Br. J. Pharmac. (1983), **79**, 429–439

Depolarization of rat isolated superior cervical ganglia mediated by β_2-adrenoceptors

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1 Depolarizations of freshly-dissected isolated superior cervical ganglia of the rat were recorded extracellularly. The following sympathomimetic amines (in order of decreasing potency) produced depolarizations of up to 0.4 mV: isoprenaline, salbutamol, adrenaline, noradrenaline. Depolarizations were lost after overnight storage, leaving only hyperpolarizing responses.

2 Depolarizations by isoprenaline were antagonized by (−)-propranolol (pA_2 8.94 ± 0.15), (±)-butoxamine (pA_2 7.36 ± 0.12) and (±)-practolol (pA_2 5.14 ± 0.13). They were not blocked by phentolamine (1 μM) or phenoxybenzamine (1 μM). Isoprenaline and salbutamol were antagonized with equal facility by practolol or butoxamine.

3 In concentrations producing ganglionic depolarization, these compounds also produced a smaller depolarization of presynaptic elements in the ganglion, but not preganglionic trunk fibres. Presynapic depolarization was blocked by 100 nM propranolol but not by 1 μM phentolamine.

4 Isoprenaline and salbutamol increased the amplitude of the compound ganglionic action potential recorded following single preganglionic nerve stimuli when transmission had been rendered submaximal by adjusting the Ca/Mg ratio, but not in normal solution.

5 Isoprenaline (0.1 μM) also increased the amount of [^{3}H]-acetylcholine released by preganglionic stimulation in low Ca/high Mg solution.

6 It is concluded that facilitatory adrenoceptors are present on pre- and postsynaptic elements in rat superior cervical ganglia, which resembles the 'β_2' subclass of β-receptors.

Introduction

De Groat & Volle (1966) and Haefely (1969) discerned two forms of potential change in cat superior cervical ganglia *in situ* following local application of catecholamines: an α-mediated hyperpolarization and a β-mediated depolarization. In previous experiments on rat isolated sympathetic ganglia, only the α-mediated hyperpolarization could be recorded and no catecholamine-induced depolarization was detected (Suzuki & Volle, 1978; Brown & Caulfield, 1979). We have now succeeded in recording such depolarization *in vitro*, and in the present paper, describe some of its pharmacological characteristics: it appears to be mediated by β-receptors resembling the 'β_2' subclass of β-receptors (see Lands, Arnold, McAuliff, Luduena & Brown, 1967; Daly & Levy, 1979). We also describe some experiments suggesting the presence of a similar type of receptor at presynaptic terminals in the ganglion. An abstract of some of these observations has been presented (Dunn, 1982).

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Methods

Superior cervical ganglia were excised from male Wistar rats (250–400 g) anaesthetized with urethane (1.5 g kg^{-1} i.p.) and their connective tissue capsules removed.

Ganglion depolarization

This was recorded extracellularly *in vitro* as described by Brown & Caulfield (1979), with the following modifications. (1) The three-chambered grease-sealed perfusion bath described by Brown & Marsh (1978) was used instead of the air-gap superfusion

method. The ganglion body was placed in the central chamber (volume ~ 0.5 ml) immersed in Krebs solution flowing at 2 ml min^{-1}, and the pre- and postganglionic trunks drawn through into proximal and distal chambers, also filled with Krebs solution. Ganglionic depolarization was measured differentially across the partition separating the ganglion and postganglionic trunk, using Ag/AgCl saline bridge electrodes coupled to a potentiometric recorder (input impedance $\geq$ 100 kΩ off null-potential). The inflow was grounded and high-frequency noise filtered with a 1 μF capacitor. (2) In the earlier study of Brown & Caulfield (1979), the ganglia were stored overnight at 4°C to allow the demarcation potential to subside and so improve stability for recording low-amplitude d.c.-potentials using the 'air-gap' system. In this system, the inter-electrode shunting fluctuates with the drop-perfusion so that a large demarcation potential produces large transient fluctuations in baseline d.c. potential. The present system obviated this difficulty, so freshly-dissected ganglia could be and were used throughout. Drug-induced potential changes were corrected for slow baseline drifts, as shown in Figure 1.

Preganglionic depolarization

This was recorded using the above bath assembly but with the reference electrode in the chamber containing the preganglionic trunk (see Brown, Adams, Higgins & Marsh, 1979).

Transmission

Ganglionic compound action potentials recorded between the ganglion and postganglionic nerve trunk were 'held' with a peak-height detector (Courtice, 1977) before display on a chart-recorder (see Brown & Marsh, 1978). The preganglionic nerve was stimulated through bipolar Pt electrodes, using 'supramaximal' currents at (normally) 0.2 Hz.

Acetylcholine release

Ganglionic acetylcholine stores were labelled with tritium by pre-incubating the ganglion for 30 min in Krebs solution containing 47 mM KCl, and then for a further 30 min in normal Krebs solution containing 1 μM [^{3}H]-choline chloride (Higgins & Neal, 1978). The preparation was then placed in the above bath assembly, with the central (ganglion) chamber reduced to 200 μl, and the ganglion perfused with Krebs solution containing 5 μM neostigmine at 0.5 ml min^{-1}. After 1 h wash-perfusion, the bath effluent was collected at 4 min intervals. Three ml scintillant ('Aquasol', New England Nuclear Corporation) was added to each fraction and tritium measured by liquid scintillation spectrometry. The preganglionic nerve was stimulated supramaximally at 1 Hz for 2 min every 20 min. Previous experiments have established that, under these experimental conditions, the extra tritium released during the 4 min period including the stimulation period is due entirely to the release of [^{3}H]-acetylcholine from the preganglionic fibres (Higgins, 1981).

