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Role of N-, P- and Q-type voltage-gated calcium channels in transmitter release from sympathetic neurones in the mouse isolated vas deferens

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1 N-type voltage-gated calcium channels are known to play an important role in transmitter release from autonomic neurones, and recent studies have demonstrated that non-N-type calcium channels are also involved. The calcium channels coupled to transmitter release from sympathetic neurones in the mouse isolated vas deferens were investigated in the present study.

2 Contractions of the mouse vas deferens were evoked by electrical stimulation at 1–50 Hz. The contractions were entirely nerve-mediated, since they were abolished by tetrodotoxin, and were used as an indirect measure of transmitter release.

3 The N-type calcium channel blocker, ω-conotoxin GVIA, inhibited contractions in a concentration-dependent manner, with a maximal effect at 30 nM. Contractions evoked by stimulation frequencies less than 10 Hz were abolished, and those evoked by 20 and by 50 Hz stimulation were decreased in amplitude by $51.3 \pm 13.9\%$ and $9.3 \pm 2.6\%$, respectively.

4 The N-, P- and Q-type channel blocker, ω-conotoxin MVIIC, inhibited contractions in a concentration-dependent manner and caused greater maximum inhibition than ω-conotoxin GVIA, suggesting an action on P- and/or Q-type channels, in addition to N-type.

5 The P-type channel blocker, ω-agatoxin IVA, alone did not have a significant effect at concentrations up to 300 nM, but inhibited contractions in the presence of ω-conotoxin GVIA. Subsequent addition of ω-conotoxin MVIIC abolished the remaining contractions. Identical results were obtained when the three toxins were tested cumulatively on the purinergic and noradrenergic components of the contraction in the presence of 0.3 μM prazosin and following desensitization to 10 μM α,β-methylene adenosine 5′-triphosphate (α,β-MeATP), respectively.

6 The results suggest that N-, P- and Q-type channels are involved in the release of noradrenaline and ATP from sympathetic neurones in the mouse vas deferens.

Keywords: Agatoxin; autonomic; calcium channel; conotoxin; sympathetic; transmitter release; vas deferens

Introduction

Regulated release of neurotransmitter is dependent on calcium influx into the nerve terminal through voltage-gated calcium channels (VGCCs). Research in recent years has demonstrated numerous subtypes of VGCCs, which can be differentiated on the basis of their pharmacological, electrophysiological and molecular properties. N-type VGCCs are sensitive to ω-conotoxin GVIA (GVIA), whereas P-type channels are blocked by low concentrations of ω-agatoxin IVA (agatoxin) and by ω-conotoxin MVIIC (MVIIC). Q-type channels are blocked by MVIIC and by higher concentrations of agatoxin (Hillyard *et al*., 1992; Mintz *et al*., 1992; Stea *et al*., 1994; Wheeler *et al*., 1994; Dunlap *et al*., 1995).

The vas deferens is innervated by sympathetic neurones which release adenosine 5′-triphosphate (ATP) and noradrenaline, in addition to peptides such as neuropeptide Y (reviewed in Morris & Gibbins, 1992). Contraction of the vas deferens is mediated by both ATP and noradrenaline. However, the excitatory junction potential (e.j.p.) is mediated only by ATP (reviewed in Morris & Gibbins, 1992). Until recently, it was accepted that transmitter release from these neurones depends only on N-type calcium channels, since GVIA abolishes the e.j.p. and neuronally evoked contractions to low frequency stimulation (up to 5 Hz) in the rat and guinea-pig vas deferens (Maggi *et al*., 1988; Brock *et al*., 1989; Lundy & Frew, 1993; 1994; Boot, 1994). However, De Luca *et al*. (1990) found that mechanical responses of the epididymal end of the rat vas deferens to electrical stimulation at 1–10 Hz were reduced by less than 50% by 50 nM GVIA, suggesting that a significant component of transmitter release depends on non-N-type VGCCs. Smith & Cunnane (1996) have shown that non-N-type channels are also involved in transmitter release from neurones in the guinea-pig vas deferens following stimulation at 3–50 Hz. These channels were not blocked by agatoxin or by MVIIC, suggesting that they were not P- or Q-type. However, they were sensitive to the non-specific calcium channel toxin, ω-grammotoxin SIA.

A growing number of studies also indicates roles for non-N-type channels in transmitter release from other autonomic neurones. Q-type channels are coupled to transmitter release from parasympathetic neurones in the rat bladder (Frew & Lundy, 1995) and channels of the P/Q family are coupled to acetylcholine release from parasympathetic neurones innervating the guinea-pig atria (Hong & Chang, 1995). In the rat anococcygeus, noradrenaline and ATP release are coupled to N-, P- and Q-type channels (De Luca *et al*., 1990; Lundy & Frew, 1994; Smith *et al*., 1995). N-, P- and Q-type channels subserve transmitter release from parasympathetic neurones in the mouse bladder (Waterman, 1995).

