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Endothelin modulates calcium channel current in neurones of rabbit pelvic parasympathetic ganglia

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1 The effects of endothelin were studied, *in vitro*, on neurones contained in the rabbit vesical pelvic ganglion by use of intracellular and single-electrode voltage clamp techniques under conditions where sodium and potassium channels were blocked.

2 In the current-clamp experiments, endothelin (1 μM) caused a depolarization followed by a hyperpolarization of the membrane potential. In the voltage-clamp experiments, endothelin (0.01–1 μM) caused an inward current followed by an outward current in a concentration-dependent manner.

3 Membrane conductance was increased during the endothelin-induced depolarization and inward current. Membrane conductance was decreased during the endothelin-induced hyperpolarization and outward current.

4 The endothelin-induced inward and outward currents were not altered by lowering external sodium concentration or raising external potassium concentration.

5 The endothelin-induced inward current was depressed (mean 72%) in a Krebs solution containing nominally zero calcium and high magnesium. These results suggest that a predominent component of the endothelin-induced inward current is mediated by calcium ions.

6 The calcium-insensitive component of the inward current was abolished by a chloride channel blocker, 4-acetamide-4'-isothiocyanostilbene-2,2'-disulphonic acid. The mean reversal potential for the calcium-insensitive component of the inward current was −18 mV. This value is near the equilibrium potential for chloride. Thus, it is presumed that the calcium-insensitive component of the inward current is carried by chloride ions.

7 Endothelin caused an initial depression followed by a long lasting facilitation of both rapidly and slowly decaying components of high-threshold calcium channel currents (N- and L-type).

8 In summary, the data show that for neurones in the vesical pelvic ganglia, endothelin causes membrane depolarization and activates an inward current. The ionic mechanisms involve receptor-operated calcium and chloride currents. Also, endothelin causes an initial depression followed by a long-lasting facilitation of the voltage-dependent calcium current.

Keywords: Endothelin-1; parasympathetic ganglia; single neurones; depolarization; calcium current; single electrode voltage-clamp

Introduction

Endothelin is a potent vasoconstrictor peptide recently isolated from porcine and human vascular endothelial cells (Itoh *et al.*, 1988; Yanagisawa *et al.*, 1988). In porcine coronary artery smooth muscle, endothelin augmented the dihydropyridine-sensitive voltage-dependent calcium channel current (Goto *et al.*, 1989; Silberberg *et al.*, 1989) whereas in portal vein smooth muscle, endothelin augmented both the T- and L-type calcium channel current (Inoue *et al.*, 1990).

Endothelin may also function as a neuropeptide (Masaki, 1989) in the central and peripheral nervous systems. Endothelin binding sites and immunoreactivity have been identified and depolarizing responses to endothelin have been reported for neurones (Giaid *et al.*, 1989; Koseki *et al.*, 1989; Yoshizawa *et al.*, 1989a,b; MacCumber *et al.*, 1990). Little is known, however, regarding the action of endothelin on neuronal ionic conductances and how activation of the endothelin receptors alters the operation of neuronal voltage-dependent calcium channels. The present study was undertaken to determine whether endothelin regulates the ionic conductance and voltage-dependent calcium channels of mammalian autonomic neurones, by use of intracellular current clamp and single-electrode voltage clamp techniques. A preliminary account of some of this work has been published (Nishimura *et al.*, 1989; 1990).

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Methods

Male white rabbits weighing 2.0–3.0 kg were anaesthetized with sodium pentobarbitone (40–50 mg kg^{-1}, i.v.). The methods for isolation of vesical pelvic ganglia were described previously (Nishimura *et al.*, 1988b). After removal of the pelvic ganglia, rabbits were killed by a large dose (100–150 mg kg^{-1}, i.v.) of sodium pentobarbitone. Individual ganglia were pinned onto Sylgard at the bottom of a small chamber (0.5 ml) and continuously superfused with Krebs solution (3 ml min^{-1}) having the following composition (mM): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11. Solutions were gassed with 95% O_2, 5% CO_2 and preheated to 35–37°C.

Microelectrodes were filled with 2 M CsCl unless stated otherwise. Tip resistances of microelectrodes were 20–40 MΩ. Membrane currents were recorded by the single-electrode voltage-clamp method with an Axoclamp 2A (Axon Instruments). Sampling frequency ranged between 3 and 5 kHz with a 70–30 duty cycle. Signals from the microelectrode were displayed on an oscilloscope with digitized memory (Nihon Kohden, VC-11) and recorded on a pen-writing chart recorder (Nihon Kohden, RJG-3022 and RJG-4124). Data were also stored on a video cassette data recorder (SONY KS609) for later analysis.

To block voltage-dependent sodium and potassium currents, a modified Krebs solution was used which contained tetrodotoxin (TTX, 300 nM) and tetraethylammonium (TEA, 50 mM) and where external sodium was reduced from 143.2 mM to 93.2 mM. To suppress potassium currents, caesium

ions (Cs^+) were also ionophoretically injected into neurones through a recording microelectrode filled with 2 M CsCl. In the current clamp experiments, injection of Cs^+ causes membrane depolarization (range, −35 mV to −10 mV; Akasu *et al.*, 1990). To record action potentials from these neurones the depolarization was nullified by injection of a constant hyperpolarizing current through the microelectrode. Efficiency of Cs^+-injection was judged by the broadening of the action potential, the complete abolition of the afterhyperpolarization and the appearance of an afterdepolarization following the action potential (Nishimura *et al.*, 1988b). Voltage-dependent inward calcium and barium currents were recorded where sodium and potassium currents were suppressed (see above). Leak currents were determined by applying hyperpolarizing voltage-commands of magnitudes equal to depolarizing commands used to evoke the inward current. Leak currents were also recorded during depolarizing commands equivalent to those used to evoke inward currents after block of voltage-dependent calcium and barium currents by the removal of calcium or barium or by cobalt application. Leak currents were subtracted from relevant currents to yield the calcium and barium currents by use of a Nicolet memory oscilloscope (Nicolet 4094).

Calcium- and barium-free Krebs solution contained nominally zero calcium and barium and 12 mM magnesium. Nominally zero calcium and high magnesium solutions have been shown to block fast excitatory postsynaptic potentials recorded from neurones in vesical pelvic ganglia (Nishimura *et al.*, 1988a). Drugs were dissolved in Krebs solution and applied by changing the flow with a three-way stopcock.

