British Journal of Pharmacology (1997) 120, 1513–1521

Contribution of α-adrenoceptors to depolarization and contraction evoked by continuous asynchronous sympathetic nerve activity in rat tail artery

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1 The effects of continuous but asynchronous nerve activity induced by ciguatoxin (CTX-1) on the membrane potential and contraction of smooth muscle cells have been investigated in rat proximal tail arteries isolated *in vitro*. These effects have been compared with those produced by the continuous application of phenylephrine (PE).

2 CTX-1 (0.4 nM) and PE (10 μM) produced a maintained depolarization of the arterial smooth muscle that was almost completely blocked by α-adrenoceptor blockade. In both cases, the depolarization was more sensitive to the selective α_2-adrenoceptor antagonist, idazoxan (0.1 μM), than to the selective α_1-adrenoceptor antagonist, prazosin (0.01 μM).

3 In contrast, the maintained contraction of the tail artery induced by CTX-1 (0.2 nM) and PE (2 and 10 μM) was more sensitive to prazosin (0.01) μM, than to idazoxan (0.01 μM). In combination, these antagonists almost completely inhibited contraction to both agents.

4 Application of the calcium channel antagonist, nifedipine (1 μM), had no effect on the depolarization induced by either CTX-1 or PE but maximally reduced the force of the maintained contraction to both agents by about 50%.

5 We conclude that the constriction of the tail artery induced by CTX-1, which mimics the natural discharge of postganglionic perivascular axons, is due almost entirely to α-adrenoceptor activation. The results indicate that neuronally released noradrenaline activates more than one α-adrenoceptor subtype. The depolarization is dependent primarily on α_2-adrenoceptor activation whereas the contraction is dependent primarily on α_1-adrenoceptor activation. The links between α-adrenoceptor activation and the voltage-dependent and voltage-independent mechanisms that deliver Ca^{2+} to the contractile apparatus appear to be complex.

Keywords: Ciguatoxin; postjunctional activity; electrophysiology; rat tail artery; noradrenaline; neuroeffector transmission; α-adrenoceptors

Introduction

Activation of α-adrenoceptors on arterial smooth muscle by exogenously applied noradrenaline (NA) initiates contraction both by releasing Ca^{2+} from intracellular stores and by depolarization of the muscles leading to voltage-dependent Ca^{2+} entry (Itoh *et al*., 1992; Nilsson *et al*., 1994). However, the mechansim(s) whereby neuronally released NA activates arterial smooth muscle remain unclear. In addition, there is evidence *in vivo* and *in vitro* that neurally evoked vasoconstriction is mediated in part by non-α-adrenoceptor mediated mechanisms (see Hirst & Edwards, 1989). In arterial vessels isolated and stimulated electrically, the relative contribution of α- and non-α-adrenoceptors to neurally-evoked contraction depends on the stimulus frequency and the train duration (Sjöblom-Widfeldt *et al*., 1990; Evans & Cunnane, 1992), the non-α-adrenoceptor-mediated component being of greater importance in smaller diameter arteries and arterioles (Bao *et al*., 1989; Evans & Suprenant, 1992). As the non-α-adrenoceptor-mediated component is blocked by P_{2X}-purinoceptor antagonists (e.g. suramin), it has been taken to result from the action of adenosine 5′-triphosphate (ATP) released together with NA from the sympathetic nerve endings (Evans & Surprenant, 1992; Bao & Stjärne, 1993; Morris, 1994).

Electrophysiological studies of small muscular arteries and arterioles have shown that synchronous activation of the perivascular nerves evokes an excitatory junction potential (e.j.p.) (see Hirst & Edwards, 1989). The e.j.p. is resistant to α-adrenoceptor blocking agents but is sensitive to purinoceptor antagonists (e.g. Sneddon & Burnstock, 1984; Evans & Surprenant, 1992; McLaren *et al*., 1995), which implies that it is mediated by the co-transmitter ATP. Single e.j.p.s are not normally associated with contraction but, during trains of stimuli, the summation of e.j.ps can open voltage-dependent Ca^{2+} channels, thereby initiating contraction. In several cutaneous arteries (e.g. rat tail artery, Cassell *et al*., 1988), perivascular stimuli evoke in addition an α_2-adrenoceptor-mediated slow depolarization that follows the e.j.ps. This depolarization has usually not been detected in vessels supplying non-cutaneous beds (see Hirst & Edwards, 1989).

In vivo, the vasoconstrictor axons discharge continuously and asynchronously at a mean firing rate of 1–2 Hz (Jänig, 1988). Thus the form of the postjunctional potential changes will differ from those recorded in *in vitro* experiments in which electrical stimuli activate axons synchronously. Recently, we have used the marine neurotoxin, ciguatoxin-1 (CTX-1), to investigate *in vitro* the electrical activity generated by continuous asynchronous nerve activity in the arterial smooth muscle of the rat proximal tail artery (Brock *et al*., 1995). This toxin makes the unmyelinated nerve fibres discharge action potentials, which sometimes occur in short bursts (Hamblin *et al*., 1995), due to a shift in the voltage-dependence of the tetrodotoxin (TTX)-sensitive Na^+ channels so that they open close to the resting membrane potential (Benoit *et al*., 1986). All the potential changes elicited in the arterial smooth muscle of rat tail artery by CTX-1 were accounted for by the postjunctional actions of NA and ATP released from the sympathetic nerve terminals. At concentrations of CTX-1 < 0.1 nM, bursts of e.j.ps were

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recorded which were due to activation of purinoceptors. Higher concentrations of CTX-1 (0.2–0.4 nM) increased the overall level of nerve activity and produced a maintained depolarization of about 20 mV that was largely blocked by the α-adrenoceptor antagonist phentolamine. In addition, these higher concentrations of CTX-1 produced a maintained constriction of the artery. We assume that the behaviour of the arterial smooth muscle in the presence of CTX-1 resembles that which occurs during natural activity of sympathetic nerves *in vivo*.

Contractions of the rat proximal tail artery evoked by electrical stimulation of the nerves are mediated primarily by neuronally released NA but α-adrenoceptor blockade reveals a small residual response that is mediated by neuronally released ATP (see Bao, 1993). Previous studies have shown that neuronally released NA produces constriction of the rat proximal tail artery solely through activation of α_1-adrenoceptors (Medgett & Langer, 1984; Medgett & Rajanayagam, 1984; Medgett, 1985) although exogenously applied NA activates both α_1- and α_2-adrenoceptors (Megdgett & Langer, 1984; Medgett & Rajanayagam, 1984; Rajanayagam & Rand, 1993). However, recently, Bao *et al.* (1993) have concluded, based on the sensitivity for neurally-evoked contractions to antagonists selective for α_1- and α_2-adrenoceptors, that both receptor subtypes are involved and that the relative importance of α_2-adrenoceptor activation decreases as the frequency of stimulation is raised. In the present study, we have investigated the actions of the α_1-adrenoceptor-selective antagonist, prazosin, and the α_2-adrenoceptor-selective antagonist, idazoxan, on the maintained depolarization and on the constriction produced by CTX-1. These data are compared with the effects of bath application of the α_1-adrenoceptor selective agonist, phenylephrine (PE).