Solutions

Krebs solution contained the following salts (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.18, $MgSO_4.7H_2O$ 1.18, D-glucose 11 and ascorbic acid, 0.5. The pH was maintained at 7.4 by bubbling with 95% O_2:5% CO_2. In some experiments $NaHCO_3$ was replaced with 15 mM Tris (hydroxymethyl) aminomethane (Tris-buffered) and 10 mM NaCl, adjusted to pH 7.4 with HCl, and bubbled with O_2. The normal perfusion temperature was maintained at 22°C.

Drugs

Sources were: (−)-adrenaline bitartrate, (−)-noradrenaline bitartrate, (±)-isoprenaline hydrochloride, (±)-propranolol hydrochloride (all from Sigma). (+) and (−)-Propranolol and (±)-practolol were gifts from I.C.I., salbutamol sulphate from Allen & Hanbury, and (±)-butoxamine hydrochloride from Burroughs Wellcome. The latter was dissolved as a 1 mM solution in distilled water or 0.9% w/v NaCl solution, and practolol in 1 M HCl, before diluting. [^{3}H]-choline chloride was purchased from the Radiochemical Centre, Amersham (specific activity 15 Ci $mmol^{-1}$).

Results

Ganglion depolarization

In agreement with Brown & Caulfield (1979), brief (2 min) applications of noradrenaline (0.1–100 μM) produced reversible ganglion hyperpolarizations, which were blocked by 1 μM phentolamine. In contrast, isoprenaline (1–100 nM) produced a depolarization of up to 400 μV lasting about 10 min (Figure 1). This was not affected by phentolamine (Figure 1a) but was reduced by 0.1 μM (±)-propranolol (Figure 1b). Adrenaline (10 nM–1 μM) produced either a small ($<$ 400 μV) hyperpolarization (Figure 1a) or an initial hyperpolarization followed by a depolarization (Figure 1b). In the presence of phentolamine (or phenoxybenzamine, 1 μM) the hyperpolarization was blocked, leaving only a depolarization (Figure 1a). In contrast, in propranolol solution adrenaline produced a pure hyperpolarization (Figure 1b).

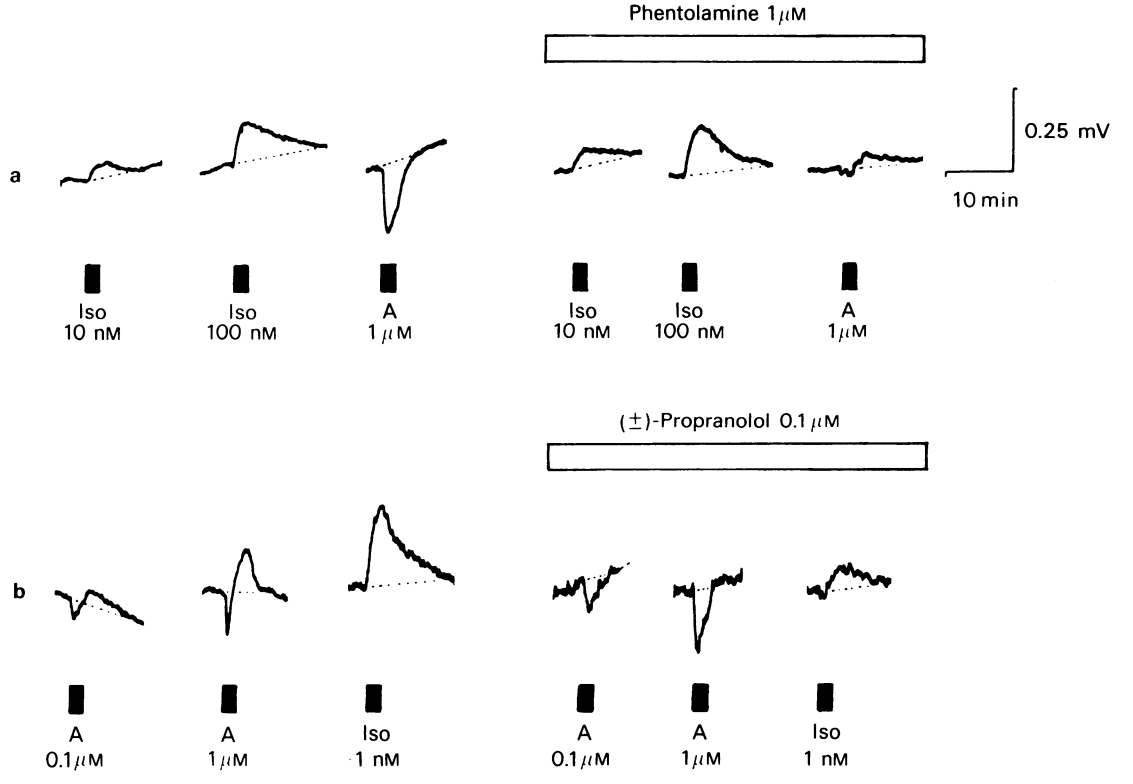

Figure 1 Effects of isoprenaline (Iso) and adrenaline (A) on the d.c. potential recorded between the ganglion and the postganglionic trunk in two isolated rat superior cervical ganglia designated (**a**) and (**b**). Drugs were perfused through the recording chamber containing the ganglion for 2 min periods (at the bars) at 15–20 min intervals in the concentrations indicated. An upward deflection of the potential record indicates ganglion depolarization.

