Inhibition of Ca-spikes in rat preganglionic cervical sympathetic nerves by sympathomimetic amines

¹P. Elliott, ²S.J. Marsh & ²D.A. Brown

M.R.C. Neuropharmacology Research Group, Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX

1 Propagated Ca-spikes were recorded from isolated cervical sympathetic nerve trunks of the rat when bathed in a solution containing 5 mM Ca^{2+} , 0.5 or 1 μM tetrodotoxin (to block Na currents) and 1 mM 4-aminopyridine (to reduce K currents).

2 Spikes persisted when external Ca^{2+} was replaced with Sr^{2+} or Ba^{2+} , but were blocked by the addition of the following inorganic Ca-channel blockers (in descending order of potency): $\text{Cd}^{2+} > \text{La}^{3+} > \text{Ni}^{2+} > \text{Co}^{2+} > \text{Mn}^{2+} > \text{Mg}^{2+}$.

3 Ca-spike amplitude was reduced by up to 90% by (–)-noradrenaline (IC_{50} 1.5 μM). The following sympathomimetic amines imitated this effect (in descending order of potency): clonidine $\geq$ (–)-adrenaline $\geq$ [(–)-noradrenaline] $\geq$ dopamine $>$ (–)-phenylephrine $\geq$ (±)-amidephrine.

4 Ca-spike inhibition by (–)-noradrenaline was antagonized by phentolamine (pA_2 6.5). Yohimbine was about 10 times weaker than phentolamine; (±)-propranolol (1 μM) and prazosin (10 μM) had no clear effect.

5 (–)-Noradrenaline reduced the amplitude of the compound action potential recorded from the superior cervical sympathetic ganglion following supramaximal preganglionic trunk stimulation when recorded in normal Krebs solution and hyperpolarized the ganglion with respect to the postganglionic trunk. Depression of the transmitted ganglionic action potential was antagonized by phentolamine (5 μM) but not by yohimbine (1 μM); in contrast 1 μM yohimbine completely prevented the ganglionic hyperpolarization. (–)-Noradrenaline did not hyperpolarize the preganglionic cervical sympathetic nerve trunk under these recording conditions.

6 It is suggested that inhibition of transmitter release from sympathetic preganglionic fibres produced by noradrenaline results from a depression of the voltage-gated Ca current in the fibres and/or their terminals, and that this action is mediated by an α -adrenoceptor which does not fully conform to either α_1 or α_2 subtypes.

Introduction

Catecholamines inhibit transmitter release from cervical sympathetic preganglionic nerves (see, for examples, Birks & MacIntosh, 1961; Christ & Nishi, 1971a,b; Dawes & Vizi, 1973; Dun & Nishi, 1974; Dun & Karczmar, 1977; Brown & Caulfield, 1981). The mechanism responsible for this is not yet clear. One possibility is that they inhibit voltage-gated Ca currents in the preganglionic nerve fibres or their terminals, since inhibition of these currents has been

detected in postganglionic sympathetic neurones (Minota & Koketsu, 1977; Horn & MacAfee, 1980; Koketsu & Akasu, 1982; Galvan & Adams, 1982; Lipscombe & Tsien, 1987) and in some other peripheral neurones (e.g., sensory ganglion cells: Dunlap & Fischbach, 1978; 1981; Forscher & Oxford, 1985; neuroblastoma hybrid cells: Docherty & McFadzean, 1987).

It is difficult to test this in sympathetic fibres since the normal conducted action potential does not show a component which can clearly be attributed to a Ca current. However, we have found that a propagated Ca-spike can be generated in rat preganglionic cervical sympathetic fibres when the Na current is blocked with tetrodotoxin and K-currents

¹ Author for correspondence at present address: Department of Physiology, University College Cardiff, Cathays Park, P.O. Box 78, Cardiff CF1 1XL.

² Present address: Department of Pharmacology, University College London, Gower Street, London WC1E 6BT.

diminished with 4-aminopyridine (cf. Kuan *et al.*, 1985). We have therefore used this preparation to assess the possible effect of noradrenaline and other sympathomimetic amines on the presynaptic Ca-current. Some of our results have been published in abstract form (Elliott, 1986).

Methods

Preganglionic cervical sympathetic nerves, with or without attached superior cervical ganglia, were excised from male Wistar rats (200–400 g weight) anaesthetized with urethane (1.5 g kg^{-1}). Their connective tissue sheaths were stripped off and the preparations were maintained in Krebs solution bubbled with 95% O_2 plus 5% CO_2 (see Brown & Caulfield, 1979, for composition).

To record compound action potentials from the preganglionic trunk, the preparation was mounted horizontally in a three-chambered 'perspex' bath as described by Brown & Marsh (1978). The chambers were insulated with silicon grease and filled with oxygenated Krebs solution. The centre chamber was superfused with oxygenated Krebs solution at 5 ml min^{-1} , at ambient temperature ($18\text{--}22^\circ\text{C}$). Drugs were applied to the centre chamber by superfusion at known concentrations. The preganglionic nerve was stimulated between the proximal and centre chambers through twin Pt wires, the centre chamber being grounded. The normal parameters were 20 V, 1 ms, 0.2 Hz. Evoked compound action potentials were recorded differentially between the centre and distal chambers with Ag/AgCl/agar-saline electrodes. In experiments where transmission through the superior cervical ganglion was measured, the ganglion was placed in the centre chamber and evoked potentials recorded with respect to the postganglionic trunk in the distal chamber. Evoked potentials were monitored on an oscilloscope, photographed on a Medelec u.v. recorder and amplitude recorded and displayed on a peak-height detector (Courtice, 1977). To record Ca-spikes, the normal Krebs solution was replaced by a HEPES-buffered solution containing $0.5\text{--}1 \mu\text{M}$ tetrodotoxin (TTX), 1 mM 4-aminopyridine (4-AP) and 5 mM CaCl_2 , and the stimulus frequency reduced to 0.017 Hz (see Results). Drug-induced spike depression was analysed by fitting a logistic curve to the experimental data (Parker & Waud, 1971).