Methods

All experiments were performed on tissues isolated from female rats (70–120 g) anaesthetized with pentobarbitone (80 mg kg^{-1}, i.p.) and decapitated. A segment of the main ventral caudal artery was dissected from 5–25 mm distal to the base of the tail. For the electrophysiology experiments, a 10 mm length of artery was pinned to the Sylgard (Dow Corning) coated base of a small organ bath (volume < 0.8 ml) on the stage of an inverted microscope. For the contraction experiments, a 1.5–2.5 mm length of the artery was mounted isometrically in a myograph. In each case the preparation was superfused with physiological saline of the following composition (mM): Na^+ 151, K^+ 4.7, Ca^{2+} 2, Mg^{2+} 1.2, Cl^-144, $H_2PO_4^-$ 1.3, HCO_3^- 16.3, glucose 9.8, gassed with 95% O_2 and 95% CO_2 (pH 7.2) and warmed to 35°C. Usually two pieces of artery from the same animal were used in an experiment, with the second being held in oxygenated physiological saline at room temperature until required. No difference could be detected in the properties or responses of the two arterial segments.

Electrophysiology

Intracellular recordings were made with microelectrodes filled with 0.5 M KCl (resistance 80–160 MΩ) in smooth muscle cells located close to the adventitial-medial border of the artery. Records were collected on a PC based data acquisition system with sampling frequencies of 0.1–1 kHz as described previously (Cassell *et al.*, 1986); criteria for accepting impalements were the same as in Cassell *et al.* (1988). When the membrane was depolarized by drug application, reimpalements were accepted if the electrode noise was the same as in the bathing solution. Perivascular nerves were stimulated either through a suction electrode, into which approximately 5 mm of the proximal end of the artery was introduced, or through a pair of platinum wires (125 μm diameter) placed vertically in the Sylgard coated base of the organ bath, 100–150 μm from either side of the artery and 1–2 mm from the mouth of the suction electrode. Recordings were made at sites between the transmural electrodes where the amplitude and time course of responses evoked through the suction electrode were generally similar between preparations (Sittiracha *et al.*, 1987). Stimuli of pulse width 1 ms and voltage between 1 and 20 V were presented; larger voltages were avoided so that slow depolarizing potentials arising from non-neural sources did not distort the decay phase of the excitatory junction potentials (see Jobling & McLachlan, 1992).

In the experiments investigating the pharmacological sensitivity of the depolarizations induced by CTX-1 and PE, the antagonists were applied cumulatively, starting 20 min after the continuous application of CTX-1 or PE had commenced.

Mechanical responses

The vessels were mounted isometrically between two stainless steel wires (50 μm diameter) in a myograph. The resting wall tension was set, by use of the method described by Mulvany & Halpern (1977), at a value approximately equivalent to that produced by a distending pressure of 70 mmHg. After mounting, the vessels were left to equilibrate for 30 min before an experiment was started. The output from the myograph was recorded with a bridge amplifier connected to a MacLab recording system (ADInstruments, Castle Hill, NSW, Australia).

In the experiments in which the pharmacological sensitivity of the CTX-1-induced contractions were investigated, the antagonists were added cumulatively, starting 20 min after the continous application of CTX-1 had commenced. The force of the CTX-1-induced contraction was measured with respect to the basal level of tone remaining after application of tetrodotoxin (TTX, 0.3 μM) at the end of each experiment.

To investigate whether the pharmacological sensitivity of PE-induced contractions was affected by the duration of exposure to the agonist, PE was either applied continuously or for a one minute period every 30 min. In the former experiments, the antagonists were added cumulatively, starting 20 min after the commencement of a continuous application of PE, while in the latter experiments the antagonists were added 20 min before the fourth and fifth applications of PE. In the experiments with continuous application of PE, the force of contraction was measured with respect to basal level of tone remaining following removal of PE at the end of each experiment. The force of contraction to each brief exposure to PE was measured with respect to the basal level of tone existing just before applying this agent.

Statistical analysis

Data are presented as mean ± s.e.mean. Unless otherwise stated data were compared by paired *t* tests. *P* values < 0.05 were considered significant.

Drugs

CTX-1 was supplied by R.J. Lewis (Drug Design and Development Centre, University of Queensland, Qld 4072, Australia) and was extracted from moray eel (*Lycodotis javanicus*) as previously described by Lewis *et al.* (1991). The other drugs used were idazoxan (Sigma Chemical Company, Castle Hill, NSW, Australia), nifedipine (Sigma), phenylephrine HCl (Sigma), phentolamine mesylate (Regitine, Ciba-Geigy Australia Ltd, Pendle Hill, NSW, Australia) and prazosin HCl (Sigma).

A stock solution of CTX-1 (0.4 μM) was prepared in 50% (v/v) aqueous methanol and stored at −20°C. Nifedipine was prepared as a stock solution (10 mM) in ethanol and prazosin as a stock solution (1 mM) in 10% (v/v) dimethyl sulphoxide in water. The remaining drugs were prepared as concentrated solutions in distilled water. Drugs were applied by adding them

to the solution superfusing the tissues. For the CTX-1 experiments, a recirculation system of 20 ml volume was used to minimize the amount of toxin used (see Hamblin *et al*., 1995).

Results

Effects of CTX-1 on membrane potential

Before drug treatment, resting membrane potentials (RMPs) ranged from −58 to −75 mV, with a mean value of −67.0±0.3 mV (71 preparations from 36 rats) and spontaneous junction potentials (s.e.j.ps) were recorded at a low frequency in cells near the medial-adventitial border (<0.1 Hz, see Jobling & McLachlan, 1992). Following introduction of 0.4 nM CTX-1, there was an initial marked increase in the frequency of occurrence of events resembling s.e.j.ps, with occasional high frequency bursts. After 1–2 min, the spontaneous activity became superimposed on a gradually developing, maintained depolarization (see Figure 2, Brock *et al*., 1995). At the onset of this depolarization, transient depolarizations occasionally reached the threshold for muscle action potentials, each of which was associated with a transient constriction of the vessel. However, as the magnitude of the depolarization continued to increase, further action potentials were not observed and it became difficult to resolve individual s.e.j.ps. Instead, the membrane potential continually varied up and down in an unstable manner over about 5 mV (see Brock *et al*., 1995).

The membrane potential reached a maximum level of depolarization 5–10 min after the addition of the toxin. At this time, the membrane was depolarized by 28.9±0.8 mV ($n=34$ preparations, range 18 to 39 mV) compared to the RMP in the absence of the toxin and the vessels were markedly constricted. In most preparations, the depolarization (and the associated constriction) then partially subsided so that, 20 min after the addition of CTX-1 the membrane was depolarized by 20.9±1.1 mV ($n=34$, range 6 to 33 mV). The instability of the membrane potential also decreased over the same time period. In 6 preparations, the effects of 0.4 nM CTX-1 on membrane potential were followed for 60 min. In these preparations the level of depolarization measured at 20 and 60 min was, respectively, 27±1.5 mV and 25.4±2.0 mV. This change of −1.6±1.7 mV ($n=6$) was not statistically significant.

