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CALCIUM CHANNELS CONTROLLING ACETYLCHOLINE RELEASE FROM PREGANGLIONIC NERVE TERMINALS IN RAT AUTONOMIC GANGLIA

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Abstract

Little is known about the nature of the calcium channels controlling neurotransmitter release from preganglionic parasympathetic nerve fibres. In the present study, the effects of selective calcium channel antagonists and amiloride were investigated on ganglionic neurotransmission. Conventional intracellular recording and focal extracellular recording techniques were used in rat submandibular and pelvic ganglia, respectively. Excitatory postsynaptic potentials and excitatory postsynaptic currents preceded by nerve terminal impulses were recorded as a measure of acetylcholine release from parasympathetic and sympathetic preganglionic fibres following nerve stimulation. The calcium channel antagonists v-conotoxin GVIA (N type), nifedipine and nimodipine (L type), v-conotoxin MVIIC and v-agatoxin IVA (P/Q type), and Ni21 (R type) had no functional inhibitory effects on synaptic transmission in both submandibular and pelvic ganglia. The potassium-sparing diuretic, amiloride, and its analogue, dimethyl amiloride, produced a reversible and concentration-dependent inhibition of excitatory postsynaptic potential amplitude in the rat submandibular ganglion. The amplitude and frequency of spontaneous excitatory postsynaptic potentials and the sensitivity of the postsynaptic membrane to acetylcholine were unaffected by amiloride. In the rat pelvic ganglion, amiloride produced a concentration-dependent inhibition of excitatory postsynaptic currents without causing any detectable effects on the amplitude or configuration of the nerve terminal impulse.

These results indicate that neurotransmitter release from preganglionic parasympathetic and sympathetic nerve terminals is resistant to inhibition by specific calcium channel antagonists of N-, L-, P/Q- and R-type calcium channels. Amiloride acts presynaptically to inhibit evoked transmitter release, but does not prevent action potential propagation in the nerve terminals, suggesting that amiloride may block the pharmacologically distinct calcium channel type(s) on rat preganglionic nerve terminals.

Key words: neurotransmitter release, calcium channels, preganglionic nerves, acetylcholine, amiloride, calcium channel blockers.

Voltage-sensitive calcium channels (VSCCs) have been classified, according to their electrophysiological, molecular and pharmacological properties, into at least five groups, termed L, N, P/Q, R and T types.[7, 15 and 16] Several studies have investigated the nature of the VSCCs involved in the release of neurotransmitters from vertebrate postganglionic autonomic nerve terminals and it is clear that release of a particular transmitter is not coupled to the same calcium channel type in all neurons. In most mammalian peripheral nerve terminals, Ca^{2+} enters mainly through N-type calcium channels[1, 4, 6, 20, 21, 29 and 30] and some P/Q-type calcium channels. [2 and 9] In contrast, little information is available on the type of calcium channel(s) controlling the release of acetylcholine (ACh) from preganglionic autonomic neurons. [13] It is known, for example, that neurotransmission in the rat parasympathetic submandibular ganglia is resistant to blockade of N- and L-type calcium channels. [19] At preganglionic sympathetic lumbar neurons of guinea-pigs, N- and P-type calcium channels have recently been reported to contribute to neurotransmitter release, [10] and at preganglionic sympathetic neurons of the guinea-pig

hypogastric ganglia, the non-selective calcium channel antagonist, ω-grammotoxin SIA, is the only toxin with an ability to inhibit transmitter release. [22] In the present study, we used two electrophysiological approaches, intracellular and focal extracellular recording, to determine the type of VSCCs controlling neurotransmitter release from the intact preganglionic parasympathetic and sympathetic nerves that innervate the rat submandibular and pelvic ganglia, respectively. A preliminary account of these findings has been communicated to the Australian Neuroscience Society. [23]

Experimental procedures

Preparation: submandibular ganglia

Two-week-old rats were anaesthetized with halothane and killed by cervical fracture prior to removal of the submandibular ganglia. Experimental procedures were in accordance with the guidelines of the University of Queensland Animal Experimentation Ethics Committee, and all efforts were made to minimize the number of animals used and their suffering. Individual preparations were pinned to the Sylgard (Dow-Corning)-covered base of a 2-ml Perspex organ bath. Preparations were perfused with a Krebs solution of the following composition (mM): NaCl 118.4, $NaHCO_3$ 25.0, NaH_2PO_4 1.13, $CaCl_2$ 1.8, KCl 4.7, $MgCl_2$ 1.3 and glucose 11.1, gassed with a mixture of 95% O_2/5% CO_2 to pH 7.4, and maintained at 36–37°C.

