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RESEARCH PAPER

The effect of epibatidine on spontaneous and evoked neurotransmitter release in the mouse and guinea pig isolated vas deferens

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Background and purpose: Nicotinic agonists increase sympathetic field-stimulus-evoked contraction of the rodent vas deferens, presumably by increasing evoked neurotransmitter release. This presumption was tested in two species.
Experimental approach: The effect of the nicotinic acetylcholine receptor (nAChR) agonist epibatidine on neurotransmitter release in mouse and guinea pig isolated vas deferens was investigated using contraction studies and conventional intracellular recording techniques.
Key results: In 12 of 14 mouse vasa deferentia, slow bath application of epibatidine (100 nM) had no significant effect on excitatory junction potential (EJP) amplitude and spontaneous EJP (SEJP) frequency. However, rapid application of epibatidine to the mouse vas deferens caused an increase in SEJP frequency (by 530%), with no effect on EJP amplitude. Despite the absence of an effect on EJPs, electrically-evoked contractions of the mouse vas deferens were significantly increased in the presence of epibatidine (by 50%). A transient contraction was reliably induced by a higher epibatidine concentration (1 μM). This contraction was significantly reduced in the presence of prazosin, tetrodotoxin, or α,β-methyleneATP. Epibatidine did not induce a contraction in the presence of a combination of prazosin, α,β-methyleneATP and cyclopentolate. In guinea pig vasa deferentia, bath-applied epibatidine potentiated EJP amplitude in a biphasic pattern, lasting for at least 30 minutes.
Conclusion and implications: The nAChR-mediated augmentation of neurogenic contraction is indeed prejunctional, but in the mouse arises from an increase in spontaneous neurotransmitter release that primes smooth muscle for subsequent contraction, while in the guinea pig there is a direct augmentation of evoked neurotransmitter (ATP) release.
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Keywords: prejunctional; nicotinic; epibatidine; intracellular recording; mouse; guinea pig; vas deferens; sympathetic; electrophysiology; neurotransmission

Abbreviations: α,β-MeATP, α, β-MethyleneATP; EJPs, excitatory junction potentials; nAChRs, nicotinic acetylcholine receptors; NA, noradrenaline; PSS, physiological saline solution; RMP, resting membrane potential; SEJPs, spontaneous excitatory junction potentials; TTX, tetrodotoxin

Introduction

The increase in neurotransmitter release that follows the activation of nicotinic acetylcholine receptors (nAChRs) on central (Pidoplichko *et al*., 1997) and peripheral (Rose *et al*., 1999) nerve terminals contributes to nicotine dependence. Furthermore, the activation of nAChRs located on sympathetic nerves is thought to be partially responsible for the detrimental effects of nicotine on cardiac function (Haass and Kubler, 1997). Despite these powerful effects, the mechanisms involved in the nicotinic potentiation of neurotransmitter release remains to be fully elucidated.

The rodent vas deferens has a rich innervation of sympathetic nerves (Sjöstrand, 1965) and exposure of the vas deferens to nAChR agonists has previously been shown to evoke neurotransmitter release. This increase in neurotransmitter release leads to both a transient contraction and a potentiation of electrically evoked contraction in guinea pig (Todorov *et al*., 1991; von Kügelgen and Starke, 1991b) and a potentiation of electrically evoked contraction in mouse (Brain *et al*., 2001). For these reasons, the rodent vas deferens provides a good model to investigate the effect of nAChR activation on sympathetic neurotransmitter release in an intact tissue. In this study, epibatidine, a potent and

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specific nAChR nicotinic agonist with no other known actions (Badio and Daly, 1994), was chosen to investigate nAChR activation.

Methods

Tissue preparation

Vasa deferentia were removed from 8–12-week-old Balb/c mice or 300–600 g guinea pigs which were killed by cervical dislocation in accordance with the guidelines of the UK Animal (Scientific Procedures) Act 1986. The prostatic quarter of each vas deferens was removed to ensure that no sympathetic ganglia were present in the preparation. The bathing physiological saline solution (PSS) contained: NaCl 118.4 mM, $NaHCO_3$ 25.0 mM, NaH_2PO_4 1.13 mM, $CaCl_2$ 1.8 mM, KCl 4.7 mM, $MgCl_2$ 1.3 mM and glucose 11.1 mM. The solution was gassed with a mixture of 95% O_2 and 5% CO_2 to pH 7.4 and maintained at a temperature of 35–37°C.