Effects of prazosin and idazoxan on the CTX-1-induced depolarization

In our previous study (Brock *et al*., 1995), the maintained depolarization was almost completely blocked by the non-selective α-adrenoceptor antagonist, phentolamine (1 μM). Here we have investigated the effects of the α_1-adrenoceptor-selective antagonist, prazosin, and the α_2-adrenoceptor-seletive antagonist, idazoxan. Two concentrations of these antagonists were used (0.01 μM and 0.1 μM prazosin, 0.1 μM and 1 μM idazoxan) which were approximately 10 and 100 fold higher than those corresponding to the estimated pA_2 values for prazosin at α_1-adrenoceptors (8.8 in rat tail artery, Medgett & Langer, 1984) or idazoxan at α_2-adrenoceptors (8–8.5 in rat vas deferens, Chapleo *et al*., 1981; Doxey *et al*., 1983).

Figure 1 shows the effects of prazosin and idazoxan on the depolarization induced by 0.4 nM CTX-1. When applied 20 min after the application of CTX-1 had commenced, 0.01 μM prazosin in some preparations produced a small inhibitory effect on the depolarization; overall the reduction (14±15%, $n=7$) was not statistically significant (Figure 1a). However, the subsequent application of 0.1 μM prazosin significantly reduced the depolarization by 58±8% ($n=4$, $P<0.01$, Figure 1a). In comparison, application of idazoxan at 0.1 and 1 μM significantly reduced the depolarization by 45±7% ($n=6$, $P<0.01$, Figure 1b) and 94±7% ($n=7$, $P<0.01$, Figure 1b), respectively. In the presence of 1 μM idazoxan, the membrane potential did not differ significantly

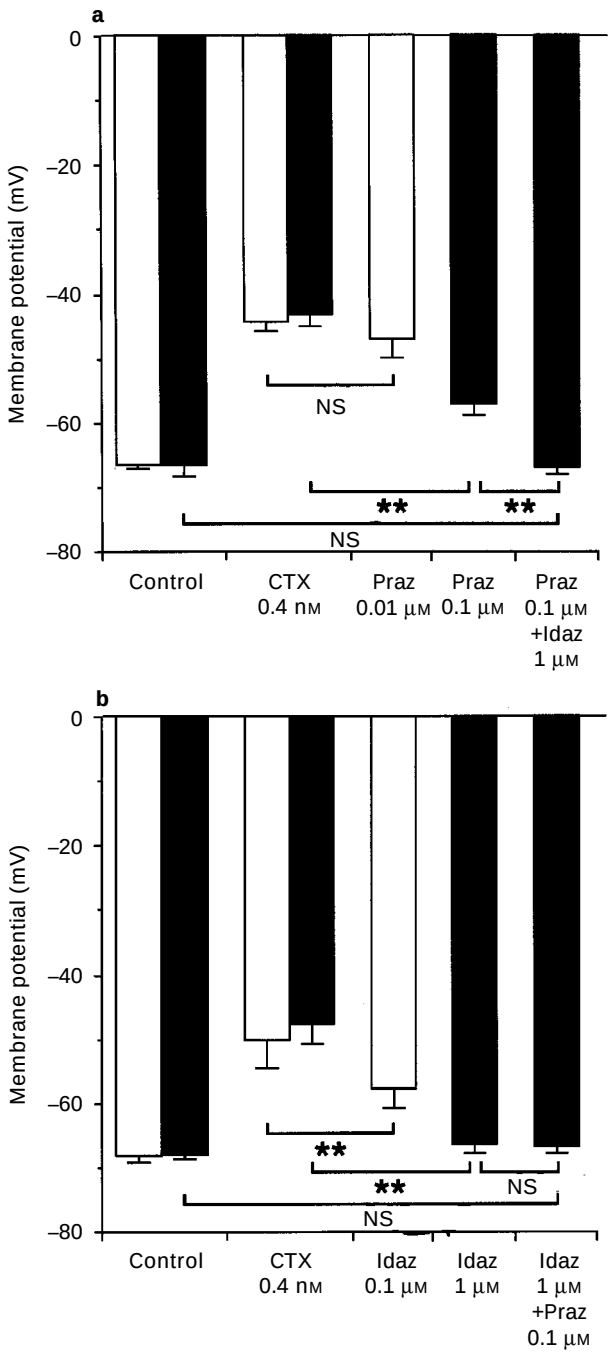

Figure 1 Effects of prazosin (Praz) and idazoxan (Idaz) on the ciguatoxin (CTX-1)-induced depolarization. The effects of 0.4 nM CTX-1 were followed sequentially by (a) 0.01 and 0.1 μM prazosin and 1 μM idazoxan and (b) 0.1 and 1 μM idazoxan and 0.1 μM prazosin. The open and solid columns indicate data for different groups of tissues. In (a), $n=7$ for the open columns and 4 for the solid columns whereas, in (b), $n=6$ for the open columns and 7 for the solid columns. Statistical comparisons (paired t tests) were made for the inhibitory effect of each successive addition of antagonist. $^{**}P<0.01$; NS = not significant.

from pretreatment control values (difference = 1.4±1.2 mV). Furthermore, the subsequent addition of 1 μM idazoxan to tissues treated with 0.1 μM prazosin returned the membrane potential to pretreatment resting values (difference = 0.7 ±0.3 mV, $n=3$, Figure 1a). Prazosin at 0.1 μM had no additional effect on the membrane potential when added after 1 μM idazoxan (Figure 1b). In the presence of 0.1 μM prazosin and 1 μM idazoxan, the addition of 0.3 μM tetrodotoxin produced a small but significant repolarization of the membrane (−1.9±0.6 mV, $n=9$, $P<0.05$) but the level

