0.5			
4.8 = 1	0.8	0.5	5	767

*Dose by retrograde injection into the femoral artery to cause a just submaximal contraction

TABLE 13

ADULT RABBITS

Ratio of the concentration of sympathomimetic amine to the equiactive concentration of l-noradrenaline.

in vitro experiments

Excitatory response

*Conc^{n} l-Nor x 10^{-7}	Ratio sympathomimetic amine/l-Nor			
	l-Adr	l-N-EtNor	l-α-EtNor	dl-Iso
0.2	0.1			4,000
2.0	0.2			4,000
4.0	0.05			250
0.8				2,000
0.8				1,400
12.0				200
2.0				900
0.4	0.25	5	50	
0.8	0.15	17	360	
1.0	0.10	8		
0.2	0.2	10		
0.25	0.3	5		
2.0	0.1			
0.6	0.07			
1.9 = 1	0.15	9	205	1,820

*Concentration to cause a just submaximal contraction.

TABLE 14

IMMATURE RABBITS + HORMONE TREATMENT

Ratio of the concentration of sympathomimetic amine to the equiactive concentration of l-noradrenaline.

in vitro experiments

Treatment	Concⁿ. l-Nor x 10^{-7}		Ratio sympathomimetic amine/l-Nor							
			l-Adr		l-N-EtNor		l-α-EtNor		dl-Iso	
	*E	*I	E	I	E	I	E	I	E	I
U	-	2.0	-	2	-	0.7				0.002
	8.0	4.0	0.5	2	-	0.3			-	0.02
	40.0	40.0	1.0	5					-	0.0005
	20.0	4.0	0.2	5						0.05
	-	0.8	-	25						0.1
	-	1.0	-	2.5						0.0025
	200.0	4.0	0.02	-						
	12.0	4.0	0.33	-						
	56=1		0.41							
		7.5=1		6.9		0.5				0.03
O	0.8	-	0.1	-						1×10^{-9}
	0.04	-	0.2	-						4×10^{-9}
	2.0	-	0.4	-					8,000	4×10^{-9}
	0.4	-	0.8	-	8	-	1,000	4×10^{-7}	8,000	4×10^{-8}
	0.8=1		0.38		8		1,000		8,000	
P	3.0	-	1.0	-	10	2×10^{-7}	-		-	2×10^{-8}
	25.0	-	0.2	-	3	4×10^{-7}	-	4×10^{-5}	-	1×10^{-8}
	14.0=1		0.6		6.5		-		-	
O + P	4.5	-	0.13	-	1.9	2×10^{-8}	80	-	267	4×10^{-9}
	1.7	0.4	0.12	-	2.6	0.25	120	50	1,180	0.025
	3.1=1		0.13		2.3		100		724	

Concentration to cause:

*E = a just submaximal contraction or
*I = a 50% reduction in height of spontaneous or acetylcholine-induced uterine contractions.

- = amine used but no action observed.

TABLE 15

OVARIECTOMISED RABBITS + HORMONE TREATMENT

Ratio of the dose of sympathomimetic amine to the equiactive dose of l-noradrenaline.

in vivo experiments

Hormone treatment	Dose (µg) l-Nor		Ratio sympathomimetic amine/l-Nor							
			l-Adr		l-N-EtNor		l-α-EtNor		dl-Iso	
	*E	*I	E	I	E	I	E	I	E	I
Untreated	5	2	0.5	1.0	-	0.25				0.1
	5	2	0.4	1.0			-	-		
	-	-	20µg	-						
	-	-	-	-	-	-	-	-	-	-
	5=1		0.45							
		2=1		1.0		0.25				0.1
Oestrogen	4	-	0.13	-	10	-	-	-		
Pro-gesterone	3	-	0.67	-	2.7	20µg	6.7	-	-	5µg
Oestrogen + Pro-gesterone	4	-	0.5	-	25	20µg			-	1µg

Dose by retrograde injection into the femoral artery to cause:

*E = a just submaximal contraction or
*I = a 50% reduction in height of spontaneous or acetylcholine-induced uterine contractions.

- = amine used but no action observed.

TABLE 16

OVARIECTOMISED RABBITS + HORMONE TREATMENT

Ratio of the concentration of sympathomimetic amine to the equiactive concentration of l-noradrenaline.

in vitro experiments

Treatment	Conc^{n} l-Nor x 10^{-7}		Ratio sympathomimetic amine/l-Nor							
			l-Adr		l-N-EtNor		l-α-EtNor		dl-Iso	
	*E	*I	E	I	E	I	E	I	E	I
U	4.0	0.2	0.05	-	4	0.01	200	3	-	0.0005
		0.1		-		0.10		3		0.0002
	4.0	0.8	0.25	-	7	0.005	200	2.5	-	0.001
	4.0		0.25							
	4.0	2.0	0.50	-						0.01
	40.0	0.08	0.05	-						0.02
	20.0	0.04	0.04	5						0.005
		0.04		10						0.1
	14.0=1		0.19		5.5		200		-	
		0.47=1		7.5		0.04		2.8		0.02
O	4.0	-	0.2	-	3	-	150	-	800	2×10^{-8}
	0.2	-	0.4	-			200	4×10^{-6}	4,800	4×10^{-8}
	2.1=1		0.3		3		175		2,800	
P	24.0	-	0.67	-	1.3	-	-	-	-	8×10^{-8}
	24.0	-	0.33	-	4.1	4×10^{-7}	-	4×10^{-5}	-	4×10^{-8}
	24.0=1		0.5		2.7		-		-	
O + P	0.24	-	0.67	-	10	-	417	8×10^{-7}	1,667	8×10^{-8}
	0.32	-	0.5	-	10	-	250	4×10^{-7}	2,500	8×10^{-9}
	1.6		0.5		12.5		250		2,500	
	0.72=1		0.56		10.8		306		2,222	

Concentration to cause:

*E = a just submaximal contraction or
*I = a 50% reduction in height of spontaneous or acetylcholine-induced uterine contractions.

- = amine used but no action observed.

TABLE 17

CORRELATION OF THE SEXUAL STATE OF THE RABBIT WITH THE RESPONSE OF THE UTERUS TO SYMPATHOMIMETIC AMINES

in vitro experiments

Sexual state	Concentration (x 10^{-8}) to cause excitation (E) or inhibition (I)							
	l-Adr		l-Nor		l-N-EtNor		dl-Iso	
	E	I	E	I	E	I	E	I
Adult	1.3	-	12	-	73	i	24,000	1.0
Ovariect. + oestrogen + progesterone	3.7	-	6.7	-	74	-	15,000	4.4
Immature + oestrogen + progesterone	4.0	-	32	4.0	72	1.5	24,000	0.25
Ovariect. + oestrogen	4.4	-	17	-	66	-	35,000	3.0
Immature + oestrogen	1.2	-	5.6	-	12	-	18,000	1.2
Ovariect. + progesterone	120	-	270	-	870	40	-	6.0
Immature + progesterone	40	-	120	-	500	30	-	1.5
Ovariect. untreated	12	ii	150	4.7	650	0.087	-	0.038
Immature untreated	112	67	1,400	40	-	17	-	0.40
*Total E and I	9	1	9	3	8	5	5	9

* = number of sexual states in which amine caused an E or I effect.

i = l-N-EtNor caused an inhibitory effect in one of five experiments.

ii = l-Adr caused an inhibitory effect in two of seven experiments.

TABLE 18

RELATIONSHIP BETWEEN THE AGE OF IMMATURE RABBITS AND THE NATURE OF THE UTERINE RESPONSE TO SYMPATHOMIMETIC AMINES

in vitro experiments

Condition of rabbit	Age (weeks)	Body weight (Kg)	Whole uterus weight (mg)	Uterine response to		
				l-Adr	l-Nor	l-N-EtNor
Immature New Zealand White	6	0.84	108	-	-	
	6	0.68	114	-	-	
	7	0.62	154	*I	I	
	8	1.08	94	EI	EI	
	8	1.68	134	EI	EI	
	9	1.31	96	EI	EI	
	11	1.25	234	I	I	
	11	1.85	834	EI	EI	
	12	1.75	750	E	E	
Immature Dutch	4	0.57	57	-	-	-
	4	0.65	72	-	-	-
	5	0.83	90	I	I	I
	5	0.70	97	I	I	I
	6	0.78	106	I, EI	I	I
	6	0.85	72	I	I	I
	7	0.69	110	E	I, E	I
	8	0.94	208	E	E	EI
	9	1.23	247	E	E	E
Immature Dutch + oestrogen (4 expts)	5 to 8	0.60 to 1.30	630 to 2,014	E	E	E
Adult Dutch (14 expts)	at least 20	at least 2.00	at least 5,000	E	E	E

*E = excitatory response; I = inhibitory response;
EI = biphasic response of excitation followed by inhibition.
- = amine used but no action observed.

TABLE 19

EFFECTS OF PHENTOLAMINE ON THE INHIBITORY ACTIVITY OF SYMPATHOMIMETIC AMINES ON THE UTERUS OF THE RABBIT IN DIFFERENT SEXUAL STATES.

in vitro experiments

Phentol-amine 10^{-6}	Sexual state	**Conc.^{n} l-Nor x 10^{-7}	Ratio of the concentration of sympathomimetic amine/ l-Nor		
			l-Adr	l-N-EtNor	dl-Iso
Before	adult	*E	*E	*E	4 x 10^{-9}
	immature	4.0	1.7	0.4	0.01
	ovariect.	0.05	6.7	0.3	0.03
After	adult	8.0	2.0	0.25	0.05
	immature	2.0	4.0	0.2	0.02
	ovariect.	0.08	5.0	0.03	0.0005

*E = amine produced excitatory action only.

** = concentration to cause a 50% reduction in height of spontaneous or acetylcholine-induced uterine contractions.

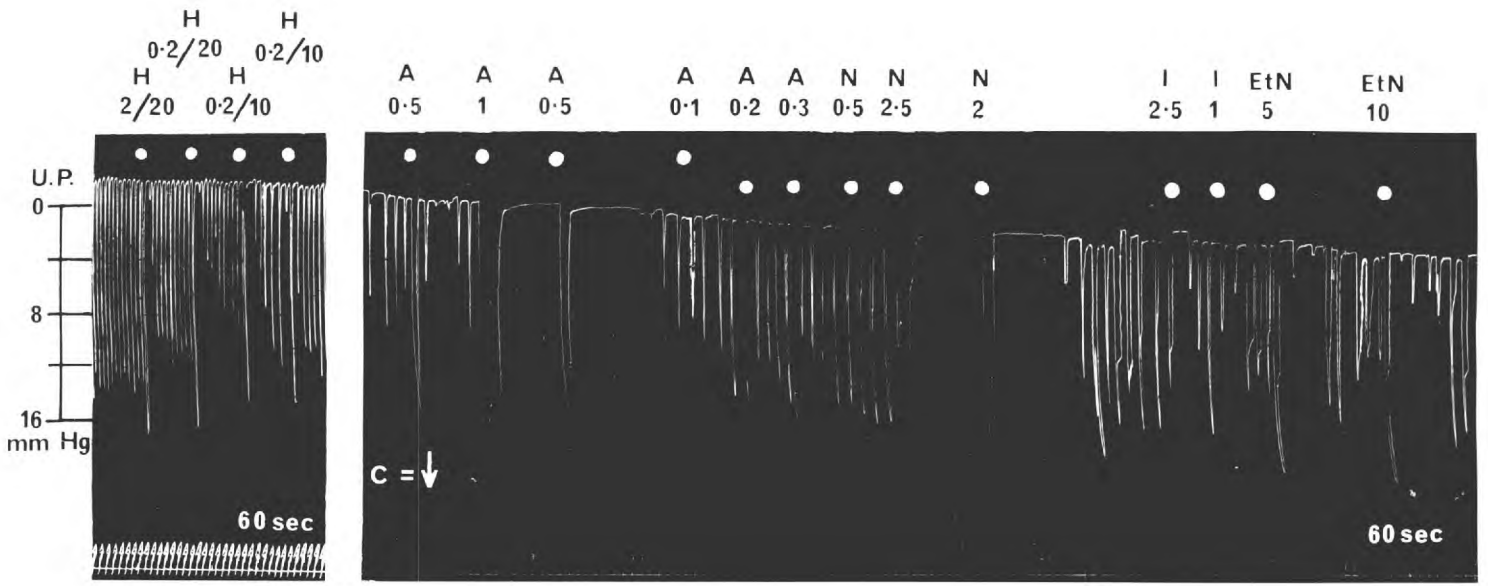


FIGURE 24. Biphasic effects of stimulation of the hypogastric nerve and of µg doses (i.a.) of adrenaline, noradrenaline and N-ethylnoradrenaline, and inhibitory action of isoprenaline on the spontaneous activity of the uterus.

Adult rabbit, 2.4 kg.
in vivo

H = hypogastric nerve stimulation for 10 sec at 1 V with e.g. 2/20 = 2 msec pulse width/20 pulses per sec.

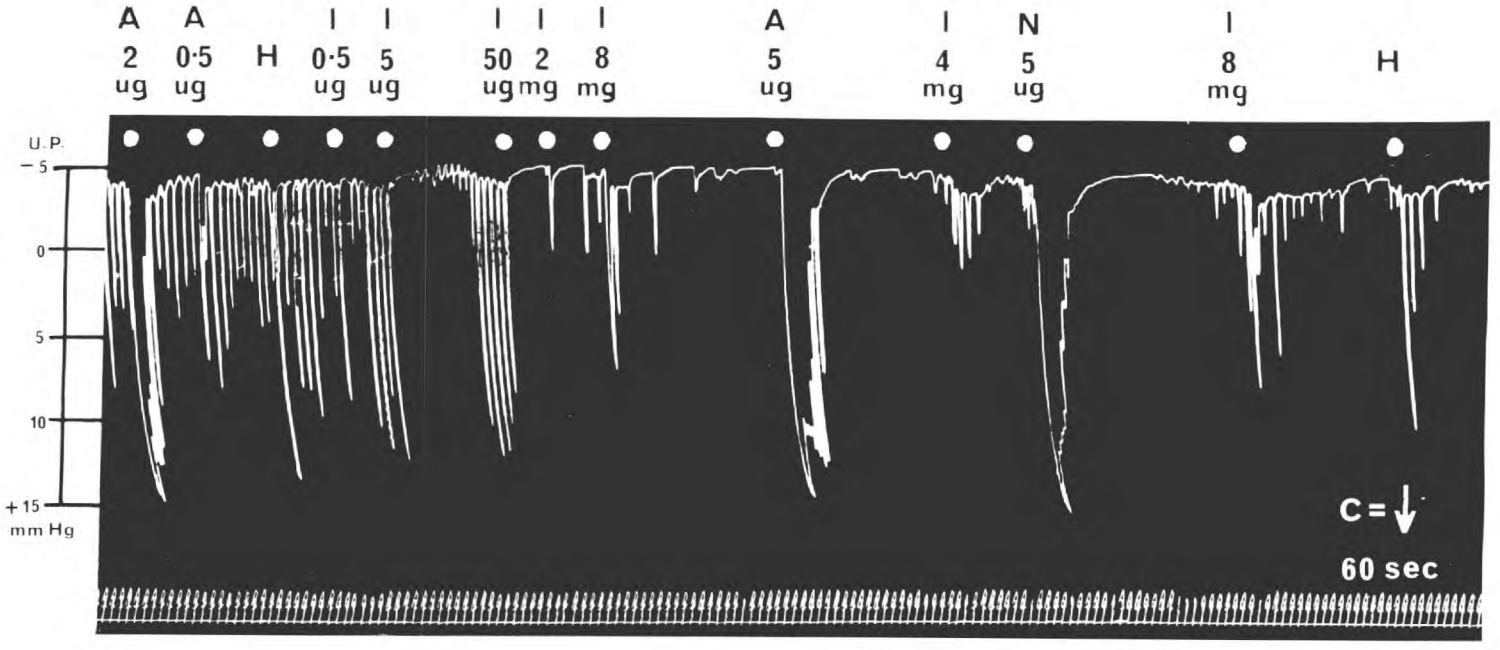


FIGURE 25. Reversal of the inhibitory effect on the uterus of µg doses (i.a.) of isoprenaline to an excitatory action with mg doses.

Adult rabbit, 2.6 kg.
in vivo

H = hypogastric nerve stimulation for 20 sec at 2 V with 2 msec pulse width and 50 pulses per sec.