The aim of the present study was to investigate the subtypes of VGCC mediating transmitter release from postganglionic sympathetic neurones in the mouse vas deferens, and to determine whether these subtypes are the same for both noradrenaline and ATP release.

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Methods

Male albino (MF1) mice (20–30 g) were killed by raised atmospheric CO_2 and cervical dislocation. The vas deferentia were excised and placed in Krebs solution, gassed with 95% O_2/5% CO_2/pH 7.4 at 37°C. The composition of the Krebs solution was (mM): NaCl 119, KCl 4.7, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.5, D-glucose 11.0 and $CaCl_2$ 2.5. After surrounding connective tissue had been removed, whole vasa deferentia was mounted in 5 ml organ baths and connected to Kent TRN001/TRN002 isometric transducers (ADInstruments, Sydney, Australia) under an initial tension of 10 mN to record contractions of the longitudinal muscle layer. Contractions were recorded on a Macintosh computer by use of Chart v3.4/s software and a MacLab/8s data acquisition system (ADInstruments). Preparations were allowed to equilibrate for 20 min before capsaicin (10 μM) was added for 20 min to desensitize sensory nerves (Holzer, 1991). The preparations were then washed. Hexamethonium (500 μM) was added to block nicotinic ganglionic transmission. Responses to electrical field stimulation which were tetrodotoxin-sensitive, were considered to be mediated by transmitter release from postganglionic sympathetic neurones. Electrical field stimulation was made by means of platinum wire electrodes placed at the top and bottom of the organ bath and connected to a digital stimulator (Applegarth Electronics, Oxford). Trains of 50 pulses (0.3 ms duration, supramaximal voltage) at frequencies of 1, 2, 5, 10, 20 and 50 Hz were delivered every 90 s. An interval of 30 min was left between consecutive frequency-response curves. Toxins were added 30 min before their effect was measured. This time is sufficient for the toxins to reach a steady-state concentration (Turner *et al.*, 1995). Preliminary experiments showed that consecutive control curves were superimposable. To confirm that the calcium channel toxins acted only prejunctionally, the response of the vas deferens smooth muscle to direct stimulation with purinoceptor (10 μM α,β-methylene ATP (α,β-MeATP) and 3 mM ATP) and adrenoceptor agonists (10 μM noradrenaline) was tested before and after addition of the toxins. Agonist responses did not show tachyphylaxis if the tissues were washed as soon as the maximum contractile response was obtained (Ellis & Burnstock, 1989).

Drugs

Drugs used were: α,β-methylene ATP (α,β-MeATP), ATP, arterenol (noradrenaline), capsaicin, hexamethonium, prazosin, tetrodotoxin, yohimbine (Sigma, St. Louis, MO, U.S.A.), GVIA (RBI, Natick, MA, U.S.A.; Peninsula Laboratories, Belmont, CA and Peptide Institute, Osaka, Japan), agatoxin (Peptide Institute and Alomone Laboratories, Jerusalem) and MVIIC (Peptide Institute). ω-Agatoxin IVA was dissolved as per instructions in 0.9% NaCl. Capsaicin and prazosin were dissolved in ethanol. Ethanol at a final concentration of 0.1% did not alter significantly the contraction amplitude. All other drugs were dissolved in distilled water. Solutions of ATP and noradrenaline were made up on the day of the experiment.

Analysis of results

Data were analysed by two-way ANOVA followed by preplanned comparisons of either sequential frequency-response curves or comparisons with control. Paired *t*-tests were used to compare responses of smooth muscle to direct stimulation before and after addition of the toxins. Probabilities less than 0.05 were considered significant.

Results

The mouse vas deferens contracted in a frequency-dependent manner, with half maximal contractions occurring at approximately 7 Hz. The contraction amplitude at all frequencies was increased in the presence of the α_2-adrenoceptor antagonist, yohimbine (0.3 μM), with half maximal contractions occurring at a frequency of approximately 5.4 Hz. All contractions were blocked by 0.1 μM tetrodotoxin. Contractions of the vas deferens are mediated by a combination of noradrenaline and ATP (see Introduction and below). Initially, the effects of calcium channel toxins were examined on the whole contraction produced by the combined effects of noradrenaline and ATP.