Contraction studies

Each vas deferens was mounted in a 5 ml organ bath (Letica Scientific Instruments, Hospitalet, Spain) under a tension of 9.8 mN and the isometric contraction of the longitudinal smooth muscle layer was measured using a force transducer (Letica Scientific Instruments, Spain). The preparations were washed with fresh PSS every 20 min and left to equilibrate for 60 min before the experiment. Field stimuli were applied to the preparation through platinum ring electrodes positioned at the prostatic end of the vas deferens. Contractions were evoked by five stimuli at 10 Hz (95 V amplitude and 0.5 ms pulse width) and were delivered every 30 s using a custom-built digital stimulator (Department of Pharmacology, Oxford, UK). To ensure that the contractions were neurogenic, tetrodotoxin (TTX) sensitivity was tested. Following incubation with TTX (300 nM) for 40 min, electrically evoked contractions were abolished (number of preparations (n_v) = 6; see Figure 4aii). Drugs were added directly to the organ bath as a bolus in a small volume ($<100\,\mu$l). Contractions were recorded using a PowerLab 4SP data acquisition system (AD Instruments, Chalgrove, Oxfordshire, UK) connected to a G3 computer (Apple) running Chart 4 software (AD Instruments, UK).

Electrophysiological studies

Conventional intracellular recording techniques were used to record excitatory junction potentials (EJPs) in smooth muscle cells. The vas deferens was carefully pinned to the Sylgard (Dow-Corning, Allesley, Coventry, UK) covered base of a 5-ml Perspex organ chamber perfused with PSS at a rate of 2 ml/min. Drugs were added to the preparations either by slow bath application or rapid application. For slow bath application, drugs were added to the organ chamber by exchanging the perfusing PSS to one containing the drugs at the required final bath concentration. For rapid application, a 2-ml solution of warmed and gassed PSS containing the drug was added using a Gilson pipette directly to the organ chamber. To ensure adequate mixing, the volume of the superfusate was reduced to a minimum (1 ml) and the epibatidine solution was prepared and added to the organ bath at the highest volume possible (2 ml). To prevent dilution of epibatidine, the superfusion system was stopped while recording in the presence of epibatidine.

EJPs were evoked by electrical stimuli (pulse width 0.1 ms, 15 V amplitude and supramaximal for axon recruitment) were delivered through a pair of platinum electrodes positioned around the prostatic end of the vas deferens. The stimuli were generated with a digital stimulator (Applegarth Instruments, Oxford, UK) coupled to an optically isolated stimulus unit.

The membrane potential of individual smooth muscle cells was recorded using a sharp microelectrode. Microelectrodes were connected by an Ag/AgCl wire to the input headstage of an Axoclamp 2B (Molecular Devices, Sunnyvale, CA, USA). The data were digitised using a PowerLab 4SP (AD Instruments, UK) and recorded on a G4 computer (Apple) with Chart 5 software (AD Instruments, UK). Microelectrodes were pulled from borosilicate glass tubing containing an inner glass filament (outer diameter 1.5 mm, inner diameter 0.86 mm; Clark Electromedical, USA) using a Flaming-Brown P87 electrode puller (Sutter Instruments, USA). The microelectrodes were filled with 5 M potassium acetate and had tip resistances of 30–90 MΩ.

EJP amplitude was measured from the resting membrane potential (RMP) to the peak amplitude following field stimulation. The RMP was calculated by averaging the membrane potential 100 ms before stimulation. Spontaneous excitatory junction potentials (SEJPs) were automatically detected using the 'template' function of Chart 5. This function uses a correlation algorithm to compare the trace with a selected typical SEJP in a given experiment. The template was redefined for each cell. The algorithm applies a rolling normalisation so that the correlation coefficient was independent of the baseline and amplitude of any SEJP. Trace segments with correlation coefficients of >0.8 (where 1 is an exact match) and amplitudes of >2 mV were counted as SEJPs; below an amplitude of 2 mV, SEJPs were difficult to distinguish from baseline noise.

Statistical analysis

Data were analysed using Prism 4 software (GraphPad Software, San Diego, CA, USA). Paired or unpaired Student's *t*-tests were carried out as appropriate.

