

STUDIES ON VASOPRESSIN SECRETION IN THE RAT AND IN MAN

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Being a thesis for the degree of Doctor of Medicine
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To my wife Mary and our daughter Antonia who was born
during the early part of this work

ABSTRACT

Aspects on the central control of the neurohypophyseal hormone arginine vasopressin (AVP) have been studied in the rat and in man with particular reference to the novel peptide atrial natriuretic factor (ANF). In the rat studies on AVP and oxytocin (OT) release were performed in vivo and in vitro. Intracerebroventricular (ICV) administration of atriopeptin III had no effect on heart rate or mean arterial pressure of the anaesthetised rat. Further studies were performed in conscious rats and following the ICV administration of atriopeptin III, plasma levels of AVP and OT were determined in a) control rats, b) rats whose drinking water had been replaced by 2% saline and c) rats who had been haemorrhaged. The release of both AVP and OT were inhibited when basal levels were elevated by saline treatment or by haemorrhage. In in vitro experiments the stimulated release of AVP and OT from the isolated neuro-intermediate lobe of the rat was shown to be inhibited by atriopeptin III. These experiments suggest that ANF acts within the hypothalamo-neurohypophyseal axis to inhibit the stimulated release of both AVP and OT.

Clinical studies were performed both in normal subjects and in patients with two different disorders of the central nervous system. Changes in urine flow have been reported previously during spontaneous migraine. This was confirmed and evidence is presented for the inhibition of

AVP secretion during migraine. A possible role for ANF in the mechanism is also discussed.

Postural changes in urine flow have been observed in tetraplegic patients. This has been confirmed and the cardiovascular regulation of AVP secretion in normal and tetraplegic man examined. Head-up tilt resulted in greater release of AVP in the tetraplegics than in the controls probably due to the additional hypotensive response in the tetraplegics. Osmotic regulation of AVP secretion was normal in the tetraplegics, however infusion of hypertonic saline caused a marked rise in blood pressure in the tetraplegics but not in the controls.

In order to ascertain whether the increased secretion of AVP in tetraplegics resulted in any change in peripheral AVP receptor mediated responses, the sensitivity to pressor effects of AVP was examined in normal controls, tetraplegics and healthy subjects who had been treated with alpha and beta adrenoceptor blockers. Infusion of AVP at physiological concentrations had no effect on blood pressure in either the normal or the healthy subjects who had been alpha and beta blocked. In contrast AVP infusion resulted in a marked rise in blood pressure in the tetraplegic subjects. The reason for this increased pressor sensitivity to AVP in tetraplegics is discussed.

PUBLICATIONS

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CONTENTS

	Page
Acknowledgements	(i)
Declaration	(ii)
Chapter 1 <u>Introduction</u>	
1.1 Plasma osmolality, osmoreceptors, sodium sensitive receptors, blood volume and blood pressure	1
1.2 Neural pathways controlling the secretion of vasopressin	6
1.3 Biosynthesis and intraneuronal transport of vasopressin	7
1.4 Neuroendocrine control of vasopressin secretion	10
1.5 Atrial natriuretic factor (ANF) and its potential role in the control of vasopressin secretion	15
Chapter 2 <u>Methodology</u>	
2.1 Animals	23
2.2 Anaesthetics	23
2.3 Stereotaxy	24
2.4 Plasma extraction	27
2.5 Radioimmunoassay (vasopressin, oxytocin and atrial natriuretic factor)	28
2.6 High performance liquid chromatography	35
2.7 Perifusion	38
2.8 Statistics	43

Chapter 3 Studies with Atrial Natriuretic Factor
in anaesthetised rats

3.1	The effect of peripherally administered ANF on urinary volume and urinary sodium	45
3.2	Studies on urine flow in rats placed in a stereotaxic frame	50
3.3	The effect of centrally administered ANF on blood pressure and heart rate	56

Chapter 4 The effect of centrally administered ANF on the stimulated release of vasopressin and oxytocin in vivo

4.1	Introduction	62
4.2	Materials and Methods	63
4.3	Results	66
4.4	Discussion	74

Chapter 5 The effect of ANF on the stimulated release of vasopressin and oxytocin in vitro

5.1	Introduction	80
5.2	Materials and Methods	80
5.3	Results	81
5.4	Discussion	84

	Page
Chapter 6 <u>Inhibition of vasopressin secretion during</u> <u>migraine</u>	
6.1 Introduction	86
6.2 Subjects and Methods	87
6.3 Results	89
6.4 Discussion	95
 Chapter 7 <u>Neuroendocrine control of vasopressin</u> <u>secretion in tetraplegic man</u>	
7.1 Introduction	98
7.2 Subjects and Methods	99
7.3 Results	103
7.4 Discussion	111
 Chapter 8 <u>The effects of vasopressin infusion on blood</u> <u>pressure and heart rate in a) tetraplegic man</u> <u>and b) alpha and beta-adrenoceptor blocked man</u>	
8.1 Introduction	116
8.2 Subjects and Methods	118
8.3 Results	121
8.4 Discussion	128
 Conclusions and implications of this work	131
 Bibliography	133

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DECLARATION

I declare that the studies presented in this thesis are the result of my own independent investigation with the exception of:

(i) the reverse phase high performance liquid chromatography (chapter 2) which was performed by Dr S Biswas.

(ii) some of the radioimmunoassays for vasopressin and oxytocin in the perfusion experiment (chapter 5) which were performed by Mr J Farrah.

(iii) the assays for atrial natriuretic factor were performed by Miss E Pitts.

(iv) the hypertonic saline infusions in healthy control subjects (chapter 7) which were performed by Dr TDM Williams.

This work has not been and is not being concurrently submitted for candidature for any other degree.

Candidate

Yool.

Supervisor

Stafford Lightman

The physiological control of arginine vasopressin (AVP) secretion is achieved by two major mechanisms: 1) changes in plasma osmolality and 2) changes in blood volume and blood pressure.

1.1 Plasma Osmolality

Verney, 1947 was the first to show that various hyperosmolar solutions when injected into the carotid artery of conscious dogs resulted in a rapid fall in urine volume, thereby suggesting a relationship between extracellular fluid tonicity and AVP release. This led to the hypothesis that there were cells in the territory of the common carotid artery sensitive to changes in tonicity of extracellular fluid which controlled the release of antidiuretic hormone. As a result of unilateral carotid artery infusions and various surgical procedures on the carotid artery, Jewell and Verney (1957) postulated the existence of osmoreceptors within the anterior hypothalamus. Subsequent work has shown that there is a linear relationship between extracellular fluid tonicity and AVP release in several different species (rats - Dunn et al, 1973; goats - Andersson, 1977; sheep - McKinley et al, 1978; monkeys - Swaminathan, 1980; dogs - Ramsay et al, 1983; humans - Zerbe and Robertson, 1983; Lightman and

Everitt, 1986). In man plasma osmolality is normally maintained between 286 and 294 mOsm/kg through the release of AVP.

There is still uncertainty as to the exact site of the osmoreceptors despite all the work that has been done to try and locate them. Within the anterior hypothalamus, the anteroventral part of the 3rd ventricle (AV3V) has been proposed as one of the sites for osmoreceptors. For example, rats with lesions of the AV3V region exhibit hypernatraemia and deficits in drinking (Buggy and Johnson, 1977) and goats with lesions in this region develop chronic dehydration and lack a significant AVP response to hypernatraemia (Andersson, 1977). The circumventricular organs, discrete areas where the blood - brain barrier is defective, such as the organum vasculosum of the lamina terminalis (which is within the AV3V), the subfornical organ and the neurosecretory neurones themselves have been shown to be sensitive to changes in plasma osmolality (Miselis & Eng, 1981; Sladek & Armstrong, 1985).

Andersson (1977) formulated the hypothesis of periventricular sodium sensitive receptors. He observed that intracerebroventricular (ICV) infusions in goats of hypertonic saline induced drinking and inhibited urine output, whereas hypertonic sucrose did not. McKinley et al (1978) extended this work by comparing the effects of

intracarotid infusions of 1M NaCl, 2M sucrose and 4.6M urea solutions on urine flow and CSF sodium concentration in sheep. Hypertonic saline and sucrose produced a rapid onset of drinking and antidiuresis whereas urea caused a relatively slow antidiuresis and no stimulation of drinking despite the fact that it caused the greatest rise in CSF sodium concentration. As stated by Schrier et al (1979, F325) these results "provide a near fatal blow to the CSF sodium receptor hypothesis for ADH release. And Fitzsimons (1986) writes that the distinction between osmoreceptors and sodium sensitive receptors may be unimportant, as increases in osmolality arising from lack of water also cause an increase in plasma sodium concentration.

Blood Volume and Blood Pressure

Ryden and Verney (1938) were the first to obtain evidence that haemorrhage is a stimulus for the release of AVP. They found that a moderate haemorrhage in the unanaesthetised dog resulted in inhibition of water diuresis. Hypo- and hypervolaemia were subsequently shown to affect the release of AVP. Share (1961) using peritoneal lavage with a hypertonic solution in dogs showed that a 15% reduction in extracellular fluid volume and no significant change in mean arterial pressure or plasma osmolality caused a six fold increase in plasma AVP. Zehr et al (1969) expanded extracellular fluid volume

in the unanaesthetised ewe by the infusion of isotonic saline and observed a fall in plasma AVP concentration as the extracellular fluid volume was increased.

The realisation that there is a haemostatic regulation of blood volume led to considerable speculation and research directed towards identifying the "volume receptors". Gauer, Henry and others in the 1950s reported how an increase in intrathoracic blood volume or rise in left atrial pressure produced a diuresis. Gauer and Henry (1963) described two types of receptor: low pressure receptors in the atria, ventricles and great veins which respond to changes in blood volume; high pressure receptors located in the carotid sinuses and aortic arch which respond to changes in blood pressure. Cooling the vagi was shown to abolish the diuretic response to atrial distension and negative pressure breathing. Cutting the vagi caused a rise in the plasma concentration of AVP (Share & Levy, 1962; Clark and Silva, 1967). ^{Experiments in} ~~Physiological~~ ^{demonstrated also suggested a physiological role for} ~~importance of atrial receptors is derived from studies on~~ ^{receptor.} ~~cardiac denervation.~~ Cardiac denervation prevents the diuretic response to atrial distension; reduced release of AVP in response to haemorrhage or dehydration. The possibility that the diuretic response to left atrial distension cannot be explained entirely by inhibition of AVP release is discussed in chapter 1.5.

In addition to the atrial stretch receptors, baroreceptors

can participate in the control of AVP release. Occlusion of both common carotid arteries in anaesthetised, vagotomised dogs results in a marked, rapid increase in plasma AVP concentration (Share & Levy, 1962). This manoeuvre was ineffective if the vagi were intact. That the receptors are located in the carotid sinuses is indicated by the observation that denervation of the carotid sinuses several weeks before the experiment blocked the release of AVP.

Chemoreceptors, as well as ^{atrial stretch receptors} ~~stretch~~ and baroreceptors, may be involved in the release of AVP. In open chest dogs in which respiration was maintained constant and the vagi cut, the perfusion of an isolated carotid sinus on one side with deoxygenated blood caused an increase in plasma concentration of AVP (Share & Levy 1966; Share 1976).

In summary, it has been shown that changes in the activity of atrial stretch receptors, baroreceptors and chemoreceptors regulate the release of AVP.

Other Stimuli Affecting AVP Secretion

A variety of stimuli in addition to plasma hypertonicity, hypovolaemia and hypotension are known to increase AVP secretion by actions on undefined central pathways. Nausea is a very potent stimulus to AVP secretion in man (Robertson, 1977) which may be a contributory factor in the very high levels observed during syncope. Hypoglycaemia induced by insulin administration results in a moderate rise in AVP (Baylis and Robertson, 1981) which is independent of alterations in plasma osmolality, blood pressure and blood volume. An oropharangeal reflex has been reported recently in which the act of drinking causes a rapid fall in plasma AVP in dehydrated dogs (Thrasher et al, 1981) and in man given water to drink (Geelen et al, 1984). This fall in plasma AVP occurs within 10 minutes and precedes any postabsorptive changes in plasma osmolality or volume.

1.2 Neural Pathways controlling the secretion of AVP

Afferent fibres from high and low pressure receptors (section 1.1) within the cardiovascular system project in the glossopharyngeal and vagus cranial nerves and terminate in the nucleus of the tractus solitarius within the dorsal vagal complex (DVC). From here pathways project by direct and indirect routes to the paraventricular (PV) nucleus as well as the supraoptic (SO) nucleus (figure 1.1). Sawchenko and Swanson (1982) demonstrated a

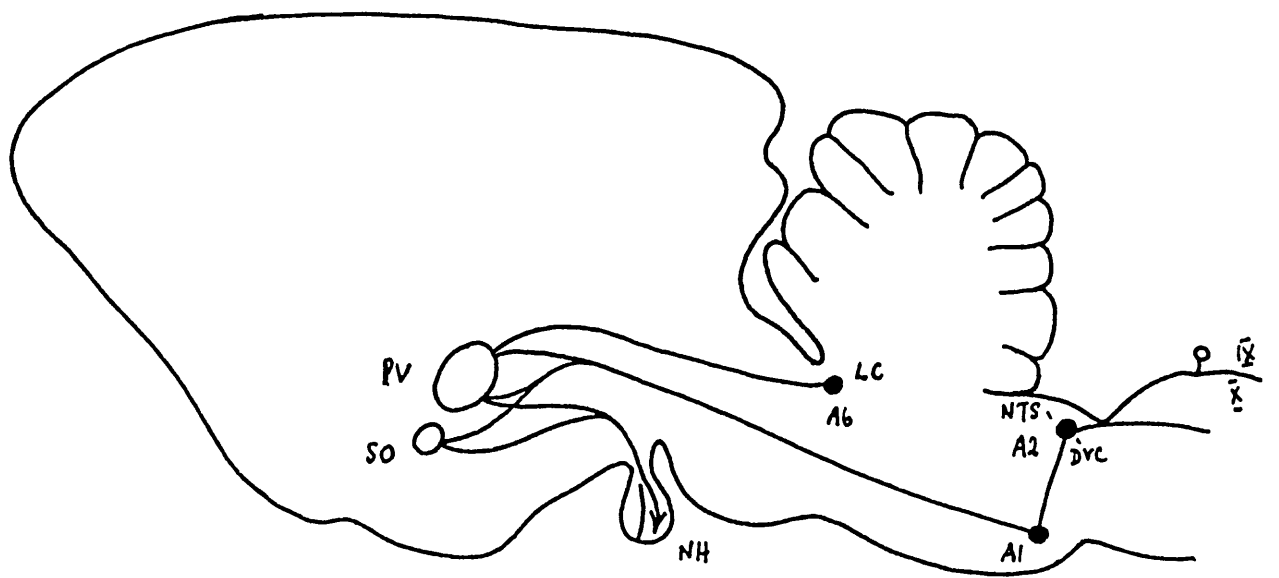
non-noradrenergic projection from the DVC to the A1 group of noradrenergic neurones which in turn project to cells in the PV and SO nuclei preferentially secreting AVP. In addition noradrenergic cells in the locus coeruleus (A6) send a projection to the hypothalamus, although their major projection is to higher structures including the cerebral cortex. The PV nucleus is divided into two parts - the magnocellular neurones which project to the neurohypophysis and the parvocellular neurones which project to the median eminence, brain stem and spinal cord where they may influence anterior pituitary function and the autonomic nervous system (Swanson & Sawchenko, 1983). The SO nucleus contains only magnocellular neurones which project to the neurohypophysis.

1.3 Biosynthesis and intraneuronal transport of vasopressin

AVP, like other peptides, is synthesised as part of a larger precursor molecule prepropressophysin. Synthesis is thought to take place in the perikarya of the PV and SO nuclei in cells distinct from those producing oxytocin (Bindler et al, 1967). The precursor molecules are transported by axoplasmic flow down the neurosecretory fibres to endings in the neurohypophysis. Cleavage of the precursor molecule to AVP and neurophysin occurs during transport. The AVP is stored in granules at the nerve

Figure 1.1

Diagrammatic representation of the rat brain showing the major inputs and outputs to the paraventricular (PV) and supraoptic (SO) nuclei. Dorsal vagal complex (DVC); locus coeruleus (LC); neurohypophysis (NH); nucleus of the tractus solitarius (NTS); glossopharyngeal (IX) and vagal (X) cranial nerves.



endings until subsequently secreted into the circulation in episodic bursts by Ca^{2+} dependent exocytosis in response to both osmotic and non-osmotic stimuli (Douglas and Poisner, 1964).

1.4 Neuroendocrine control of vasopressin secretion

The release of AVP into the circulation appears to be largely determined by the combined effect of excitatory and inhibitory inputs to the magnocellular neurons in the SO and PV nuclei of the hypothalamus. The nature of the interaction between these inputs is unknown but it has been proposed that the magnocellular neurons receive incoming information from both osmotic and non osmotic stimuli (Schrier et al, 1979).

Most of the known neurotransmitters and biologically active peptides have been implicated in the central control of vasopressin release. Some of the more important neurotransmitters will be discussed:

1) Acetylcholine

Following previous experiments with intravenous injections of acetylcholine in dogs, Pickford (1945) demonstrated that an antidiuresis also occurred in response to injections of acetylcholine directly into the supraoptic nucleus. Inhibition of urine flow was not seen after

removing the neurohypophysis suggesting that the antidiuresis was due to the release of AVP. Intravenous injections of acetylcholine into atropinised dogs similarly resulted in an antidiuresis suggesting that the nicotinic receptors were involved in the release of AVP. Burn, Truelove & Burn (1945) reported antidiuresis in man and rats in response to i.v. nicotine as well as antidiuresis after smoking cigarettes. Milton & Paterson (1974) performed direct injections of nicotine into the supraoptic nucleus and observed a rise in jugular AVP concentration, an effect prevented by pretreatment with hexamethonium or pempidine.

In the water loaded rat Kuhn (1974) demonstrated an excitatory role for the muscarinic receptor in AVP release. However Bisset and Chowdrey (1984) in addition to confirming Kuhn's observation demonstrated that the release of AVP in response to a physiological stimulus involves a cholinergic link through a nicotinic receptor. Immunocytochemical techniques have demonstrated the presence of the enzyme choline acetyltransferase, a specific marker of cholinergic neurons, in the neurohypophysis and immediate vicinity (lateral hypothalamus) of the SO nucleus (for review see Sladek, 1983). More recently using monoclonal antibodies, nicotinic cholinergic receptors have been demonstrated in regions of the hypothalamus associated with vasopressinergic neurons (Mason, 1985).

2) Catecholamines

The first effect of catecholamines to be reported was an inhibitory one. Early experiments showed that the inhibition of diuresis induced by emotional stress could be reduced by neurohypophysectomy. The same workers, O'Connor & Verney (1945), reported that adrenaline inhibited the antidiuretic response to emotional stress. Later work has shown that noradrenaline acting on one or other type of receptor may either stimulate or inhibit the release of AVP. For example, phentolamine or phenoxybenzamine will inhibit the antidiuresis produced by intracerebroventricular injections of noradrenaline, in the water loaded rat (Kuhn, 1974; Hoffman, Phillips & Schmid, 1977) and propranolol will inhibit the diuresis produced by isoprenaline and adrenaline in dogs (Bhargava et al. 1972). The effects of injections on urine flow or the concentration of AVP in plasma are variable, possibly depending on such factors as the dose of noradrenaline, the species, the state of hydration of the animal and whether the animal was conscious or anaesthetised.

Immunocytochemical techniques have separated ascending noradrenergic pathways into the so-called ventral noradrenergic bundle (VNAB) and dorsal noradrenergic bundle (DNAB). Injections of 6-hydroxydopamine into the DNAB, but not VNAB, of rats has been reported as producing a significant reduction in the release of AVP in response

to 0.5% body weight haemorrhage (Lightman, Todd & Everitt, 1984). Whereas similar injections into the VNAB, but not DNAB, produced a significant reduction in 1.5 M saline-induced rise in plasma AVP (Lightman, Todd & Everitt, 1983). These observations suggest, firstly that the noradrenergic projections involved in the AVP response to increased osmolality are different from those implicated in the AVP response to haemorrhage and, secondly, that noradrenaline has an excitatory role in these pathways.

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3) Opioid Peptides

Immunoreactive Leu-enkephalin is present in the neurointermediate lobe of the rat pituitary and an enkephalinergic pathway arising in the SO and PV nuclei has been traced to the neurohypophysis (Rossier et al, 1979). Opiate receptors have also been observed in the neural lobe (Simantov and Snyder, 1977; Lightman et al, 1983); predominately kappa in type (Bunn et al, 1985; Gerstberger & Barden, 1986). Homozygous Brattleboro rats (lacking AVP) have significantly less opioid peptides in their neuro-intermediate lobes than heterozygous rats. Coexistence of dynorphin with AVP immunoreactivity in the same magnocellular neurones has been shown (Coulter et al, 1981) as has coexistence of Leu-enkephalin and oxytocin immunoreactivity (Martin & Voigt, 1981). Several studies have now been done in animals and man to investigate the functional role of opioid peptides in the control of AVP

secretion and it appears that endogenous enkephalins exert an inhibitory effect on stimulated AVP release (for review see Carter and Lightman, 1985).

4) Angiotensin II

Several studies have shown AVP release after ICV administration of angiotensin II and in one study (Keil et al 1975) the release of AVP was directly related to the dose of angiotensin II injected into the cerebral ventricles. Peripheral infusion of high doses of angiotensin II have been shown to increase plasma AVP concentrations (Padfield and Morton, 1977) but a physiological role for angiotensin II in the central control of AVP release seems unlikely.

5) Other Peptides

Other peptides such as Substance P and corticotrophin-releasing factor (CRF) have been localised in the PV nucleus. Intracerebroventricular administration of Substance P has been reported to stimulate the release of AVP in anaesthetised rats (Haldar et al 1979) and high doses of intravenous CRF have been shown to release AVP in conscious dogs (Klingbeil et al 1988) but their physiological role in the central control of AVP release is unknown.

1.5 Atrial Natriuretic Factor and its potential role in the control of vasopressin secretion

In 1961 de Wardener et al published experimental work as evidence for the existence of a humoral agent or natriuretic factor. This "third factor" was thought to act in association with the glomerular filtration rate and the renin-angiotensin-aldosterone system to regulate sodium loss from the kidney. In 1984 a novel group of peptides were isolated from atrial cardiocytes of animals and found to have powerful natriuretic, diuretic and hypotensive properties. This family of peptides is collectively known as atrial natriuretic factor (ANF).

Historical background to ANF

It was Kisch, 1956 who reported granules in the cytoplasm of guinea pig atrial cardiocytes between the myofibrils which he called osmophilic bodies. Similar granules were subsequently described by Jamieson and Palade, 1964 in the atrial but not ventricular cardiocytes of 10 mammalian species. Their function however was unknown.

Gauer et al, 1954 showed that dogs exposed to constant negative pressure breathing have an increase in urine flow. They postulated that negative pressure breathing increased intrathoracic blood volume, which stimulated stretch receptors within the pulmonary circulation or heart, to cause an increase in urine volume. Further

experimental work by Henry, Gauer and Reeves, 1956 on anaesthetized dogs suggested that volume receptors were located in the left atrium and terminal pulmonary veins. In the same year Henry and Pearce, 1956 suggested that atrial stretch rather than intra-atrial pressure was responsible for changes in urine flow rates and blood volume. None of this work however contained data on urinary sodium.

Ledsome et al, 1961 showed that the diuresis produced by left atrial distention would still occur despite the infusion of up to 0.1 μ g/kg/min of AVP in conscious dogs, suggesting that the diuresis was not due to the inhibition of AVP. They also showed that the diuresis was associated with a natriuresis and that the diuresis could be prevented by prior sectioning of the vagus nerve but not by renal denervation. Meanwhile, Baisset et al, 1959 had reported in the French literature that balloon distension of the right atrium produced a natriuresis and a fall in the aldosterone concentration of adrenal venous blood in anaesthetized dogs.

It was de Wardener, 1961 working with cross circulated anaesthetized dogs who reported a natriuresis and diuresis of glomerular filtration rate and aldosterone secretion. The same group postulated that this increase in sodium excretion was related to a humoral agent of short half

life. Carswell et al, 1968 reported a diuresis and natriuresis in the isolated perfused dog kidney in response to left atrial distension. At about this time several groups of workers performed experiments in which the blood volume expanded and deoxycorticosterone treated animals induced a rise in urinary sodium excretion in a denervated or isolated 'assay' kidney suggesting the role of a circulating factor other than aldosterone (for review see de Wardener, 1977). Many groups of workers tried to extract this natriuretic substance from plasma, urine, lymph, kidney and liver but with little agreement about the structure or chemical properties of the various substances that were found. Raghavan and Gonick, 1980 purified and characterised a natriuretic factor from rat kidney which inhibited Na^+/K^+ ATPase activity and increased plasma glucose-6-phosphate activity but this was not to be the same natriuretic factor subsequently isolated from mammalian atria.

Huet and Cantin, 1974 reported that the atrial granules originally observed by Kisch contained protein. Subsequent work by Marie et al, 1976 showed that there were more of these granules in the right than left atrium of the rat and that water restriction increased the number of granules in the right but not left atrium. Further experiments by De Bold, 1979 confirmed the observation of Marie et al. and also showed that sodium loading and the administration of deoxycorticosterone decreased the number

of granules suggesting that they had a role in salt and water homeostasis.

It was Sonnenberg working with de Bold (1980) who performed the crucial experiments (communicated at the American Physiological Society) which demonstrated that crude homogenates of rat atria when injected into normally hydrated rats, produced a 10 fold diuresis and 30 fold natriuresis of rapid onset (5-10 minutes) and short duration (20-30 minutes). It was soon shown that this natriuretic and diuretic activity of crude rat atrial extract was localised in the atrial granules (Garcia et al, 1982; De Bold, 1982).

Within the next two years several low molecular weight peptides from atrial extracts were purified by different groups and shown to have natriuretic, diuretic and smooth muscle relaxing properties. A variety of names for these peptides have been used in the literature: auriculin; atriopeptin; cardionatrin; atrial natriuretic polypeptide; atrial natriuretic factor. The term atrial natriuretic factor (ANF) is used in this thesis. Several review articles on ANF have been published in the last 2 years, they include: Lancet, 1984 & 1986; Sagnella and MacGregor, 1984; Cantin and Genest, 1985; De Bold, 1985; Maack et al, 1985; Palluk et al, 1985; Atlas and Laragh, 1986; Anderson and Bloom, 1986; Needleman, 1986.

Using rat atrial mRNA the DNA encoding for a 152-amino acid precursor of ANF was isolated. This was found to contain the amino acid sequences of all the ANF peptides that had been reported to date. Some of these peptides are thought to be artifactual cleavage products generated during isolation, however it appears that a disulphide-looped sequence of 17 amino acids plus various COOH and NH₂ terminal extensions are necessary for biological activity.

Intravenous injection of ANF into rat or man is followed by an increase in cyclic guanosine monophosphate (cGMP) in both plasma and urine (Hamet et al, 1984; Gerzer et al, 1985). The addition of ANF to preparations of dog glomeruli is reported to cause a marked rise in cGMP, occurring at 1-2 minutes after the addition of ANF (Tremblay et al, 1985). This stimulation occurs in the presence of a phosphodiesterase inhibitor supporting previous experimental evidence that the effects of ANF are exerted at the level of guanylate cyclase stimulation rather than cGMP phosphodiesterase inhibition. ANF has also been shown to inhibit adenylate cyclase in homogenates of the rat pituitary (Anand-Scrivastava, 1985) suggesting that the effects of ANF are mediated through selective activation of guanylate cyclase and inhibition of adenylate cyclase.

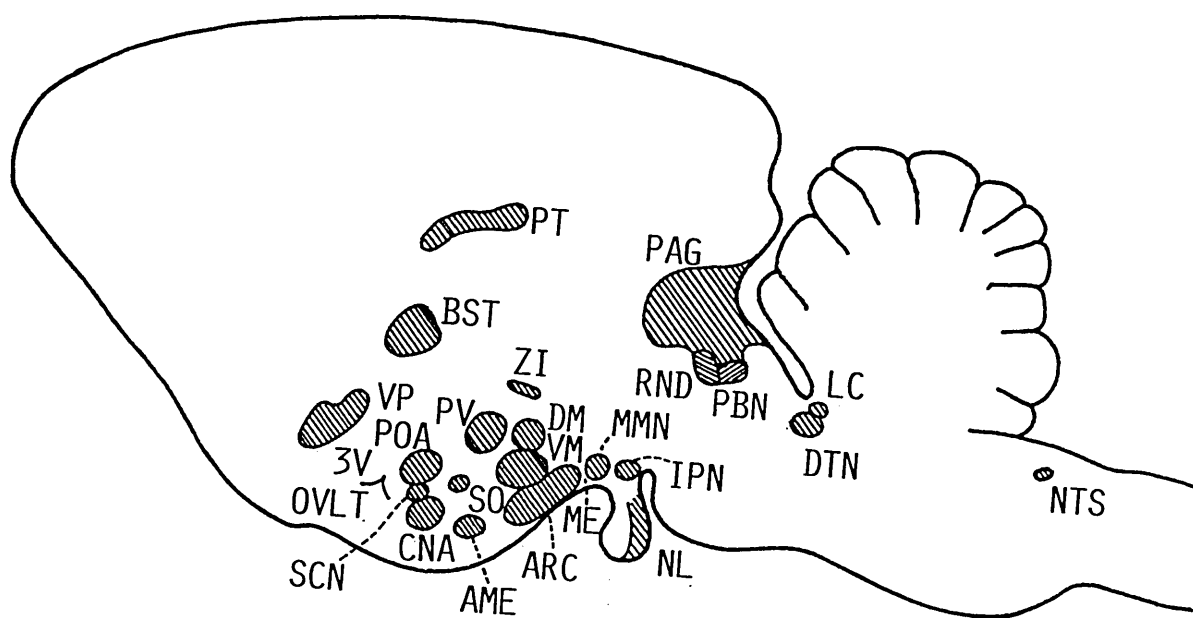
¹²⁵I-labelled ANF binding sites have been localised in the

zona glomerulosa of the adrenal cortex, the adrenal medulla, hepatocytes and bowel (Bianchi, et al, 1985). In addition binding sites have been localised in the renal glomerular endothelial cells, vasculature of the adrenal cortex, lung, liver and brain. Immunoreactive ANF in addition to being localised to the secretory granules in atrial cardiocytes has also been demonstrated in the rat brain. The principal immunoreactive ANF areas are shown in figure 1.2. Other areas not shown are the caudate nucleus, dorsal raphe nuclei, hippocampus, nucleus accumbens, nucleus of the diagonal band, the subfornical organ, substantia nigra as well as the cerebral cortex, cerebellum and cervical spinal cord. Several of these areas; in particular the AV3V are involved in electrolyte, water and blood pressure homeostasis.