These observations confirm, in essence, the original reports of De Groat & Volle (1966) and Haefely (1969) that there are two types of postsynaptic response to catecholamines in sympathetic ganglia, an α-mediated hyperpolarization and a β-mediated depolarization. However, in previous tests on rat isolated superior cervical ganglia, only the α-mediated hyperpolarization was observed and no depolarizing responses to adrenaline or isoprenaline were detected (Caulfield, 1978; Brown & Caulfield, 1979). Why the difference? One possibility is that the earlier experiments were preceded by overnight storage of the ganglia at 4°C *in vitro*, whereas freshly-dissected ganglia were used for the present experiments (see Methods). To check this, the responses of a number of freshly-dissected and stored ganglia to adrenaline and isoprenaline were compared (Figure 2). The depolarization produced by isoprenaline was considerably reduced (by $76 \pm 12\%$ at 10 nM; $n = 6$) in stored ganglia, whereas the initial hyperpolarizing response to adrenaline was approximately doubled in amplitude. This change was a consequence of the prolonged maintenance *in vitro*, rather than the period of low temperature storage *per se*, since similar changes occurred after 24 h incubation at 22°C (Figure 2c). The effect of prolonged incubation *in vitro* was essentially similar to that of adding propranolol (*cf.* Figure 1b): the depolarization produced by isoprenaline was reduced to very low levels, while the enhanced hyperpolarization by adrenaline appeared to result from the reduction or abolition of the secondary depolarizing component. This suggests that, after prolonged isolation, there is a reduction or loss of the β-mediated component of response. This does not appear to reflect a 'conversion' to α-receptors (Kunos, 1977), since α-hyperpolarizing responses to noradrenaline are not changed by storage (Caulfield, 1978). Another possibility is that the β-depolarization is generated indirectly through the release of acetylcholine from presynaptic nerve-endings, and that loss of β-responses reflects the degeneration of the terminals. This can be excluded because the depolarizing action of isoprenaline on freshly-dissected ganglia was not depressed by

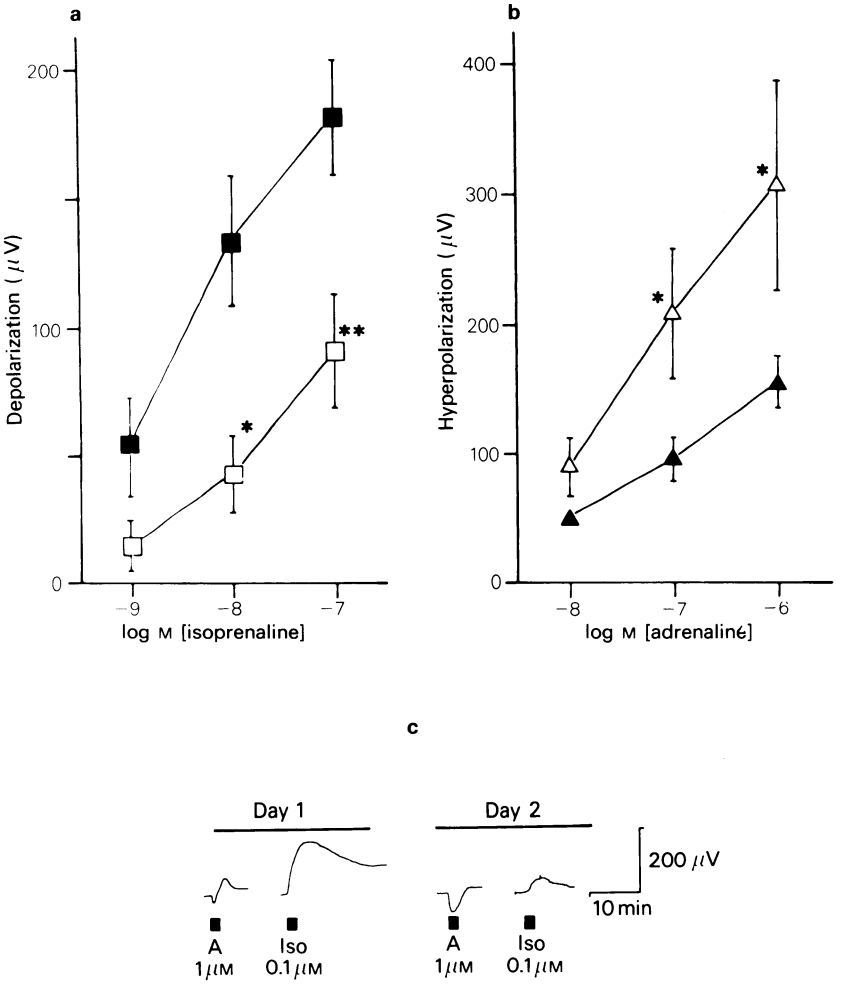

Figure 2 Effects of overnight storage on the responses of rat superior cervical ganglia to isoprenaline and adrenaline. The graphs in (**a**) and (**b**) show the amplitude of the depolarizations produced by isoprenaline (■, □) and the initial hyperpolarizations produced by adrenaline (▲, △) (see Figure 1 for representative responses) recorded on the day of excision (■, ▲) or after overnight storage in oxygenated Krebs solution at 4°C (□, △; cf. Brown & Caulfield, 1979). Each point shows the mean amplitude of the responses recorded in at least 6 ganglia; bars show s.e.mean. Responses of the two groups were significantly different at $P = 0.05$ (*) or $P = 0.02$ (**) by Student's two-tailed t test. The records in (**c**) show responses of the same ganglion to adrenaline (A) and isoprenaline (Iso) recorded on the day of excision (day 1) and then after overnight storage at 22°C in oxygenated Tris-buffered Krebs maintained at pH 7.4.

acetylcholine antagonists (hexamethonium 1 mM, plus atropine 1 μM), indicating a direct postsynaptic action.