Intracellular recording and analysis

The lingual nerve was field stimulated by rectangular voltage pulses via bare platinum wires delivered from a digital stimulator (Pulsar 7^+; Frederick Haer & Company, Brunswick, ME, U.S.A.) coupled to an optically isolated stimulation unit (Model DS2; Digitimer, Welwyn Garden City, U.K.). Intracellular recordings were made from individual ganglion cells using glass microelectrodes filled with 5 M potassium acetate (resistances 80–120 MΩ). Conventional intracellular recording techniques were employed as described previously.[3 and 19] Membrane potentials were recorded through a headstage connected to an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA, U.S.A.) in bridge mode and stored on a digital tape recorder (DTR-1204; BioLogic Science Instruments, Claix, France). Evoked events were digitized at 5 kHz and transferred to a Pentium computer using an analogue-to-digital converter (TL-1 DMA interface) and Axotape software (Axon Instruments). The amplitude, frequency, rise time, and latency of evoked and spontaneous events were analysed using the program Axograph 2 (Axon Instruments). The mean resting membrane potential (RMP) of the submandibular ganglion neurons was −63.9±0.7 mV (n=157; mean±S.E.M.). The mean baseline was determined by averaging the initial part of the digitized signal between the stimulus artifact and the onset of the response. Data are expressed as the mean±S.E.M. and n values refer to the number of preparations. Data were analysed statistically using Student's paired t-test with the level of significance being taken as $P<0.05$.

Preparation: pelvic ganglia

Rats (Wistar) aged between two and four weeks postnatal were anaesthetized with halothane and killed by cervical fracture. Both pelvic ganglia were dissected free from the surrounding tissues and mounted in a 3-ml bath. The preparations were continuously perfused at the rate of 3 ml/min with a Krebs solution bubbled with 95% O_2/5% CO_2 (pH 7.4). The temperature of the bath solution was maintained at 32–34°C, which was the optimum temperature range for obtaining stable recordings in longer experiments. The capsule and other connective tissue were carefully removed under the dissecting microscope using a pair of fine forceps.

The preparations were bathed in 3,3′-diethyloxardicarbocyanine iodide [0.1 μM; $DiOC_2(5)$][11] for 30 s and washed with Krebs solution (0.2 mM extracellular Ca^{2+} concentration) for 3 min. An image intensifier camera (Panasonic WV 1900/B) attached to an Olympus (BH2) microscope equipped with a rhodamine filter and ULWD ×50 objective was used to display the image on a video monitor. The arrangement of $DiOC_2(5)$-fluorescing boutons on the ganglion cells was traced on to the video monitor screen. The excitation wavelength (540 nm) was then turned off to avoid photobleaching.

Focal extracellular recording from individual synaptic boutons

The rat pelvic ganglion was chosen for the extracellular studies because the postganglionic cell bodies are large (15–30 μm) and have no dendritic arborization of their spherical or ovoid shapes.[24 and 25] Most of the cells are innervated by single preganglionic axons which form short strings or clusters of synaptic boutons. [5 and 18] $DiOC_2(5)$ fluorescence of boutons aided the precise placement of the recording pipette (3–5 μm diameter) over single boutons.[28] The interior of the pipette was constantly perfused with Krebs solution at the rate of 0.2 ml/h using a micropump. Drugs and toxins were added directly to the intrapipette perfusing solution. Intrapipette perfusion with zero Ca^{2+} or Cd^{2+} (100 μM) abolished excitatory postsynaptic currents (EPSCs) and greatly reduced the frequency of spontaneous EPSCs. These control experiments were conducted to check that the intrapipette perfusion technique was successful in rapidly changing the solution bathing the boutons enclosed within the recording electrode.

Following placement of the electrode over $DiOC_2(5)$ fluorescence-visualized single boutons, the hypogastric nerve was stimulated. Evoked nerve terminal impulses (NTIs) and EPSCs were amplified using an Axoclamp-2B amplifier, digitized by a MacLab 4e with Scope software, stored on a Macintosh computer (PowerMac 7500/120) and analysed using IgorPro software. Between 100 and 200 NTIs and EPSCs were obtained and analysed per recording site. Histograms of the amplitude of EPSCs versus number of observations were constructed, including the number of failures.

