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HYPERPOLARIZING 'α_2'-ADRENOCEPTORS IN RAT SYMPATHETIC GANGLIA

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1 Receptors mediating catecholamine-induced hyperpolarization of isolated superior cervical sympathetic ganglia of the rat have been characterized by means of an extracellular recording method.

2 (−)-Noradrenaline (EC_{50}, 1.7 ± 0.6 μM) produced an immediate low-amplitude (<400 μV) hyperpolarization. The hyperpolarization was increased on removal of external Ca^{2+} or on reduction of external K^+ from 6 to 2 mM. Hyperpolarization was unaffected by changing the temperature from 25° to 37°C.

3 Hyperpolarization was also produced by the following agonists (potencies relative to (−)-noradrenaline): (−)-noradrenaline 1; (±)-isoprenaline 0.41; (−)-phenylephrine 0.40; (+)-noradrenaline 0.13; 2-amino-6,7-dihydroxy tetrahydronaphthalene (ADTN) 0.25; dopamine 0.1; methoxamine 0.012; amidephrine 0.0015.

4 Responses were antagonized by phentolamine (1 μM) but not by (±)-propranolol (1 μM), haloperidol (10 μM) or α-flupenthixol (1 μM). This suggested that hyperpolarization was mediated solely through α-receptor stimulation not through stimulation of β-receptors or dopamine-receptors.

5 Dose-ratio shifts produced by phentolamine varied with different agonists. The shift increased in inverse proportion to the ability of the agonists to inhibit [^{3}H]-(−)-noradrenaline uptake, suggesting that uptake of agonists limited the dose-ratio shift. Cocaine and nortriptyline reduced catecholamine-induced hyperpolarization in concentrations (10 μM and 1 μM respectively) necessary to inhibit [^{3}H]-(−)-noradrenaline uptake.

6 Clonidine (0.01 to 1 μM), oxymetazoline (0.01 to 1 μM) and ergometrine (0.1 to 10 μM) produced a persistent, low-amplitude hyperpolarization, as though they were partial agonists. Responses to the agonists were blocked by yohimbine (1 μM) but not be prazosin (1 μM).

7 It is concluded that the adrenergic cell bodies in the ganglion were hyperpolarized through activation of the same type of α-receptor ('α_2-receptors') as those present at adrenergic nerve terminals.

Introduction

De Groat & Volle (1966) and Haefely (1969) described two clear effects of catecholamines on sympathetic ganglion cells: a depolarization mediated through β-receptors and a hyperpolarization apparently mediated through α-receptors. However, others have suggested, or implied, that ganglion cell hyperpolarization may also be initiated through activation of some form of dopamine receptor (Greengard, 1976; Dun, Kalibara & Karczmar, 1977). This is of interest in connection with the proposal that release of dopamine by interneurones is responsible for the 'slow inhibitory postsynaptic potentials' which can be generated under certain conditions by preganglionic nerve stimulation (Libet & Owman, 1974), and also with the postulated relationship between the responses of sympathetic ganglion cells and those of central neurones to dopamine and other catecholamines (Greengard, 1976).

In the present experiments a more detailed pharmacological characterization of the hyperpolarizing action of catecholamines on the isolated superior cervical ganglion of the rat has been attempted. The results suggest that the receptors are neither dopamine receptors nor conventional α-receptors, but in-

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stead resemble the 'α_2-receptors' associated more usually with sympathetic nerve terminals (Langer, 1974; Starke, 1977; Berthelsen & Pettinger, 1977).

Methods

Superior cervical ganglia were excised from male Wistar rats (200 to 300 g) under light anaesthesia (1.5 g/kg urethane i.p.) and their connective tissue sheaths removed.

Electrical recording

Potential changes were recorded extracellularly using the 'air-gap' superfusion method of Brown & Marsh (1975). Ganglia were mounted vertically with the postganglionic trunk uppermost and Ag/AgCl electrodes were placed in contact with the body of the ganglion and the cut end of the postganglionic trunk, contact with the tissue being established through an agar/saline bridge. The assembly was enclosed in a moist chamber maintained at 24 to 26°C and the tissue was superfused with Krebs solution (previously equilibrated with 95% O_2 and 5% CO_2) at a rate of 1 ml/min. Drugs were perfused through separate, pre-filled channels. The deadtime for solution changes was <20 s; mixing of drug was precluded by a deliberately-introduced air bubble, so that the appearance of the air-bubble at the outlet signified the moment at which the drug reached the preparation in its final concentration. Since the d.c. potential changes produced by the catecholamines were very small ($\leqslant 400$ μV), the ganglia were stored overnight at 4°C in pre-oxygenated Krebs solution before use. This allows the initial demarcation potential to subside (as the cut ends of the nerves reseal), and thereby improves d.c. stability (Brown, Brownstein & Scholfield, 1972). The low operating temperature (25°C) also helped to stabilize the recording system. Neither overnight storage, nor low temperature, caused any qualitative change in the responses of the ganglia to catecholamines. D.c. potentials were displayed on a potentiometric chart recorder (Bryans 28,000) damped to minimize drop-artefacts.