The diuretic, vasorelaxant and hypotensive properties of peripherally administered ANF are in direct contrast to the antidiuretic, vasoconstrictive and pressor effects of AVP, which together with the presence of immunoreactive ANF within the rat brain would make it not unreasonable to propose a hypothesis that ANF acts centrally to inhibit the secretion of AVP. It is the potential role of ANF in the central control of AVP secretion in the rat that is addressed in chapters 4 and 5.

Figure 1.2

My own schematic drawing of the rat brain showing the immunoreactive atrial natriuretic factor (ANF) positive areas: medial nucleus of the amygdala (AME); arcuate nucleus (ARC); bed nucleus of the stria terminalis (BST); central nucleus of the amygdala (CNA); dorsomedial nucleus of the hypothalamus (DM); dorsal tegmental nucleus (DTN); interpeduncular nucleus (IPN); locus coeruleus (LC); medial mammillary nucleus (MMN); median eminence (ME); neurohypophysis (NL); organum vasculosum of the lamina terminalis (OVLT); parabrachial nucleus (PBN); paraventricular nucleus (PV); periaqueductal grey (PAG); perifornical region (PFR); periventricular nucleus of the thalamus (PT); preoptic area (POA); raphe nucleus dorsalis (RND); suprachiasmatic nucleus (SCN); supraoptic nucleus (SO); third ventricle (3V); ventral pallidum (VP); ventromedial nucleus of the hypothalamus (VM); zona incerta (ZI). Based on information from Bianchi et al, 1985; Quirion et al, 1984 and 1986; Saper et al, 1985a; Skofitsch et al, 1985; Zamir et al, 1986.



2.1 Animals

Male Sprague-Dawley rats 230-320 g were used for all animal experiments. They were housed under controlled lighting (lights on 06.00 and off 18.00) and temperature (22°C). Access to food (41B, Pitsbury, Birmingham) and tap water were freely available. Animals undergoing recovery experiments were housed singly after surgery with free access to food and tap water.

2.2 Anaesthetics

Three anaesthetics were used:

1. Urethane: a 10% solution in saline was used at a dose of 0.2 ml/100 g body weight injected intraperitoneally.
2. Equithesin: a solution containing 4.25 g chloral hydrate and 2.12 g magnesium sulphate in 30 ml distilled water with 16.2 ml pentobarbitone (60 mg/ml), 39.6 ml propylene glycol and 10 ml ethanol. A dose of 0.45 ml/100 g body weight was injected intraperitoneally.
3. Ethanol: a 12% solution equivalent to 5% of the animal's weight was given in 2 divided doses 30 minutes apart. Hydration and anaesthesia were maintained by an i.v. infusion of 1.5% ethanol in 0.4 % saline at 6.0

ml/hour (Braun Perfusor VI, Scientific Instruments Ltd, Bredon, Gloucestershire).

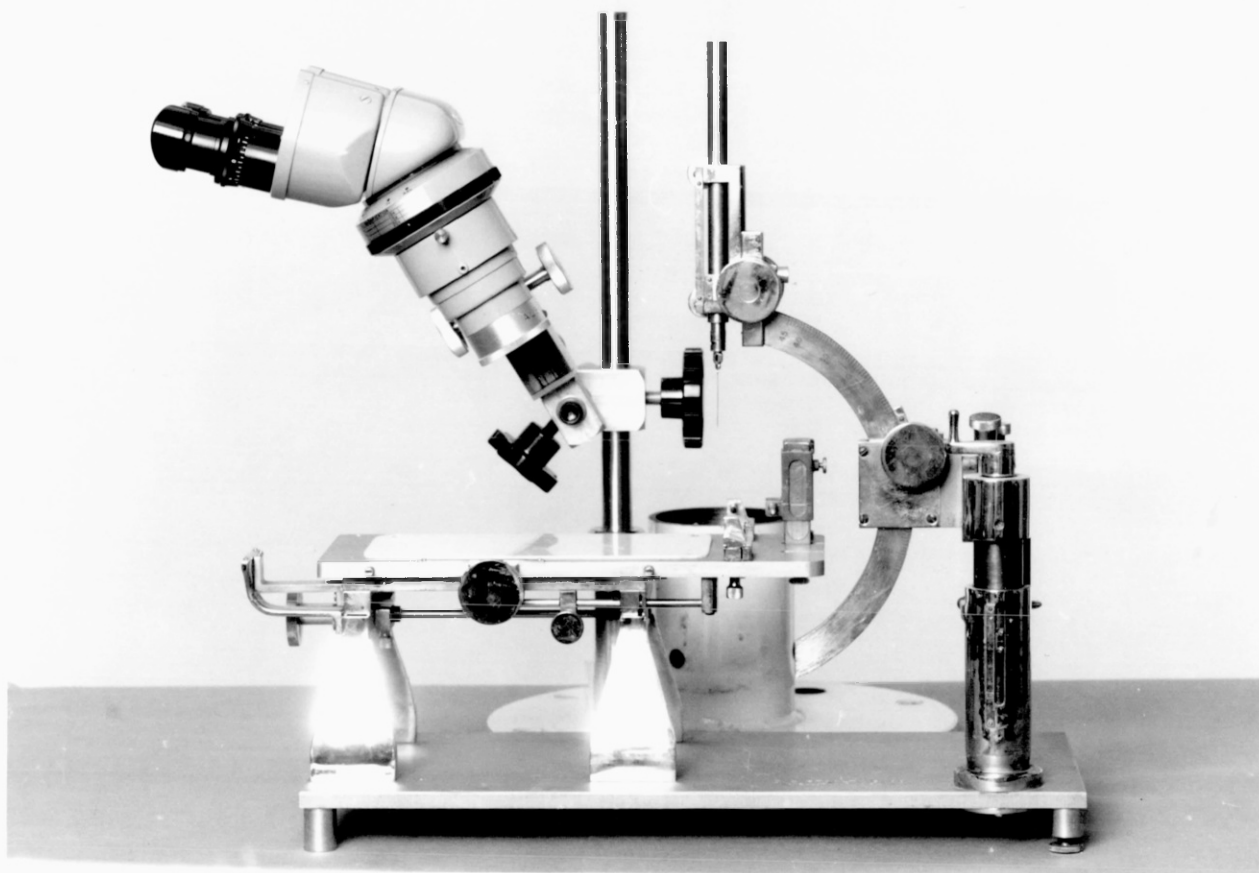
2.3 Stereotaxy

Intracerebroventricular injections were made in rats using a Stoelting stereotaxic frame and Nikon (40X) microscope (photo). The animals were placed prone and held in position by two ear bars, an upper incisor and nose bar. The co-ordinates were taken from Pellegrino et al, 1979. For the lateral ventricle they were: upper incisor bar + 5 mm; lateral to the bregma 1.5 mm; anterior to the bregma 0.2 mm; ventral to the dura 4.0 mm. Third ventricle: upper incisor bar + 5 mm; 10° to the vertical; lateral to the bregma 1.4 mm, anterior to the bregma 0.2 mm; ventral to the dura 7.9 mm.

Burr holes were made using a high speed dental drill. Lateral ventricle injections were given through 0.4mm internal diameter polythene cannulae (Portex, Hythe, Kent) held in place with dental cement and brass screws. Glass pipettes, made with a glass electro-puller (PE-2 Narishige Scientific Instrument Laboratory, Tokyo, Japan) were used for 3rd ventricle injections. Intracerebroventricular injections were given with a 10 µl glass syringe (Hamilton, Bonaduz, Switzerland).

Photograph

Stoelting stereotaxic frame and Nikon microscope with modified incisor bar and microscope stand.



2.4 Plasma Extraction

Rat and human blood were collected into heparinized plastic tubes and kept on ice. Samples were centrifuged at 3000 g for 15 minutes at 4°C and the plasma removed with a Pasteur pipette into plastic storage tubes and kept at -20°C until extracted.

Plasma samples (0.5 to 1.0 ml) were acidified with an equal volume of 0.1M formic acid, centrifuged at 3000 g for 15 minutes at 4°C decanted and extracted using a modification of the Sep-Pak method described by La Rochelle et al, 1980 for AVP. Octadecylsilane columns (C18 Sep-Paks, Waters Associates, Massachusetts, USA) were washed with 3 ml 95% methanol / 5% 0.1M formic acid followed by 3 ml 0.1M formic acid using a multichannel pump (Watson-Marlow, Falmouth, England). Acidified, separated plasma samples were passed into the columns at 1 ml/minute and washed with 3 ml 0.1M formic acid (polar solvent). This was followed by elution with 3 ml 95% methanol / 5% 0.1M formic acid (less polar solvent) and then evaporation in a vacuum centrifuge (Speed Vac Concentrator, Uniscience, London).

The residue was taken up in 500 µl RIA buffer (0.1M Tris acidified to pH 7.4 with HCl and containing 2 g/litre Bovine Serum Albumin) for subsequent assay of AVP and OT in 100 µl duplicates. Extraction yield for AVP, calculated

from extracting ^{125}I -labelled AVP added to plasma, was $94 \pm 2\%$ (mean $\pm$ standard deviation, $n=10$).

2.5 Radioimmunoassay

The principle of radioimmunoassay is the competition between labelled and unlabelled antigen for a limited number of molecules of specific antibody. The amount of labelled antigen that is bound to antibody decreases as the concentration of unlabelled antigen is increased and by comparing the degree of binding with the binding produced by a set of known standards, an estimate of the unknown concentration of antigen can be made. The technique was originally described by Yalow and Berson, 1960 and Ekins, 1960; also Chard, 1982.

All samples, standards and quality controls were aliquoted into LP3 tubes (Luckham Ltd, Dagenham, Essex) using a Microlab dilutor dispenser (Hamilton, Switzerland). Standards were assayed in triplicate and samples in duplicate for all assays. The AVP assay had been validated previously by comparison with a rat biological assay. Serial dilutions of a large sample of rat plasma were diluted in parallel with standard dilutions of synthetic AVP and OT.

AVP Assay

AVP was measured by a highly specific and sensitive RIA using 100 μ l each of 1) An International Standard, National Institute of Biological Standards (NIBS) for AVP, 2) ^{125}I -labelled AVP (3000-4000 cpm) and 3) specific AVP antiserum (A8 from Ferring, Malmo, Sweden or Jo from Dr PH Baylis, Royal Victoria Infirmary, Newcastle) at a final dilution of 1: 1,200,000 for A8 and 1: 300,000 for Jo. Simultaneous addition of sample or standard, antibody and label was incubated at 4°C for 48 hours before counting (see figure 2.1 for representative standard curve) except in the perfusion experiments. Here extra sensitivity was sometimes required to determine low concentrations of AVP and so a 24 hour sample and antibody preincubation period was followed by addition of label and counting 24 hours later. Bound hormone was separated from free hormone by the rapid addition of chilled absolute alcohol, centrifuging and decanting the supernatant. The bound hormone was counted using an automatic Packard Crystal gamma counter. The detection limit for the assay was taken to be the lowest standard distinguishable from zero.

OT Assay

OT was measured by RIA as described by Robinson, 1980 using 100 μ l each of: 1) An International Standard (NIBS) for OT, 2) ^{125}I -labelled OT (3000-4000 cpm), and 3)

Figure 2.1

Standard curve of a representative assay for vasopressin with simultaneous addition of antibody (A8) and label to standard. Each point represents the mean of triplicates $\pm$ standard deviation. Maximum specific binding (B_0) 32.7%, non specific binding 6.5%.

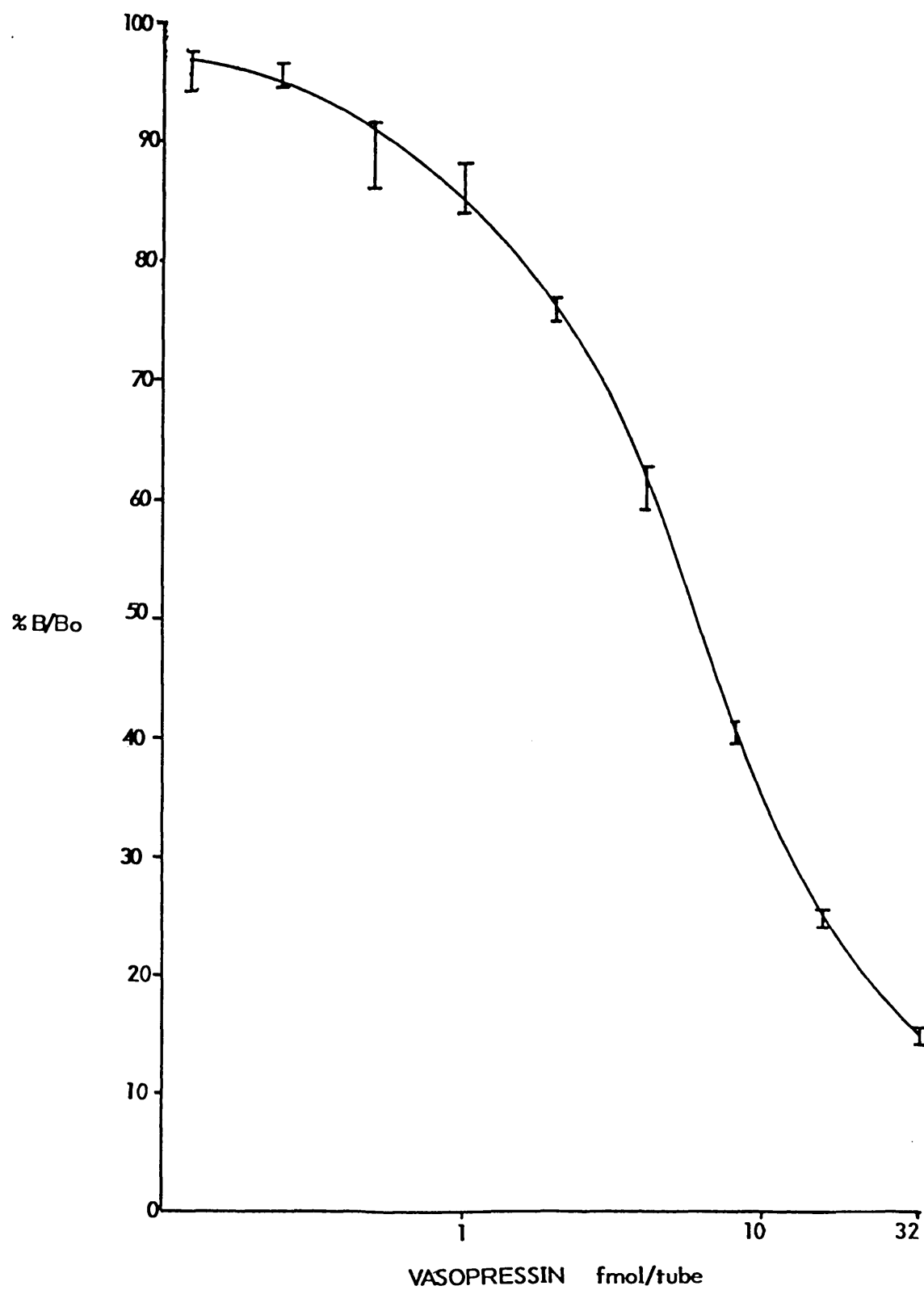
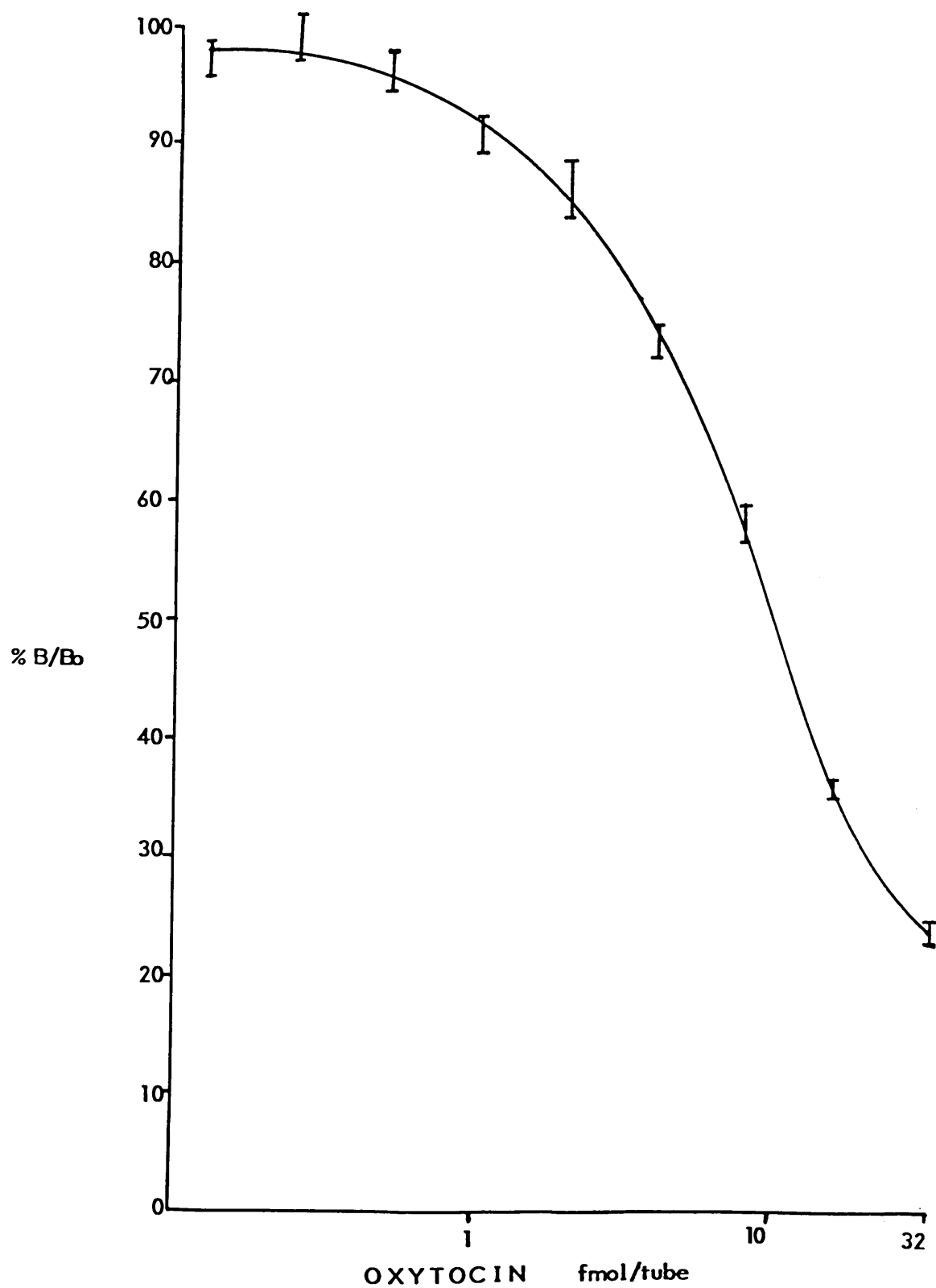


Figure 2.2

Standard curve of a representative assay for oxytocin with simultaneous addition of antibody (RIII5) and label to standard. Each point represents the mean of triplicates $\pm$ standard deviation. Maximum specific binding (Bo) 40.5%, non specific binding 3.3%.



specific OT antiserum (RIII5, ICAF Robinson, National Institute for Medical Research, Mill Hill, London) at a final dilution of 1: 600,000. Simultaneous addition of sample or standard, antibody and label was incubated at 4°C for 48 hours before counting (see figure 2.2 for representative standard curve) except where extra sensitivity was required to measure low concentrations of OT. Twenty four hours of preincubation would then be followed by addition of label and counting 24 hours later. Separation and counting were as above.

ANF assay

1ml samples of either rat or human plasma were extracted as in 2.4 and reconstituted in 500 μ l RIA buffer (0.1M 'phosphate', 0.05M NaCl, 0.1% BSA, 0.1% sodium azide, 0.01% triton X 100 alkalised to pH 7.4 with NaOH. The extraction yield for ANF, calculated from extracting ^{125}I -labelled ANF added to plasma, was $83 \pm 2.0\%$ (mean $\pm$ SE, n=6).

ANF was measured by radioimmunoassay using 100 μ l of: 1) standard atriopeptin 111 (Bachem, Cambridge) - 100% cross reactivity with alpha human ANP; 2) ^{125}I -labelled ANF (8000 cpm, Amersham, Buckinghamshire) and 3) specific ANF antiserum at a working dilution as instructed by Peninsula, St Helens. A 24 hour sample or standard and antibody preincubation period at 4°C was followed by

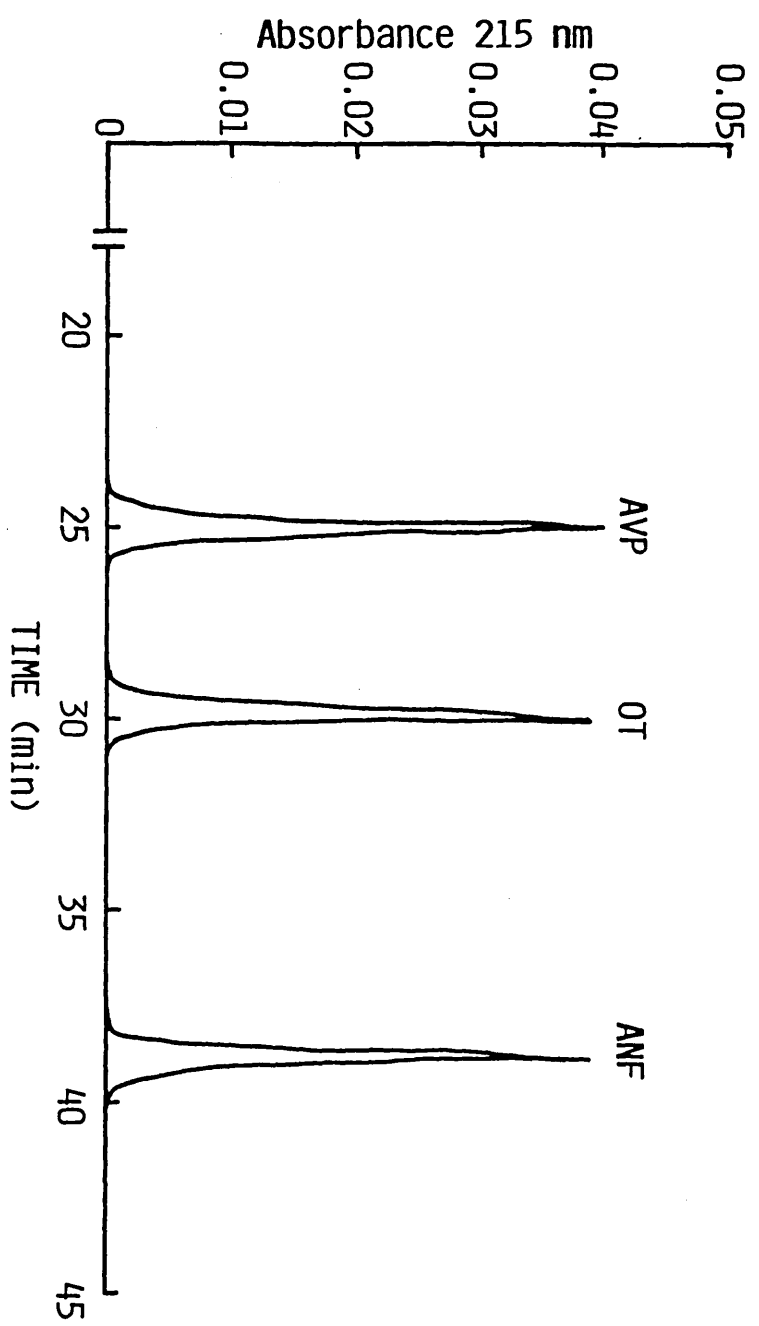
addition of label and counting 24 hours later. Bound and free hormone were separated by adding 250 ul of a solution containing 3% activated charcoal, 0.3% dextran T70; diluted 1:5 with the above RIA buffer. The free hormone was counted using an automatic Packard crystal gamma counter. The minimal detectable concentration in all assays was 4 pg/tube. The coefficient of variation for control ANF samples (62.5 pg) was 6% (intra-assay) and (31.25) 26% interassay.

2.6 High Performance Liquid Chromatography (HPLC)

This technique separates molecules according to their relative affinity for a liquid mobile phase and a solid adsorbent phase. Constituents emerge from the column (stationary phase) at characteristic times after injection. The purity of the atrial natriuretic factor (atriopeptin III₅₋₂₈ Bachem, Cambridge) used in the following experiments was examined by reverse-phase HPLC using a Spectroflow 400 (Kratos Analytical, Manchester) solvent delivery system; Spectroflow 757 absorbance detector (Kratos Analytical) and a octylsilane 5 um particle column (Nucleosil 5 - C₈, Machergy-Nagel, Duren, GFR). The column was equilibrated with a solution of 12% acetonitrile in 0.1% trifluoroacetic acid (TFA) and eluted with a linear gradient of 12-48% acetonitrile containing 0.1% TFA at a flow rate of 1ml/min for 1 hour (Dr S.

Figure 2.3

Reverse-phase high performance liquid chromatography elution profile for 10 μg atriopeptin III (ANF), 10 μg vasopressin (AVP) and 10 μg oxytocin (OT).



Biswas). The chromatographic profile of 10ug atriopeptin III, with 10µg AVP and 10µg OT for comparison, is shown in figure 2.3.

2.7 Perifusion

This is a technique for studying the effect of chemicals, drugs or hormones on isolated tissue perifused with a physiological medium at a constant temperature. The system used is shown in figure 2.4 and is essentially the same as that described by Jessell, 1978.

The perifusion medium was Krebs bicarbonate (2.4M NaCl, 0.5M NaHCO₃, 0.48M KCl, 0.12M MgSO₄ 7H₂O, 0.12M KH₂PO₄, 0.25M CaCl₂ 2H₂O) with glucose (2 g/l) and bovine serum albumin (2.5 g/l) (KRBG) gassed with 95% O₂ / 5% CO₂ (V/V). Bovine Serum Albumin (BSA) was used in the perifusion medium to prevent the absorption of atriopeptin III, AVP or oxytocin to the silicone-rubber tubing. The inner water-bath was maintained at 37°C and the flow rate at 0.2 ml/min. Silicone-rubber tubing (Esco Rubber, Feltham, Middlesex) was used throughout as this is selectively permeable to oxygen and carbon dioxide thus maintaining saturation of the KRBG without mechanical disruption of the tissue by direct gassing of the perifusion chamber. Test substances were introduced into the perifusion system by transferring the inlet tube from the medium reservoir to the test solution. During the

Figure 2.4

Diagrammatic representation of the perfusion apparatus. One chamber only is shown; normally four are used simultaneously in each experiment. A peristaltic pump (502 S, Watson Marlow) and fraction collector (7000 Ultrorac, LKB, Bromma) were used. Krebs bicarbonate with glucose and bovine serum albumin (KRBG).