Stimulation of the hypogastric nerve was achieved by gently sucking the nerve in a glass pipette, which had one chlorided silver wire inside and another outside the pipette tip. The hypogastric nerve was stimulated continually at 0.2 Hz using pulses of 0.05 ms duration and 10–20 V amplitude, while searching for the extracellular signals of the NTI and the EPSC produced by released neurotransmitter activating the postsynaptic nicotinic ACh receptors. Once a suitable bouton was located, stimulation was stopped for 10 min. Following this rest period, stimulation was resumed at 0.1 Hz.

Drugs

Drugs were dissolved in the Krebs solution perfusing the preparation and the effects evaluated after it reached equilibrium (≥5 min). The following drugs were used: ω-agatoxin IVA, ω-conotoxin (ω-CTX) GVIA, ω-CTX MVIIC (Alamone Labs, Jerusalem, Israel), cadmium chloride, nickel chloride hexahydrate (Aldrich), amiloride hydrochloride dihydrate, dimethyl amiloride, hexamethonium chloride, mecamylamine, nifedipine, nimodipine, tetrodotoxin (Sigma) and mibefradil dihydrochloride (Hoffmann-La Roche, Basel, Switzerland). ω-Agatoxin IVA was dissolved in a stock solution containing cytochrome C (1 mg/ml) to prevent non-specific binding of the peptide to chamber walls and tubing. Drugs were bath perfused for up to 60 min to ensure that the maximum effect was obtained.

Results

Basic characteristics of ganglionic transmission: submandibular ganglia

Stimulation of the lingual nerve with trains of stimuli (0.1–50 Hz, 4–50 V, pulse width 0.05–0.25 ms) evoked excitatory postsynaptic potentials (EPSPs), which could initiate action potentials in the cell bodies of the postsynaptic neurons of the rat submandibular ganglion. The postsynaptic neurons studied (n>150) can be classified into three types by their responses to these trains of stimuli. (1) Neurons in which supramaximal stimulation of the preganglionic nerve fibres at 0.1 Hz evokes a suprathreshold EPSP and action potential in response to every stimulus (strong input synapse, approximately 50% of the total number of cells studied). (2) Neurons where the EPSP evoked by supramaximal stimulation does not usually reach threshold for the initiation of an action potential (weak input synapse, approximately 25% of the total number of cells studied). Approximately 50% of the weak input synapses exhibit frequency-dependent facilitation, i.e. a change in the frequency of stimulation from 0.1 to 10 Hz increased the probability of an action potential being initiated. (3) Neurons that receive multiple synaptic inputs (approximately 25% of the total number of cells studied), cell bodies being innervated by two or more preganglionic axons. EPSPs recorded from cells receiving strong or weak inputs were abolished by either hexamethonium (30–100 μM) or mecamylamine (10 μM), indicating that EPSPs were mediated by ACh acting at nicotinic receptors.

Effects of N-, P/Q- and L-type calcium channel antagonists on excitatory postsynaptic potentials

The effects of the following calcium channel antagonists, applied alone and in combination, were investigated on evoked and spontaneous transmitter release in cells that received strong and weak inputs: 300 nM ω-CTX GVIA (N type), 100 nM ω-agatoxin IVA (P type), 300 nM ω-CTX MVIIC (Q type) and 30 μM nifedipine or 10 μM nimodipine (L type). In contrast to mammalian postganglionic autonomic nerves (for review see Ref. [13]), EPSPs were resistant to blockade by the N-type calcium channel blocker ω-CTX GVIA (300 nM; Fig. 1A). In all strong input neurons studied (n=10), no functional change in evoked release was detected after the application of ω-CTX GVIA, i.e. ω-CTX GVIA could not functionally reduce the EPSP below the threshold for the initiation of an action potential.