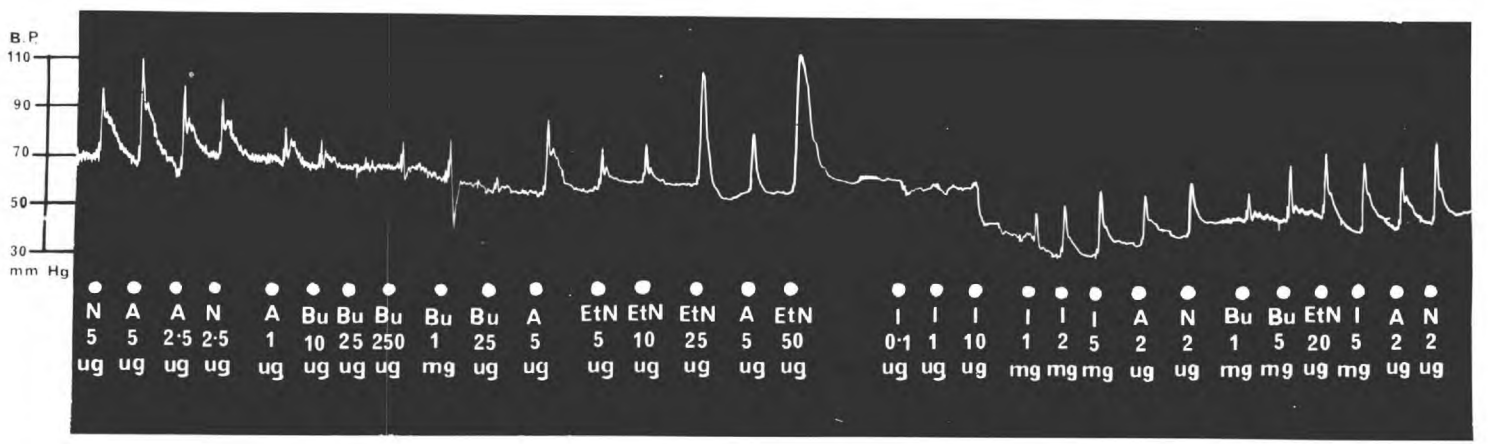


FIGURE 26. Effects of catecholamines (i.v.) upon carotid arterial blood pressure, showing the reversal of the depressor action of µg doses of isoprenaline to a pressor effect with mg doses.

Adult rabbit, 2.6 kg.
in vivo

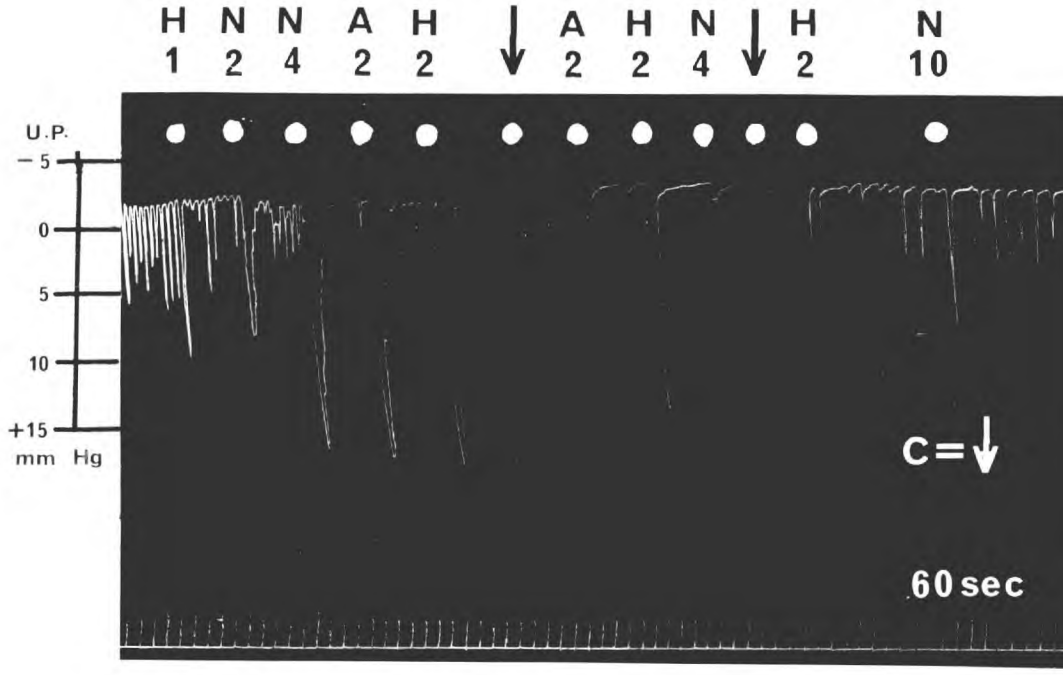


FIGURE 27. Blockade by phentolamine of the excitatory effects on the uterus of µg doses (i.a.) of adrenaline and noradrenaline and of stimulation of the hypogastric nerve.

Adult rabbit, 2.6 kg.
in vivo

↓= intravenous injection of 3 mg phentolamine.

H = hypogastric nerve stimulation for 15 sec with 1 msec pulse width and 20 pulses per sec at e.g. 1 = 1 V.

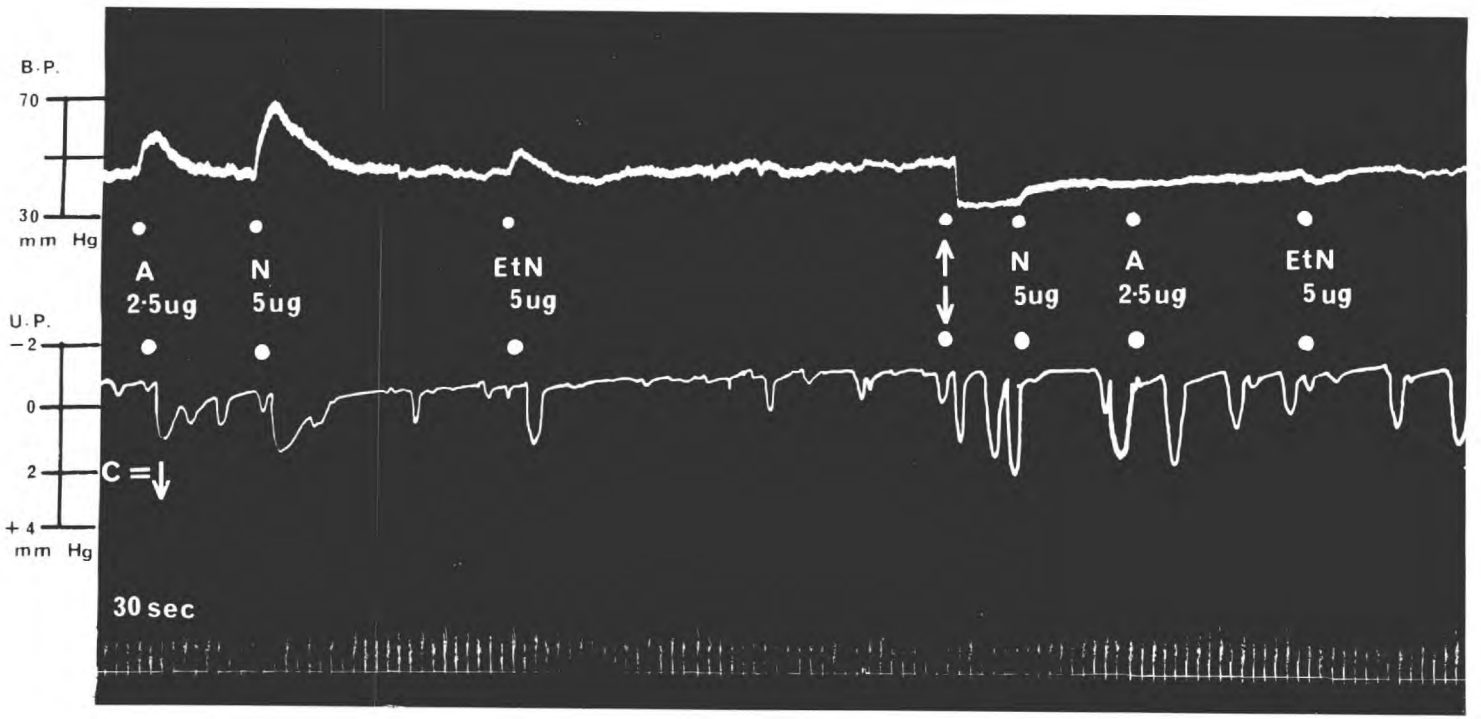


FIGURE 28. Blockade by phentolamine of the pressor effects of adrenaline, noradrenaline and N-ethylnoradrenaline; blockade by phentolamine of the motor effect of adrenaline and reversal of the motor effects of noradrenaline and of N-ethylnoradrenaline on the uterus.

Adult rabbit, 2.4 kg.
in vivo

↑ = i.v. injection of 1 mg phentolamine. Other doses given i.a.

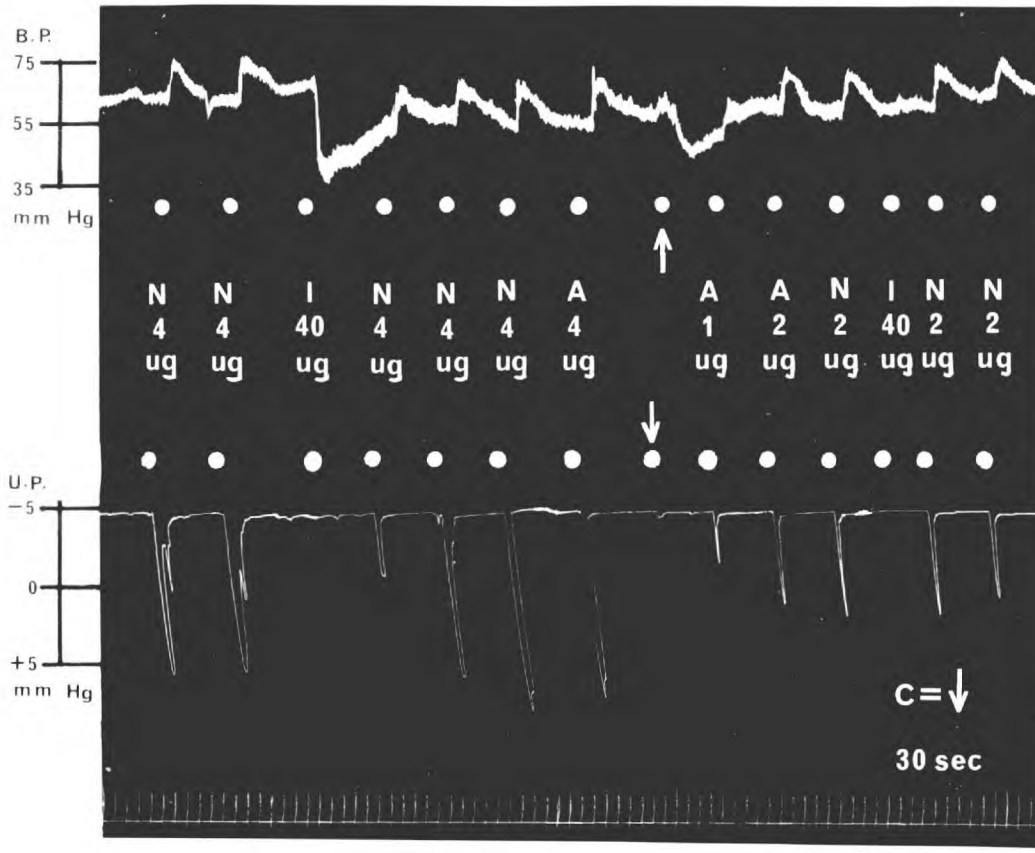


FIGURE 29. Blockade by propranolol of the depressor effect of isoprenaline and of the inhibitory effect of isoprenaline on the uterus.

Adult rabbit, 2.4 kg.
in vivo

↑= i.v. injection of 1 mg propranolol.
Other doses given i.a.

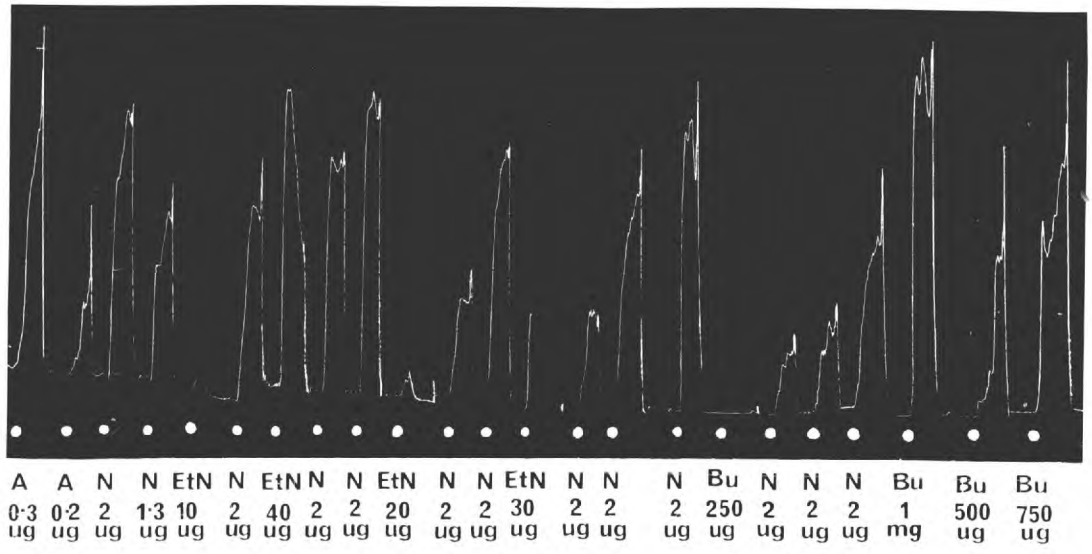


FIGURE 30a. Excitatory effects of catecholamines, biphasic effects of N-ethylnoradrenaline, and the inhibitory effects of α-ethylnoradrenaline on the uterus.

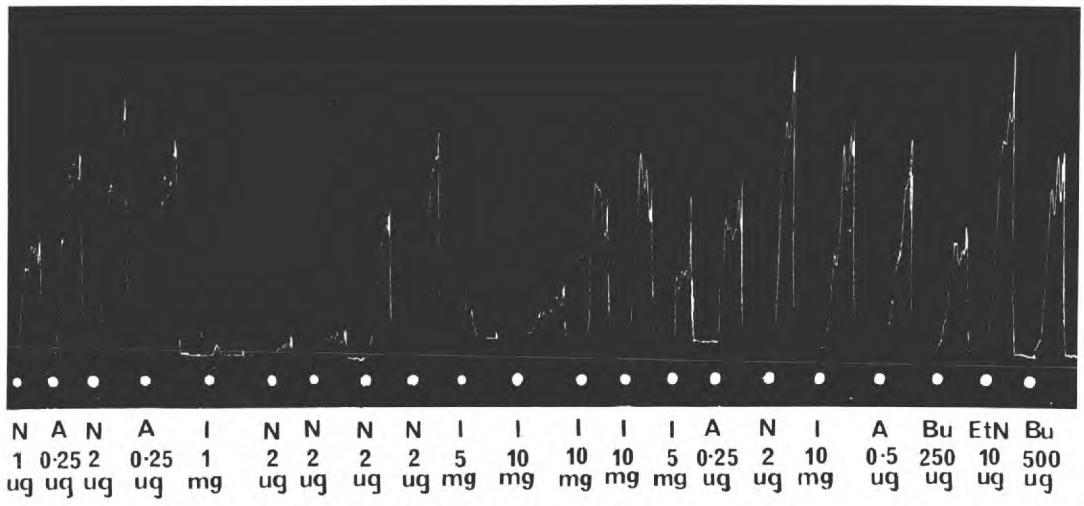


FIGURE 30b. Biphasic and excitatory effects of isoprenaline and the enhancement by isoprenaline of the excitatory effects of N-ethylnoradrenaline and α-ethylnoradrenaline on the uterus.

Adult rabbit, 3.5 kg.
in vitro; Krebs solution; 38°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

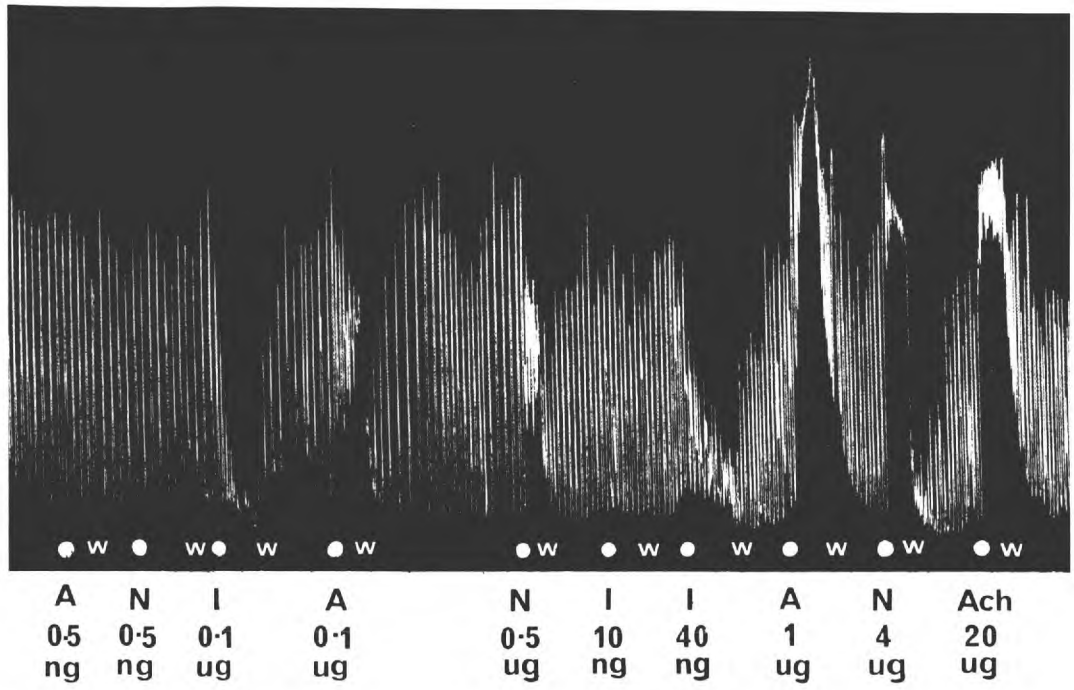


FIGURE 31. Failure of low concentrations of adrenaline and noradrenaline to produce inhibitory effects, and the inhibitory effect of isoprenaline on the spontaneous activity of the uterus.