Effect of GVIA on the whole contraction

The N-type voltage-gated calcium channel blocker, GVIA, was tested on electrically-evoked contractions of the vas deferens at concentrations of 10, 30, 100 and 300 nM. Contractions evoked at each stimulation frequency were reduced in amplitude by all concentrations of the toxin (Figure 1). The maximal effect of the toxin was reached at 30 nM; higher concentrations did not produce further significant inhibition. A concentration of 30 nM was therefore used in all subsequent experiments. The toxin abolished contractions evoked by 1–5 Hz. However, at higher frequencies, contractions could still be evoked in the presence of the toxin. There was no effect of GVIA on contractions produced by 10 μM α,β-MeATP, an agonist at P_{2X} purinoceptors on the smooth muscle, confirming that the toxin acts prejunctionally. Thus, N-type VGCCs alone can mediate transmitter release at low frequencies of stimulation; at higher frequencies, other VGCC subtypes are likely to be involved.

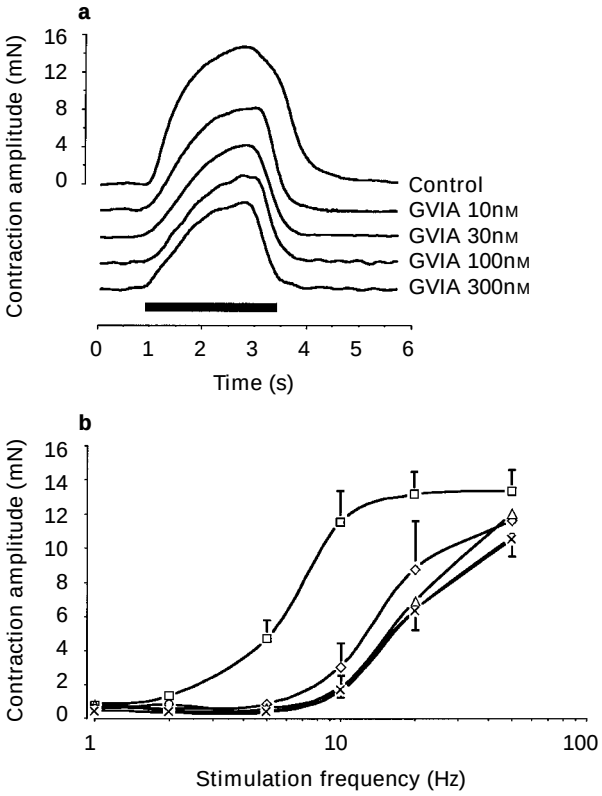

Figure 1 Effect of GVIA on the whole contraction of the mouse vas deferens. Vas deferentia were stimulated with 50 pulses at frequencies of 1–50 Hz. Cumulative concentrations of 10, 30, 100 and 300 nM GVIA were tested. Thirty minutes were allowed between periods of stimulation for the toxin to equilibrate. (a) Example of the effect of the toxin on contractions evoked by 20 Hz stimulation. The bar shows the period of electrical stimulation. (b) Summary of the effect of 10–300 nM toxin on 5 preparations from different animals. (□) Control; (◇) 10 nM GVIA; (△) 30 nM GVIA; (○) 100 nM GVIA; (×) 300 nM GVIA. Two-way ANOVA indicated that each concentration produced significant inhibition compared with control (10 nM, $P<0.05$; 30 nM, $P<0.02$; 100 and 300 nM, $P<0.01$). In (b), vertical lines show s.e.mean.

Effect of MVIIC on the whole contraction

To begin to define the non-N-type VGCC involved in transmitter release, the effect of MVIIC, which blocks N-, P- and Q-type VGCCs, was tested (Hillyard *et al*., 1992; Mintz *et al*., 1992; Wheeler *et al*., 1994). There was no significant effect of the toxin at concentrations of 10 or 30 nM. However, the contraction amplitude was reduced by 100 and 300 nM (Figure 2). A concentration of 300 nM was used in all further experiments. Contractions produced by 10 μM α,β-MeATP were unaffected by 300 nM MVIIC, indicating that the toxin acts prejunctionally.

Table 1 shows a comparison of the concentrations of GVIA and MVIIC required to reduce the contraction amplitude by 50% at frequencies of 5–50 Hz. It also shows the maximum inhibition produced by 30 nM GVIA and by 300 nM MVIIC. Both toxins abolished the contraction produced by 5 and 10 Hz stimulation, although GVIA was more potent. At 20 and 50 Hz, the maximum inhibition produced by MVIIC was 30–40% greater than that produced by GVIA, suggesting that at these frequencies, P- and/or Q-type channels mediate a proportion of transmitter release. At these higher stimulation frequencies, MVIIC was more potent than GVIA.