Solutions

Normal Krebs solution contained the following (mM): NaCl 118, KCl 4.8, CaCl_2 2.5, NaHCO_3 25, KH_2PO_4 1.18, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.18, D-glucose 11. The pH was maintained at pH 7.4 by continuously

gassing the solution with 95% O_2 : 5% CO_2 . To record Ca-spikes the following solution was used (mM): NaCl 118, KCl 6, CaCl_2 5, MgCl_2 1.2, D-glucose 11, HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid, Sigma) 10, $0.5\text{--}1 \mu\text{M}$ TTX, 1 mM 4-AP. The pH was adjusted to pH 7.4 with 1 M NaOH and the solution continuously bubbled with 100% O_2 . In experiments involving catecholamines, 0.5 mM ascorbic acid was added to prevent oxidation. Na-free Tris solution had the following composition (mM): Tris(trihydroxymethylamino-methane)-base 143, KCl 4.8, CaCl_2 5, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.2, KH_2PO_4 1.2 and D-glucose 11; titrated to pH 7.4 with HCl, and gassed with 100% O_2 .

Drugs

The following drugs were obtained from commercial sources: tetrodotoxin (TTX), 4-aminopyridine (4-AP), tetraethylammonium chloride (TEA), (–)-noradrenaline bitartrate, (–)-adrenaline bitartrate, dopamine HCl, (–)-phenylephrine HCl, adenosine, ($\pm$)-propranolol HCl, yohimbine HCl (Sigma), urethane (Fisons). Amidephrine mesylate was supplied by Mead Johnson, clonidine HCl by Boehringer Ingelheim, phentolamine methanesulphonate by CIBA and prazosin HCl by Pfizer.

BaCl_2 , SrCl_2 , CdCl_2 , LaCl_3 , NiCl_2 , CoCl_2 , MnCl_2 and the salts used to make normal Krebs solution were all AnalaR grade reagents from BDH.

Agonist drugs were dissolved in Krebs solution or the HEPES buffered solution to give stock solutions of 10 mM . The antagonists were dissolved in distilled water to give stock solutions of 1 mM or dissolved directly in the appropriate recording medium. The inorganic calcium antagonists were dissolved in distilled water to give stock solutions of $10 \text{ mM--}1 \text{ M}$.

Results

Ca-spikes

The normal compound action potential recorded from the cervical sympathetic trunk was rapidly and completely blocked by $1 \mu\text{M}$ tetrodotoxin (TTX: Figure 1) or by Na-free Tris-solution (see Methods), indicating that it was generated by a conventional TTX-sensitive Na-current. However, on adding to the HEPES-buffered Krebs solution a further 2.5 mM Ca^{2+} (bringing the total concentration to 5 mM) and 1 mM 4-aminopyridine (4-AP) (Kuan *et al.*, 1985) a large, slowly-conducted compound action potential of about 50 to 100 ms duration could be evoked (Figure 1). This tended to run down quite quickly ($t_{1/2} = 5\text{--}10 \text{ min}$) at high ($\geq 0.2 \text{ Hz}$) stimulus

Figure 1 Evoked compound action potentials recorded from an isolated preganglionic cervical sympathetic nerve trunk preparation of the rat bathed in normal Krebs solution ('Na spike') and then after adding tetrodotoxin (TTX, 0.5 and 1 μ M) and replacing the normal Krebs solution with a HEPES-buffered Krebs solution containing 1 μ M TTX, 1 mM 4-aminopyridine (4-AP) and 5 mM CaCl_2 ('Ca spike'). (See text.) The stimulus frequency was 0.2 Hz in normal Krebs solution and 0.017 Hz (1 min⁻¹) in the modified solution. Upper trace: continuous display of peak action potential amplitude, recorded with a peak height detector (Courtice, 1977); note the change of recorder speed. Lower records: single evoked action potentials in the two solutions recorded on an oscilloscope; note the change in time-base.

rates but run-down could be slowed by using a stimulus rate of 1 min⁻¹ (0.017 Hz). The properties of the spikes recorded in normal Krebs solution and in this modified solution (hereafter referred to as 'Na-spikes' and 'Ca-spikes' respectively, see Discussion) are summarized in Table 1.