Adult rabbit, 2.4 kg.
in vitro; Krebs solution; 37°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

W = amine washed out.

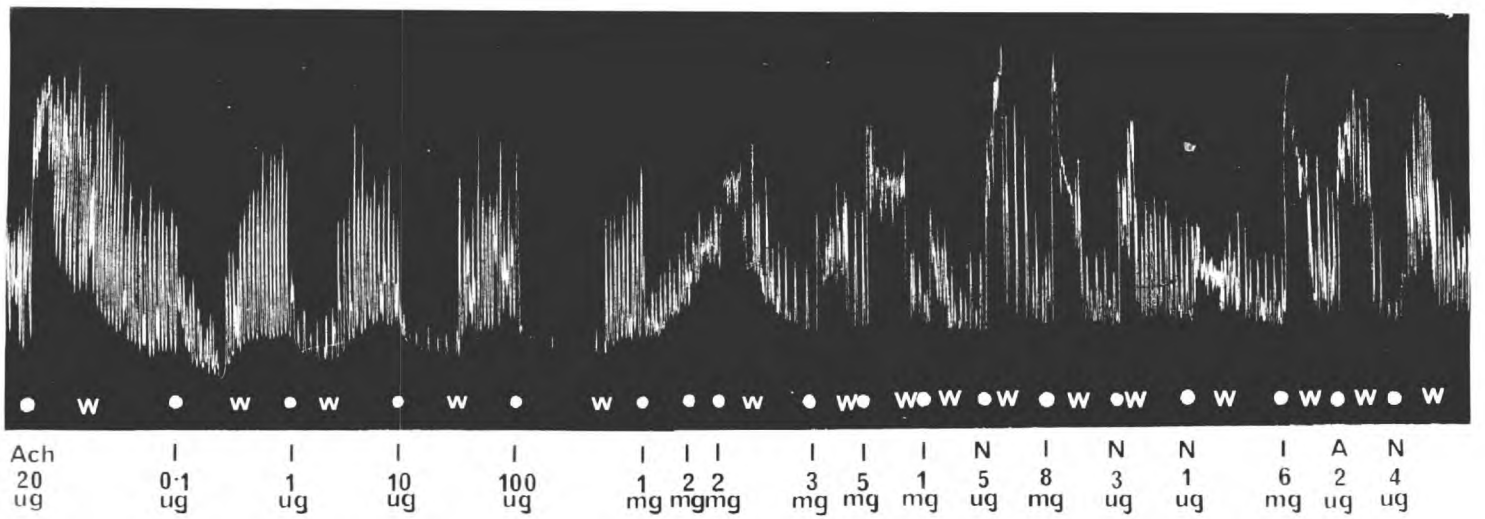


FIGURE 32. Reversal of the inhibitory actions of µg concentrations of isoprenaline to excitatory actions with mg concentrations on the spontaneous activity of the uterus.

Adult rabbit, 3.1 kg.
in vitro; Krebs solution; 38°C; air; isotonic lever.

W = amine washed out.

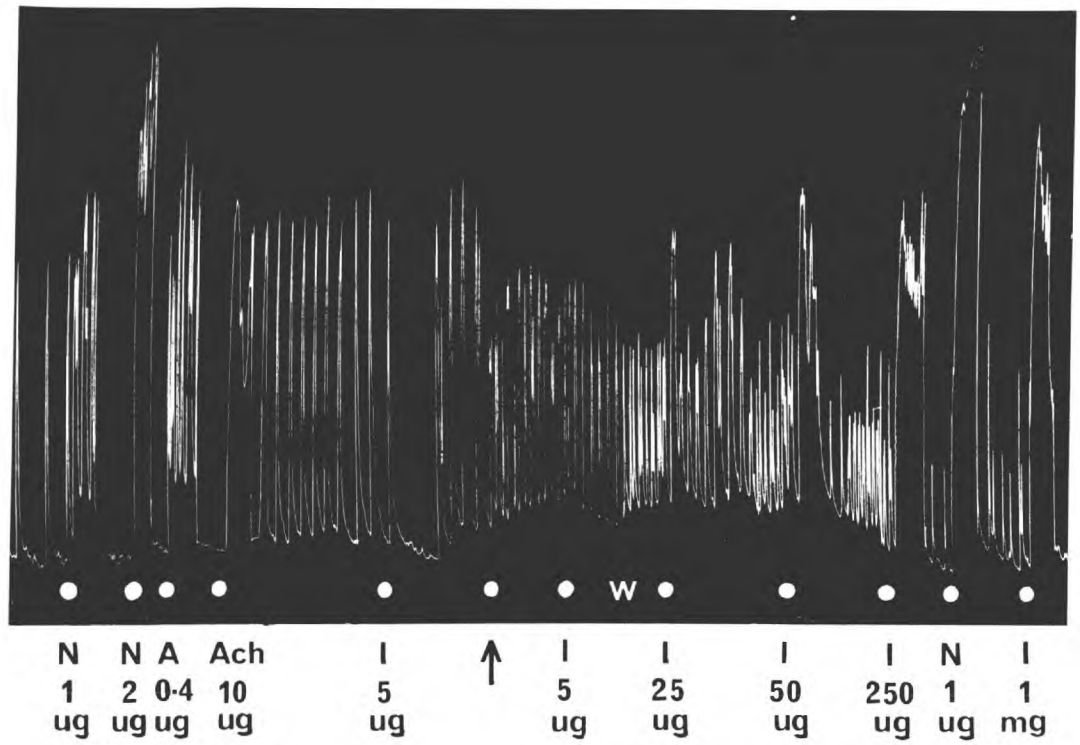


FIGURE 33. Blockade by propranolol of the inhibitory activity of isoprenaline and potentiation of the excitatory activity on the spontaneous movements of the uterus.

Adult rabbit, 3.1 kg.
in vitro; Krebs solution; 37.5°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

↑ = 2×10^{-8} propranolol added to the bathing solution.

W = amine washed out after no effect recorded.

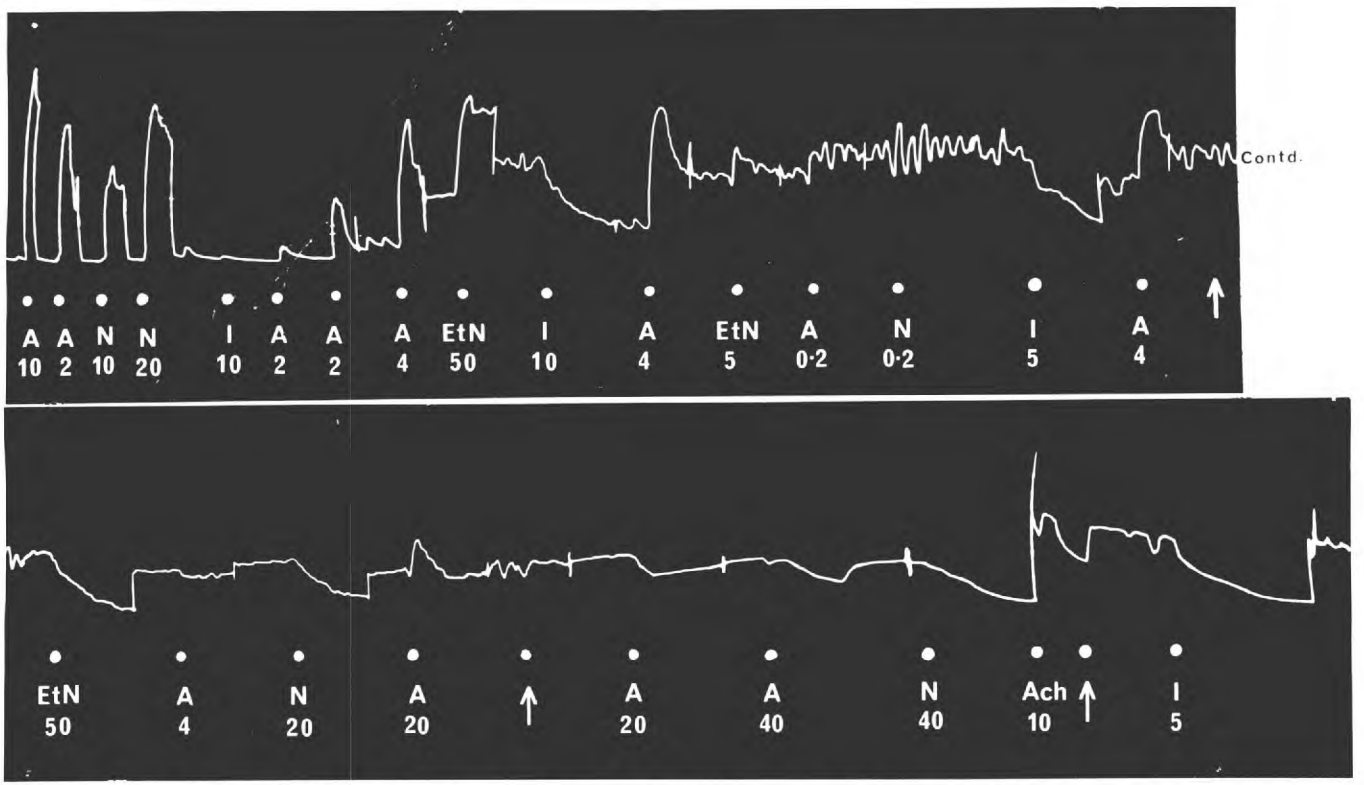


FIGURE 34. Reversal by phentolamine of the excitatory effects of µg doses of adrenaline, noradrenaline and N-ethylnoradrenaline, and persistence, after phentolamine, of the inhibitory action of isoprenaline on the uterus.

Adult rabbit, 2.2 kg.
in vitro; Krebs solution; 37.5°C; 95% oxygen + 5% carbon dioxide; isometric lever.

↑ = 1×10^{-6} phentolamine added to the bathing solution.

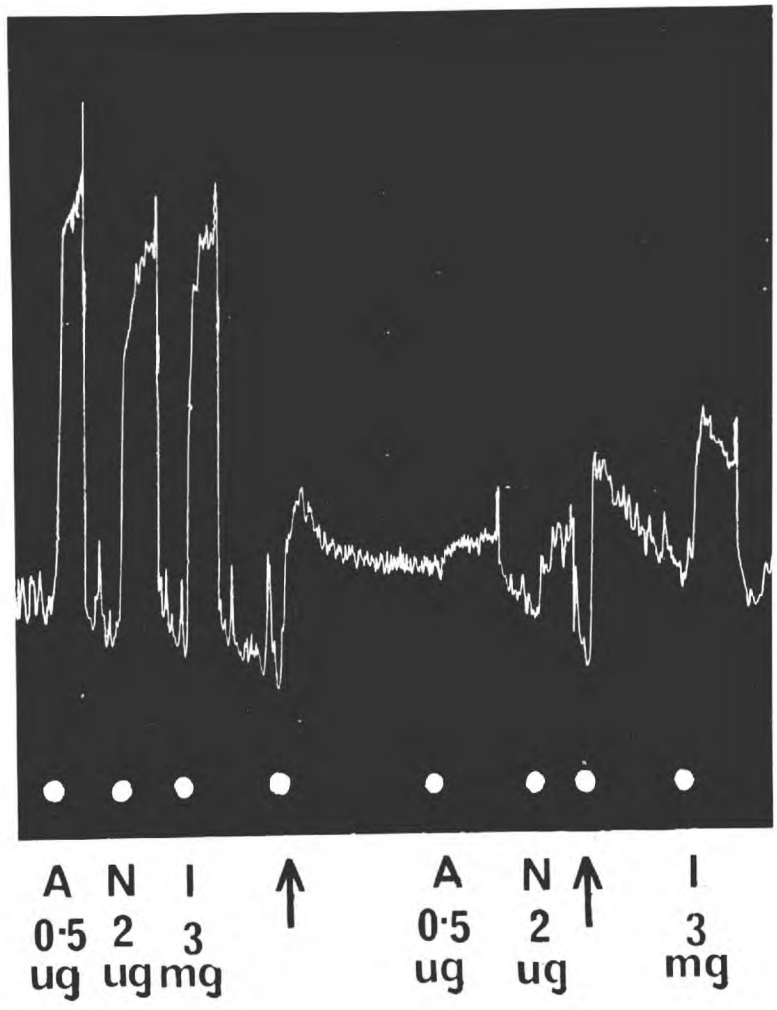


FIGURE 35. Blockade by tolazoline of the motor effects of adrenaline, noradrenaline and isoprenaline on the uterus.

Adult rabbit, 4.4 kg.
in vitro; Krebs solution; 38°C; air; isotonic lever.

↑ = 4×10^{-5} tolazoline added to the bathing solution.

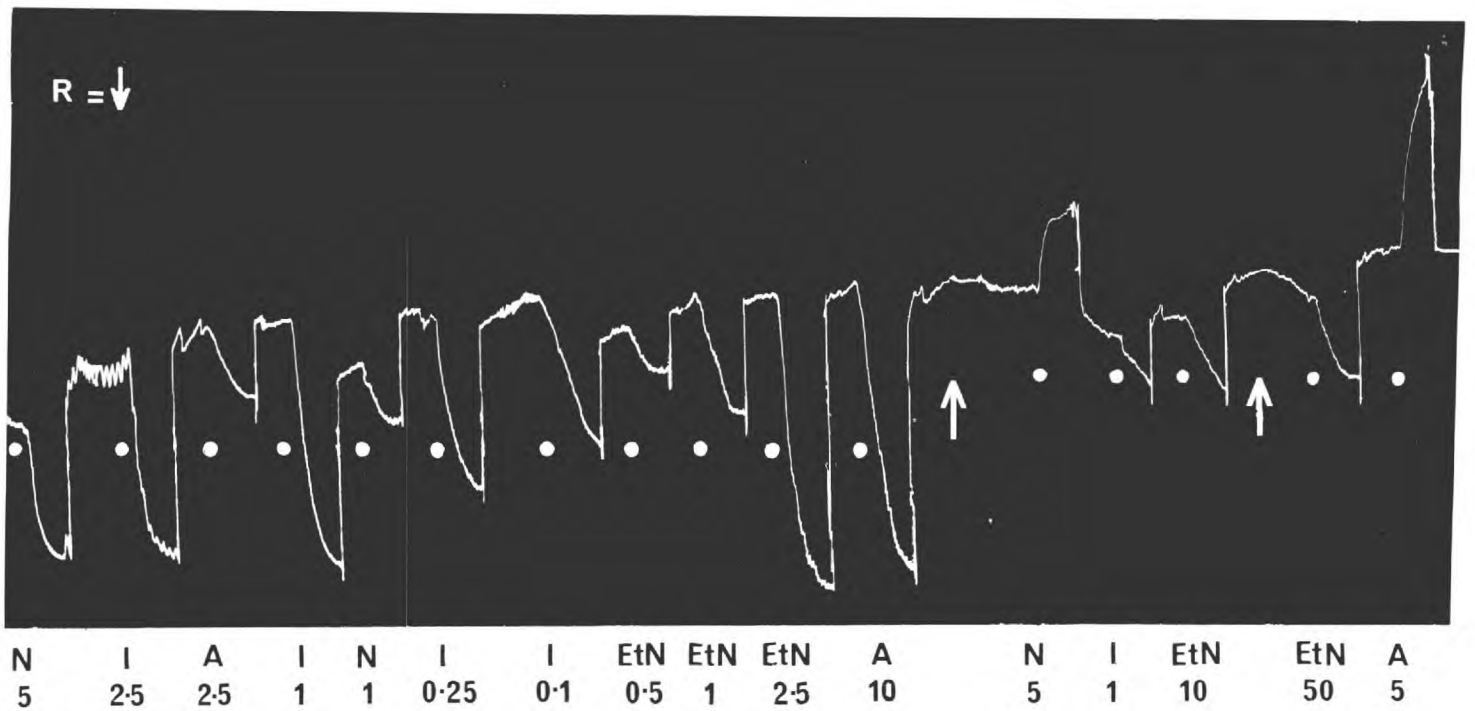


FIGURE 36. Blockade by propranolol of the inhibitory actions of μg concentrations of N-ethylnoradrenaline and isoprenaline, and reversal by propranolol of the inhibitory actions of adrenaline and noradrenaline on the uterus.

Immature, untreated Dutch rabbit, 0.84 kg, 5 weeks old.
in vitro; Krebs solution; 38°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

↑ = 4×10^{-8} propranolol added to the bathing solution.