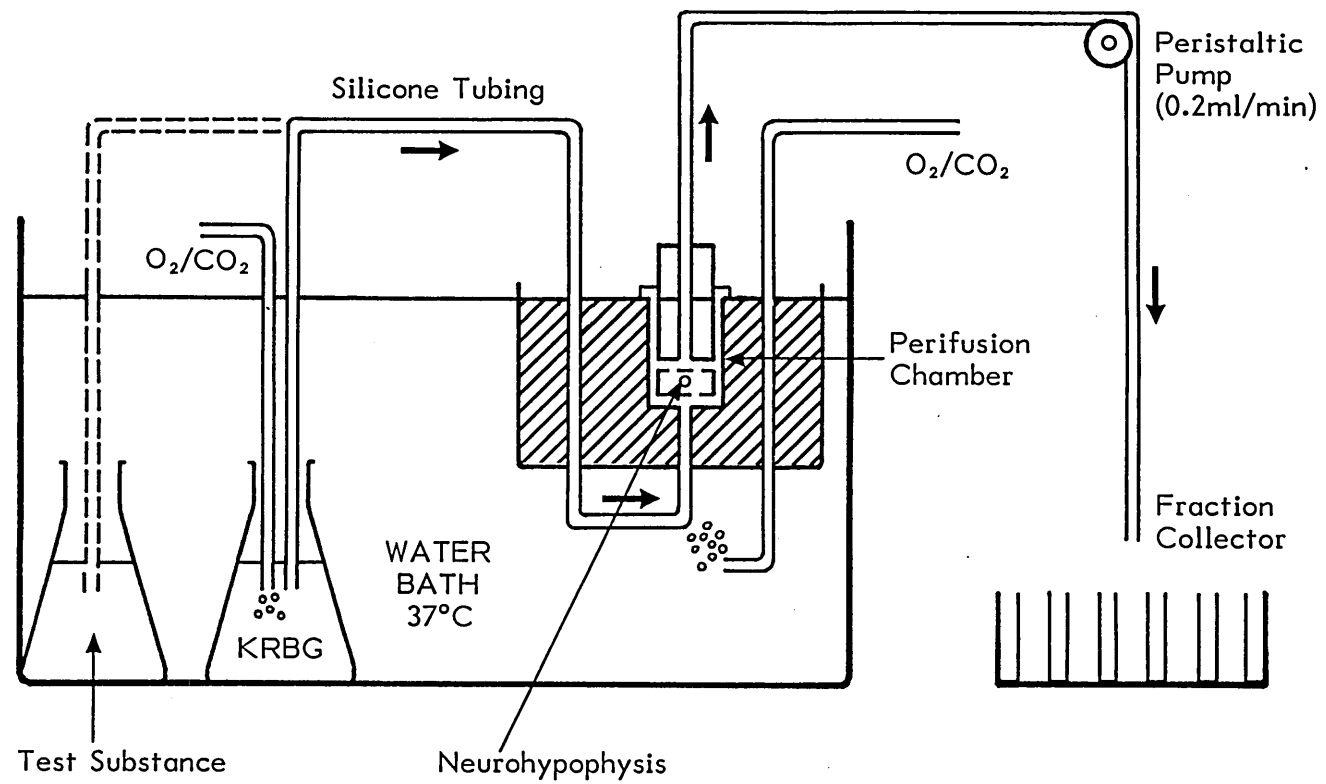
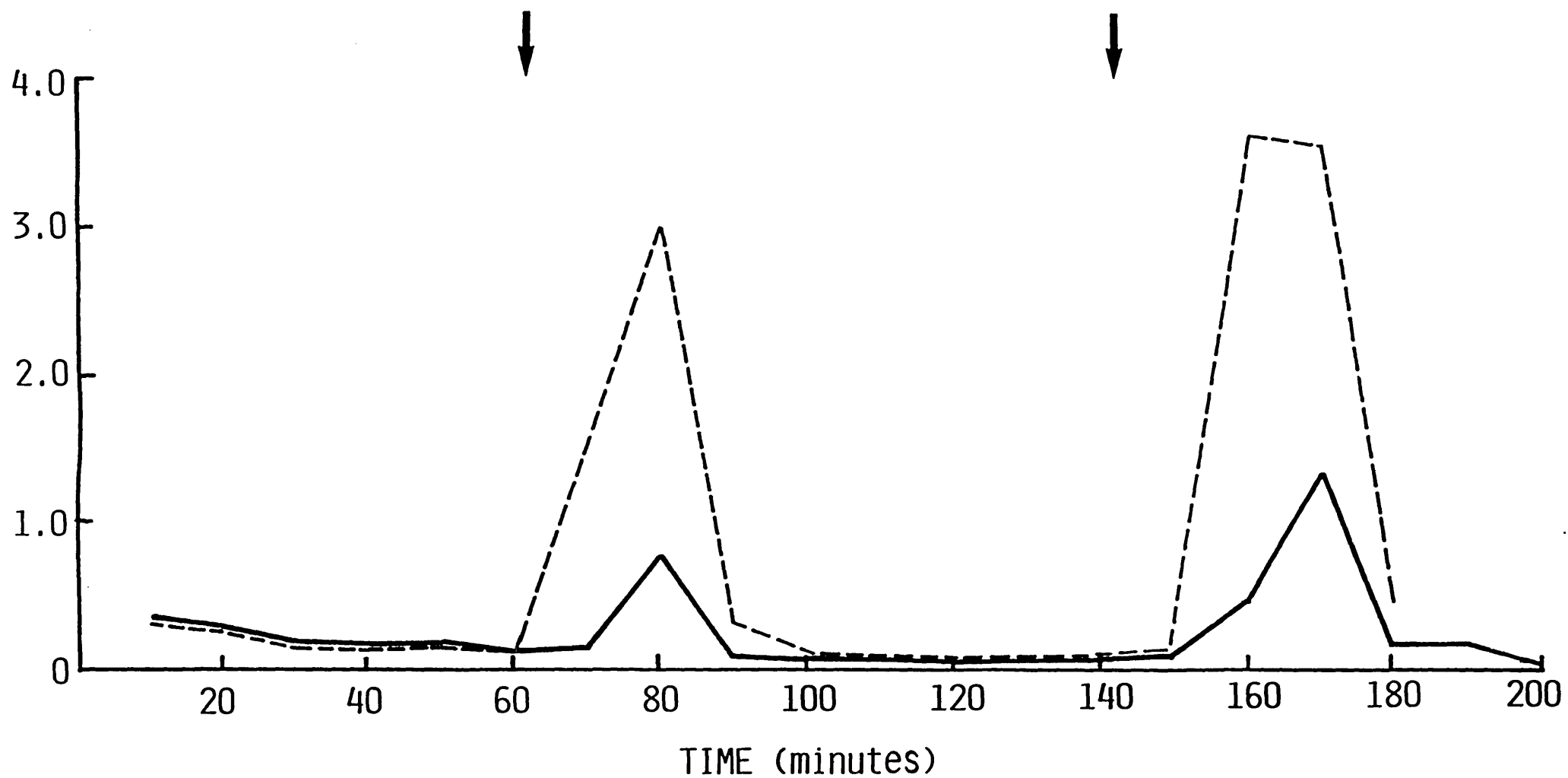


Figure 2.5

Concentrations (pmol/l) of vasopressin (continuous line) and oxytocin (broken line) in 10 minute collections of perfusate from an isolated neurohypophysis stimulated twice for 5 minutes with 25 uM buffered veratrine (arrows).



transfer the pump was halted to prevent air bubbles entering the system. Perfusate samples were collected in LP4 tubes (Luckham Ltd, Dagenham), frozen immediately and stored at -20°C . Pituitary glands for the perfusion were obtained in the following manner. Rats were killed by decapitation, the brain removed from the skull and the pituitary gland excised from the sella turcica. The neurointermediate lobe was picked off with fine forceps, placed in KRBG at 37°C and transferred to one of four perfusion chambers. In all experiments four chambers were perfused simultaneously. The lag time for a test substance to flow through the complete system had been estimated previously in this department (Dr D.A. Carter) and was approximately eight minutes. The pH, pO_2 and pCO_2 had also been estimated previously and found to be suitable for the maintenance of tissue viability. A typical pattern of basal and stimulated release of AVP and OT from the isolated neurohypophysis is shown in figure 2.5. Two 5 minute stimulation periods with either 25 μM veratrine in KRBG or 45 mM K^+ KRBG 80 minutes apart were used. Fractions of perfusate were collected at 10 minute intervals for 3 hours 20 minutes.

2.8 Statistics

Data was assumed to be normally distributed so parametric tests (Students t-test, Duncans multiple range test, two way analysis of variance) were used to determine

significance between groups. An unpaired Students t-test was used for comparison between two groups and Duncans multiple range test for comparisons between multiple groups. Significance was accepted for $p < 0.05$.

CHAPTER 3 STUDIES WITH ATRIAL NATRIURETIC FACTOR
IN ANAESTHETISED RATS

3.1 The Effects of peripherally administered Atrial
Natriuretic Factor on urinary volume and urinary sodium

Introduction

Diuretic, natriuretic and hypotensive properties have been reported after the peripheral infusion of atrial natriuretic factor (ANF) in dogs (Maak et al, 1984) in rats (Atlas et al 1984) and after slow intravenous injection in man (Richards et al, 1985). To confirm that the sample of ANF (atriopeptin III 5-28, Bachem, Cambridge) used in the following experiments was biologically active, experiments were designed to observe its diuretic and natriuretic actions in anaesthetised rats.

Materials and Method

The peptide (1 mg) was dissolved in 1 ml saline (0.9% sodium chloride in distilled water) and stored in 100 μ l aliquots (each containing 100 μ g atriopeptin III) at -20°C. The purity of the peptide was confirmed by reverse phase high-performance liquid chromatography (vide supra).

Adult male Sprague-Dawley rats weighing between 260 and

350 g with free access to food and water were lightly anaesthetised with ether. Whilst semi-conscious a loading dose of tap water (5% body weight) was given with a 10 ml syringe and polythene oro-gastric tube. The animals were then anaesthetised with equithesin (0.45 ml/100 g body weight) and an indwelling cannula (Polythene tubing, Portex, Hythe, Kent) inserted into a jugular vein and a similar cannula inserted suprapubically into the urinary bladder. Hydration was maintained with an intravenous infusion of 5% Dextrose at 6 ml/hour for the duration of the experiment. Twenty minute fractions of urine were collected in weighed LP4 tubes (Luckham Ltd., Dagenham, Essex).

After 3 basal urine collections 0.1 ml rat atriopeptin III (5 µg/kg) or 0.1 ml vehicle (0.9% saline) was given over 10 seconds by intravenous injection into the jugular vein. Urine collections were continued for a further 60 minutes. As each urine sample was passed the volume was determined and then frozen at -20°C for subsequent determination of urinary sodium (Flame Photometer).

Results from atriopeptin III and saline treated rats were compared by Duncan's multiple range test. p values > 0.05 were taken as being non-significant (NS).

Results

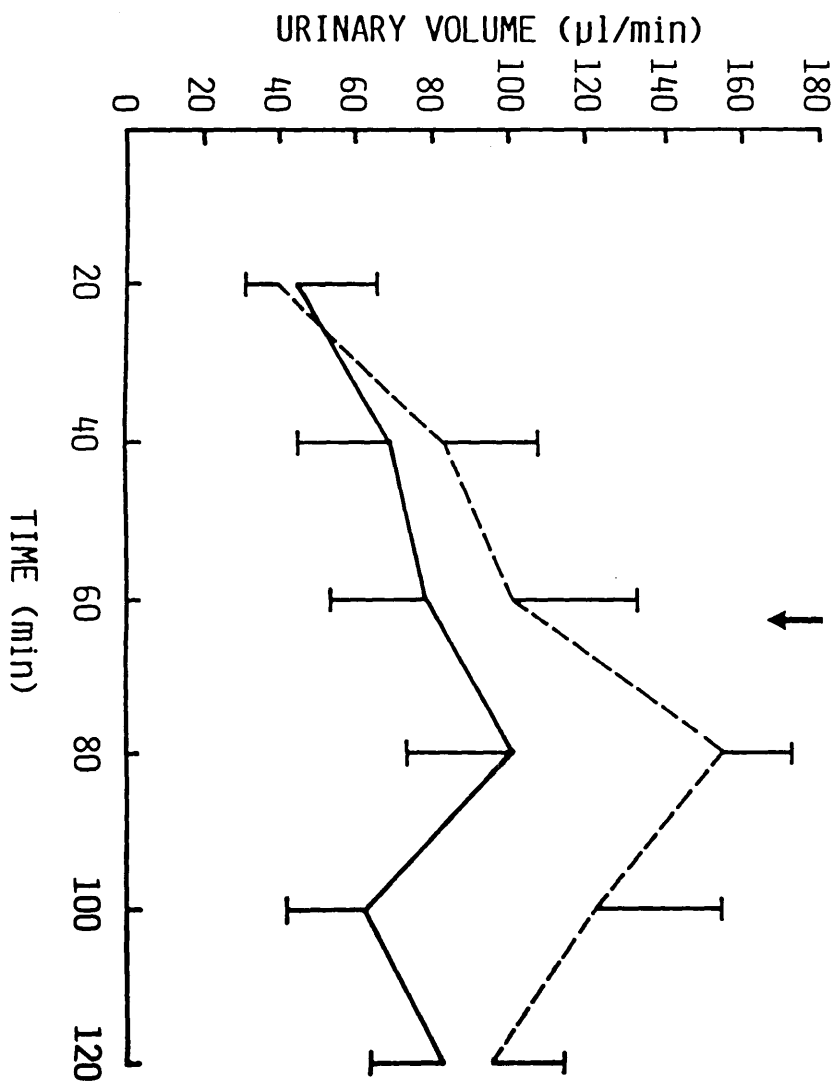
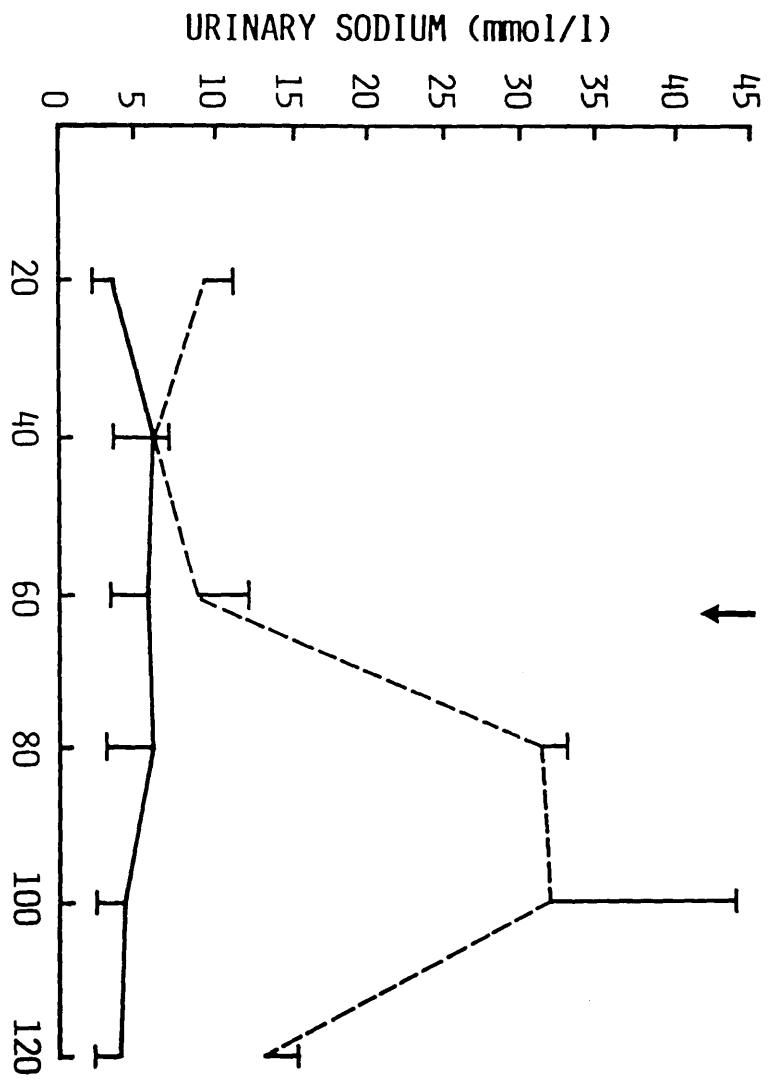
Mean urinary volumes (U_{vol}) rose from a basal of 76.5 ± 22.5 μ l/min to a maximum of 99.0 ± 26.1 μ l/min after 20 minutes in the vehicle treated rats (NS) and from 99.5 ± 32.5 to 154 ± 19.5 μ l/min in the atriopeptin III treated rats ($p < 0.06$) (figure 3.1). Mean urinary sodium (U_{Na}) was 5.75 ± 2.25 mmol/l preceding vehicle treatment and 6.0 ± 2.9 mmol/l 20 minutes later (NS). In the atriopeptin III treated rats however, U_{Na} rose from a basal of 8.8 ± 3.4 mmol/l to 31.3 ± 1.9 after 20 minutes ($p < 0.02$) and 32.0 ± 12.1 after 40 minutes (NS) and 13.4 ± 2.4 mmol/l after 60 minutes ($p < 0.02$) (figure 3.1).

Discussion

The data confirms that our sample of atriopeptin III when given peripherally causes a natriuresis and is therefore biologically active. There was an increase in urinary volume but this did not reach significance. The specificity of action of the peptide was confirmed in a series of experiments by inactivating it with performic acid and observing its effect on the stimulated release of AVP and OT in vivo (chapter 4).

Figure 3.1

Urinary sodium and urinary volume in anaesthetised rats hydrated with 5% dextrose (6 ml/hour). Vehicle (0.9% NaCL) treated rats (continuous line, n=4), atriopeptin III (5 µg/kg) treated rats (broken line, n=5). The timing of intravenous injection with either vehicle or atriopeptin III is shown by the arrow. Mean $\pm$ SEM.



3.2 Studies on urine flow in rats placed in a stereotaxic frame

Introduction

Experiments were designed with the intention of investigating the possible effects of intracerebro-ventricular atriopeptin III in anaesthetised rats, on AVP secretion as monitored by recording urinary output. Using antisera to atriopeptin III, immunoreactivity has been found in the rat brain (vide supra). Heavily stained areas have been found in the anteroventral part of the 3rd ventricle, PV, SO and arcuate nuclei all of which are important areas for the maintenance of fluid and electrolyte balance (Jacobowitz et al 1985; Saper et al, 1985; Skofitsch et al, 1985). In rats deprived of water reductions in immunoreactive ANF have been reported in the neurohypophysis, OVLT, suprachiasmatic and SO nuclei. It has been reported that peripherally administered ANF can inhibit dehydration and haemorrhage-induced AVP release (Samson, 1985a). Ethanol was used as the anaesthetic to produce a good basal urine flow.

Materials and Method

Adult Sprague-Dawley rats weighing between 250 and 300 g with free access to food and water were anaesthetised with ethanol (Forsling, 1968). A loading dose of 15% ethanol

(5% body weight) was given in two doses 30 minutes apart. An indwelling cannula (polythene tubing, Portex, Hythe, Kent) was inserted into a jugular vein and a similar cannula suprapubically into the urinary bladder. Hydration was maintained with an infusion of 1.5% ethanol in 0.4% saline at 6 ml/hour via the jugular cannula for the duration of the experiment. Fifteen minute fractions of urine were collected in weighed LP4 tubes (Luckham Ltd., Dagenham, Essex).

After 3 basal collections of urine with the animal supine on the bench it was transferred to a Stoelting stereotaxic frame where the ear bars were inserted, in the usual way, in preparation for the drilling of a burr hole and injection into the 3rd ventricle. These experiments were repeated at a later date with homozygous Brattleboro (AVP deficient) rats.

Results

Urine volume (U_{vol}) in supine Sprague-Dawley rats rose from 80.7 ± 22.7 to 104.7 ± 12.0 $\mu\text{l}/\text{min}$ 105 minutes later (figure 3.2). Urine volume in the Sprague-Dawley's placed in the stereotaxic frame fell from 104.0 ± 11.3 to 44.7 ± 11.3 $\mu\text{l}/\text{min}$ within 15 minutes of insertion of the ear bars and to 4.7 ± 0.7 $\mu\text{l}/\text{min}$ within 30 minutes. Urine flow was seen to fall within seconds of inserting the ear bars. Urine volume in the Brattleboro rats fell from 80.0 ± 13.3

to 10.7 ± 8.7 $\mu\text{l}/\text{min}$ within 15 minutes of inserting the ear bars (table).

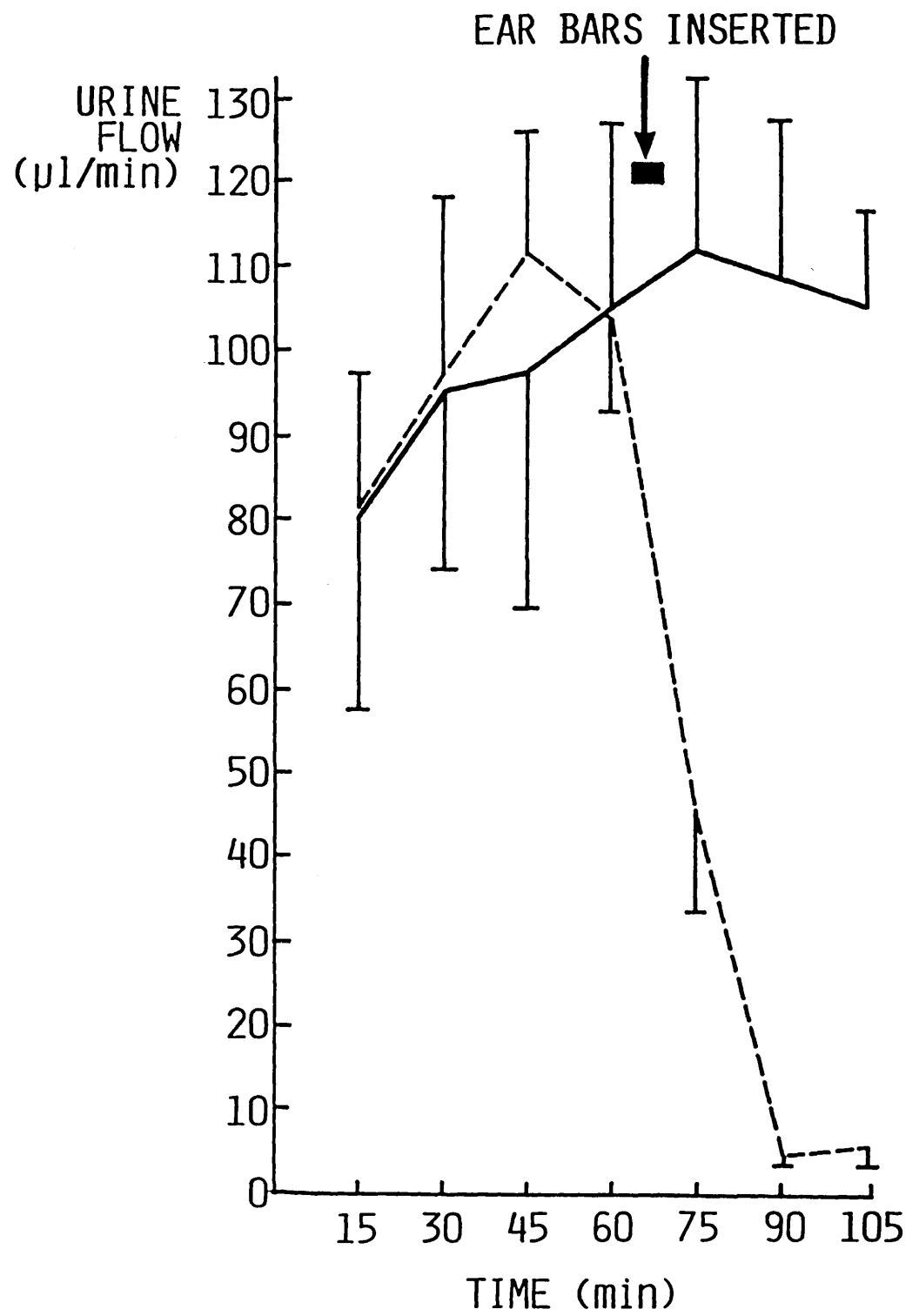
Discussion

These experiments show that insertion of ear bars into the ethanol anaesthetised rat causes an abrupt reduction in the rate of flow of urine. However unknown to us at the time, this is a well known effect. The mechanism for this observation is unknown, however it is interesting that there was a similar rapid and marked fall in urinary volume in Brattleboro (AVP deficient) rats on insertion of ear bars. This provides strong evidence that this observation is not due to secretion of AVP.

In view of the problems associated with the investigation of AVP secretion in anaesthetised rats in stereotaxic frames, further studies on the central control of AVP release were performed using conscious rats (chapter 4). However it was possible to use this technique to observe the effects of centrally administered atriopeptin III on mean arterial pressure and heart rate, but facilities were not available to study urine flow in conscious rats.

Figure 3.2

Urine flow rates in ethanol anaesthetised rats lying supine on a bench (continuous line, n=4) or prone in a stereotaxic frame (broken line, n=5). The point at which the ear bars were inserted is shown. Mean $\pm$ SEM.



TABLE

Brattleboro rats no.1 no.2 no.3 no.4 Mean SEM

Time (min)	U vol (ml)				ul/min	
0 - 15	0.5	1.4	1.0	0.7	60.0	11.3
15- 30	1.3	1.7	1.3	1.3	93.3	6.0
30- 45	1.1	0.9	0.8	1.8	80.0	13.3
ear bars						
inserted						
45- 60	0.6	0.03	0.0	0.0	10.7	8.7
60- 75	0.5	0.05	0.19	0.0	12.7	6.7
75- 90	0.5	0.32	0.15	0.09	18.0	5.3

3.3 The effects of centrally administered Atrial Natriuretic Factor on Heart Rate and Mean Arterial Blood Pressure

Introduction

Peripherally administered atriopeptin III (24 amino acids) causes hypotension in conscious dogs (Maak et al, 1984) and peripherally administered synthetic human alpha-ANF (28 amino acids) causes hypotension in conscious man (Richards et al, 1985). Immunoreactive ANF has been found in the anteroventral third ventricle (as noted above) a region not only involved with fluid and electrolyte balance, but with the development and maintenance of experimental hypertension (Brody et al, 1980). Experiments were designed to investigate the effects of intracerebroventricularly (ICV) administered rat atriopeptin III on heart rate (HR) and mean arterial pressure (MAP) in anaesthetised rats.

Material and method

An indwelling cannula, internal diameter 0.58 mm, external diameter 0.96 mm (polythene tubing, Portex, Hythe, Kent) was inserted into a carotid artery of adult male Sprague-Dawley rats (250 to 320 g) under urethane anaesthesia (0.2 ml/100g). 3 µl of vehicle (0.9% saline) or either 0.2, 2.0, 20 nmol atriopeptin III were injected

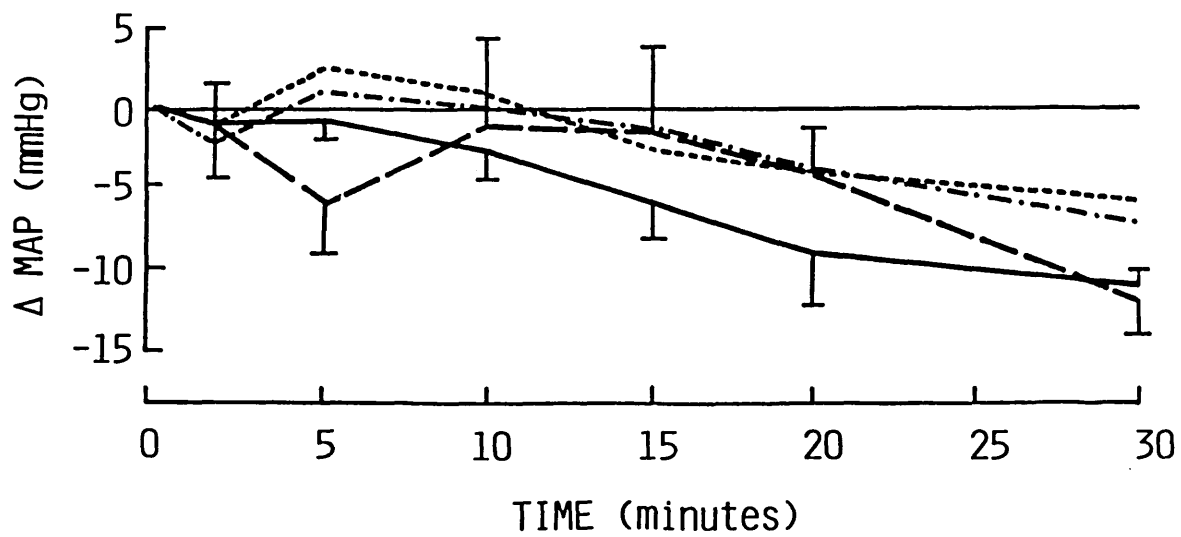
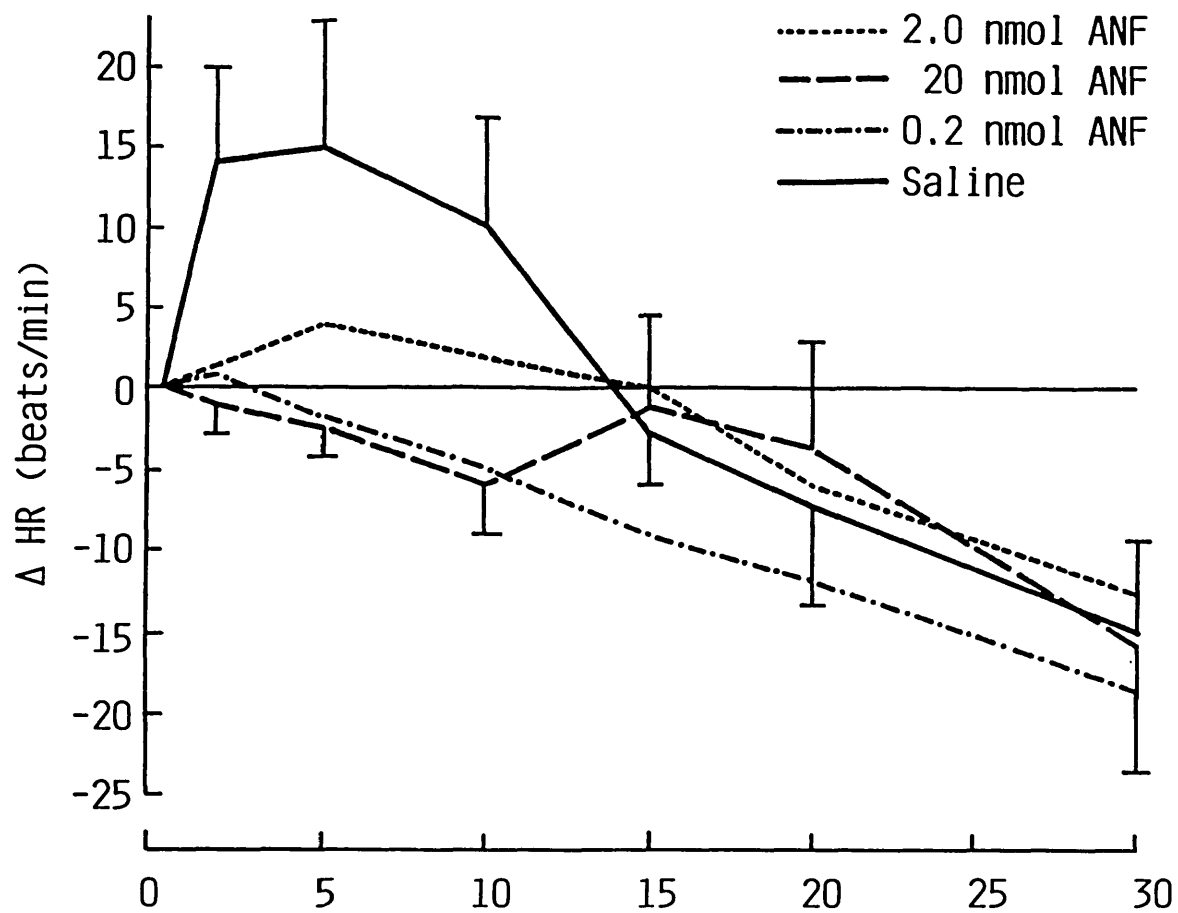
slowly (2-3 secs) into the 3rd ventricle via a glass micropipette placed according to stereotaxic co-ordinates (for details see methodology). Injections (Hamilton Microliter Syringe) were given 30 minutes after surgery and the micropipette left in situ for the duration of the experiment. Arterial blood pressure and heart rate were recorded continuously via a Statham transducer (P23AC) on a Rikadenki recorder (R-OX) before and for 30 minutes after ICV injection. Results for atriopeptin III and saline treated rats were compared by Duncan's multiple range test. p values ≥ 0.05 were taken as being non significant (NS).