[^{3}H]-noradrenaline uptake

Ganglia were incubated individually in 1 ml aliquots of Krebs solution at 25°C bubbled with 95% O_2 and 5% CO_2, containing 0.1 μM (−)-[^{3}H]-noradrenaline, specific activity 5.85 Ci/mmol (New England Nuclear) and 10 μM nialamide, unless otherwise stated. After incubation, ganglia were briefly rinsed in Krebs solution, to remove adhering radioactivity, blotted and weighed (to within 5 μg) on an electrotorsion microbalance (Beckman/R.I.I.C.). Readings were taken at 1 and 2 min and extrapolated to zero time to estimate fresh weight (Brown, Halliwell & Scholfield, 1971). Ganglia (or 10 μl aliquots of incubation medium) were dissolved in solubilizer (0.5 ml Soluene, Packard), neutralized with 1.5 M HCl, 10 ml scintillant (Aquasol, New England Nuclear) added and radioactivity counted by liquid scintillation spectrometry. Samples were individually corrected for quenching. The amount of radioactivity in the ganglion was expressed as the tissue/medium ratio, i.e., (d min^{-1} mg^{-1} ganglion)/(d min^{-1} μl^{-1} incubation medium).

In control experiments, ganglia (8) incubated as above for 2 h were homogenized in 50 μl ice-cold 0.001 N HCl, centrifuged at 10,000 rev/min for 10 min and the supernatant subjected to paper chromatography, using phenol/HCl as the solvent, to separate metabolites (Langer, 1970). In the presence of nialamide, unchanged [^{3}H]-noradrenaline accounted for 87% of the extracted radioactivity.

Drugs and solutions

All drugs were dissolved in Krebs solution (containing 1 mM ascorbic acid to suppress oxidation of catecholamines) at a concentration of 1 to 10 mM, as needed, unless otherwise indicated. The drugs used were: (−)-noradrenaline, (−)-phenylephrine hydrochloride, (+)-propranolol hydrochloride, yohimbine hydrochloride, (all obtained from Sigma Chemical Corp.), apomorphine (Macfarlane Smith) and methoxamine hydrochloride (Burroughs Wellcome). We are very grateful to the following companies for supplying: (±)-amidephrine hydrochloride (Mead Johnson), fluphenazine hydrochloride (Squibb), phentolamine mesylate (CIBA), oxymetazoline hydrochloride (Allen & Hanbury), ergometrine maleate (Sandoz), nortriptyline hydrochloride (Lilly), nialamide (Pfizer), prazosin hydrochloride (Pfizer), practolol (ICI), clonidine hydrochloride (Boehringer Ingelheim), and (+)-noradrenaline bitartrate (Chemie Linz AG); and to Dr G.N. Woodruff and Dr L.L. Iversen respectively for samples of 2-amino-6,7-dihydroxy tetrahydronaphthalene (ADTN) and of α-flupenthixol.

Yohimbine and prazosin were dissolved in 30% ethanol as 1 mM stock solutions and appropriate controls carried out in the presence of 0.3% ethanol (the highest concentration present in the final solution) to ensure that agonist responses remained constant.

The composition of the Krebs solution used was (in mEquiv/l): Na^+ 143, K^+ 5.9, Cl^- 128, HCO_3^- 25, Mg^{2+} 1.2; SO_4^{2-} 1.2, Ca^{2+} 2.5, $H_2PO_4^-$ 1.2 and glucose, 11. For 'potassium-free' or low potassium solutions, potassium chloride was omitted and potassium dihydrogen phosphate was substituted with an equimolar concentration of sodium dihydrogen phosphate. The required potassium concentration was obtained by addition of potassium chloride. In ex-

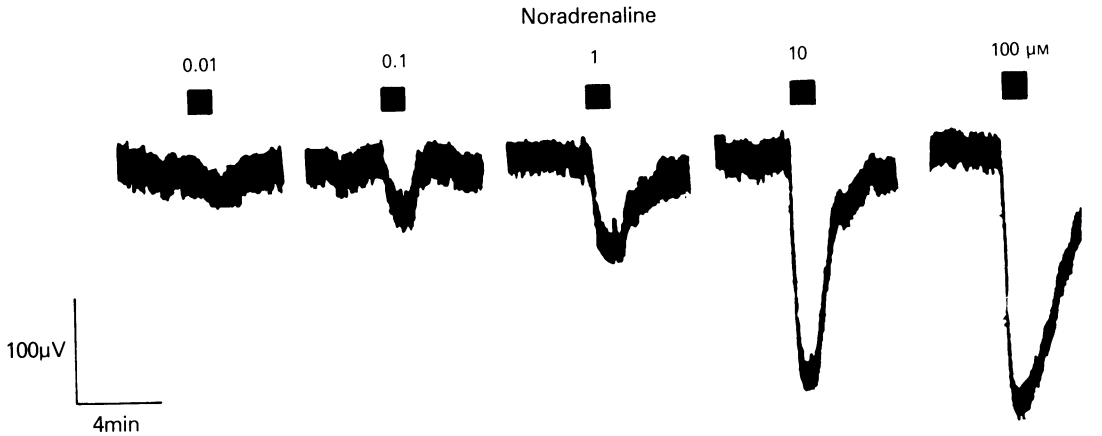

Figure 1 Hyperpolarizations of isolated superior cervical ganglion of the rat produced by 1 min applications (solid bars) of increasing concentrations of (−)-noradrenaline (NA), at 15 min intervals. The signal bars show the duration for which NA solution was in contact with the preparation, the perfusion dead-time having been subtracted (see Methods).

periments where calcium concentration was varied, a solution buffered (to pH 6.6) with Hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid, Sigma), and bubbled with O_2, was used. The composition was that of the Krebs solution described above, except that 10 mM Hepes was substituted for bicarbonate. Initially, only 118 mM Na^+ was added (as NaCl); the pH was then adjusted to 6.6 by adding a measured amount of 1 M NaOH. After taking into account the Na^+ added as NaOH, the remaining Na^+ was made up (to 143 mM) with sodium isethionate. Appropriate volumes of a 1 M $CaCl_2$ solution were added to obtain the desired Ca^{2+} concentration.