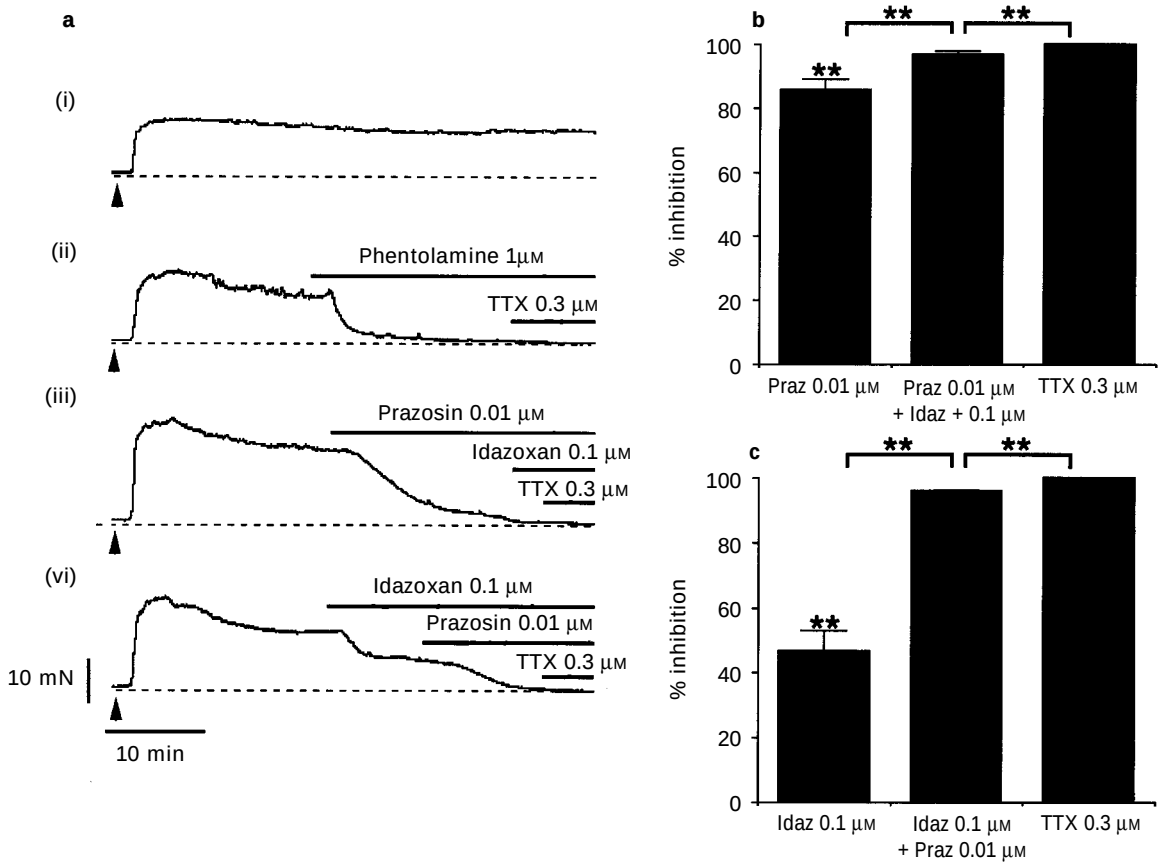

Figure 2 Effects of phentolamine (1 μM), prazosin (Praz, 0.01 μM) and idazoxan (Idaz, 0.1 μM) on ciguatoxin (CTX-1, 0.2 nM)-induced contraction. (a) Representative traces showing (i) the force of contraction produced by CTX-1 and its inhibition by (ii) phentolamine, (iii) the addition of prazosin followed by idazoxan and (iv) the addition of idazoxan followed by prazosin. In each trace, CTX-1 was added at the time indicated by the arrowhead. (b and c) Histograms showing the % inhibition of the CTX-1 induced contraction by the addition of (b) prazosin followed by idazoxan ($n=5$) and of (c) idazoxan followed by prazosin ($n=5$). Statistical comparisons (paired t tests) were made for the inhibitory effect of each successive addition of antagonist. $^{**}P<0.01$.

of membrane potential did not differ from pretreatment resting values (difference = -0.8 ± 0.7 mV).

Effects of CTX-1 on contraction

In the contraction experiments, CTX-1 was applied at a concentration of 0.2 nM, as preliminary experiments indicated that the contractile response had peaked at this concentration. In 12 control experiments, the force of the contraction induced by 0.2 nM CTX-1 increased relatively rapidly during the first 5 min after its addition to the perfusion system and then over the next 5–15 min slowly reached a plateau level which was normally maintained for over 40 min (Figure 2a (i)). The force of contraction measured 20 min after the addition of CTX-1 was 14.9 ± 0.1 mN ($n=38$) and in the control experiments the force of contraction measured at 35 and 50 min (i.e. at the time points used in the antagonist experiments) differed respectively by $5.1\pm5.5\%$ ($n=12$) and $-10.6\pm13.5\%$ ($n=12$) from values measured at 20 min. None of these differences was statistically significant.

Effects of prazosin and idazoxan on CTX-1-induced contraction

The effects of phentolamine, prazosin and idazoxan on the CTX-1-induced contraction were investigated (see Figures 2a (ii–iv)). In comparison with values measured 20 min after the addition of CTX-1, phentolamine (1 μM), prazosin (0.01 μM) and idazoxan (0.1 μM) significantly reduced the force of contraction measured 15 min later by $95.5\pm0.3\%$ ($n=4$, $P<0.01$), $85.5\pm3.6\%$ ($n=5$, $P<0.01$, see Figure 2b) and $46.4\pm6.5\%$ ($n=5$, $P<0.01$, see Figure 2c), respectively. Application of a combination of 0.01 μM prazosin and 0.1 μM idazoxan reduced the force of the CTX-1-induced contraction by $96.4\pm0.5\%$ ($n=10$, see Figures 2b and c). In the presence of phentolamine (1 μM) or a combination of prazosin (0.01 μM) and idazoxan (0.1 μM), TTX (0.3 μM) produced a small additional decrease in the force of contraction (see Figure 2a (ii–iv), b and c).

Effects of nifedipine on CTX-1-induced depolarization and contraction

To determine the role of voltage-dependent Ca^{2+} channels in mediating the depolarization and contraction induced by CTX-1, the effects of applying the Ca^{2+} channel antagonist, nifedipine, were investigated. Nifedipine did not affect the CTX-1-induced depolarization: the membrane potential measured 20 min after application of 0.4 nM CTX-1 was -39.5 ± 1.7 mV ($n=4$) and 20 min following the further addition of 1 μM nifedipine was -39.5 ± 1.6 mV ($n=4$). In contrast, nifedipine (1 μM) produced a rapid decrease in the force of the CTX-1-induced contraction (see Figure 3a (i and ii)). The force of contraction measured 15 min after the application of nifedipine was signficantly reduced by $46.8\pm5.1\%$ ($n=8$, $P<0.01$). Increasing the concentration of nifedipine to 5 ($n=3$) or 10 μM ($n=4$) had no further inhibitory effects.

The effects of prazosin and idazoxan on the nifedipine-resistant component of the CTX-1 induced contraction were also investigated (see Figures 3b and c). Addition of prazosin (0.01 μM) and idazoxan (0.1 μM) significantly reduced the force of the nifedipine-resistant contraction by $85.5\pm3.8\%$ ($n=5$, $P<0.01$, Figure 3b) and $46.4\pm3.8\%$

($n=5$, $P<0.01$, Figure 3c), respectively. Application of a combination of 0.01 μM prazosin and 0.1 μM idazoxan reduced the force of the nifedipine-resistant contraction by $95.5\pm0.8\%$ ($n=10$). The magnitude of the inhibition produced by the α-adrenoceptor antagonists was therefore virtually identical to that observed in the absence of nifedipine (cf. Figure 2b, c).

Effects of PE on membrane potential

Application of 0.1 – 10 μM PE resulted in a maintained depolarization of the muscle, the amplitude of which was concentration-dependent. At 10 μM, PE depolarized the membrane by 18.8 ± 2.0 mV ($n=17$, range = 5 to 34 mV). The magnitude of this PE-induced depolarization did not differ significantly from the maintained depolarization induced by 0.4 nM CTX-1 (i.e. measured 20 min after the commencement of the application of CTX-1). In 5 tissues, the effect of 10 μM PE on membrane potential was followed for up to 90 min, during which a stable depolarization was maintained; the membrane potential measured 60 min following the introduction of PE differed from that measured at 20 min by -0.2 ± 1.0 mV ($n=5$). This difference was not statistically significant.