Drugs used were: endothelin-1 from Peptide Institute (Japan); tetrodotoxin (TTX) from Sankyo; tetraethylammonium chloride (TEA) from Tokyo Kasei (Japan); 4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonic acid disodium salt (SITS) from Research Organics (U.S.A.); (+)-tubocurarine chloride, yohimbine hydrochloride and prazosin hydrochloride from Sigma; atropine sulphate from Merck; [3α-tropanyl]-1H-indole-3-carboxylic acid ester (ICS 205-930) from Sandoz. Results are expressed as the mean ± standard error of the mean (s.e.mean).

Results

Endothelin-induced membrane responses

Superfusion of the pelvic ganglia with endothelin (1 μM for 2–3 min) produced a slow depolarization in neurones with a mean time to peak of 1.1 ± 0.1 min ($n = 5$) and a mean peak amplitude of 19 ± 3 mV (Figure 1a). When endothelin was removed from the Krebs solution the depolarization recovered and was followed by membrane hyperpolarization with a mean peak amplitude of 10 ± 6 mV ($n = 5$). The hyperpolarization occurred for time periods ranging from 20 to 30 min. In three neurones the depolarizing and hyperpolarizing action of endothelin was associated with a decrease and an increase in membrane input resistance, respectively. The endothelin-induced depolarization and the subsequent hyperpolarization after washout and associated changes in membrane input resistance were not altered by the cholinoceptor antagonists, (+)-tubocurarine (10 μM) and atropine (1 μM) ($n = 2$) (Gallagher *et al.*, 1982), by the adrenoceptor antagonists, yohimbine (1 μM) and prazosin (1 μM) ($n = 2$) (Akasu *et al.*, 1985) or by the 5-hydroxytryptamine (5-HT) antagonist, ICS 205-930 (10 nM) ($n = 2$) (Akasu *et al.*, 1987). Thus, cholinoceptors, adrenoceptors and 5-HT_3 receptors were not involved in mediating the response to endothelin.

Endothelin-induced membrane currents

Voltage-clamp methods were used to examine the ionic mechanisms underlying the endothelin-induced responses, under conditions where voltage-dependent sodium and potassium currents were blocked (see Methods). Superfusion of the ganglia with endothelin (10 nM to 1 μM) induced an inward current with amplitudes ranging from 0.2 to 3.3 nA, at holding potentials ranging from −50 to −75 mV (Figure 2a). When endothelin was removed from the superfusing solution the inward current, which slowly declined, was followed by an outward current (amplitude range, 0.1–2.2 nA). The outward current lasted for time periods ranging between 15 to 30 min before recovery. Endothelin induced inward and outward currents in a concentration-dependent manner (Figure 2b). The calculated EC_{50} values were 60 nM and 90 nM for the inward and outward currents, respectively. The current responses were reproducible, when endothelin was applied to neurones for times ranging between 10 to 30 s and intervals between applications were longer than 30 min. Current responses to endothelin showed tachyphylaxis, when applied for a period of 5 to 10 min. Subsequent application of endothelin caused no current response even after neurones were superfused with an endothelin-free Krebs solution for more than 30 min.

Conductance changes associated with endothelin-induced currents

Endothelin-induced inward and outward currents were associated with an increase and a decrease, respectively, in membrane conductance (Figure 3a,b). Membrane conductance was

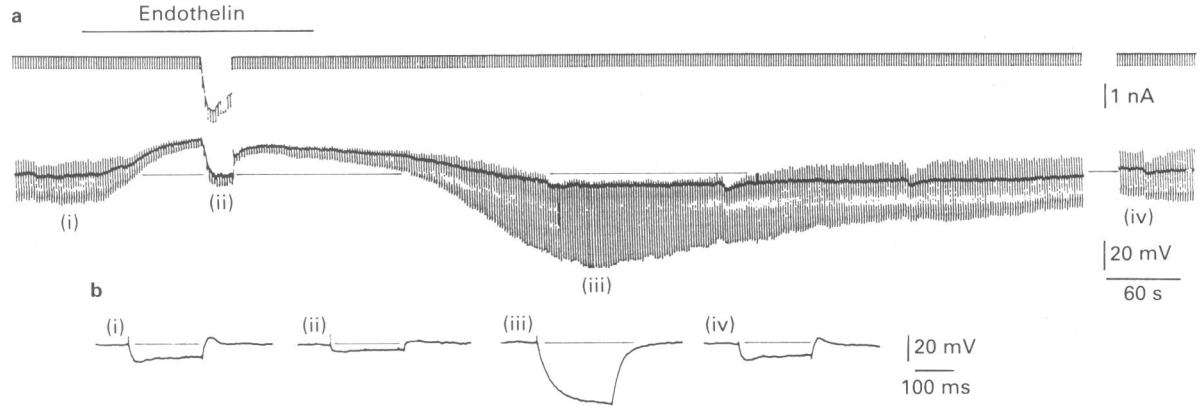

Figure 1 (a) Effect of endothelin on resting membrane potential and membrane input resistance of vesical pelvic neurone. The neurone was superfused with Krebs solution containing 1 mM caesium and impaled by a microelectrode containing 3 M KCl. Endothelin (1 μM) induced a depolarization followed by a hyperpolarization of the membrane potential. Input resistance was calculated from the amplitude of the hyperpolarizing electrotonic potentials (lower trace) evoked by injection of hyperpolarizing current pulses (upper trace) through the recording microelectrode. Endothelin-induced depolarization was partially nullified by hyperpolarizing direct current. Horizontal bar indicates time period of endothelin application. (b) Expanded records of the electrotonic potential. All records were taken at the times marked by numbers in (a). Record (iv) was taken 20 min after removal of endothelin from the perfusate. The resting membrane potential was −56 mV.