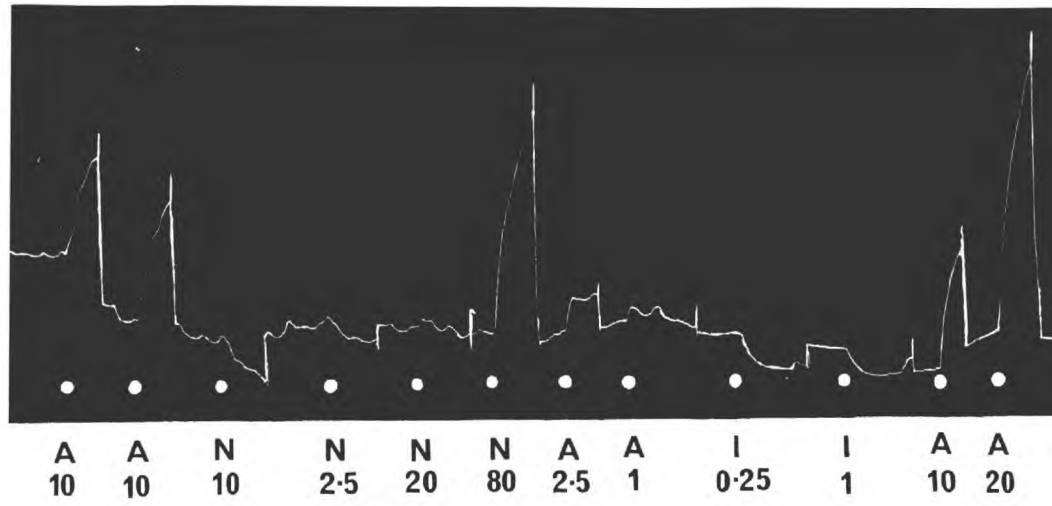


FIGURE 37. Excitatory action of μg concentrations of adrenaline; excitatory and inhibitory actions of noradrenaline, and inhibitory action of isoprenaline on the uterus.

Immature, untreated Dutch rabbit, 0.99 kg, 7 weeks old. in vitro; Krebs solution; 38°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

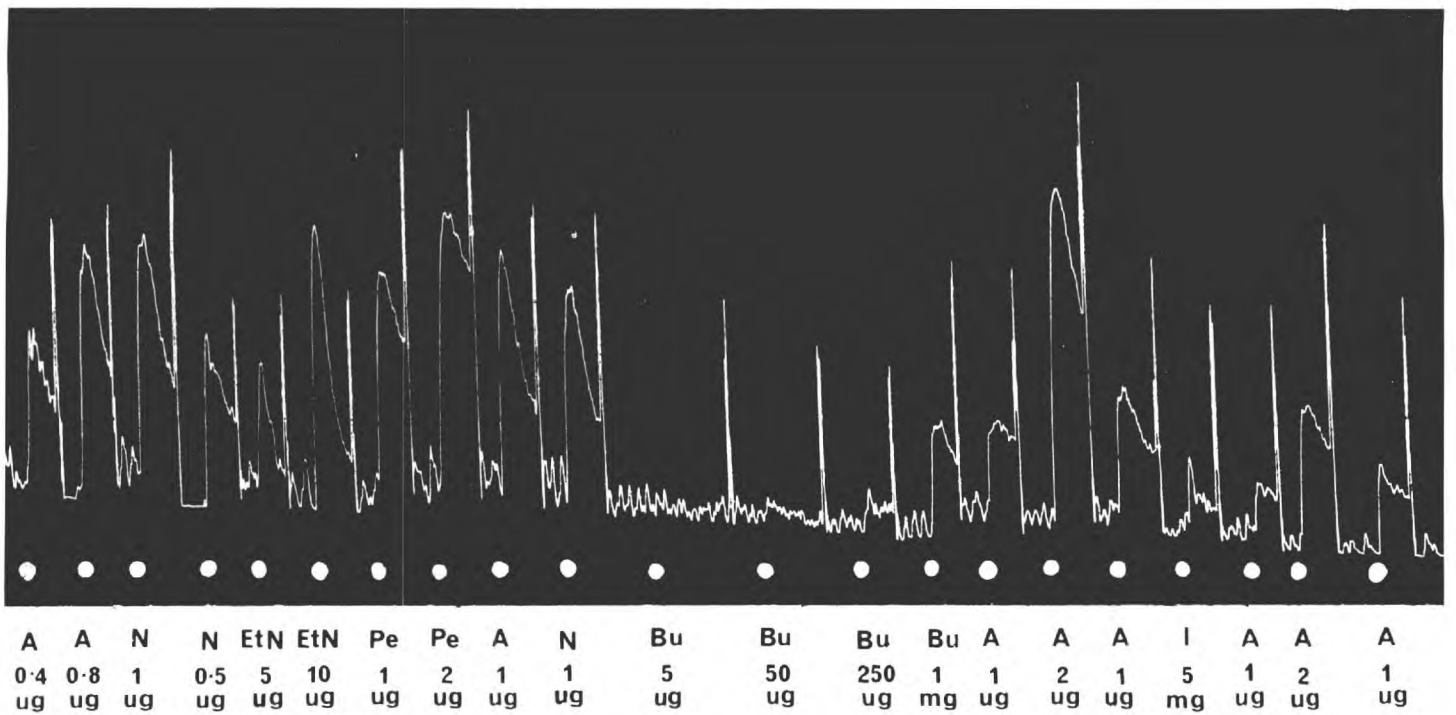


FIGURE 38. Excitatory actions of sympathomimetic amines on the uterus.

Immature, oestrogen-treated Dutch rabbit, 0.63 kg, 5 weeks old.
in vitro; Krebs solution; 37.5°C; 95% oxygen + 5% carbon dioxide;
isotonic lever.

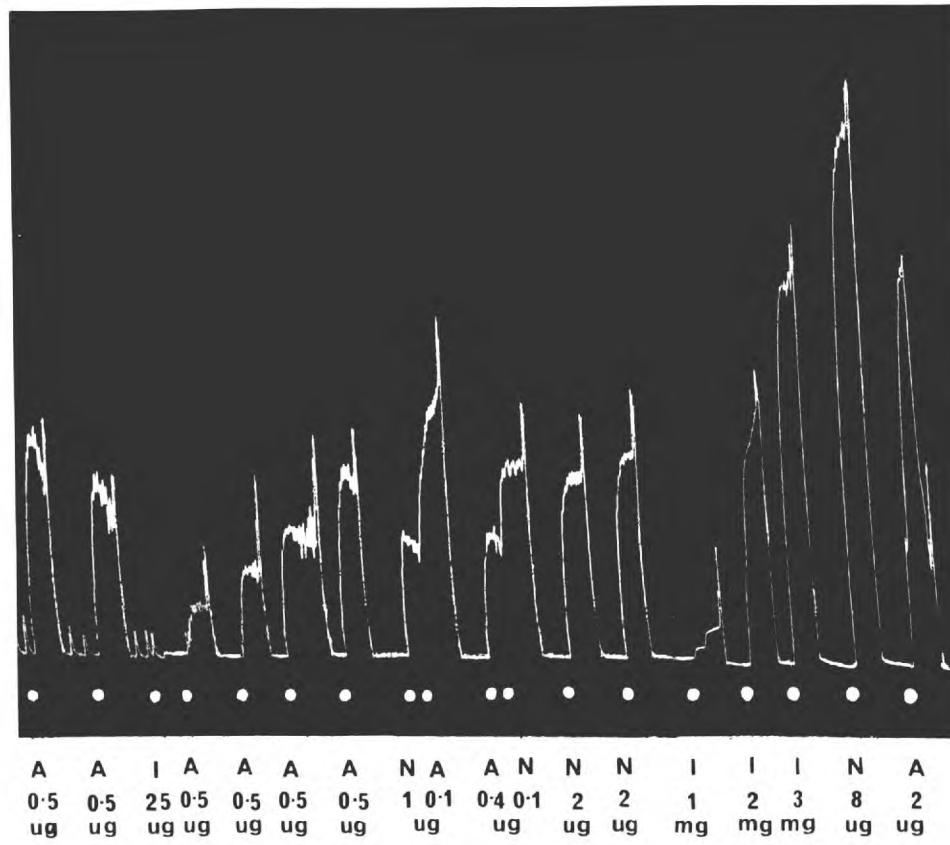


FIGURE 39. Inhibitory effect of a low concentration of isoprenaline; excitatory effects of adrenaline, noradrenaline and of high concentrations of isoprenaline on the uterus.

Immature, oestrogen-treated New Zealand White rabbit; 1.55 kg; 8 weeks old.
in vitro; Krebs solution; 37.5°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

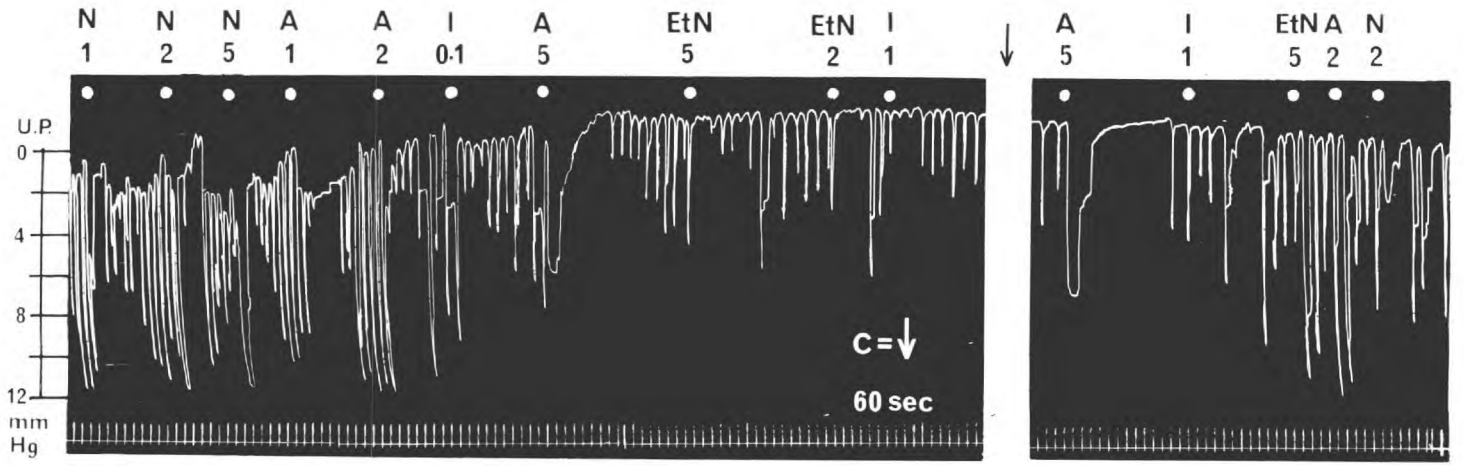


FIGURE 40. Blockade by propranolol of the inhibitory actions of µg doses (i.a.) of adrenaline, noradrenaline, N-ethylnoradrenaline and isoprenaline on the spontaneous activity of the uterus.

Ovariectomised, untreated rabbit, 2.2 kg.
in vivo

↓ = intravenous injection of 1 mg propranolol.

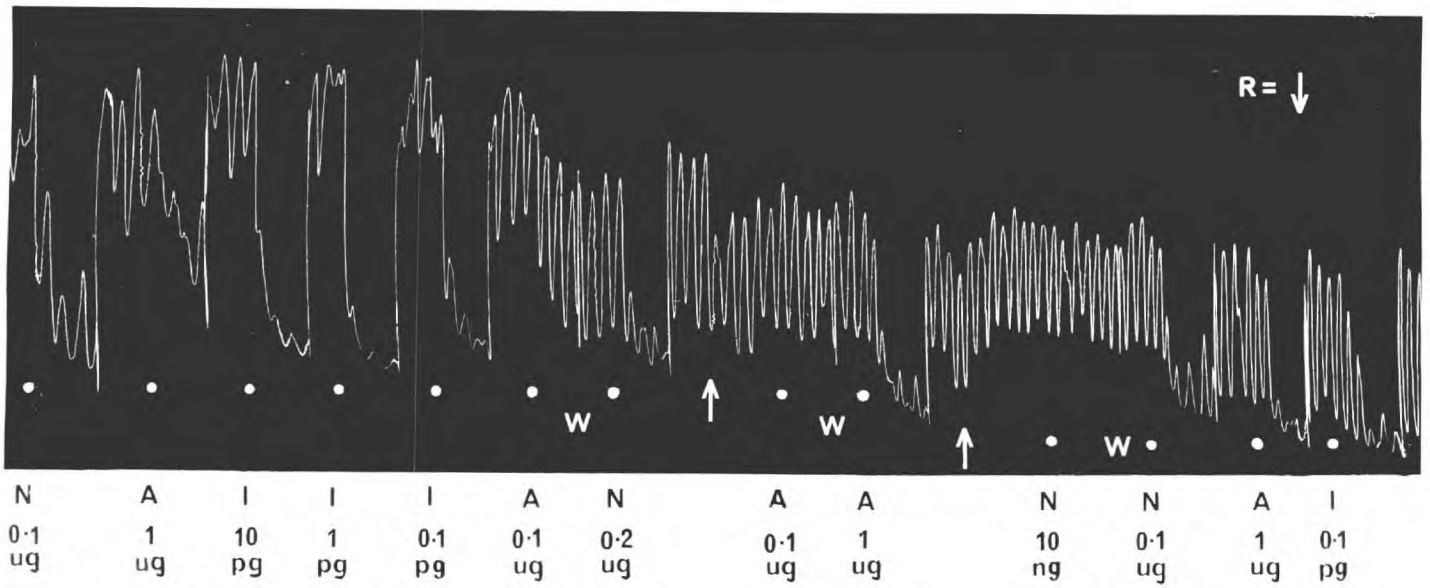


FIGURE 41. Persistence of the inhibitory actions of adrenaline, noradrenaline and isoprenaline on the uterus in the presence of phentolamine.

Ovariectomised, untreated rabbit, 2.1 kg.
in vitro; Krebs solution; 37.8°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

↑ = 4×10^{-7} phentolamine added to the bathing solution.

W = amine washed out after no effect recorded.

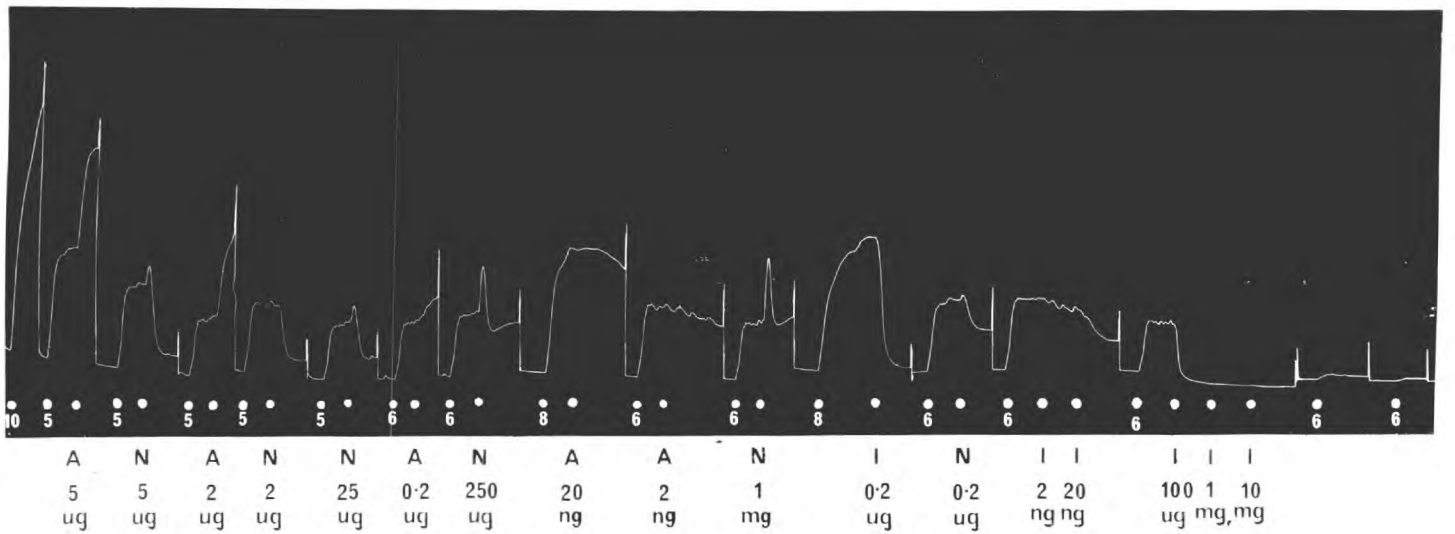


FIGURE 42. Excitatory actions of adrenaline, excitatory and inhibitory actions of noradrenaline, and inhibitory actions of isoprenaline on the uterus in the presence of acetylcholine.

Ovariectomised, untreated rabbit, 2.1 kg.
in vitro; Krebs solution; 37.5°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

Numbers in white = μg concentrations of acetylcholine.