Drugs

Stock solutions of α,β-methylene ATP (α,β-MeATP) (lithium salt), prazosin (hydrochloride), TTX (citrate), hexamethonium (chloride) and cyclopentolate (hydrochloride) were dissolved in distilled water. Epibatidine (hydrochloride) was dissolved in DMSO. The maximum final concentration of DMSO was 0.1%. All solutions were prepared and aliquotted before storing at −20°C. All drugs passed through a maximum of one freeze–thaw cycle. Epibatidine (dihydrochloride) was obtained from Tocris, UK; all other drugs were obtained from Sigma-Aldrich (Dorset, UK). The drug vehicle alone was without effect.

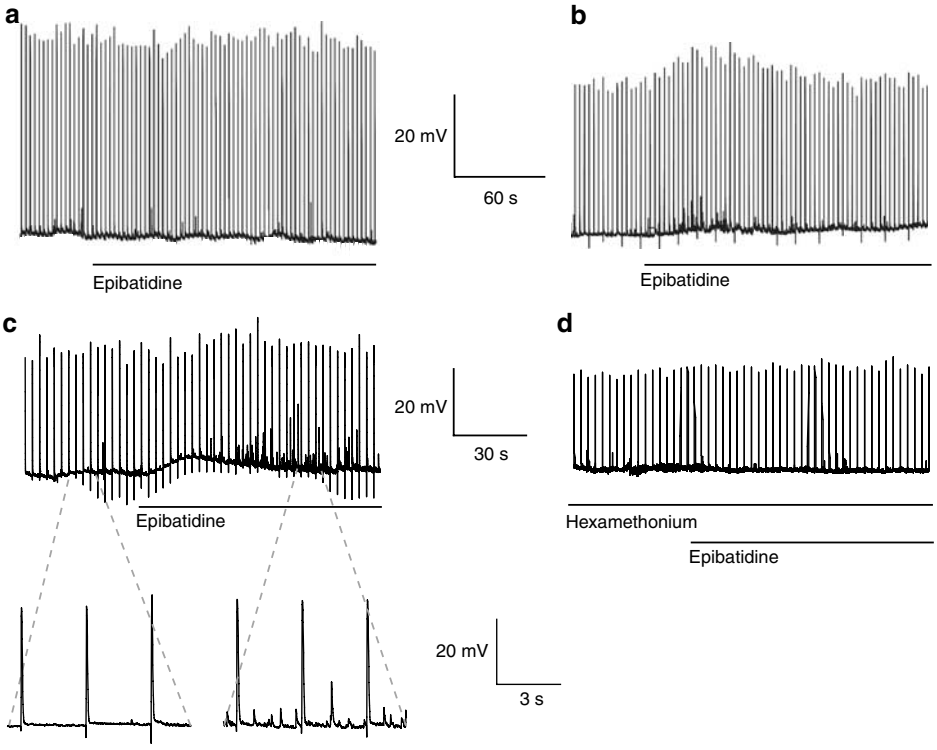

Figure 1 The effect of slow and rapid application of epibatidine (100 nM) on neurotransmitter release in the mouse vas deferens. (**a**) A typical trace of the membrane potential following slow, bath application of epibatidine (100 nM). EJPs were evoked at a frequency of 0.33 Hz. In most experiments, there was no clear effect of epibatidine on EJP amplitude or SEJP frequency. (**b**) In two experiments, a weak potentiation of EJPs and a small increase in SEJP frequency was observed following addition of epibatidine. (**c**) A typical electrophysiological trace showing that the rapid application of 100 nM epibatidine had no significant effect on EJP amplitude but clearly increased SEJP frequency. Portions of the trace before and following the addition of epibatidine are expanded to show SEJPs more clearly. The apparent depolarisation of the RMP shown in (**c**) was not consistently observed. (**d**) A typical trace showing that following incubation with hexamethonium (100 μM) for 60 min, the increase in SEJP induced by rapid application of epibatidine is abolished.