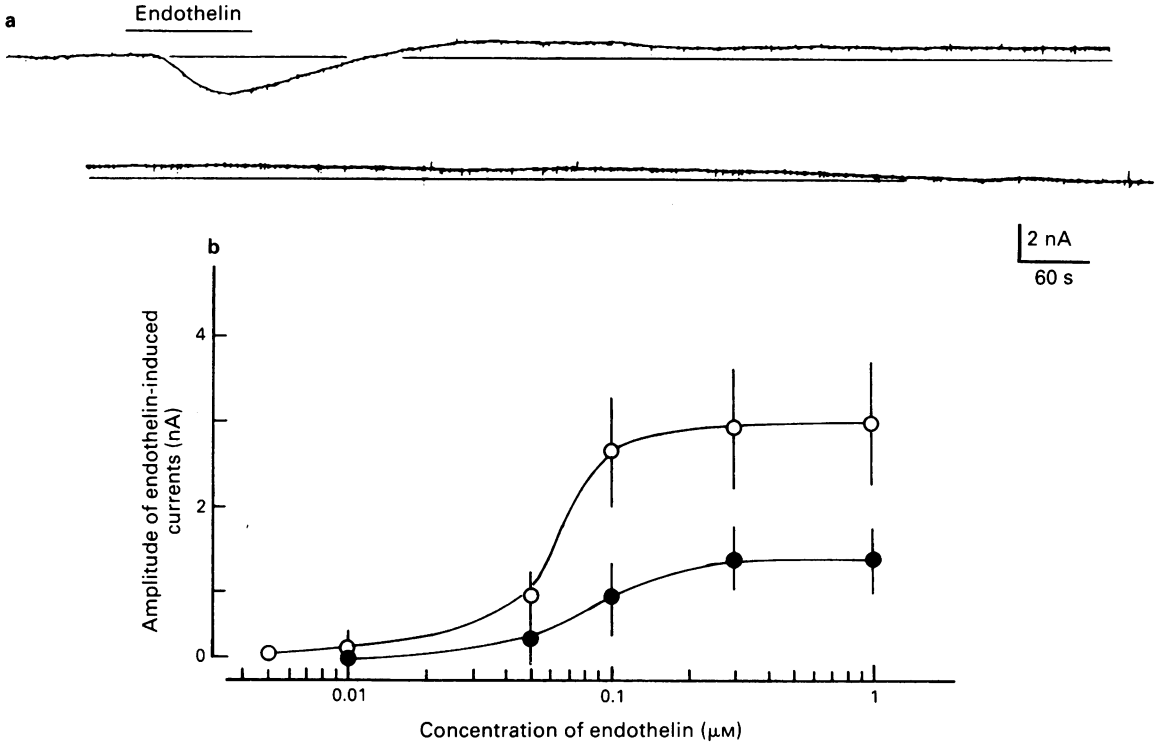

Figure 2 Effect of endothelin on membrane current. The modified Krebs solution contained tetrodotoxin (300 nM) and tetraethylammonium (50 mM) and sodium ions were reduced to 93.2 mM. Caesium ions had been injected into the cell. (a) Endothelin (100 nM) caused inward current followed by outward current. The horizontal bar indicates the time period of endothelin-application. (b) Relationship between concentration of endothelin (abscissa scale) and amplitude of current responses evoked by endothelin (ordinate scale). Points and vertical lines represent mean of 3 to 5 responses and s.e.mean, respectively: (○) inward current; (●) outward current.

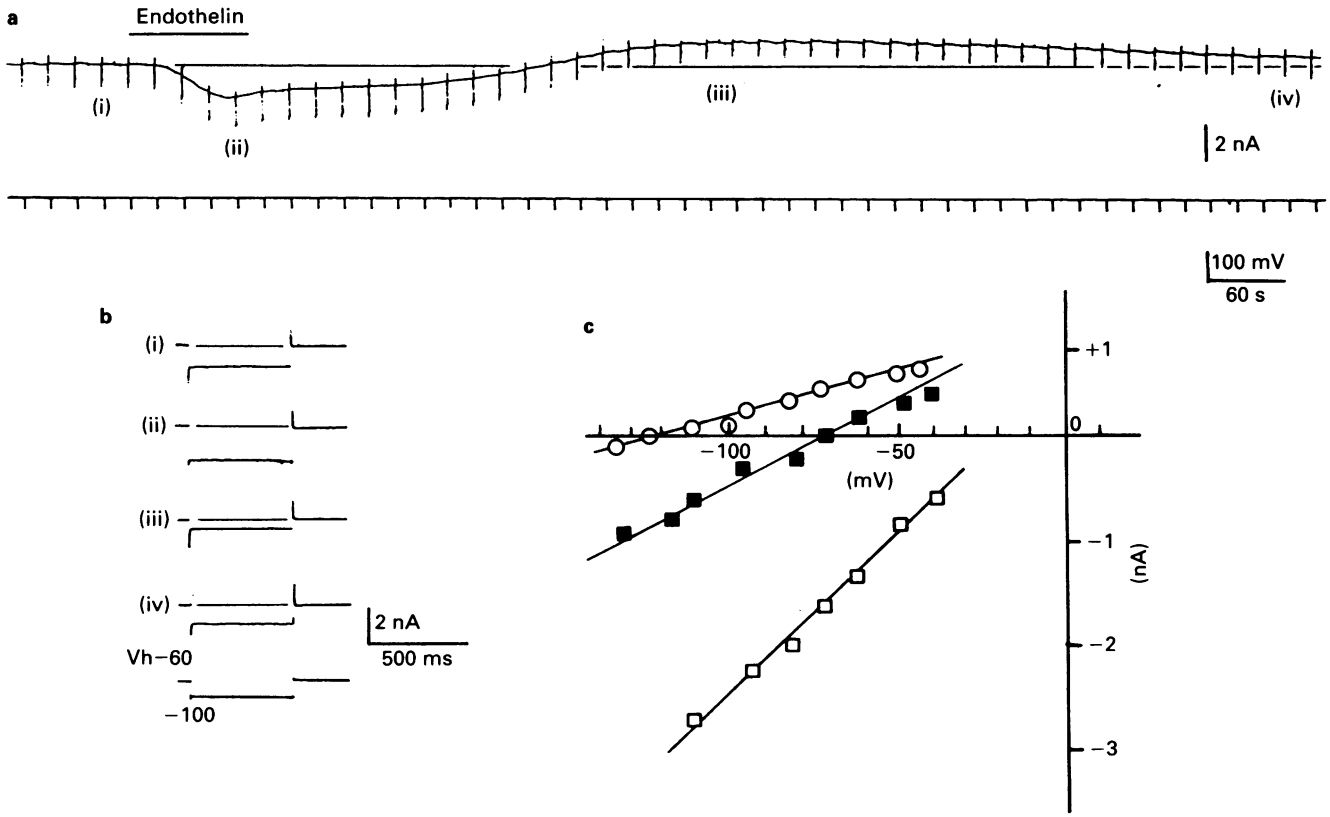

Figure 3 (a) Conductance changes during endothelin-induced (100 nM) current responses in a caesium-loaded neurone. The superfusate contained tetrodotoxin (300 nM) and tetraethylammonium (50 mM). The neurone was voltage-clamped at −60 mV and subjected to hyperpolarizing voltage jumps to −100 mV to measure membrane conductance. (b) Expanded records of inward currents produced by the hyperpolarizing command pulse of 500 ms duration. Records (i)–(iv) were taken at the times marked by numbers in (a). (c) Current-voltage relation (I–V curve) made by step commands with a duration of 200 ms at 0.2 Hz. The holding potential was −72 mV. The perfusate also contained caesium (2 mM) to block inward rectification. I–V curve before application of endothelin (100 nM) (■); I–V curve during endothelin-induced inward (□) and outward (○) currents, respectively.

determined by measuring the steady-state inward current induced by a 500 ms step command from a holding potential of −60 mV to −100 mV. In modified Krebs solution the calculated membrane conductance for this neurone was 25 nS. During the endothelin-induced inward and outward currents calculated membrane conductances were 43 nS and 11 nS, respectively (Figure 3b).