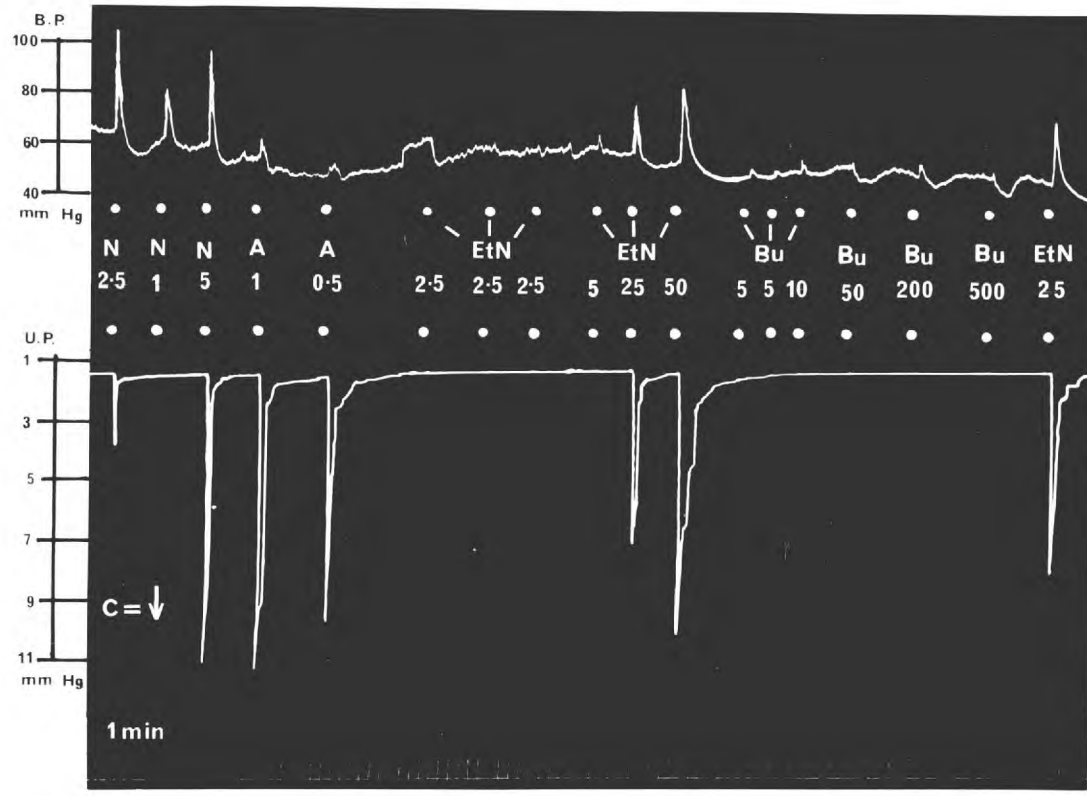


FIGURE 43. Effects of μg doses (i.a.) of catecholamines on the blood pressure and uterus. Pressor effects were produced by adrenaline, noradrenaline and by high doses of N-ethylnoradrenaline, and depressor effects by low doses of N-ethylnoradrenaline and by high doses of α-ethylnoradrenaline. Excitatory effects were produced on the uterus by adrenaline, noradrenaline and N-ethylnoradrenaline.

Ovariectomised, oestrogen-treated rabbit, 1.8 kg.
in vivo

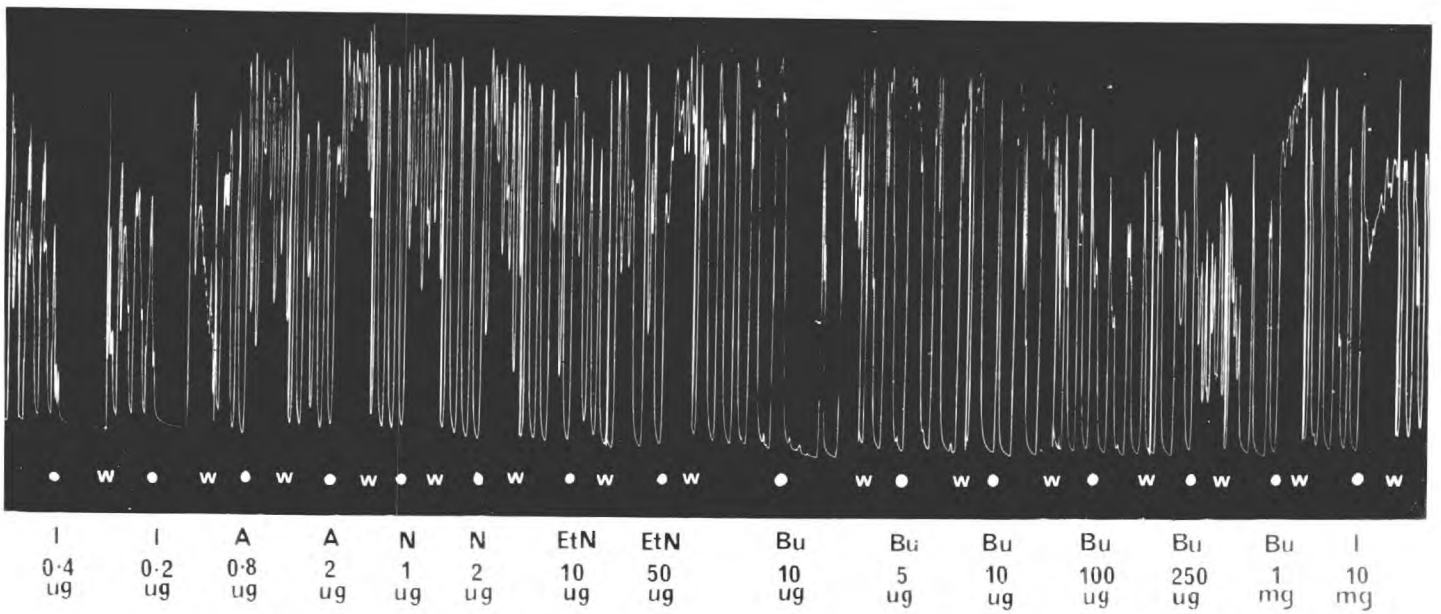


FIGURE 44. Excitatory effects of adrenaline, noradrenaline and N-ethylnoradrenaline, and inhibitory and excitatory effects of α-ethylnoradrenaline and isoprenaline on the spontaneous activity of the uterus.

Ovariectomised, oestrogen + progesterone-treated rabbit, 2.6 kg.
in vitro; Krebs solution; 38°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

W = amine washed out.

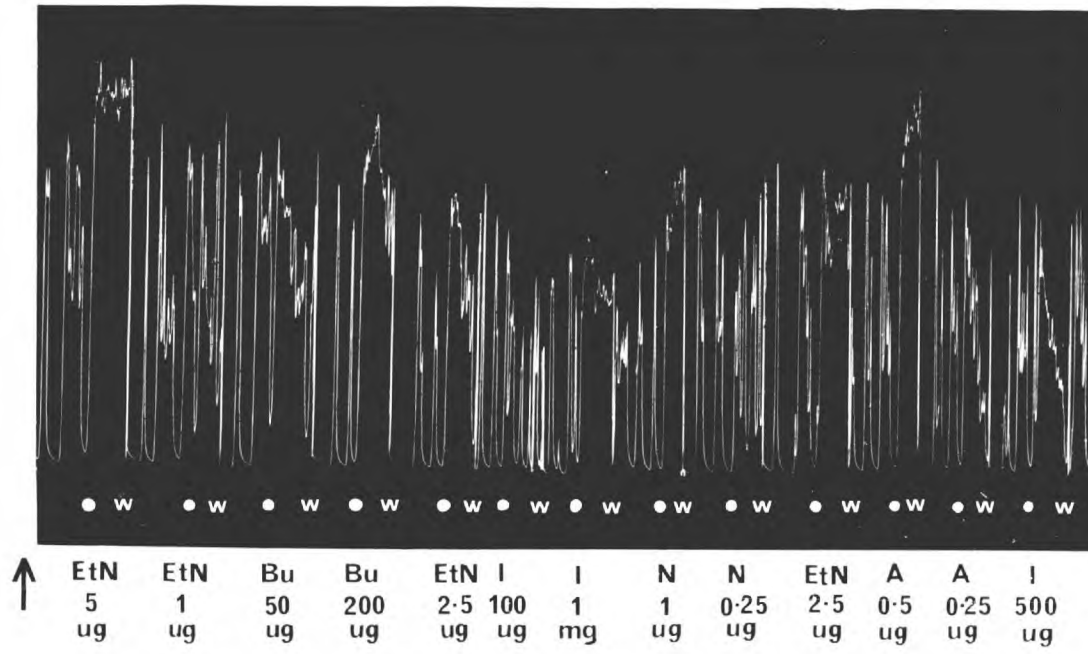


FIGURE 45. (Continuation of Fig. 44). Enhancement by propranolol of the excitatory effects of catecholamines on the uterus.

Ovariectomised, oestrogen + progesterone-treated rabbit, 2.6 kg. in vitro; Krebs solution; 38°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

↑ = 4×10^{-8} propranolol added to the reservoir bathing solution.

THE RESPONSES OF THE HUMAN UTERUS

TO SYMPATHOMIMETIC AMINES

SECTION III

HISTORICAL

RESPONSES TO SYMPATHOMIMETIC AMINES

Kehrer (1907) and Rübsamen and Kligermann (1912) found that small concentrations of adrenal gland extracts caused powerful contractions of isolated strips of human uterus taken at Caesarean section. In 1914 Gunn suspended uterine strips, and in one instance the complete uterus and Fallopian tubes from a non-pregnant woman, in a Ringer solution and found that an adrenal extract caused a contraction of the tissue. He suggested that the sympathetic fibres to the uterus exerted an excitatory role. Subsequent studies using the isolated tissue showed that adrenaline invariably produced motor effects, whether the uterus was from a pregnant or from a non-pregnant woman (Adair and Haugen, 1939; Garrett, 1955a; Quadros and Sinha, 1960; Lehrer, 1965; Stander and Barden, 1966; Wansbrough, Nakanishi and Wood, 1967).

In contrast, the results obtained from in vivo studies were not consistent, even though the recording methods used by the different investigators were similar. Basically, the methods entailed the insertion of a balloon into the uterus to record intrauterine pressure. Most studies were carried out on pregnant women near term, under which conditions adrenaline caused motor effects (Brown and Wilder, 1943), inhibitory effects (Bourne and Burn, 1927; Kaiser, 1951;

Cibils, Pose and Zuspan, 1962a; Stander, 1966), or both types of activity, depending upon the route of administration and upon the dose administered (Woodbury and Abreu, 1944; Kaiser and Harris, 1950). In vivo studies of the responses of uteri of non-pregnant women showed that adrenaline produced excitatory effects in the pre-ovulatory phase of the menstrual cycle and inhibitory effects in the post-ovulatory phase (Garrett, 1955b). Wansbrough et al. (1967) however, found that adrenaline caused inhibitory effects throughout the cycle.

A number of authors attempted to explain these discrepancies between the in vivo and the in vitro results. Garrett (1959) noticed that the small, frequent spontaneous contractions recorded from the uteri of non-pregnant women, A-waves (Moir, 1944), were similar in nature and origin to the Alvarez-waves (Alvarez and Caldeyro Barcia, 1950) observed when uterine recordings were taken from pregnant patients. Similarly Moir's B-waves, the large slow contractions seen in the secretory phase of the menstrual cycle, were very similar to the Braxton Hicks major contractions of late pregnancy. Both these larger waves involved coordinated contractions over the whole uterus. Garrett considered that uterine contractility consisted of two elements which he termed A-type and B-type contractions. He noticed that adrenaline enhanced the A-type contractions but inhibited the B-type contractions. Thus earlier workers had recorded both types of action from the intact

uterus depending upon the nature of the uterine contractions and the sensitivity of the recording apparatus in detecting A-type contractions. The contractions of isolated myometrial strips were of the A-type and were myogenic in origin (Garrett, 1955a). Thus the excitatory action of adrenaline on isolated myometrial strips corresponded with its action on the A-type contractions of the intact organ. The B-type waves, which were a coordinated movement involving the whole uterus, could not occur in the isolated strips of tissue.

Recently Stander and Barden (1966) forwarded an alternative explanation for the different *in vivo* and *in vitro* results obtained with adrenaline. They found that the excitatory effect of adrenaline *in vitro* was not influenced by propranolol and that no inhibitory activity was revealed after α-receptor blockade with phentolamine. Isoprenaline produced only a very weak inhibitory action. From these results they considered that, in the preparation of uterine tissue for *in vitro* study, the β-receptors were "broken down" or "inactivated", a possibility which they thought was due to the enzymic nature of the receptors. Their *in vivo* studies (Barden and Stander, 1968a, b; Stander, 1966, 1967) showed the existence of β-receptors in the human uterus. Therefore it was in the intact uterus only where the inhibitory action of adrenaline was seen. Wansbrough *et al.* (1967) proposed that this discrepancy between *in vivo* and *in vitro* responses to

adrenaline was because *in vivo* the predominant inhibitory action was observed due to a previous selective occupation of the α-receptors by endogenous noradrenaline, allowing injected adrenaline to act mainly on the β-receptors.

In contrast to the dual action of adrenaline, noradrenaline invariably caused a motor response, regardless of whether the experiment was performed on isolated or intact uteri from either pregnant or non-pregnant women. This was true even when adrenaline caused an inhibitory action. Kaiser (1951) and Garrett (1954) found that noradrenaline and adrenaline were equiactive in producing their respective effects of contraction and relaxation of the intact uterus at term. Cibils *et al.* (1962a, b) however observed that noradrenaline was twice as active in causing excitation as adrenaline was in producing inhibition. All these workers used similar intravenous infusion rates for both amines.

Garrett (1955b) studied the *in vivo* actions of noradrenaline and of adrenaline in non-pregnant patients and found the amines to be approximately equiactive in producing uterine contraction and relaxation respectively, whether adrenaline enhanced the intensity of the A-type waves or inhibited the B-type waves.

On isolated strips of uteri from non-pregnant women Garrett (1955a) found that noradrenaline was 2.4 times more

potent than adrenaline in causing a contraction. Sandberg, Ingelman-Sundberg and Lindgren (1958) however, found them equipotent, while Lehrer (1965) observed adrenaline to be the more potent.

The results obtained using isoprenaline have not been consistent. Quadros and Sinha (1960) stated that concentrations of the order of 10^{-5} produced only motor effects on the excised uterus from non-pregnant patients. Under similar conditions Lehrer (1965) found that concentrations of 10^{-6} - 10^{-5} produced no effect, while 2.5×10^{-5} produced either no effect, slight inhibition, or slight excitation followed by slight inhibition. Stander (1966) and Stander and Barden (1966) reported that isoprenaline produced a "transient reduction in maximum tension" of the myometrium in both <u>in vivo</u> and <u>in vitro</u> studies using uterine tissue from patients at term. Mahon, Reid and Day (1967) also studied the effects of isoprenaline on the intact uterus at term. Under these conditions intravenous infusions inhibited either natural labour or Syntocinon-induced contractions. Thus as with adrenaline, it appears that isoprenaline can produce opposite effects on the isolated and on the intact uterus.

THE EFFECT OF ADRENERGIC BLOCKING AGENTS

In an examination of catecholamine activity on human uterine tissue Garrett (1955a) found that the excitatory effects produced both by adrenaline and by noradrenaline could be blocked by dihydroergotamine. In a few preparations the action of adrenaline was reversed. Although Levy and Ahlquist (1961) reported that dihydroergotamine possessed a weak β-receptor blocking activity they stated that it produced a more complete blockade at α-receptors. The existence of α-receptors was confirmed by Stander and Barden (1966) using isolated uterine preparations, and by Althabe, Schwarcz, Sala and Fisch (1968) who studied uterine activity in vivo in pregnant women. They found that increased uterine activity produced by noradrenaline was antagonised by phentolamine. Similar findings were reported by Wansbrough et al. (1968) on the uterus of both pregnant and non-pregnant women.

Stander and Barden (1966) found that propranolol blocked, and sometimes reversed, the inhibitory action of isoprenaline in vitro and concluded that the effect of the amine was mediated through β-receptors. Similar conclusions were reached by Mahon et al. (1967), who showed that an intravenous injection of propranolol antagonised the uterine inhibition produced by isoprenaline in vivo in patients at term. Further evidence for the existence of β-adrenergic receptors has been found (Eskes, Stolte, Seelen, Moed and Vogelsang, 1965;

Wansbrough, et al., 1968) by observing the action of β-receptor blocking agents on the isoprenaline derivative C_c25 (p. 281). Similarly, Barden and Stander (1968a) found that pronethalol and propranolol antagonised the action of isoxsuprine (p. 281) in vivo. However, Stander (1966) found that MJ-1999 was ineffective as an antagonist of isoxsuprine in vitro although the action of isoprenaline was antagonised by this agent.

Thus both α-excitatory and β-inhibitory receptors have been demonstrated in human uterine tissue, in both in vivo and in vitro studies. It appears however, that the actions of the catecholamines on the intact organ are not always similar to their actions on the isolated preparation.

INTRODUCTION

Since the opportunity arose to make use of specimens of human uterine tissue taken at operation, it was considered worthwhile to examine the activity of sympathomimetic amines, especially with regard to a comparison of their effects on this tissue and on the uterus of the rat and the rabbit.

The activity of a series of catecholamines has been studied and the influence of adrenergic blocking agents upon the responses to the amines has been assessed, in order to determine more precisely the nature of adrenergic receptors in human myometrium. Attention has been paid to the action of isoprenaline as *in vitro* studies to date have produced conflicting results.