Conducted spikes could also be recorded in TTX/4-AP solution in the presence of 5 mM Sr^{2+} or Ba^{2+} (Figure 2). Spikes were broader in these solutions,

Table 2 Potencies of inorganic calcium channel antagonists on the Ca-spike

	ID_{50} (mean $\pm$ s.e.mean)	n
Cd^{2+}	$3.0 \pm 0.67 \mu\text{M}$	3
La^{3+}	$10.23 \pm 3.23 \mu\text{M}$	4
Ni^{2+}	$54.67 \pm 19.9 \mu\text{M}$	3
Co^{2+}	$0.51 \pm 0.01 \text{ mM}$	3
Mn^{2+}	$1.07 \pm 0.16 \text{ mM}$	4
Mg^{2+}	$15.7 \pm 1.86 \text{ mM}$	3

particularly so in Ba^{2+} solution where they lasted several hundred ms. This accords with previous reports that Sr^{2+} and Ba^{2+} act as charge carriers for voltage-activated Ca-currents (see, for examples, Hagiwara & Byerly, 1981). The prolonged and larger spike in Ba^{2+} solution may reflect additional block of residual K-currents (e.g., Werman & Grundfest, 1961; Arhem, 1980).

Ca-spikes evoked by 5 mM external Ca^{2+} were completely blocked by a number of divalent cations previously reported to block voltage-activated Ca-currents (Hagiwara & Byerly, 1981) (Figure 3). In order of descending potency, these were: $\text{Cd}^{2+} > \text{La}^{3+} > \text{Ni}^{2+} > \text{Co}^{2+} > \text{Mn}^{2+} > \text{Mg}^{2+}$ (see Table 2).

Action of sympathomimetic amines

A range of sympathomimetic amines was tested for their ability to reduce the amplitude of the Ca-spike. Representative effects are illustrated in Figure 4 and pooled dose-response curves from a number of preparations shown in Figure 5. A fresh preparation was used for each drug, because of the tendency of the Ca-spike to run down with time; with this caveat, effects of all drugs tested were readily reversible on washing. Table 3 lists the maximum depression attained and mean IC_{50} values for 6 amines in descending order of potency (clonidine > (-)-adrenaline > (-)-noradrenaline > dopamine > (-)-phenylephrine > amidephrine).

Table 1 Differences between Na- and Ca-spikes in rat preganglionic cervical sympathetic nerves

	Amplitude (mV)	Delay (ms)	Duration (ms)	After-hyperpolarization (mV)
Na-spike	6.8 ± 0.46	17.1 ± 0.48	24.4 ± 0.83	—
Ca-spike	8.8 ± 0.58	90.2 ± 2.4	72.8 ± 1.96	1.76 ± 0.16

Comparison of Na- and Ca-spikes from 26 separate experiments. Values are mean $\pm$ s.e.mean. The amplitude of the Ca-spike was measured after the initial run-down (see text). The delay is an indication of the conduction velocity: it was measured from the stimulus artifact to the peak of the spike. The duration of the spike was measured at baseline potential. The peak amplitude of the after-hyperpolarization following the spike was also measured. For details of the recording conditions see Figure 1.

Figure 2 Representative compound action potentials recorded from three different nerve trunk preparations bathed in HEPES-buffered Krebs solution containing $1 \mu\text{M}$ tetrodotoxin, 1 mM 4-aminopyridine and 5 mM of either Ca^{2+} , Sr^{2+} or Ba^{2+} as indicated. Note the slower time base in Ba^{2+} solution.

At sufficiently high concentration (10 to $30 \mu\text{M}$) the more potent group of agonists (clonidine, adrenaline and noradrenaline) produced up to 90% inhibition of the Ca -spike. The effect of clonidine was notice-

ably more slow to reverse on washing than that of adrenaline or noradrenaline (see Figure 4a). The dose-response curves for dopamine and phenylephrine were rather shallow (slopes 0.54 and 0.81 respec-

Figure 3 Depression of the cervical sympathetic Ca -spike by some inorganic Ca -channel blockers. Action potentials were recorded in HEPES-buffered Krebs solution containing 0.5 or $1 \mu\text{M}$ tetrodotoxin, 1 mM 4-aminopyridine and 5 mM Ca^{2+} (see Figure 1) and increasing concentrations of the antagonists applied cumulatively. Graphs show mean % reduction of peak amplitude (bars show s.e.mean). The numbers beside the points give the number of preparations used.

Table 3 Pooled data for agonist inhibition of Ca -spikes in rat preganglionic cervical sympathetic nerves

Agonist	Maximum depression (%)	IC_{50} (μM)	Slope factor	n
Clonidine	76.1 ± 4.9	0.44 ± 0.11	0.739 ± 0.246	5
(-)-Adrenaline	92.6 ± 12.1	1.3 ± 0.74	0.633 ± 0.315	7
(-)-Noradrenaline	88.8 ± 5.6	1.52 ± 0.32	1.057 ± 0.315	4
Dopamine	66.9 ± 6.8	45.9 ± 23.6	0.541 ± 0.258	3
(-)-Phenylephrine	61.6 ± 5.7	154 ± 50.4	0.814 ± 0.401	3
Amidephrine	< 50	$> 10^{-2} \text{ M}$	—	2
Adenosine	38.5 ± 1.2	3.1 ± 0.5	0.654 ± 0.150	3

The dose-response curves were analyzed by the method of Parker & Waud (1971). Numbers are means $\pm$ s.e.mean.

Figure 4 Examples of the inhibition of the Ca-spike (see Figure 1, legend) produced by increasing concentrations of some sympathomimetic amines: (a) clonidine, (b) dopamine, (c) (–)-adrenaline, (d) phenylephrine, (e) (–)-noradrenaline and (f) amidephrine. Each record is from a different experiment.

tively, see Table 3) and block was incomplete at 1 mM or more. Amidephrine was very weak, 10 mM being required for appreciable (up to 30%) block.