Measurements of membrane conductance (chord conductance) were also obtained from the slope of the linear portion of a current-voltage relation (I–V curve). Figure 3c shows the I–V curve of a neurone in a modified Krebs solution containing 2 mM caesium. The calculated chord conductance was 21 nS at potentials ranging between −50 mV and −110 mV. Endothelin-induced (100 nM) inward current was associated with an increase in the slope of the linear portion of the I–V curve (Figure 3c); the chord conductance was 40 nS in the presence of endothelin. In contrast, the slope of the linear I–V curve was decreased during the endothelin-induced outward current (Figure 3c). The chord conductance was 16 nS during the outward current. The mean chord conductance of four neurones in a modified Krebs solution was 23 ± 4 nS. The mean chord conductance during the endothelin-induced inward and outward currents was 39 ± 6 nS and 12 ± 3 nS, respectively.

Calcium dependency of endothelin-induced inward currents

In caesium-loaded neurones superfused with a modified Krebs solution containing TTX (300 nM) and TEA (50 mM), lowering the external calcium concentration to a nominal zero value did not change the holding current. Under these conditions the amplitude of the endothelin-induced currents was reduced and the outward current was totally inhibited (Figure 4a). The mean decrease in peak amplitude of the inward current for six neurones was 72 ± 3%.

In caesium-loaded neurones superfused with a modified Krebs solution, lowering the external sodium to 26.2 mM (67 mM sodium chloride was replaced with equimolar choline chloride) did not alter the amplitude and the time course of the endothelin-induced inward currents. In three neurones the range of peak amplitudes for the endothelin-induced (100 nM) inward and outward currents in a low sodium (26.2 mM) solution was 2 to 3 nA and 0.5 to 1 nA, respectively. In two other caesium-loaded neurones, raising the external potassium to 10 mM did not alter the holding current or the endothelin-induced inward and outward currents. These results suggest that the endothelin-induced inward current is mediated in part by calcium ions.

Reversal potential for endothelin-induced inward current

Experiments were next conducted to determine the reversal potential for the endothelin-induced inward current in the presence and absence of external calcium. Figure 4b shows that effects of endothelin (100 nM) on a current-voltage relation (I–V curve) for a neurone when the external calcium concentration was 2.5 mM (Figure 4b(i)) and nominally zero (Figure 4b(ii)). In both panels, the reversal potentials of the inward currents were estimated from the intersection of I–V

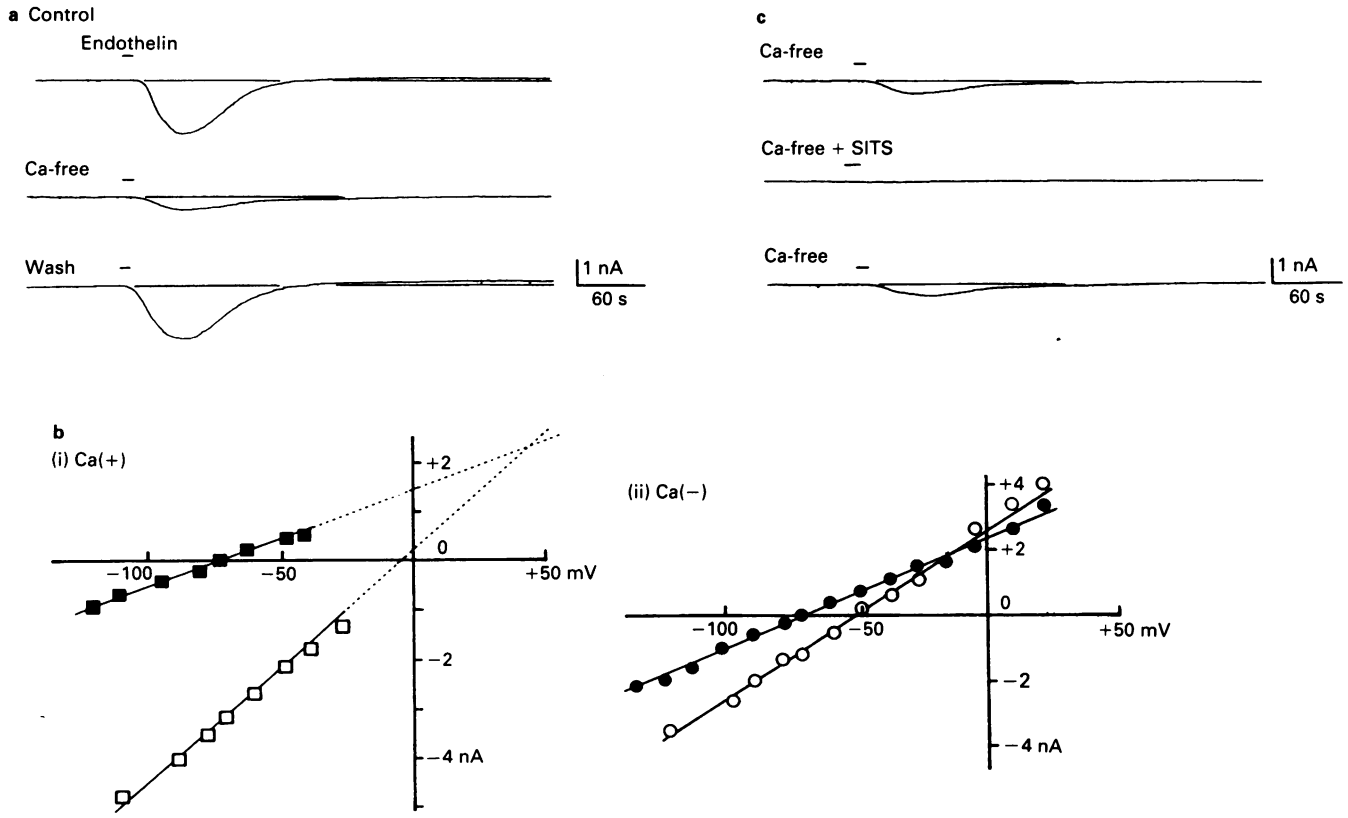

Figure 4 (a) Effects of removal of calcium ions on endothelin-induced (100 nM) current responses. The holding potential was −60 mV. Modified Krebs solutions contained tetrodotoxin (300 nM) and tetraethylammonium (50 mM). The calcium-free solution contained nominally zero calcium and 12 mM magnesium. Horizontal bars above each trace represent the time period of endothelin-application. (b) Effect of endothelin (100 nM) on the steady state I–V curve obtained in the presence (i) and absence (ii) of calcium. Step commands with a duration of 200 ms were applied from a holding potential of −70 mV at 0.2 Hz. Filled and open symbols were obtained before and during application of endothelin, respectively. I–V curves were obtained from two cells. (c) Effect of SITS (500 μM) on calcium-insensitive inward current produced by endothelin. Holding potential was −62 mV. Endothelin (100 nM) was applied to the perfusate at a time period indicated by short horizontal bars.