An attempt has been made to determine the effects of catecholamines on the uterus from the human foetus since it was considered that this would afford some comparison with the preparations from immature rats and rabbits.

The study has been limited by the number of specimens available, especially of uteri from the human foetus.

METHODS

In vitro experiments only have been performed.

1. ADULT HUMAN

Uteri were obtained at operation from patients undergoing hysterectomy, usually for fibroids.

Strips of uterine muscle from the outer muscle coat (stratum supravasculare) of the corpus region, 3 - 4 cm in length; 4 - 5 mm in width; and 1.5 - 2.0 mm in thickness were set up in a 25 ml. isolated organ bath. The bathing medium was either oxygenated Lehrer solution (Appendix, p. 283) or Krebs solution aerated with 95% oxygen + 5% carbon dioxide. The bath temperature was maintained at 37 - 38°C. Movements of the muscle strip were recorded with an isotonic frontal writing lever on a smoked drum.

Each preparation was set up as soon as possible after operation. Alternatively, tissue specimens were stored for up to 48 hours in Lehrer solution at 1 - 4°C before being used in experiments.

2. FOETAL HUMAN

The uterus and cervix were removed from foetuses which were 16 - 22 weeks old (crown-rump length 117 - 190 mm). The complete uterus was set up in the organ bath in Krebs solution

aerated with 95% oxygen + 5% carbon dioxide. Since the preparations were small, measuring from 5 - 16 mm in length, the responses were magnified approximately twenty times using an isotonic frontal writing lever.

RESULTS

ADULT HUMAN

The results of these studies are described separately for each specimen of uterine tissue obtained (Table 20, p. 258) and a subsequent correlation of the results has been attempted.

1. aet 31, hysterectomy for dysmenorrhoea

A uterine strip was set up after storage for 44 hours.

Adrenaline and noradrenaline, in concentrations from 4×10^{-7} - 2×10^{-5}, caused contractions of the uterine muscle. Adrenaline was approximately twice as potent as noradrenaline in producing these effects. Isoprenaline in concentrations from 4×10^{-9} - 4×10^{-4} failed to produce any effects. The motor actions of adrenaline and noradrenaline were unaffected by the addition of 2×10^{-5} propranolol to the bathing solution but were abolished by 2×10^{-5} phentolamine. Isoprenaline was still without effect after treatment either with propranolol or with phentolamine.

2. aet 44, hysterectomy for fibroids

A uterine strip was set up after storage for 20 hours.

Adrenaline and noradrenaline were approximately equiactive in causing uterine contractions and submaximal effects were

obtained with concentrations of the order of 10^{-6}. Isoprenaline ($1.0 - 4.0 \times 10^{-4}$) also produced uterine excitation and possessed about a 25th of the activity of adrenaline. The three amines were devoid of inhibitory activity, even after blockade of their motor effects with 2×10^{-5} phentolamine (Fig. 46, p. 259).

3. aet 48, hysterectomy for fibroids

A uterine strip was set up after storage for 20 hours.

Adrenaline and noradrenaline were approximately equipotent in producing contractions of the uterus with concentrations of the order of 10^{-6}. Isoprenaline also contracted the preparation but possessed approximately a 1,000th of the potency of adrenaline. Even after these motor responses had been antagonised with 2×10^{-5} phentolamine, no inhibitory effects were produced by any of the three amines.

4. aet 49, hysterectomy for fibroids

A uterine strip was set up after storage for 18 hours. A second experiment was carried out using this tissue after storage for 42 hours.

In both experiments adrenaline and noradrenaline, in concentrations from $2 \times 10^{-7} - 1 \times 10^{-6}$, were approximately equiactive in causing uterine excitation. Isoprenaline was devoid of excitatory effects, but concentrations of the order

of 10^{-5} inhibited the excitatory actions of adrenaline and noradrenaline, although no inhibition of spontaneous movements occurred. After 4×10^{-5} propranolol the inhibitory action of isoprenaline was antagonised, but no reversal of response occurred with concentrations of up to 4×10^{-4}. The motor actions of adrenaline and noradrenaline were not altered after propranolol.

No appreciable differences, either qualitative or quantitative, were noticed between the results obtained using the preparation stored for 18 hours and those from the preparation stored for 42 hours.

5. aet 49, hysterectomy for fibroids

A uterine strip was set up 2 hours after operation. A further experiment was carried out using a second strip of this tissue after storage for 21 hours.

In both experiments only excitatory effects were produced by adrenaline, noradrenaline and isoprenaline, and the approximate ratios of equiactive concentrations to cause excitation were, in terms of noradrenaline = 1.0 (at a concentration of 0.8×10^{-8}); adrenaline = 1.0; isoprenaline = 1,000. Difficulty was experienced in the assessment of blockade with phentolamine, for although the excitatory actions of the amines were antagonised, the spontaneous activity of the preparation was depressed.

Propranolol (4×10^{-5}) also depressed the uterine spontaneity, but did not appreciably affect the excitatory activity of the three amines.

6. aet 50, hysterectomy for fibroids

A uterine strip was set up 2 hours after operation. A further experiment was carried out using a second strip of this tissue after storage for 24 hours.

In both experiments only excitatory effects were produced by the amines (Fig. 47, p. 260), and the approximate ratios of equiactive concentrations to cause excitation were, in terms of noradrenaline = 1.0 (at a concentration of 1.0×10^{-6}); adrenaline = 1.0; N-ethylnoradrenaline = 10; isoprenaline = 200. Phentolamine (1×10^{-5}), when added to the bathing solution, itself caused a slow but large increase in the uterine tone so that a precise estimation of the degree of blockade of the excitatory actions of the amines was not possible. Dihydroergotamine (2×10^{-5}) did not influence the uterine activity but had little effect however, on the excitatory actions of the amines.

7. aet 52, hysterectomy for fibroids

A uterine strip was set up after storage for 18 hours.

Adrenaline and noradrenaline were approximately equiactive in producing contractions of the uterus and submaximal effects

were obtained with concentrations of the order of 10^{-6}. Isoprenaline also produced uterine excitation but its activity was about a 200th of that of noradrenaline. Phentolamine (4×10^{-5}) antagonised these excitatory actions but also depressed the uterine spontaneity and the contractions caused by acetylcholine.

8. aet 76, hysterectomy for prolapse of uterus

A uterine strip was set up after storage for 18 hours. A further experiment was carried out using a second strip of this tissue after storage for 42 hours.

In the first experiment adrenaline, noradrenaline and isoprenaline caused inhibition of the spontaneous contractions and a fall in base-line tone (Fig. 48, p. 261). The approximate ratios of equiactive concentrations to cause inhibition were, in terms of noradrenaline = 1.0 (at a concentration of 4×10^{-6}); adrenaline = 2.5; isoprenaline = 0.2. After treatment with 4×10^{-5} propranolol these inhibitory effects were antagonised but in no instance did any reversal of effect, to produce a contraction of the uterus, occur with concentrations of adrenaline and noradrenaline of up to 2×10^{-5}. Uterine contractions were produced however, with acetylcholine (4×10^{-6}). In contrast, in the second experiment, both adrenaline and noradrenaline caused excitatory effects in concentrations which in the first

experiment had produced uterine inhibition. Isoprenaline however, still caused inhibitory effects using the same concentration range as in the first experiment. Propranolol (1×10^{-7}) effectively blocked the inhibition caused by isoprenaline but did not reveal any excitatory effects (Fig. 49, p. 262) even with concentrations of isoprenaline as great as 2×10^{-4}. The excitatory actions of adrenaline and noradrenaline were not appreciably altered by the treatment with propranolol but were abolished by 4×10^{-5} phentolamine.

The results of these experiments on uterine tissue from the human adult are summarised in Table 20, p. 258. The mean values for the approximate ratios of equiactive concentrations to cause excitation were, in terms of noradrenaline = 1.0 (at a concentration of 2.1×10^{-6}); adrenaline = 0.8; N-ethylnoradrenaline = 10; isoprenaline = 518. Out of a total of twelve experiments using uterine tissue from eight patients, adrenaline and noradrenaline caused excitatory effects in eleven experiments, and isoprenaline in seven experiments. Adrenaline and noradrenaline produced inhibitory effects in only one of these twelve experiments, while a similar action was produced by isoprenaline in four experiments on uterine tissue from two patients. In no experiment did any amine produce both excitatory and inhibitory effects. Phentolamine antagonised the excitatory effects of the catecholamines, but

concentrations of phentolamine necessary to produce the blockade caused a depression of the uterine spontaneity and a depression of the response of the uterus to acetylcholine. Propranolol antagonised the inhibitory activity of the catecholamines but did not influence their excitatory actions. In no experiment was it possible to expose a secondary component in an adrenergic response after the use of either α- or β-blocking agents.

FOETAL HUMAN

In only one out of five preparations of foetal uteri was it possible to record responses to the catecholamines. In this experiment the uterus was larger than the other preparations, measuring 16 mm in length, including the cervix. The foetus was approximately 22 weeks old. The preparation was devoid of spontaneous activity.

Adrenaline and noradrenaline were found to contract the uterus in concentrations similar to those necessary to excite the adult preparation and responses were obtained with 4×10^{-7} and 4×10^{-6} of each amine (Fig. 50a, p. 263). Isoprenaline in a concentration as low as 2×10^{-8} caused uterine inhibition. This was observed as an inhibition of the contractile effect of adrenaline added to the bathing solution while the isoprenaline was still in the tissue bath (Fig. 50b, p. 263). The responses to concentrations of isoprenaline

greater than 2×10^{-7} could not be ascertained, and the effect of α- and β-blocking agents could not be determined, since the uterus became insensitive to the amines within one hour of setting up the preparation.

DISCUSSION

The existence of α-excitatory and β-inhibitory adrenergic receptors has been shown in in vitro preparations of strips of adult human myometrium. The order of the amines when arranged according to their relative activities to cause uterine contraction was adrenaline > noradrenaline > N-ethylnoradrenaline > isoprenaline. This order, together with the observation that uterine excitation was antagonised by phentolamine, indicated that the excitatory receptors were of the α-type. The evidence for the existence of β-inhibitory receptors was that in four preparations out of twelve isoprenaline caused relaxation of uterine strips and in one of these experiments adrenaline and noradrenaline also produced similar but weaker inhibitory actions. These responses were antagonised by propranolol. In no experiment did any amine produce both excitation and inhibition even at widely different concentrations.

The number and/or activity of the β-receptors is very small compared to the α-receptors in in vitro preparations of human uterine tissue. This is indicated by the observations that (i) the predominant response to isoprenaline was a contraction of the uterus and, even after blockade of the α-receptors, no β-receptor activation was noticed; (ii) the ratio of the excitatory activity of noradrenaline to that of

adrenaline approached a figure of one, a value obtained in most tissues only after blockade of β-receptors; (iii) the ratios of the excitatory activities of noradrenaline, N-ethylnoradrenaline and isoprenaline to adrenaline resembled closely those ratio values for excitatory activities on the uterus of the rabbit after β-receptor blockade with propranolol.

The results obtained in these experiments support those of Quadros and Sinha (1960) and Lehrer (1965), who reported that isoprenaline could induce uterine contractions as do adrenaline and noradrenaline. However, Stander (1966) and Stander and Barden (1966) found that isoprenaline produced only uterine inhibition unless propranolol had first been used, when the response was reversed to excitation. Lehrer (1965) found that, as in these studies, the responses to isoprenaline were different in apparently similar preparations. The reasons for these different results are not clear, but it would appear that they are not related to the age of the patient from which the tissue originated. It is not known precisely how many of the uterine specimens used in this thesis were removed from patients of a pre-menopausal age and if so at what stage of the menstrual cycle the hysterectomy was performed. However other workers have reported that the catecholamines exert similar effects on isolated uteri from both pre- and post-menopausal women

(Garrett, 1955a; Quadros and Sinha, 1960) and during both the secretory and the proliferative stages of the menstrual cycle *in vitro* (Garrett, 1955a; Wansbrough *et al.*, 1967). It appears that storage of the tissue for a period of up to two days also does not influence the response of the tissue to isoprenaline. One factor which may be important in determining the response is the pathological condition of the uterus at the time of operation. None of the preparations could be considered to be physiologically normal, otherwise no operation would have been necessary, and it may be as a result of this, that different responses to the catecholamines occurred especially if the action of the amine was normally weak and therefore easily altered.

The modifications of the activities of the catecholamines by α- and β-blocking agents reported in this work are in agreement with results recently reported (Stander and Barden, 1966; Wansbrough *et al.*, 1967). One of the difficulties experienced in assessing the activities of the amines at α- and β-receptors was that the adrenergic blocking agents themselves caused an alteration in myometrial activity. Phentolamine, in concentrations greater than 10^{-5}, depressed the contractility in human as well as in rat and in rabbit uteri *in vitro* and also non-specifically antagonised the action of uterine stimulants other than catecholamines. Similar findings were reported by Stander and Barden (1966) and

Wansbrough et al. (1967) using in vitro preparations of human uterus. Propranolol did not greatly affect the contractility of the human uterus in vitro, but in vivo it enhanced the spontaneous activity of the uterus (Stander, 1967; Barden and Stander, 1968b). These results parallel those reported in this work on studies of the uterus of the rat (p. 101) where propranolol exerted a stimulatory action in vivo but not in vitro.

The great difference between the activities of the catecholamines on the foetal human uterus and on the preparation from the adult was the sensitivity of the former to isoprenaline in producing inhibition. Only in a few experiments did isoprenaline inhibit in vitro preparations from the adult in which concentrations of the order of 10^{-6} - 10^{-5} were required, while a concentration of 2×10^{-8} was effective in producing inhibition of the foetal preparation. It appears therefore that, as in the rabbit, the foetal or immature uterus is more likely to be inhibited by catecholamines than the adult preparation. Thus, the possibility arises as to whether or not the responses of the human uterus to catecholamines are regulated by the hormonal influence governing the uterus. Oestrogens are secreted in the urine of new-born infants but in considerably smaller quantities than in the adult human female (Zucconi, Lisbon, Simonitson, Roth, Hagen and Diczfalusy, 1967). This greater

inhibitory potency of isoprenaline on the foetal preparation may be explained if this lack of oestrogen domination in the foetal uterus results in a decreased α-activity or increased β-activity of the uterine adrenergic receptors, as has been suggested for the uterus of the immature rat and rabbit.

SUMMARY

1. The predominant effect of adrenaline, noradrenaline, N-ethylnoradrenaline and isoprenaline on *in vitro* preparations of strips of uterus from the adult human was a contraction of the myometrium. This action was elicited via adrenergic α-receptors.

2. On a few preparations isoprenaline, and on one occasion adrenaline and noradrenaline, produced uterine relaxation. This action was elicited via β-adrenergic receptors.

3. On a preparation of a uterus from a human foetus, adrenaline and noradrenaline produced excitatory effects in concentrations similar to those necessary to excite preparations from the uterus of the adult. Isoprenaline failed to produce a contractile effect, but was able to inhibit myometrial contractile activity in concentrations considerably lower than those required to exert such an effect on adult preparations.

TABLE 20

FIGURES 46 - 50

Where the abbreviations and symbols used are not explained at the foot of the Table or Figure, an explanation is given in the Appendix on page 278.

TABLE 20

ADULT HUMAN

Ratio of the concentration of sympathomimetic amine to the equiactive concentration of l-noradrenaline.

in vitro experiments

State of Uterus			Conc^{n} l-Nor x 10^{-6}		Ratio sympathomimetic amine/ l-Nor					
					l-Adr		l-N-EtNor		dl-Iso	
**No.	Age of patient (years)	Storage time (hours)	*E	*I	E	I	E	I	E	I
1	31	44	4.0	-	0.5	-			-	-
2	44	20	3.2	-	1.0	-			25	-
3	48	20	2.0	-	1.0	-			1,000	-
4	49	18	0.4	-	1.0	-			-	$4x10^{-5}$
		42	0.4	-	0.5	-			-	$8x10^{-5}$
5	49	2	0.8	-	1.0	-			1,000	-
		21	0.8	-	1.0	-			1,000	-
6	50	2	1.0	-	1.0	-	10	-	200	-
		24	1.0	-	1.0	-	10	-	200	-
7	52	18	2.0	-	0.5	-			200	-
8	76	18	-	4	-	2.5			-	0.2
		42	8.0	-	0.5	-			-	$8x10^{-7}$
Mean excitatory ratios			2.1=1		0.8		10		518	

Concentration to cause:

*E = a just submaximal contraction or
*I = a just submaximal inhibition of spontaneous uterine contractions.

** Number corresponds to numbering of uterine specimens in Results Section (p. 243).

- = amine used but no action observed.