Agonist potency

When α-receptors were blocked with phenoxybenzamine, all three catecholamines produced depolarizations of equivalent amplitudes, in the potency order isoprenaline > adrenaline > noradrenaline (Figure 3). Responses to isoprenaline were noticeably more prolonged than those to noradrenaline and adrenaline.

The effects of isoprenaline were closely replicated by salbutamol (Figure 4). The mean ratio of equieffective concentrations of salbutamol and isoprenaline in 9 experiments was about 35:1. This dose-ratio is reasonably close to that expected for a β_2-

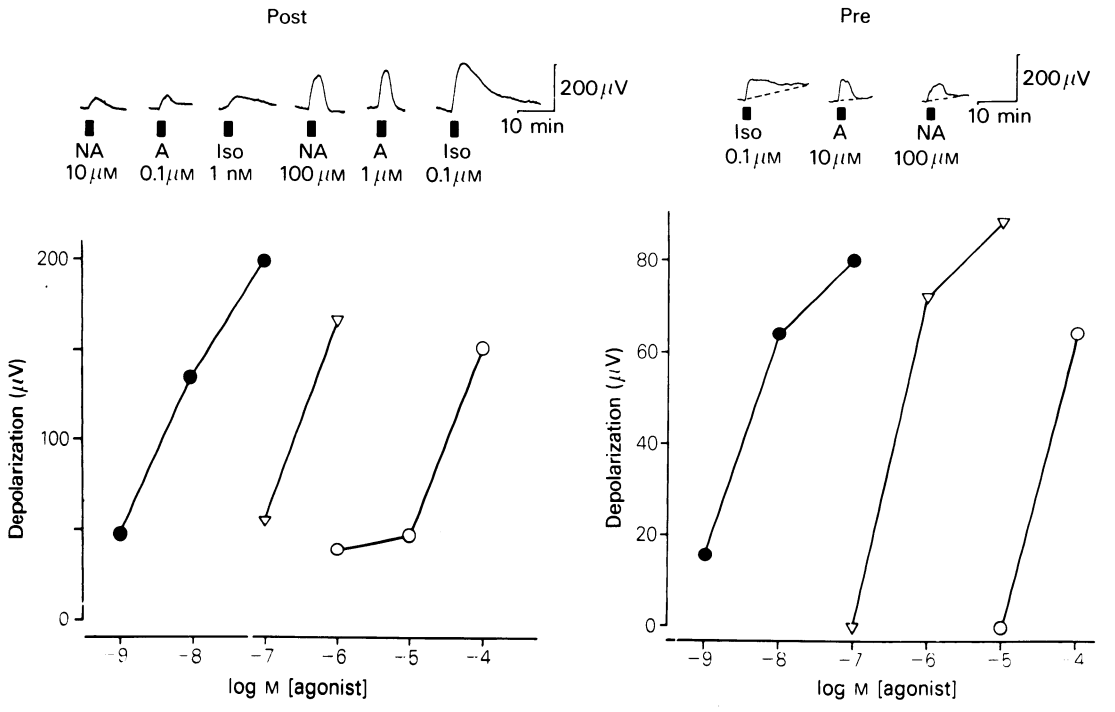

Figure 3 Depolarizations of a single isolated superior cervical ganglion of rat by isoprenaline (Iso, ●), adrenaline (A, ▽) and noradrenaline (NA, ○) recorded with reference to the postganglionic (Post) and preganglionic (Pre) trunks. The ganglion was pre-incubated for 60 min with 1 μM phenoxybenzamine, to block α-mediated effects.

receptor but very much less than that associated with β_1-receptors (see Daly & Levy, 1979: 26 in rat aortic strip versus 581 in guinea-pig atrium).

Antagonists

Responses to isoprenaline were antagonized by (−)-propranolol (at >1 nM), (±)-butoxamine (>0.5 μM) and (±)-practolol (>100 μM). (+)-Propranolol had no effect at 100 nM. Schild plots (Arunlakshana & Schild, 1959) were linear, but the slope of the line for butoxamine (0.52) was significantly less than 1 (Figure 5). Extrapolation to the abscissa at log (DR − 1) = 0 (where slope differences have minimal influence: Tallarida, Cowan & Adler, 1979) yielded the following estimates of pA_2 values (± s.e.mean): (−)-propranolol, 8.94 ± 0.15 ($n = 6$); (±)-butoxamine, 7.36 ± 0.12 ($n = 9$); and (±)-practolol, 5.14 ± 0.13 ($n = 7$).