E.j.ps evoked by single electrical stimuli in the presence of PE (10 μM) had significantly longer time courses of decay than in control solution (control e.j.p. time constant of decay = 215 ± 13 ms, PE e.j.p. time constant of decay = 685 ± 93 ms, $n=11$, $P<0.01$). As the time constant of decay of the e.j.p. provides a measure of the membrane conductance of the arterial smooth muscle (see Cassell *et al*., 1988), the depolarization induced by PE must be associated with a decrease in smooth muscle membrane conductance.

Effect of prazosin and idazoxan on the PE-induced depolarization

When applied 20 min after the commencement of a continual application of 10 μM PE, 0.01 μM prazosin produced a small but statistically signficant reduction in the level of depolarization ($9\pm11\%$, $n=4$, $P<0.05$, Figure 4a). However, application of 0.1 μM prazosin significantly reduced the depolarization by $68\pm17\%$ ($n=5$, $P<0.01$, Figure 4a). The remaining depolarization was sensitive to 1 μM idazoxan, which returned the membrane potential to pretreatment control values (difference = -1.0 ± 0.4 mV, $n=5$, Figure 4a). Idazoxan alone at 0.1 and 1 μM significantly reduced the depolarization by $56\pm3\%$ ($n=4$, $P<0.01$) and $93\pm1\%$ ($n=5$, $P<0.01$), respectively (Figure 4b) and, in the presence of the higher concentration, the membrane potential did not differ significantly from pretreatment control values (difference = 0.8 ± 0.2 mV). In the presence of 1 μM idazoxan, addition of 0.1 μM prazosin had no further effect on the membrane potential (Figure 4b). The sensitivity of the PE-induced depolarization to prazosin and idazoxan was therefore virtually identical to that produced by CTX-1 (cf. Figure 1).

Effects of PE on contraction

Two experimental protocols were used to investigate the pharmacological sensitivity of the PE-induced contractions. In the first series of experiments, 2 or 10 μM PE was applied continuously. The higher of these concentrations is maximally effective for producing contraction of the tail artery (see Abe *et al*., 1987). When applied continuously, the force of contraction increased rapidly during the first 2 – 3 min of exposure to PE and then more slowly to reach a plateau level after 15 – 20 min

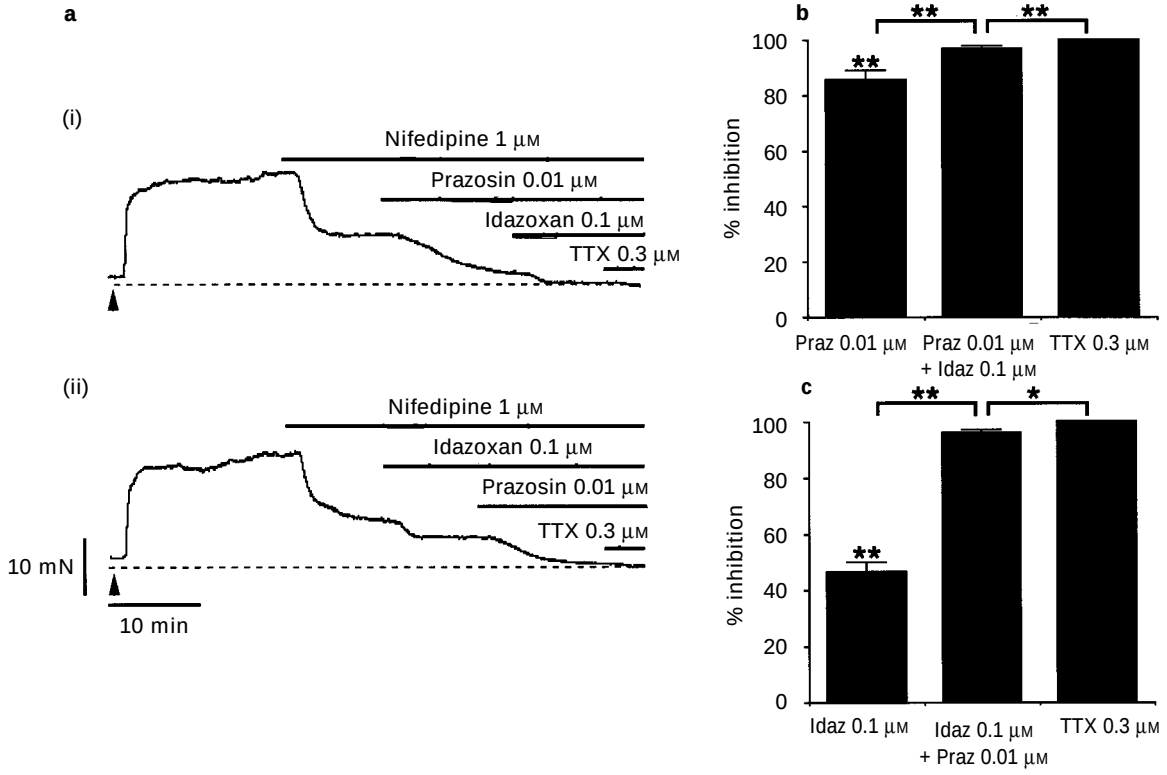

Figure 3 Effects of nifedipine (1 μM) followed by prazosin (Praz, 0.01 μM) and idazoxan (Idaz, 0.1 μM) on ciguatoxin (CTX-1, 0.2 nM)-induced contraction. (a) Representative traces showing the force of contraction produced by CTX-1 and its inhibition by addition of nifedipine followed by (i) prazosin and idazoxan and by (ii) idazoxan and prazosin. In each trace, CTX-1 was added at the time indicated by the arrowhead. (b and c) Histograms showing the % inhibition of the nifedipine-resistant component of the CTX-1-induced contraction by the addition of (b) prazosin followed by idazoxan ($n=5$) and of (c) idazoxan followed by prazosin ($n=5$). Statistical comparisons (paired t tests) were made for the inhibitory effect of each successive addition of antagonist. $^*P<0.05$, $^{**}P<0.01$.

(see Figure 5a (i)). The force of contraction measured 20 min after the start of an application of 2 and 10 μM PE was 16.3 ± 0.9 mN ($n=15$) and 23.6 ± 0.1 mN ($n=18$), respectively. In comparison with the force of contraction induced by 0.2 nM CTX-1 (measured 20 min after the commencement of an application of CTX-1), that induced by 2 μM PE did not differ significantly but that induced by 10 μM PE was significantly greater in magnitude ($P<0.01$, unpaired t test). In control experiments, the force of contraction measured 60 min after applying 2 or 10 μM PE did not differ significantly from that measured at 20 min (2 μM PE, difference $= -3.3 \pm 6\%$, $n=7$; 10 μM PE, difference $= -1.3 \pm 2.0\%$, $n=5$). In the second series of experiments, 10 μM PE was applied for only 1 min every 30 min. In these experiments the force of contraction to the third application of PE was 16.3 ± 0.8 mN ($n=16$) and did not differ significantly in magnitude (unpaired t test) from that induced by a continual application of 0.2 nM CTX-1. In 4 control experiments, the forces of contraction to the fourth (at 90 min) and the fifth (at 120 min) applications of PE increased, respectively, by $9.2 \pm 3.3\%$ and by $6.6 \pm 4.3\%$ compared to that evoked by the third application (at 60 min). Neither of these changes was statistically significant.

