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A role for Q type Ca^{2+} channels in neurotransmission in the rat urinary bladder

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1 In isolated bladder strips of the rat, a substantial component (46%) of the Ca^{2+}-dependent contractile response to electrical field stimulation (5 Hz) was resistant to combined block of both N and P type Ca^{2+} channels by ω-conotoxin-GVIA (300 nM) and ω-agatoxin-IVA (100 nM) respectively.

2 The resistant portion (non-N, non-P) was sensitive to ω-conotoxin-MVIIC (3 μM), which in addition to N and P also blocks Q type channels at this concentration. ω-Conotoxin-MVIIC administered alone, inhibited the neurogenic response to the same degree as that observed in the combined presence of ω-agatoxin-IVA, ω-conotoxin-GVIA and ω-conotoxin-MVIIC.

3 ω-Agatoxin-IVA (100 nM), a concentration that fully inhibits P type channels, had a negligible effect on the neurogenic response. Following blockade of N type Ca^{2+} channels with ω-conotoxin-GVIA (300 nM), ω-agatoxin-IVA (3 μM) (a concentration well above that used to block P channels, inhibits Q type channels, but spares N type channels), inhibited the residual response to the same degree as ω-conotoxin-MVIIC alone.

4 Results suggest that neurotransmission in rat urinary bladder is supported by both N and Q type Ca^{2+} channels.

Keywords: Voltage-sensitive Ca^{2+} channels; ω-conotoxin-MVIIC; ω-agatoxin-IVA; ω-conotoxin-GVIA; urinary bladder; neurotransmission

Introduction

Synaptic transmission in the CNS is dependent on the influx of extracellular Ca^{2+} via multiple types of voltage-sensitive Ca^{2+} channels (VSCCs) under physiological conditions (Wheeler *et al.*, 1994). In sympathetic and parasympathetic peripheral autonomic nerves, transmitter release is highly sensitive to the N type Ca^{2+} channel blocker, ω-conotoxin-GVIA, where N type Ca^{2+} channels appear to be the predominant channel type supporting neurotransmission (Hirning *et al.*, 1988; Lundy & Frew, 1988; 1993; De Luca *et al.*, 1990).

Non-adrenergic, non-cholinergic (NANC) transmission, in contrast, appears to exhibit various degrees of sensitivity to ω-conotoxin-GVIA depending on the tissue and species studied (Maggi *et al.*, 1988; Lundy & Frew, 1994). In the rat urinary bladder, Ca^{2+}-dependent motor responses to stimulation of parasympathetic postganglionic fibres which are resistant to the muscarinic antagonist, atropine (Taira, 1972), also display resistance to ω-conotoxin-GVIA (Maggi *et al.*, 1988), and to P channel blockade by ω-agatoxin-IVA (Lundy & Frew, 1994), suggesting a novel Ca^{2+} channel may be involved in translocating extracellular Ca^{2+} necessary for triggering neurotransmitter release in this preparation.

We have studied the effects of the above omega-toxins, and of ω-conotoxin-MVIIC, a 26 amino acid peptide deduced and synthesized from a c-DNA clone derived from the marine snail *Conus magus* (Hillyard *et al.*, 1992), on parasympathetic stimulation in rat bladder in order to elucidate a possible role for Q type channels. ω-Conotoxin-MVIIC, in addition to blocking N and P channels, also blocks recently identified Q channels (Wheeler *et al.*, 1994).

Methods

Urinary bladders obtained from male Sprague Dawley rats (250–300 g) were placed in cold Krebs-Henseleit solution. Four detrusor strips 0.5 cm long and 1–2 mm wide were prepared for contractility studies from each bladder, and mounted under 1 g tension in 5 ml organ baths containing oxygenated (95%O_2 : 5%CO_2) Krebs solution of the following composition (mM): NaCl 116, KCl 5.4, $CaCl_2$ 1.5, $MgSO_4$ 1.1, NaH_2PO_4 1.1, $NaHCO_3$ 25 and D-glucose 11, pH 7.4 and maintained at 37°C. Following a 60 min equilibration period, with frequent changes of the bath fluid, tissues were electrically stimulated with trains of square wave pulses (5 Hz, 1 ms pulse width, for 1 s every 30 s at supramaximal voltage) using parallel platinum electrodes connected to a Grass S88 stimulator. Responses to nerve stimulation and to drugs were recorded auxotonically with Harvard smooth muscle transducers connected to a Rikadenki chart recorder.