curves. In modified Krebs solution containing 2.5 mM calcium the estimated reversal potential for the endothelin-induced inward current was +45 mV. The mean reversal potential was +42 ± 3 mV ($n = 4$). In the calcium-free solution, endothelin induced an inward shift of membrane current and increased the slope of the I–V curve. The estimated reversal potential for the calcium-insensitive component for the cell was −14 mV. The mean reversal potential was −18 ± 4 mV ($n = 4$). The reversal potential for the calcium-insensitive component for the endothelin-induced inward current was near the equilibrium potential for chloride ions (Akasu *et al.*, 1990).

Effect of SITS on the endothelin-induced inward current

Pharmacological experiments were next conducted to determine whether chloride ions mediate the calcium-insensitive component of the endothelin-induced inward current. 4-Acetamido-4′-isothiocyanostilbene-2,2′-disulphonic acid (SITS) has been reported to block chloride current in squid axon (Inoue, 1985), cultured astrocytes (Gray & Ritchie, 1986), sensory neurones (Bader *et al.*, 1987), endocrine cells (Korn & Weight, 1987) and parasympathetic neurones (Akasu *et al.*, 1990). Addition of SITS (0.5 mM) to a calcium-free solution completely inhibited the calcium-insensitive component of the inward current (Figure 4c) ($n = 3$). The results are consistent with chloride being a charge carrier for a component of the inward current.

Voltage-dependent calcium (I_{Ca}) and barium (I_{Ba}) current

Voltage-dependent I_{Ca} and I_{Ba} were recorded from caesium-loaded neurones superfused with a Krebs solution containing Ca^{2+} (2.5 mM) or Ba^{2+} (2.5 mM), TTX (300 nM) and TEA (50 mM) (see Methods). When calcium was the charge carrier, application of a step command from a holding potential of −55 mV to −5 mV evoked an inward current with amplitudes ranging between 1–3 nA followed by an inward tail current (Figure 5). Tail currents are due to deactivation of calcium-dependent chloride channels (Akasu *et al.*, 1990). In contrast when barium was the charge carrier, application of a step command from a holding potential of −60 to −10 mV evoked inward currents with slower decay time than that of I_{Ca} and the absence of tail currents (Figure 6). Both the I_{Ca} and I_{Ba} were blocked by application of either cobalt (1 mM) or ω-conotoxin (500 nM) (Akasu *et al.*, 1990). During the endothelin-induced inward current, the I_{Ca} and tail current were reduced (Figure 5). When endothelin was removed from the perfusate, both I_{Ca} and the tail current recovered and then increased in amplitude as long as the outward current occurred.

Figure 6 shows the effect of endothelin (100 nM) when barium was the charge carrier for the calcium channel current. Endothelin also caused both inward and outward currents. The amplitude of the I_{Ba} was also depressed and then facilitated by endothelin. Partial recovery of the I_{Ba} was evident in the continued presence of endothelin (Figure 6c). The facilitation of the I_{Ba} lasted for approximately 20 min in an endothelin-free modified Krebs solution. The action of endothelin on the I_{Ba} was concentration-dependent (Figure 7).

Current-voltage relation of the I_{Ba}

Figure 8a(i) shows the current-voltage relation (I–V curve) of net membrane currents obtained before and during the endothelin-induced inward current. The neurone was initially clamped at −60 mV and subjected to step commands which ranged between −100 mV and +50 mV. Endothelin (100 nM) caused a 2 nA shift of the holding current at −60 mV and depressed the amplitude of the I_{Ba}. This resulted in a nearly linear I–V curve for the net membrane currents during the endothelin-induced inward current. Amplitudes of the net I_{Ba} obtained by subtraction of leak currents were plotted against command voltage in Figure 8a(ii). The I_{Ba} activated near −30 mV and the peak of the I_{Ba} occurred at −10 mV to +10 mV (see Akasu *et al.*, 1990). Endothelin depressed the amplitude of I_{Ba} at all potentials tested without producing any change in the threshold and the peak voltage of the I_{Ba} (Figure 8a(ii)). Figure 8b(i) shows the I–V curve of net membrane currents obtained during the outward current. Endothelin increased the amplitude of the I_{Ba} at each potential tested, but did not change the threshold membrane potential and the peak voltage of the I_{Ba} (Figure 8b(ii)).

Effect of endothelin on two types of voltage-dependent calcium current

Two types of high-threshold calcium currents, comparable to N- and L-type of calcium currents observed in cultured sensory neurones of the chick embryo (Nowycky *et al.*, 1985; Fox *et al.*, 1987), were recorded in neurones of rabbit vesical pelvic ganglia (Akasu *et al.*, 1990). At holding potentials between −70 and −50 mV, the I_{Ba} evoked at potentials more positive than −30 mV consists of the sum of two components of decay; a rapid decay with a mean time constant of 150 ms and a slow decay with a mean time constant of 560 ms (Akasu *et al.*, 1990). In addition, a slowly decaying component of