Assuming the (+)-isomer to be ineffective, the pA_2 value for (−)-propranolol corresponds to a value of 8.64 for the racemic mixture. This agrees with values reported for rat uterus (8.56) and rabbit atrium (8.61; Daly & Levy, 1979). The low activity of practolol and high activity of butoxamine accords with a β_2-receptor (Wasserman & Levy, 1972; Levy & Apperly, 1978; Daly & Levy, 1979; Larson, 1979). As far as could be ascertained, there was no consistent difference between the sensitivities of isoprenaline and other agonists to these antagonists. Thus, as illustrated in Figure 6, equieffective concentrations of isoprenaline and salbutamol were antagonized with equal facility by butoxamine or practolol. This suggests that these two agonists acted on a single pharmacologically-defined receptor.

Preganglionic depolarization

A small (<200 μV) depolarization was also recorded following application of the catecholamines to the ganglion when the reference electrode was transferred from the postganglionic chamber to the chamber containing the preganglionic nerve trunk (Figure 3). Although effects recorded with reference to retrograde postganglionic fibres cannot be excluded entirely, such fibres appear to be very sparse in this preparation (Bowers & Zigmond, 1979); also, no comparable hyperpolarizing responses to α-agonists could be recorded, so it seems more probable that these depolarizations reflect responses of presynaptic

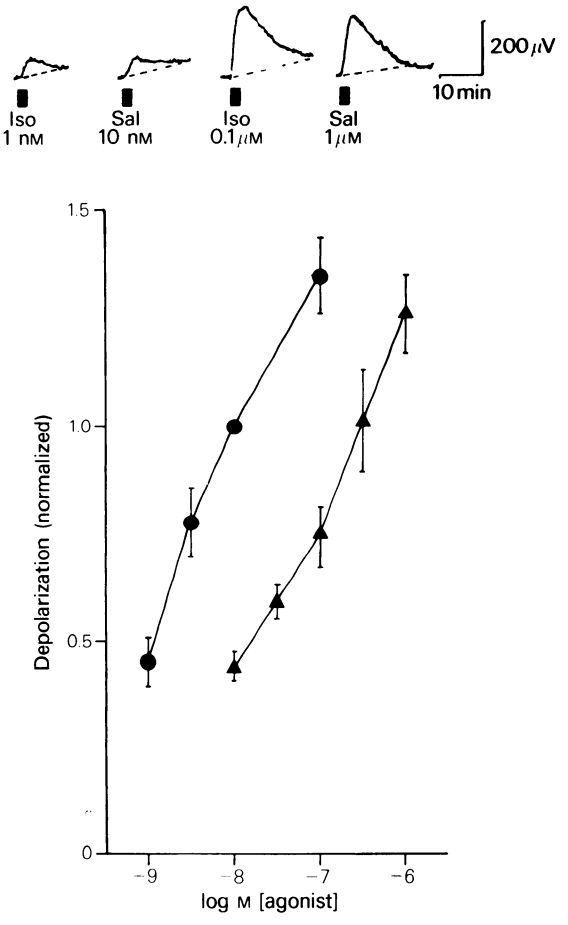

Figure 4 Comparative depolarizations to isoprenaline (Iso, ●) and salbutamol (Sal, ▲). The traces show representative responses of a single ganglion to these two agonists. The plots show the concentration-dependence of the depolarizations in 9 ganglia, expressed as a ratio of that produced by 10 mM isoprenaline. Bars show s.e.mean.

elements in the ganglion rather than postsynaptic elements. No such response could be recorded following application of catecholamines to the preganglionic trunk, so the presynaptic receptors appear to be concentrated at, or near to, the preganglionic fibre terminals.

As shown in Figure 3, the potencies of the three catecholamines, isoprenaline, adrenaline and noradrenaline, on these presynaptic elements closely matched those recorded postsynaptically. Salbutamol was between 10 and 100 times less effective than isoprenaline. The depolarizations were not blocked by phentolamine but were antagonized by propranolol, 100 nM producing about a 100 fold shift of the isoprenaline dose-response curve. Thus the presynaptic receptors were pharmacologically very similar to the postsynaptic receptors.

Facilitation of ganglionic transmission

The evoked postganglionic action potential produced by single supramaximal preganglionic nerve stimuli in normal Krebs solution was usually depressed by all three catecholamines, as previously reported in this preparation by Caulfield (1978), Quenzer, Yahn, Alkadhi & Volle (1979), and Brown & Caulfield (1981). This may imply that the availability of Ca^{2+} for transmitter release was already supramaximal under these experimental conditions. To reduce Ca^{2+} availability, external Ca^{2+} was lowered to 1.25 mM and Mg^{2+} raised to 11.2 mM. This reduced the compound action potential to less than half its original amplitude; isoprenaline (1 to 100 nM) and salbutamol (0.01–1 μM) then produced a consistent increase in the amplitude of the compound action potential (Figure 7), of up to 26 ± 3% (mean maximum increase in 15 experiments). This facilitation was blocked by 0.1 μM propranolol and hence appears to be β-mediated. Under the same experimental conditions noradrenaline and adrenaline still reduced the spike height, as in normal Krebs solution.

This facilitation accords with earlier observations on the actions of isoprenaline on cat superior cervical ganglia *in vivo* (Pardo, Cato, Gijon & Alonzo de Florida, 1963; De Groat & Volle, 1966; Haefely, 1969; Krstić, 1971) and on rabbit superior cervical ganglia *in vitro* (Elliott & Quilliam, 1964). Facilitation of transmission by adrenaline has also been reported by Bülbring (1944) and Malmejac (1955). Since the effective concentrations of isoprenaline or salbutamol produced simultaneous, and time-related, depolarizations of both pre- and postsynaptic elements, the pre- or postsynaptic origin of the facilitation is uncertain.