Results

Noradrenaline

Application of (−)-noradrenaline (NA) produced a concentration-dependent ganglionic surface-positivity, ranging from 60 to 400 μV peak amplitude in different preparations (Figure 1). The voltage-deflection began within 5 s of the time when the NA reached the ganglion, attained a peak amplitude within 30 s and was sustained for at least 60 s with concentrations ≤ 10 μM; at higher concentrations some 'fade' occurred during superfusion of NA. Resting potential was restored within 5 min of washing off the NA. Repeatable responses could be obtained with 60 s applications of NA at 15 to 20 min intervals.

These surface-positive deflections were replicated when NA was applied locally to the body of the ganglion, perfused separately from the pre- and postganglionic nerve trunks by means of transverse partitions (see Bowery & Tulett, 1975): hence they resulted from ganglion hyperpolarization, rather than depolarization of the postganglionic trunk (see Brown *et al.*, 1972). Ganglia in which the preganglionic nerve trunk had been sectioned 10 to 20 days previously were also hyperpolarized by NA.

Responses to NA or to its antagonists (see below) were not obviously altered by raising the perfusion temperature from 25° to 37°C.

Omission of Ca^{2+} from the superfusion fluid, or reduction in external K^+ concentration, increased the maximum amplitude of the hyperpolarizing responses to NA (Figure 2). Conversely, elevation of external $[Ca^{2+}]$ to 5 mM or of external $[K^+]$ to 18 mM depressed responses. Responses were unaffected by hyoscine (1 μM) and/or hexamethonium (1 mM).

Other agonists

The mean EC_{50} for (−)-NA was 1.7 μM (Table 1); (+)-NA was about 8 times weaker. Isoprenaline, phenylephrine, 2-amino-6,7-dihydroxytetralin (ADTN), dopamine and amidephrine (in order of decreasing potency: Table 1) produced hyperpolarizations of comparable peak amplitude and onset rate. The response to isoprenaline declined more slowly (10 to 15 min) than that to NA. None of these agonists depolarized the ganglion.

β-Receptor antagonists

The high potency of isoprenaline, coupled with the weak effect of the α-agonist amidephrine (Dungan,

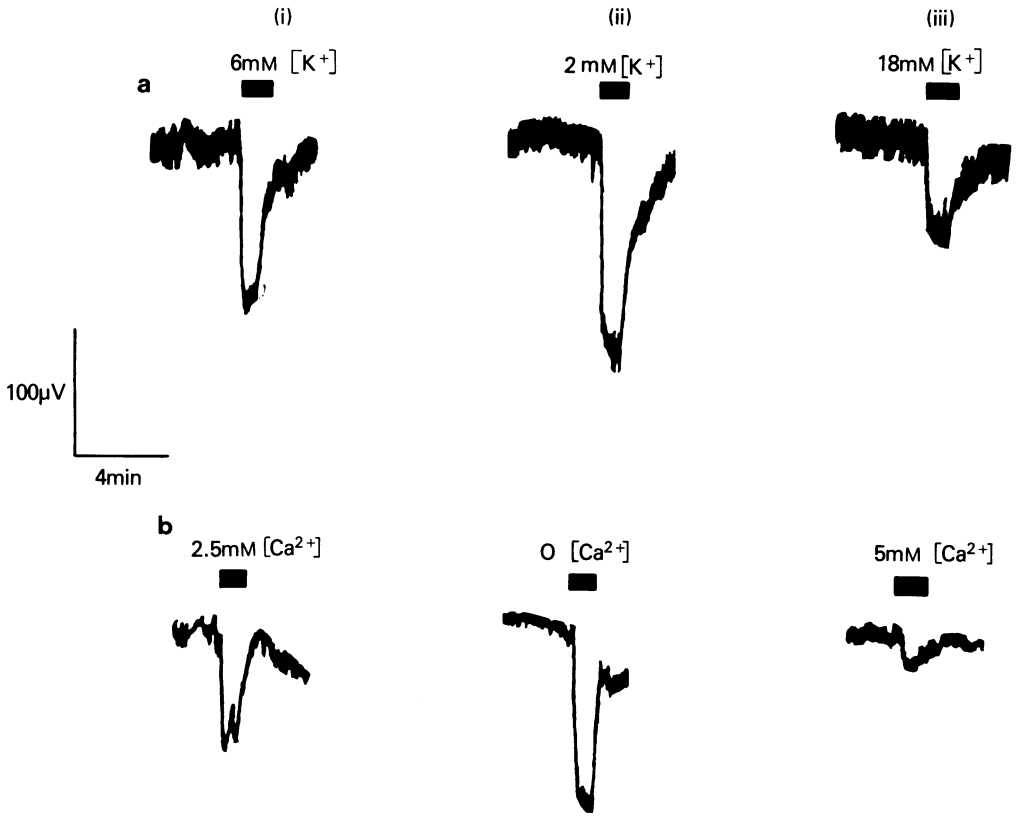

Figure 2 Effects of changing (a) external K^+ concentration and (b) external Ca^{2+} concentration on the hyperpolarizing responses to 10 μM (−)-noradrenaline. Responses in column (i) are controls in normal Krebs solution; responses in column (ii) and (iii) were obtained 30 min after solution changes.