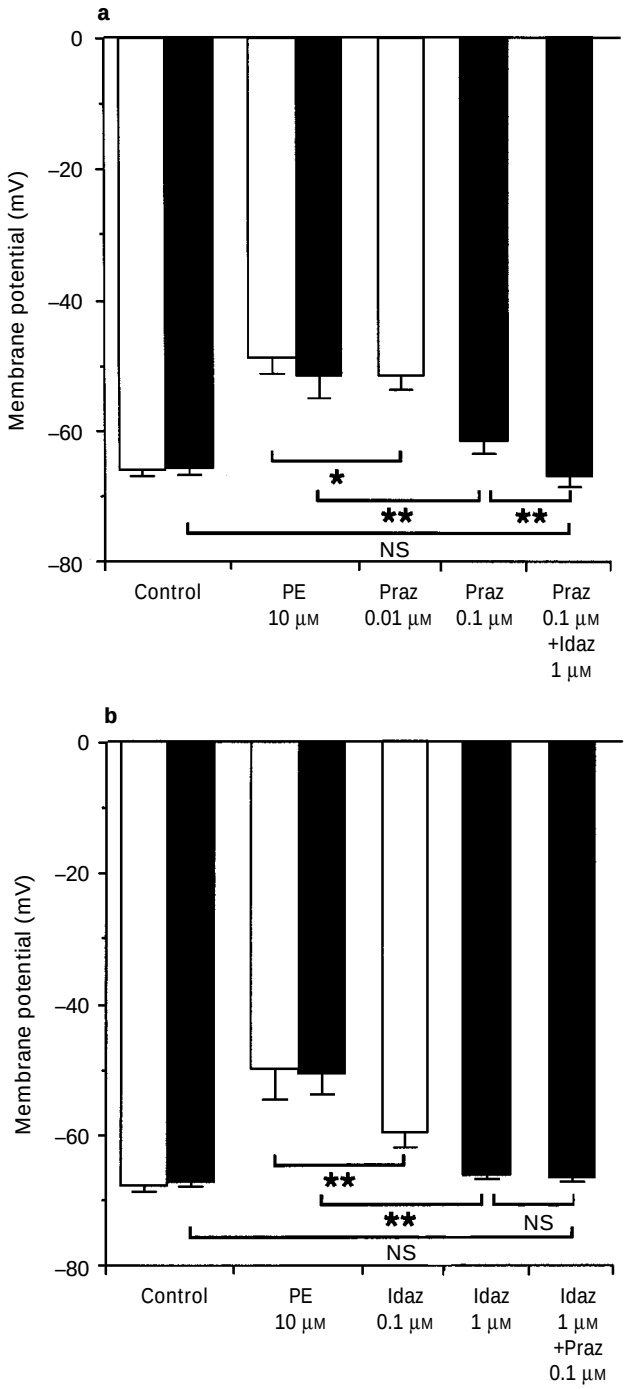

Figure 4 Effects of prazosin (Praz) and idazoxan (Idaz) on depolarization induced by phenylephrine (PE). Histograms showing the effects of 10 μM PE followed sequentially by (a) 0.01 and 0.1 μM prazosin and 1 μM idazoxan and (b) 0.1 and 1 μM idazoxan and 0.1 μM prazosin. The open and solid columns indicate data for different groups of tissues. In (a) and (b), $n=4$ for the open columns and 5 for the solid columns. Statistical comparisons (paired t tests) were made for the inhibitory effect of each successive addition of antagonist. $^*P<0.05$, $^{**}P<0.01$; NS = not significant.

Effects of prazosin and idazoxan on PE-induced contraction

When applied 20 min after the start of the continuous application of 2 and 10 μM PE, prazosin (0.01 μM) significantly reduced the force of the PE-induced contraction measured 15 min later by $93.1 \pm 2\%$ ($n=5$, $P<0.01$, Figure 5a (ii) and b) and $48.5 \pm 5.5\%$ ($n=5$, $P<0.01$), respectively. In comparison, 0.1 μM idazoxan applied at this time significantly reduced the force of contraction to 2 μM PE by $59.8 \pm 12.2\%$ ($n=5$, $P<0.01$, Figure 5a (iii) and c) and to 10 μM PE by $20.9 \pm 1.4\%$ ($n=5$, $P<0.01$). The subsequent combined application of prazosin (0.01 μM) and idazoxan (0.1 μM) reduced the force of contraction induced by 2 and 10 μM PE by $99.5 \pm 0.5\%$ ($n=10$) and $78 \pm 1.3\%$ ($n=10$), respectively.

In the experiments in which the pharmacological sensitivity of contractions induced by 1 min applications of 10 μM PE were investigated, prazosin (0.01 μM) and idazoxan (0.1 μM), applied 20 min before the fourth application of PE, significantly reduced the force of contraction by $61.6 \pm 11.5\%$ ($n=4$, $P<0.01$) and $34.9 \pm 8.9\%$ ($n=4$, $P<0.01$), respectively. The subsequent application of a combination of prazosin (0.01 μM) and idazoxan (0.1 μM) reduced the force of contraction to the fifth application of PE by $90.2 \pm 2.2\%$ ($n=8$).

Effects of nifedipine on PE-induced depolarization and contraction

Nifedipine (1 μM) did not affect the PE-induced depolarization: the membrane potential measured 20 min after the application of 10 μM PE was -39.3 ± 2.5 mV ($n=4$) and 20 min following the further addition of 1 μM nifedipine was -39.0 ± 2.6 mV ($n=4$). In contrast, 1 μM nifedipine significantly inhibited contractions evoked by continuous application of 2 and 10 μM PE and by 1 min applications of 10 μM PE. In the former experiments, the force of contraction induced by 2 and 10 μM was reduced respectively by $56.0 \pm 2.7\%$ ($n=7$, $P<0.01$) and $36.4 \pm 4.6\%$ ($n=4$, $P<0.01$), whereas in the latter experiments the force of contraction to the fourth application of PE was reduced by $27 \pm 4\%$ ($n=4$, $P<0.01$).

Discussion

In the present study the postjunctional responses to NA release from nerve terminals (by CTX-1) and to exogenously applied PE were compared in the rat proximal tail artery. The finding that phentolamine or a combination of prazosin and idazoxan almost totally inhibited CTX-1-induced contractions indicates that the action of released NA on α-adrenoceptors is primarily responsible for constriction during continuous asynchronous nerve firing. This finding contrasts with a number of studies which have shown that contractions evoked by short trains of electrical stimuli are mediated by postjunctional actions of both NA and ATP (see Bao, 1993). However, when train duration is increased, the component of contraction sensitive to purinoceptor blockade wanes, the sustained component of contraction being almost entirely accounted for by the activation of α-adrenoceptors (Bao *et al.*, 1993; see also Muir & Wardle, 1989). Thus it is possible that ATP contributes to the