Increased acetylcholine release

Catecholamines have usually been observed to reduce the release of acetylcholine from sympathetic ganglia (Paton & Thompson, 1953; Birks & McIntosh, 1961; Dawes & Vizi, 1973) although Birks & MacIntosh (1961) detected an increased release of acetylcholine from plasma-perfused cat superior cervical ganglia on adding a low concentration (10 ng ml^{-1}) of adrenaline. Adrenaline, noradrenaline and dopamine also reduced the quantal content of the excitatory postsynaptic potential in rabbit and guinea-pig superior cervical ganglia (Christ & Nishi, 1971a, b; Dun & Nishi, 1974; Dun & Karczmar, 1977); in bullfrog ganglia this depression is followed by a long-lasting increase in quantal con-

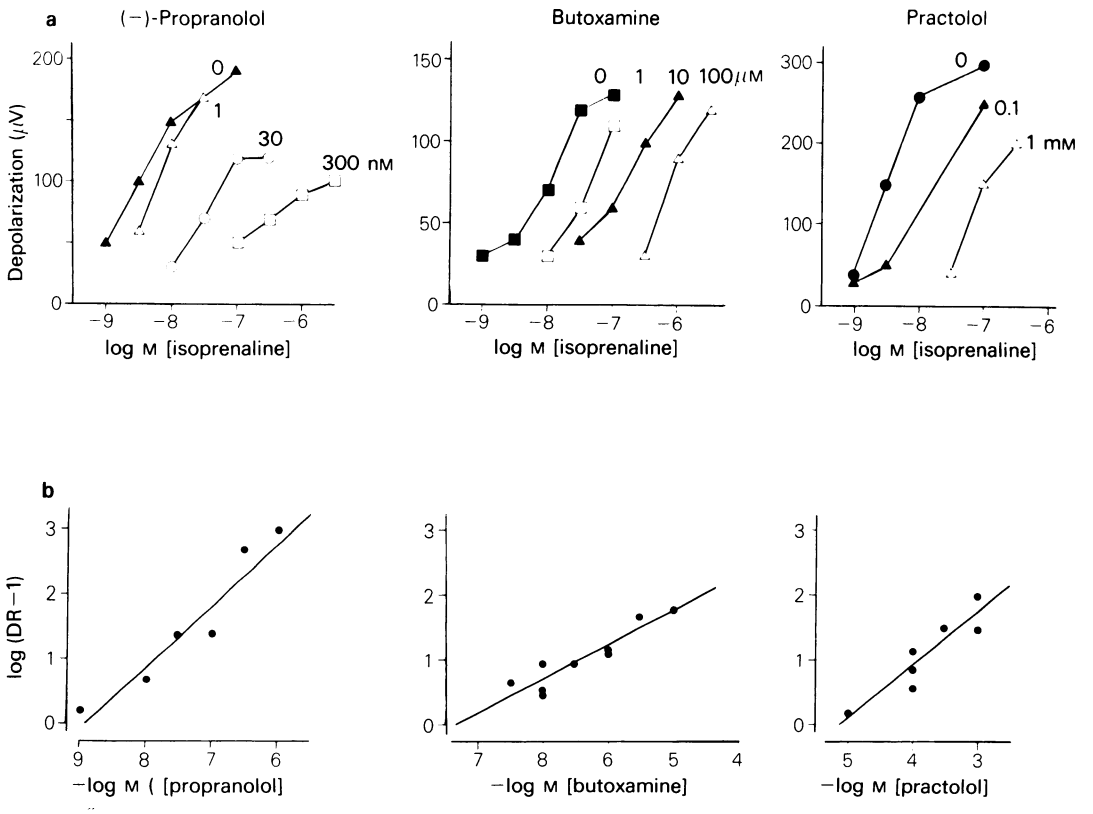

Figure 5 Antagonism of isoprenaline by (−)-propranolol, (±)-butoxamine and (±)-practolol. The graphs in (**a**) show representative dose-response relationships in single ganglia recorded in the presence of increasing concentrations of antagonist. (The flattening of the dose-response curve for isoprenaline at $\geq$ 10 nM (−)-propranolol was a consistent observation). The plots in (**b**) show Schild plots constructed from similar dose-response curve shifts in at least 3 ganglia for each antagonist. The dose-ratios (DR) were calculated from the shifts measured at a response level corresponding to about 30% of the maximal depolarization, to minimize the effect of the flattening at high (−)-propranolol concentrations. Each point represents a single measurement. Lines are least-squares regressions.

tent (Kuba, Kato, Kumamoto, Koketsu & Hirai, 1981).

In previous experiments on isolated superior cervical ganglia of the rat, in which the acetylcholine stores were radiolabelled with [^{3}H]-choline, adrenaline was observed to reduce the amount of [^{3}H]-acetylcholine released by preganglionic nerve stimulation (Caulfield, 1978; Brown & Caulfield, 1981). Under the same experimental conditions, 1 Hz supramaximal stimulation in normal Krebs solution containing 50 μM neostigmine, isoprenaline (0.1 μM) had no discernible effect on either tritium overflow or compound postganglionic action potential. However, 50 μM neostigmine itself depolarized the ganglion and occluded the ganglionic depolarization produced by isoprenaline. When the neostigmine concentration was reduced to 5 μM, and external Ca^{2+} reduced to 1.25 mM in the presence of 11.2 mM Mg^{2+} (to limit presynaptic Ca^{2+}-availability, as described above), isoprenaline increased the amplitude of evoked action potentials and produced a consistent increase of about 20% in the overflow of tritium (Figure 8).