Stanton & Lish, 1965; Buchthal & Jenkinson, 1970) suggested the presence of β-receptors. However, the β-receptor antagonists propranolol (≤ 10 μM) and practolol (≤ 100 μM) failed to antagonize responses to isoprenaline.

α-Receptor antagonists

In contrast to the ineffectiveness of propranolol, 1 μM phentolamine shifted the dose-response curve for isoprenaline to the right, by a factor of 150. Accurate estimates of the inhibitor constant (K_i) from Schild plots (Figure 3) were impracticable, because the relationship between dose-ratio and antagonist concentration became non-linear, with slope less than unity, above 1 μM phentolamine. Extrapolation from lower concentrations (slope = 1.27 ± 0.32) suggested a K_i for phentolamine of about 20 nM.

Effects of phentolamine on the other agonists, measured as the agonist dose-ratio in the presence of 1 μM phentolamine, are shown in Table 2. There was a considerable variation with different agonists: isoprenaline and phenylephrine showed large (though different) shifts; noradrenaline was antagonized to a lesser extent and dopamine and ADTN hardly at all.

Table 1 Concentrations of agonists required to produce half-maximal (EC_{50}) hyperpolarization of ganglia

Agonist	(EC_{50} (M)) *Mean*	±	*s.e.*	(*n*)
(−)-Noradrenaline	1.7×10^{-6}	±	0.6	(5)
(±)-Isoprenaline	4.1×10^{-6}	±	0.8	(6)
(−)-Phenylephrine	4.2×10^{-6}	±	0.4	(4)
ADTN[1]	6.2×10^{-6}	±	0.4	(3)
Dopamine	1.7×10^{-5}	±	0.5	(4)
(±)-Amidephrine	1.1×10^{-3}	±	3.1	(3)

[1] 2-Amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene.

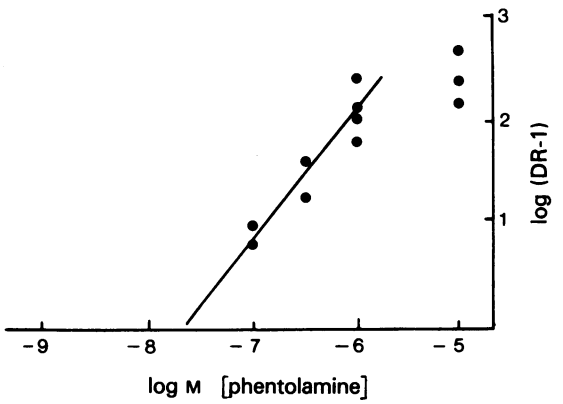

Figure 3 The antagonism of responses to (±)-isoprenaline by phentolamine, expressed by a Schild plot (cf. Arunlakshana & Schild, 1959). Dose-ratio shifts for isoprenaline were determined (using submaximal agonist doses) in the presence of each concentration of phentolamine after at least 40 min exposure to the antagonist. The (dose-ratio − 1) is plotted on a logarithmic scale against the logarithm of molar phentolamine concentration. Each point represents a separate experiment. The best fit line (computed by least squares regression analysis) is drawn through the points representing phentolamine concentrations <1 μM and has a slope of 1.27, which is not significantly different from 1 ($P < 0.05$ by Student's t test). The projected line intercepts the abscissa scale at a phentolamine concentration of 21 nM.

Dopamine receptors?

Notwithstanding the hyperpolarizing effects of dopamine or ADTN (reported to be a selective dopamine-receptor agonist: Woodruff, 1971; Volkmann, Kohli, Goldberg, Cannon & Lee, 1977), the presence of specific dopamine-receptors was not confirmed by other tests. Firstly, apomorphine, another dopamine-receptor agonist (Rekker, Engel & Nys, 1972), failed to hyperpolarize the ganglion at concentrations up to 100 μM. Secondly, the effects of dopamine and ADTN were not antagonized by haloperidol (10 μM), methysergide (10 μM), fluphenazine (1 μM) or α-flupenthixol (1 μM). (These are potent antagonists of dopamine at other sites: Seeman, Lau Wong, Tedesco & Wong, 1975; Dray, Gonye & Oakley, 1976; Burt, Creese & Snyder, 1976; Ginsborg, House & Silinsky, 1976.) Control responses to phenylephrine or noradrenaline were also unaffected by these drugs. Clearly, if dopamine-induced hyperpolarization does result from activation of 'dopamine-receptors', these are pharmacologically quite different from those previously reported in other tissues.

Table 2 The antagonist effect of phentolamine on various agonists, expressed as the shift of the dose-response curve to the right in the presence of 1 μM phentolamine

	Phentolamine (1 μM) dose-ratio			
Agonist	*Mean*	±	*s.e.*	(*n*)
(−)-Noradrenaline	5.6	±	0.9	(4)
(±)-Isoprenaline	157.3	±	48.1	(4)
(−)-Phenylephrine	24.7	±	3.8	(5)
Dopamine	2.3	±	0.1	(3)
ADTN	1.3	±	0.3	(4)

Influence of catecholamine uptake

An alternative explanation to the presence of multiple receptor types for the variable agonist dose-ratios in the presence of phentolamine might be variable agonist uptake (see Langer & Trendelenburg, 1969). To test this, the affinity of different agonists for the noradrenaline carrier was estimated by their ability to inhibit the uptake of [^{3}H]-(−)-NA by isolated ganglia.