Tissues were randomly treated according to the following experimental protocols: (i) Strips were treated with ω-agatoxin-IVA (100 nM), ω-conotoxin-GVIA (300 nM), and ω-conotoxin-MVIIC (3 μM) added sequentially, in that order; (ii) with ω-conotoxin-MVIIC (300 mM) followed by ω-agatoxin-IVA (3 μM); or (iii) with either ω-conotoxin-GVIA (300 nM), ω-conotoxin-MVIIC (3 μM), or atropine (1 μM) each administered alone. Tissues were exposed to the peptides for 30 min, or until the inhibitory effect of each peptide on the response to nerve stimulation had fully plateaued. Drug effects were expressed as percentage inhibition of the response immediately prior to drug addition. Data were analyzed by one way analysis of variance, followed by Dunnett's test for multiple comparisons (SigmaStat, Jandel, California). A P value of < 0.05 was considered statistically significant.

ω-Agatoxin-IVA was kindly supplied by Dr N Saccomono, Pfizer, Groton, Conn., U.S.A. ω-Conotoxin-GVIA, and ω-conotoxin-MVIIC were obtained from Bachem, Torrance, California, U.S.A. Stock solutions (0.1 mM) of the peptides were prepared in distilled H_2O, and aliquots stored at –20°C until use. Atropine sulphate, tetrodotoxin (TTX), and mecamylamine hydrochloride were obtained from Sigma, St Louis, MO, U.S.A.

Results

Ca^{2+}-dependent, tetrodotoxin-sensitive motor responses to electric field stimulation of strips of rat bladder detrusor were

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unaffected by a 30 min exposure to ω-agatoxin-IVA (100 nM), and only partially inhibited by atropine (1 μM) or by a maximally effective concentration of ω-conotoxin-GVIA (300 nM). Atropine, ω-agatoxin-IVA, and ω-conotoxin-GVIA insensitive components were 80.1±1.5 (n=4), 91.1±2.1 (n=12) and 56.9±4.3 (n=5) percent of control responses (5 Hz) respectively. The residual ω-agatoxin-IVA, ω-conotoxin-GVIA insensitive component of the response to parasympathetic stimulation was 45.9±5.7% (n=8) of control values, which was further inhibited to 18.3±3.7% (n=9) of control values by the subsequent addition of ω-conotoxin-MVIIC (3 μM). ω-Conotoxin-MVIIC administered alone, in the absence of ω-agatoxin-IVA and ω-conotoxin-GVIA inhibited responses to field stimulation to 18.7±4.0% (n=6) of control.

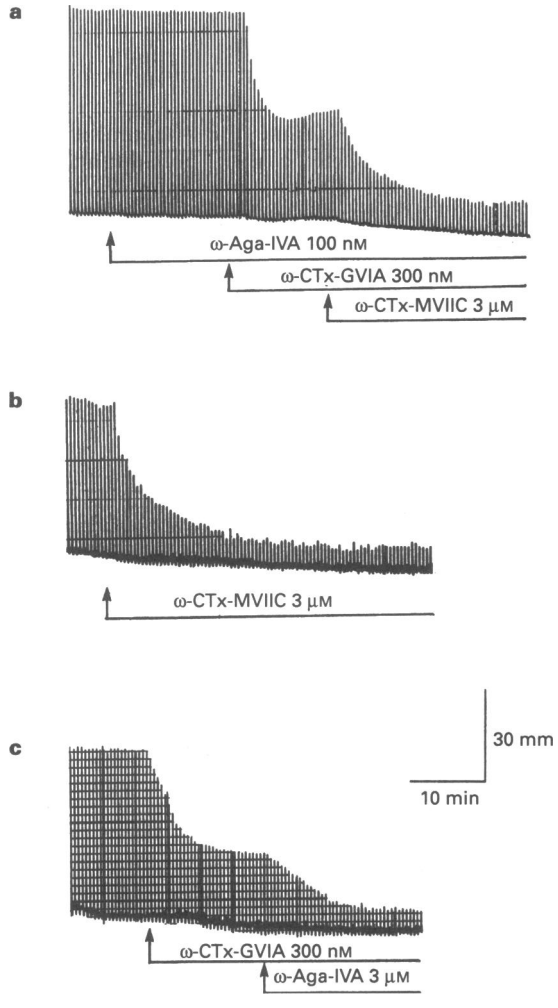

Figure 1 The effects of ω-toxins on neurotransmission in the rat urinary bladder. (a) Effects of ω-agatoxin-IVA (ω-Aga -IVA) 100 nM, ω-conotoxin-GVIA (ω-CTx-GVIA) 300 nM, and ω-conotoxin-MVIIC (ω-CTx-MVIIC) 3 μM, added sequentially, on responses to nerve stimulation, train 5 Hz, for 1 s every 30 s, supramaximal voltage. (b) Effect of ω-CTx-MVIIC alone, or (c) ω-conotoxin-GVIA (ω-CTx-GVIA) 300 nM, followed by ω-Aga -IVA (3 μM).