Results

Effect of bath-applied epibatidine on EJPs and SEJPs in the mouse vas deferens

The effect of nAChR activation on sympathetic neurotransmitter release was first investigated using slow bath application of the nAChR agonist epibatidine on the mouse vas deferens. EJPs were evoked at a frequency of 0.33 Hz and monitored using conventional intracellular recording. Following slow-bath application of epibatidine (100 nM), EJP amplitude was not significantly increased; 2 min after epibatidine addition, the average EJP amplitude was $106 \pm 3\%$ of the control EJP amplitude (compared with 2 min before epibatidine addition; $P = 0.13$; $n_v = 14$; Figure 1a). In two experiments, a weak potentiation of EJP amplitude occurred following the application of epibatidine. This potentiation reached a maximum 1 min after epibatidine addition and decreased back to control levels within 2 min (Figure 1b). The frequency of SEJP occurrence was not significantly different in the presence of epibatidine, $128 \pm 26\%$ of the control ($P = 0.82$, $n_v = 14$; Figure 1a).

The effect of rapid addition of epibatidine on EJPs and SEJPs in the mouse vas deferens

Desensitisation of nAChRs during slow application of epibatidine may account for the lack of effect on EJPs. In order to add epibatidine more rapidly in the electrophysiological experiments, epibatidine was added directly into the bath (producing a final bath concentration of 100 nM). When epibatidine was added, the amplitude of EJPs (during the 120 s after addition) was $100 \pm 5\%$ of the control EJP amplitude (during the 60 s before epibatidine addition; $P = 0.82$, $n_v = 8$, number of cells (n_c) = 14; Figure 1c). In contrast to the lack of effect on EJPs, rapid application of epibatidine (100 nM) significantly increased the frequency of SEJP occurrence to $629 \pm 189\%$ of the control frequency ($P < 0.05$, $n_v = 8$, $n_c = 14$; Figure 1c). In the presence of hexamethonium, rapid application of 100 nM epibatidine had no effect on SEJP frequency: $90 \pm 14\%$ of the control frequency ($P = 0.31$, $n_v = 8$, $n_c = 21$; Figure 1d).

Effect of bath-applied epibatidine on EJPs and SEJPs in the guinea pig vas deferens

In the guinea pig isolated vas deferens, epibatidine (100 nM) induced a significant increase in EJP amplitude (11 of 12 tissues), reaching a maximum of $285 \pm 35\%$ of the control amplitude 6 min after addition of epibatidine. The EJP amplitude then decreased to about 175% of the control and remained significantly greater than the control for at least 30 min in the continued presence of epibatidine ($P < 0.05$; $n_v = 11$; Figure 2a(i) and b). When the guinea pig