Results

The basal MAP and HR of the rats were 99.6 ± 2.7 mm Hg and 379 ± 10.1 beats/min respectively ($n=24$). Mean changes in HR and MAP are shown in figure 3.3. For clarity only the SEMs for the vehicle and 20 nmol atriopeptin III treated rats are shown. There was no significant difference in HR or MAP between the vehicle and atriopeptin III treated rats at 5, 10, 15, 20 or 30 minutes after ICV injection. For the first 10 minutes HR increased in the vehicle treated animals but not in the atriopeptin III treated animals (NS). Heart rate and MAP fell similarly in all groups of rats during the course of the experiment.

Figure 3.3

Change in heart rate (HR) and Mean Arterial Pressure (MAP) after intracerebroventricular administration of either vehicle (0.9% saline) or atriopeptin III (0.2, 2.0, 20 nmol). Numbers of rats were between 5 and 7 in each treatment group. Mean $\pm$ SEM.



Discussion

Centrally administered ANF had no effect on HR or MAP in the urethane anaesthetised rat. This is in keeping with the findings of Iitake et al (1986) who reported no effect on MAP in conscious rats after ICV injection of atriopeptin III. Bonham et al (1983) reported in abstract form, no effect on MAP and HR in conscious intact rats after ICV injection of unspecified ANF; in rats that were sino-aortic baroreceptor denervated however they observed a dose-dependent increase in arterial pressure (actual dose not given), suggesting that the baroreflex normally buffers a pressor response when ANF is centrally administered. Unfortunately HR, an indicator of baroreceptor activation, was not reported in these experiments. Sills et al (1985) have reported the effects of microinjections of atriopeptin III into the preoptic suprachiasmatic nucleus which is located in the AV3V region (see figure 1.2). Doses in the pmol range produced a dose dependent increase in MAP and HR within 15-45 minutes of injection; lasting 60-150 minutes suggesting that this effect is mediated via a metabolite of atriopeptin III or, via the synthesis of another peptide.

In my own studies there was a trend for HR to increase in the vehicle treated animals but not in the atriopeptin III treated animals during the first 10 minutes after ICV injection of atriopeptin III (although this was not

statistically significant). A fall in MAP with no change in HR during an intravenous infusion of atriopeptin III was noted by Maack et al, 1984 in their experiments with conscious dogs. Metzler et al, 1985 made a similar observation with dogs but Richards et al, 1985 using a bolus injection of human alpha-ANP in man, reported a rise in HR associated with a fall in MAP. A fall in blood pressure is normally associated with baroreceptor activation and increased HR. These observations indicate that the effect of ANF on HR is variable and that atriopeptin III may have an inhibitory effect on the baroreflex in some species. In the absence of significant central effects, experiments to investigate the peripheral effects of ANF on MAP and HR were not performed.

Chapter 4 THE EFFECTS OF CENTRALLY ADMINISTERED ANF ON
THE STIMULATED IN VIVO RELEASE OF AVP AND
OXYTOCIN IN THE RAT

4.1 Introduction

In addition to the cardiac atria, immunoreactive ANF has been found in the rat brain (chapter 1.5). ANF-positive fibres and cell bodies have been demonstrated in areas important for the maintenance of fluid and electrolyte balance (Jacobowitz et al, 1985; Saper et al, 1985a; Skofitsch et al, 1985). These areas include the PV and SO nuclei of the hypothalamus, ant^eroventral region of the of the 3rd ventricle (AV3V), neurohypophysis and tegmentum of the pons. At the tip of the 3rd ventricle is the organum vasculosum of the lamina terminalis, one of the circumventricular organs, a structure which acts as a "window" in the blood-brain barrier allowing free exchange of substances in either direction. The ANF immunoreactive neurons here probably enjoy relatively direct access to the circulation and could respond to alterations in blood composition. An ANF immunoreactive pathway has been demonstrated running from the AV3V to the magnocellular areas of the PV and SO nuclei (Saper et al, 1985b) and this pathway is ideally situated to respond to changes in blood osmolality with a resultant change in neurohypophyseal hormone secretion. ¹²⁵I ANF binding sites have also been reported in the rat brain and

neurohypophysis (Quirion et al, 1984 and 1986).

These binding sites are particularly dense in two other circumventricular organs: the subfornical organ and the area postrema as well as the nucleus tractus solitarius; all of which could be relevant to the potential actions of ANF in electrolyte and water homeostasis.

The central role of ANF is unknown but recent findings indicate that it may function in the control of AVP release (Januszewicz et al, 1985a; Samson, 1985a). However the specificity of the neuroendocrine effects of ANF has not been studied. The relative importance of actions at the hypothalamic and pituitary levels is also unknown. In this chapter the central effects of ANF on both AVP and oxytocin (OT) release are addressed in the conscious rat.

4.2 Material and Methods

Effect of ANF on AVP and OT release in conscious rats

An indwelling cannula, internal diameter 0.40mm, external diameter 0.80mm (Polythene tubing, Portex, Hythe, Kent) was implanted into the lateral ventricle of adult male Sprague-Dawley rats (250 to 320 g) under freshly made equithesin anaesthesia (0.45 ml/100 g). Cannulae were sited using a stereotaxic frame according to co-ordinates (for technique see chapter 2.3) and fixed in position with

dental cement and brass screws. The following day one group of rats was given tap water and another group 2% NaCL (w/v) to drink. Three days later 5 μ l of vehicle (0.9% saline) or either 0.2, 2.0 or 20 nmol atriopeptin III (Bachem, Cambridge, UK) were injected ICV (Hamilton Microliter Syringe) into the lightly restrained conscious animal. Rats were decapitated 10 minutes after ICV injection. Other rats who had been given 2% NaCL to drink were injected with vehicle or 20 nmol atriopeptin III ICV and decapitated 5, 20, 40 and 80 minutes after injection. Trunk blood was collected in chilled heparinised tubes and the separated plasma was stored at -20°C.

The specificity of action of atriopeptin III was investigated using inactivated peptide. This was done by oxidation with performic acid to cleave the disulphide linkage (Hirs, 1967). Previous studies have shown that the disulphide linkage is essential for biological activity (Misono et al, 1984). Rats were prepared for ICV injection and given 2% NaCL. After 3 days 5 μ l of either oxidized atriopeptin III (20 nmol) or vehicle were injected and trunk blood collected as above.

In a further experiment the possibility that an ICV bolus dose as large as 20 nmol may have crossed the blood brain barrier, was investigated by preparing two groups of rats for ICV injection as above. After 3 days, with the rats drinking tap water, 5 μ l of either 20 nmol atriopeptin III

or vehicle were injected ICV and trunk blood collected 5 minutes later for subsequent ANF assay.

Effect of ANF on AVP and OT release in conscious haemorrhaged rats

A cannula, was implanted into the lateral ventricle of adult male Sprague-Dawley rats as above. Two days later an indwelling cannula, internal diameter 0.58 mm, external diameter 0.96 mm (Polythene tubing, Portex, Hythe) was inserted into a carotid artery under equithesin anaesthesia (0.45 ml/100 g). The was firmly secured with 3/0 silk sutures and passed subcutaneously to project from the dorsum of the neck. Patency of the cannula was maintained with 40% polyvinylpyrrolidone in 0.9% (w/v) NaCl containing 50 iu/ml heparin. The next day rats were haemorrhaged (1 ml/100g body weight) 10 minutes after a 5 µl icv injection of either vehicle or 5 µl 20 nmol atriopeptin III whilst lightly restrained. This dose was used on the basis of the results from the previous experiments with saline drinking rats (p 63-64). Rats were decapitated 10 minutes after haemorrhage. Control rats were not haemorrhaged but injected ICV with either 5 µl vehicle or 5 µl 20 nmol atriopeptin III and decapitated 20 minutes later. Trunk blood was collected and separated as above.

Radioimmunoassay

Fractions of plasma were assayed for OT and AVP by direct radioimmunoassay (for details see chapter 2.5). The coefficient of variation for control AVP samples (2 fmol) was 6% (intra-assay) and 16% (interassay). The coefficient of variation for control OT samples (2 fmol) was 6% (intra-assay) and 14% (interassay). The detection limit for both assays was 0.25 fmol/tube.

Data Analysis and Statistics

Results were expressed as the mean $\pm$ SEM. Statistical analysis was performed using Duncan's multiple range test or unpaired t tests.

4.3 Results

Effect of ANF on AVP and OT release in conscious rats

In rats drinking tap water plasma levels of AVP and OT 10 minutes after vehicle ICV were 7.9 ± 1.3 and 8.1 ± 1.3 pmol/l respectively. Atriopeptin III (0.2, 2.0 and 20 nmol) ICV had no significant effect on plasma concentrations of AVP or OT (table 4.1).

In animals drinking 2% NaCl plasma levels of AVP and OT 10 minutes after vehicle ICV were 29.3 ± 0.7 and 23.9 ± 3.2

pmol/l respectively (figure 4.1). In the group treated with 20 nmol atriopeptin III ICV the concentrations of AVP and OT were reduced compared with the vehicle treated group but no difference was found in the groups treated with 0.2 and 2.0 nmol atriopeptin III. Following 20 nmol atriopeptin III plasma concentrations of AVP were significantly reduced for 40 minutes whereas concentrations of OT were reduced for 10 minutes only (figure 4.2). There was no significant change in the plasma concentrations of AVP and OT 5 minutes after ICV injection.

Inactivated atriopeptin III (20 nmol) did not significantly modify plasma concentrations of AVP or OT following ICV injection into rats drinking 2% NaCl. Plasma concentrations of AVP and OT in vehicle injected rats were 27.7 ± 2.1 and 39.8 ± 4.2 pmol/l respectively, and in inactivated atriopeptin III injected rats 25.6 ± 2.5 and 30.2 ± 3.6 pmol/l (no significant difference between groups, unpaired t test, n=6 in each group).

The mean concentration of plasma atriopeptin III 5 minutes after ICV injection of vehicle and 20 nmol atriopeptin III was 55.2 ± 20.7 and 201.0 ± 28.8 pmol/l respectively ($p < 0.01$, unpaired t test) (table).

Table 4.1

Rats	5µl inj.	Plasma	Plasma
drinking	ICV of	AVP	OT
tap water		(pmol/l)	(pmol/l)

1	0.9% NaCL	12.2	12.5
2	"	9.4	9.0
3	"	6.4	5.8
4	"	5.9	5.8
5	"	5.4	7.3
6	0.2 nmol ANF	8.6	9.3
7	"	6.9	7.5
8	"	6.4	9.0
9	"	8.8	18.0
10	"	8.8	15.8
11	2.0 nmol ANF	6.5	16.8
12	"	11.6	8.3
13	"	6.8	7.4
14	"	6.0	11.0
15	"	10.0	6.2
16	"	10.6	5.9
17	20 nmol ANF	6.9	7.3
18	"	4.1	10.0
19	"	5.3	4.8
20	"	6.4	9.0
21	"	6.1	7.8

Figure 4.1

Plasma concentrations of a) AVP and b) OT in conscious rats drinking 2% NaCl 10 minutes after the ICV administration of either vehicle or atriopeptin III (0.2, 2.0 and 20 nmol). The group size is indicated within the bars. Mean $\pm$ SEM. * $p < 0.05$ compared with vehicle treatment.

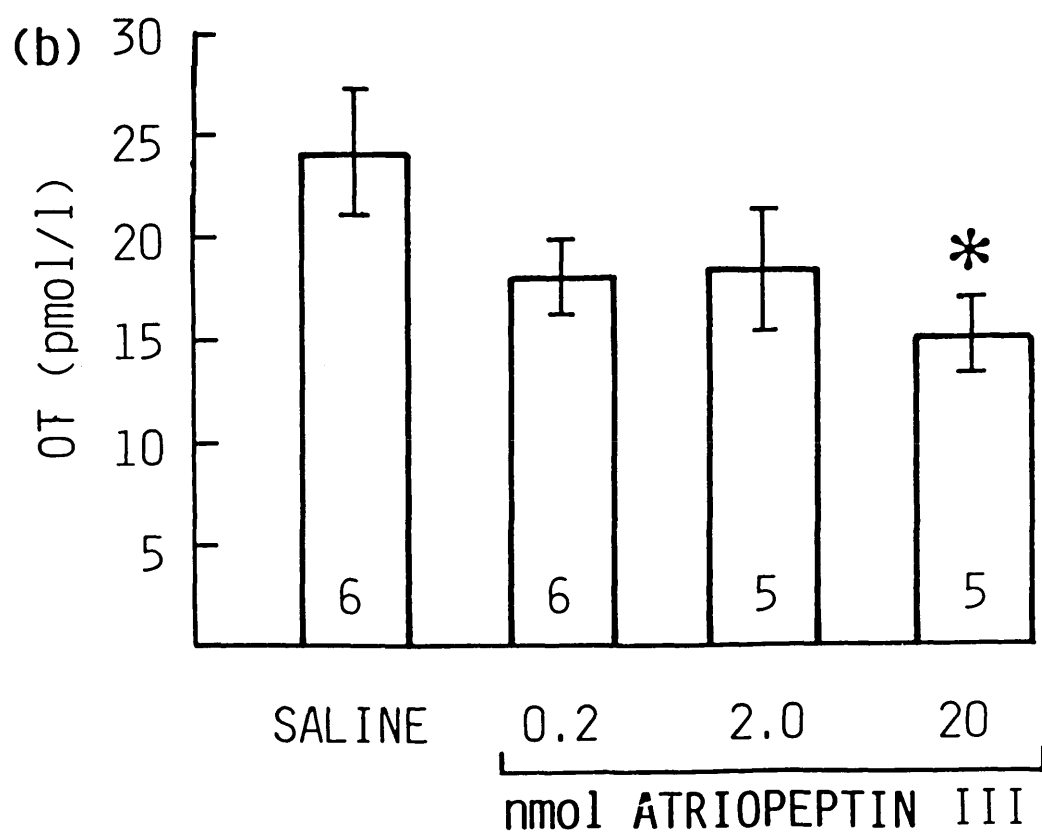
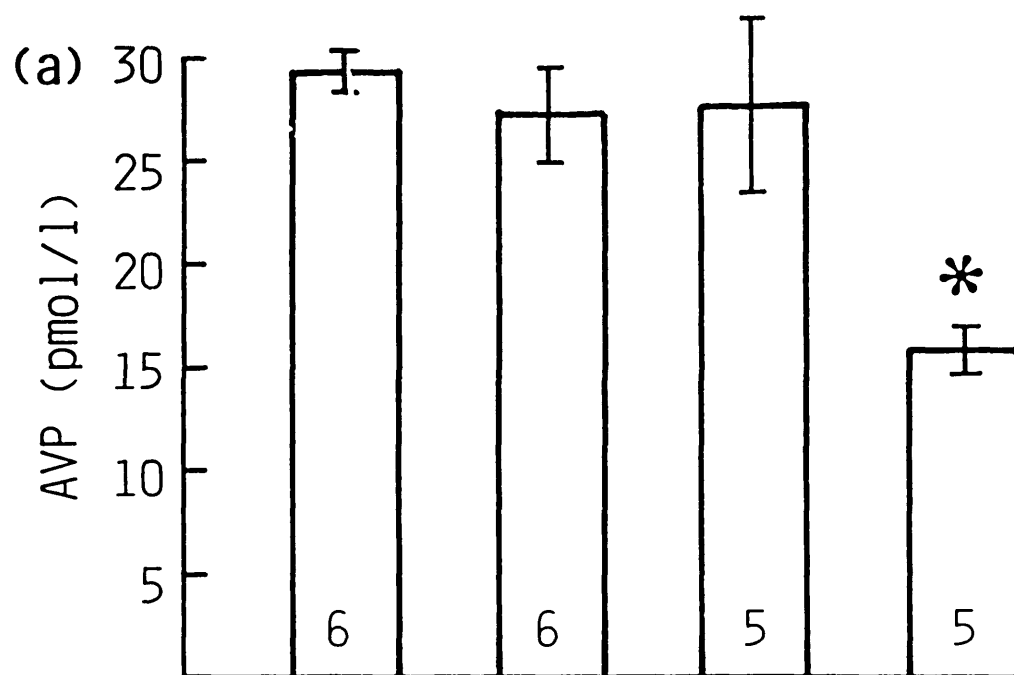


Figure 4.2

Plasma concentrations of a) AVP and b) OT in conscious 2% NaCL drinking rats after ICV administration of either vehicle (0.9% NaCL) (closed circles) or 20 nmol atriopeptin III (open circles). Number of rats between 5 and 7 in all groups. Mean $\pm$ SEM. * $p < 0.05$ compared with vehicle treatment.

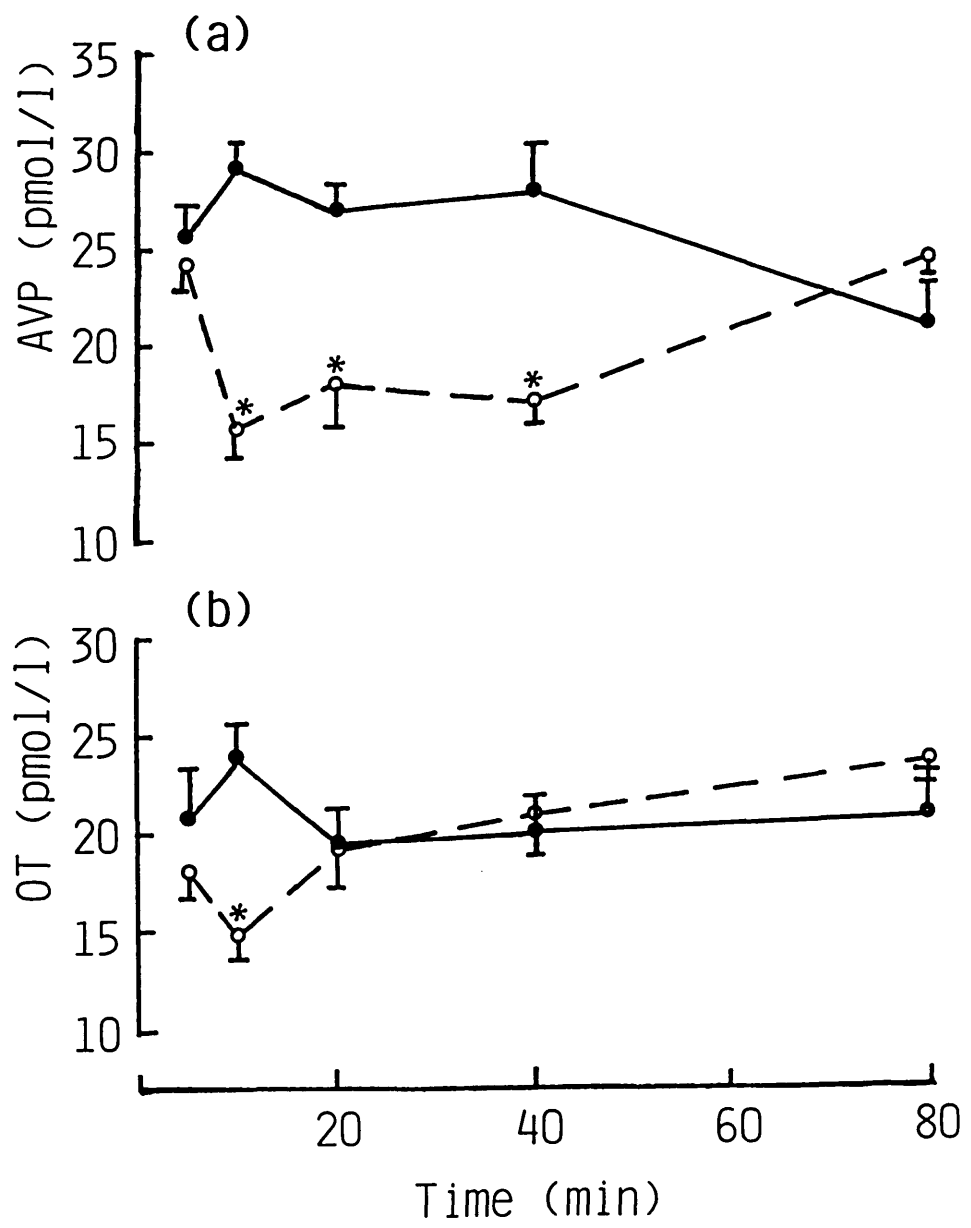


Figure 4.3

Plasma concentrations of a) AVP and b) OT in conscious haemorrhaged (hatched bars) and non haemorrhaged (open bars) rats after the ICV administration of either vehicle (0.9% NaCL) or 20 nmol atriopeptin III. The group size is indicated within the bars. Mean $\pm$ SEM. * $p < 0.01$ ** $p < 0.025$ compared with vehicle treated rats (unpaired t test).

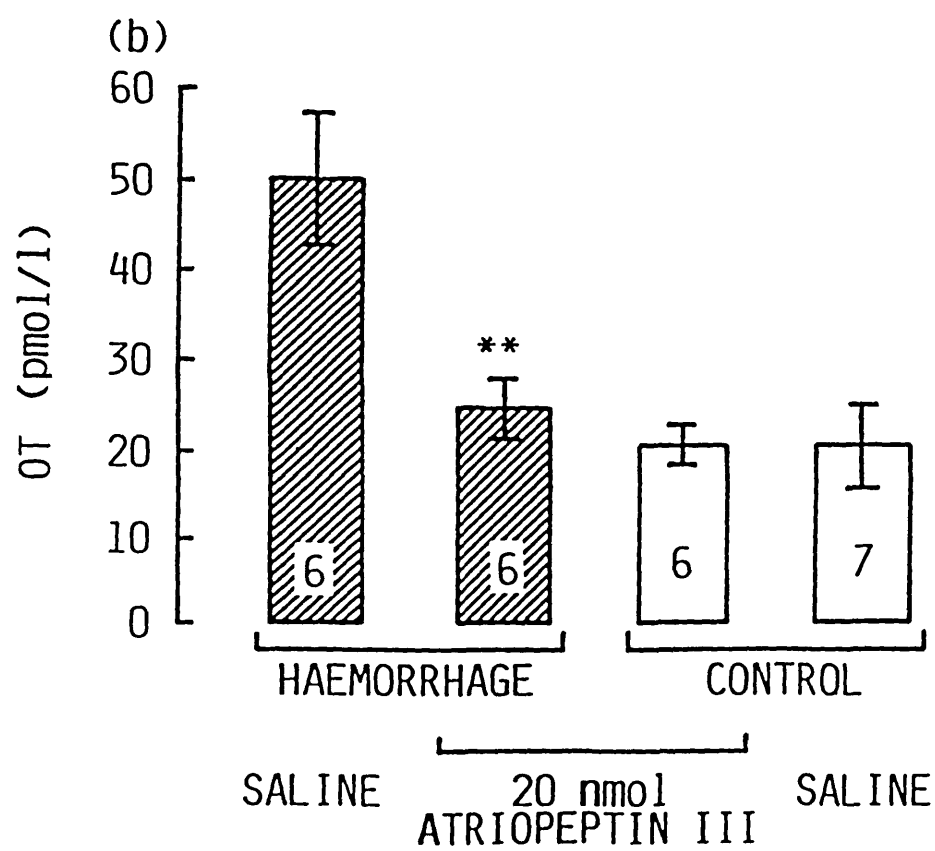
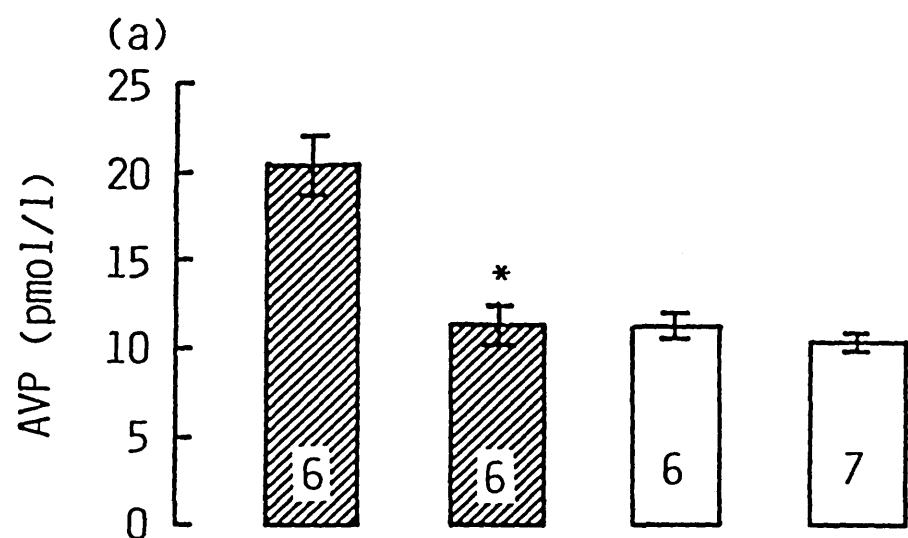


TABLE 4.2

	Rats drinking Tap water	Plasma atriopeptin III (pmol/l)
20 nmol atriopeptin III ICV	1 2 3 4 5 6	105.8 180.4 214.2 148.2 266.6 291.0
Mean $\pm$ SEM		201.0 $\pm$ 28.8
5 μ l 0.9% NaCL ICV	7 8 9 10 11 12	158.0 38.6 39.9 39.2 23.2 32.2
Mean $\pm$ SEM		55.2 $\pm$ 20.7

Effect of ANF on haemorrhage induced release of AVP and OT in conscious rats

In control rats plasma concentrations of AVP and OT after 5 μ l vehicle ICV were 10.7 ± 0.8 and 20.6 ± 4.7 pmol/l respectively (figure 4.3). In control rats plasma concentrations of AVP and OT after 5 μ l 20 nmol atriopeptin III were 12.5 ± 0.7 and 20.6 ± 2.0 pmol/l respectively (no significant difference between vehicle and atriopeptin III treated groups, unpaired t test).

Following haemorrhage plasma concentrations of AVP and OT in the vehicle injected groups were significantly elevated compared with the equivalent non-haemorrhaged controls ($p < 0.01$ in both cases). In the atriopeptin III injected group, plasma concentrations of AVP and OT after haemorrhage were not significantly different to the equivalent control groups. Plasma concentrations of AVP and OT in the atriopeptin III injected haemorrhaged rats were significantly lower than in the vehicle injected haemorrhaged rats (AVP $p < 0.025$; OT $p < 0.01$).