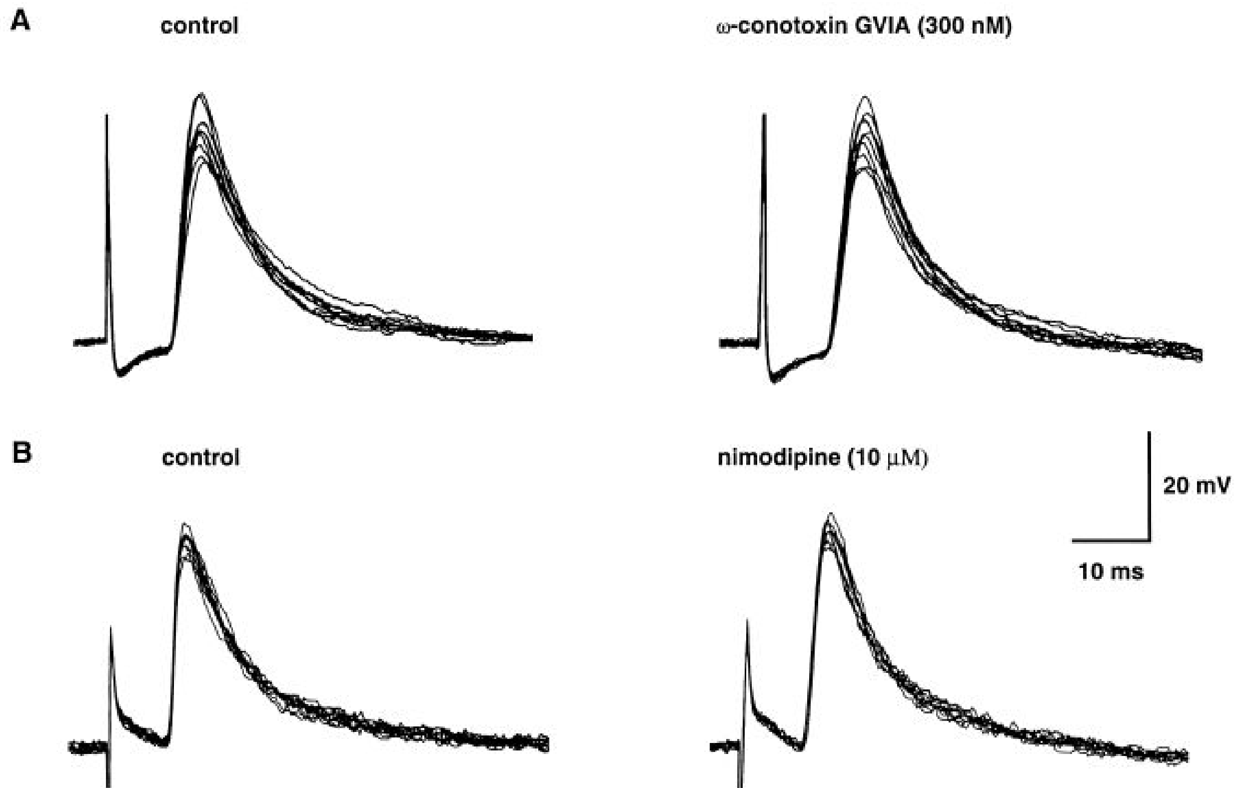

Fig. 1. Effect of ω-CTX GVIA and nimodipine on nerve-evoked responses in the rat submandibular ganglion. EPSPs were evoked by trains of 10 stimuli at 1 Hz. (A) Ten superimposed traces of EPSPs in the absence (control) and presence of ω-CTX GVIA (300 nM). RMP: −73 mV. (B) Ten superimposed traces of EPSPs in the absence (control) and presence of nimodipine (10 μM). RMP: −70 mV.

However, in weak input neurons, close examination of the recordings revealed subtle inhibitory effects of ω-CTX GVIA on the amplitude of EPSPs. In some cells, ω-CTX GVIA was able to inhibit EPSPs by up to 20% of the control amplitude (five of eight preparations; not shown), suggesting that N-type calcium channels may play a minor role in neurotransmission in the rat submandibular ganglia.

Application of ω-agatoxin IVA (100 nM), ω-CTX MVIIC (300 nM), nifedipine (30 μM) or nimodipine (10 μM), either alone or in combination with each other, produced no functional change in synaptic transmission in any of the cells studied (n=3–13 per drug; Figs 1B, 2A, B). EPSPs were, however, abolished by low concentrations of the non-specific calcium channel blocker, Cd^{2+} (30 μM; n=4; Fig. 2C). These results suggest that ACh release is evoked by Ca^{2+} entry into the preganglionic nerve terminals through calcium channels other than the N, P/Q or L types.