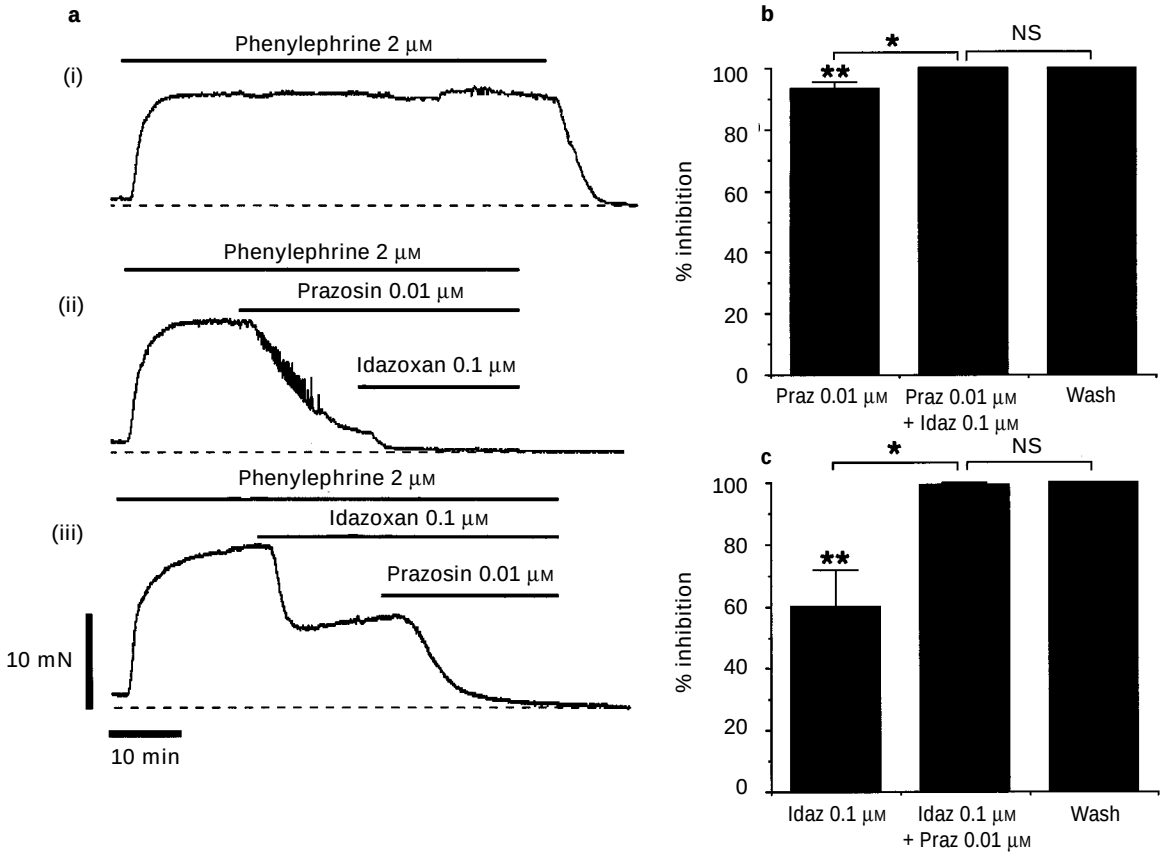

Figure 5 Effects of prazosin (0.01 μM) and idazoxan (0.1 μM) on phenylephrine (PE, 2 μM)-induced contraction. (a) Representative traces showing (i) the force of contraction produced by PE and its inhibition by (ii) prazosin followed by idazoxan and (iii) idazoxan followed by prazosin. (b and c) Histograms showing the % inhibition of the PE-induced contraction by the addition of (b) prazosin followed by idazoxan ($n=5$) and of (c) idazoxan followed by prazosin ($n=5$). Statistical comparisons (paired t tests) were made for the inhibitory effect of each successive addition of antagonist. $^*P<0.05$, $^{**}P<0.01$; NS = not significant.

Table 1 Effects of prazosin and idazoxan on depolarization and contraction produced by ciguatoxin (CTX-1) and phenylephrine

	Prazosin (0.01 μM) % *inhibition*	*Idazoxan* (0.1 μM) % *inhibition*	*Prazosin* (0.01 μM) + *Idazoxan* (0.1 μM) % *inhibition*	*Praz : Idaz*
Depolarization				
CTX-1 (0.4 nM)[a]	14	45	ND	0.31
Phenylephrine (10 μM)[a]	9	56	ND	0.16
Contraction				
CTX-1 (0.2 nM)[a]	86	46	96	2.32
Phenylephrine (2 μM)[a]	93	60	99	1.55
Phenylephrine (10 μM)[a]	49	21	78	1.84
Phenylephrine (10 μM)[b]	61	35	90	1.74

The superscript characters indicate that the agent was applied continuously (a) or for a 1 min period (b). ND = not done.

initial response to CTX-1 but that, after 20 min of continual nerve activity, this component of the response is very small. *In vivo* the sympathetic nerves supplying the tail artery are tonically active (Johnson & Gilbey, 1994) and cutting the collector nerves supplying the tail artery produces a large increase in vessel diameter (E.M. McLachlan, unpublished observations), demonstrating that the vessel is tonically constricted by nerve activity. We believe that the pharmacological sensitivity of the CTX-1-induced contraction is likely to be representative of the *in vivo* situation. While the data imply that, under the conditions of the experiment, the co-transmitters ATP and neuropeptide Y do not act directly to produce constriction, the present study does not exclude the possiblity that these agents act postjunctionally to potentiate the effects of α-adrenoceptor activation (Neild, 1987; Helliwell *et al*., 1994). However, in tail artery, purinoceptor blockade potentiates the component of nerve evoked contraction sensitive to α-adrenoceptor blockade, suggesting a postjunctional action of ATP inhibits the actions of NA (Bao & Stjärne, 1993).

The role of α_1- and α_2-adrenoceptors in producing maintained depolarization and contraction of the arterial smooth muscle was investigated. The findings are summarized in Table 1, which show the proportional inhibitions by 0.01 μM prazosin and by 0.1 μM idazoxan of the depolarizations and contractions produced by each form of activation. At these concentrations, these antagonists were expected to reveal effects mediated selectively through activation of α_1- and α_2-adrenoceptors (see Medgett, 1985). The sensitivities of the CTX-1-induced depolarizations and contractions to prazosin and to idazoxan were notably similar to those of the responses to the selective α_1-adrenoceptor agonist, PE. However, in each case, the depolarization was more sensitive to idazoxan than to prazosin, whereas the contraction was more sensitive to prazosin than to idazoxan (see Table 1). The differences are

clearly illustrated by the ratios of the inhibitory effect of 0.01 μM prazosin to that of 0.1 μM idazoxan shown in Table 1. These findings suggest that contraction to neuronally released NA and applied PE is mediated primarily through activation of α_1-adrenoceptors but questions the nature of the receptor(s) responsible for depolarization.