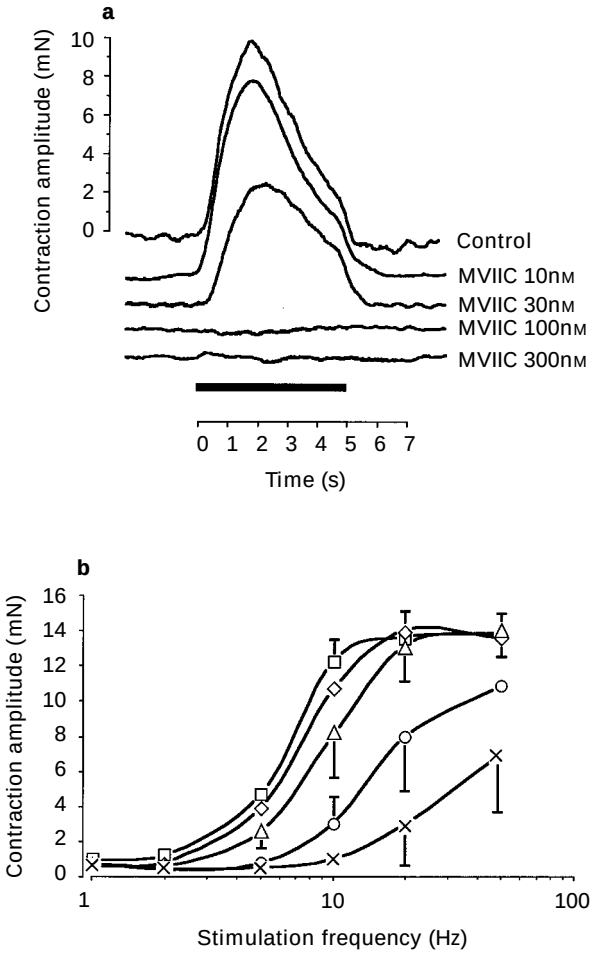

Figure 2 Effect of MVIIC on the whole contraction of the mouse vas deferens. Experiments were performed as in Figure 1. (a) Example of the effect of the toxin on contractions evoked by 10 Hz stimulation. The bar shows the period of electrical stimulation. (b) Summary of the effect of 10–300 nM toxin on 4 preparations from different animals. (□) Control; (◇) 10 nM MVIIC; (△) 30 nM MVIIC; (○) 100 nM MVIIC; (×) 300 nM MVIIC. The effect of 10 and 30 nM toxin was not significant compared to control ($P>0.05$). The contraction amplitude was reduced significantly by 100 and 300 nM toxin ($P=0.04$ and $P=0.0008$, respectively). In (b), vertical lines show s.e.mean.

Effect of agatoxin on the whole contraction

Agatoxin was initially tested at concentrations of 1 and 3 nM. Neither concentration caused significant inhibition of the contraction. Agatoxin was then tested at concentrations of 10, 30, 100 and 300 nM. There was no significant effect of the toxin at any of these concentrations (Figure 3), in agreement with previous studies with agatoxin or whole spider toxin on the guinea-pig and rat vas deferens and mouse bladder (Boot, 1994; Lundy & Frew, 1994; Waterman, 1995). Agatoxin also did not have any effect on the response of the smooth muscle to direct stimulation with 10 μM α,β-MeATP.

Effect of sequential addition of GVIA, agatoxin and MVIIC on the whole contraction

Although agatoxin alone did not have a significant effect, studies on the mouse bladder (Waterman, 1995) showed that agatoxin can have a significant effect after N-type channels are blocked. A series of experiments was performed in which agatoxin was added in the continued presence of GVIA; N- and any P-type channels which may be present were blocked and MVIIC was added to test for a possible role of Q-type channels.

Consistent with the earlier experiments, 30 nM GVIA significantly reduced the contraction amplitude in response to all frequencies of stimulation (Figure 4). Subsequent addition of 300 nM agatoxin produced further significant inhibition of the contraction. MVIIC abolished the remaining contraction. Taken together, these results suggest that at stimulation frequencies greater than 5 Hz, N-, P- and Q-type channels are involved in transmitter release.

Responses of the muscle to direct stimulation with 10 μM

Table 1 Effect of conotoxins on contraction of the mouse vas deferens

Stimulation frequency (Hz)	*GVIA* IC_{50} (nM)	*MVIIC* IC_{50} (nM)	*% inhibition produced by 30 nM GVIA*	*% inhibition produced by 300 nM MVIIC*
5	<10	20	82.8±5.7	86.4±4.6
10	<10	30	86.3±4.0	92.3±3.2
20	25	16	51.3±13.9	79.3±16.0
50	>300	250	9.3±2.6	55.2±18.9

GVIA: ω-conotoxin GVIA; MVIIC: ω-conotoxin MVIIC. IC_{50}s were interpolated from graphs of concentration versus response at a single stimulation frequency.