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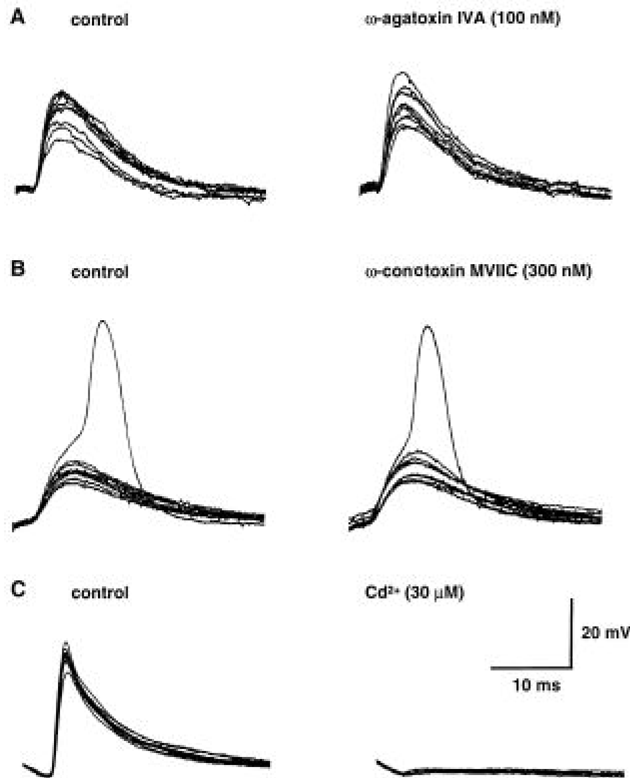

Fig. 2. Effect of ω-agatoxin IVA, ω-CTX MVIIC and Cd^{2+} on nerve-evoked responses in the submandibular ganglion. EPSPs were evoked by trains of 10 stimuli at 0.3 Hz. (A) Ten superimposed traces of EPSPs in the absence (control) and presence of ω-agatoxin IVA (100 nM). RMP: −73 mV. (B) Ten superimposed traces of EPSPs in the absence (control) and presence of ω-CTX MVIIC (300 nM). RMP: −70 mV. (C) Ten superimposed traces of EPSPs in the absence (control) and presence of Cd^{2+} (30 μM). RMP: −55 mV.

Effects of amiloride on excitatory postsynaptic potentials

The effects of the potassium-sparing diuretic, amiloride, which has been reported to inhibit T-type calcium channels in dorsal root ganglion neurons,[26] were investigated on ganglionic neurotransmission. Amiloride produced a reversible and concentration-dependent inhibition of EPSPs in the rat submandibular ganglion (Fig. 3). Control EPSPs, at weak input synapses, had a mean amplitude of 36.5±3.6 mV, and 20 min after bath application of 30 and 100 μM amiloride had mean amplitudes of 3.5±1.4 and 0.8±0.5 mV, respectively (n=4). The amplitude and frequency of spontaneous EPSPs and the sensitivity of the postsynaptic membrane were unaffected by 100 μM amiloride (not shown), suggesting that amiloride was acting presynaptically to inhibit only evoked neurotransmitter release. Similar results were observed when the effect of the amiloride analogue, dimethyl amiloride, was investigated. Dimethyl amiloride (100 μM) also caused a strong inhibition of evoked transmitter release, but the onset of the inhibition was less rapid (~5 min) than amiloride (<2 min; not shown).

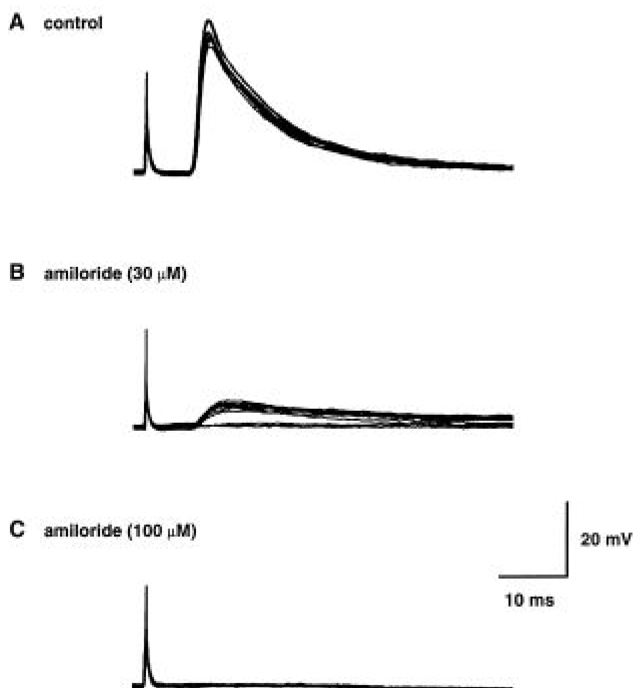

Fig. 3. Effect of amiloride on nerve-evoked EPSPs in the submandibular ganglion. (A) Control EPSPs evoked by trains of 10 stimuli at 0.4 Hz. (B) Postsynaptic responses evoked 10 min after the bath application of 30 μM amiloride. (C) Postsynaptic responses evoked 10 min after the bath application of 100 μM amiloride. RMP: −65 mV.