Notwithstanding the presence of 4-AP, it seemed likely that some residual K-currents contributed to the repolarization of the Ca-spike, as suggested by the effect of Ba described above. In this case, the inhibition of the Ca-spike produced by the catecholamines might in part have been due to an increased K-conductance, some evidence for which has been previously detected in the postganglionic neurones of this preparation (Brown & Caulfield, 1981). To obviate this, K-currents were further blocked by addition of tetraethylammonium (TEA, 10 mM) or Ba^{2+} (1 or 5 mM). Under these conditions, Ca-spikes were augmented and prolonged and a second, slower component of the waveform became apparent (Figure 6). Noradrenaline still reduced the peak amplitude of the spike, albeit by less than in 4-aminopyridine solution alone, and eliminated the second spike component. Quantitative assessment of noradrenaline action in TEA or Ba solution was pre-

cluded by the faster run-down of these spikes than of those obtained in 4-aminopyridine solution.

Antagonists Several antagonists were tested against the inhibitory action of (–)-noradrenaline. Phentolamine antagonized the effect of noradrenaline at concentrations of 1 to 10 μM (Figure 7a); at 100 μM phentolamine produced some additional depression of the Ca-spike itself. An attempt was made to estimate a pA_2 from a Schild plot (Arunlakshana & Schild, 1959) using concentrations of phentolamine up to 10 μM (Figure 8). The slope was less than unity; with this constraint, the apparent pA_2 was 6.47 ± 0.07 ($n = 3$).

The α_2 -adrenoceptor antagonist yohimbine had no antagonistic action at 1 μM but reduced the effect of 10 μM noradrenaline by about 35% at 10 μM and by 60% at 100 μM (Figure 7b; $n = 3$); since phentolamine produced 60 to 70% reduction of the response to 10 μM noradrenaline at 10 μM , yohimbine appeared to be about 10 times weaker than phentolamine. The α_1 -adrenoceptor antagonist prazosin had

Figure 5 Mean percentage reduction of the Ca-spike produced by clonidine (◇), (–)-adrenaline (▲), (–)-noradrenaline (□), dopamine (○), phenylephrine (■) and amidephrine (◆). Bars are s.e.mean; numbers give number of preparations tested at that concentration, each drug being tested on a different preparation.

no clear effect at concentrations up to 100 μM (Figure 7c) and the β -adrenoceptor antagonist ($\pm$)-propranolol had no effect up to 1 μM (not illustrated).

To control for non-specific effects, the action of these antagonists on responses to adenosine was also determined. Adenosine inhibits Ca-currents in sympathetic and sensory neurones (see Henon & McAfee, 1983; Dolphin *et al.*, 1986; MacDonald *et al.*, 1986) and, in the present experiments, reduced the amplitude of the Ca-spikes with an IC_{50} of 3.1 μM (Table 3). However, none of the adrenoceptor antagonists affected inhibitory responses to 10 μM adenosine (Figure 7).

Effects on transmission

When the superior cervical ganglion preparation was bathed in normal Krebs solution, application of 10 μM noradrenaline reduced the amplitude of the ganglionic action potential evoked by single supra-maximal preganglionic stimuli by 20 to 30% (Figure 9). This effect was accompanied by a small hyperpo-

Figure 6 Comparison of the effects of noradrenaline (NA, 10 μM) on Ca-spikes recorded using the solution in Figure 1 (i) and then (ii) on (a) adding 10 mM tetraethylammonium (TEA), (b) adding 1 mM Ba^{2+} or (c) replacing 5 mM Ca^{2+} with 5 mM Ba^{2+} . Calibration: 5 mV and 100 ms (500 ms for row c ii).

Figure 7 Effect of increasing concentrations of (a) phentolamine, (b) yohimbine and (c) prazosin on the inhibition of Ca-spikes (recorded as in Figure 1) produced by $10\ \mu\text{M}$ (—)noradrenaline (solid rectangles below trace) and $10\ \mu\text{M}$ adenosine (open rectangles below trace). (Three experiments).

larization of the ganglion, as previously reported (Brown & Caulfield, 1979; see also below). Phentolamine ($5\ \mu\text{M}$) completely antagonized the inhibition of transmission even though, at this concentration, the ganglion hyperpolarization was only slightly reduced. In contrast, $1\ \mu\text{M}$ yohimbine very effectively suppressed the hyperpolarization as noted previously (Brown & Caulfield, 1979), but only partly antago-

nized the inhibition of transmission, even though it produced some depression of transmission itself (Figure 9b); the residual effect of noradrenaline on transmission was then completely blocked by further addition of $5\ \mu\text{M}$ phentolamine.