Discussion

The principal conclusions from these experiments are that 'excitatory' β-receptors can be detected in rat superior cervical ganglia *in vitro* and that these resemble more closely the subclass of receptors previously designated as 'β_2' than 'β_1' receptors (cf. Lands *et al.*, 1967; Furchgott, 1972).

Four manifestations of this 'excitation' have been detected: ganglion depolarization, presynaptic depolarization, facilitation of submaximal transmission and enhanced acetylcholine release. The ganglion

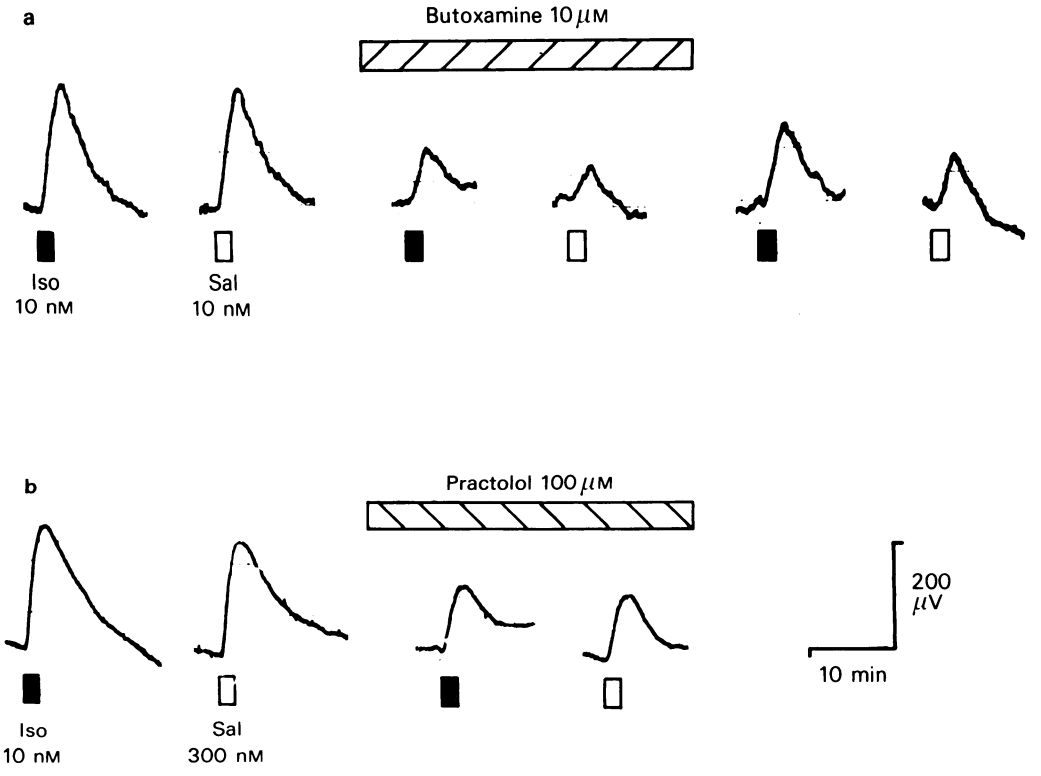

Figure 6 Effects of (**a**) butoxamine (10 μM) and (**b**) practolol (100 μM) on matched depolarization produced by isoprenaline (Iso) and salbutamol (Sal). (Two experiments). The ganglion in (**a**) showed an atypically-high sensitivity to salbutamol.

depolarization was small (< 0.4 mV) and probably corresponds to an average cell depolarization of < 4 mV, since slow changes in transmembrane potential are attenuated some 5–10 times by the low resistance extracellular path through the grease-sealed partition: for example, maximal extracellularly-recorded responses to carbachol are usually 5–7 mV, as opposed to some 40 mV recorded intracellularly (see Figure 6 in Brown, 1980). In fact, no catecholamine-induced depolarizations have previously been observed by intracellular recording in rat superior cervical ganglia *in vitro* (Suzuki & Volle, 1978; Horn & McAfee, 1980), but Christ & Nishi (1971a) reported a 5–10 mV depolarization of some neurones in rabbit isolated superior cervical ganglia following applications of a high (1 mM) concentration of adrenaline. By analogy with the depressant action of α-agonists (cf. Christ & Nishi, 1971a), it seems probably that the facilitation of submaximal transmission produced by β-agonists stems primarily from the augmented transmitter release observed under the same experimental conditions, rather than from the small postganglionic depolarization. The relationship between the presynaptic depolarization and the increased transmitter release is unclear since, in other systems, presynaptic depolarization is usually

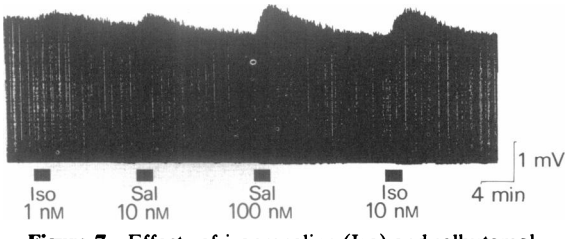

Figure 7 Effects of isoprenaline (Iso) and salbutamol (Sal) on the amplitude of the compound postganglionic action potential recorded during 0.2 Hz stimulation of the preganglionic trunk with 'supramaximal' stimuli. The ganglion was perfused with Krebs solution containing 1.25 mM Ca^{2+} and 11.2 mM Mg^{2+}, to induce a constant state of 'submaximal' transmission (the action potential amplitude being reduced to less than half that recorded in normal Krebs solution). The amplitude of the action potential was 'held' by a peak-height detector (Courtice, 1977; cf. Brown & Marsh, 1978) before being displayed on the chart-recorder.