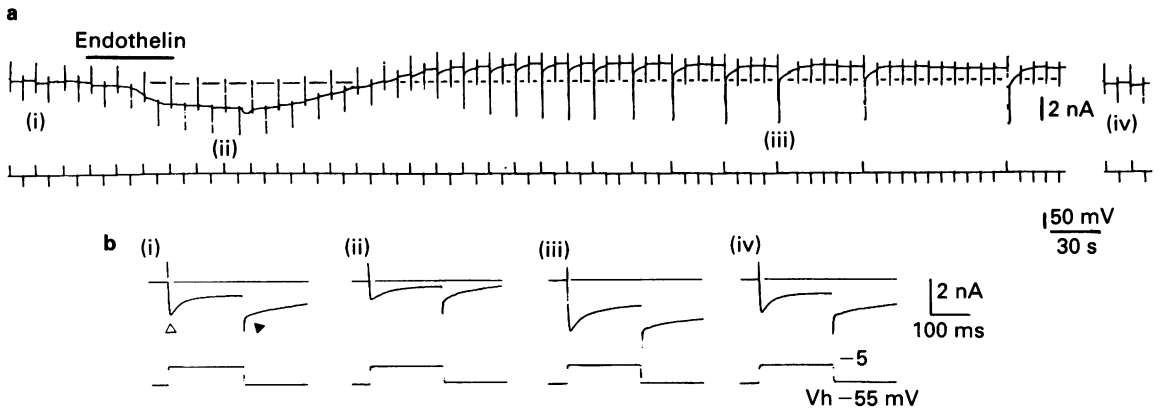

Figure 5 Effect of endothelin (100 nM) on voltage-dependent calcium currents (△) and tail currents (▲) recorded from caesium-loaded neurone. Modified Krebs solution contained tetrodotoxin (300 nM) and tetraethylammonium (50 mM). (a) Calcium and tail currents were evoked by a depolarizing step command (duration of 200 ms) from a holding potential of −55 mV to −5 mV. Upper and lower traces represent membrane current and holding voltage, respectively. Horizontal bar above current trace indicates time period of application of endothelin. (b) Expanded records (i)–(iv) were taken at the times marked by numbers in (a). Record (iv) was taken 20 min after withdrawal of endothelin from the perfusate.

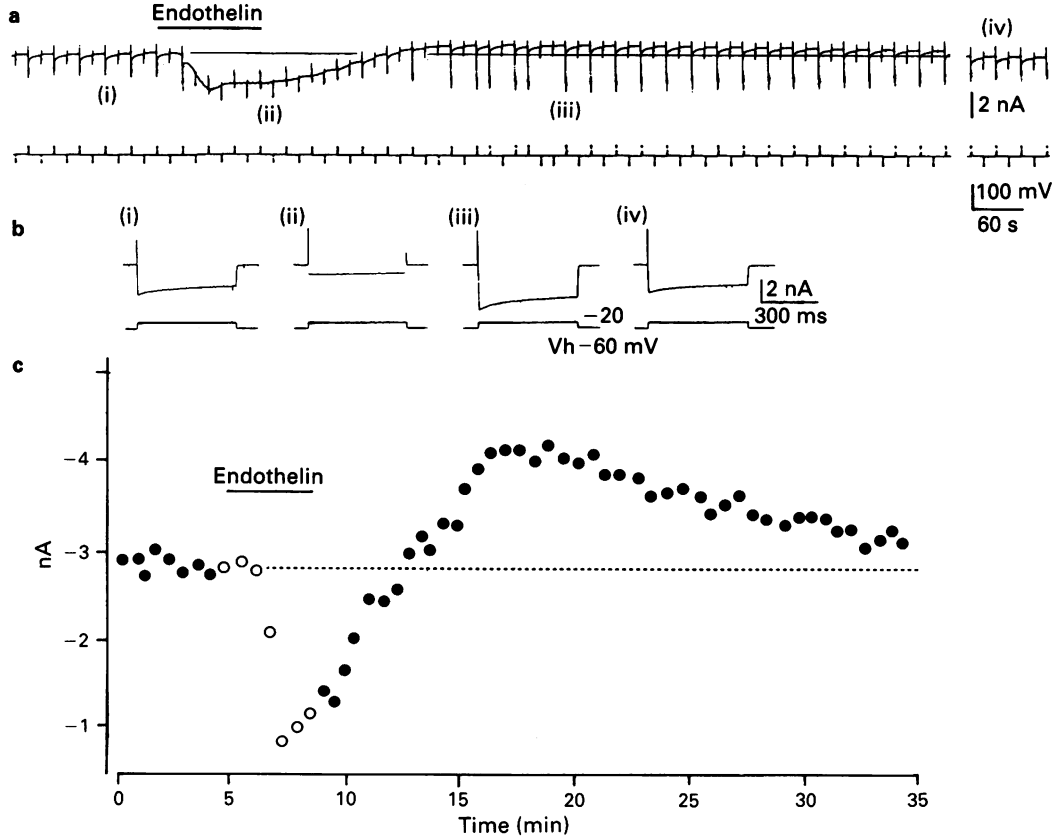

Figure 6 Effect of endothelin (100 nM) on barium inward current (I_{Ba}) recorded from a caesium-loaded neurone. Modified Krebs solution contained tetrodotoxin (300 nM) and tetraethylammonium (50 mM). Calcium (2.5 mM) was replaced with equimolar barium. (a) Upper and lower traces represent membrane current and holding voltage, respectively. The I_{Ba} was evoked by a depolarizing step command to −20 mV (duration of 500 ms) from a holding potential of −60 mV. Horizontal bar indicates the time period of endothelin-application. Downward and upward deflections in lower trace represent step commands to −100 mV and −20 mV, respectively. (b) Expanded records of depolarizing step command (lower trace) and I_{Ba} (upper trace) taken at times marked by numbers in (a). Record (iv) was obtained 20 min after withdrawal of endothelin. Leak currents obtained by hyperpolarizing step command were subtracted (see Methods). (c) Time course of inhibition and facilitation of I_{Ba} caused by endothelin (100 nM). Ordinate scale: peak amplitude of I_{Ba} in nA. Abscissa scale: time in min.

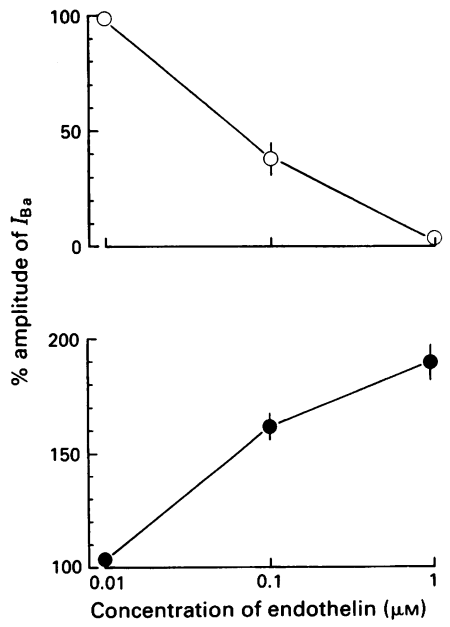

Figure 7 Relationship between concentration of endothelin (abscissa scale) and amplitude of I_{Ba} (ordinate scale). I_{Ba} was evoked by step commands to between −20 to +10 mV from a holding potential of −60 mV. Amplitude of I_{Ba} is expressed as a percentage of control amplitude. Upper and lower graphs represent endothelin-induced depression and facilitation of I_{Ba}, respectively. Circles and vertical lines represent mean and s.e.mean, respectively. Number of neurones tested were 3 for 10 nM, 8 for 100 nM and 2 for 1 μM of endothelin.

calcium current can be recorded in isolation from the rapidly decaying component by use of a pulse protocol where neurones are held at −45 to −40 mV and subjected to step commands to potentials more positive than −10 mV (Figure 9b). The time constant of these decays are similar to N- and L-type calcium currents (Fox *et al.*, 1987) or rapidly and slowly inactivating components of N-type calcium channel current (Plummer *et al.*, 1989; Seward & Henderson, 1990).