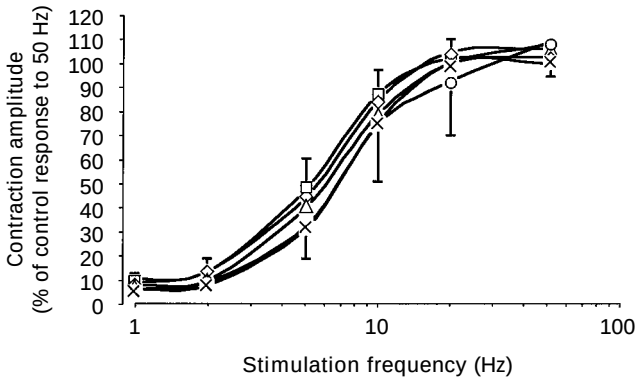

Figure 3 Effect of agatoxin on the whole contraction of the mouse vas deferens. Experiments were performed as in Figure 1. (□) Control; (◇) 10 nM agatoxin; (△) 30 nM agatoxin; (○) 100 nM agatoxin; (×) 300 nM agatoxin. Agatoxin did not have a significant effect on contraction amplitude at concentrations of 10–300 nM ($P=0.45$). Vertical lines show s.e.mean; $n=3$ preparations from different animals.

α,β-MeATP were not significantly altered in the presence of the three toxins (6.28 ± 0.86 mN before and 6.86 ± 1.31 mN after addition of the toxins; $n = 8$; $P > 0.05$). Responses to 3 mM ATP were unaffected (3.41 ± 0.34 mN before and 3.18 ± 0.08 mN after addition of the toxins; $n = 4$; $P > 0.05$), as were those to 10 μM noradrenaline (4.35 ± 1.44 mN before and 4.59 ± 0.68 mN after addition of the toxins; $n = 4$; $P > 0.05$). Thus the toxins did not act postjunctionally.

Two transmitters largely mediate contraction of the vas deferens in response to stimulation of sympathetic neurones: noradrenaline and ATP (Morris & Gibbins, 1992). After the nature of the calcium channels involved in transmitter release in the whole contraction had been investigated, the effect of the toxins was examined on the noradrenergic or purinergic contractions. First, the components of the whole contraction were characterized.

Components of the contraction of mouse vas deferens

Desensitization of the vas deferens to 10 μM α,β-MeATP reduced the amplitude of contractions at all frequencies of stimulation (Figure 5a). Subsequent addition of 0.3 μM prazosin abolished the remaining response. Similarly, prazosin alone significantly reduced the amplitude of the contraction, and subsequent desensitization of P_{2X} receptors with α,β-MeATP abolished the remaining response (Figure 5b). The amplitude of either the noradrenergic or purinergic components of the contraction was calculated once the effects of ATP and noradrenaline, respectively, had been blocked. Thus the noradrenergic component of the contraction was calculated as the difference between the second and third curves shown in Figure 5a, and the purinergic component was calculated as the difference between the second and third curves