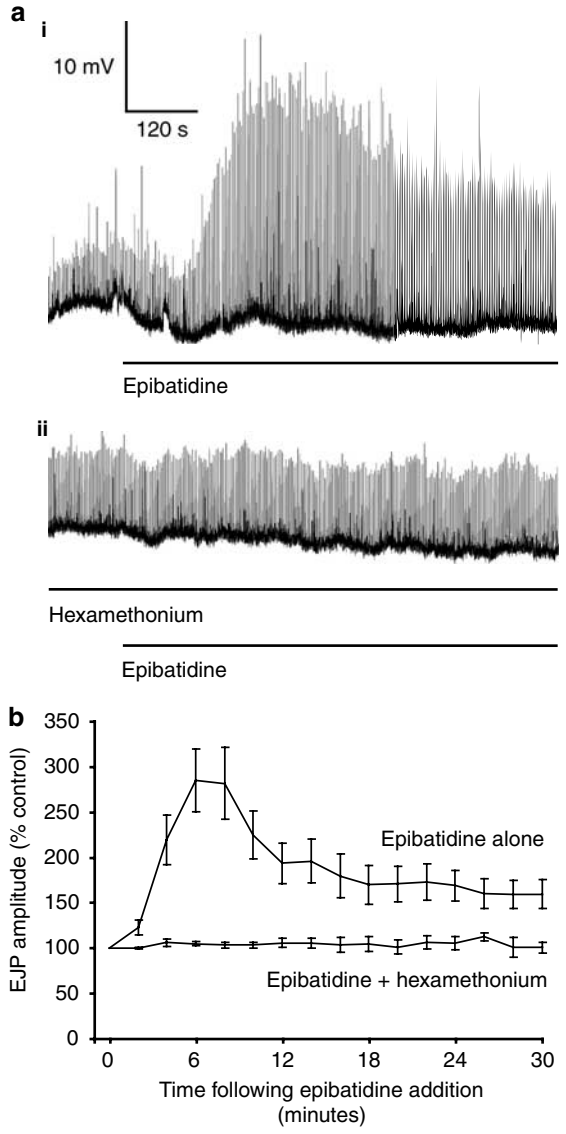

Figure 2 The effect of bath application of epibatidine (100 nM) on EJP amplitude in the guinea pig vas deferens. (**a**) Typical traces showing membrane potential of the smooth muscle cells following neuronal stimulation at 0.33 Hz. (i) After addition of epibatidine, there was a transient large potentiation in EJP amplitude followed by a more sustained, smaller potentiation of EJP amplitude. (ii) In the presence of hexamethonium (100 μM) for 60 min, the potentiating effects of epibatidine were blocked. (**b**) Graph showing the effect of epibatidine alone and in the presence of hexamethonium on normalised EJP amplitude. Results shown are means $\pm$ s.e.m. from 11 (epibatidine alone) or 13 (epibatidine + hexamethonium) experiments.

vas deferens was pretreated with hexamethonium (100 μM) for 1 h, the effect of epibatidine was almost abolished; EJP amplitude was $105 \pm 3\%$ of the control amplitude after 6 min ($P<0.05$, $n_v=13$; Figure 2a(ii) and b). In four of 12 preparations, a transient increase in SEJP frequency occurred reaching an average maximum of $427 \pm 76\%$ of the control SEJP frequency between 4 and 8 min (depending on the preparation) following epibatidine addition and lasted approximately 2 min.

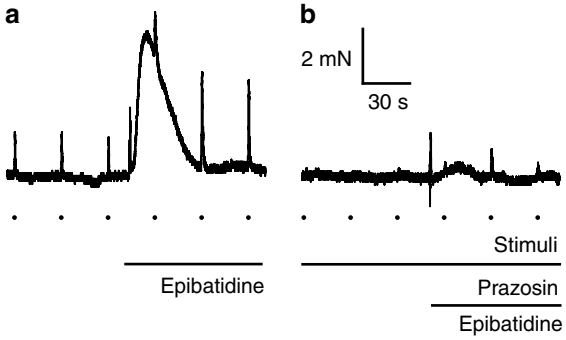

Figure 3 The effect of epibatidine (100 nM) on neurogenic contraction in the mouse vas deferens. (**a**) A trace showing the effect of epibatidine (100 nM) on neurogenic contraction in the mouse vas deferens. In 50% of the preparations, a transient contraction was observed following epibatidine addition. Neurogenic contractions, evoked every 30 s by a train of five stimuli at 10 Hz (marked by the dots below the trace) were potentiated following epibatidine addition. (**b**) Following the addition of prazosin (100 nM) for 40 min, the amplitude of neurogenic contractions was still potentiated by epibatidine. On both traces, a movement artefact is visible when epibatidine is added.

Effect of epibatidine on electrically evoked contraction in the mouse vas deferens

Although the lack of an effect of epibatidine on EJP amplitude in the mouse argues against an increase in the evoked release of ATP, it is possible that epibatidine selectively potentiates NA, release, which would not be detected by intracellular recording (Burnstock and Holman, 1964). To investigate the role of noradrenaline (NA), contraction studies were carried out. Contractions were evoked by trains of five stimuli at a frequency of 10 Hz every 30 s. The amplitude of electrically evoked contractions was measured 2 min before and 2 min after epibatidine addition. The calculated amplitude of electrically evoked contractions did not include the amplitude of any epibatidine-induced contraction. Following addition of epibatidine (100 nM), the amplitude of the electrically evoked contraction increased from an average of 1.9 ± 0.3 mN to 2.9 ± 0.3 mN ($P<0.05$, $n_v=10$; Figure 3a). A potentiation of electrically evoked contraction was observed in all preparations tested. When epibatidine exposure was brief (less than 120 s), this epibatidine-induced potentiation of the electrically evoked contraction could be consistently elicited an hour later. In the presence of the α_1-adrenoreceptor antagonist prazosin (100 nM) for 40 min, the amplitude of electrically evoked contraction was significantly reduced ($P<0.05$, Figure 3b). Following addition of epibatidine, the amplitude of the electrically evoked contraction increased from 0.3 ± 0.1 to 0.6 ± 0.1 mN ($P<0.05$, $n_v=10$; Figure 3b).