The hyperpolarizing action of noradrenaline on the superior cervical ganglion persisted in the presence of $1\ \text{mM}$ 4-aminopyridine (i.e. under conditions

Figure 8 Schild plot of $\log (\text{dose-ratio} - 1)$ against $\log$ [antagonist concentration] for the antagonism of the inhibitory effect of $(-)$ -noradrenaline on the Ca-spike by phentolamine. The graph shows pooled data from 3 experiments using 2-point dose-response curves for $(-)$ -noradrenaline in the absence and presence of 4 concentrations of phentolamine. Least-squares regression analysis yielded a slope of -0.65 ± 0.077 and an intercept (pA_2) of 6.47 ± 0.067 .

in which Ca-spikes were recorded: Figure 10a). In contrast, noradrenaline ($10 \mu\text{M}$) did not hyperpolarize the preganglionic cervical sympathetic trunk but instead produced a small ($\leq 100 \mu\text{V}$) depolarization which also persisted in 1 mM 4-aminopyridine solution (Figure 10b; $n = 3$). This action was shared by clonidine ($10 \mu\text{M}$) and persisted in the presence of $1 \mu\text{M}$ ($\pm$)-propranolol which inhibited the preganglionic depolarization of isoprenaline (see Brown *et al.*, 1982). In the same preparations adenosine produced a very small hyperpolarization ($\leq 50 \mu\text{V}$) while γ -aminobutyric acid depolarized the fibres as reported previously (Brown & Marsh, 1978).

Discussion

The main points established in these experiments are that, under conditions where the normal Na-spike is blocked with tetrodotoxin and K-currents reduced with 4-aminopyridine, propagated Ca-spikes can be recorded from preganglionic cervical sympathetic nerves, and that these can be blocked by sympathomimetic amines.

The principal evidence that the propagated spikes are true Ca-spikes, rather than TTX-resistant Na-spikes, is that spikes can be recorded with Ca^{2+} , Sr^{2+} or Ba^{2+} as charge-carriers, and that the spikes are blocked by micromolar concentrations of divalent cations known to block Ca-channels such as Cd^{2+} , La^{3+} , Ni^{2+} and Co^{2+} . This accords with the properties of Ca-spikes recorded from a variety of nerve cell somata (see Hagiwara & Byerly, 1981).

Following strong suppression of K-currents with additional TEA or Ba^{2+} , the Ca-spike showed two components (see Figure 6). At least two explanations for this are possible. Firstly, the preganglionic cervical sympathetic trunk in the rat contains more than

one population of fibres as determined by their conduction velocities and fibre diameters (Dunant, 1967). It is therefore possible that the balance of inward Ca-current and outward K-currents in the different fibre groups varies, such that the K-currents of the slower-conducting group requires greater suppression before the Ca-current becomes net inward and hence capable of producing a regenerative Ca-spike. A second possibility is that, as in postganglionic sympathetic neurones (Lipscombe & Tsien, 1987), there may be more than one kinetically-distinct type of Ca-current in individual preganglionic axons, of different magnitudes such that their expression as regenerative currents depends on the degree of K-current suppression. This interpretation has been offered for the appearance of a second, slower component of inward current in mouse motor nerve terminal bundles in the presence of high concentrations of tetraethylammonium and 3,4-diaminopyridine (Penner & Dreyer, 1986). This view stemmed from a large differential sensitivity of the two components to both inorganic and organic Ca-channel blocking agents. We have not compared the sensitivities of the two Ca-spikes in the sympathetic fibres to a variety of Ca-channel blocking agents, so cannot discriminate between these two possibilities.

Some indication that preganglionic cervical sympathetic nerves are potentially capable of generating Ca-spikes has previously been obtained by Greengard & Straub (1959), who reported that long-lasting action potentials could be recorded from desheathed rabbit cervical sympathetic nerves on replacing Locke solution with an isotonic Ba^{2+} solution. Bowers (1985) has also reported that TTX-resistant spikes can be recorded in bullfrog autonomic fibres; however, unlike the spikes recorded in the present study, these were attributed to entry of Na^{+} through TTX-resistant Na-channels, since they were lost in Na-free solution, required high concentrations (100 to $300 \mu\text{M}$) of Cd^{2+} ions for block and were not blocked by Co^{2+} or Ni^{2+} at concentrations up to 5 mM .

The inhibitory effect of the sympathomimetic amines might be attributable to a direct effect on the inward Ca-current or to an increase in K-current or leak current, thereby raising the threshold for regenerative Ca-entry. Both effects have been reported in other preparations. A direct effect on the inward Ca-current under conditions where K-currents have been eliminated has been recorded in sympathetic neurones (Galvan & Adams, 1982; Koketsu & Akasu, 1982; Lipscombe & Tsien, 1987), sensory neurones (Dunlap & Fischbach, 1981; Forscher & Oxford, 1985) and neuroblastoma hybrid cells (Docherty & McFadzean, 1987). On the other hand, increases in K-conductance induced by catecholamines have been detected in a variety of neurones

Figure 9 Effects of (a) phentolamine (5 μ M) and (b) yohimbine (1 μ M) followed by 5 μ M phentolamine on the inhibition of the transmitted compound action potential produced by noradrenaline (10 μ M; NA) and adrenaline (1 μ M; Ad). Records show the amplitude of the compound action potential recorded from the superior cervical ganglion with respect to the postganglionic trunk following single supramaximal preganglionic stimuli at 0.2 Hz. Changes in baseline reflect ganglionic hyperpolarizations (see Brown & Caulfield, 1979).

including sympathetic neurones (Brown & Caulfield, 1979 and unpublished), parasympathetic neurones of the myenteric and submucous plexus (Morita & North, 1981; North & Suprenant, 1985) and cat urinary bladder (Akasu *et al.*, 1986), spinal substantia gelatinosa neurones (North & Yoshimura, 1984)

and brainstem locus coeruleus neurones (Williams *et al.*, 1984).