Ω-Conotoxin-GVIA-insensitive neurogenic responses were further inhibited by ω-agatoxin-IVA (3 μM) to 19.6±4.7% of control. It has been reported that ω-agatoxin-IVA inhibits Q-type channels at concentrations >200 nM (Zhang *et al.*, 1993), and at 1 μM (Wheeler *et al.*, 1994), these concentrations are well above those used to block P type channels (Mintz *et al.*, 1992). Typical tracings are shown in Figure 1, and results are presented in Table 1.

Neurogenic responses were completely abolished in the absence of Ca^{2+}, and by tetrodotoxin (1 μM), but were not affected by mecamylamine (10 μM), confirming that responses were Ca^{2+}-dependent, action potential-evoked, and due to postganglionic stimulation (not shown).

Discussion

We have demonstrated in the rat urinary bladder that synaptic transmission following stimulation of postganglionic parasympathetic nerves is supported by components sensitive to ω-conotoxin-GVIA, and others insensitive to ω-conotoxin-GVIA, and to nanomolar concentrations of ω-agatoxin-IVA. Thus the neuronal channel involved in supplying Ca^{2+} for a large portion of neurotransmitter release appears to be neither an N nor P type channel.

The inhibitory effect of ω-conotoxin-GVIA in rat bladder is frequency-dependent, optimal sensitivity was reportedly observed at 2–5 Hz, resulting in a 50% reduction in the neurogenic response (Maggi *et al.*, 1988). In the present study, 46% of the bladder neurogenic response (5 Hz) was resistant to a combination of ω-conotoxin-GVIA and nanomolar concentrations of ω-agatoxin-IVA, and only 18.3% of this resistant portion (non-N, non-P) remained, following subsequent addition of ω-conotoxin-MVIIC. ω-Conotoxin-MVIIC alone inhibited the neurogenic response to 18.7% of control, consistent with its reported ability to inhibit N and Q channels (Table 1). ω-Conotoxin-MVIIC, used in conjunction with ω-conotoxin-GVIA, and nanomolar concentrations of ω-agatoxin-IVA, provides useful confirmation of possible involvement of Q type channels in neurotransmission (Hillyard *et al.*, 1992; Wheeler *et al.*, 1994). According to Zhang *et al.*

Table 1 Effects of ω-agatoxin-IVA, ω-conotoxin-GVIA, and ω-conotoxin-MVIIC on responses to electrical field stimulation in the rat urinary bladder

Treatment		*% of control response*	n
Untreated		100	
ω-Agatoxin-IVA	100 nM	91.1±2.1	12
plus ω-Conotoxin-GVIA	300 nM[a]	45.9±5.7	8
plus ω-Conotoxin-MVIIC	3 μM[a]	18.3±3.7*	9
ω-Conotoxin-GVIA	300 nM	56.9±4.3	5
plus ω-Agatoxin-IVA	3 μM[a]	19.6±4.7*	3
ω-Conotoxin-MVIIC	3 μM	18.7±4.0*	6
Atropine	1 μM	80.1±1.5	4

Values represent means±s.e.mean in the absence, or following a 30 min pretreatment and in the continued presence of the peptide, administered alone, or sequentially[a]. Stimulation parameters are as described in Methods. ANOVA $F(7,40)=57.6$, $P<0.001$. *Significantly different from ω-agatoxin-IVA *plus* ω-conotoxin-GVIA treated, $P<0.05$ (Dunnett's test).

(1994), lower concentrations of ω-conotoxin-MVIIC inhibit Q type channels, but do so very slowly ($IC_{50} < 0.15$ μM). The rapidity and effectiveness of block increases as the concentration is increased from nanomolar to micromolar (Sather *et al.*, 1993; Wheeler *et al.*, 1994). The micromolar concentrations used in the present study cause a rapidly developing and effective block of Q channels in agreement with that observed in studies by others (Randall *et al.*, 1993; Wheeler *et al.*, 1994; Lopez *et al.*, 1994). In their studies approximately 50% of the high voltage-activated Ca^{2+} current remained following N, L, and P channel blockade in CNS neurones and in bovine chromaffin cells, which was totally sensitive to subsequent Q channel block.