The NA-induced slow depolarization evoked by short trains of electrical stimuli in the rat tail artery is blocked by α_2-adrenoceptor antagonists and is insensitive to high concentrations of prazosin (up to 1 μM, see Itoh *et al*., 1983; Cassell *et al*., 1988), implying that it is mediated through activation of α_2-adrenoceptors. Thus, the simplest explanation for the relatively high sensitivity of both the CTX-1 and the PE-induced depolarization to idazoxan, is that in both cases it is mediated primarily through activation of α_2-adrenoceptors. However, as previously shown by others (Abe *et al*., 1987), we have found that PE is more potent than the α_2-adrenoceptor selective agonists UK14,304 and clonidine in producing depolarization (J.A. Brock and E.M. McLachlan, unpublished observations). A possible explanation is that co-activation of α_1- and α_2-adrenoceptors is required to produce a large depolarization. It has previously been found that low concentrations of α_2-adrenoceptor agonists augment the contractile response to α_1-adrenoceptor activation in rat tail artery by a mechanism that involves voltage-dependent Ca^{2+} influx (Xiao & Rand, 1989). The idea that activation of both receptor subtypes is necessary to produce substantial depolarization may explain why depolarization induced by a high concentration of PE, which is a weak α_2-adrenoceptor agonist (see McGrath *et al*., 1989), has a relatively high sensitivity to idazoxan. However, it does not readily explain why depolarization has a low sensitivity to prazosin, unless there is a very large α_1-adrenoceptor reserve.

An alternative explanation for the relatively high sensitivity of CTX-1 and PE-induced depolarization to idazoxan, is that they are mediated by a population of 'atypical' α_1-adrenoceptors which have a relatively low sensitivity to prazosin and a relatively high sensitivity to idazoxan. In a number of vascular tissues from the dog, contractions to noradrenaline and to PE have been shown to have a rather low sensitivity to prazosin and a rather high sensitivity to the α_2-adrenoceptor selective antagonist, yohimbine, e.g. dog mesenteric artery and vein, saphenous vein (Muramatsu *et al*., 1990; see also Flavahan & Vanhoutte, 1986). However, the sensitivity of these contractions to the imidazoline antagonist, idazoxan, has apparently not been tested. Interestingly, the rat proximal tail artery has been shown to have a small population of α_1-adrenoceptors with a very high affinity for prazosin ($-\log K_B$ affinity values of <9.4) and a larger population of α_1-adrenoceptors with a lower affinity for prazosin ($-\log K_B$ affinity values of 8.8) (Medgett & Langer, 1984). The receptors with a higher affinity for prazosin were proposed to play a major role in neurally-evoked contractions. Perhaps the receptors with a lower affinity for prazosin are the receptors responsible for depolarization.

There is one major difference between the contractions of the tail artery induced by continual application of CTX-1 and PE. At 0.2 nM CTX-1 and 10 μM PE these agents are maximally effective in producing contraction. However, contractions induced by CTX-1 generated significantly smaller force than did those induced by PE. In addition, the contraction induced by CTX-1 was more sensitive to antagonism both by prazosin and by idazoxan than was that induced by PE (see Table 1). Both these findings are most readily explained if the effective concentration of agonist at the α-adrenoceptors is lower in CTX-1-treated preparations. Indeed, this might be expected since during continual exposure to PE the agonist-receptor interaction would be at equilibrium, whereas in CTX-1-treated preparations, in which NA is released phasically from the perivascular nerves directly on to the muscle surface, the concentrations of agonist at the activated α-adrenoceptors would be expected to vary considerably over time so that equilibrium conditions are never achieved. It is even possible that not all α-adrenoceptors present on the arterial smooth muscle at the medial-adventitial border can be activated by neuronally released NA. In support of the suggestion that the levels of agonist-induced activation are different, contractions induced by continual application of 2 μM PE or by brief applications of 10 μM PE, which were similar in magnitude to those induced by continual application of CTX-1, had a greater sensitivity to antagonism both by prazosin and by idazoxan (see Table 1).

It is important to consider whether receptor desensitization during continuous application of CTX-1 and of PE changed the postjunctional sensitivity of arterial smooth muscle. Two observations suggest that this was not the case. First, the depolarizations and the contractions to both CTX-1 and PE were generally maintained throughout the period of their application. Secondly, the relative magnitudes of the inhibitory effects of prazosin and of idazoxan on contractions produced by maintained applications of PE were virtually identical to those produced by 1 min applications (see Table 1). This indicates that α-adrenoceptors in the rat tail artery do not desensitize significantly when activated continuously.

This study has also identified the role of nifedipine-sensitive Ca^{2+} channels in mediating contraction to CTX-1 and PE. In segments of tail artery (with the endothelium removed), contractions evoked by α_1-adrenoceptor agonists are dependent both on Ca^{2+} released from intracellular stores and on voltage-dependent Ca^{2+} entry (Abe *et al*., 1987), extracellular Ca^{2+} being necessary for sustained constriction. In contrast, the contractions of this vessel to α_2-adrenoceptor agonists are totally dependent on the presence of extracellular Ca^{2+} (Abe *et al*., 1987) and have a greater sensitivity to blockade of voltage-dependent Ca^{2+} channels (e.g. by dihydropyridines) than do those produced by α_1-adrenoceptor agonists. In the present study, application of nifedipine (1 μM) maximally reduced the force of the maintained contraction produced by continuous application of CTX-1 and PE by about 50%. As maintained contraction is dependent on extracellular Ca^{2+}, it is likely that Ca^{2+} influx is maintained in the presence of nifedipine. The question of whether Ca^{2+} enters through voltage-activated channels that are insensitive to nifedipine or through receptor-operated channels remains to be determined. Interestingly, the sensitivity of the contraction evoked by CTX-1 to prazosin and to idazoxan was not changed by the application of nifedipine, suggesting that both α_1- and α_2-adrenoceptors are capable of producing contraction through mechanisms which do not require Ca^{2+} influx through dihydropyridine-sensitive Ca^{2+} channels.

Prazosin (0.01 μM) had a minimal effect on the depolarization induced by CTX-1 and PE (see Table 1). Thus contraction can be substantially inhibited without greatly changing the level of membrane depolarization. This finding can be explained, at least in part, if activation of α_1-adrenoceptors increases the probability of opening of L-type Ca^{2+} channels, as demonstrated in isolated vascular smooth muscle cells (see Nelson *et al*., 1988). This suggestion is supported by the finding that nifedipine also produced a substantial reduction in the force of maintained contraction to CTX-1 and to PE without significantly affecting the level of membrane depolarization (see Table 1). As prazosin was more effective than nifedipine in reducing the CTX-1 and PE-induced contraction, much of the α_1-adrenoceptor-mediated contraction must be independent of changes in membrane potential. Such mechanisms include the release of Ca^{2+} from intracellular stores and an increase in the sensitivity of the contractile apparatus to Ca^{2+} (see Chen & Rembold, 1995).

The observations in this study are clearly not easy to interpret in terms of distinct populations of postjunctional α_1- and α_2-adrenoceptors linked to separate mechanisms that deliver Ca^{2+} to the contractile apparatus. However, α-adrenoceptor activation is responsible for virtually all of

the contraction of the tail artery induced by continuous nerve activity. It should be noted that the nerve-evoked α-adrenoceptor-mediated depolarization observed in the tail artery is not detected in vessels supplying many other vascular beds and that the relative effectiveness of nerve-released co-transmitters in producing vasoconstriction in other blood vessels has yet to be determined.

This work was supported by the National Heart Foundation of Australia.

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(Received June 24, 1996
Revised November 18, 1996
Accepted January 8, 1997)