Epibatidine-induced contraction in the mouse vas deferens

Immediately following the addition of epibatidine (100 nM), a transient contraction occurred in 5 out of 10 of the preparations (Figure 3a). Such a contraction could be induced in all preparations tested, by the addition of a higher concentration of epibatidine (1 μM; Figure 4a(i) and b). Repeated application of this higher concentration of epibatidine, even after a 3 h wash, could not reliably induce

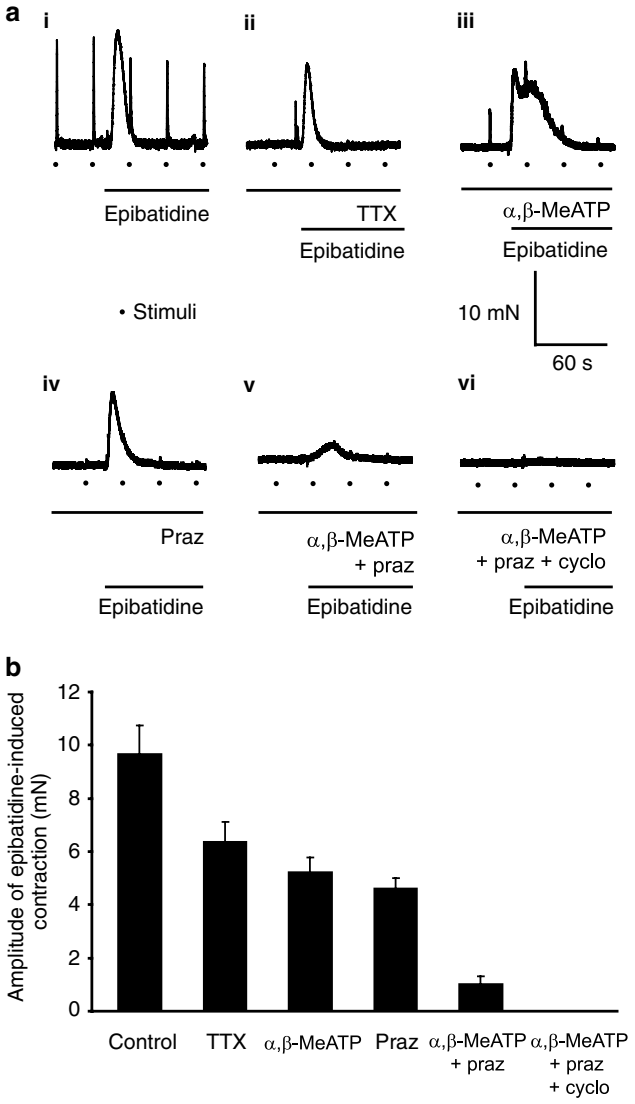

Figure 4 Epibatidine-induced contraction of mouse vas deferens. (**a**) Typical traces showing a transient contraction following the addition of epibatidine (1 μM), either (i) without other drugs or (ii) following pre-treatment with TTX 300 nM for 40 min, (iii) α,β-MeATP 1 μM for 1 h or (iv) prazosin 100 nM for 40 min. Pretreatment with (v) both α,β-MeATP and prazosin or (vi) α,β-MeATP, prazosin and cyclopentolate 1 μM for 1 h, led to further diminution of the epibatidine-induced contraction. (**b**) Bar chart showing the amplitude of epibatidine-induced contractions in the presence of various drugs. Each drug significantly reduced the amplitude of contraction in comparison with control experiments ($P<0.05$). In the presence of cyclopentolate, α,β-MeATP and prazosin, the amplitude of contraction was below the level of detection. Results shown are means$\pm$s.e.m. from 6 to 11 experiments in each group.