ω-Agatoxin-IVA, in addition to blocking P type channels at nanomolar concentrations ($IC_{50} \sim 2$–20 nM) (Mintz *et al.*, 1992), also blocks Q-type channels at > 100 fold higher concentrations (Sather *et al.*, 1993; Zhang *et al.*, 1993; Wheeler *et al.*, 1994). In this respect ω-conotoxin-MVIIC and ω-agatoxin-IVA are roughly equipotent at blocking Q-type channels (Sather *et al.*, 1993). Following block of N type channels with ω-conotoxin-GVIA, micromolar concentrations of ω-agatoxin-IVA caused further inhibition of the ω-conotoxin-GVIA-insensitive neurogenic response, to the same degree as that observed following ω-conotoxin-GVIA plus ω-conotoxin-MVIIC, or ω-conotoxin-MVIIC alone (Table 1). Since ω-agatoxin-IVA spares N type channels (Zhang *et al.*, 1993), and the involvement of P type channels in neurotransmission in bladder has been precluded (Lundy & Frew, 1994), the results strongly suggest the presence of a novel Ca^{2+} channel with a pharmacological profile similar to that of Q type channels.

A significant role for L channels in peripheral synaptic transmission has been difficult to demonstrate; thus in peripheral preparations L channel effects appear to be restricted to post-junctional elements (Wessler *et al.*, 1990). ω-Conotoxin-MVIIC and ω-agatoxin-IVA do not block L type Ca^{2+} channels (Olivera *et al.*, 1994), and ω-conotoxin-MVIIC has no effect on K^+-contracted rat aortic strips, which is a paradigm for L type Ca^{2+} channel activity (unpublished observations). This would appear to preclude either pre- or post-junctional L channel effects in the actions of ω-conotoxin-MVIIC or ω-agatoxin-IVA in our tissue preparations. The non N, L, and P type Ca^{2+} channel in rat bladder appears pharmacologically similar to the Q channel described in previous studies using cerebellar granule cells (Randall *et al.*, 1993), rat hippocampal CA3 and CA1 neurones (Wheeler *et al.*, 1994), and bovine chromaffin cells (Lopez *et al.*, 1994). Our study suggests that both N and Q type Ca^{2+} channels are coupled to transmitter release in the rat urinary bladder.

Atropine-resistance of the response to nerve stimulation of rat urinary bladder is well-documented (Taira, 1972). It is of interest that in the present study the magnitude of the inhibitory effect of ω-conotoxin-GVIA on the neurogenic response approximates that previously reported for the muscarinic antagonist, atropine, where in a number of studies (cited by Maggi *et al.*, 1985) atropine inhibition did not exceed 40–50% of the TTX-sensitive response when tested at frequences similar to those used in the present study. Since ω-conotoxin-GVIA is a potent inhibitor of peripheral cholinergic- and adrenergic- nerve mediated responses (Lundy & Frew, 1988; De Luca *et al.*, 1990), the above observations add further support to the view that the atropine-resistant response in bladder is indeed NANC in character.

In the present study ω-conotoxin-GVIA inhibited neurogenic responses to a greater degree than atropine (Table 1), suggesting that the atropine-resistant NANC response is also at least partly sensitive to the N channel blocker, ω-conotoxin-GVIA. A substantial component of the neurogenic response however is sensitive to Q channel block by micromolar concentrations of ω-conotoxin-MVIIC or ω-agatoxin-IVA. The ω-conotoxin- GVIA, ω-agatoxin-IVA, ω-conotoxin-MVIIC resistant portion (~20%) of the neurogenic response may be mediated by a Ca^{2+} channel that remains to be pharmacologically characterized.

If in rat urinary bladder, cholinergic and NANC transmitters originate from a single population of postganglionic nerve terminals (Maggi, 1991), then our results would suggest multiple Ca^{2+} channel types regulate neurotransmitter release from individual peripheral autonomic nerve terminals. Previous studies have shown the co-localization of multiple Ca^{2+} channel types on individual terminals in the CNS (Turner *et al.*, 1993; Takahashi & Momlyama, 1993). This would promote a high degree of flexibility in the regulation of neurotransmitter release under different physiological conditions.

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