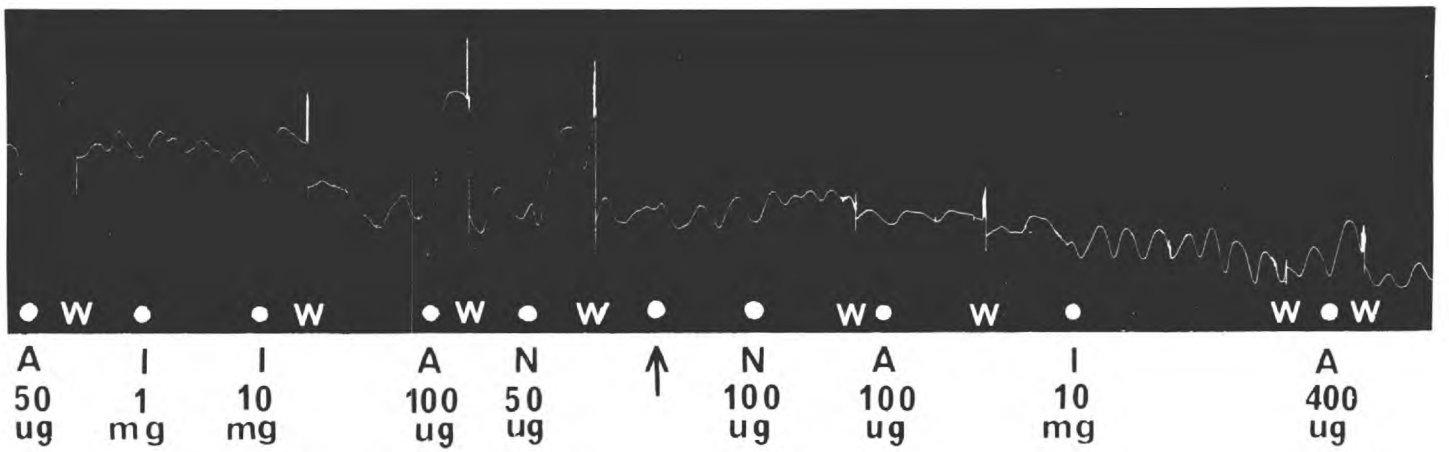


FIGURE 46. Blockade by phentolamine of the excitatory effects of adrenaline, noradrenaline and isoprenaline.

Adult human, aet 44 years.
in vitro; uterine strip stored for 20 hours; Lehrer solution; 38.5°C; 100% oxygen; isotonic lever.

↑ = 2×10^{-5} phentolamine added to the bathing solution.

W = amine washed out.

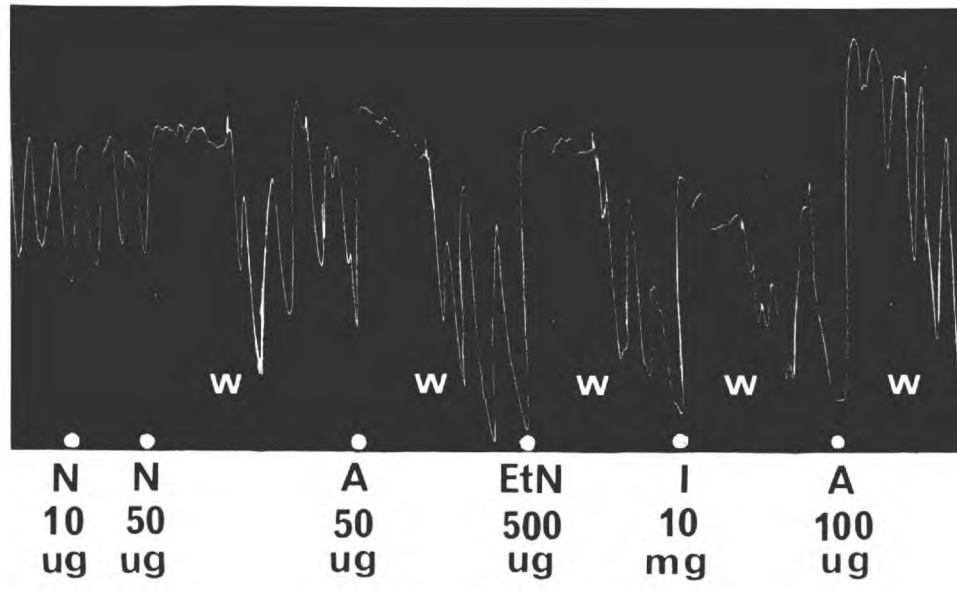


FIGURE 47. Excitatory effects of catecholamines on the uterus.

Adult human, aet 50 years.
in vitro; uterine strip stored for 24 hours; Krebs solution; 37.5°C; 95% oxygen + 5% carbon dioxide; isotonic lever.

W = amine washed out.

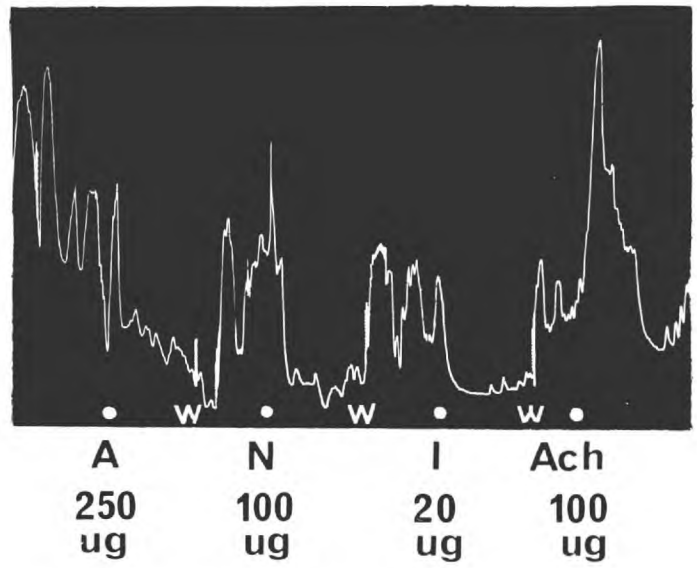


FIGURE 48. Inhibitory effects of adrenaline, noradrenaline and isoprenaline on the uterus.

Adult human, aet 76 years.
in vitro; uterine strip stored for 18 hours; Lehrer solution; 38°C; 100% oxygen; isotonic lever.

W = amine washed out.

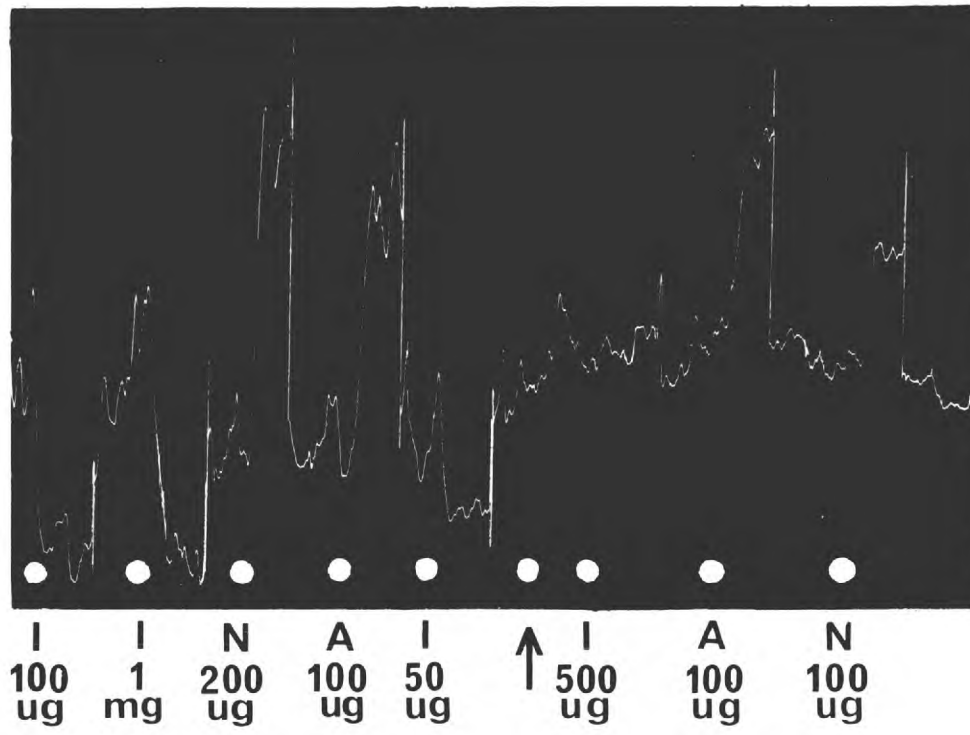


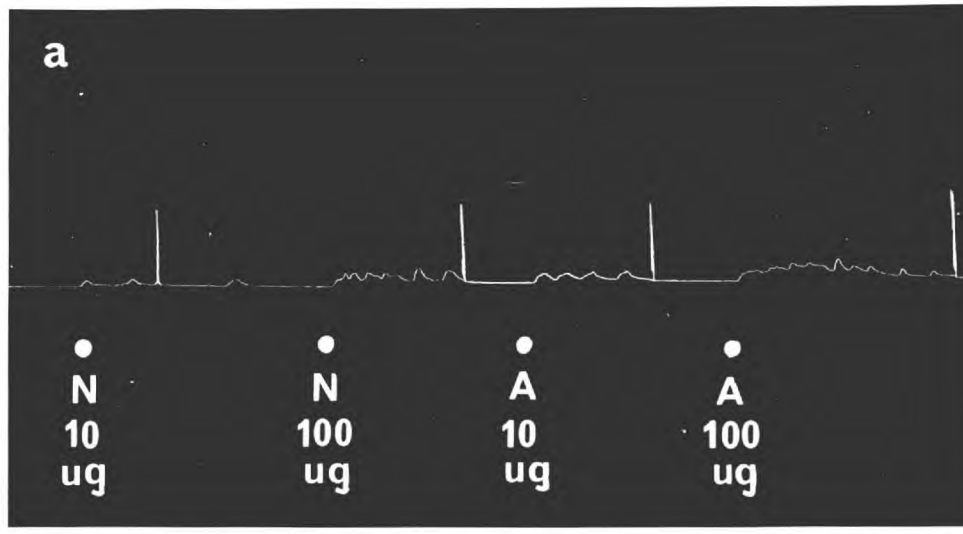
FIGURE 49. Excitatory effects of adrenaline and noradrenaline, and blockade by propranolol of the inhibitory effects of isoprenaline on the uterus.

Adult human, aet 76 years.
in vitro; uterine strip stored for 42 hours; Lehrer solution; 38°C; 100% oxygen; isotonic lever.

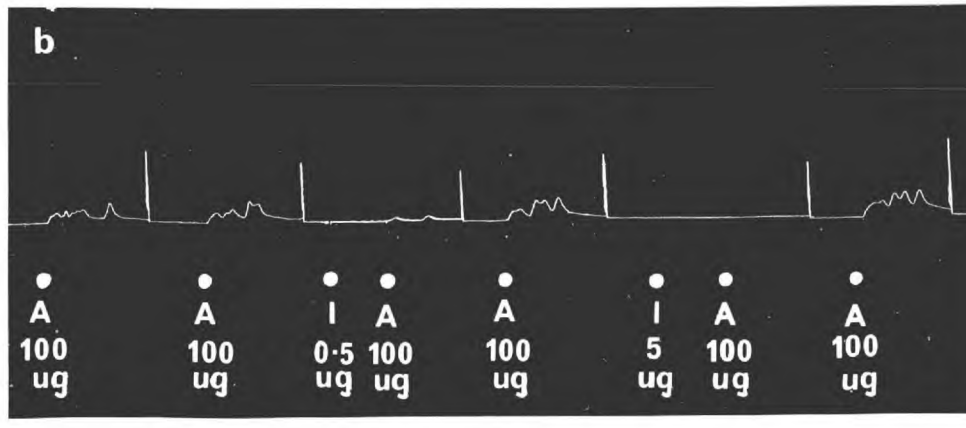
↑ = 1 x 10^{-7} propranolol added to the bathing solution.

FIGURE 50. Activity of catecholamines on the uterus from the human foetus.

Human foetus, aet 22 weeks.
in vitro; whole uterus stored for 1½ hours; Krebs solution; 37.5°C; 95% oxygen + 5% carbon dioxide; isotonic lever.



a) Excitatory actions of adrenaline and noradrenaline.



b) Inhibitory action of isoprenaline upon adrenaline-induced contractions.

G E N E R A L

D I S C U S S I O N

The work presented on the foregoing pages, employing a series of sympathomimetic amines and a number of α- and β-blocking agents, demonstrates both *in vivo* and *in vitro*, the existence of α- and β- adrenergic receptors in the smooth muscle of rabbit and human uteri. Levy and Tozzi (1963) reported that only β-receptors exist in the uterus of the rat *in vitro*. The results obtained in this thesis however, demonstrate, both *in vivo* and *in vitro*, not only the presence of β-receptors but also of α-receptors, as postulated by Ahlquist (1948, 1962). In all the three species studied the α-receptors mediated only excitatory effects and the β-receptors only inhibitory effects. The presence of α-inhibitory or β-excitatory receptors was not demonstrated and the reports by Wiqvist (1959) and Rudzik and Miller (1962) that α-inhibitory receptors exist in the uterus of the rat could not be substantiated.

In many of the experiments using the uteri of rats in oestrus or the uteri of rabbits the sympathomimetic amines exerted biphasic actions of excitation followed by inhibition. Except for the biphasic response to adrenaline *in vivo* of the uterus of the rabbit (p. 190), it was shown by the use of α- and β-blocking agents that these biphasic responses consisted of an initial excitatory action on α-receptors followed by an inhibitory action on β-receptors. The blockade

of one component usually led to the enhancement of the other component. It was observed that in any particular uterine preparation the size of the excitatory component of the response in relation to the size of the inhibitory component was not always constant, even to similar doses of the same amine. It was found that the nature of any biphasic response depended upon the following factors: (i) the sympathomimetic amine used; (ii) the dose of the amine; (iii) the species employed; (iv) the sexual state of the animal; (v) the spontaneous activity of the uterus, and (vi) whether the experiment was performed in vivo or in vitro. A change in any one of these factors could lead to an alteration in the nature of the biphasic response or further, to its conversion into solely an excitatory or an inhibitory effect.

Concerning the actions of isoprenaline, this work has shown that the amine can excite α-receptors in the myometrium of the rabbit and the human, where its potency relative to adrenaline approximated to that described by Butterworth (1963a, b) for elevation of the blood pressure of the cat. In this respect it possessed only about one thousandth of the potency of adrenaline. The low number and/or activity of α-receptors in the uterus of the rat relative to the number and/or activity of β-receptors is indicated by the fact that only those amines with a potent action at α-receptors could produce motor effects in this tissue, and this explains why

isoprenaline was unable to do so. The amine was able however, to excite α-receptors in other tissues in the rat, for it produced a pressor response similar to that described in the cat. Pressor responses to large doses of isoprenaline were observed also in the rabbit, concomitantly with the motor effect via α-receptors in the uterus.

A comparison of the relative activities of catecholamines on the uteri of mammalian species shows that the responses of the human uterus in vitro resemble most closely those produced by the rabbit uterus in vitro (compare Tables 13 and 20, pp. 203 and 258). Only isoprenaline caused inhibitory effects on the uterus of the rabbit and on that of the human prior to treatment with α-blocking agents, while the uterus of the rat in dioestrus was inhibited by all the sympathomimetic amines employed. Isoprenaline produced motor effects also on the uterus of the rabbit and on that of the human using concentrations higher than those required for uterine inhibition. It may be concluded therefore, that the uterus of the rabbit would be the preparation of choice for testing potentially useful adrenergic agents, especially in regard to the production of substances which cause relaxation of the human uterus.

In contrast to its activity on adrenergic receptors in the rat, the α-excitatory activity of adrenaline on the uterus and on the vasculature of the rabbit was greater than that of

noradrenaline. Similarly, the β-inhibitory activity of noradrenaline after α-receptor blockade with phentolamine, was greater than that of adrenaline on these tissues. Also the inhibitory activity of noradrenaline was greater on uteri from immature and from ovariectomised rabbits. Thus although both rat and rabbit uteri contain α-receptors, these receptors do not appear to be identical in the two species as judged by their sensitivity to a series of sympathomimetic amines and similarly the β-receptors also may not be identical. It is realised however, that the different activities of adrenaline and of noradrenaline in the two species may be influenced by factors other than the nature of the adrenergic receptors. There may be differences between the neuronal uptake, the storage mechanisms and/or the affinities of the enzymes responsible for catecholamine metabolism in the two species. Such factors would influence the amount of amine available to act at the adrenergic receptors. The outstanding difference between the adrenergic receptors in the uterus of the rat and the rabbit however, lies in the relative numbers and/or activity of each type, as measured by the ability of the amines to exert an action at one type of receptor with the other type blocked. For example, in the rat in dioestrus adrenaline exerted only a weak action (excitatory) at the α-receptors in the presence of propranolol, and in the rabbit only a weak action (inhibitory) at the β-receptors in the

presence of phentolamine.