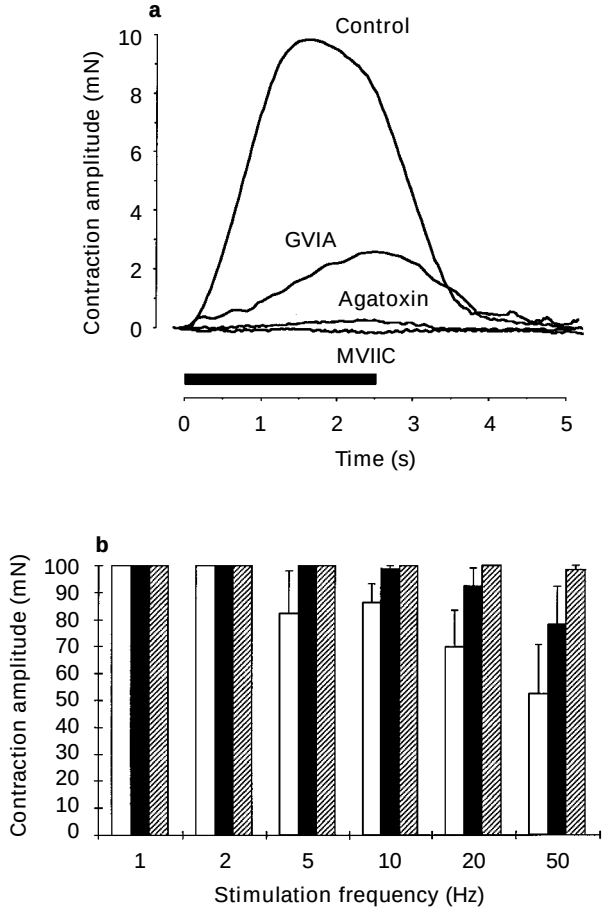

Figure 4 Effect of sequential addition of GVIA, agatoxin and MVIIC on the whole contraction of the vas deferens. (a) Examples of raw traces in which the vas deferens was stimulated with 50 pulses at 20 Hz. The bar shows the period of electrical stimulation. (b) Summary of the effect of the toxins on 5 vas deferentia from separate animals. For each stimulus parameter, the open column represents the percentage inhibition produced by GVIA alone. The solid column shows the combined effect of GVIA and agatoxin and the hatched column, the effect of all three toxins. Contractions were reduced significantly in amplitude by 30 nM GVIA ($P < 0.0001$). In the continued presence of GVIA, 300 nM agatoxin produced further inhibition of the remaining contraction at frequencies greater than 10 Hz ($P < 0.05$). Addition of 300 nM MVIIC abolished the remaining contraction ($P < 0.05$).

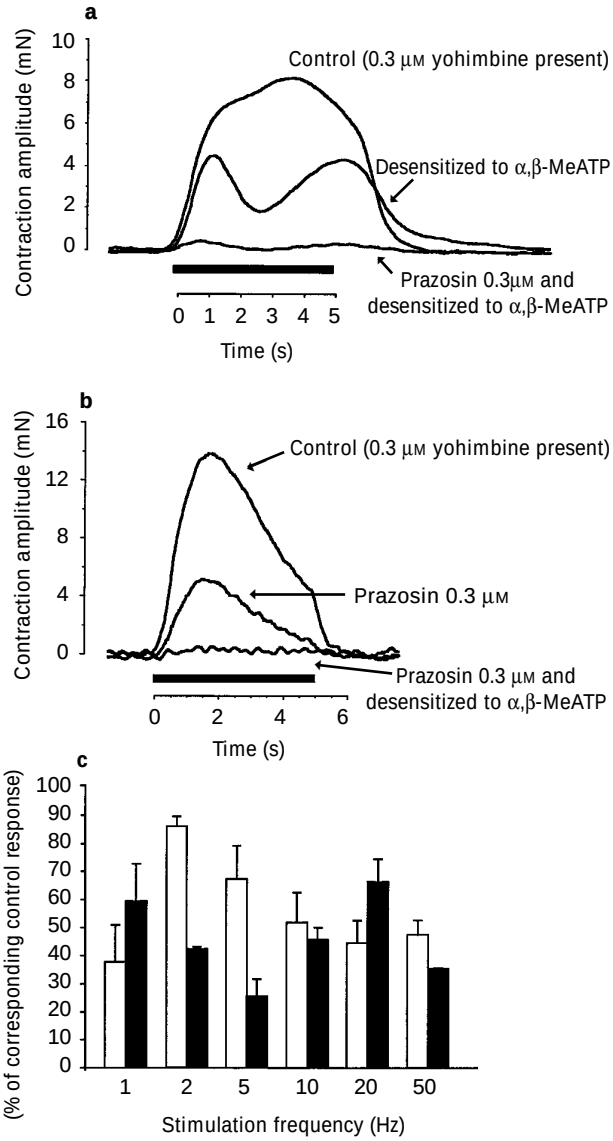

Figure 5 Components of the vas deferens contraction. (a) Example of control contractions in response to 50 pulses delivered at 10 Hz and the effect of desensitization to 10 μM α,β-MeATP and subsequent addition of 0.3 μM prazosin. The bar shows the period of electrical stimulation. (b) Example of control contractions to 50 pulses delivered at 10 Hz and the effect of 0.3 μM prazosin and subsequent desensitization 10 μM α,β-MeATP. The bar shows the period of electrical stimulation. (c) Summary of 7 experiments on tissues from different animals. The open columns show the noradrenergic response, calculated as the difference between contraction amplitude following desensitization to α,β-MeATP and after the addition of prazosin and expressed as a percentage of the control response (i.e. the difference between the second and third curves in (a). Similarly, the purinergic component (solid columns) was calculated as the difference in contraction amplitude after the addition of prazosin and following desensitization to α,β-MeATP (i.e. the difference between the second and third curves in (b)).