The effects of the neuronal T-type calcium channel blocker, mibefradil,[14, 17 and 27] were also investigated. In cerebellar Purkinje neurons, 0.1–2 μM mibefradil is sufficient to block neuronal T-type calcium channels.[14] Mibefradil (1–30 μM) had no detectable effect on either evoked (Fig. 4) or spontaneous transmitter release (*n*=4; not shown). Therefore, it appears that T-type calcium channels do not control evoked neurotransmitter release in the rat submandibular ganglion.

The R-type calcium channel blocker, Ni^{2+} (50–100 μM), also had no detectable effects on either evoked or spontaneous transmitter release (*n*=3; not shown), suggesting that R-type calcium channels do not control neurotransmitter release in the submandibular ganglion.

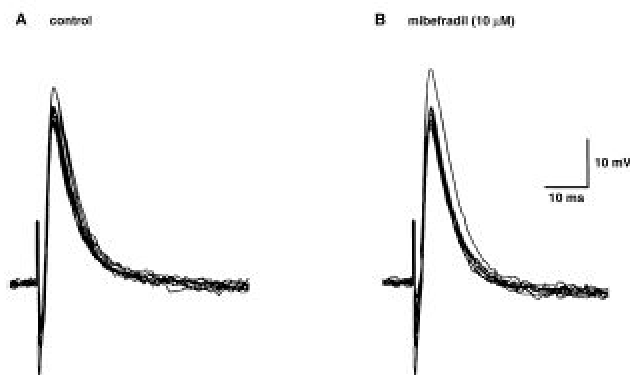

Fig. 4. Effect of mibefradil on nerve-evoked EPSPs in the submandibular ganglion. (A) Control EPSPs evoked by trains of stimuli at 0.5 Hz. (B) Postsynaptic responses evoked in the presence of 10 μM mibefradil. RMP: −70 mV.

Basic characteristics of ganglionic transmission: pelvic ganglia

Focal extracellular recording techniques were used to investigate the effects of calcium channel antagonists on ganglionic transmission in the rat pelvic ganglia. Electrical stimulation of the hypogastric nerve evoked EPSCs which were always preceded by the NTI (Fig. 5). In most extracellular recordings, EPSCs and postganglionic NTIs could be recorded when the extracellular Ca^{2+} concentration was 0.5 mM. To inhibit initiation of postganglionic action potentials, the bath concentration of Ca^{2+} was lowered to 0.25 mM. This procedure allowed the recording of EPSCs without postganglionic action potentials.

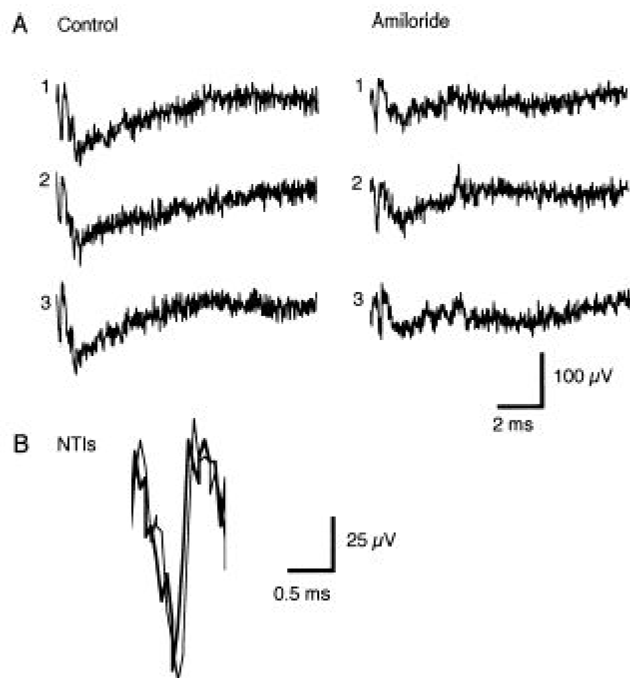

Fig. 5. Effect of amiloride on EPSCs and NTIs in the rat pelvic ganglion. (A) The traces on the left show three consecutive control responses to stimulation of the hypogastric nerve at 0.1 Hz. The traces on the right show three consecutive responses after the bath application of amiloride (30 μM). (B) Effect of amiloride on the NTI configuration shown by two superimposed NTIs. The thin line trace was recorded in the absence of amiloride, while the thick line trace was recorded after 20 min of amiloride exposure (30 μM).