At 0.1 μM [^{3}H]-(−)-NA, uptake of label (see Methods) increased linearly with time for up to 30 min in normal Krebs solution, and for longer periods in the presence of 10 μM nialamide (Figure 4), presumably reflecting the reduced backflux of deaminated metabolites (see Maxwell, Ferris & Burcsu, 1976). Addition of unlabelled (−)-NA reduced tritium uptake in the presence of nialamide, with half-saturation (IC_{50}) at 4.7 μM. IC_{50} values (μM) for other agonists were: ADTN, 2.0; dopamine, 3.8; phenylephrine, 18; and isoprenaline, 100 (Figure 5). This order of potency for inhibition of [^{3}H]-NA uptake is the inverse of the order of sensitivity of the agonists to phentolamine (as measured by the dose-ratios in Table 2). This would be expected were uptake to limit the dose-ratio shifts (Langer & Trendelenburg, 1969): under these circumstances an increase in external agonist concentration saturates the carrier and so produces a disproportionate increase in juxta-receptor concentration, limiting the effect of the antagonist.

The most obvious way of testing the influence of uptake is to eliminate it with an uptake blocker. Unfortunately, though in agreement with previous observations on sympathetic ganglia (Fischer & Snyder, 1965; Hanbauer, Johnson, Silberstein & Kopin, 1972), [^{3}H]-NA uptake by rat isolated ganglia proved relatively insensitive to conventional blocking agents such as cocaine or nortriptyline (IC_{50} values 3.7 μM and 1.7 μM respectively: Figure 6). Use of these concentrations of the uptake blockers produced a depression of the responses to all agonists, probably by a non-specific action. (Nortriptyline may block α-receptors,

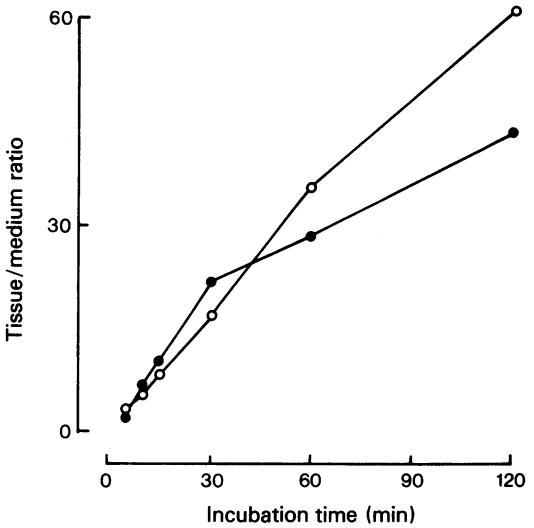

Figure 4 The effect of increasing incubation times on the uptake of 0.1 μM (−)-[^{3}H]-noradrenaline by ganglia expressed as the tissue: medium ratio (see Methods) in the absence (●, control) and presence (○) of nialamide (10 μM). Each point shows results from one ganglion.

see Hughes, Kneen & Main, 1974; U'Prichard, Greenberg, Sheehan & Snyder, 1978.)

An alternative way of minimizing the effect of uptake is to measure the antagonism of fixed, equieffective concentrations of different agonists. The proportion of agonist taken up then remains constant. As shown in Figure 7, the depressions in the responses to matching concentrations of noradrenaline and dopamine produced by a low concentration of phentolamine were similar, suggesting that the two agonists (at these particular concentrations) were equally sensitive to phentolamine and so probably acted on the same receptor. (Similar effects were seen with another α-receptor blocker, yohimbine, see below and Figure 9.)

'α_2' *Receptors?*

Thus, far, it appeared that the hyperpolarizing receptors were indeed α-receptors, in accord with the conclusions of DeGroat & Volle (1966). However, even allowing for complications introduced by agonist uptake, some anomalies persisted, for example, the weak action of amidephrine.

A clue to the cause of this arose from experiments in which ergometrine was used in an attempt to block presumptive dopamine receptors (cf. Walker, Ralph, Woodruff & Kerkut, 1971). In these experiments ergometrine exhibited an *agonist* action at concentrations between 0.01 and 10 μM. This raised the possibility

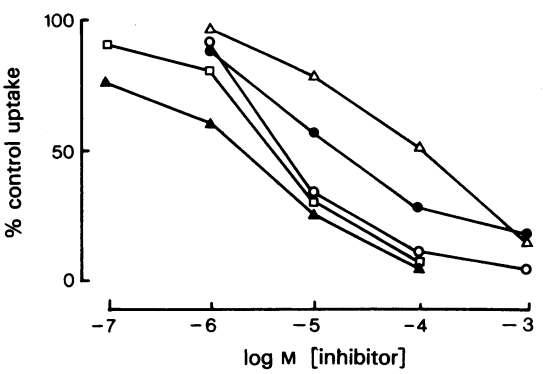

Figure 5 Inhibition of [^{3}H]-noradrenaline uptake into ganglia. Uptake was measured after 30 min incubation in 0.1 μM [^{3}H]-noradrenaline in the presence of 10 μM nialamide. Uptake measured in the presence of inhibitor is expressed as % of mean control value measured in the absence of inhibitor. Inhibitors (IC_{50} given after each substance, μM) were: (±)-isoprenaline (△), 100; (−)-phenylephrine (●), 18; (−)-noradrenaline (○), 4.7; dopamine (□), 3.8; and ADTN (▲), 2. Each point represents a single determination.