The evidence available suggests that the inhibition of the cervical sympathetic Ca-spike most probably results primarily from a direct action on the Ca-current, for the following reasons. (i) An increased

Figure 10 Extracellular d.c. potentials recorded (a) from the superior cervical ganglion with reference to the postganglionic trunk and (b) from the preganglionic trunk with reference to an adjacent segment of the same trunk (see Methods) following 1 min applications of $10\ \mu\text{M}$ (—) noradrenaline (at bars) before and after adding $1\ \text{mM}$ 4-aminopyridine (4-AP) to the perfusion fluid. Two preparations; hyperpolarization downwards. 4-AP depolarized the preganglionic trunk by $0.6\ \text{mV}$; this was backed off during the break in the two records in (b). The preparations were superfused with normal Krebs solution (see Methods). Note that noradrenaline hyperpolarized the ganglion but depolarized the preganglionic trunk. (The reduced depolarization produced by noradrenaline in 4-aminopyridine solution in b may have been due to the large depolarization induced by the 4-aminopyridine itself).

K-conductance would hyperpolarize the preganglionic fibres. No such hyperpolarization could be detected under the same recording conditions where a clear hyperpolarization of the ganglion cells was observed (cf. Brown & Caulfield, 1979). (ii) The Ca-spike was still depressed when residual K-currents retained in 4-AP solution were further blocked with Ba^{2+} ions and/or tetraethylammonium. Although the depression of the peak Ba-spike by noradrenaline was about half that of the Ca-spike, this may be due to the fact that the larger and more prolonged Ba-spike allowed a larger fraction of the Ca-channels to open, so that effects of noradrenaline were not compared at the same current densities; further, the late component of the Ba spike (see above) was annulled by noradrenaline.

The receptors responsible for the inhibition of the Ca-spike appear to be α -adrenoceptors in view of the effectiveness of phentolamine and the ineffectiveness

of propranolol as antagonists. The high potency of clonidine (Starke *et al.*, 1975), coupled with the weak effect of amidephrine (Butler & Jenkinson, 1978) suggests an α_2 -receptor. This would accord with the inability of prazosin, an α_1 -adrenoceptor antagonist (Cambridge *et al.*, 1977; Doxey *et al.*, 1977), to block the inhibitory effect of noradrenaline. However, yohimbine, a potent α_2 -antagonist on the rat vas deferens (Drew, 1977), also failed to antagonize the effect of noradrenaline at $1\ \mu\text{M}$. Further, the apparent pA_2 for phentolamine (6.5) was much lower than that in the rat vas deferens (7.5). These weak effects of phentolamine and yohimbine cannot be due to problems of access or other peculiarities of the experimental conditions since both readily block the α_2 -mediated hyperpolarization of the ganglion under these conditions (see also Brown & Caulfield, 1979). Thus, the effects of catecholamines on Ca-spikes observed in this study cannot be unequivocally attributed to an interaction with either α_1 - or α_2 -adrenoceptors. A comparable difficulty in assigning the receptor responsible for inhibition of Ca-spikes to either of these categories has been encountered in tests on sensory neurones (Canfield & Dunlap, 1984), on locus coeruleus neurones (Williams & North, 1985) and on myenteric neurones (Slack, 1986). The receptors encountered in the present study do not appear to correspond to the ' γ -receptors' reported in rat basilar arteries (Hirst *et al.*, 1982) since the latter were activated equally well by noradrenaline and dopamine and were resistant to phentolamine.

It seems reasonable to suppose that the Ca-spikes recorded from these preganglionic trunk fibres are capable of propagating into the terminals, and therefore that sympathomimetic amines are capable of exerting a comparable inhibition of the Ca-current in such terminals. The question then arises whether the well-known inhibition of transmitter release from these fibres (see Introduction) results from this action or from some other effect such as the increase in K-conductance recordable from the postganglionic cells and at many other sites (see above). Three pieces of evidence suggest that Ca-current inhibition might be the primary factor. Firstly (as mentioned above), no hyperpolarization of the preganglionic fibres could be detected under conditions where catecholamines clearly hyperpolarized the ganglion cells, suggesting that catecholamines may not significantly increase the preganglionic K-conductance. (However, it should be noted that noradrenaline can hyperpolarize the cell bodies of preganglionic axons in the cat, as well as reducing the amplitude to the Ca-spike: Yoshimura *et al.*, 1986; 1987). Secondly, the inhibition of orthodromic transmission produced by noradrenaline was fully blocked by a concentration of phentolamine that did not suppress the postgan-

glionic hyperpolarization (Figure 9a); and thirdly, and conversely, yohimbine (at concentrations that did not antagonize Ca-spike inhibition) only partly antagonized the inhibition of transmission yet fully blocked the postganglionic hyperpolarization (Figure 9b).

In conclusion: we have found that sympathomimetic amines strongly and potently inhibit the Ca-spike in the unmyelinated fibres of the rat preganglionic cervical sympathetic nerve by an

action on an α -adrenoceptor of uncertain type, and suggest that an equivalent effect on the Ca-current in the terminal of these fibres might contribute to their ability to inhibit transmitter release.