Effects of amiloride on excitatory postsynaptic currents and nerve terminal impulses

Amiloride produced a reversible and concentration-dependent inhibition of EPSCs. Control EPSCs had a mean amplitude of 326±43 μV, and 20 min after bath application of amiloride (100 μM) the amplitude was 30±23 μV (*n*=4; Fig. 5). The decrease in mean amplitude of EPSCs produced by amiloride was due to an increase in the frequency of failures of transmitter release (EPSCs) to occur. However, amiloride had no detectable effects on the amplitude or configuration of the NTI (Figs 5, 6), suggesting that amiloride does not prevent action potential propagation to the nerve terminals.

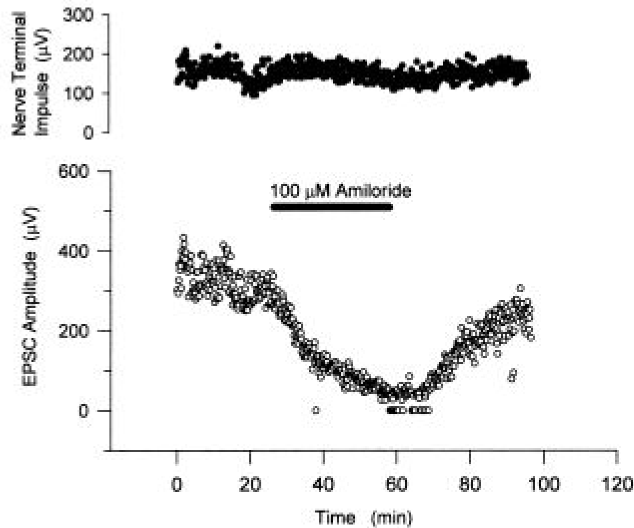

Fig. 6. Effect of amiloride on EPSC amplitude in the rat pelvic ganglion. The upper trace (filled circles) shows the amplitude of NTIs and the lower trace (open circles) shows the amplitude of EPSCs plotted against time. Application of amiloride is indicated by the solid bar. Amiloride (100 µM) reversibly decreased EPSC amplitude without affecting NTI amplitude.

Effects of N-, P/Q- and L-type calcium channel antagonists on excitatory postsynaptic currents and nerve terminal impulses

The effects of the following calcium channel antagonists, applied alone and in combination, were investigated on neurotransmitter release in the pelvic ganglia: 100 nM ω-CTX GVIA (N type), 100 nM ω-CTX MVIIC (P/Q type) and 10 µM nifedipine (L type). None of these selective calcium channel antagonists caused a significant inhibition of EPSC amplitude (*n*=6; Table 1) or any effect on the configuration or amplitude of the NTI or the frequency of recording spontaneous EPSCs (*n*=6; not shown). EPSC amplitudes were, however, substantially reduced by amiloride (100 µM) and abolished by the non-specific calcium channel blocker, Cd^{2+} (100 µM; Table 1). The frequency of spontaneous EPSCs was greatly reduced by Cd^{2+} (*n*=6), but not by amiloride (100 µM). These results suggest that ACh release is evoked by Ca^{2+} entry into the nerve terminals through calcium channels other than the N, P/Q or L types.

Table 1. Effect of calcium channel antagonists on extracellular excitatory postsynaptic current amplitude in the rat pelvic ganglion

Antagonist	Concentration (µM)	Relative EPSC amplitude	*n*
ω-CTX GVIA	0.1	0.98 ± 0.08	6
ω-CTX MVIIC	0.1	0.94 ± 0.07	6
Nifedipine	10	0.91 ± 0.07	6
Cd^{2+}	100	0	4
Amiloride	100	0.13 ± 0.10	4

Discussion

Numerous studies have investigated the nature of the calcium channels controlling transmitter release in postganglionic autonomic nerves. In contrast, there is limited information concerning the types of calcium channels controlling transmitter release in preganglionic autonomic nerves. The aim of this study was to investigate the type(s) of calcium channel that controls ACh release in the parasympathetic submandibular ganglia and the sympathetic pelvic ganglia of the rat.

The main result from this investigation was that none of the specific calcium channel antagonists at N-, L- and P/Q-type calcium channels was able to inhibit neurally evoked transmitter release in the two ganglionic preparations. ω-CTX GVIA was unable to functionally inhibit neurotransmitter release, i.e. ω-CTX GVIA could not

prevent each EPSP from triggering an action potential, in strong input neurons. However, in some weak input neurons, ω-CTX GVIA did cause a slight inhibition (~20%) of EPSP amplitude, suggesting that some N-type calcium channels are present on the presynaptic nerve terminals of the submandibular ganglia, but they play a minor role in neurotransmitter release.