Endothelin (100 nM) depressed the amplitude of the I_{Ba} measured at the beginning (mean 70 ± 6%, $n = 5$) and the end (mean 40 ± 4%) of the 500 ms step command (Figure 9a). Figure 9a also shows that during the endothelin-induced inward current the initial rapid component of I_{Ba} inactivation was not apparent whereas the slow component of inactivation still remained although it had been reduced. During the endothelin-induced outward current, the I_{Ba} increased in amplitude at the beginning (mean 58 ± 6%, $n = 4$) and the end (53 ± 5%, $n = 4$) of the 500 ms command pulses. Figure 9b shows the effect of endothelin (100 nM) on slowly decaying current (similar to the L-type current). The peak amplitude of the I_{Ba} was depressed (mean 37 ± 6%, $n = 3$) during the endothelin-induced inward current. In contrast, the amplitude of the I_{Ba} was increased (mean, 67 ± 5%, $n = 3$) at the peak of the outward current. These results indicate that both rapidly and slowly decaying components of the calcium channel currents were depressed and facilitated by endothelin.

Discussion

The results of the present study indicate that endothelin causes membrane depolarization associated with a decrease in membrane input resistance followed by membrane hyperpo-

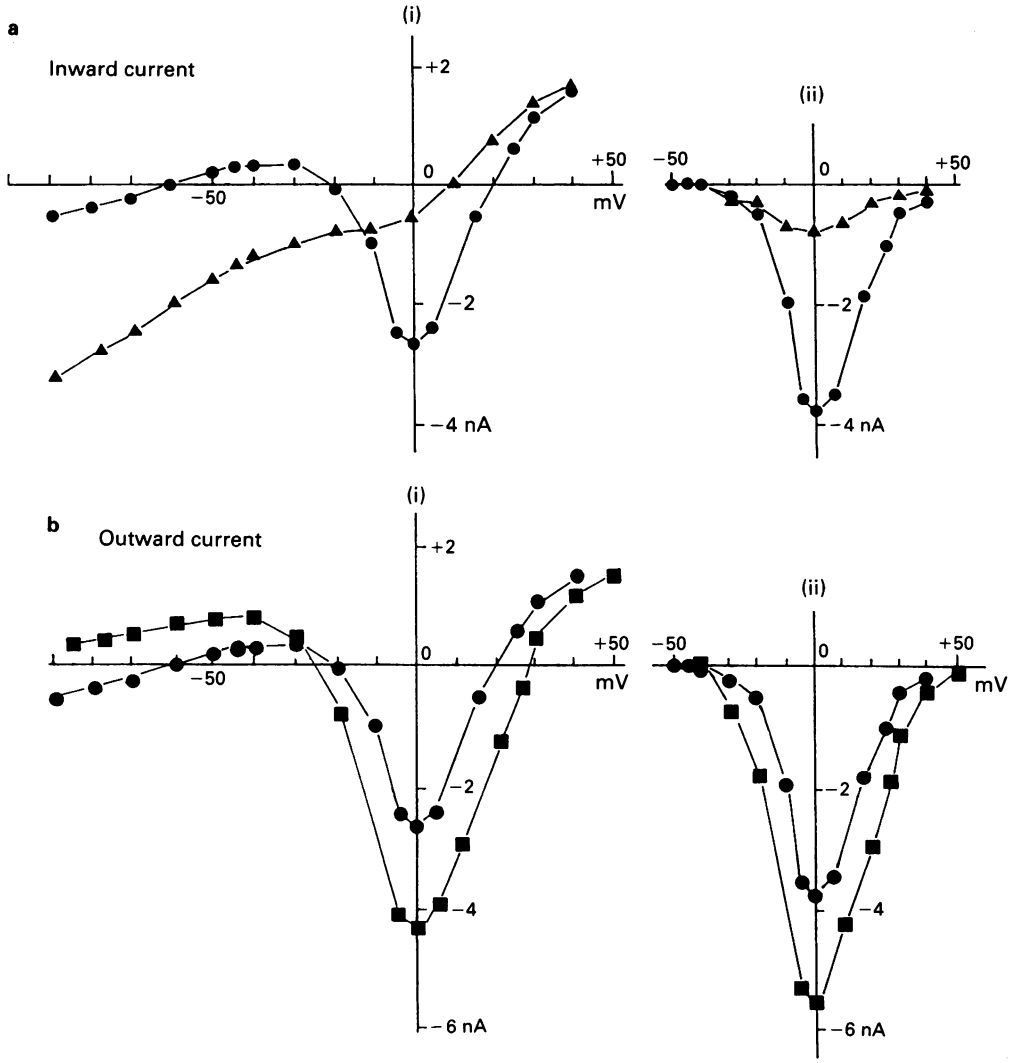

Figure 8 Current-voltage relation (I–V curve) for I_{Ba} obtained from a caesium-loaded neurone during inward current (a) and outward current (b) produced by endothelin (100 nM). I–V curves were obtained by step commands (duration of 200 ms) to potentials between −120 and +50 mV from a holding potential of −60 mV. Perfusate contained tetrodotoxin (300 nM), tetraethylammonium (50 mM) and caesium (2 mM). Graphs (ii) show net I_{Ba} obtained by subtraction of leak currents. Leak currents were obtained in barium-free solution containing 1 mM cobalt. Control I–V curve obtained before application of endothelin (100 nM) (●); I–V curves obtained during endothelin-induced inward (▲) and outward (■) currents, respectively. Data were obtained from one neurone.

larization associated with an increase in membrane input resistance of neurones of rabbit vesicle pelvic ganglia. The membrane effects of endothelin were concentration-dependent. It is likely that the effects of endothelin were due to a direct action on the postsynaptic neurone and did not involve cholinoceptors, 5-HT receptors (5-HT_3 subtypes) or adrenoceptors. Both the magnitude and time course of the depolarization and hyperpolarization and the associated changes in membrane input resistance were not altered after blockade of these three types of receptor. Furthermore, a component of the endothelin-induced inward current which was coincident with the endothelin-induced depolarization was observed after blocking presynaptic release of cholinergic transmitter (modified Krebs solution containing nominally zero calcium and high magnesium; Nishimura *et al.*, 1988a).