This work was aided by a grant from the Medical Research Council. P.E. was in receipt of a Studentship Award from the School of Pharmacy. We are indebted to the following companies for gifts of drugs: Mead Johnson, Boehringer Ingelheim, CIBA and Pfizer.

References

- AKASU, T., GALLAGHER, J.P., NAKAMURA, T., SHINNICK-GALLAGHER, P. & YOSHIMURA, M. (1985). Noradrenaline hyperpolarization and depolarization in cat vesicular parasympathetic neurones. *J. Physiol.*, **361**, 165–184.
- ARHEM, P. (1980). Effects of rubidium, caesium, strontium, barium and lanthanum on ionic currents in myelinated nerve fibres from *Xenopus laevis*. *Acta Physiol. Scand.*, **108**, 7–16.
- ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol.*, **14**, 48–58.
- BIRKS, R. & MACINTOSH, F.C. (1961). Acetylcholine metabolism of a sympathetic ganglion. *Can. J. Biochem. Physiol.*, **39**, 787–827.
- BOWERS, C.W. (1985). A cadmium-sensitive, tetrodotoxin-resistant sodium channel in bullfrog autonomic axons. *Brain Res.*, **340**, 143–147.
- BROWN, D.A. & CAULFIELD, M.P. (1979). Hyperpolarizing ' α_2 '-adrenoceptors in rat sympathetic ganglia. *Br. J. Pharmacol.*, **65**, 435–445.
- BROWN, D.A. & CAULFIELD, M.P. (1981). Adrenoceptors in ganglia. In *Adrenoceptors and Catecholamine Action*. ed. Kunos, G. pp. 99–116. New York: Wiley.
- BROWN, D.A., DUNN, P.M. & MARSH, S. (1982). Possible electrophysiological mechanisms for modulating transmitter release. In *Presynaptic Receptors: Mechanism and Function*. ed. De Belleruche, J. pp. 207–218. London: Ellis Horwood.
- BROWN, D.A. & MARSH, S. (1978). Axonal GABA-receptors in mammalian peripheral nerve trunks. *Brain Res.*, **156**, 187–191.
- BUTLER, M. & JENKINSON, D.H. (1978). Blockade by WB 4101 of α -adrenoceptors in the rat vas deferens and guinea-pig taenia caeci. *Eur. J. Pharmacol.*, **52**, 303–311.
- CAMBRIDGE, D., DAVEY, M.J. & MASSINGHAM, R. (1977). Prazosin, a selective antagonist of postsynaptic α -adrenoceptors. *Br. J. Pharmacol.*, **59**, 514–515P.
- CANFIELD, D.R. & DUNLAP, K. (1984). Pharmacological characterization of amine receptors on embryonic chick sensory neurones. *Br. J. Pharmacol.*, **82**, 557–563.
- CHRIST, D.D. & NISHI, S. (1971a). Site of adrenaline blockade in the superior cervical ganglion of the rabbit. *J. Physiol.*, **213**, 107–117.
- CHRIST, D.D. & NISHI, S. (1971b). Effects of adrenaline on nerve terminals in the superior cervical ganglion of the rabbit. *Br. J. Pharmacol.*, **41**, 331–338.
- COURTICE, C.J. (1977). A circuit for recording evoked action potential amplitudes. *J. Physiol.*, **268**, 1–2P.
- DAWES, P.M. & VIZI, E.S. (1973). Acetylcholine release from the rabbit isolated superior cervical ganglion preparation. *Br. J. Pharmacol.*, **48**, 225–232.
- DOCHERTY, R.J. & McFADZEAN, I. (1987). Noradrenaline decreases voltage-dependent calcium current in neuroblastoma $\times$ glioma hybrid (NG108-15) cells. *J. Physiol.*, **390**, 81P.
- DOLPHIN, A.C., FORDA, S.R. & SCOTT, R.H. (1986). Calcium-dependent currents in cultured rat dorsal root ganglion neurones are inhibited by an adenosine analogue. *J. Physiol.*, **373**, 47–61.
- DOXEY, J.C., SMITH, C.F.C. & WALKER, J.M. (1977). Selectivity of blocking agents for pre- and postsynaptic α -adrenoceptors. *Br. J. Pharmacol.*, **60**, 91–96.
- DREW, G.M. (1977). Pharmacological characterization of the presynaptic α -adrenoceptor in the rat vas deferens. *Eur. J. Pharmacol.*, **42**, 123–130.
- DUN, N. & KARCZMAR, A.G. (1977). The presynaptic site of action of norepinephrine in the superior cervical ganglion of guinea-pig. *J. Pharmacol. Exp. Ther.*, **200**, 328–335.
- DUN, N. & NISHI, S. (1974). Effects of dopamine on the superior cervical ganglion of the rabbit. *J. Physiol.*, **239**, 155–164.
- DUNANT, Y. (1967). Organization topographique et fonctionnelle du ganglion cervical supérieur chez le rat. *J. Physiol., Paris*, **59**, 17–38.
- DUNLAP, K. & FISCHBACH, G.D. (1978). Neurotransmitters decrease the calcium component of sensory neurone action potentials. *Nature*, **276**, 837–839.
- DUNLAP, K. & FISCHBACH, G.D. (1981). Neurotransmitters decrease the calcium conductance activated by depolarization of embryonic chick sensory neurones. *J. Physiol.*, **317**, 519–535.
- ELLIOTT, P. (1986). Inhibition of catecholamines of Ca-spike in rat preganglionic cervical sympathetic nerves. *Br. J. Pharmacol.*, **88**, 459P.
- FORSCHER, P. & OXFORD, G.S. (1985). Modulation of calcium channels by norepinephrine in internally dialyzed avian sensory neurones. *J. Gen. Physiol.*, **85**, 743–763.
- GALVAN, M. & ADAMS, P.R. (1982). Control of calcium current in rat sympathetic neurons by norepinephrine. *Brain Res.*, **244**, 135–144.
- GREENGARD, P. & STRAUB, R.W. (1959). Restoration by barium of action potentials in sodium-deprived mammalian B and C fibres. *J. Physiol.*, **145**, 562–569.
- HAGIWARA, S. & BYERLY, L. (1981). Calcium channel. *Ann.*