contraction of similar amplitude. For this reason, each experiment was conducted on a separate tissue. Following the addition of 1 μM epibatidine, the amplitude of electrically evoked contractions was reduced (Figure 4a(i)); this reduction was not investigated further. The average amplitude of the epibatidine-induced contraction was 9.6 ± 1.1 mN ($n_v=6$; Figure 4a(i) and b). The epibatidine-induced contraction was significantly reduced by the voltage-gated Na^+ channel blocker TTX (300 nM, a concentration that abolished electrically evoked contractions) to an average of 6.3 ± 0.8 mN ($P<0.05$, $n_v=6$; Figure 4a(ii) and b). Following desensitisation of P2X receptors by the addition of α,β-MeATP (1 μM) for 1 h, the average epibatidine-induced contraction was significantly reduced from the control value to 5.1 ± 0.5 mN ($P<0.05$, $n_v=11$; Figure 4a(iii) and b). It is noteworthy that in the presence of α,β-MeATP, the epibatidine-induced contraction was typically prolonged (see Figure 4a(iii)), although this phenomenon was not investigated further in the present study. In the presence of prazosin (100 nM), the average amplitude of the epibatidine-induced contraction was 4.5 ± 0.4 mN ($P<0.05$, $n_v=9$; Figure 4a(iv) and b). In the presence of both prazosin and α,β-MeATP, a residual contraction of 1.0 ± 0.3 mN still remained ($P<0.05$, $n_v=9$; Figure 4a(v) and b). No contraction was present after pretreating with a mixture of prazosin, α,β-MeATP and the muscarinic receptor antagonist cyclopentolate (1 μM) for 1 h ($n_v=9$; Figure 4a(vi) and b).

Discussion and conclusions

This investigation has shown that activation of nAChRs can induce neurotransmitter release in both the mouse and guinea pig vas deferens, but that the potentiation of field stimulus-induced neurotransmitter release cannot explain the action of epibatidine on neuronally evoked contraction in the mouse vas deferens, as epibatidine had no significant effect on the EJP in this species. Previous studies measuring NA release in either rat or guinea-pig vas deferens (Jayasundar and Vohra, 1977; Todorov *et al*., 1991) have demonstrated an increase in NA release, while in the mouse, a combination of contraction and electrophysiological studies have suggested that ATP release is augmented by nicotine (Brain *et al*., 2001). In the present work, however, the effect of epibatidine on EJPs, and hence the release of ATP, was weak and only detectable in some cells. This discrepancy might be explained by the different nAChR-subtype affinities of epibatidine and nicotine (Gerzanich *et al*., 1995); for example, activation of different nAChRs subtypes mediate different effects in other preparations such as hippocampal slices (Alkondon *et al*., 1997).

It seems unlikely that a selective potentiation of NA release over that of ATP, as previously shown in the guinea pig vas deferens (von Kügelgen and Starke, 1991a), could explain the enhanced contraction without an effect on (purinergic) EJPs in the mouse because a proportionally similar potentiation of electrically evoked contractions remained when α_1-adrenoceptors were antagonised with prazosin. This observation also means that the mechanism of epibatidine-induced potentiation of purinergic contraction does not require postjunctional α_1-adrenoceptors. Contraction studies are a relatively indirect measurement of neurotransmitter release and augmentation of contraction could involve a postjunctional mechanism. The epibatidine-induced potentiation of the electrically evoked contraction with no effect on EJP amplitude could be explained by an increase in the sensitivity of smooth muscle cells to neurotransmitter action. An increase in sensitivity of the contraction apparatus by a Rho kinase-dependent pathway following

neurotransmitter-receptor activation has been established in many smooth muscle preparations, including the vas deferens (Büyükafsar *et al*., 2003; Somlyo and Somlyo, 2003). However, the potential role of the Rho kinase pathway in epibatidine-induced neurotransmitter release (which leads to a potentiation of the electrically evoked contraction) has yet to be investigated.

TTX reduced but did not abolish the epibatidine-induced contraction suggesting that epibatidine-induced neurotransmitter release does not solely depend on neuronal action potential generation. It has previously been reported that epibatidine and nicotine-induced Ca^{2+} transients in nerve terminals of the mouse vas deferens, which are thought to be responsible for the potentiation of neurotransmitter release, are insensitive to TTX (Brain *et al*., 2001). Furthermore, nicotine did not induce synchronous Ca^{2+} signals along the nerve terminal; such synchronous Ca^{2+} transients are characteristic of nerve action potentials, at least those induced by field stimulation (Brain and Bennett, 1997) or drugs such as oxaliplatin (Webster *et al*., 2005). In the mouse vas deferens, the purinergic component of smooth muscle contraction is TTX-sensitive: the smooth muscle action potential, unusually for a smooth muscle, is generated by voltage-gated Na^{+} channels (Holman *et al*., 1977). For this reason, a reduction in the amplitude of the epibatidine-induced contraction in the presence of TTX might be entirely explained by a postjunctional effect.