It is possible, however that endothelin may also release neuropeptides (i.e. substance P, vasoactive intestinal polypeptide (VIP) and enkephalin) from presynaptic sites. For example in rat spinal cord the effect of endothelin was considered to be mediated by substance P (Yoshizawa *et al.*, 1989a). A prejunctional release of substance P and/or VIP by endothelin is, however, unlikely for neurones in vesical parasympathetic ganglia as in these neurones the administration of substance P (unpublished observations) and VIP (Akasu *et al.*, 1986) caused membrane depolarization associated with an increase in membrane input resistance. In contrast, endothelin-induced depolarization was associated with a decrease in membrane input resistance. Also, for neurones in parasympathetic colonic ganglia the administration of δ, μ and κ opioid receptor agonists did not cause membrane depolarization and changes in membrane input resistance (Kennedy & Krier, 1987).

Endothelin caused an initial inward followed by an outward current in caesium-loaded neurones superfused with modified Krebs solution containing TTX (300 nM) and TEA (50 mM). The inward current was blocked by approximately 72% by removal of calcium from the perfusate, while it was not altered by lowering external sodium ions or by increasing extracellular potassium ions. These results suggest that endothelin-induced inward current is primarily carried by calcium ions. Furthermore, the reversal potential of the calcium-insensitive component of the inward current obtained by I–V curves was near the equilibrium potential for chloride ions (Akasu *et al.*, 1990). A chloride channel blocker, SITS, completely eliminated the calcium-insensitive component of the inward current produced by endothelin. It is presumed that the endothelin-induced inward current also involves activation of chloride current.

The results of the present study show that endothelin also modulates both rapidly and slowly decaying components of

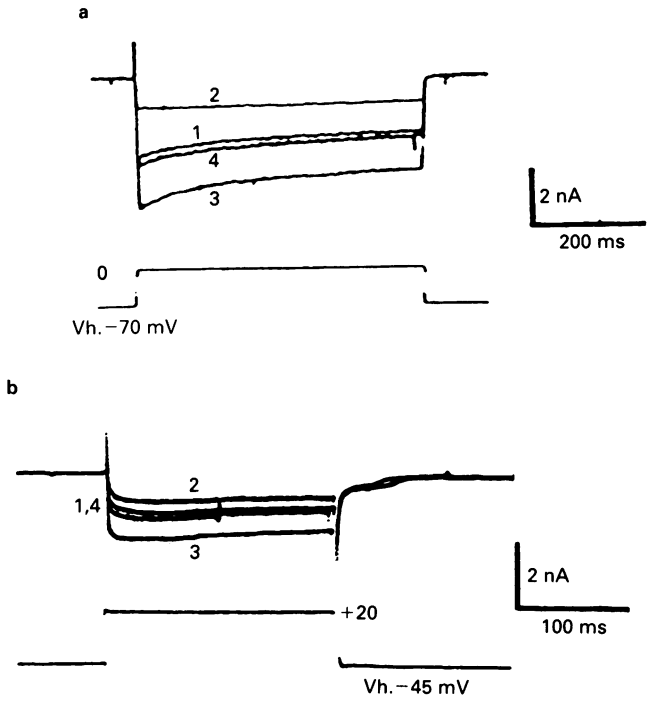

Figure 9 Effect of endothelin (100 nM) on the high-threshold calcium currents, sum of N- and L-type (a) and isolated L-type (b) I_{Ba}, obtained from caesium-loaded neurone in the presence of tetrodotoxin (300 nM) and tetraethylammonium (50 mM). In (a) N- and L-type currents were evoked by step commands to 0 mV from a holding potential of −70 mV. In (b) the L-type current was evoked by step commands to +20 mV from a holding potential of −45 mV. Duration of step commands were 500 ms and 200 ms for (a) and (b), respectively. Leak currents were determined by hyperpolarizing step commands (see Methods). Records (1) in (a) and (b) were obtained before application of endothelin (100 nM). Records (2) and (3) were taken during the inward and outward currents produced by endothelin, respectively. Records (4) were taken 20 min after withdrawal of endothelin. Data in (a) and (b) were obtained from different cells.

voltage-dependent calcium channel currents. These two components were comparable to N- and L-type calcium currents (Nowycky *et al.*, 1985; Fox *et al.*, 1987; Akasu *et al.*, 1990). Endothelin depressed the peak of the I_{Ba} evoked at the beginning of the depolarizing command pulses and to a lesser extent depressed the I_{Ba} measured at the end of voltage command pulse with duration of 500 ms.

In mammalian parasympathetic neurones, the depression of the voltage-dependent calcium channel current by endothelin may be related to elevation of intracellular calcium. In the present study, endothelin activates receptor-operated calcium channels and this may increase the intracellular concentration of calcium (or barium). Alternatively, endothelin may increase intracellular calcium by mobilizing the release of calcium from intracellular stores via the formation of inositol triphosphate; an action reported for glioma cells (Zhang *et al.*, 1990) and anterior pituitary cells (Stojiković *et al.*, 1990). Increased intracellular calcium may cause suppression of the voltage-dependent calcium channels through a 'calcium-induced calcium block' (Brehm & Eckert, 1978; Tillotson, 1979; Lee *et al.*, 1985). In addition the influx of barium ions also leads to an elevation of intracellular calcium due to activation of a calcium-induced calcium release mechanism (Scott *et al.*, 1988).

Endothelin also produced a long-lasting facilitation of voltage-dependent high threshold calcium channel currents (N- and L-type) in neurones of vesical pelvic ganglia. This effect was consistently observed when endothelin was removed from the external Krebs solution. A similar augmentation of dihydropyridine-sensitive L-type calcium channel current by endothelin has been reported for porcine coronary artery (Goto *et al.*, 1989; Silberberg *et al.*, 1989). In the hepatic portal vein, endothelin augments both T- and L-type calcium currents (Inoue *et al.*, 1990). Thus, vesical pelvic neurones represent another site where endothelin facilitates the voltage-dependent calcium channel current.

The mechanism for the endothelin-induced facilitation of the voltage-dependent calcium channel current is not known for either smooth muscle cells or for peripheral autonomic neurones. It is likely that the facilitating action of endothelin in the present study is due to an indirect action involving second messengers in view of the long period required to observe facilitation of the calcium channel current.

In summary, the present data show that endothelin causes membrane depolarization and hyperpolarization of mammalian autonomic neurones. These actions are associated with alterations in receptor-operated calcium and chloride currents and also the voltage-gated calcium channel current. If endothelin is released from urinary bladder vascular endothelial cells, it may modulate the calcium-dependent release of neurotransmitters in autonomic ganglia (Miller, 1987).

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