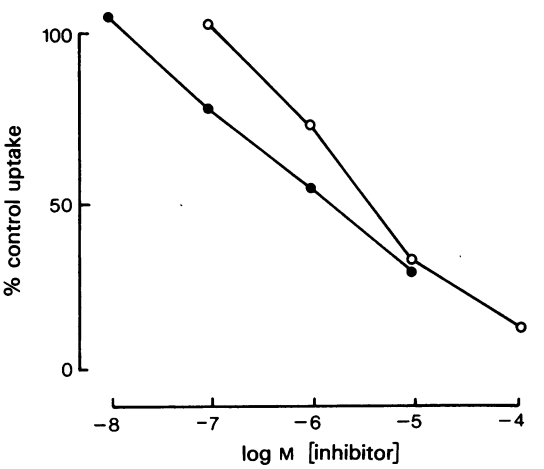

Figure 6 Inhibition of the uptake of 0.1 μM [^{3}H]-noradrenaline into ganglia by cocaine (○) and nortriptyline (●). Uptake in the presence of inhibitor is expressed as % of uptake in its absence (control), as in Figure 5. Ganglia were pre-incubated with the inhibitors for 60 min before adding [^{3}H]-(−)-noradrenaline. The IC_{50}s were 3.7 μM for cocaine and 1.7 μM for nortriptyline.

that the ganglion α-receptors might belong to the 'α_2' subclass (Berthelsen & Pettinger, 1977), since Marshall, Nasmyth, Russell & Shepperson (1977) found that ergometrine inhibited noradrenaline release from sympathetic nerve terminals in the mouse vas

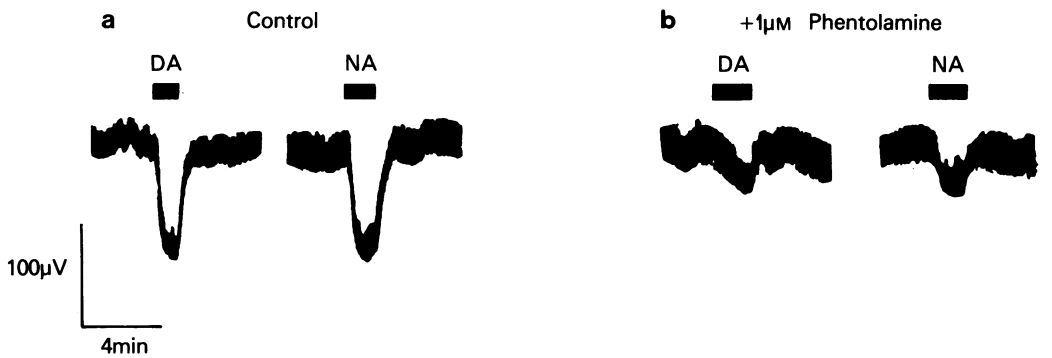

Figure 7 Depression of matched submaximal responses to 10 μM dopamine (DA) and 1 μM noradrenaline (NA) by phentolamine. Application of agonists is indicated by the solid bars. Responses in (b) were obtained after at least 40 min in the presence of 1 μM phentolamine.

deferens, through an agonist action on the presynaptic α_2-receptors (cf. Langer, 1974; Starke, 1977). This possibility was explored with a further range of agonists and antagonists selective for the two α-receptor subclasses.

Agonists

The α_2-stimulants oxymetazoline and clonidine (Starke, Endo & Taube, 1975a) hyperpolarized the ganglion at very low concentrations (1 nM–1 μM). The response produced by each was of lower (~30%) maximum amplitude than that produced by NA, was more sustained and reversed only slowly on washing (cf. Drew, 1977) over periods up to 2 h (Figure 8). This prolonged effect appeared to result from persistent receptor activation, since it was rapidly reversed by the addition of phentolamine. Thus clonidine, oxymetazoline and ergometrine exhibited characteristics associated with potent partial agonists (see Starke, 1977). In contrast, the selective α_1-stimulant methoxamine (Starke *et al.*, 1975a) was much less potent (EC_{50} 300 μM).

Antagonists

Responses to all α-agonists were markedly reduced in the presence of 1 μM yohimbine (Figure 9), a powerful antagonist at α_2-receptors (Starke, Borowski & Endo, 1975). Prazosin, a selective α_1-receptor antagonist (Cambridge, Davey & Massingham, 1977; Doxey, Smith & Walker, 1977), was ineffective at concentrations up to 10 μM (Figure 9). Determination of K_1 values for these antagonists was not attempted for the same reasons as those mentioned in connection with the studies using phentolamine.