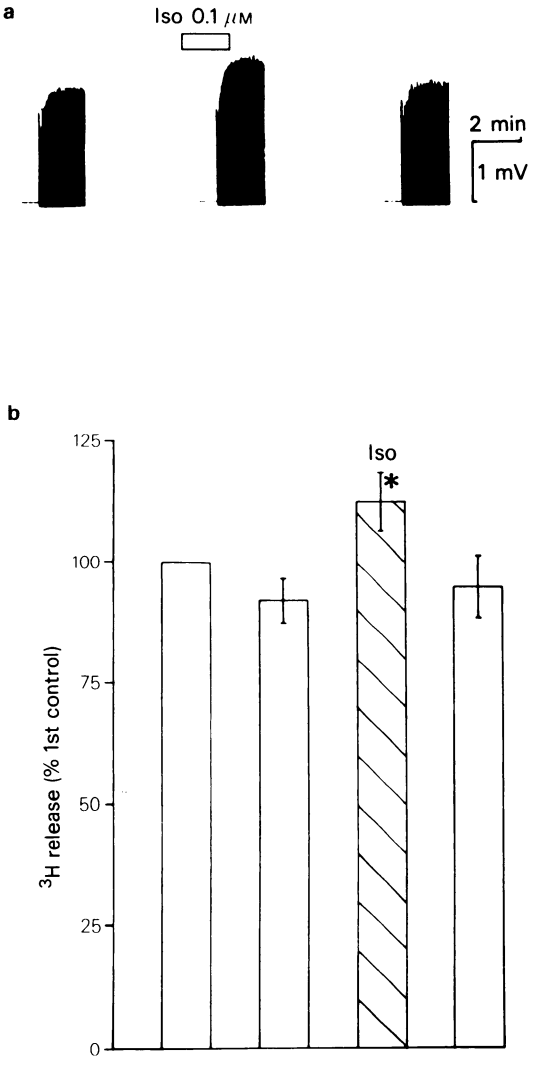

Figure 8 Effect of isoprenaline (Iso, 0.1 μM) on (**a**) ganglionic transmission and (**b**) [^{3}H]-acetylcholine release following 2 min trains of orthodromic stimulation at 1 Hz. The ganglia were perfused with Krebs solution containing 1.25 mM Ca^{2+}, 11.2 mM Mg^{2+} and 5 μM neostigmine. The records in (**a**) show peak ganglionic action potential amplitude (cf. Figure 7) recorded from a single ganglion before, during and after application of isoprenaline for 2 min (at bar). The histograms in (**b**) show the extra amount of tritium released by the preganglionic stimuli after subtraction of resting release, expressed as a percentage of that released by the first period of stimulation (see Methods). The hatched column represents release in the presence of 0.1 μM isoprenaline. *Significantly different from the preceding release peak ($P < 0.001$, by Student's t test).

associated with a reduced release of transmitter, rather than an increased release (Hubbard, Schmidt & Willis, 1967; Gage, 1967; Kusano, Livengood & Werman, 1967; Nicoll & Alger, 1979). No presynaptic depolarization by adrenaline could be detected in rabbit superior cervical ganglia (Christ & Nishi, 1971b).

Pharmacological information on these 'excitatory' β-receptors is most complete regarding the ganglion depolarization. Evidence that this results from β_2-stimulation may be summarized as follows. (i) The order of agonist potency was isoprenaline > salbutamol > adrenaline > noradrenaline. (ii) Salbutamol was about 35 times less potent than isoprenaline. (iii) Responses were readily blocked by propranolol (pA_2 8.94), butoxamine (pA_2 7.36) but weakly by practolol (pA_2 5.14). These observations accord broadly with those expected for β_2-receptors but differ radically from β_1-effects (see Daly & Levy, 1979). Although information is less complete, both the presynaptic depolarization and facilitation of transmission probably involve the same type of receptor since their sensitivities to isoprenaline and salbutamol were similar to that of the postganglionic response.

Interestingly, the β-autoreceptors at the terminals of sympathetic nerve fibres also appear to conform to the β_2-subtype (Stjarne & Brundin, 1976; Dahlof, Ljung & Bengt, 1978; Majewski, Rand & Tung, 1981; Dahlof, 1982). This implies that the same type of β-receptor is present at both proximal and distal ends of the sympathetic fibres, an analogous situation to that for sympathetic α-receptors (cf. Brown & Caulfield, 1979). This may be helpful in future investigations into the mechanism of β-autoreceptor function.

P.M.D. was in receipt of an S.E.R.C. CASE studentship, in collaboration with Pfizer (U.K.) Ltd. This research was also aided in part by a grant from the Medical Research Council. We thank I.C.I., Allen & Hanbury and Burroughs Wellcome for gifts of drugs.

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(Received October 18, 1982. Revised January 31, 1983.)