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Modulation of transmission in rat sympathetic ganglia by activation of presynaptic α- and β-adrenoceptors

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1 Conditions under which transmission in rat isolated superior cervical ganglia may be affected by activation of presynaptic α- and β-adrenoceptors have been investigated by means of an extracellular recording method.

2 Clonidine caused a small hyperpolarization of the ganglia (mean EC_{50} ~ 2 nM) in unstimulated preparations; with continuous preganglionic stimulation at 0.2 Hz, clonidine markedly decreased the height of the compound action potential (mean EC_{50} ~ 18 nM).

3 Phentolamine (0.1–3 μM) *per se* increased the height of the compound action potential by up to 15%, and antagonized the inhibitory effects of adrenaline and clonidine.

4 Using a higher frequency of stimulation (0.5 Hz), the effect of phentolamine (1 μM) was unchanged, whereas the inhibitory effectiveness of adrenaline on the height of the compound action potential was reduced.

5 (±)-Propranolol (0.1 μM) did not affect the height of the compound action potential, whereas the inhibitory effects of high concentrations of adrenaline were enhanced.

6 During an infusion of clonidine (1 μM), adrenaline (1–100 μM) and, less effectively, noradrenaline (10–100 μM) increased the height of the compound action potential by up to 14%; these effects were antagonized by propranolol (0.1 μM).

7 In the presence of noradrenaline (10 and 30 μM) adrenaline (100 μM) caused a small (up to 5%) enhancement of the height of the compound action potential.

8 The results obtained are consistent with the existence of presynaptic α- and β-adrenoceptors on preganglionic terminals. The α-adrenoceptor may be part of a trans-synaptic inhibitory feedback mechanism; however the functional role of the facilitatory β-adrenoceptor is not clear.

Introduction

Catecholamines have been reported to cause either facilitation or depression of transmission in sympathetic ganglia; these effects appear to be mediated by β- and α-adrenoceptors, respectively (reviewed by De Groat & Volle, 1966; Haefely, 1969; Kosterlitz & Lees, 1972; Volle, 1980; Brown & Caulfield, 1981). Depression is more frequently observed (especially *in vitro*), and appears to be unequivocally presynaptic in origin in rabbit, guinea-pig and rat superior cervical ganglia (see Brown & Caulfield, 1981). Recently, Kuba, Kato, Kumamoto, Koketsu & Hirai (1981) demonstrated a direct facilitatory effect of adrenaline and dibutyryl cyclic AMP on transmitter acetycholine release in bullfrog sympathetic ganglia which may involve a β-adrenoceptor.

In view of these findings, the purpose of the present study was to investigate the possibility that negative (α-adrenoceptor-mediated) and positive (β-adrenoceptor-mediated) feedback mechanisms might modulate the release of transmitter acetylcholine from preganglionic terminals in mammalian sympathetic ganglia; such mechanisms have been shown to exist at peripheral noradrenergic and cholinergic neuroeffector sites (for reviews, see Starke, 1977; Rand, McCulloch & Story, 1980; Langer, 1981). Some of the results have been presented to the British Pharmacological Society (Brown & Medgett, 1982).

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Methods

Superior cervical ganglia were excised from Wistar rats of either sex (200–350 g) under light anaesthesia (1.5 g/kg urethane, i.p.) and their connective tissue sheaths removed.

Electrical recording

Ganglia were mounted in a three-chambered bath as described by Brown & Marsh (1978). The chambers were insulated from one another with grease-sealed partitions and were filled with Krebs solution (previously equilibrated with 95% O_2 and 5% CO_2). The middle chamber, which contained the ganglion body, was perfused at a rate of 3 ml/min with oxygenated Krebs solution at room temperature (22–25°C). Drugs were added to the perfusion solution in their final concentration as required. The outer chambers contained the pre- and postganglionic nerve trunks. In most experiments the preganglionic nerves were stimulated using simple shielded bipolar platinum electrodes. D.c potential changes (unstimulated preparations), and compound action potentials (evoked by preganglionic nerve stimulation with 0.3 ms pulses at supramaximal voltage) were recorded differentially between the ganglion body and the postganglionic trunk, using Ag/AgCl electrodes (Brown & Caulfield, 1978; 1979), and monitored with a Tektronix storage oscilloscope; d.c. potential changes were displayed on a potentiometric chart recorder (Bryans 28–000).

In order to have a slow time-base permanent recording of the amplitude of compound action potentials, a peak height detector (Courtice, 1977) was used, which operated by sampling the input during a short variable period initiated by a trigger pulse (synchronized with the stimulus) from the stimulator: a built-in variable delay allowed separation of the sample period and the stimulus artefact. During the sample period, the detector recorded the highest voltage deflection (i.e. the spike height) occurring, and this was then displayed on the chart recorder, the sample and hold time being long enough to allow the recorder to respond without any selective attenuation of the signal. The peak height detector also provided a direct output of the recorded signal (after amplification) which was displayed on the oscilloscope. Comparison of photographs of oscilloscope screen compound action potentials, and recorder peak heights showed no differences in amplitude irrespective of the height or shape of the compound action potentials.

In the experiments in which compound action potentials were elicited by preganglionic stimulation, it was considered essential that the 'control' level of the amplitude of the compound action potentials remained constant during the period when drugs were to be applied. Thus the total duration of any one experiment was limited to 2 to 3 h, which included an initial period of approximately 30 min to allow for stabilization of peak heights.