A small proportion of the epibatidine-induced contraction was sensitive to cyclopentolate, which suggests that some of the epibatidine-induced contraction is cholinergic in origin. There is a functional cholinergic innervation in the mouse vas deferens (Kaschube and Zetler, 1989) and activation of prejunctional nAChRs can potentiate electrically evoked cholinergic neurotransmitter release in the presence of the adrenergic nerve blocker bretylium (Cuprian *et al*., 2005).

The effect of nAChR activation on neurotransmitter release was highly dependent on the rate of nAChR agonist application. In the mouse vas deferens, epibatidine only caused a consistent potentiation of SEJP frequency when a rapid method of application of the drug was used. During slow-bath application of epibatidine, the PSS perfusing the preparation was gradually replaced with one containing epibatidine (100 nM). The nAChRs may have become desensitised by a concentration of epibatidine insufficient to induce neurotransmitter release. Similar desensitisation of nAChRs by low concentrations of nAChR agonists occurs in mouse forebrain synaptosomes (Lu *et al*., 1999) and rat hippocampal slices (Dani *et al*., 2000). However, more rapid activation of nAChRs initiates an increase in Ca^{2+} concentration in presynaptic terminals that enhances the release of many neurotransmitters (Wonnacott, 1997). Hence, it is important to consider the rate of agonist application when assessing the physiological relevance of any nAChR-agonist mediated effect.

In the guinea pig vas deferens, potentiation of neurotransmitter release by bath-applied epibatidine appeared to be made up of two phases: a transient large increase in neurotransmitter release followed by a second, smaller and more sustained potentiation. Many subtypes of nAChRs desensitise rapidly (Quick and Lester, 2002); if such receptor subtypes were present, continuous exposure to nAChR agonists would cause a transient effect on neurotransmitter release (Grady *et al*., 1994; Lu *et al*., 1999). The potentiation of neuronally-evoked neurotransmitter release may be a result of influx of Ca^{2+} through the nAChR (as most subtypes of nAChRs are highly permeable to Ca^{2+}; (Fucile, 2004). This increase in basal Ca^{2+} concentration produced by nAChR activation results in a higher concentration of nerve terminal Ca^{2+} being obtained during action potential invasion; such a basal increase in Ca^{2+} concentration has been observed in nerve terminals in the mouse vas deferens in response to nicotine (Brain *et al*., 2001).

A sustained potentiation of electrically evoked neurotransmitter release has been reported in other preparations; for example, in mouse hippocampal slices, the potentiation persisted following the wash out of the nAChR agonist (Radcliffe and Dani, 1998). In the rat interpeduncular nucleus (Covernton and Lester, 2002) and the chick lateral spiroform nucleus (Zhu and Chiappinelli, 2002), a sustained (>15 min) potentiation of neurotransmitter release in the continual presence of nAChR agonists was reported; a second messenger cascade was implicated in this prolonged phase of neurotransmitter release. The mechanism of the prolonged potentiation in the guinea pig vas deferens remains to be determined, but a possible cause is a population of nAChRs which are not rapidly desensitised in the continued presence of agonists (Fenster *et al*., 1997). Alternatively, influx of Ca^{2+} through the nAChR could lead to changes in the intraneuronal Ca^{2+} concentration, which has the potential to affect synaptic plasticity in the long term (Berridge *et al*., 2000). Unfortunately, it is not possible, at present, to monitor Ca^{2+} in sympathetic terminals in the guinea-pig vas deferens (unlike the mouse; Brain and Bennett, 1997).

In summary, these studies reveal surprising differences in the response of the mouse and guinea pig vas deferens to epibatidine; whereas while neurogenic contraction is increased in both preparations, the mechanism is either potentiation of spontaneous transmitter release (in the mouse) to prime the muscle for contraction or an increase in evoked release (guinea pig). Both of these mechanisms need to be considered whenever nicotinic agonists are shown to enhance neurogenic contractions.

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Conflict of interest

The authors state no conflict of interest.

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