The results presented have shown that although both α-excitatory and β-inhibitory receptors exist in the uterus of the rat, rabbit and human during the different sexual states, the responses of these uteri to sympathomimetic amines and to hypogastric nerve stimulation vary according to the sexual condition of the animal. The actions of the sympathomimetic amines on the uteri from immature rats and rabbits and on the uterus from the human foetus differed qualitatively from their actions on the uteri from the adult of that species. Further, the effects of the amines and of stimulation of the hypogastric nerve on the uteri of adult rats varied according to the stage of the oestrous cycle. In the adult rabbit, which does not exhibit a sexual cycle but remains in an oestrous state until mated (Parkes, 1956), no appreciable variation in responses to the amines occurred between different adult animals. Thus the differences observed between responses of the uteri from adult rabbits and from immature rabbits may be regarded as being similar to the contrasting results obtained between the action of the amines on the uteri of adult rats in oestrus and on the uteri of immature rats. Responses of the uteri of a number of other mammalian species to sympathomimetic amines also have been reported to vary according to the sexual state of the animal. These studies however, have been well documented only using the cat (Dale,

1906; Kennard, 1937; Van Dyke and Chen, 1939; Graham and Gurd, 1960; Tsai and Fleming, 1962, 1963).

It has been shown that changes in the uterine response to amines, similar to those which occur during these different sexual states, may be induced by the injection of ovarian hormones. After treatment with oestradiol, the excitatory activities of the amines were enhanced so that the responses of the uteri from animals which had received this treatment resembled those of uteri from adult animals. Administration of progesterone with the oestradiol did not modify the effect produced by the latter hormone. Treatment with progesterone alone did not alter qualitatively the actions of the amines. Hence it has been suggested that the responses of the uterus to adrenergic stimuli are controlled by the fluctuating levels of ovarian hormones which occur during the different sexual conditions. Thus it is possible that if the sympathetic fibres innervating the uterus of the various mammalian species exert a physiological role of regulation of the activity of the uterine muscle, then this function in turn may be controlled by the ovarian hormones.

Further evidence for the involvement of ovarian hormones in modifying uterine responses to sympathomimetic amines would be the detection of changed levels of the hormones in the circulating blood during the different sexual states when the alteration in the responses to the amines occur. The

assumption involved in this type of conclusion is that the levels of ovarian hormones measured at a given time reflect the intensity of their action at that moment. In regard to the well-known action of these hormones in modifying the endometrium, it has been reported in the rat that there is a delay of about 24 - 26 hours between the secretion of the oestrogen causing vaginal cornification and the observation of that response (Zondek and Sklow, 1942; Schwartz, 1964; Barnea, Gershonowitz and Shelesnyak, 1968). Recently it has been possible to measure in the rat the minute levels of oestrogen secreted into the ovarian venous blood (Presl, Horský, Herzmann, Mikuláš and Henzl, 1967; Hori, Ide and Miyake, 1968) and changes in the amounts secreted have shown that the highest levels of oestrogen are reached some 20 - 25 hours before vaginal cornification occurs. Peak secretion rates of progesterone occur in proestrus and dioestrus (Telegdy and Endröczi, 1963; Hashimoto and Melampy, 1967; Hashimoto, Hendricks, Anderson and Melampy, 1968). However, no changes in the effects of the amines occur during dioestrus subsequent to the peak progesterone secretion at this time. Thus it appears that the early proestrous rise in oestrogen seems more likely to be related to the changed effects of the amines than to the increased secretion of progesterone during dioestrus.

As yet the mechanisms through which the ovarian hormones

act remain to be elucidated, but the similarity of the responses of the uterus to amines after treatment of animals with ovarian hormones and after treatment with adrenergic blocking agents has been noted. The responses of uteri from rats in oestrus were similar to the responses from rats in dioestrus and proestrus after treatment with propranolol. The responses of uteri from oestrogen-treated, immature and from oestrogen-treated, ovariectomised rats were similar to the responses from untreated animals after β-receptor blockade with propranolol. Similarly, the responses of uteri from immature and from ovariectomised rabbits after oestradiol treatment were similar to the responses obtained after β-receptor blockade in the untreated animals. These results raise the possibility that the ovarian hormones may modify the uterine responses by inducing a change in the activity and/or the availability of α- and β- adrenergic receptors, so that an increase in the predominance of one receptor over the other will determine the nature of the response to the amines. This conclusion has been reached in regard to responses of the uterus of the rat (p. 105), rabbit (p. 197), cat (Tsai and Fleming, 1962, 1963) and guinea-pig (Vander-Pol, 1956; Cieciorowska, 1962; Deis and Pickford, 1964; Rossignol et al., 1965).

Recently however Bülbring, Casteels and Kuriyama (1968) have postulated that a different mechanism of action may be involved. These workers have suggested that in the cat

the changed uterine response to adrenaline which occurs during pregnancy may be brought about by an increase in the intracellular chloride content of the uterine smooth muscle cells which occurs during early pregnancy. The influence of ovarian hormones on the electrical activity and ionic content of the myometrium has received considerable attention (Jung, 1956; Goto and Csapo, 1960; Csapo, 1962; Marshall, 1962; Casteels and Kuriyama, 1965), and Bülbring et al. (1968) have considered that the increased chloride content in the myometrial cells which occurs during early pregnancy may be brought about by the increased progesterone secretion which occurs at this time. Adrenergic blocking agents influence the activities of catecholamines in altering membrane permeabilities to ions (Jenkinson and Morton, 1967) and this may explain the parallelism of action of the effects of adrenergic blockade and of ovarian hormones in altering uterine responses to catecholamines.

A further possible mechanism of action of the ovarian hormones is that they may affect the subcellular levels of noradrenaline and adrenaline in uterine tissue and thus alter the activity of both endogenous and exogenous sympathomimetic amines. The experiments performed so far however, have produced conflicting results. Alterations in amine levels in the uterus in different sexual states of the animal or alterations induced by hormone treatment have been reported

in the rat (Rudzik and Miller, 1962a, b; Wurtman, Axelrod and Potter, 1964; Cha, Lee, Rudzik and Miller, 1965), rabbit (Cha et al., 1965; Rosengren and Sjöberg, 1968; Sjöberg, 1968) and human (Cha et al., 1965). In contrast, other workers have reported no such variations in the rat (Oskarsson, 1960; Gutman and Weil-Malherbe, 1967), rabbit (Miller and Marshall, 1965) and human (Gaffney, Burkett and Woronkow, 1965; Owman, Rosengren and Sjöberg, 1967).

It would be interesting to investigate whether the hormonal state of an animal was decisive not only for changes in the responses of the uterus to sympathomimetic amines but also whether it exerts an influence on adrenergic mechanisms in other organs. Ahlquist, Taylor, Rawson and Sydow (1954) noted that in female dogs the pressor response to adrenaline was greater than in males. Boxhill and Brown (1955) showed that, in female dogs in oestrus or in oestrogen-treated, ovariectomised dogs, adrenaline produced greater pressor responses than in males or in metoestrous or ovariectomised females.

Further studies on all these possible mechanisms of action of ovarian hormones are required before any firm conclusions can be reached as to how the changed responses of the uterus to sympathomimetic amines are brought about.

MATERIALS USED

ACETYLCHOLINE - 100 mg in 0.005 ml acetic acid and triethylene glycol to 1 ml, "Acécoline" (Labs. Lematte et Boinot, Paris).

l-ADRENALINE - as acid tartrate, B.P., 1 mg/ml (Gale & Baiss).

DICHLOROISOPRENALINE - as hydrochloride (Lilly).

DIHYDROERGOTAMINE - 1 mg/ml "DHE -45" (Sandoz).

ETHER - diethyl ether anaesthetic reagent (B.D.H.).

l-N-ETHYLNORADRENALINE - as hydrochloride (Sterling-Winthrop).

l-α-ETHYLNORADRENALINE - as hydrochloride, "Butanephrine" (Sterling-Winthrop).

HEPARIN - B.P. (Boots Pure Drug Co.).

dl-ISOPRENALINE - as sulphate, B.P. (B.D.H.).

l-NORADRENALINE - as acid tartrate, 1 mg/ml, "Levophed" (Bayer).

OESTRADIOL BENZOATE - as injection, B.P., 5 mg/ml in 20% ethyl oleate and 80% arachis oil (B.D.H.).

17β-OESTRADIOL-3-SODIUM SULPHATE - (Organon).

PENTOBARBITONE SODIUM - B.P., 60 mg/ml, "Nembutal" (Abbott).

PHENTOLAMINE - as mesylate, B.P., 5 mg/ml, "Rogitine" (Ciba).

l-PHENYLEPHRINE - as hydrochloride, U.S.P. (Sigma).

PROGESTERONE IN AQUEOUS SOLVENT - 20 mg/ml, "Primolut-Aqueous" (Schering).

PROGESTERONE IN OIL - as injection, B.P., 10 mg/ml in ethyl oleate, "Progestin" (B.D.H.).

PRONETHALOL - as hydrochloride, "Alderlin" (I.C.I.).

PROPRANOLOL - as hydrochloride, 1 mg/ml, "Inderal" (I.C.I.).

SUTURES - sterilised surgical sutures, B.P., number 2/0, non-boilable (Allen & Hanburys).

TOLAZOLINE - as hydrochloride in aqueous buffered solution, 5 mg/ml, "Priscol" (Ciba).

URETHANE - ethyl carbamate, 25% w/v aqueous solution (B.D.H.).

Dilutions

Stock solutions of the sympathomimetic amines were prepared by making dilutions to 1 mg/ml in 0.01 N hydrochloric acid. These were stored at $4^{o}C$ and renewed each month. Further dilutions were made immediately before use in 0.9% sodium chloride solution.

Dilutions of oestradiol and progesterone in oil were made in arachis oil.

Dilutions of all other substances were made in 0.9% sodium chloride solution immediately before use.

ABBREVIATIONS USED:

a) THROUGHOUT THE THESIS

d	= dextro	mg	= milligramme
Fig.	= Figure	min	= minute
g	= gramme	ml.	= millilitre
i.a.	= intra-arterial	msec	= millisecond
i.v.	= intravenous	p.	= page; pp. = pages
kg	= kilogramme	sec	= second
l	= laevo	µg	= microgramme
l.	= litre	V	= volt

b) IN THE TABLES

l-Adr	= l-adrenaline
l-α-EtNor	= l-α-ethylnoradrenaline
l-N-EtNor	= l-N-ethylnoradrenaline
dl-Iso	= dl-isoprenaline
l-Nor	= l-noradrenaline
l-Phe	= l-phenylephrine
O	= oestrogen
ovariect.	= ovariectomised
P	= progesterone
U	= untreated

c) IN THE FIGURES

A = l-adrenaline

Ac = acetylcholine

B.P. mm Hg = blood pressure in millimetres of mercury

Bu = l-α-ethylnoradrenaline

C = ↓ = direction of contraction of uterine muscle

DCI = dichloroisoprenaline

EtN = l-N-ethylnoradrenaline

H = hypogastric nerve stimulation

I = dl-isoprenaline

i.a. = intra-arterial

i.v. = intravenous

N = l-noradrenaline

Pe = l-phenylephrine

Pn = pronethalol

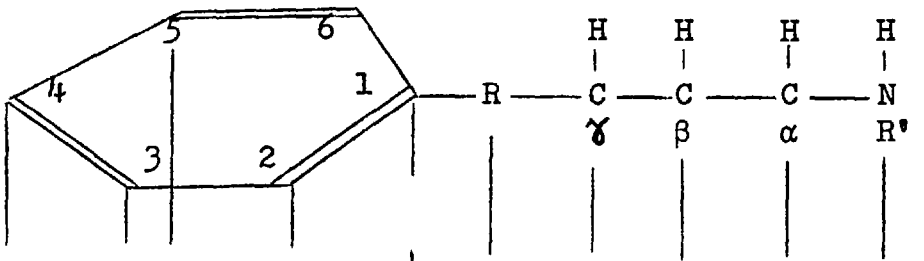
Pr = propranolol

R = ↑ = direction of relaxation of uterine muscle

U.P. mm Hg = uterine pressure in millimetres of mercury

CHEMICAL FORMULAE OF β-RECEPTOR AGONISTS AND ANTAGONISTS

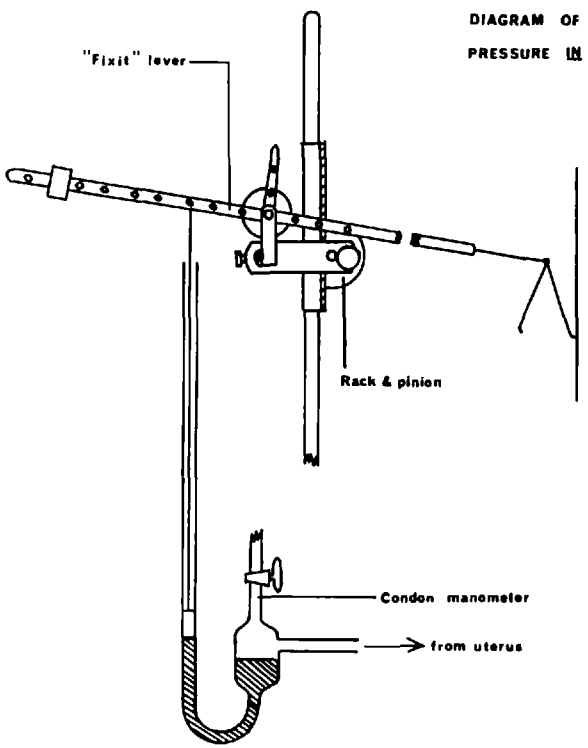
The formulae shown overleaf are based upon the phenylethylamine or phenylpropylamine skeleton.

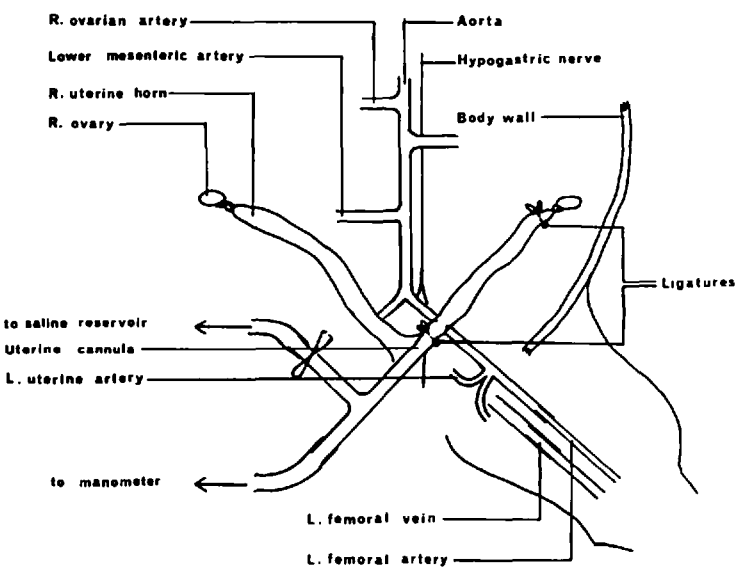


Noradrenaline	OH	OH			OH	H	H
Adrenaline	OH	OH			OH	H	CH_3
N-ethylnoradrenaline	OH	OH			OH	H	C_2H_5
α-ethylnoradrenaline	OH	OH			OH	C_2H_5	H
Isoprenaline	OH	OH			OH	H	$CH(CH_3)_2$
Phenylephrine		OH			OH	H	CH_3
Dichloroisoprenaline	Cl	Cl			OH	H	$CH(CH_3)_2$
INPEA	NH_2				OH	H	$CH(CH_3)_2$
H 13/57	CH_3	CH_3			OH	H	$CH(CH_3)_2$
MJ 1999	NH SO_2CH_3				OH	H	$CH(CH_3)_2$

	4	3	5	2	R	γ	β	α	R'
Pronethalol	⟵ naphthyl ⟶						OH	H	$CH(CH_3)_2$
Propranolol	⟵ naphthyl ⟶				O	H	OH	H	$CH(CH_3)_2$
I.C.I.50172	NH $\vert$ $COCH_3$				O	H	OH	H	$CH(CH_3)_2$
Kö 592) I.C.I.45763)		CH_3			O	H	OH	H	$CH(CH_3)_2$
H 56/28				CH_2 $\vert$ $CH=CH_2$	O	H	OH	H	$CH(CH_3)_2$
N-isopropyl-methoxamine (IMA)			OCH_3	OCH_3			OH	CH_3	$CH(CH_3)_2$
Dimethylisopropyl-methoxamine	CH_3			CH_3			OH	CH_3	$CH(CH_3)_2$
Butoxamine			OCH_3	OCH_3			OH	CH_3	$C(CH_3)_3$
α-methyldichloro-isoprenaline	Cl	Cl					OH	CH_3	$CH(CH_3)_2$
Isoxsuprine	OH						OH	CH_3	$CH.CH_3$ $\vert$ $CH_2CH_2C_6H_5$
C_c25	OH						OH	H	$CH(CH_3)_2$

DIAGRAM OF APPARATUS FOR RECORDING UTERINE PRESSURE IN VIVO IN THE RAT AND RABBIT





PHYSIOLOGICAL SALT SOLUTIONS

Composition in g/litre

	Tyrode (1910)	Krebs-Henseleit (1932)	de Jalon (1945)	Lehrer (1965)
NaCl	8.00	6.87	9.00	9.00
KCl	0.20	0.40	0.42	0.40
$MgCl_2$	0.10	-	-	-
$MgSO_4.7H_2O$	-	0.30	-	-
$CaCl_2$	0.20	0.28	0.06	0.24
NaH_2PO_4	0.05	0.14	-	-
$NaHCO_3$	1.00	2.10	0.50	0.40
Glucose	1.00	2.00	0.50	0.10

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