Discussion

With this type of extracellular recording method, nicotinic agonists and γ-aminobutyric acid (GABA) produce peak depolarizaations of 4 to 8 mV and 1 to 2 mV respectively (Brown & Marsh, 1975, and unpublished observations). Comparison with intracellularly-recorded responses of rat ganglion cells to these agonists (Adams & Brown, 1975; Brown, 1978) suggests a 5 to 10 fold attenuation of the membrane potential change by the low-resistance extracellular fluid layer. Thus, the low-amplitude extracellular response to catecholamines (up to 400 μV) would correspond to a neuronal hyperpolarization of, at most, 2 to 4 mV, if evenly spread among the ganglion cell population. This accords with previous observations on single rabbit ganglion cells (Kobayashi & Libet, 1970; Dun & Nishi, 1974). In agreement with this, we (Adams & Brown; and Caulfield, Constanti & Brown: unpublished) have detected small hyperpolarizations in some individual rat ganglion cells of up to 3 mV during perfusion with 10 μM noradrenaline, 100 μM dopamine or 1 μM adrenaline, unaccompanied by any clear reduction in cell input resistance. Also in agreement with published experiments on rabbit ganglia (Kobayashi & Libet, 1970; Dun & Nishi, 1974; Dun *et al.*, 1977) not all cells showed a clear response to noradrenaline or dopamine: this might reflect variations in cell sensitivity or in impalement shunts (see Adams & Brown, 1975).

Adrenoceptors

The receptor mediating the hyperpolarizing response of the isolated superior cervical ganglion of the rat to catecholamines resembles most closely the 'α_2' type of receptor (Berthelsen & Pettinger, 1977), normally

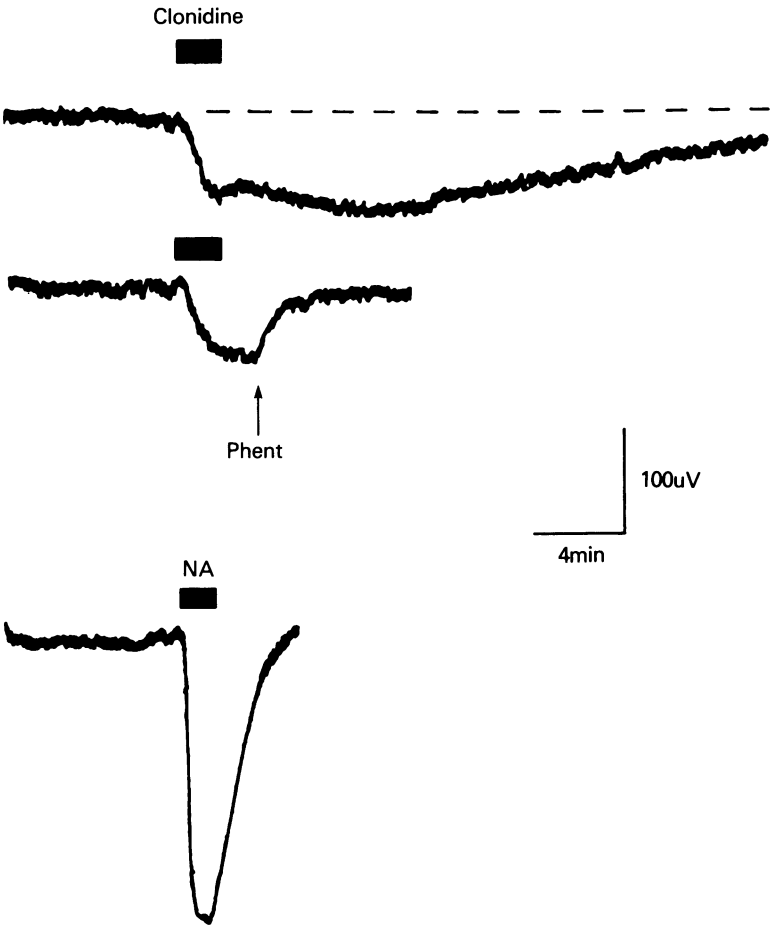

Figure 8 A comparison of the maximum hyperpolarization produced by clonidine (1 μM) and noradrenaline (NA; 10 μM), showing the different rates of recovery. The agonists were applied only for the period shown by the solid bar. Addition of phentolamine (Phent 1 μM, at the arrow) during the maintained hyperpolarization following washout of clonidine produced a rapid restoration of the potential to normal levels.

associated with adrenergic nerve terminals. Thus, clonidine and oxymetazoline were potent agonists, whereas methoxamine was not (cf. Starke *et al.*, 1975a; Drew, 1977). The weak hyperpolarizing action of amidephrine is explicable by the presence of an α_2-receptor since this appears to be a selective α_1-receptor stimulant (Butler & Jenkinson, 1978). The differential effectiveness of yohimbine and prazosin as antagonists (see Doxey *et al.*, 1977) provide support for an α_2-mediated hyperpolarization.

This implies that the same type of adrenoceptor is present on both somatic and terminal ends of adrenergic nerves. There have been two previous reports indicating the presence of the terminal α_2-type of adrenoceptor on adrenergic cell somata: Schumann & Werner (1971) reported inhibition of catecholamine-release from adrenal medullary cells by the imidazoline α_2-agonist, BAY a 6781; and Cederbaum & Aghajanian (1977) observed inhibition of spike discharges in locus coeruleus neurones by other α_2-agonists such as clonidine, which were antagonized by piperoxane.