The L-type (nifedipine and nimodipine), P/Q-type (ω-agatoxin IVA and ω-CTX MVIIC) and R-type (Ni^{2+}) calcium channel antagonists had no detectable inhibitory effects on transmitter release, indicating that L-, P/Q- and R-type calcium channels are not involved in mediating neurally evoked transmitter release in these preganglionic terminals. The lack of effect of Ni^{2+} on evoked transmitter release in the rat submandibular ganglion is consistent with results reported previously in this preparation.[19] In contrast, R-type Ca^{2+} currents inhibited by Ni^{2+} (100 μM) contribute to evoked transmitter release in the calyx-type synapse of the rat medial nucleus of the trapezoid body.[31] Cadmium ions (30–100 μM) rapidly and reversibly abolished neurotransmitter release in both preparations, demonstrating that ACh release is dependent on Ca^{2+} influx into the nerve terminal through VSCCs.

However, nerve-evoked transmitter release from these terminals was rapidly and reversibly abolished by amiloride, which has been reported to block T-type calcium channels in dorsal root ganglion neurons.[26] Amiloride markedly inhibited evoked transmitter release without affecting spontaneous transmitter release or the amplitude or configuration of the nerve terminal impulse. These results suggest that amiloride acts presynaptically to inhibit neurotransmitter release, but does not prevent action potential propagation in the nerve terminals. Similar results were observed using the amiloride analogue, dimethyl amiloride, but the T-type calcium channel blocker, mibefradil (1–30 μM), had no detectable effects on synaptic transmission. This observation suggests that amiloride does not inhibit neurotransmitter release by blocking T-type calcium channels, but may inhibit one or more pharmacologically distinct calcium channel types on the synaptic boutons.

Similarly, the hypogastric boutons of the rat pelvic ganglia were shown to be insensitive to N-, P/Q- and L-type VSCC antagonists. Amiloride, however, inhibited neurotransmitter release by causing a decrease in EPSC amplitude and a decrease in the number of release events that occurred. On closer examination of the amplitude and time-course of the NTI, we could not detect any significant alteration to the size or configuration of the NTI. This observation suggests that amiloride does not affect action potential propagation in the nerve terminals. The decrease in neurotransmitter release observed in the presence of amiloride may be caused by an increase in intraterminal pH due to a suppression of Na^+–H^+ exchange, by some alteration of the calcium sensitivity of the vesicular secretory mechanism or by an inhibition of VSCCs.[15] It is unlikely that amiloride is inducing its inhibitory effects by inhibiting the Na^+–H^+ exchanger, since the potent Na^+–H^+ exchange inhibitor, dichlorobenzamil, does not suppress EPSP amplitude in rat submandibular ganglia.[19] The lack of effect of amiloride on spontaneous EPSPs suggests that amiloride does not disrupt the transmitter secretory mechanism. Interestingly, it has been shown that amiloride causes a concentration-dependent but incomplete inhibition of depolarization-activated Ba^{2+} currents through the pore-forming α_{1B} subunit of the N-type calcium channel[12] expressed in *Xenopus* oocytes (Luchian T. and Adams D. J., unpublished observations). Therefore, it is possible that amiloride causes an inhibition of evoked transmitter release by blocking one or more types of VSCC.

Conclusions

On the presynaptic nerve terminals of the rat parasympathetic submandibular ganglia and the sympathetic pelvic ganglia, neurotransmitter release is controlled by pharmacologically distinct calcium channels which are resistant to blockade by N-, L-, P/Q-, R- and T-type calcium channel blockers. Amiloride and dimethyl amiloride are the only drugs to date (except for cadmium ions) which inhibit neurally evoked transmitter release from synaptic boutons on postganglionic neurons, suggesting that they are able to block the pharmacologically distinct calcium channel(s) on the preganglionic nerve terminals of rat autonomic ganglia. These results are similar to those reported in the guinea-pig anterior pelvic ganglion,[22] but differ from those reported in the rat superior cervical ganglion [8] and the guinea-pig paravertebral ganglion, [10] where P-type Ca^{2+} channels are proposed to play a substantial role in controlling nerve-evoked transmitter release. These data suggest that there is considerable heterogeneity in the expression and function of VSCCs in presynaptic nerve terminals between peripheral ganglia and species.

Abbreviations

ACh, acetylcholine; ω-CTX, ω-conotoxin; $DiOC_2(5)$, 3,3′-diethyloxardicarbocyanine iodide; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; NTI, nerve terminal impulse; RMP, resting membrane potential; VSCC, voltage-sensitive calcium channel

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