- Rev. Neurosci.*, **4**, 69–125.
- HENON, B.K. & McAFEE, D.A. (1983). The ionic basis of adenosine receptor actions on post-ganglionic neurones in the rat. *J. Physiol.*, **336**, 607–620.
- HIRST, G.D.S., NEILD, T.O. & SILVERBERG, G.D. (1982). Noradrenaline receptors on the rat basilar artery. *J. Physiol.*, **328**, 351–360.
- HORN, J.P. & McAFEE, D.A. (1980). Alpha-adrenergic inhibition of calcium-dependent potentials in rat sympathetic neurones. *J. Physiol.*, **301**, 191–204.
- KOKETSU, K. & AKASU, T. (1982). Modification of the slow inward Ca^{2+} current by adrenaline in bullfrog sympathetic ganglion cells. *Jpn. J. Physiol.*, **32**, 137–140.
- KUAN, Y.F., SCHOLFIELD, C.N. & STEEL, L. (1985). Effects of Ca antagonists on axonal Ca/Ba-spikes in slices of guinea-pig brain. *J. Physiol.*, **362**, 35P.
- LIPSCOMBE, D. & TSIEN, R.W. (1987). Noradrenaline inhibits N-type Ca channels in isolated frog sympathetic neurones. *J. Physiol.*, **390**, 84P.
- MACDONALD, R.D., SKERRITT, J.H. & WERZ, M.A. (1986). Adenosine agonists reduce voltage-dependent calcium conductance of mouse sensory neurones in cell culture. *J. Physiol.*, **370**, 75–90.
- MINOTA, S. & KOKETSU, K. (1977). Effects of adrenaline on the action potential of sympathetic ganglion cells in bullfrogs. *J. Physiol.*, **27**, 353–366.
- MORITA, K. & NORTH, R.A. (1981). Clonidine activates potassium current in myenteric neurones. *Br. J. Pharmacol.*, **74**, 419–428.
- NORTH, R.A. & SURPRENANT, A. (1985). Inhibitory synaptic potentials resulting from α_2 -adrenoceptor activation in guinea-pig submucous plexus neurones. *J. Physiol.*, **358**, 17–33.
- NORTH, R.A. & YOSHIMURA, M. (1984). The actions of noradrenaline on neurones of the rat substantia gelatinosa *in vitro*. *J. Physiol.*, **349**, 43–55.
- PARKER, R.B. & WAUD, D.R. (1971). Pharmacological estimation of drug-receptor dissociation constants. Statistical evaluation. I Agonists. *J. Pharmacol. Exp. Ther.*, **177**, 1–12.
- PENNER, R. & DREYER, F. (1986). Two different presynaptic calcium currents in mouse motor nerve terminals. *Pflügers Arch.*, **406**, 190–197.
- SLACK, B.E. (1986). Pre- and postsynaptic actions of noradrenaline and clonidine on myenteric neurones. *Neuroscience*, **19**, 1303–1309.
- STARKE, K., ENDO, T. & TAUBE, H.D. (1975). Relative pre- and postsynaptic potencies of α -adrenoceptor agonists in the rabbit pulmonary artery. *Naunyn-Schmiedeberg Arch. Pharmacol.*, **291**, 55–78.
- WERMAN, R. & GRUNDFEST, H. (1961). Graded and all-or-none electrogenesis in arthropod muscle. I. The effects of alkali-earth cations on the neuromuscular system of *Romalea microptera*. *J. Gen. Physiol.*, **44**, 979–995.
- WILLIAMS, J.T., HENDERSON, G. & NORTH, R.A. (1985). Characterization of α_2 -adrenoceptors which increase potassium conductance in rat locus coeruleus neurones. *Neuroscience*, **14**, 95–101.
- WILLIAMS, J.T. & NORTH, R.A. (1985). Catecholamine inhibition of calcium action potentials in rat locus coeruleus neurones. *Neuroscience*, **14**, 103–109.
- YOSHIMURA, M., POLOSA, C. & NISHI, S. (1986). Noradrenaline modifies sympathetic preganglionic neuron spike and afterpotential. *Brain Res.*, **362**, 370–374.
- YOSHIMURA, M., POLOSA, C. & NISHI, S. (1987). Slow IPSP and the noradrenaline-induced inhibition of the cat sympathetic preganglionic neuron *in vitro*. *Brain Res.*, **419**, 383–386.

(Received March 21, 1988

Revised June 27, 1988

Accepted July 25, 1988)