The responses of sympathetic ganglion cells to α-agonists further resembles that of the nerve terminals in their sensitivity to K^+ and Ca^{2+} ions. Thus, α-mediated inhibition of noradrenaline release is enhanced in low-Ca^{2+} solution (Marshall, Nasmyth & Shepperson, 1977a) and reduced in high-K^+ solution (Dismukes, de Boer & Mulder, 1977); and ganglionic hyperpolarization is affected likewise. This

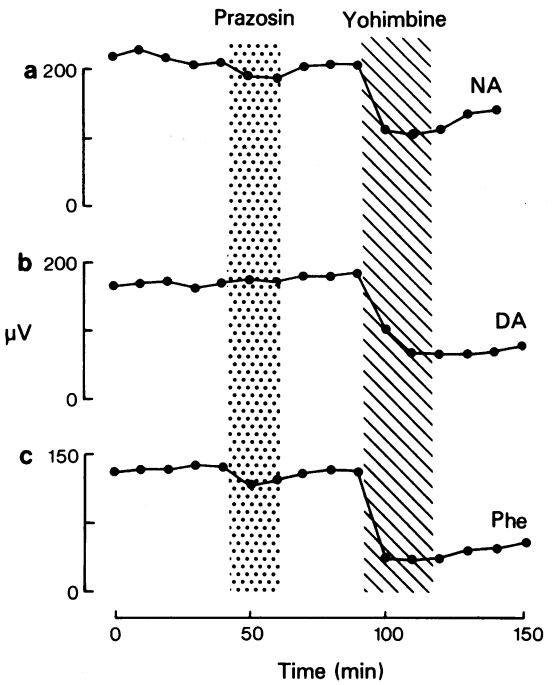

Figure 9 The effects of prazosin (10 μM stippled area) and yohimbine (1 μM striped area) on hyperpolarizing responses to (a) 10 μM noradrenaline (NA), (b) 30 μM dopamine (DA) and (c) 10 μM phenylephrine (Phe) (3 separate experiments). Agonists were applied in approximately equi-effective submaximal doses for 1 min, at 10 min intervals; points show the maximal hyperpolarization produced. Antagonists were applied sequentially for the durations indicated.

might be considered as offering support for the view (Starke, 1977; Dismukes *et al.*, 1977) that inhibition of transmitter release is connected with hyperpolarization of adrenergic nerve terminals. It may be pertinent that adrenaline has been reported to hyperpolarize unmyelinated sympathetic nerve fibres (Goffart & Holmes, 1952).

Dopamine-receptors

This study began as an attempt to define 'dopamine receptors' in ganglia. In the event, none were found. Dopamine itself, and apomorphine, proved relatively weak agonists, and were not antagonized by conventional dopamine-receptor antagonists such as haloperidol, fluphenazine or α-flupenthixol. Instead, they were antagonized by yohimbine, suggesting the hyperpolarization produced by dopamine resulted from an effect on the same (α_2) receptor as that activated by noradrenaline and other α-agonists.

The initial proposal for hyperpolarizing dopamine-receptors on sympathetic ganglion cells stemmed from experiments on rabbit ganglia (Libet, 1970), but pharmacological evidence is unconvincing. Libet (1970) described dopamine as 'equally effective' with noradrenaline without further defining their relative activities. Dun & Nishi (1974), using intracellular recording, reported that the small hyperpolarizations of individual rabbit ganglion cells produced by 100 μM dopamine were abolished by prior treatment with phenoxybenzamine. Subsequently, Dun *et al.* (1977) observed antagonism to iontophoretically-applied dopamine by 0.1 to 1 μM haloperidol: this is inconclusive because haloperidol is a strong α-antagonist in the rabbit (pA_2 8.36 on rabbit aortic strips: Gothert, Lox & Rieckesmann, 1977).

Inferences of specific dopamine-receptors drawn from adenyl cyclase activation (Kebabian & Greengard, 1971) or synaptically-mediated hyperpolarization (Libet, 1970) are indirect and pharmacologically unsupported. The strongest evidence for a ganglionic dopamine-receptor is that of Willems (1973), who reported that dopamine and apomorphine depressed transmission through dog paravertebral ganglia *in vivo*, and that this effect was antagonized selectively (*vis-a-vis* that of noradrenaline) by haloperidol and pimozide. However, depression of ganglionic transmission probably results from a presynaptic action rather than from ganglion-cell hyperpolarization (Dun & Nishi, 1974).

It is, of course, possible that a sub-population of ganglion cells (perhaps those directly innervated by adrenergic interneurones) may possess specific dopamine-receptors. However, the present results indicate that this could apply only to a small minority of cells, at best: hyperpolarization of the majority of rat ganglion cells as represented by the surface-potentials is clearly not mediated by dopamine-receptors, and evidence for dopamine-receptors on ganglionic neurones in other species is hardly more convincing. This limits the extent to which the ganglion can be used as a model for interpreting the action of dopamine on central neurones (cf. Greengard, 1976).

Note added in proof

T. Suzuki & R.L. Volle (1978: *Naunyn Schmiedebergs Arch. Pharmac.*, **304**, 15–20) have recently described the effects of isoprenaline on the isolated superior cervical ganglion of the rat recorded with intracellular electrodes. They report that 10 to 100 μM isoprenaline hyperpolarized the ganglion cells by 4 mV and depressed excitatory postsynaptic potentials. The latter effect was not antagonized by practolol (10 μM); propranolol (10 μM) and phentolamine (10 to 50 μM) depressed transmission *per se*. Effects of these antagonists on the hyperpolarization are not described.

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