Drugs and solutions

All drugs were dissolved in Krebs solution (containing 1 mM ascorbic acid to suppress oxidation of catecholamines) at the required concentrations. The following drugs were used: (−)-adrenaline bitartrate (Sigma); clonidine hydrochloride (Boehringer Ingelheim); (−)-noradrenaline bitartrate (Sigma); phentolamine mesylate (Ciba); and (±)-propranolol hydrochloride (ICI).

The composition of the Krebs solution used was (mM): 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 mM.

Results

Hyperpolarizing effect of clonidine

In agreement with previous observations (Brown & Caulfield, 1979), clonidine caused a low-amplitude ganglion hyperpolarization which was antagonized by phentolamine (1 μM). The concentration-effect curve (Figures 1a and c) lies between 0.1 nM and 1 μM. The EC_{50} value for clonidine's hyperpolarizing effect was estimated as 2 nM.

Effect of clonidine on the compound action potential

Figures 1b and c indicate that clonidine (1 nM–1 μM) also caused a marked, concentration-dependent decrease in the height of the compound action potential with stimulation at a frequency of 0.2 Hz.

In Figure 1b, responses to clonidine (1 nM and 0.1 μM) and adrenaline (1 μM), which also inhibited the compound action potential, are shown. Even at a concentration of 100 μM, adrenaline never totally abolished transmission (see also Brown & Caulfield, 1978), the maximum effect being a $23 \pm 1\%$ ($n = 30$) reduction in the peak height. The inhibitory effect of a supramaximal concentration of clonidine (1 μM; see Figure 1c) was $19 \pm 3\%$ ($n = 10$), which is not significantly different from the maximum effect of adrenaline.

From Figure 1b it can be seen that after a concentration of clonidine (0.1 μM) which produces almost the maximum level of inhibition for the experiment, the effect of adrenaline (1 μM; applied during the period of recovery from the clonidine-induced inhibition) is to inhibit further the compound action po-

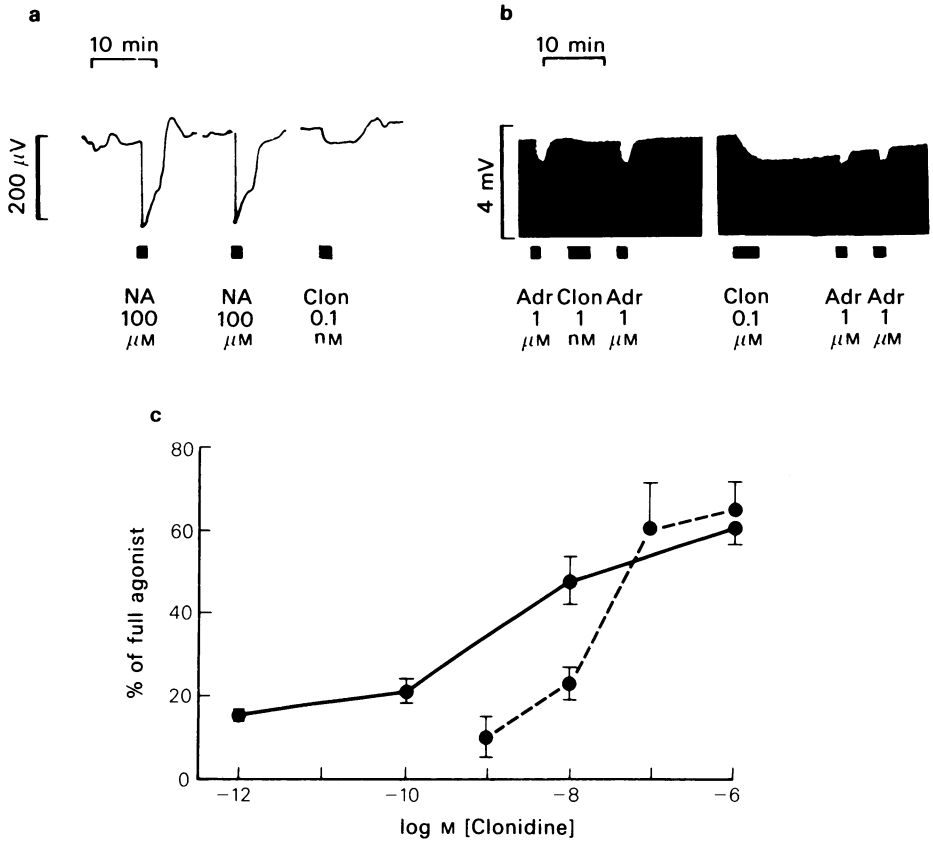

Figure 1 (a) Hyperpolarizations of the superior cervical ganglion of the rat produced by 2-min applications (solid bars) of either noradrenaline (NA) or clonidine (Clon). (b) Effects of adrenaline (Adr) and clonidine on the compound action potentials elicited by single supramaximal preganglionic stimuli at 0.2 Hz. The experimental record shows the magnitude of the compound action potentials: individual spikes cannot be distinguished due to the contracted time-scale, however the inhibitory effects of clonidine and adrenaline are clearly observed. Clonidine was perfused until the maximum effect had developed; adrenaline was applied for 2 min as in (a). (c) Concentration-response curves for the effects of clonidine in producing hyperpolarizations in unstimulated preparations (solid lines) and in inhibiting the compound action potentials elicited by 0.2 Hz stimulation (broken lines). Responses were expressed as % of the hyperpolarizations produced by noradrenaline (100 μM), and the spike inhibitions produced by adrenaline (100 μM), respectively, in individual experiments. Each point represents the mean of 4 to 10 observations.

tential up to the maximum level obtainable in the experiment; i.e. there is apparently no antagonism of the effect of a submaximal concentration of adrenaline by a near-maximal concentration of clonidine. From Figure 1c, the EC_{50} value for clonidine's inhibitory effect on the compound action potential was estimated as 18 nM.