Discussion

These experiments show that ICV injection of atriopeptin III inhibits the stimulated release of both AVP and OT in conscious rats after either substituting their water with 2% NaCl or after haemorrhage. Iitake et al (1986) have

recently reported a significant reduction in plasma AVP concentration from 5 to 30 minutes after similar doses of ICV ANF in normally hydrated and dehydrated rats but there was no data for OT secretion. Angiotensin II or dehydration stimulated water intake has been reported to be significantly reduced by comparable doses of ANF both ICV and intravenously in rats (Antunes-Rodrigues et al, 1985; Nakamura et al, 1985). Since ANF immunoreactivity (Jacobowitz et al, 1985; Saper et al, 1985a; Skofitsch et al, 1985) and ANF binding sites (Quirion et al, 1984 and 1986) have been localised in brain areas involved in the control of neurohypophyseal function these results indicate that a central or pituitary site of action may be involved in a functional role for ANF in the control of AVP and OT secretion.

Samson (1985a) has reported the effect of intravenous infusion of atriopeptin III on plasma AVP concentrations in conscious dehydrated and haemorrhaged rats. He found that doses as low as 0.2 and 2.0 nmol atriopeptin III inhibited AVP release in dehydrated and haemorrhaged rats suggesting a pituitary site of action for ANF in the control of AVP secretion. Evidence in support of a central role for ANF has come also from the demonstration of an ANF immunoreactive pathway running from the AV3V to the magnocellular areas of the PV and SO nuclei (Saper et al, 1985b) and the demonstration of a reduction in immunoreactive ANF in response to dehydration in areas of the

brain involved in salt and water homeostasis (Samson 1985c).

In these experiments both osmotic stimulation and haemorrhage resulted in release of AVP and OT. In cats and goats a selective release of AVP is the usual response to osmotic stimulation (Bisset, 1985; Thornton et al. 1986). With regards to haemorrhage, Fabian et al. 1969 reported release of AVP and OT into the circulation of rats when blood loss exceeded 40% of total blood volume. Ginsburg & Smith, 1959 found a selective increase in plasma antidiuretic activity in response to haemorrhage in rats, but only 4 animals were studied. As discussed by Fabian et al. (1969) there appears to be a marked variation in neurohypophyseal hormone release between animals as well as between species.

This study has shown that centrally administered atriopeptin III inhibits the stimulated release of both AVP and OT. However, only a high dose of atriopeptin III (20 nmol bolus ICV) was effective; a dose in the same order of magnitude as that used by Samson 1985b (2 nmol infused ICV) which suppressed basal secretion of AVP. The active concentration of ANF reaching the relevant receptor sites in these experiments is of course unknown. Some of the 20 nmol ICV bolus dose did cross the blood brain barrier and it is possible that the atriopeptin III acted peripherally, at the level of the pituitary, to inhibit

the release of AVP and OT. Unlike Samson, I did not find any significant effect of atriopeptin III on basal concentrations of AVP or OT possibly due to differences in experimental design, such as the use of ICV injection compared with ICV infusion of peptide.

In conclusion, I have demonstrated that atriopeptin III inhibits the stimulated release of both AVP and OT in vivo. The effects were similar on both AVP and OT, although a degree of selectivity in the duration of action was evident following central administration of ANF in rats drinking 2% saline.

CHAPTER 5 THE EFFECT OF ANF ON THE STIMULATED RELEASE OF VASOPRESSIN AND OXYTOCIN IN VITRO

5.1 Introduction

It has been reported previously that ANF stimulates the release of AVP in an in vitro system (Januszewicz et al, 1985a). This contrasts with the results of chapter 4 and those of Samson (1985a and 1985b) and more recently Iitake et al, 1986 who reported inhibition of AVP release by ANF. So this chapter addresses the effect of ANF on the stimulated release of AVP and OT from the isolated neuro-intermediate lobe of the rat.

5.2 Materials and Methods

Adult male Sprague-Dawley rats (250 to 320 g) were killed by decapitation and the neuro-intermediate lobes dissected out. The lobes were transferred to individual chambers in a perfusion system and perfused with Krebs bicarbonate solution containing glucose and bovine serum albumin (KRBG) (for details see chapter 2.7). After pre-incubation for 30 minutes, fractions of perfusate were collected at 10 minute intervals for 3 hours 20 minutes. Two 5 minute stimulation periods with either a) 45 mM K^+ KRBG or b) 25 μ M veratrine (Sigma, Bournemouth, UK) 80 minutes apart were introduced. Atriopeptin III (0.001, 0.1 and 10 nM) was added to the medium for 40 minutes starting 20 minutes

before stimulation. In all experiments, half of the pituitaries had atriopeptin III added to the medium before the first stimulation and half before the second stimulation.

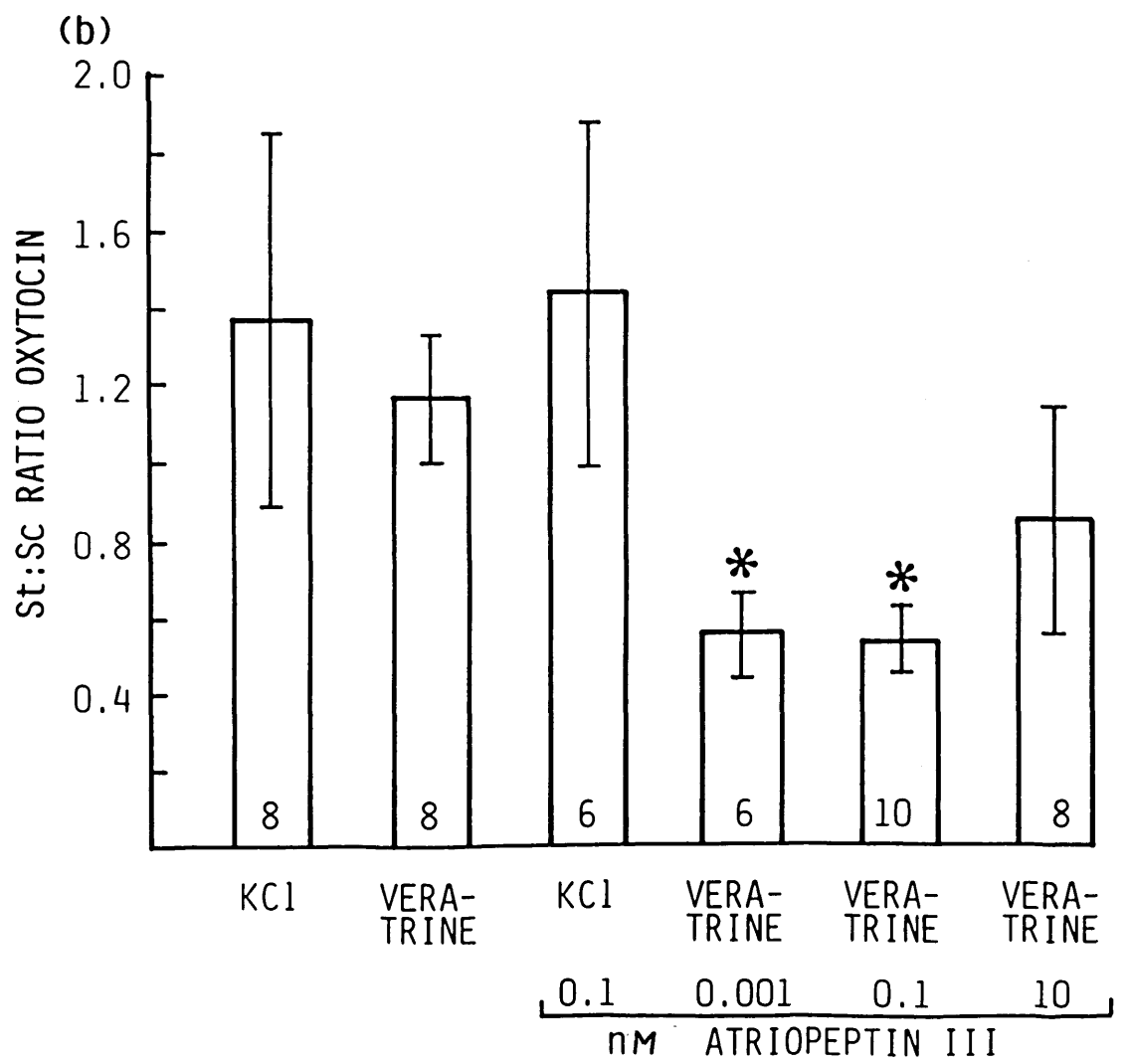
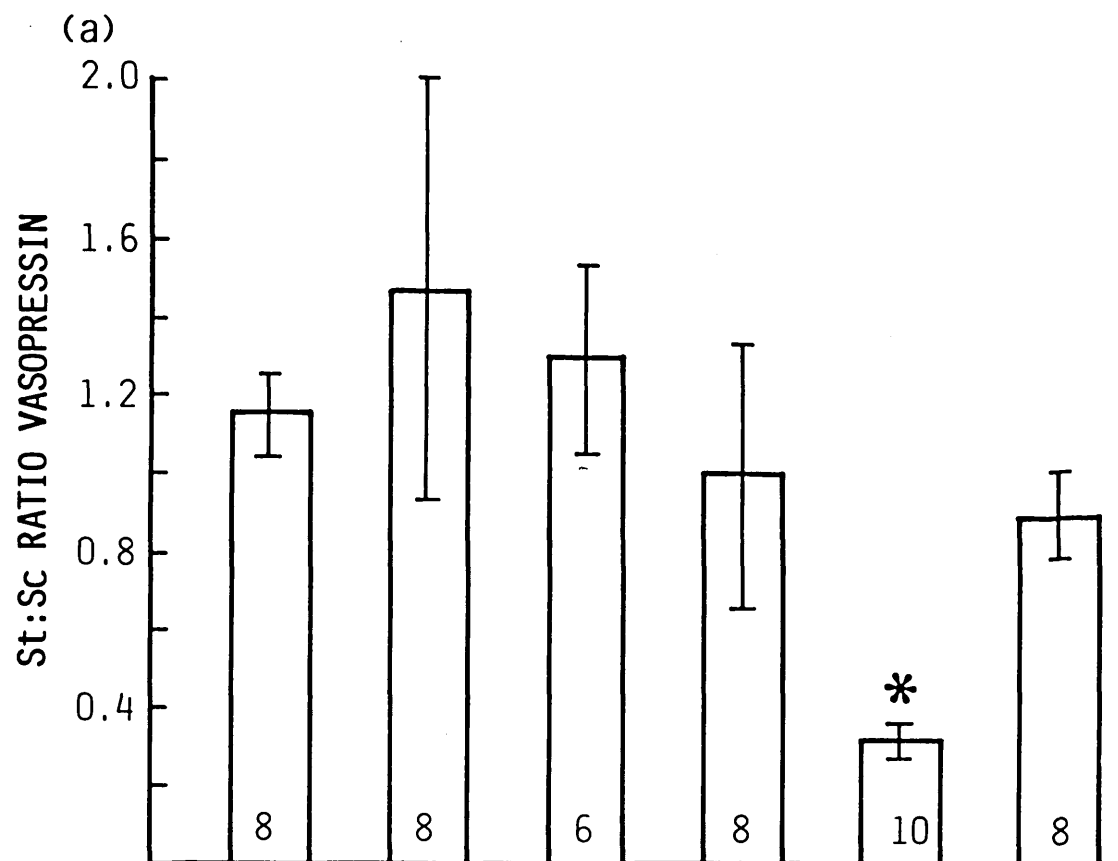
Fractions of perfusate were assayed for AVP and OT by direct radioimmunoassay (chapter 2.5). The coefficients of variation and detection limits for the assays were as in chapter 4. Because of marked variation in both basal and stimulated concentrations of AVP and OT in the perfusate, results were expressed as a ratio of AVP and OT released during the test stimulation period (St) to AVP or OT released during the control stimulation period (Sc) when no atriopeptin III was present. Results are expressed as the mean $\pm$ SEMs. Statistical analysis was performed using Duncan's multiple range test.

5.3 Results

Atriopeptin III (0.001, 0.1 or 10 nM) failed to modify the basal secretion of either AVP or OT (data not shown), however 0.1 nM ($p < 0.05$) but not 0.001 or 10 nM atriopeptin III inhibited the release of veratrine stimulated AVP release but failed to inhibit KCl stimulated AVP release (figure 5.1). 0.001 ($p < 0.05$) and 0.1 nM ($p < 0.05$) but not 10 nM atriopeptin III inhibited the release of veratrine stimulated OT release. 0.1 nM atriopeptin III failed to inhibit KCl stimulated

Figure 5.1

Effect of atriopeptin III (0.001, 0.1 or 10 nM) on veratrine (25 μ M) or KCL (45 mM) stimulated release of a) vasopressin and b) oxytocin from perifused neuro-intermediate lobes. Results are expressed as a ratio of peptide released during the test stimulation period (St) compared to release during the control stimulation period (Sc) when no atriopeptin III was present. The group size is indicated within the bars. Mean $\pm$ SEM. *p < 0.05 compared with veratrine stimulation alone.



OT release.

5.4 Discussion

In contrast to the high dose of ANF required to modify neurohypophyseal hormone secretion after central administration, this experiment shows that low doses of ANF inhibit the stimulated release of AVP and OT from isolated neurointermediate lobes. These in vitro findings are consistent with previous studies (Januszcwicz et al, 1985b; Obana et al, 1985) and indicates that a pituitary site of action may be of primary importance in the effect of ANF on neurohypophyseal function. It may be inferred that the inhibitory effects of intravenous ANF on AVP secretion (Samson, 1985a) are most likely to be due to an effect at the pituitary level. Whether circulating levels of endogenous ANF (approximately 100 pg/ml; Eskay et al, 1986) exert a similar action is unknown. An inhibitory effect of ANF on the stimulated release of AVP from the isolated neurohypophysis has been reported previously (Januszcwicz et al, 1985b; Obana et al, 1985) although Januszcwicz et al (1985a) initially reported a stimulatory effect of ANF on AVP secretion in a stationary in vitro system.

I have shown that the stimulated release of both neurohypophyseal hormones, AVP and OT, can be inhibited by atriopeptin III; in fact atriopeptin III was a more potent

inhibitor of OT than AVP. In contrast to previous work (Januszewicz, et al. 1985a; Obana et al, 1985) I did not find any significant effect of ANF on basal levels of AVP. Similarly no effect on basal levels of OT was seen. The reason for this difference is unknown but differences in tissue preparation, such as the use of sectioned neurointermediate lobes in the cited studies and variations in experimental design may be relevant. For example, Obana et al failed to vary the sequence in which ANF with KCL and KCL alone were added to the perfusate and this could give misleading results. My finding that veratrine but not KCL stimulated release was inhibited by atriopeptin III may be related to the selective effect of veratrine on Na⁺ channels, compared with the non-selective depolarising action of KCL.

In conclusion, I have demonstrated that atriopeptin III also inhibits the stimulated release of both AVP and OT in vitro. Comparison of doses required to inhibit hormone secretion suggests that a pituitary site of action is potentially of greater importance in a regulatory action of endogenous ANF on neurohypophyseal function.

Chapter 6 INHIBITION OF VASOPRESSIN SECRETION
DURING MIGRAINE

6.1 Introduction

Headache in the form of migraine may be associated with changes in urine flow. The passing of large volumes of dilute urine during migraine was noted as long ago as 1873 by Liveing and in a recent study 24% of migraineurs (migraine sufferers) reported either a change in urinary frequency or symptoms suggestive of fluid retention during their migraines (Blau, 1982). Increased urinary frequency is the most common symptom although oliguria, weight gain, increased girth and tightness of rings may be experienced. Schottstaedt and Wolff (1955) reported decreased water and sodium clearance during the early phase of migraine; followed by increased water and sodium clearance during the recovery phase, however attempts to modify the headache by inducing a diuresis during the early part of the migraine were unsuccessful. Campbell et al, 1951 had made similar observations and postulated alterations in the secretion of vasopressin and aldosterone during migraine.

The aim of this study was to determine whether the reported changes in urine flow in spontaneous migraine are

Amendment

Migraine is more common in women than in men (Green, 1977). The protocol required considerable co-operation by the subject during a spontaneous migraine and this was most likely to be achieved whilst the subject was at home rather than at work and so housewives were most likely to participate in this study.

due to changes in AVP secretion. To achieve this aim the urine of 6 migraineurs has been studied during spontaneous migraines. In addition it was possible to study the plasma of one patient during a migraine. This was practically a difficult project to undertake as by their nature, migraines are unpredictable in their timing and the cooperation of the patient during such a distressing event was going to be essential.

6.2 Subjects and Methods

Subjects were recruited from outpatient clinics at the National Hospital for Nervous Diseases and the Princess Margaret Migraine Clinic, Charing Cross Hospital. Having ascertained that they had migraine as previously defined (Poole, 1986) those who had also noticed a change in urinary frequency during their migraines were invited to participate in the study. Subjects were asked not to take any medication either prophylactically or acutely during this study.

As soon as the migraineur was aware of the onset of a migraine she voided discarding the urine. Then at two hourly intervals, for a total of 12 hours each subject voided into a measuring beaker, recorded the volume passed and poured 20ml into a labelled screw-topped specimen bottle. Fluid input during the migraine was carefully

recorded; one cup was taken as 150ml for the purposes of this study. Specimen urine bottles were kept in a fridge at 4°C until the next morning when they were either collected or sent by Red Star post to a London main line station where they were met on arrival. Urine osmolality (Osmomat 030, Gonotec, Berlin) and sodium concentration (flame photometer) were measured before storing at -20°C for subsequent assay of AVP and OT. Control urine was obtained in a similar manner from the same 6 migraineurs during a headache free day at least 3 days after their last migraine. Urine from 6 non-migrainous subjects acted as an additional control.

For one subject both plasma and urine were obtained simultaneously during a spontaneous attack of common migraine. In this case the first blood sample was taken 45 minutes from when the subject became aware of the onset of the migraine and then at 30 minute intervals for 4 hours. 10ml aliquots of blood were collected into heparinised tubes, centrifuged at 3000 rpm for 10 minutes in a portable centrifuge, separated and the plasma divided into two aliquots. One was transported in a plastic storage tube at ambient temperature for measurement of sodium and osmolality; the other was transported in a plastic storage tube in dry ice and stored at -20°C for subsequent measurement of AVP, oxytocin and alpha human ANP (ANF). Urine was collected 2 hourly and a careful record of fluid input charted as above for 12 hours. A convalescent blood

sample was taken 3 days after the end of the previous migraine.

Radioimmunoassay

Fractions of urine and plasma were assayed for AVP and oxytocin by direct radioimmunoassay as described in chapter 2.5. Fractions of plasma were assayed also for alpha human ANP.

Data analysis and statistics

Results are expressed as means $\pm$ SEM. The Student's t test and Wilcoxon matched pairs test were used where appropriate. Significance was accepted for $p < 0.05$. Excretion of urinary AVP and oxytocin are expressed as pmol/2 hours and urine volumes as ml/2 hours.

6.3 Results

All migraineurs studied were female, mean age 36 years (range 28 to 49); 3 had classic and 3 common migraine. One migraineur was premenstrual when studied and another was taking a combined oral contraceptive pill. All attacks occurred during the day with headaches lasting from 7 to 12 hours. During migraine there was a marked increase in urine volume ($p < 0.001$) and fall in the excretion of AVP (fig 6.2). The mean nadir of urinary AVP was 1.1 ± 0.24

Figure 6.1

Urine volume (U_{vol}) and urinary sodium (U_{Na}) at 2 hourly intervals in migraineurs during a) spontaneous migraine and b) control period at least 72 hours from the last migraine. Mean + SEM. $n = 6$ for both a) and b).

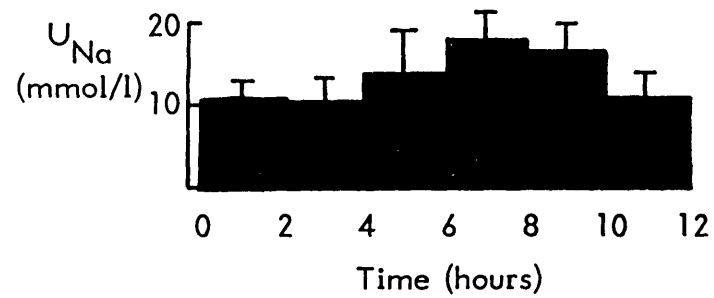
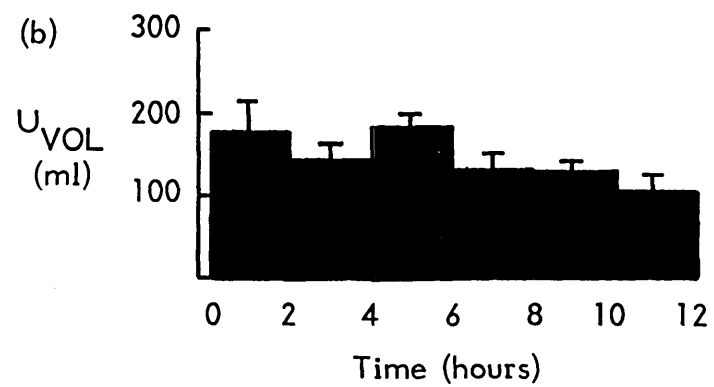
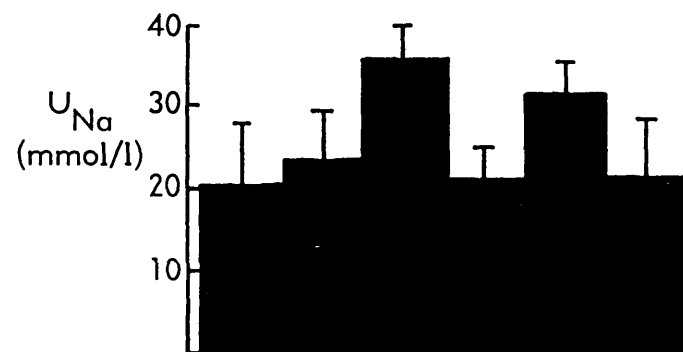
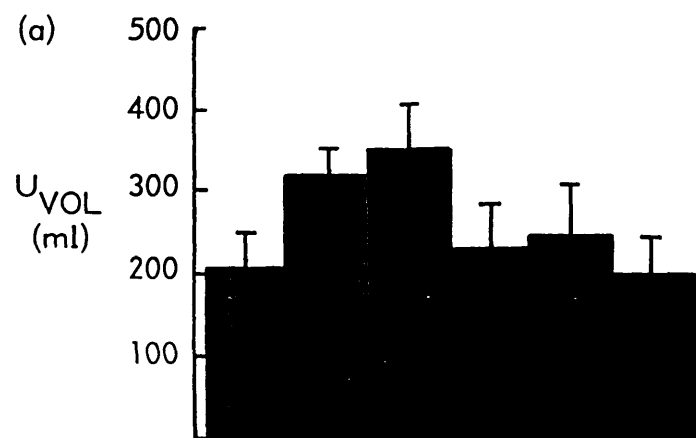
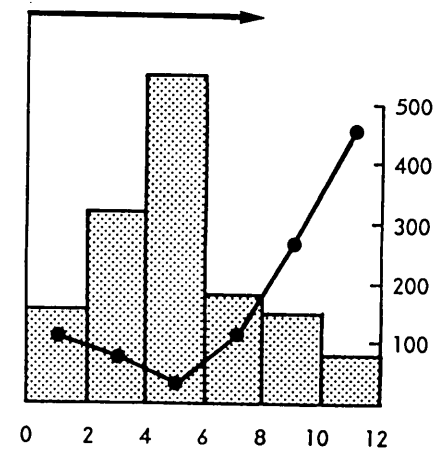
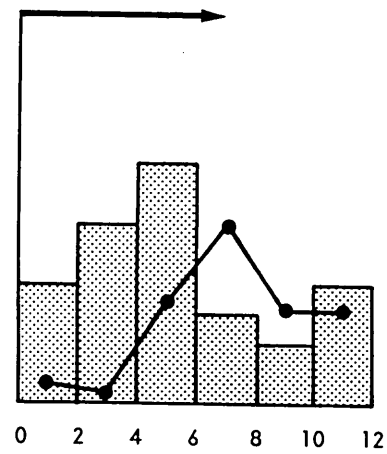
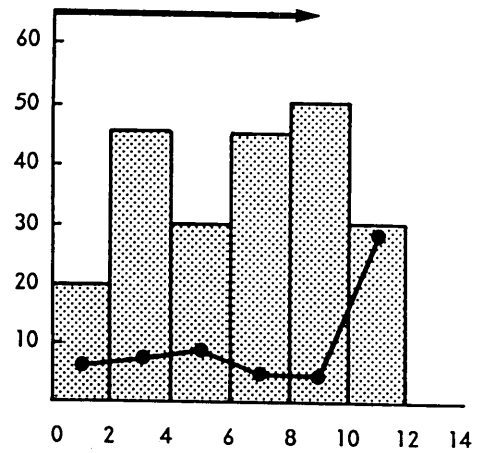
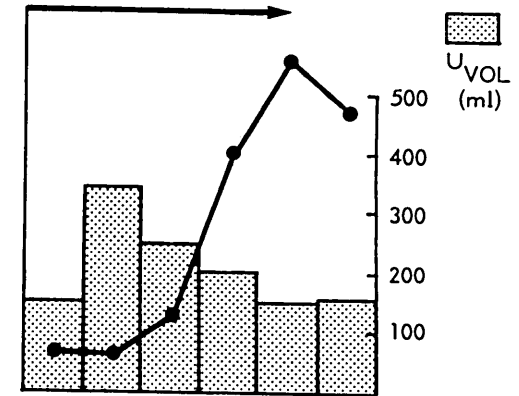
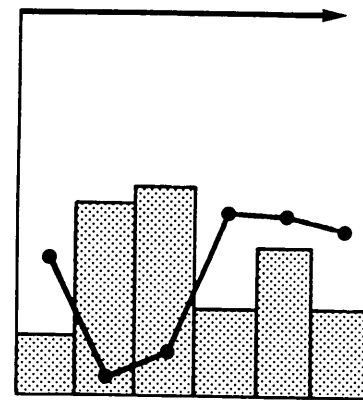
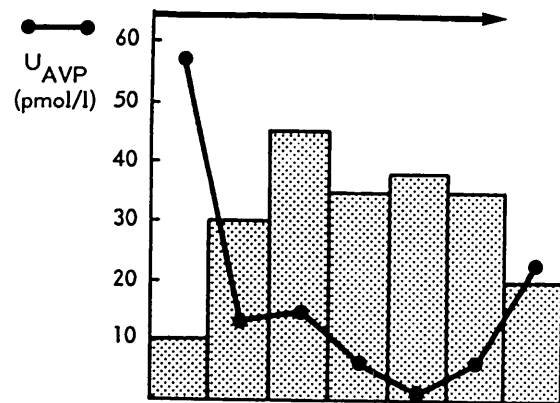


Figure 6.2

Urinary AVP (U_{AVP}) pmol/l and urine volumes (stippled bars) ml at 2 hourly intervals in 6 migraineurs during spontaneous migraines. The duration of the headache is shown by the arrow.



Time (hours)

TABLE

Time	Plasma				
	Na ⁺	Osmolality	AVP	OT	ANF
	(mmol/l)	(mosmol/kg)	(pmol/l)	(pmol/l)	(pmol/l)

13.00	131	284	0.75	3.1	72
30	135	298	1.19	2.7	132
14.00	144	303	0.99	4.8	140
30	153	303	1.19	3.3	101
15.00	150	296	1.66	8.1	96
30	147	285	1.77	8.7	99
16.00	137	285	1.06	2.3	105
30	138	288	1.11	4.3	64
convaless-					
cent	141	291	2.30	7.3	57

normal	130-148	285-295	1.0-4.0	1.0-8.0	20-40
basal lab					
range					

Fluid intake 09.00-21.00 = 700 ml

Urine output 09.00-21.00 = 1870 ml

pmol/2 hr during migraine and 2.8 ± 0.45 pmol/2 hr migraine free ($p < 0.01$); both figures are corrected for the effect of dilution. The corresponding urinary oxytocin was 1.8 ± 0.51 and 2.1 ± 0.46 pmol/2 hr (NS) The excretion of urinary sodium increased significantly during migraine ($p < 0.001$), (fig 6.1). Fluid intake and urine output 89 during migraine were 971 ± 162 and 1543 ± 186 ml/12 hours respectively ($p < 0.01$). Fluid intake in migraineurs between migraines was 1050 ± 118 ml/12 hours (NS compared with intake during migraine) and urine output between migraines was 910 ml/12 hours ($p < 0.01$ compared with output during migraine). There was no significant difference in urine volumes, urinary AVP or urinary oxytocin between controls and migraine free migraineurs (data not shown). In the one patient who provided plasma as well as urine specimens, there was a negative fluid balance of at least one litre during the first 12 hours of the migraine (see table). Plasma sodium, osmolality and alpha human ANP all increased, AVP remained inappropriately low and oxytocin did not change. Heart rate and blood pressure (portable ward sphygmomanometer) remained in the range 64-72 bpm and 100/60-110/80 mm Hg respectively throughout the attack.

6.4 Discussion

The finding of a diuresis and natriuresis during migraine is in keeping with previous observations (Liveing, 1873;

Schottstaedt and Wolff, 1955). A negative fluid balance of 600ml and in some cases greater than one litre suggests that thirst and prostration the following day may be related to fluid and electrolyte loss. These results suggest that the mechanism for the diuresis is inhibition of AVP secretion. Urinary AVP, and in the one subject studied plasma AVP concentrations, were significantly and inappropriately reduced during migraine, however oxytocin remained unchanged suggesting a selective inhibitory mechanism for AVP release. This finding is particularly remarkable as nausea or vomiting was experienced by all the migraineurs and is normally a potent stimulus for AVP secretion (Robertson, 1977).

Opiates (chapter 1.4), dopamine, and ANP (chapters 4 and 5) may all have inhibitory effects on AVP release. Opiates may be released centrally during pain and inhibition of vasopressin secretion could be secondary to non-specific activation of endogenous opioid peptides during migraine. This would be amenable to experimental assessment using an opiate antagonist such as naloxone.

It is of interest that in the one patient who took a dopamine antagonist (domperidone) as treatment for her migraine she noticed a reduction in urine volumes during those attacks when she took domperidone. Unfortunately this could not be confirmed but suggests a role for dopamine as an inhibitor of AVP release during migraine.

Atrial natriuretic factor may also inhibit the release of AVP as well as cause a natriuresis and is therefore another candidate for a role in migraine associated diuresis and natriuresis. It is of note that in the one patient from whom plasma was obtained, concentrations of ANF reached 130-140 pmol/l (table), which is well within the range of ANF associated with natriuresis and diuresis (Anderson et al, 1987). If this were to be a repeatable observation, one could then speculate as to whether the ANF was of cerebral origin, which seems unlikely as the total concentration of ANF in the brain is relatively small; on the other hand it seems equally unlikely that it is of cardiac origin as this evokes the need for a cerebral-cardiac mechanism during migraine.

The changes in AVP in particular, suggest that in these patients a modification of hypothalamic activity occurs during migraine. It seems unlikely that migraine is a secondary result of a primary hypothalamic change; it would be more logical to assume that a depression of hypothalamic supraoptic and paraventricular nucleus activity is a result of local changes in neurotransmitters or electrical activity. This would be compatible with the idea of spreading electrical depression, as described by Leao in 1944, in the pathogenesis of migraine.

7.1 Introduction

Tetraplegics with bladder catheters are often aware of reduced urine volumes whilst sitting and increased urine volumes whilst in bed at night. Tetraplegics who are not catheterised may be aware of reduced urinary frequency whilst in their wheel-chair and increased urinary frequency whilst supine in bed, occasionally to the extent of incontinence. The reason for this clinical observation is unknown. It was my hypothesis that the postural hypotension to which tetraplegics are prone leads to increased secretion of AVP which may in turn contribute to or cause the antidiuresis associated with sitting.

It has been shown previously that subjects with progressive autonomic failure (PAF) have a normal release of AVP to hypertonic saline infusion (Williams et al, 1985b) but a defective cardiovascular regulation of AVP secretion (Puritz et al, 1983). These patients are disabled by postural hypotension, urinary incontinence, defective sweating and impotence usually in association with spasticity, parkinsonism or signs of cerebellar disease. Neuropathological examination shows degeneration in catecholamine containing cerebral nuclei such as the

caudate putamen, locus coeruleus, tractus solitarius and dorsal vagal complex as well as cell loss in the inter-mediolateral columns and sympathetic ganglia (Spokes et al, 1979; Oppenheimer, 1983). Since they have lesions in both their afferent and efferent cardiovascular pathways it is difficult to define the cause of their defective release of AVP. Tetraplegic subjects with a lesion above C7 however, have interruption of their sympathetic outflow, whilst in contrast to PAF subjects, they have normally functioning glossopharyngeal and vagal cranial nerves and normal central catecholaminergic pathways. Therefore a study was performed in subjects with mid cervical cord transection to assess their osmotic and cardiovascular control of AVP secretion in an attempt to answer two questions: 1) why are tetraplegics sometimes oliguric whilst sitting; 2) why did head-up tilt not produce a rise in AVP in the PAF subjects?

7.2 Subjects and Methods

Eight male subjects, with a median age of 31 years (range 23 to 53 years) were studied in the Spinal Unit, Stoke Mandeville Hospital. They all had complete traumatic cervical (C5 to C6) spinal cord transection as evidenced by no detectable somatic motor or sensory function below the level of the lesion on physical examination. Four had lesions of less than 1 year duration (range 3 to 8 months) and 4 had lesions of more than 8 years duration (range 8

to 18 years). They were all free of other significant medical problems and were not on any medication that might have affected their blood pressure or 'flow' of urine. Subjects gave their informed consents and the study was approved by the ethics committee at Stoke Mandeville Hospital.

The control group consisted of 7 healthy subjects with a median age of 35 years (range 26 to 57 years) none of whom were taking any medication. Their responses to hypertonic NaCL (Williams et al, 1985b) had already been evaluated. Three separate studies were undertaken:

1) Postural changes in urine flow

After a normal nights sleep 6 catheterised tetraplegics had their catheter bags emptied. Urine was then collected at hourly intervals from 09.30 to 17.30 into a measuring beaker and the volume recorded. At 11.30 the subject was lifted into his wheel-chair where he remained until 14.30 whence he was returned to his bed. Throughout the course of the experiment each subject drank 180 ml of water hourly (equivalent to 2.2 l over 12 hours); eat a light breakfast and light lunch. Heart rate (HR) and blood pressure (BP) were recorded at 60 minute intervals, except for the first 30 minutes after transfer from bed to chair or chair to bed when they were recorded more frequently using an automatic monitor (Sentron, Bard Biomedical,

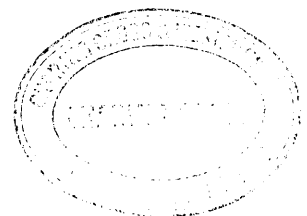
Illinois, USA). Blood was taken at similarly timed intervals from a venous catheter in the opposite arm and the separated plasma used to measure AVP concentrations. There were no controls for this part of the experiment.

2) Hypertonic NaCL infusion

When the subjects had rested supine for 30 minutes a catheter was inserted into a forearm vein and an intravenous infusion of hypertonic (0.85M) NaCL (0.05 ml/kg/min) was commenced and continued for 2 hours. Heart rate and BP were recorded at 15 minute intervals using an automatic monitor. Blood was taken at 30 minute intervals from a venous catheter in the opposite arm and the separated plasma subsequently used to measure sodium, osmolality and AVP.

3) Head-up tilt

Three days before this study the tetraplegics were tested on a tilt table to determine what angle of head-up tilt resulted in a 25% fall in systolic blood pressure without causing any symptoms of cerebral hypoperfusion. On the day of the study, after resting supine for 30 minutes a catheter was inserted in a forearm vein. The tetraplegics were tilted to the pre-determined angle (between 30 and 45°) and the normal controls to 45°. This position was maintained for 30 minutes during which time pulse and



blood pressure were recorded at 5 minute intervals. Blood was also taken at 5 minute intervals for subsequent estimation of haematocrit and plasma AVP. Blood samples were collected into lithium heparin or potassium EDTA tubes and immediately put on ice. The lithium heparin tubes were centrifuged at 3000 rpm at 4°C and the separated plasma used to measure sodium (flame photometer) and osmolality (Osmomat 030, Gonotec, Berlin, FRG). Haematocrit was measured after microcentrifugation (Hawkesley microcentrifuge). Plasma was stored for less than one month at -20°C before subsequent Sep-Pak extraction and estimation of AVP by radioimmunoassay (Williams et al, 1985a). The detection limit for the assay was 0.25 fmol/tube. The coefficients of variation for control AVP samples (2 fmol) were 6% (intra-assay) and 17% (interassay).

Mean arterial pressure (MAP) was calculated by adding one-third of the pulse pressure to the diastolic pressure. Basal HR and blood pressure were taken as the mean of 3 consecutive readings whilst supine. Results were expressed as mean $\pm$ SEM. The significance of changes were assessed by two way analysis of variance and comparisons between the 2 groups of subjects were made using Duncan's multiple comparison test.

7.3 Results

1) Postural changes in urine flow

In all subjects studied urine volumes fell whilst seated to a nadir of 29 ± 11 ml/hour during the second hour, followed by a rebound diuresis of 372 ± 78 ml/hour ($p < 0.05$) during the second hour after return to supine (figure 7.1). Mean arterial pressure (MAP) fell to a nadir of 68 mm Hg within 5 minutes of sitting and remained between 68 and 81 mm Hg for the remainder of the seated period. Supine MAPs were significantly higher varying between 83 and 94 mm Hg ($p < 0.05$). Plasma AVP rose from 2.3 ± 0.5 pmol/l supine to 11.3 ± 4.9 pmol/l sitting ($p < 0.05$) within 30 minutes of transfer; AVP concentrations then quickly returned to basal levels.

2) Hypertonic NaCL infusion

The changes in osmolality (Table 6.1) were associated with a significant rise in plasma AVP ($p < 0.01$) in both the controls and the tetraplegics (fig 6.1). Plasma levels of AVP in the tetraplegics reached 7.1 ± 1.0 pmol/l compared with 4.5 ± 1.0 pmol/l in the controls but this difference was not statistically significant.

In the control group there was a small but insignificant rise in MAP (from 79 ± 2.5 mm Hg at the onset to 84 ± 3.2

Figure 7.1

Changes in urinary volume (U vol), mean arterial pressure (MAP) and plasma AVP with posture (shown diagrammatically) in tetraplegic man (n = 6; mean $\pm$ SEM).

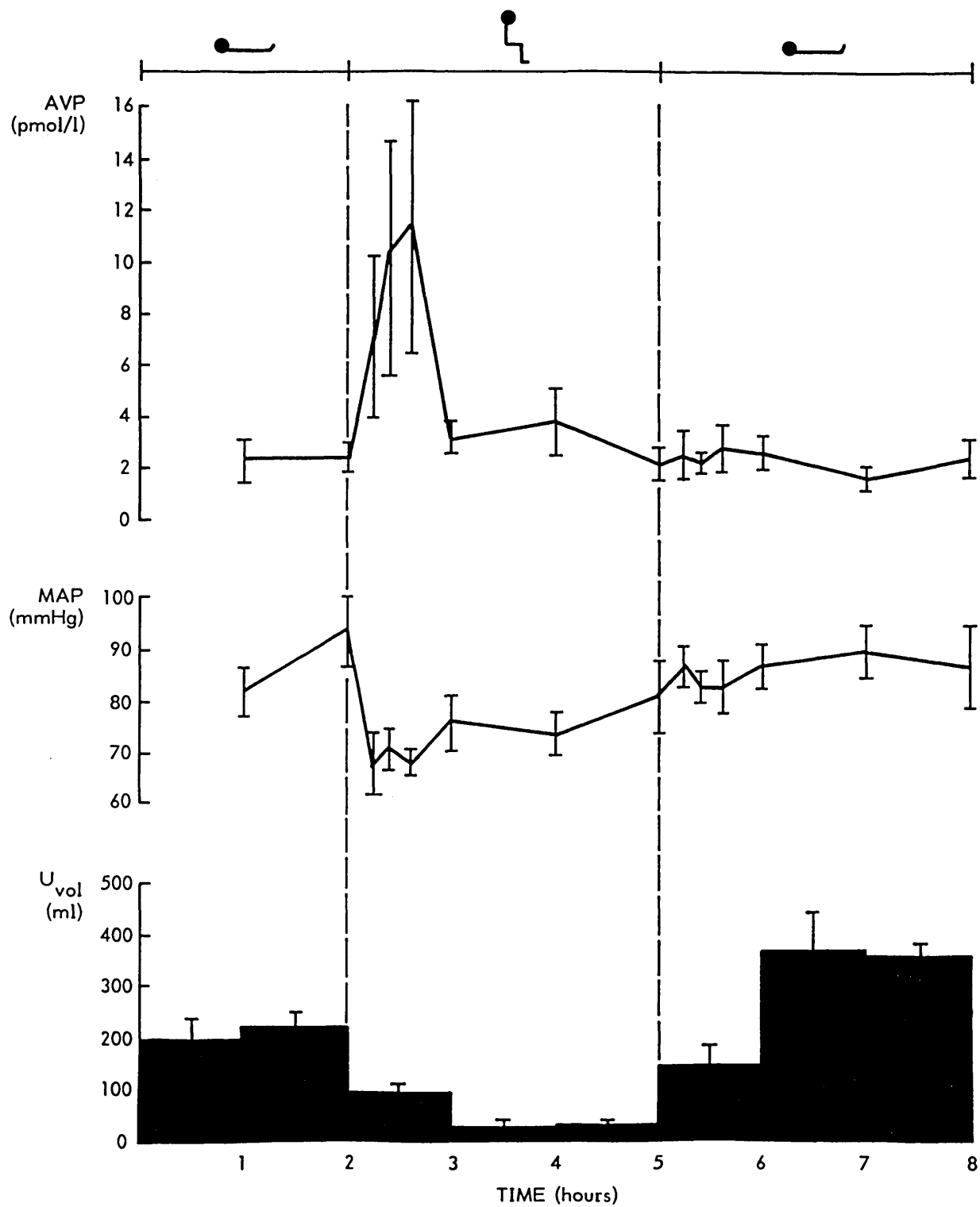


Figure 7.2

Changes in mean arterial pressure (MAP), heart rate (HR) and plasma AVP during intravenous infusion of 0.85M NaCL at 0.05 ml/kg/min over 2 hours in controls (solid line) and tetraplegics (broken line). Mean $\pm$ SEM, n=6 in each group. * $p < 0.05$ compared with controls.

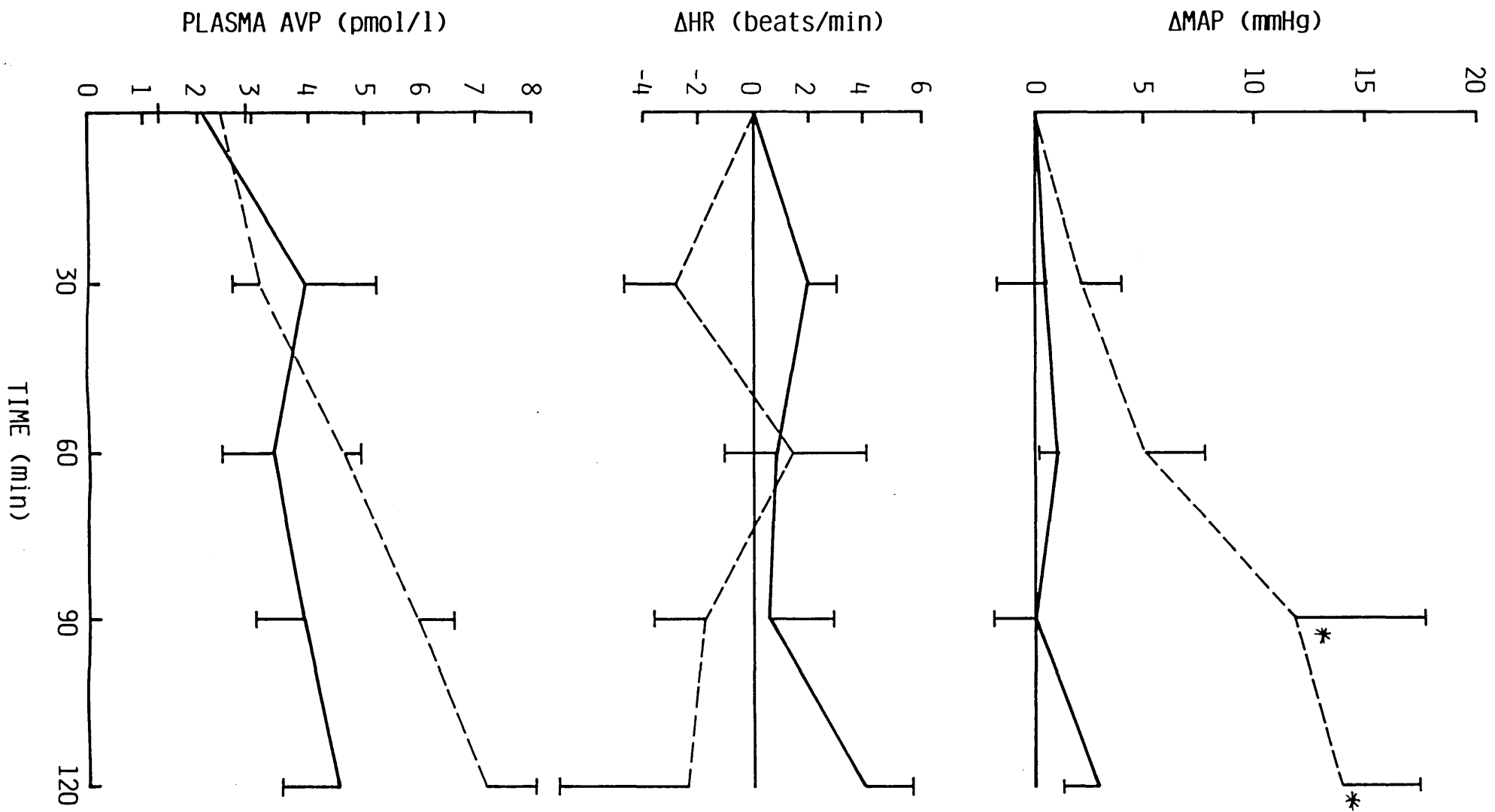
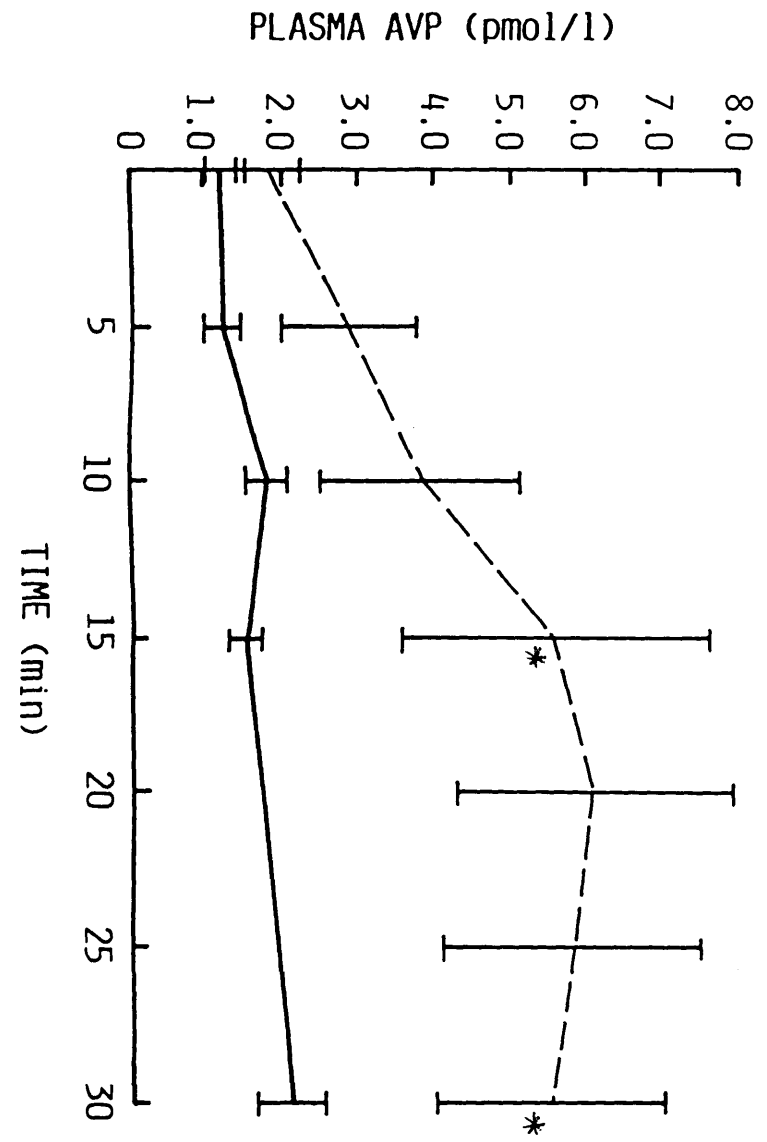
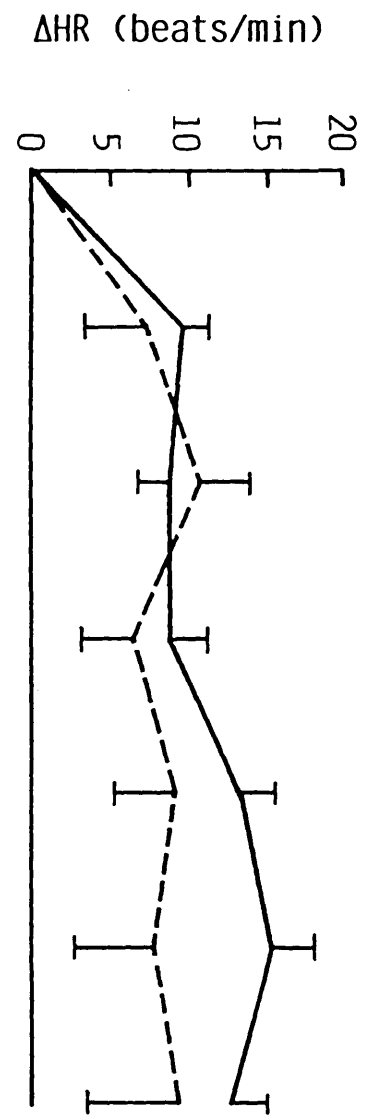
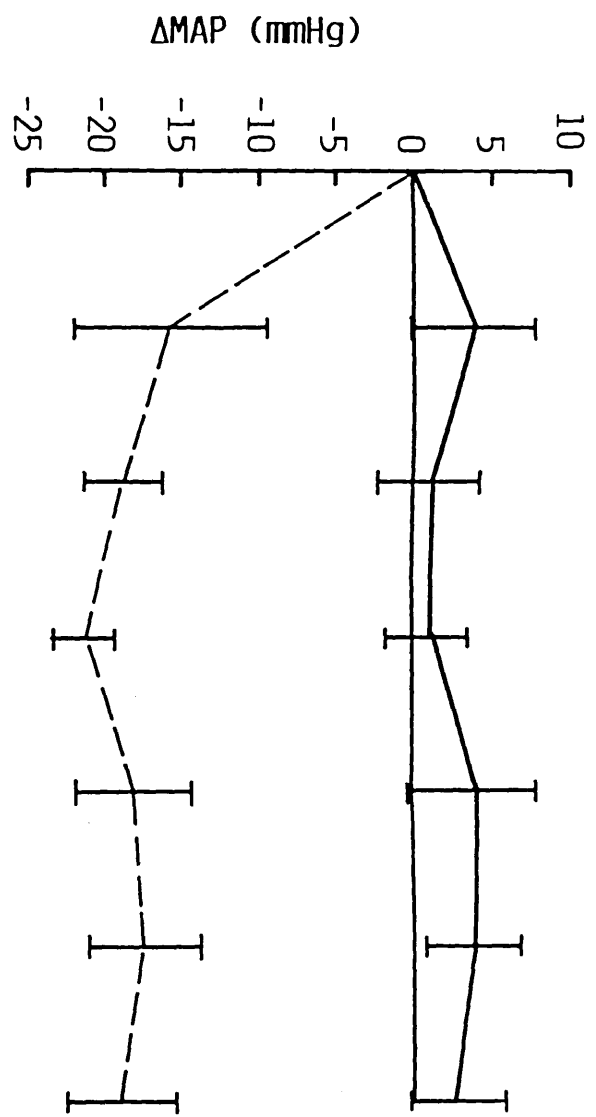


Figure 7.3

Changes in mean arterial pressure (MAP), heart rate (HR) and plasma AVP during head-up tilt over 30 minutes in controls (solid line, n=6) and tetraplegics (broken line, n=8). Mean $\pm$ SEM. * $p < 0.05$ compared with controls.



mm Hg at the end of the infusion) and HR (from 56 ± 4.0 to 59 ± 3.8 beats/min (fig 6.1). The tetraplegic group however, showed an increase in MAP from 87 ± 5.3 to 101 ± 5.2 mm Hg ($p < 0.05$). This rise was significantly greater than that in the control group both at 90 ($p < 0.05$) and 120 minutes ($p < 0.05$) but was not associated with any significant change in HR 73 ± 5.9 beats/min at onset and 70 ± 4.8 beats/min at end of infusion.

3) Head-up tilt

In the control subjects 45° head-up tilt was not associated with any significant change of MAP but HR rose from 67 ± 0.9 beats/min at rest to a maximum of 82 ± 2.3 beats/min ($p < 0.01$), (fig 6.2). In the tetraplegics MAP fell by 16 mm Hg at 5 minutes and remained between 17 and 22 mm Hg below basal values for the duration of the tilt (fig 6.2). HR rose from 73 ± 7.2 beats/min at rest to a maximum of 83 ± 6.4 beats/min at 10 minutes (NS). Haematocrit in the controls rose from $41.7 \pm 1.2\%$ at rest to $43.5 \pm 1.1\%$ at 30 minutes ($p < 0.01$) and in the tetraplegics from 40.8 ± 1.9 to $41.7 \pm 2.3\%$ respectively (NS).

Plasma AVP rose from 1.2 ± 0.23 pmol/l at rest to 2.1 ± 0.38 pmol/l at 30 minutes ($p < 0.05$) in the controls and from 1.8 ± 0.43 to 5.5 ± 1.46 pmol/l ($p < 0.05$) in the

tetraplegics. The rise in AVP in the tetraplegics was significantly greater than that found in the normal controls at 15 ($p < 0.05$) and 30 minutes ($p < 0.05$). Basal AVP in the controls was 1.2 ± 0.2 pmol/l and in the tetraplegics 1.8 ± 0.4 pmol/l (NS).

7.4 Discussion

This work confirms the clinical observation in tetraplegics that a change in posture, from supine to sitting, may be associated with a fall in urine volume. The results of this study also indicate that tetraplegics have normal osmotic and appropriate cardiovascular control of AVP secretion. The secretion of AVP in response to hypertonic saline was not significantly different from that of the control or PAF subjects. The secretion of AVP in response to head-up tilt was increased commensurate with the simultaneous fall in mean arterial blood pressure due to their loss of sympathetic vasomotor control. The previously studied PAF subjects also had a significant fall in MAP on head-up tilt (Puritz et al, 1983), but in spite of this added stimulus to AVP secretion they showed no increase in plasma AVP after this procedure. The difference between the PAF and the tetraplegic subjects is most likely to be due to lesions in the afferent pathways (glossopharyngeal or vagal cranial nerves) or to lesions in the central catecholaminergic pathways rather than a defect of the sympathetic outflow (intermediolateral

columns and sympathetic ganglia).

The rise in plasma AVP in response to head-up tilt in the tetraplegic is in agreement with the results reported by Sved et al, 1985 who studied 5 subjects with complete or partial spinal cord transection between C5 and C7 some of whom were taking anti-hypertensive medication. They were all tilted to 70° both rapidly (in 15 seconds) for 5 minutes during which time their systolic blood pressures fell precipitously (90 mm Hg in one case) and again slowly (4.8°/min) for 15 minutes. Our subjects all had complete cervical spinal cord transection at levels C5 and C6 and none of them were taking medication which might affect blood pressure. There was wide variation in the release of AVP in response to tilt in both our study and that of Sved et al. There was no association between the level of cervical spinal cord transection, the duration of tetraplegia, the age of the subject and the observed rise in plasma AVP. The explanation for this variable rise in AVP is unknown.

The increased postural response of AVP may be an important cause of oliguria often seen in patients with high spinal cord transection during long periods of sitting, especially since glomerular filtration rate has been reported not to fall with head-up tilt in these patients (Ragnarsson et al, 1981). The latter finding is rather surprising as MAP may fall particularly low, albeit

transiently, and some reduction in renal blood flow would be expected. Bladder ultrasound or catheterization often reveals an empty or near empty bladder in these patients (Dr Hans Frankel, personal observation), an undesirable situation in patients already prone to recurrent urinary tract infections, bladder calculi and chronic renal failure.

The pressor response to hypertonic NaCl in the tetraplegics but not controls is interesting as this occurred at plasma concentrations of AVP (5.9 ± 0.7 and 7.1 ± 1.0 pmol/l) which were not significantly different from the controls. It will be seen from chapter 8 that these concentrations of AVP are associated with a pressor response in tetraplegics but not in controls. This suggests that tetraplegics are supersensitive to the pressor effects of endogenous AVP. Another explanation could be that 400 ml 0.85M NaCl equivalent to $2.2 \pm 0.9\%$ (w/v) NaCl in mmol Na⁺ causes a rise in circulating blood volume, the pressor effect of which cannot be buffered adequately in a cardiovascular system with defective baroreflexes.

The postural hypotension to which tetraplegics are prone occurs in both recently injured tetraplegics in spinal shock and in chronic tetraplegics, especially during the early stages of rehabilitation. Passive head-up tilt studies have shown an immediate fall in both systolic and

diastolic blood pressure which may drop extremely low but may not always result in loss of consciousness (Corbett et al, 1971). The hypotension is due to the inability to reflexly increase sympathetic nervous activity in response to postural change, as would occur normally. This is reflected in levels of plasma noradrenalin which do not change in tetraplegics in the early phase following head-up tilt (Mathias and Frankel, 1983). If tilt is prolonged the blood pressure often tends to partially recover and it has been suggested that this is related to activation of the renin-angiotensin system (Johnson et al, 1971; Mathias et al, 1975). However in the light of the above finding it is possible that AVP also plays a part in the recovery of blood pressure in tetraplegics after head-up tilt.

The rise in heart rate during head-up tilt is well recognised and inversely related to the fall in blood pressure (Mathias and Frankel, 1983). It appears to be partly a vagal withdrawal^a response to baroreceptor activation as it is attenuated, although not abolished, by atropine (Corbett et al, 1971) and partly due to beta-adrenoceptor stimulation, presumably from circulating catecholamines from the adrenal medulla or via spinal reflex pathways as it is also attenuated by propranolol (Mathias et al, 1980).

As an extension of this work it would be interesting to

observe the changes in plasma AVP, renin and aldosterone that occur in tetraplegics when transferring from bed to wheelchair and whether these changes vary with the level of cervical lesion and to what extent they persist with time. Confirmation of the report by Ragnarsson et al that glomerular filtration rate does not fall with head-up tilt in tetraplegics would also be important. To explore the possible role of AVP in the adaption to postural hypotension that occurs in the first few weeks and months after tetraplegia it would be necessary to see how the release of AVP in response to head-up tilt varies with time following cervical transection and at the same time to determine whether any effect that AVP may have on blood pressure (see chapter 8) changed with time after cervical transection.

Chapter 8 THE EFFECTS OF VASOPRESSIN INFUSION ON BLOOD
PRESSURE AND PULSE IN a) TETRAPLEGIC MAN AND
b) ALPHA AND BETA-ADRENOCEPTOR BLOCKED MAN

8.1 Introduction

In vitro, vasopressin is one of the most potent vasoconstrictors known (Altura and Altura, 1977). Despite this its role in systemic arterial blood pressure regulation is controversial. When AVP is infused incrementally into conscious dogs, to levels that may be found in physiological states, there is an increase in MAP, fall in HR and fall in cardiac output (CO) (Cowley et al, 1974; Montani et al, 1980). The rise in MAP however is relatively modest compared with the potent peripheral vasoconstrictor effects of AVP and for this reason attention has focused on the baroreflex as possibly buffering the expected rise in arterial pressure. Montani et al (1980) showed that a rise in MAP occurred at a rate of AVP infusion in the range 0.04 to 5.0 pmol/kg/min with a corresponding rise in total peripheral resistance (TPR) of 119.5 ± 28.1 to 940.1 ± 190.2 mm Hg/kg/min/l. After sino-aortic baroreceptor denervation the pressor sensitivity to AVP was increased and there were no longer any significant effects on HR or CO. These results indicate that the cardiac depressant effects of AVP were reflexly mediated. The pressor sensitivity to AVP in conscious dogs can also be enhanced by ganglion blockade (Pullan et al, 1980; Matsuguchi and Schmid, 1982) and

pretreatment with atropine (Liard, 1984). Montani et al (1980) also found that the increase in TPR in response to i.v. AVP was similar in intact and sino-aortic baroreceptor denervated dogs suggesting that sympathetic vasomotor tone was not involved in buffering the pressor effects of AVP. Thus there is evidence both for and against the pressor response to AVP being moderated by the baroreflex.

There is great interspecies variation in response to AVP (Bennett and Gardiner, 1986). Most studies in man report that infused AVP has either a small or transient pressor effect (Graybiel and Glendy, 1941; Wagner and Braunwald, 1956; Mohring, 1981; Ebert et al, 1984; Simpson et al 1986; Williams et al, 1986a) which is sometimes associated with baroreceptor activation as evidenced by decreased HR at lower doses of AVP and increased HR at higher doses of AVP. The most likely explanation for the small pressor effect of AVP in the face of obvious cutaneous vasoconstriction is that vasodilatation is occurring in other vascular beds. To complicate matters further if a selective V_2 agonist is infused into man, MAP actually falls (Williams et al, 1986b). Subjects with PAF (see chapter 7) also have an increased pressor response to AVP (Williams et al, 1986a) but because of their central and peripheral lesions it is difficult to define the cause of their altered sensitivity to AVP. In the light of the increased release of AVP in the tetraplegic in response to head-up tilt (chapter 7) it would be interesting to

investigate the possible effect of exogenous AVP on blood pressure in these subjects. A study was therefore performed in subjects with a) mid cervical spinal cord transection and b) alpha and beta-adrenoceptor blockade to try to define further the role of the sympathetic nervous system in the haemodynamic responses to AVP in man.

8.2 Subjects and Methods

a) Tetraplegic man

Six male subjects with a median age of 30 years (range 23 to 34 years) were studied. All had complete traumatic cervical (C5 to C6) spinal cord transection as evidenced by no detectable somatic motor or sensory function below the level of the lesion on physical examination. Three had lesions of less than 1 year duration (range 4 to 8 months) and 3 had lesions of more than 8 years duration (range 8 to 12 years). They were all free of other significant medical problems and were not on any medication that might have affected their blood pressure. Subjects gave their informed consents and the study, which was done in conjunction with that in chapter 6, was approved by the ethics committee at Stoke Mandeville Hospital. The control group consisted of 7 healthy subjects with a mean age of 35 years (range 26 to 57 years). Their response to AVP infusion had already been evaluated (Williams et al, 1986a).

After resting supine for 30 minutes a catheter was inserted into a forearm vein. All subjects received incremental 5 minute infusions of AVP (Ferring Pharmaceutical, Malmo, Sweden) at 0.05, 0.2, 0.5 and 2.0 pmol/kg/min. At 5 minute intervals pulse and blood pressure were recorded. Blood was drawn from a catheter in the contralateral arm at 5 minute intervals for subsequent measurement of plasma AVP.

b) Alpha and beta-adrenoceptor blocked man

Six male subjects with a median age of 24 years (range 23 to 25 years) were studied. They were all free from any medical problems and were on no medication. Subjects gave their written and informed consents and the study was approved by the ethics committee at Westminster Hospital.

On the day prior to study a test dose of 40mg propranolol (a competitive beta 1 and beta 2 adrenoceptor antagonist) was given orally. To assess the efficacy of beta blockade a Bruce treadmill exercise ECG was performed (to 4.2 mph at a gradient of 16°) before and again 2 hours after an oral dose of 160mg propranolol. Resting and maximum heart rates were compared before and after the beta blocker. Effective beta blockade was taken as failure of maximum heart rate in the second ECG to rise by 20% of maximum heart rate in the first ECG. Immediately following the

second exercise ECG all subjects lay supine for 30 minutes during which time a catheter was inserted in a forearm vein. A pressor dose of noradrenaline (Winthrop Laboratories, Guildford, Surrey) was then infused (1.2 nmol/kg/min) i.v. over 5 minutes and blood pressure and heart rate were recorded at 2½ minute intervals. This was followed 15 minutes later, by a 20 minute infusion (0.5 mg/min) of phentolamine (Ciba Laboratories, Horsham, Sussex), a competitive alpha 1 and alpha 2 antagonist, into the same forearm vein via a three-way tap. The infusion of noradrenaline was then repeated as above, 15 minutes after the start of the phentolamine infusion to determine whether its pressor effect had been abolished or significantly reduced. After resting supine for a further 30 minutes a catheter with three-way tap was inserted in a vein in the contralateral forearm. Phentolamine was then infused at 0.5 mg/min for 45 minutes. After 15 minutes, incremental 5 minute infusions of AVP (Parke-Davis & Company, Pontypool, Gwent) were given at 0.05, 0.1, 0.2, 0.5, 2.0 and 5.0 pmol/kg/min. Pulse and blood pressure were recorded at 2½ minute intervals and blood drawn for subsequent measurement of plasma AVP at 5 minute intervals.

All blood samples were collected and processed as in 6.2 for radioimmunoassay of AVP. The detection limit and coefficients of variation were as in 6.2.

MAP, basal HR and blood pressure were calculated as before. Results were expressed as mean $\pm$ SEM. The significance of changes were assessed by two way analysis of variance and comparisons between groups were made by Duncan's multiple comparison test.

8.3 Results

a) Tetraplegic man

Basal plasma AVP concentrations were 1.18 ± 0.23 in controls and 1.80 ± 0.43 in tetraplegics (NS). The rise in plasma AVP concentration was similar in both the control and tetraplegic groups in response to AVP infusion (fig 8.1). This rise was not associated with any significant change in either MAP or HR in the normal controls whilst in the tetraplegics MAP rose markedly in response to AVP, by 13.7 ± 3.1 mm Hg at an infusion rate of 0.5 pmol/kg/min ($p < 0.01$) when plasma AVP was 10.9 ± 2.2 pmol/l and by 15.3 ± 3.2 mm Hg at 2.0 pmol/kg/min ($p < 0.01$) when plasma AVP was 22.3 ± 3.3 pmol/l (fig 6.1). This was associated with a fall in HR from 71 ± 6.5 to 62 ± 7.0 beats/min ($p < 0.01$).

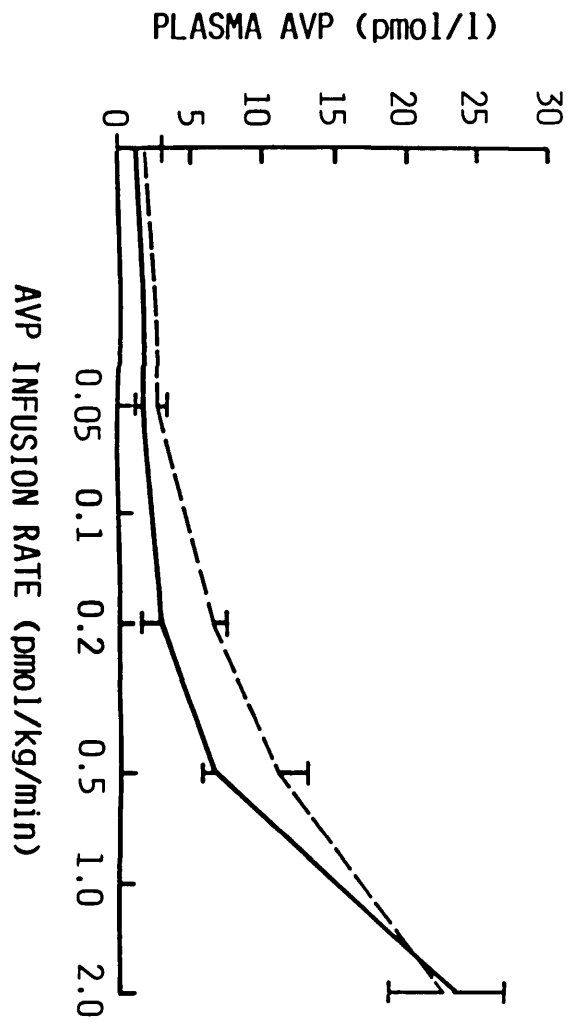
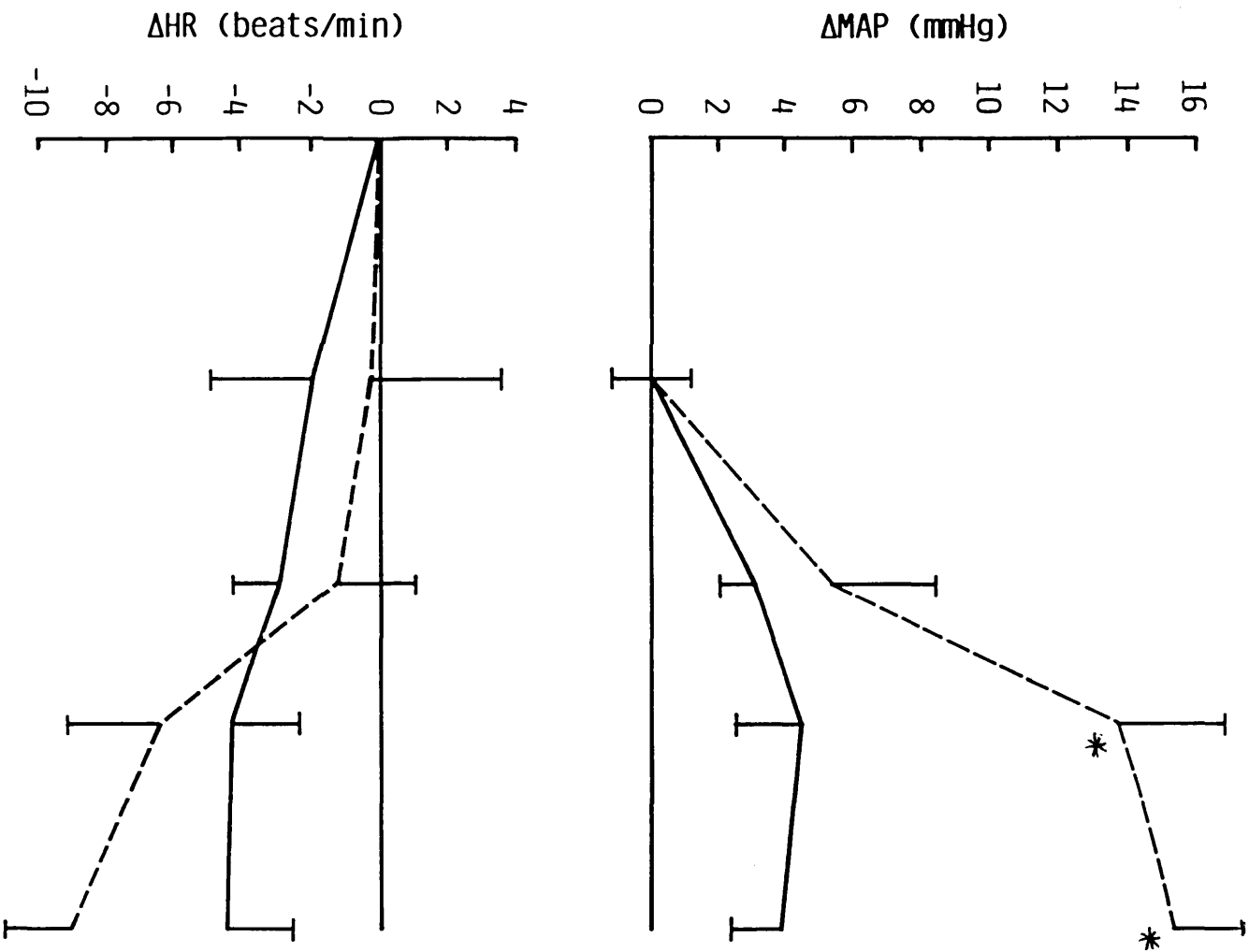
b) Alpha and beta-adrenoceptor blocked man

160 mg oral propranolol reduced mean resting HR by 16.6% from 67.5 ± 5.2 to 56.3 ± 3.2 beats/min ($p < 0.05$) and mean

maximum HR by 19.1% from 126.7 ± 7.2 to 102 ± 4.2 beats/min ($p < 0.05$) (table). 0.5 mg/min phentolamine reduced the rise in MAP by 16.7% from 114.7 ± 4.3 to 95.5 ± 5.6 ($p < 0.05$) in response to a 5 min infusion of 0.2 $\mu\text{g/kg/min}$ noradrenaline. The rise in plasma AVP was similar in both the control and alpha and beta blocked group in response to AVP infusion. Neither the MAP nor the mean HR changed significantly in the control group (fig 8.2). In the alpha and beta adrenoceptor blocked group there was no pressor response to AVP, infact MAP fell significantly at 0.2 ($p < 0.05$) and at 0.5 ng/kg/min ($p < 0.05$) compared with controls. Mean HR fell significantly during the AVP infusion from 62 ± 3.4 to 56 ± 2.6 beats/min ($p < 0.05$) but this fall was not significantly different from the fall in HR that occurred in the control group.

Figure 8.1

Changes in mean arterial pressure (MAP), heart rate (HR) and plasma AVP during incremental 5 minute intravenous infusions of AVP in controls (solid line) and tetraplegics (broken line). Mean $\pm$ SEM, n=8 in each group. *p 0.05 compared with control group.



TABLE

Subjects	Resting Heart Rate		Maximum Heart Rate	
	Before	After	Before	After
AG	49	45	120	94
TC	65	54	140	118
SW	88	67	130	99
MG	62	53	100	90
WM	71	63	150	110
CM	70	56	120	104

Heart Rate: beats/min

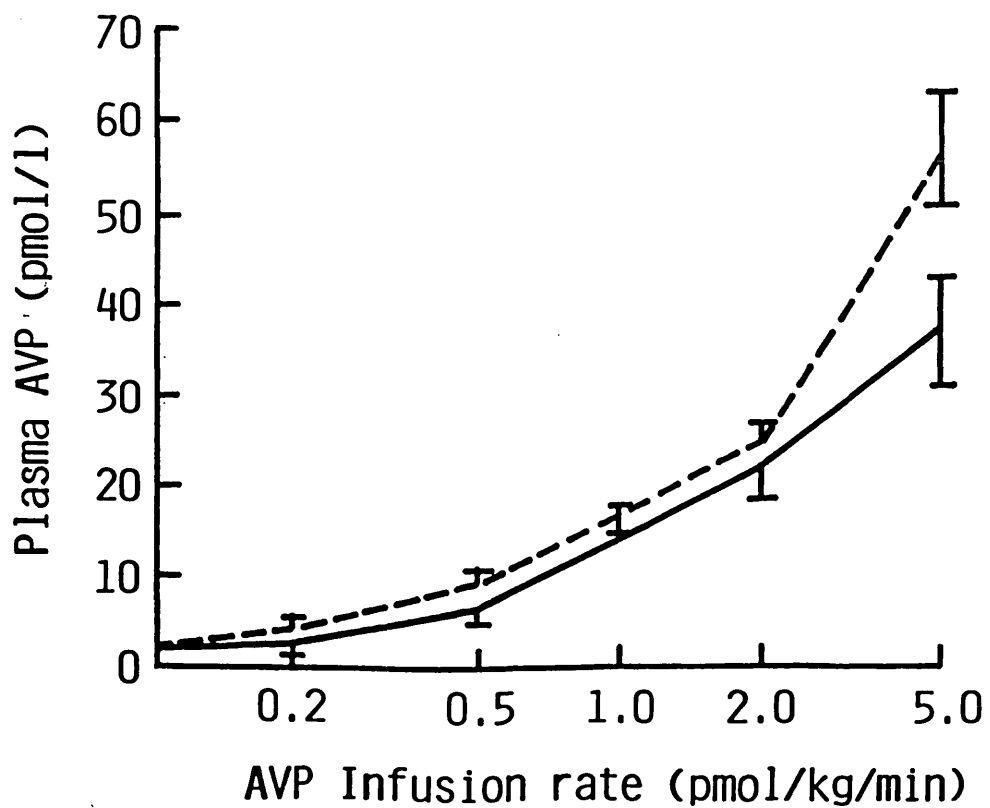
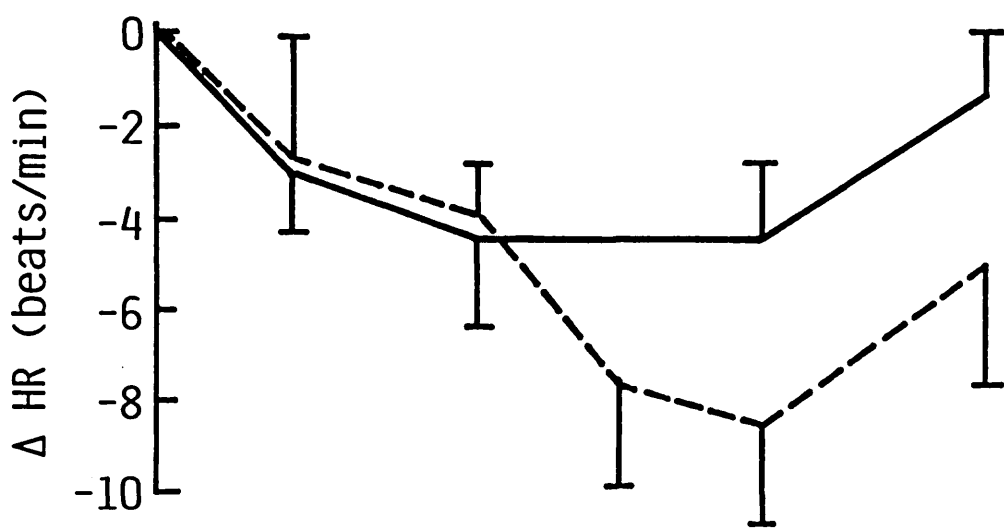
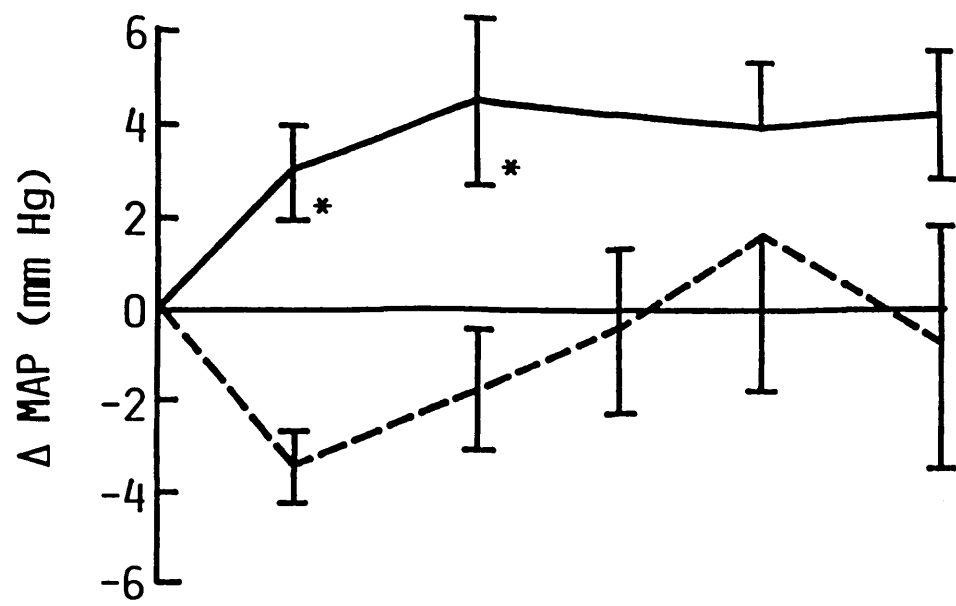
Resting and maximum heart rate before and 2 hours after
160mg oral propranolol

Subjects	Mean Arterial Pressure	
	Before	After
AG	133	117
TC	117	103
SW	107	87
MG	117	97
WM	111	92
CM	103	77

Mean Arterial Pressure: mm Hg in response to a 5 minute
infusion of 0.2 μ g/kg/min noradrenaline before and after 15
minute infusion of 0.5 mg/min phentolamine

Figure 8.2

Changes in mean arterial pressure (MAP), heart rate (HR) and plasma AVP during incremental 5 minute intravenous infusions of AVP in controls (solid line) and alpha and beta-adrenoceptor blocked man (broken line). Mean $\pm$ SEM, n=6 in each group. *p<0.05 compared with control group.



8.4 Discussion

The results of these experiments indicate that tetraplegic man quite unlike normal man has increased sensitivity to the pressor response of AVP at plasma levels within the physiological range. This pressor response was associated with a significant bradycardia presumably mediated via the baroreflex and intact vagal outflow. The rise in blood pressure in response to AVP was similar in magnitude in both the tetraplegic and PAF subjects. This suggests that the pressor sensitivity of PAF is related to the sympathetic outflow lesions rather than any lesions in the glossopharyngeal, vagal cranial nerves or central catecholaminergic pathways. Clearly the difference in pressor response to AVP between controls and tetraplegics cannot be ascribed to a loss of baroreceptor function and suggests either an increase in the sensitivity to the pressor vascular effects of AVP or a failure of the baroreflex mediated changes in sympathetic tone in response to AVP.

There is evidence of differential effects of AVP on different arteries (Monos et al, 1978) and it is possible that sympathetic denervation, whether due to cervical spinal cord transection, PAF, or other causes results in local changes in AVP receptor sensitivity. This may be a supersensitivity phenomenon, a change in the ratio of V_1 to V_2 receptors or even a change in the importance of

different vascular beds in blood pressure control after sympathetic denervation. It is interesting to speculate whether the increased pressor response to AVP plays any role in tetraplegics such as preventing them from excessive postural hypotension. Certainly the plasma levels of AVP reached during head-up tilt (6.1 ± 1.8) and hypertonic saline infusion (7.1 ± 1.0) (Chapter 7) are in the range associated with a pressor effect during infusion of AVP. The effect of AVP on cardiovascular parameters in normal man whilst supine and 50° head-up has been reported by Simpson et al (1986). Whilst supine a moderate dose infusion of AVP produced a fall in forearm blood flow and a rise in systemic vascular resistance. Cardiac output (CO) and MAP were not significantly affected. On tilting the same dose of AVP produced an increased fall in forearm blood flow and an increased rise in systemic vascular resistance; CO fell significantly and MAP rose significantly. So not only does the posture of the tetraplegic affect the release of AVP it may also contribute to the cardiovascular effects of the released AVP.

There was no increased pressor sensitivity to AVP in the healthy subjects whose adrenergic receptors had been competitively blocked with propranolol and phentolamine suggesting that the pressor sensitivity to AVP in the tetraplegic occurs independently of alpha and beta receptor occupancy. In fact MAP fell initially during the

AVP infusion the cause of which remains unknown to me. There were a number of relevant differences between this experiment and the AVP infusion in tetraplegics which may have contributed to the different findings. Firstly, in the former experiment healthy subjects were given drugs to competitively block adrenergic receptors whereas in the tetraplegics these receptors were free to interact with circulating catecholamines. Secondly, the normal subjects had no cervical sympathetic lesion whereas the tetraplegics all had a preganglionic sympathetic lesion. Thirdly, the normal subjects were all studied within a few hours of adrenergic receptor blockade whereas the shortest interval between the onset of tetraplegia and this study being performed was 3 months and potential changes in vasopressin receptors may vary with time after cervical spinal cord transection. Finally an AVP-like peptide has been demonstrated in sympathetic nerves (Hanley et al, 1984) which may itself exert post ganglionic effects. Sympathetic denervation may produce a diminution of this peptide which may in turn result in increased sensitivity of the vasopressin receptor to exogenous AVP.

Conclusions and Implications of this work

In this thesis I have studied factors implicated in the secretion and effects of the neurohypophyseal hormone vasopressin. A novel peptide which may be of importance in modulating vasopressin secretion is ANF. Work is presented showing an inhibitory role, both in vivo and in vitro, for ANF in the secretion of vasopressin, although its site of action (above or below the blood-brain barrier) is uncertain.

In addition to my work with rats I have performed clinical studies to assess aspects of vasopressin in two neurological conditions as well as in normal controls. In migraine, despite pain and nausea which would normally stimulate vasopressin secretion, a sub-group of patients with pol^guria had a marked inhibition of vasopressin release. This suggests that the exhaustion and prostration felt by some migraineurs during their migraines may be due in part to dehydration.

In the other disorder of the central nervous system, I confirmed the clinical observation of a reduction in urine flow in tetraplegics whilst sitting, to be associated with a brisk rise in vasopressin secretion. This is in contrast to normal subjects who have only a small and gradual postural change in plasma AVP. Subjects with multiple system atrophy, in spite of a similar postural drop in

blood pressure, have no significant change in vasopressin secretion due to their afferent or central pathway lesions. Since this work was performed tetraplegic patients with oliguria whilst sitting, are being advised to lie down for an hour or two, rather than undergo surgery to their bladder necks *in* the mistaken belief that their oliguria was due to obstruction to the outflow of urine.

I have also investigated whether the enhanced release of vasopressin in response to a postural stimulus is associated with any changes in receptor mediated cardiovascular responses. Not only did I find in tetraplegics that the rise in vasopressin secretion in response to an osmotic stimulus was associated with a pressor response, not found in normal controls, but infusion of exogenous AVP was also associated with a pressor response. I further investigated whether the pressor sensitivity to AVP in the tetraplegic occurs independently of alpha and beta receptor occupancy.

In normal man alpha and beta blockade did not alter pressor sensitivity, suggesting that the lack of alpha and beta receptor occupancy alone was not the reason for the increased sensitivity to vasopressin in tetraplegic man.

Bibliography

Altura BM, Altura BT (1977). Vascular smooth muscle and neurohypophyseal hormones. Federation Proceedings, 36, 1853-1859.

Anand-Srivastava MB, Cantin M, Genest J (1985). Inhibition of pituitary adenylate cyclase by atrial natriuretic factor. Life Sciences, 36, 1873-1879.

Anderson JV, Bloom SR (1986). Atrial natriuretic peptide: what is the excitement all about? Journal of Endocrinology, 110, 7-17.

Anderson JV, Donckier J, Payne NN, Beacham J, Slater JD, Bloom SR (1987). Atrial natriuretic peptide: evidence of action as a natriuretic hormone at physiological plasma concentrations in man. Clinical Science, 72, 305-312.

Andersson B (1977). Regulation of body fluids. Annual Review of Physiology, 39, 185-200.

Anonymous (1984). Atrial natriuretic peptides (editorial). Lancet, 2, 328-329.

Anonymous (1986). Atrial natriuretic peptides (editorial). Lancet, 2, 371-372.

Antunes-Rodrigues J, McCann SM, Rogers LC, Samson WK (1985). Atrial natriuretic factor inhibits dehydration- and angiotensin II-induced water intake in the conscious, unrestrained rat. Proceedings of the National Academy of Science, 82, 8720-8723.

Antunes-Rodrigues J, McCann SM, Samson WK (1986). Central administration of atrial natriuretic factor inhibits saline preference in the rat. Endocrinology, 118, 1726-1728.

Atlas SA, Kleinert HD, Camargo MJ, Januszewicz A, Sealey JE, Laragh JH, Schilling JW, Lewicki JA, Johnson LK, Maack T (1984). Purification, sequencing and synthesis of natriuretic and vasoactive rat atrial peptide. Nature, 309, 717-719.

Atlas SA, Laragh JH (1986). Atrial natriuretic peptide: a new factor in hormonal control of blood pressure and electrolyte homeostasis. Annual Review of Medicine, 37, 397-414.

Baisset A, Douste-Blazy L, Montastruc P (1959). Reduction de la secretion d'aldosterone sous l'effet d'une distension auriculaire. Journal de Physiologie 51, 393-394.

Balfour WE (1985). Blood volume regulation. On the right lines at last? Nature, 314, 226-227.

Baylis PH, Robertson GL (1981). Arginine vasopressin response to insulin-induced hypoglycaemia in man. Journal of Clinical Endocrinology and Metabolism, 53, 935-940.

Bennett T, Gardiner SM (1986). Influence of exogenous vasopressin on baroreflex mechanisms. Clinical Science, 70, (4), 307-315.

Bhargava KP, Kulshrestha VK, Srivastava YP (1972). Central cholinergic and adrenergic mechanisms in the release of antidiuretic hormone. British Journal of Pharmacology, 44, 805-806.

Bianchi C, Gutkowska J, Thibault G, Garcia R, Genest J, Cantin M (1985). Radioautographic localization of ^{125}I -atrial natriuretic factor (ANF) in rat tissues. Histochemistry, 82, 441-452.

Bindler E, Labella FS, Sanwal M (1967). Isolated nerve endings (neurosecretosomes) from the posterior pituitary. Partial separation of vasopressin and oxytocin and the isolation of microvesicles. Journal of Cell Biology, 34, 185-205.

Bisset GW, Walker JM (1957). The effects of nicotine, hexamethonium and ethanol on the secretion of the antidiuretic and oxytocic hormones of the rat. British Journal of Pharmacology and Chemotherapy, 12, 461-467.

Bisset GW, Chowdrey HS (1984). A cholinergic link in the reflex release of vasopressin by hypotension in the rat. Journal of Physiology, 354, 523-545.

Bisset GW, Chowdrey HS, Feldberg W (1985). An osmosensitive zone on the dorsal surface of the medulla in the cat. Journal of Physiology, 365, 30P.

Blau JN (1982). Resolution of migraine attacks: sleep and the recovery phase. Journal of Neurology, Neurosurgery and Psychiatry, 45, 223-226.

Bonham A, Barron K, Allen M, Pollock D, Banks R, Brody MJ. (1983). Centrally-mediated pressor effect of atrial natriuretic factor in sino-aortic baroreceptor denervated (SAD) rats. Federation Proceedings, 42, (3), 494.

Brody MJ, Haywood JR, Touw KB (1980). Neural mechanisms in hypertension. Annual Review of Physiology, 42, 441-453.

Buggy J, Johnson AK (1977). Preoptic-hypothalamic periventricular lesions: thirst deficits and hypernatraemia. American Journal of Physiology, 233, R44-52.

Bunn SJ, Hanley MR, Wilkin GP (1985). Evidence for a kappa-opioid receptor on pituitary astrocytes: an autoradiographic study. Neuroscience Letters, 55(3), 317-323.

Burn JH, Truelove LH, Burn I (1945). Anticholinergic action of nicotine and of smoking. British Medical Journal, 1, 403-406.

Cambell DA, Hay KM, Tonks EM (1981). An investigation of the salt and water balance in migraine. British Medical Journal, 1424-1429.

Cantin M, Thibault G, Gutkowska J, Garcia R, Hamet P, Anand-Srivastava MB, Genest J (1985a). The heart as an endocrine gland. Regulatory Peptides, Suppl, 4, 83-88.

Cantin M, Genest J (1985b). The Heart and the Atrial Natriuretic Factor. Endocrine Reviews, 6, (2), 107-127.

Carswell F, Hainsworth R, Ledsome JR (1968). A diuretic response to left atrial distension in the isolated perfused kidney. Journal of Physiology, 195, 20-21P.

Carter DA, Lightman SL (1985). Neuroendocrine control of vasopressin secretion. In: Baylis PH, Padfield PL, eds. The Posterior Pituitary. Marcel Dekker, Inc., New York, pp 53-118.

Chard T (1982). An introduction to radioimmunoassay and related techniques. Elsevier Biomedical Press, Amsterdam.

Clark BJ, Silva MR (1967). An afferent pathway for the selective release of vasopressin in response to carotid occlusion and haemorrhage in the cat. Journal of Physiology, 191, 529-542.

Corbett JL, Frankel HL, Harris PJ (1971). Cardiovascular responses to tilting in tetraplegic man. Journal of Physiology, 215, 411-431.

Coulter HD, Elde RP, Unverzagt SL (1981). Co-localization of neurophysin- and enkephalin-like immunoreactivity in cat pituitary tetraplegic man. Journal of Physiology, 215, 411-431.

Cowley AW Jr., Monos E, Guyton AC (1974). Interactions of vasopressin and the baroreceptor reflex system in the regulation of arterial blood pressure in the dog. Circulation Research, 34, 505-514.

Currie MG, Geller DM, Cole BR, Siegel NR, Fok KF, Adams SP, Eubanks SR, Galluppi GR, Needleman P (1984). Purification and sequence analysis of bioactive atrial peptides (atriopeptins). Science, 223, 67-69.

De Bold AJ (1979). Heart atria granularity effects of changes in water-electrolyte balance. Proceedings of the Society for Experimental Biology and Medicine, 161, 508-511.

De Bold AJ, Borenstein HB, Veress AT, Sonnenberg H (1981).
A rapid and potent natriuretic response to intravenous
injection of atrial myocardial extracts in rats. Life
Sciences, 28, 89-94.

De Bold AJ (1982). Tissue fractionation studies on the
relationship between an atrial natriuretic factor and
specific atrial granules. Canadian Journal of Physiology
and Pharmacology, 60, 324-330.

De Bold AJ (1985). Atrial natriuretic factor: a hormone
produced by the heart. Science, 230, 767-770.

de Wardener HE, Mills IH, Clapham WF, Hayter CJ (1961).
Studies on the efferent mechanism of the sodium diuresis
which follows the administration of intravenous saline in
the dog. Clinical Science, 21, 249-258.

de Wardener HE (1977). Natriuretic hormone. Clinical
Science and Molecular Medicine, 53, 1-8.

Douglas WW, Poisner AN (1964). Stimulus-secretion coupling
in a neuro-secretory organ: the role of calcium in the
release of vasopressin from the neurohypophysis. Journal
of Physiology, 172, 1-18.

Dunn FL, Brennan TJ, Nelson AE, Robertson GL (1973). The role of blood osmolality and volume in regulating vasopressin secretion in the rat. Journal of Clinical Investigation, 52, 3212-3219.

Ebert TJ, Cowley AW, Skelton M, Smith JJ (1984). Physiologic vasopressin (AVP) infusion in man alters resting hemodynamics and reflex responses to low-level lower body negative pressure (LBNP). Federation Proceedings, 43, (4), 896.

Ekins RP (1960). The estimation of thyroxine in human plasma by an electrophoretic technique. Clinica Chimica Acta, 5, 453-459.

Eskay R, Zukowska-Grojec Z, Haass M, Dave JR, Zamir N (1986). Circulating atrial natriuretic peptides in conscious rats: regulation of release by multiple factors. Science, 232, 636-639.

Fabian M, Forsling ML, Jones JJ, Lee J (1969). The release, clearance and plasma protein binding of oxytocin in the anaesthetised rat. Journal of Endocrinology, 43, 175-189.

Fitts DA, Thunhorst RL, Simson JB (1985). Diuresis and Reduction of salt appetite by lateral ventricular infusions of atriopeptin II. Brain Research, 348, 118-124.

Fitzsimons JT (1986). Thirst and sodium appetite. In Lightman SL, Everett BJ, eds. Neuroendocrinology. Blackwell Scientific Publications, Oxford, pp 207-228.

Flynn TG, De Bold ML, De Bold AJ (1983). The amino acid sequence of an atrial peptide with potent diuretic and natriuretic properties. Biochemical and Biophysical Research Communications, 117, 859-865.

Forsling ML, Jones JJ, Lee J (1968). Factors influencing the sensitivity of the rat to vasopressin. The Journal of Physiology, 196, 495-505.

Garcia R, Cantin M, Thibault G, Ong H, Genest J (1982). Relationship of specific granules to the natriuretic and diuretic activity of rat atria. Experientia, 38, (19), 1071-1073.

Gauer OH, Henry JP (1963). Circulating basis of fluid volume control. Physiological Reviews, 43, 423-481.

Gauer OH, Henry JP, Sieker HO, Wendt WE (1954). Effect of negative pressure breathing on urine flow. Journal of Clinical Investigation, 33, 287-296.

Geelen G, Keil LC, Kravik SE, Wade CE, Thrasher TN, Barnes PR, Pyka G, Nesvig C, Greenleaf JE (1984). Inhibition of plasma vasopressin after drinking in dehydrated humans. American Journal of Physiology, 247, R968-971.

Geller DM, Currie MG, Wakitani K, Cole BR, Adams SP, Fok KF, Siegel NR, Eubanks SR, Galluppi GR, Needleman P (1984). Atriopeptins: a family of potent biologically active peptides derived from mammalian atria. Biochemical and Biophysical Research Communications, 120, (2), 333-338

Gerstberger R, Barden N (1986). Dynorphin 1-8 binds to opiate kappa receptors in the neurohypophysis. Neuroendocrinology, 42, 376-382.

Gerzer R, Witzgall H, Tremblay J, Gutkowska J, Hamet P (1985). Rapid increase in plasma and urinary cyclic GMP after bolus injection of atrial natriuretic factor in man. Journal of Clinical Endocrinology and Metabolism, 61, 1217-1219.

Gilmore JP (1968). Contribution of cardiac nerves to the control of body salt and water. Federation Proceedings, 27, (2), 1156-1159.

Ginsburg M, Smith MW (1959). The fate of oxytocin in male and female rats. British Journal of Pharmacology, 14, 327

Graybiel A, Glendy RE (1941). Circulatory effects following the intravenous administration of pitressin in normal persons and in patients with hypertension and angina pectoris. American Heart Journal, 21, 481-489.

Hamet P, Tremblay J, Pang SC, Garcia R, Thibault G, Gutkowska J, Cantin M, Genest J (1984). Effect of native and synthetic atrial natriuretic factor on cyclic GMP. Biochemical and Biophysical Research Communications, 123, (2), 515-527.

Hainsworth R, Kidd C, Linden RJ (1979). Cardiac Receptors. Cambridge University Press, Cambridge.

Hanley MR, Benton HP, Lightman SL, Todd K, Bone EA, Fretten P, Palmer S, Kirk CJ, Michell RH (1984). A vasopressin-like peptide in the mammalian sympathetic nervous system. Nature, 309, 258-261.

Henry JP, Gauer OH, Reeves JL (1956). Evidence of the atrial location of receptors influencing urine flow. Circulation Research, 4, 85-90.

Henry JP, Pearce JW (1956). The possible role of cardiac atrial stretch receptors in the induction of changes in urine flow. Journal of Physiology, 131, 572-585.

Hirs CHW (1967). Methods in enzymology, 11. Academic Press, New York, pp 197-199.

Huet M, Cantin M (1974). Ultrastructural cytochemistry of atrial muscle cells. II.Characterization of the protein content of specific granules. Laboratory Investigation, 30, 525-532.

Iitake K, Share L, Crofton JT, Brooks DP, Ouchi Y, Blaine EH (1986). Central atrial natriuretic factor reduces vasopressin secretion in the rat. Endocrinology, 119, 438-440.

Jacobowitz DM, Skofitsch G, Keiser HR, Eskay RL, Zamir N (1985). Evidence for the existence of atrial natriuretic factor-containing neurons in the rat brain. Neuroendocrinology, 40, 92-94.

Jamieson JD, Palade GE (1964). Specific granules in atrial muscle cell. The Journal of Cell Biology, 23, 151-172.

Januszewicz P, Gutkowska J, De Lean A, Thibault G, Garcia R, Genest J, Cantin M (1985a). Synthetic atrial natriuretic factor induces release (possibly receptor-mediated) of vasopressin from rat posterior pituitary. Proceedings of the Society for Experimental Biology and Medicine, 178, 321-325.

Januszewicz P, Gutkowska J, De Lean A, Thibault G, Garcia R, Genest J, Cantin M (1985b). The effect of synthetic atrial natriuretic factor on in vitro vasopressin release. Federation Proceedings, 44, 1200.

Januszewicz P, Thibault G, Garcia R, Gutkowska J, Genest J, Cantin M (1986). Effect of synthetic atrial natriuretic factor on arginine vasopressin release by the rat hypothalamo-neurohypophyseal complex in organ culture. Biochemical and Biophysical Research Communications, 134, 652-658.

Jessell TM (1978). Substance P release from the rat substantia nigra. Brain Research, 151, 469-478.

Jewell PA, Verney EB (1957). An experimental attempt to determine the site of the neurohypophyseal osmoreceptors in the dog. Philosophical Transactions of the Royal Society of London, 240, B, 197-324.

Johnson RH, Park D, Frankel HL (1971). Orthostatic hypotension and the renin-angiotensin system in paraplegia. Paraplegia, 9, 146-152.

Kimura T, Abe K, Shoji M, Tsunoda K, Matsui K, Ota K, Inoue M, Yasujima M, Yoshinaga K (1986). Effect of human atrial natriuretic peptide on renal function and vasopressin release. American Journal of Physiology, 250, R 789-794.

Kisch B (1956). Electron microscopy of the atrium of the heart. I. Guinea Pig. Experimental Medicine and Surgery, 14, 99-112.

Kuhn ER (1974). Cholinergic and adrenergic release mechanisms for vasopressin in the male rat: A study with injections of neurotransmitters and blocking agents into the third ventricle. Neuroendocrinology, 16, 255-264.

Kumada M, Sagawa K (1970). Aortic nerve activity during blood volume changes. American Journal of Physiology, 218, 961-965.

LaRoche Jr FT, North WG, Stern P (1980). A new extraction of arginine vasopressin from blood: the use of octadecasilyl- silica. Pflugers Archiv, 387, 79-81.

Leao AAP (1944). Spreading depression of activity in cerebral cortex. Journal of Neurophysiology, 7, 359-390.

Ledsome JR, Linden RJ, O'Connor WJ (1961). The mechanisms by which distention of the left atrium produces diuresis in anesthetized dogs. Journal of Physiology, 159, 87-100.

Liard JF. Atropine potentiates the pressor effect of arginine-vasopressin in conscious dogs. Journal of Cardiovascular Pharmacology, 6, 867-871.

Lightman SL, Todd K, Everitt B (1983a). Role for lateral tegmental noradrenergic neurons in the vasopressin response to hypertonic saline. Neuroscience Letters, 42, 55-59.

Lightman SL, Ninkovic M, Hunt SP, Iversen LL (1983b). Evidence for opiate receptors on pituicytes. Nature, 305, 235-237.

Lightman SL, Everitt BJ, Todd K (1984). Ascending noradrenergic projections from the brain stem: Evidence for a major role in the regulation of blood pressure and vasopressin secretion. Experimental Brain Research, 55, 145-151.

Lightman SL, Everitt BJ (1986). Water excretion. In Neuroendocrinology. eds. Lightman SL, Everitt BJ. Blackwell Scientific Publications, Oxford pp 197-

Living E (1873). On megrim, sick-headache and some allied disorders: a contribution to the pathology of nerve storms. J and A Churchill, London.

Maack T, Marion DN, Camargo MJF, Kleinert HD, Laragh JH, Darracott Vaughan E Jr., Atlas SA (1984). Effects of auriculin (atrial natriuretic factor) on blood pressure, renal function, and the renin-aldosterone system in dogs. The American Journal of Medicine, 77, 1069-1075.

Maack T, Camargo MJF, Kleinert HD, Laragh JH, Atlas SA (1985). Atrial natriuretic factor: Structure and functional properties. Kidney International, 27, 607-615.

Marie JP, Guillemot H, Hatt PY (1976). Le degre de granulation des cardiocytes auriculaires. Etude planimetrique au cours de differents apports d'eau et de sodium chez le rat. Pathologie Biologie, 24, 549-554.

Martin R, Voigt KH (1981). Enkephalins co-exist with oxytocin and vasopressin in nerve terminals of rat neurohypophysis. Nature, 289, 502-504.

Mason WT (1985). Staining of the magnocellular nuclei of the rat hypothalamus by a monoclonal antibody directed against the alpha-subunit of the nicotinic cholinergic receptor. Neuroscience Letters, 59, 89-95.

Mathias CJ, Christensen NJ, Corbett JL, Frankel HL, Goodwin TJ, Peart WS (1975). Plasma catecholamines, plasma renin activity and plasma aldosterone in tetraplegic man, horizontal and tilted. Clinical Science and Molecular Medicine, 49, 291-299.

Mathias CJ, Frankel HL, Christensen NJ, Spalding JMK (1976). Enhanced pressor response to noradrenaline in patients with cervical spinal cord transection. Brain, 99, 757-770.

Mathias CJ, Christensen NJ, Frankel HL, Peart WS (1980). Renin release during head-up tilt occurs independently of sympathetic nervous activity in tetraplegic man. Clinical Science, 59, 251-256.

Mathias CJ, Frankel HL, (1983). Autonomic failure in tetraplegia. In: Bannister R, ed. Autonomic Failure. Oxford University Press, Oxford, pp 453-488.

Matsuguchi H, Schmid PG (1982). Pressor response to vasopressin and impaired baroreflex function in DOC-salt hypertension. Americal Journal of Physiology, 242, H44-49.

Metzler CH, Thrasher TN, Keil LC, Ramsay DJ (1986). Inhibition of the baroreceptor reflex in conscious dogs by rat atrial peptide 1-28 (ANP). Federation Proceedings, 45, (4), 1024.

McKinley MJ, Denton DA, Weisinger RS (1978). Sensors for antidiuresis and thirst - osmoreceptors or CSF sodium detectors? Brain Research, 141, 89-103.

Milton AS, Paterson AT (1974). A microinjection study of the control of antidiuretic hormone release by the supraoptic nucleus of the hypothalamus in the rat. Journal of Physiology, 241 (3), 607-28.

Miselis RR, Eng R (1981). Disturbances in water balance after transection of subfornical organ efferent projections. Society for Neuroscience Abstracts, 7, 638.

Misono KS, Fukumi H, Grammer RT, Inagami T (1984). Rat atrial natriuretic factor: complete amino acid sequence and disulfide linkage essential for biological activity. Biochemical and Biophysical Research Communications, 119, 524-529.

Mohring J, Kintz J, Schoun J, McNeill JR (1981). Pressor responsiveness and cardiovascular reflex activity in spontaneously hypertensive and normotensive rats during vasopressin infusion. Journal of Cardiovascular Pharmacology, 3, 948-957.

Monos E, Cox RH, Peterson LH (1978). Direct effect of physiological doses of arginine vasopressin on the arterial wall in vivo. American Journal of Physiology, 234, H167-172.

Montani JP, Liard JF, Schoun J, Mohring J (1980). Haemodynamic effects of exogenous and endogenous vasopressin at low plasma concentrations in conscious dogs. Circulation Research, 47, 346-355.

Nakamura M, Katsuura G, Nakao K, Imura H (1985). Antidipsogenic action of alpha-human atrial natriuretic polypeptide administered intracerebroventricularly in rats. Neuroscience Letters, 58, 1-6.

Needleman P (1986). The expanding physiological roles of atrial natriuretic factor. Nature, 321, 199-200.

Obana K, Naruse M, Inagami T, Brown AB, Naruse K, Kurimoto F, Sakurai H, Demura H, Shizume K (1985). Atrial natriuretic factor inhibits vasopressin secretion from rat posterior pituitary. Biochemical and Biophysical Research Communications, 132, 1088-1094.

O'Connor WJ, Verney EB (1945). Effects of increased activity of sympathetic system in inhibition of water diuresis by emotional stress. Quarterly Journal of Experimental Physiology, 33, 77-90.

Oppenheimer D (1983). Neuropathology of progressive autonomic failure. In: Bannister R, ed. Autonomic Failure. Oxford University Press, Oxford, pp 267-283.

Palluk R, Gaida W, Hoefke W (1985). Minireview: atrial natriuretic factor. Life Sciences, 36, 1415-1425.

Pellegrino LJ, Pelligrino AS, Cushman AJ (1979). A stereotaxic atlas of the rat brain. Plenum Press, New York.

Pickford M (1947). Action of acetyl choline in supraoptic nucleus of chloralosed dog. Journal of Physiology, 106, 264-270.

Poole CJM (1986). Migraine. British Journal of Hospital Medicine, August, 90-93.

Pullan PT, Johnston CI, Anderson WP, Korner PI (1980). Plasma vasopressin in blood pressure homeostasis and in experimental renal hypertension. American Journal of Physiology, 239, H81-87.

Puritz R, Lightman SL, Wilcox CS, Forsling M, Bannister R (1983). Blood pressure and vasopressin in progressive autonomic failure. Brain, 106, 503-511.

Quillen EW Jr, Cowley AW Jr (1983). Influence of volume changes on osmolality-vasopressin relationships in conscious dogs. American Journal of Physiology, 244, H73-79.

Quirion R, Dalpe M, De Lean A, Gutkowska J, Cantin M, Genest J (1984). Atrial natriuretic factor (ANF) binding sites in brain and related structures. Peptides, 5, 1167-1172.

Quirion R, Dalpe M, Dam TH (1986). Characterization and distribution of receptors for the atrial natriuretic peptides in mammalian brain. Proceedings of the National Academy of Science, 83, 174-178.

Ragnarsson KT, Krebs M, Naftchi NE, Demeny M, Sell GH, Lowman EW, Tuckman J (1981). Head-up tilt effect on glomerular filtration rate, renal plasma flow and mean arterial pressure in spinal man. Archives of Physical Medicine and Rehabilitation, 62, 306-310.

Raghavan SRV, Gonick HC (1980). Partial purification and characterization of natriuretic factor from rat kidney. Proceedings of the Society for Experimental Biology and Medicine, 164, 101-104.

Ramsay DJ, Thrasher TN, Keil LC (1983). The organum vasculosum laminae terminalis: a critical area for osmoreception. In: The neurohypophysis structure, function and control. Eds. BA Cross, G Leng. Progress in Brain Research, 60, Elsevier, Amsterdam, pp 91-98.

Richards AM, Nicholls MG, Ikram H, Webster MWI, Yandle TG, Espiner EA (1985). Renal, haemodynamic, and hormonal effects of human alpha atrial natriuretic peptide in healthy volunteers. Lancet, 1, 545-549.

Robertson GL (1977). The regulation of vasopressin function in health and disease. Recent Progress in Hormone Research, 33, 333-385.

Robinson ICAF (1980). The development and evaluation of a sensitive and specific radioimmunoassay for oxytocin in unextracted plasma. Journal of Immunoassay, 1, 323-347.

Rossier J, Battenberg E, Pittman Q, Bayon A, Koda L, Miller, R, Guillemin R, Bloom R (1979). Hypothalamic enkephalin neurones may regulate the neurohypophysis. Nature, 277, 653-655.

Ryden H, Verney EB (1938). The inhibition of water diuresis by emotional stress and by muscular exercise. Quarterly Journal of Experimental Physiology, 27, 343-375.

Sagnella GA, MacGregor GA (1984). Cardiac peptides and the control of sodium excretion. Nature, 309, 666-667.

Samson WK (1985a). Atrial Natriuretic factor inhibits dehydration and haemorrhage-induced vasopressin release. Neuroendocrinology, 40, 277-279.

Samson WK (1985b). Atrial Natriuretic factor inhibits vasopressin release. Federation Proceedings, 44, 1200.

Samson WK, (1985c). Dehydration induced alterations in rat brain vasopressin and atrial natriuretic factor immunoreactivity. Endocrinology, 117, 1279-1281.

Saper CB, Standaert DG, Currie MG, Schwartz D, Geller DM, Needleman P (1985a). Atriopeptin-immunoreactive neurons in the brain: presence in cardiovascular regulatory areas. Science, 227, 1047-1049.

Saper CB, Standaert DG, Needleman P (1985b). Origin of atriopeptin-immunoreactive (AP-IR) innervation of the paraventricular nucleus of the hypothalamus in the rat. Neuroscience Letters, Suppl, 22, 445.

Sawchenko PE, Swanson LW (1982). The organisation of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat. Brain Research Reviews, 4, 275-325.

Schottstaedt WW, Wolff HG (1955). Variation in fluid and electrolyte excretion in association with vascular headache of the migraine type. AMA Archive Neurology and Psychiatry, 73, 158-164.

Schrier RW, Berl T, Anderson RJ (1979). Osmotic and non osmotic control of vasopressin release. American Journal of Physiology, 236, (4), F321-332.

Share L, Levy MN (1962). Cardiovascular receptors and blood titre of antidiuretic hormone. American Journal of Physiology, 203, 425-428.

Share L (1974). Blood pressure, blood volume, and the release of vasopressin. In: Handbook of Physiology, Section 7, Endocrinology IV, Part I, Chapter 11, pp 243-255.

Share L (1976). Role of cardiovascular receptors in the control of ADH release. Cardiology, 61 Suppl. 1, 51-64.

Sills MA, Nguyen KQ, Jacobowitz DM (1985). Increase in heart rate and blood pressure by microinjections of atrial natriuretic factor into the AV3V region of rat brain. Peptides, 6, 1037-1042.

Simantov R, Snyder SH (1977). Opiate receptor binding in the pituitary gland. Brain Research, 124, 178-184.

Simpson HCR, Zubillaga JE, Collier JG, Bennett ED, Ang VTY, Mehta N, Jenkins JS (1986). Haemodynamic effects of vasopressin in man are related to posture. Clinical Science, 70, 177-184.

Skofitsch G, Jacobowitz DM, Eskay RL, Zamir N (1985). Distribution of atrial natriuretic factor-like immunoreactive neurons in the rat brain. Neuroscience, 16, 917-948.

Sladek CD (1983). Regulation of vasopressin release by neurotransmitters, neuropeptides and osmotic stimuli. In: The neurohypophysis, structure, function and control. Eds. BA Cross and G Leng. Progress in Brain Research, 60, Elsevier, Amsterdam, pp 71-90.

Sladek CD, Armstrong WE (1985). Osmotic control of vasopressin release. Trends in Neurosciences, 8, 166-169.

Sonnenberg H, Veress AT, Borenstein HB, de Bold AJ (1980). Rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Physiologist, 23, (4), 13.

Spokes EGS, Bannister R, Oppenheimer DR (1979). Multiple system atrophy with autonomic failure: clinical, histological and neurochemical observations on four cases. Journal of Neurological Sciences, 43, 59-82.

Sved AF, McDowell FH, Blessing WW (1985). Release of antidiuretic hormone in quadriplegic subjects in response to head-up tilt. Neurology, 35, 78-82.

Swaminathan S (1980). Osmoreceptors or sodium receptors: an investigation into ADH release in the rhesus monkey. Journal of physiology, 307, 71-83.

Swanson LW, Sawchenko PE (1980). Paraventricular nucleus: a site for the integration of neuroendocrine and autonomic mechanisms. Neuroendocrinology, 31, 410-417.

Swanson LW, Sawchenko PE (1983). Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. Annual Review Neuroscience, 6, 269-324.

Thrasher TN, Nistal-Herrera JF, Keil LC, Ramsay DJ (1981). Satiety and inhibition of vasopressin secretion after drinking in dehydrated dogs. American Journal of Physiology, 240, E394-401.

Tremblay J, Gerzer R, Vinay P, Pang SC, Beliveau R, Hamet P (1985). The increase of cGMP by atrial natriuretic factor correlates with the distribution of particulate guanylate cyclase. FEBS letters, 181, 17-22.

Verney EB (1947). The antidiuretic hormone and the factors which determine its release. Proceedings of the Royal Society of London, B, 135, 25-106.

Wagner HN Jr., Braunwald E (1956). The pressor effect of the antidiuretic principle of the posterior pituitary in orthostatic hypotension. Journal of Clinical Investigation, 35, 1412-1418.

Williams TDM, Carter DA, Lightman SL (1985a). Sexual dimorphism in the posterior pituitary response to stress in the rat. Endocrinology, 116, 738-740.

Williams TDM, Lighthman SL, Bannister R (1985b). Vasopressin secretion in progressive autonomic failure: evidence for defective afferent cardiovascular pathways. Journal of Neurology, Neurosurgery and Psychiatry, 48, 225-228.

Williams TDM, Carter DA, Bannister R, Lightman SL, Mathias CJ (1986a). Pressor effect of arginine vasopressin infusion in autonomic failure. Clinical Science, 71, 173-178.

Williams TDM, Lightman SL, Leadbeater MJ (1986b). Hormonal and cardiovascular responses to DDAVP in man. Clinical Endocrinology, 24, 89-96.

Yalow RS, Berson SA (1960). Immunoassay of endogenous plasma insulin in man. Journal of Clinical Investigation, 39, 1157-1175.

Zamir N, Skofitsch G, Eskay RL, Jacobowitz DM (1986). Distribution of immunoreactive atrial natriuretic peptides in the central nervous system of the rat. Brain Research, 365, 105-111.

Zehr JE, Johnson JA, Moore WW (1969). Left atrial pressure, plasma osmolality and ADH levels in the unanaesthetised ewe. American Journal of Physiology, 217, 1672-1680.

Zerbe RL, Robertson GL (1983). Osmoregulation of thirst and vasopressin secretion in human subjects: effect of various solutes. American Journal of Physiology, 244, E607-614.