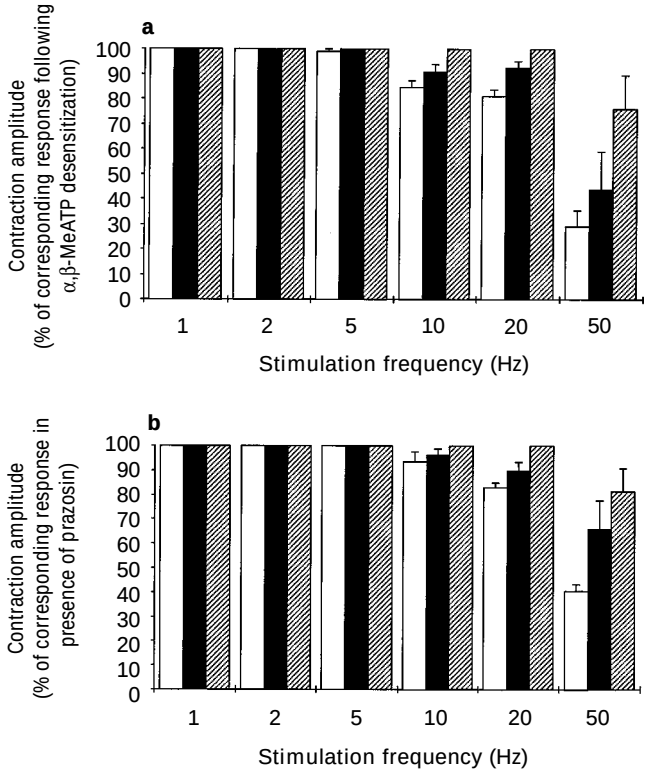

Figure 6 Effect of sequential addition of GVIA, agatoxin and MVIIC on the noradrenergic and purinergic components of the vas deferens contraction. (a) A summary of the effect of the toxins on the noradrenergic contraction and (b) the effect on the purinergic component. For each stimulus parameter, the open column represents the percentage inhibition produced by ω-conotoxin GVIA alone. The solid column shows the combined effect of ω-conotoxin GVIA and ω-agatoxin IVA and the hatched column, the effect of all three toxins. GVIA reduced the amplitude of both the cholinergic and purinergic contractions ($P<0.001$ for each), as did agatoxin ($P=0.02$ and $P=0.06$, respectively; comparison with GVIA). MVIIC abolished the remaining noradrenergic and purinergic contractions ($P=0.07$ and $P=0.02$, respectively); $n=4$ preparations from different animals for each type of experiment.

shown in Figure 5b. Figure 5c shows the average amplitudes of the noradrenergic and purinergic contractions at each stimulation frequency.

The role of different neuronal VGCC subtypes in mediating the noradrenergic and purinergic contraction (following desensitization to α,β-MeATP and in the presence of prazosin, respectively) was investigated by sequentially adding GVIA, agatoxin and MVIIC as described above for the whole contraction.

Effect of sequential addition of GVIA, agatoxin and MVIIC on the noradrenergic and purinergic contractions

Figure 6 shows the results of experiments in which the calcium channel toxins were tested on the noradrenergic and purinergic contractions respectively. The results were the same in each case and were similar to those obtained when the toxins were tested on the whole contraction. Thus, GVIA abolished the contractions at stimulation frequencies up to 5 Hz, indicating that N-type channels alone are sufficient to mediate the release of both noradrenaline and ATP. At higher stimulation frequencies, small noradrenergic and purinergic contractions remained in the presence of GVIA. These were reduced in amplitude by agatoxin, and the small resistant contractions remaining at 20 and 50 Hz were abolished by MVIIC.

Discussion

The present study confirms that N-type VGCCs are coupled to transmitter release from postganglionic sympathetic neurones in the mouse vas deferens at low frequencies of stimulation, and demonstrates that P- and Q-type channels are also involved at higher stimulation frequencies.

The role of N-type channels in transmitter release in the vas deferens

Numerous studies have demonstrated that N-type VGCCs play a crucial role in transmitter release from many autonomic neurones. At stimulation frequencies less than 10 Hz, most studies found that N-type channels are solely responsible for transmitter release from postganglionic sympathetic neurones in the rat and guinea-pig vas deferens (Maggi *et al*., 1988; Brock *et al*., 1989; Lundy & Frew, 1993; 1994; Boot, 1994). The present study demonstrates that this is also the case in the mouse vas deferens. Boot (1994) found that the IC_{50} for the inhibition of contraction of the rat vas deferens by ω-conotoxin GVIA was 20 nM; at stimulation frequencies less than 10 Hz, the IC_{50} in the mouse vas deferens was less than 10 nM (present study).

When the sympathetic neurones in the vas deferens are stimulated at frequencies of 10 Hz or greater, transmitter release is only partly inhibited by GVIA, suggesting that other subtypes of VGCC are involved. This has been demonstrated in the guinea-pig (Smith & Cunnane, 1996) and in the mouse vas deferens (present study). Two studies have also shown partial inhibition by GVIA of transmitter release from sympathetic neurones in the rat and guinea-pig vas deferens at stimulation frequencies from 1–5 Hz (De Luca *et al*., 1990; Smith & Cunnane, 1996). The nature of these channels is addressed below.

The role of P-type channels in the vas deferens

Agatoxin blocks P-type channels and at higher concentrations, Q-type channels (see Introduction). In these experiments, a saturating concentration for P-type channels (300 nM; Dunlap *et al*., 1995) was used, so any P-type channels present should be blocked. However, at this concentration, one might anticipate an additional effect of agatoxin on Q-type channels. A previous study demonstrated that in these conditions, agatoxin and MVIIC appear to act at different channels, most likely P- and Q-type channels, respectively (Waterman, 1995).

In the presenst study agatoxin alone did not have a significant effect, which could indicate that P-type channels are not involved in transmitter release from neurones in this tissue. Previous studies have similarly been unable to demonstrate an effect of P-type channel blockers (Boot, 1994; Lundy & Frew, 1994). In a study on the mouse bladder, agatoxin also did not have an effect when tested alone. However, when the toxin was added in the presence of GVIA, it significantly reduced transmitter release (Waterman, 1995). The effect of agatoxin was therefore tested following the addition of GVIA. Under these conditions agatoxin significantly reduced the contraction amplitude, without altering the responses of the muscle to direct stimulation by α,β-MeATP, ATP and noradrenaline. Thus agatoxin reduced transmitter release, and this could only be revealed after N-type VGCCs had been blocked. It is likely, therefore, that P-type as well as N-type VGCCs play a role in transmitter release from sympathetic neurones in the mouse vas deferens.

The role of Q-type channels in the vas deferens

Q-type VGCCs, like P- and some N-type channels, are sensitive to MVIIC (Boot, 1994). In the present study, MVIIC alone significantly reduced contractions of the mouse vas deferens at all frequencies of stimulation. This effect was prejunctional, since the toxin did not alter the response to α,β-

MeATP, ATP and noradrenaline. The IC_{50} for this effect varied between 16 and 30 nM for stimulation frequencies of 20 Hz or less. When the preparation was stimulated at 50 Hz, the IC_{50} was 250 nM. The inhibitory effect of MVIIC exceeded that of GVIA, suggesting that at least part of the effect of MVIIC must be on channels other than N-type.

In another series of experiments, MVIIC was added after N- and P-type channels had been blocked by GVIA and agatoxin. Under these conditions, MVIIC still reduced the amplitude of contractions of the mouse vas deferens. This indicates that Q-type channels are involved in transmitter release from sympathetic nerve terminals in this preparation.

Whilst no effect of MVIIC has been obtained in the guinea-pig and rat vas deferens (Boot, 1994; Smith & Cunnane, 1996), the toxin has been shown to inhibit transmitter release from autonomic neurones in other preparations. Acetylcholine release from enteric neurones innervating the longitudinal muscle of the guinea-pig ileum was inhibited with an IC_{50} of 26 nM (Boot, 1994). However, this effect was attributed to an action of the toxin on N-type channels, since it did not occur in the presence of GVIA. On the other hand, release of noradrenaline and ATP from sympathetic neurones innervating the rat anococcygeus (Smith *et al*., 1995) was inhibited by MVIIC, both in the absence and presence of GVIA. Thus, MVIIC appears to act on non-N-type VGCCs in the rat anococcygeus as well as in the mouse bladder (Waterman, 1995) and vas deferens (present study). However, it is evident that there is considerable species and tissue variation in the type of VGCCs coupled to transmitter release from autonomic neurones.

Multiple subtypes of calcium channel control both noradrenaline and ATP release

This study suggests that N-, P- and Q-type channels are involved in transmitter release from sympathetic neurones in the mouse vas deferens. By desensitizing to α,β-MeATP or adding prazosin, it was possible to measure the contraction of the vas deferens mediated by ATP or noradrenaline, respectively. The effects of the toxins on the noradrenergic contraction were almost identical to those on the purinergic contraction and on the whole contraction. Thus N-, P- and Q-type channels are involved in the release of both noradrenaline and ATP. This finding contrasts with those in the mouse bladder (Waterman, 1995) in which the combinations of VGCCs involved in acetylcholine- and ATP-mediated contractions release differed.

In conclusion, this study demonstrates a role for N-, P- and Q-type calcium channels in the release of noradrenaline and ATP from postganglionic sympathetic neurones in the mouse vas deferens.

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