Thus there seems to be a clear difference in potency of about 10 fold between the hyperpolarizing effect of clonidine in unstimulated preparations, and its effect in causing inhibition of the compound action potentials evoked by continuous preganglionic nerve stimulation at 0.2 Hz.

Effects of adrenaline

Like clonidine, adrenaline (10 nM–100 μM) reduced the height of the compound action potential in a concentration-dependent manner as previously reported (Figure 2a; see also Brown & Caulfield, 1978; 1981). The effects of adrenaline (10 nM, 1 μM and 100 μM) were also assessed at a higher frequency of stimulation (0.5 Hz; Figure 2b). The threshold concentration was unchanged, whereas the inhibitory effects of 1 and 100 μM adrenaline were significantly ($P<0.05$) less than at 0.2 Hz.

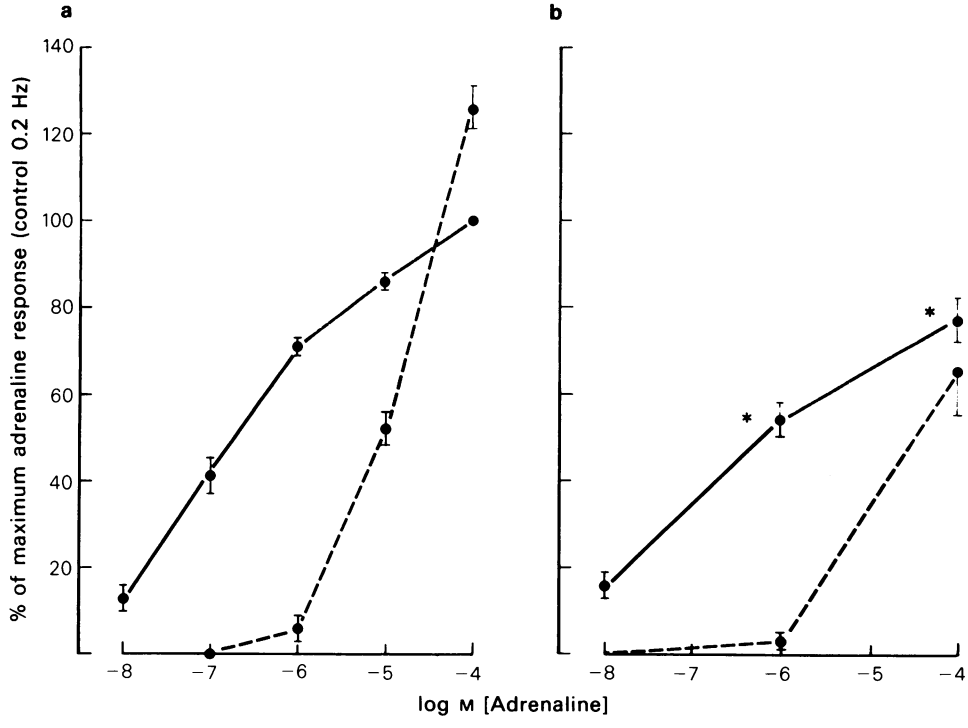

Figure 2 Concentration-response curves for the effects of adrenaline in the absence (solid lines) or in the presence of phentolamine (1 μM; broken lines) on the compound action potentials elicited by preganglionic stimulation at frequencies of either 0.2 (a) or 0.5 Hz (b). Responses were expressed as % of the maximum inhibitory effect of adrenaline (100 μM) at 0.2 Hz, in individual experiments. Each point represents the mean of 6 to 30 observations. *Significant differences from the corresponding effects at 0.2 Hz: $P<0.05$, unpaired t test.

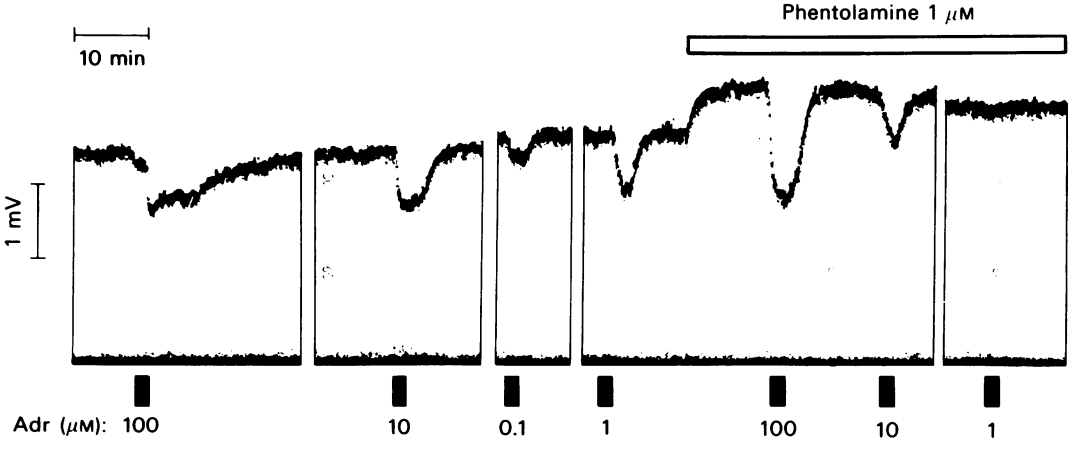

Figure 3 Effects of adrenaline (Adr) and phentolamine on compound action potentials elicited at a frequency of 0.2 Hz. Note that phentolamine increases the spike height, and that the effects of 1 and 10 μM adrenaline are reduced (cf. Figure 2a).

Effects of phentolamine

Phentolamine (1 μM) appeared to antagonize the effects of adrenaline at 0.5 Hz to a similar extent to those at 0.2 Hz (compare Figures 2a and b). Phentolamine (1 μM) also antagonized the inhibitory effect of clonidine (1 μM): in control experiments at 0.2 Hz, the inhibitory effect of clonidine (1 μM) was 72±7% ($n=10$) and that of adrenaline (1 μM) 71±2% ($n=34$) of the maximum response to adrenaline (100 μM); after phentolamine (1 μM) these effects were significantly reduced to 11±1% ($n=4$) and 6±3% ($n=6$, Figure 2a) respectively.

In addition to antagonizing the inhibitory effects of adrenaline and clonidine, phentolamine (0.1–3 μM) itself caused an *increase* in the height of the compound action potential using stimulation at 0.2 Hz. Figure 3 shows the effect of phentolamine (1 μM) in a typical experiment; subsequent responses to adrenaline (10 μM or less) are inhibited. Increases in peak heights caused by 0.1, 1 and 3 μM amounted to respectively, 8±3% ($n=3$), 10±1% ($n=12$) and 15±5% ($n=3$) of control values. The effect of 3 μM phentolamine, unlike those of the lower concentrations, was not maintained and the height of the compound action potential tended to return to pre-phentolamine levels despite the continued presence of the drug. The effect of phentolamine (1 μM) was unchanged when the frequency of stimulation was increased to 0.5 Hz (increase of 9±1%, $n=6$). Concentrations of phentolamine above 10 μM caused marked depression of the compound action potential, probably due to a non-specific effect.

Effects of propranolol

In view of the reported β-adrenoceptor-mediated facilitatory effects on ganglionic transmission (see Introduction for references), the effects of adrenaline were reassessed in the presence of propranolol (0.1 μM) to block any underlying component of β-adrenoceptor activation in the observed α-adrenoceptor-mediated inhibition of transmission by adrenaline.

As illustrated in Figure 4a, after the addition of propranolol (0.1 μM), the inhibitory effects of high concentrations of adrenaline (10 and 100 μM) are enhanced; however, propranolol itself did not significantly affect the height of the compound action potential.

The curves illustrated in Figure 4b were obtained from the data of 6 experiments of the type shown in Figure 4a; the enhancement of the inhibitory effect of adrenaline becomes statistically significant at 100 μM adrenaline. However, as shown in Figure 4a, propranolol was only present for 10 min before responses to adrenaline were again elicited; it might be that

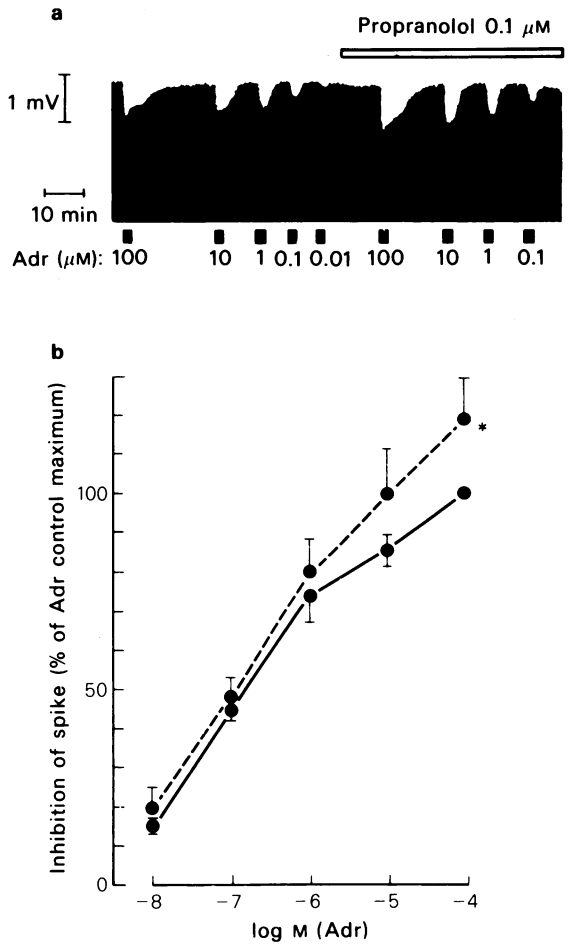

Figure 4 (a) Effects of adrenaline (Adr) and propranolol on compound action potentials elicited at a frequency of 0.2 Hz. Note that propranolol does not itself affect the spike height, but that the effects of 10 and 100 μM adrenaline are increased. (b) Concentration-response curves for the effects of adrenaline in the absence (solid line) or in the presence of propranolol (0.1 μM; broken line). Each point represents the mean of 6 observations. The control curve is obtained from the set of control responses to adrenaline immediately before propranolol was applied; the curve in the presence of propranolol is obtained from the set of responses immediately after. *Significant differences from the control value: ($P<0.05$, paired t test).

insufficient contact time with propranolol was allowed for the maximum effect to be obtained. Due to the limitation on the time for experimentation (see Methods), a longer incubation period with propranolol was not practicable; thus another series of experiments was performed in which propranolol (0.1 μM) was present in the Krebs solution from the

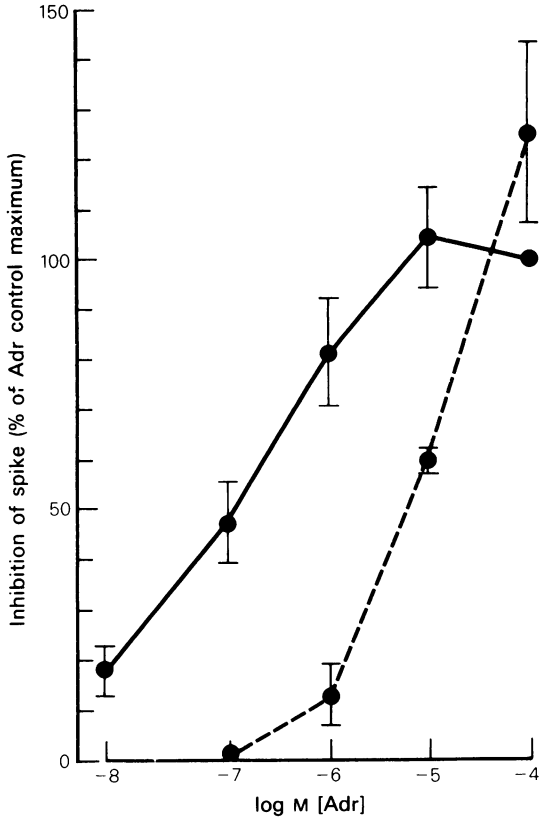

Figure 5 Concentration-response curves for the effects of adrenaline on compound action potentials elicited at a frequency of 0.2 Hz, in the absence (solid line) or in the presence of phentolamine (1 μM; broken line). Each point represents the mean of six observations. Propranolol (0.1 μM) was present throughout the experiments. Responses were expressed as a % of the maximum inhibitory effect of adrenaline (100 μM) in individual experiments.

start of the experiment. Full sets of responses to adrenaline were obtained as in previous experiments; after responses were constant the effect of phentolamine (1 μM) was assessed. The data from 6 experiments are shown in Figure 5.

Since, from the data given in Figure 4, the effect of propranolol is modest, the variation in the sensitivity of individual preparations to adrenaline precludes a comparison between the magnitude of responses obtained in experiments performed entirely in the presence of propranolol and those in its absence. However, comparing Figure 2 with Figure 5 it is apparent that the *shapes* of the adrenaline concentration-response curves are different. In Figure 5 the control concentration-response curve is steeper, more clearly sigmoidal and there is a clear plateau, in contrast to Figure 2a. The shift to the right produced by phentolamine (1 μM) is similar in both cases, but is somewhat closer to parallelism with the control curve in Figure 5. Thus an EC_{50} value of 0.2 μM can be obtained for the inhibitory α-adrenoceptor-mediated effect of adrenaline with stimulation at 0.2 Hz.

The effect of phentolamine (1 μM) *per se* of increasing the height of the compound action potential was unchanged in the presence of propranolol.

Effects of adrenaline and noradrenaline in the presence of clonidine

The experiments with propranolol suggested that, at least with the higher concentrations of adrenaline, there might be a component in the overall response resulting from β-adrenoceptor activation. In order to try and demonstrate direct, β-adrenoceptor-mediated, facilitation, the effects of adrenaline and noradrenaline were re-examined in the presence of clonidine (1 μM): it seemed possible that with near maximal α-adrenoceptor occupation and markedly reduced peak height, β-adrenoceptor-mediated facilitatory effects of adrenaline and noradrenaline might be most readily observed.

Figure 6 shows that, in the presence of clonidine (1 μM), adrenaline (1–100 μM) causes concentration-dependent biphasic effects on the height of the compound action potential. An initial further inhibitory effect is succeeded, towards the end of the 2 min perfusion period with adrenaline, by a more pronounced facilitatory effect which persists (in the case of 100 μM adrenaline) for several minutes after the termination of the adrenaline perfusion. After propranolol (0.1 μM), the initial inhibitory phase is enhanced, whereas the subsequent facilitatory effects of 1 and 10 μM adrenaline are antagonized. Higher concentrations of propranolol caused depression of the compound action potential and thus could not be used; at these concentrations propranolol is known to possess local anaesthetic activity.

Figure 7a shows the effects in the form of a graph: propranolol (0.1 μM) significantly reduces the facilitatory effects of 1 and 10, but not 100 μM adrenaline. Figure 7b shows that noradrenaline produces similar responses to those of adrenaline, but less effectively, the threshold concentration being around 10 μM. The maximum effects of adrenaline and noradrenaline at 100 μM were similar, the height of the compound action potential being increased by $14 \pm 3\%$ ($n = 5$) and $14 \pm 2\%$ ($n = 6$), respectively.

These observations of a direct, β-adrenoceptor-mediated facilitatory action of adrenaline on the compound action potential may explain the anomalous effects illustrated in Figures 2(a), 3 and 5, in which it can be clearly seen that after phentolamine (1 μM) the inhibitory effect of adrenaline (100 μM) is

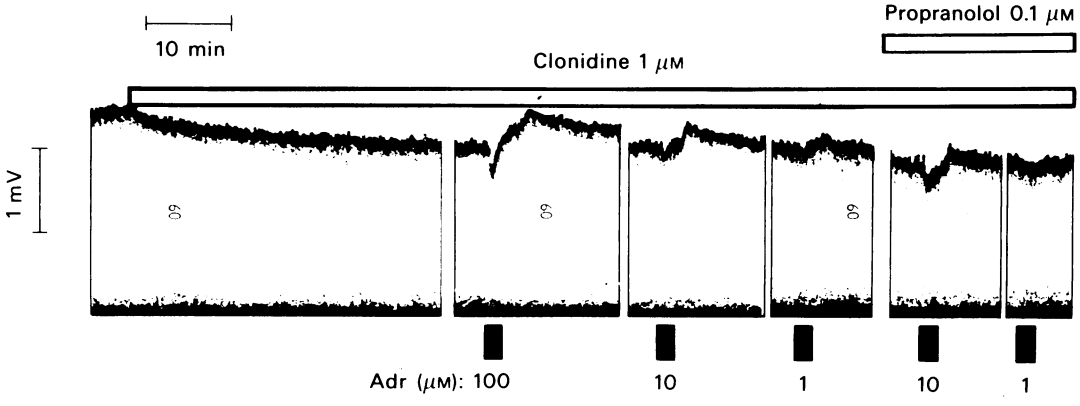

Figure 6 Effects of adrenaline (Adr) on compound action potentials elicited by stimulation at a frequency of 0.2 Hz, in the presence of clonidine alone, or clonidine and propranolol. Note that propranolol reduces the facilitatory effect of 10 μM adrenaline and abolishes the facilitatory effect of 1 μM adrenaline. The facilitatory effect of 100 μM adrenaline in the presence of clonidine was unchanged after propranolol (not shown).

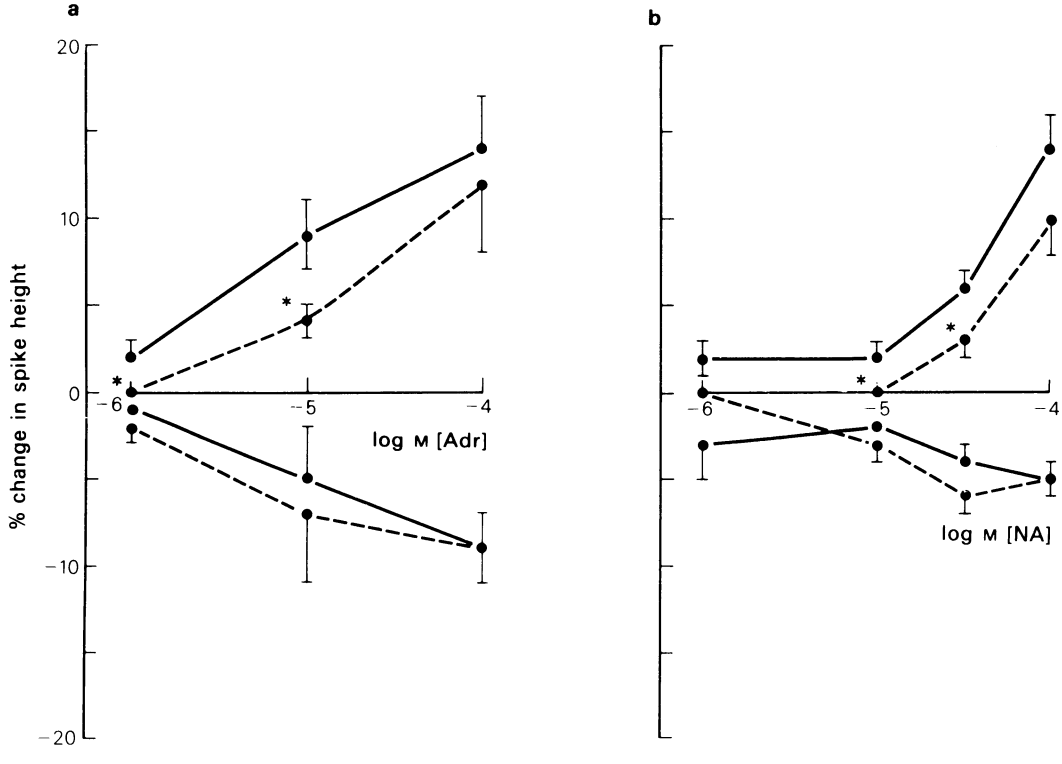

Figure 7 Concentration-response curves for the effects of adrenaline (a) and noradrenaline (b) on compound action potentials elicited by 0.2 Hz stimulation, in the presence of clonidine (1 μM; solid lines) or clonidine (1 μM) plus propranolol (0.1 μM; broken lines). Data for the initial inhibitory phase (% decrease in spike height) as well as the subsequent facilitatory phase (% increase in spike height) are given (cf. text and Figure 6). Each point represents the mean of 6 observations. *Significant decreases compared to control values: ($P<0.05$, paired t test).

significantly *enhanced* (rather than inhibited, as with lower concentrations of adrenaline). From Figure 4 it was seen that propranolol enhanced the inhibitory effect of 100 μM adrenaline. In the absence of other drugs, the inhibitory effect of high (10–100 μM) concentrations of adrenaline plateaus, and begins to reverse, before the end of the 2 min perfusion period. This failure to maintain the maximum effect may be attributed to the facilitatory β-adrenoceptor-mediated component whose onset is clearly *later* than the α-adrenoceptor-mediated inhibition (Figure 6). In the presence of phentolamine (1 μM), a surmountable antagonist, the response to adrenaline (100 μM) is not reduced, however the time taken for it to develop is increased (due to the contracted time scale this is not clearly seen in Figure 3). Thus the maximum α-adrenoceptor-mediated inhibition may now be *coincident* with the β-adrenoceptor-mediated facilitatory phase such that the latter component does not limit the inhibitory effect as it did in the absence of phentolamine.

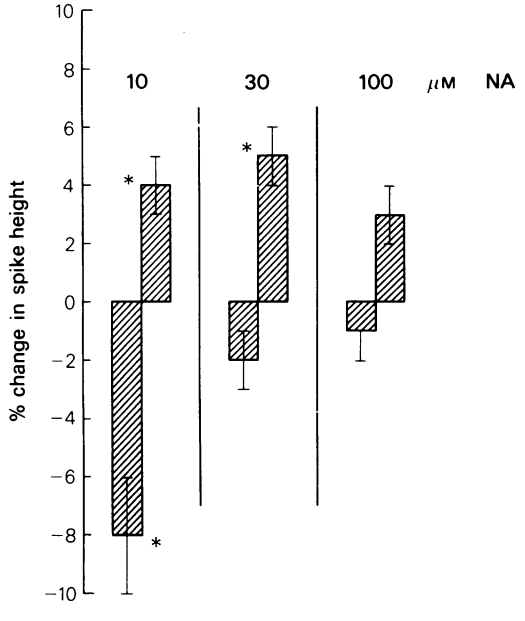

Figure 8 Effects of adrenaline (100 μM; columns) on compound action potentials elicited at a frequency of 0.2 Hz, in the presence of 10, 30 or 100 μM noradrenaline (NA). These concentrations of NA produced spike inhibitions amounting to respectively, 12 ± 2% ($n = 5$), 17 ± 3% ($n = 5$) and 21 ± 5% ($n = 4$). Data for the initial inhibitory phase (% decrease in spike height) as well as the subsequent facilitatory phase (% increase in spike height) of the response to 2 min applications of adrenaline (100 μM; cf. Figure 6) are given. Each column represents the mean of 4 to 5 observations. *Significant changes in spike height: ($P<0.05$, paired t test).

Since adrenaline appeared to be more effective than noradrenaline in producing a facilitatory effect, it seemed possible that adrenaline might also produce an increase in peak height in the presence of appropriate concentrations of noradrenaline. Figure 8 shows that adrenaline (100 μM) causes small but significant increases in the height of the compound action potential in the presence of 10 or 30 μM noradrenaline but not 100 μM noradrenaline. The maximum increase, in the presence of 30 μM, amounted to 5 ± 1% ($n = 5$). The initial inhibitory phase of the adrenaline response is seen only in the presence of 10 μM noradrenaline; higher concentrations of noradrenaline probably produce maximal α-adrenoceptor activation.

Discussion

Several observations of the present study are consistent with the existence of a trans-synaptic α-adrenoceptor-mediated negative feedback mechanism for release of acetylcholine from preganglionic terminals in mammalian sympathetic ganglia. Such a mechanism has been proposed by other workers (Noon, McAfee & Roth, 1975; Noon & Roth, 1975; Martinez & Adler-Graschinsky, 1980a, b).

In the present study, phentolamine enchanced the height of the compound action potential in concentrations which cause selective and potent effects on α-adrenoceptors, and which have been shown to cause marked enhancement of stimulation-induced noradrenaline release from peripheral noradrenergic terminals by disrupting α-adrenoceptor-mediated inhibitory feedback loops (for references, see Starke, 1977; Langer, 1981). Clonidine appeared to be more potent in causing hyperpolarization of the ganglia in experiments in which the preganglionic nerves were not stimulated, than in inhibiting the compound action potentials evoked by continuous preganglionic stimulation at 0.2 Hz. Adrenaline appeared to be more effective in inhibiting the compound action potentials evoked by 0.2 Hz stimulation than at the higher frequency of 0.5 Hz.

It can be assumed that the effects of adrenaline and clonidine on transmission are *presynaptic in origin*, i.e. they cause inhibition of release of transmitter acetylcholine. Thus, transmission failure in rabbit and guinea-pig ganglia can occur without postsynaptic hyperpolarization, and without any reduction in postsynaptic excitability or in the response to iontophoretic acetylcholine (Christ & Nishi, 1971; Dun & Nishi, 1974; Dun & Karczmar, 1977): there is depression of the excitatory postsynaptic potential and a fall in its quantal content. In rat superior cervical ganglia, adrenaline reduces the stimulus-evoked release of [^{3}H]-acetylcholine, and the height

of the compound action potential, in spite of an almost negligible ganglionic hyperpolarization (Brown & Caulfield, 1981).

The single most attractive explanation for the effects of phentolamine, clonidine and adrenaline which were obtained in the present study, is that preganglionic nerve stimulation in rat superior cervical ganglia releases catecholamines, which in turn activate α-adrenoceptors on cholinergic terminals resulting in decreased transmitter release. Thus if these receptors are occupied to some extent at 0.2 Hz stimulation, clonidine might be expected to be less effective than in the absence of any pre-existing receptor activation. The latter situation applies in the case of the postsynaptic α-adrenoceptor-mediated hyperpolarization produced by clonidine. Similarly if receptor activation by endogenous catecholamines is increased by increasing the stimulation frequency, exogenously applied adrenaline would be expected to appear less effective, as was observed. The effect of phentolamine in disrupting the feedback loop and increasing transmitter release is thus manifested as an increase in the height of the compound action potential. The observation that phentolamine did not produce a greater enhancement of the peak height at 0.5 Hz, as might have been predicted from the reduced effectiveness of adrenaline, may indicate that with stimulation at 0.2 and 0.5 Hz, the height of the compound action potential is already near-maximal. That this might be the case is suggested by the finding that increasing the frequency of stimulation from 0.2 to 0.5 Hz had no significant effect on the height of the compound action potential, whereas with higher frequencies of stimulation (>1 Hz) the peak height began to decline (unpublished observations).

It has been previously suggested (Brown & Caulfield, 1978, 1979) that the α-adrenoceptor mediating both the postsynaptic (hyperpolarization) and presynaptic (reduction in transmitter release) effects of catecholamines is of the α_2-type. The high potency of clonidine obtained in the present study for both effects (activity in the nanomolar range) supports this suggestion; in the periphery, at α_1-adrenoceptors, clonidine appears as a weak partial agonist (activity in the micromolar range) whereas at α_2-adrenoceptors potency and intrinsic activity are considerably higher (Medgett, McCulloch & Rand, 1978; Starke & Docherty, 1980).

From a study carried out in rabbit superior cervical ganglia, Noon *et al.* (1975) proposed noradrenergic nerve terminals within the ganglia as the source of the catecholamines activating the postulated feedback mechanism. In a later study using cat superior cervical ganglia, Martinez & Adler-Graschinsky (1980a) proposed that, in addition to the noradrenergic terminals, the dendrites of the postganglionic neurones were likely to be the sites from which noradrenaline is released by preganglionic stimulation.

Steinberg & Keller (1978) however, in contrast to the results obtained in other studies, reported that preganglionic stimulation failed to release catecholamines from superior cervical ganglia of the rat, the species used in the present study. Methodological differences may explain the discrepancy: Noon *et al.* (1975) showed that rabbit ganglia had an extremely active catechol-*O*-methyltransferase and pointed out that, because only small amounts of radiolabelled catecholamines were released on preganglionic stimulation, it was necessary to inhibit their catabolism as much as possible. In the study of Steinberg & Keller (1978) however, catechol-*O*-methyltransferase inhibitors were not used, and the bathing medium which was collected after stimulation of radiolabelled ganglia was not assayed for non-catechol metabolites of noradrenaline.

It has recently been reported (Bright, Gower & Webb, 1982) that in 48-h cultured rat superior cervical ganglia, an *in vitro* model for adrenergic neurones, electrical field stimulation causes noradrenaline release which is reduced in low Ca^{2+}–high Mg^{2+} solution or by bretylium and augmented by phenoxybenzamine.

Thus, from the literature it would appear that the necessary elements of a trans-synaptic α_2-adrenoceptor-mediated inhibitory feedback mechanism for release of acetylcholine from preganglionic terminals are present; the results of the present study suggest that, under the stimulation conditions used (0.2 and 0.5 Hz continuous preganglionic stimulation with 0.3 ms pulses at supramaximal voltage), the mechanism operates, and that consequently there exists a tonic inhibition of acetylcholine release. On disruption of the feedback loop with phentolamine, ganglionic transmission is enhanced.

The present study does not provide any information regarding the functional significance of presynaptic β-adrenoceptors. However, their presence on cholinergic terminals where they mediate enhancement of transmitter release is strongly indicated, from the results of Kuba *et al.* (1981). These authors obtained biphasic effects of adrenaline and dibutyryl cyclic AMP, (inhibition followed by facilitation) on transmitter release (measured as increases in amplitude and quantal content of fast excitatory postsynaptic potentials and the frequency of miniature excitatory postsynaptic potentials) in bullfrog paravertebral sympathetic ganglia. Essentially compatible effects of adrenaline which were antagonized by propranolol, were obtained in the present study on rat superior cervical ganglia; however, direct β-adrenoceptor-mediated effects of adrenaline and noradrenaline were only seen if the height of the compound action potential was reduced, using

clonidine. Since clonidine is a partial agonist, a greater proportion of the presynaptic α-adrenoceptors should be occupied to cause the same degree of inhibition as with an equieffective concentration of a full agonist such as noradrenaline; the question arises whether total α-adrenoceptor occupancy is a necessary requirement for β-adrenoceptor effects to be manifested.

It was observed that adrenaline (100 μM) also caused a (small) facilitatory effect in the presence of noradrenaline (in concentrations (10 and 30 μM) less effective than adrenaline in producing facilitation in the presence of clonidine). At these concentrations of noradrenaline it can be assumed that only a small percentage of the total α-adrenoceptor population is occupied, which suggests that the β-adrenoceptor-mediated facilitatory effect might also be observed using other procedures which decrease the height of the compound action potential. Thus Kuba *et al.* (1981) observed facilitatory effects of adrenaline in a low Ca^{2+}–high Mg^{2+} solution in bullfrog sympathetic ganglia; and in rat superior cervical ganglia, if the height of the compound action potential is reduced (stimulation at 0.2 Hz) using a low Ca^{2+}–high Mg^{2+} solution, isoprenaline causes a marked facilitatory effect which is blocked by propranolol (P. M. Dunn, unpublished observations). However if submaximal pulse widths and voltages were used (Brown and Caulfield, unpublished observations), facilitatory effects of catecholamines were not observed. This procedure reduces the number of nerve endings which are activated, rather than the amount of transmitter released per nerve impulse (as is the case using clonidine or low Ca^{2+}–high Mg^{2+}), suggesting that this latter parameter is important for the manifestation of β-adrenoceptor-mediated facilitatory effects.

Since propranolol did not itself affect the height of the compound action potential, either in the presence or absence of clonidine, it would appear that with preganglionic stimulation at 0.2 Hz, presynaptic β-adrenoceptors are not tonically activated by endogenous catecholamines; it is thus not clear what, if any, functional significance they might possess. Possibly, as suggested by the observation that adrenaline increases the peak height in the presence of noradrenaline, if the inhibitory presynaptic α-adrenoceptor receptor mechanism is operating, circulating adrenaline (e.g. from the adrenal gland) might be capable of enhancing ganglionic transmission. However, it may be that rather than mediating direct facilitatory effects, activation of presynaptic β-adrenoceptors may function to limit the degree to which ganglionic transmission is inhibited by the action of catecholamines at presynaptic α-adrenoceptors. