Modulation of cholinergic contraction by cathinone: beneficial effects in airway diseases

Véronique C. Freund-Michel¹, Mark A. Birrell¹, Hema J. Patel¹,
Iain M. Murray-Lyon², Maria G. Belvisi¹

¹ Respiratory Pharmacology Group, Airway Disease Section, Imperial College, National Heart and Lung Institute, London, UK; ² Chelsea and Westminster Hospital, London, UK

Corresponding author:
Professor Maria G. Belvisi
Respiratory Pharmacology
Airway Disease Section
National Heart and Lung Institute
Imperial College School of Medicine
Guy Scadding Building
Dovehouse Street
LONDON SW3 6LY, UK
Phone: + 44 2073578270
Fax: + 44 2073518173
E-mail: m.belvisi@imperial.ac.uk

Running head: Cathinone modulates cholinergic tone
Abstract

Infusion of khat leaves is an African traditional remedy used to treat airway diseases. The beneficial effects of Khat are thought to be due to the activity of its main active component cathinone.

Cathinone inhibited EFS-induced ACh release and contractions of smooth muscle and this activity could be responsible for the beneficial effects seen in airway disease. The mechanism of action of this natural product appears to be via the activation of both pre-junctional α2 adrenergic and 5-HT7 receptors.

This is a novel study describing how cathinone modulates airway tone, and may go someway to explaining the traditional use of khat as a remedy to alleviate respiratory disease symptoms.

In conclusion, cathinone may have beneficial effects in airway diseases with heightened cholinergic tone. There is some rationale in following up on these observations given previous experience of other traditional remedies being developed for therapeutic use.

Keywords: anticholinergic therapies, cathinone, chronic obstructive pulmonary diseases (COPD), parasympathetic innervation
Introduction

*Catha edulis* (khat) is an evergreen shrub which grows along the Eastern coast of Africa and in the Arabian Peninsula. Because of their stimulant properties, fresh khat leaves are commonly chewed in these countries [1]. These properties are thought to be due to its main active ingredient cathinone [2]. Interestingly, Khat leaves are also traditionally considered to be a herbal remedy for airway diseases [3, 4], but the mechanism behind this activity is as yet unknown. We have hypothesised that cathinone may contribute to the beneficial effects of khat through modulation of airway neural control.

In this work, we have hypothesised that cathinone modulates the neural control of airway tone and that this activity may be responsible for some of the beneficial effects seen. Elucidating the mechanism of action of cathinone it may lead to the development of new therapeutic entities for the treatment of respiratory diseases.
Material and methods

Preparation of human and/or guinea pig tracheal smooth muscle strips and Vas deferens
Lung tissues were obtained from human healthy donors as previously described [5]. Male Dunkin-Hartley guinea-pigs (300-500g, Harlan, Bicester, Oxon, UK) were killed by cervical dislocation, and *Vas deferens* and/or tracheal strips were prepared as previously described [10, 11].

Contractile/relaxant effects of cathinone per se
Cathinone (10⁻⁹ to 10⁻⁴M) or vehicle (0.1% dH₂O) was added to the baths with tracheal strips subjected to a resting tension of 1g, or pre-contracted with carbachol (10⁻⁵M).

Contractile responses evoked by exogenous acetylcholine
Cumulative concentration-response curves to acetylcholine (10⁻⁹ to 10⁻²M) were compared before and after incubation of the tissues with cathinone or vehicle for 3h.

EFS-induced contractile responses
EFS was elicited as previously described [6]. Cathinone (10⁻⁶ to 10⁻⁴M), nisoxetine (3x10⁻⁵M) or vehicle was incubated until maximal inhibition was observed. In some experiments, tracheal tissues were pre-incubated with the α₂ antagonists yohimbine or idazoxan and/or the 5-HT₇ antagonist SB269970 at least 30 min before addition of cathinone (10⁻⁵M). For electrically-evoked eNANC contractions, preparations of hilar bronchi were prepared as previously described [5].
Measurements of acetylcholine release from tracheal parasympathetic nerves

The release of ACh from cholinergic nerves was measured as previously described [5].

Expression of results and statistical analysis

Data are expressed as mg tension or as percentage change and presented as mean ± S.EM. of n independent observations. Parametric data were analysed by a two-tailed Student’s t test when comparing two groups, or by a one-way ANOVA followed by a Dunnett’s test when comparing several groups to the same control. Non parametric data were analysed by a Kruskal-Wallis test followed by a Dunn’s multiple comparison test. All data were analysed using GraphPad PRISM at a p<0.05 level of significance.
Results

**Contractile/relaxant activity of cathinone per se**
Cathinone had neither contractile activity on guinea pig tracheal smooth muscle strips subjected to a resting tension of 1g nor relaxant activity on strips pre-contracted with carbachol (10^{-5}M), as compared to the β_2 adrenergic agonist isoprenaline (10^{-9}-10^{-4}M) (Figure 1A).

**Effect of cathinone on EFS-induced contractile responses**
EFS induced NANC contractions of hilar bronchi of were unaffected by cathinone (Figure 1B). EFS induced cholinergic contractions of the tracheal strips were inhibited by cathinone in a concentration-dependent manner (Figure 1C). The maximal inhibition of 44.9 ± 3.1% was achieved with cathinone at 10^{-5}M after 138 ± 11 min of incubation.

**Effect of cathinone on contractile responses evoked by exogenous ACh**
ACh (10^{-9} to 10^{-2}M) induced contractions of guinea-pig tracheal strips in a concentration-dependent manner which was not modified after pre-incubation (3h) either with vehicle or with cathinone (Figure 1D).

**Effect of cathinone on EFS-induced cholinergic contractions of human tracheal strips**
EFS induced cholinergic contractions of the human tracheal strips that were inhibited by cathinone at 10^{-5}M (38.4 ± 5.8% inhibition, p<0.05, n=4) after 92 ± 15 min of incubation (Figure 2A and B).

**Modulation by cathinone of ACh release by tracheal parasympathetic nerves**
Cathinone (10^{-5}M) inhibited EFS-induced ACh release (26.2 \pm 10.2\% inhibition, n=4, p<0.05) (**Figure 3A and B**). The non selective muscarinic agonist oxotremorine M (10^{-6}M), used as a positive control [7], induced a 72.3 \pm 6.2\% inhibition of EFS-induced [^3H]ACh release from guinea pig tracheal strips (n=4, p<0.01).

**Modulation of guinea pig vas deferens contractile responses induced by EFS**

One possible mechanism of action for cathinone is on the airway sympathetic innervation. We studied the effect of cathinone on the *Vas deferens*, a tissue with a “classical” sympathetic innervation. Incubation with nisoxetine, an inhibitor of the noradrenaline transporter (10^{-9}-10^{-4}M) induced a concentration-dependent increase in the EFS-induced contractions of the guinea pig *Vas deferens*, with a maximal increase of 54.9 \pm 3.2\% at 3x10^{-5}M after 34 \pm 10 min of incubation. Incubation with cathinone induced a concentration-dependent inhibition of EFS-induced contractions of the guinea pig *Vas deferens*, with a maximal inhibition of 55.8 \pm 6.5\% at 10^{-5}M after 46 \pm 9 min of incubation (**Figure 4A and B**) suggesting that the mechanism of action is not via inhibition of the noradrenaline transporter.

**Effects of \(\alpha_2\) adrenergic and 5-HT\(_7\) antagonists**

The effect of cathinone on EFS-induced cholinergic contractions of guinea pig tracheal smooth muscle strips was reversed by the selective \(\alpha_2\) adrenergic antagonist yohimbine or by the selective 5-HT\(_7\) antagonist SB269970. The effect of yohimbine was confirmed by use of idazoxan, another \(\alpha_2\) adrenergic antagonist (10^{-5}M, 51.3 \pm 1.6\% inhibition, n=6, p<0.01). A combination of these two antagonists further inhibited the effect of cathinone (n=6, p<0.001) (**Figure 5A**). On human tracheal smooth muscle strips, the effect of cathinone was inhibited in a similar manner by yohimbine or by SB269970 (**Figure 5B**), and a combination of these two antagonists totally inhibited the effect of cathinone. No effect of yohimbine and/or
SB269970 was observed *per se* on EFS-induced cholinergic contractions of neither guinea pig nor human tracheal smooth muscle strips. The lack of effect of yohimbine on EFS-induced cholinergic contractions in guinea-pig trachea is consistent with previous data from Stretton and Barnes (1988) [20].
Discussion

The aim of this study was to determine whether cathinone had any beneficial activity on airway tone which may contribute to the beneficial properties of khat reported through its use as a traditional African remedy for the treatment of airway diseases. We report here for the first time that this natural product modulates the cholinergic control of the tracheal smooth muscle through concomitant activation of $\alpha_2$ adrenergic and 5-HT$_7$ presynaptic receptors and inhibition of acetylcholine release from parasympathetic nerves innervating the airways (Figure 6).

Cathinone has been demonstrated to be the main active constituent in fresh khat leaves [8] and increases the release of catecholamines in the brain and, as such, is thought to be responsible for the stimulant effect obtained by chewing khat [9]. A number of studies have shown that cathinone also affects the peripheral nervous system [10] [11]. Our new finding that cathinone targets the airways is therefore in accordance with these other reported peripheral actions of cathinone.

This is the first study to show that cathinone acts presynaptically to inhibit cholinergic contractions of the tracheal smooth muscle. Cathinone had been previously shown to induce its central [9] and some of its peripheral [17, 18] effects mainly via a sympathomimetic mechanism. We therefore investigated the effect of cathinone on the Vas deferens, a “classical” sympathetic system. In this organ, EFS-induced contractions are mainly due to the release of noradrenaline from presynaptic stores and activation of postsynaptic $\alpha_1$ adrenergic receptors [12]. A sympathomimetic effect in this system would result in increased EFS-induced contractions as exemplified in our experiments using nisoxetine, an inhibitor of the noradrenaline transporter. In contrast, cathinone inhibited EFS-induced contractions of the
guinea pig Vas deferens consistent with other recent studies demonstrating sympathomimetic-independent effects of cathinone \cite{13}.

Interestingly, a previous study had reported that cathinone only exhibited affinity for two receptors: \(\alpha_2\) adrenergic and 5-HT\(_7\) receptors when screened against a large battery of cloned human receptors and transporters \cite{14}. Interestingly, the existence of presynaptic \(\alpha_2\) adrenergic and 5-HT\(_7\) receptors has previously been suggested on parasympathetic nerves innervating the trachea, with their activation reducing EFS-induced contractions through inhibition of acetylcholine release \cite{15} \cite{16}. However, functional effects of cathinone via activation of these receptors have never been reported. We therefore investigated this as a possible mechanism and showed that the inhibitory effect of cathinone was inhibited in human tissues by a combination of \(\alpha_2\) and 5-HT\(_7\) antagonists.

In conclusion, we have shown for the first time that cathinone presynaptically inhibits acetylcholine release from airway parasympathetic nerves through activation of both \(\alpha_2\) and 5-HT\(_7\) presynaptic receptors. This effect may contribute to the beneficial effects of khat that have been traditionally reported in airway diseases \cite{3, 4}. In addition, the dual mechanism of action revealed for cathinone in this study may be particularly beneficial in airway diseases displaying a heightened cholinergic tone, such as asthma associated with gastro-oesophageal reflux \cite{17}, nocturnal asthma \cite{18} or chronic obstructive pulmonary diseases (COPD) \cite{19}. The development of new anticholinergic molecules based on mechanisms of action other than antagonising muscarinic receptors may be particularly interesting in the treatment of such airway diseases with heightened cholinergic tone.

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References

Figures legends

Figure 1. Effects of cathinone on the parasympathetic neural control of the airway tone
A) Relaxant effect of cathinone (10^{-9} to 10^{-4}M) was investigated on guinea pig tracheal smooth muscle strips pre-contracted with the stable muscarinic agonist carbachol (10^{-5}M), in the presence of indomethacin (non-selective COX inhibitor, 10^{-5}M). The $\beta_2$ adrenergic agonist isoprenaline (10^{-9} to 10^{-4}M) was used as a positive control. Data are expressed as a percentage of relaxation and are presented as means ± S.E.M. of 3 independent experiments.

B) Electrically-evoked eNANC contractions were obtained on preparations of hilar bronchi in presence of indomethacin (non-selective COX inhibitor, 10^{-5}M), propranolol (non-selective $\beta$-adrenergic antagonist, 10^{-6}M) and atropine (non-selective muscarinic antagonist, 10^{-6}M). eNANC contractions were studied in the presence or absence of cathinone (10^{-5}M) or vehicle (0.1% dH2O final concentration), incubated to the baths during 20min. Data are expressed as absolute values in mg contraction and are presented as means (blocks) ± S.D. (bars) of 3 independent experiments.

C) Electrically-evoked cholinergic contractions were induced in guinea pig tracheal smooth muscle strips (300mA at source, 0.5ms pulse width, 4Hz, 15s every 4min), in presence of indomethacin (non-selective COX inhibitor, 10^{-5}M). Cholinergic contractions were studied in the presence or absence of cathinone (10^{-6} to 10^{-5}M) or vehicle (0.1% dH2O final concentration), incubated in the baths until maximal inhibition was observed. Data are expressed as a percentage of inhibition compared to the control and are presented as means ± S.E.M. of 6 independent experiments. Data were analysed by a one-way ANOVA followed by a Dunnett’s test. **: p<0.01. A representative trace of EFS-induced contractions of a guinea pig tracheal strip inhibited by cathinone (Cath, 10^{-5}M) is presented on the right, as compared to the effect of the vehicle (Veh, 0.1% dH2O final concentration).

D) Exogenous acetylcholine dose-response (10^{-9} to 10^{-2}M) was performed before and after cathinone (10^{-5}M) pre-incubation (3h), in presence of indomethacin (10^{-5}M). Data are
expressed as absolute values in mg contraction and are presented as means ± S.E.M. of 6 independent experiments.
Figure 2. Effect of cathinone on EFS-induced contractions of human tracheal strips

A) Electrically-evoked cholinergic contractions were induced in human tracheal strips (300mA at source, 0.5ms pulse width, 8Hz, 15s every 4min), in the presence of indomethacin (non-selective COX inhibitor, $10^{-5}$M). Cholinergic contractions were studied in the presence...
or absence of cathinone (10^{-5}M) or vehicle (0.1% dH2O final concentration), incubated to the baths until maximal inhibition was observed. Data are expressed as a percentage of inhibition compared to the control and are presented as means ± S.E.M. of 4 independent experiments. Data were analysed by a two-tailed Student’s t test. *: p<0.05.

B) Representative trace of EFS-induced cholinergic contractions of a human tracheal smooth muscle strip inhibited by cathinone (Cath, 10^{-5}M), in comparison with the effect of the vehicle (Veh, 0.1% dH2O final concentration).
A) Effect of cathinone ($10^{-5}$M) on EFS-induced $[^3]$H]ACh release from guinea pig tracheal strips. Each tissue acted as its own control such that the data presented reflect the percentage change in EFS-induced $[^3]$H]ACh output after drug administration, relative to the first control stimulation. Data are presented as means ± S.E.M. of 4 independent experiments. Data were analysed by a two-tailed Student’s t test for paired data. *: p<0.05.
B) Representative data of the inhibition of EFS-induced $[^3]$H]ACh release from an individual guinea-pig tracheal strip by vehicle (dH$_2$O, 0.1% final concentration) or cathinone ($10^{-5}$M). Each point represents the rate coefficient of ACh output, which is a measure of the fractional quantity of tritium released per unit time.
A) Electrically-evoked contractions were induced in guinea pig Vas deferens portions (300mA at source, 1ms pulse width, 20Hz, 1s every min), in presence of indomethacin (non-selective COX inhibitor, $10^{-5}$M). Contractions were studied in the presence or absence of nisoxetine (inhibitor of the noradrenaline transporter, $3x10^{-5}$M), cathinone ($10^{-5}$M) or vehicle.
(dH₂O, 0.1% final concentration), incubated to the baths until maximal inhibition was observed. Data are expressed as absolute values in mg contraction and are presented as means ± S.E.M. of 6 independent experiments. Data were analysed by a one-way ANOVA followed by a Dunnett’s test. **: p<0.01.

B) Representative traces of EFS-induced contractions of the guinea pig Vas deferens potentiated by nisoxetine (3x10⁻⁵M) or inhibited by cathinone (10⁻⁵M) as compared to the effect of the vehicle.
EFS-induced contractions were induced in guinea pig (A) or human (B) tracheal smooth muscle strips. The effect of cathinone ($10^{-5}$M) was assessed in presence or absence of yohimbine (Yohimb, $\alpha_2$ adrenergic selective antagonist, $10^{-5}$M) and/or SB269970 (SB, 5-HT$_7$ selective antagonist, $10^{-5}$M). Both antagonists were incubated 30min with tissues before adding cathinone, and indomethacin (non-selective COX inhibitor, $10^{-5}$M) was present throughout the experiment. Data are expressed as a percentage of inhibition compared to the control and are presented as means ± S.E.M. of 6 and 3 independent experiments in guinea pig and human tracheal smooth muscle strips respectively. Data were analysed by
Kruskal-Wallis test followed by a Dunn’s multiple comparison test. *: p<0.05 and ***: p<0.001 as compared with the controls. #: p<0.05, ##: p<0.01 and ###: p<0.001 as compared with cathinone.
On the left, Electrical field stimulation (EFS) induces acetylcholine release from parasympathetic nerves innervating the tracheal smooth muscle, which activates its postsynaptic M₃ muscarinic receptors and induces contractions of the airway smooth muscle. On the right, in presence of cathinone, presynaptic α₂ adrenergic and 5-HT₇ receptors are activated and inhibit EFS-induced acetylcholine release from airway parasympathetic nerves, therefore reducing acetylcholine-induced contractions of the airway smooth muscle through reduced postsynaptic muscarinic M₃ receptors activation.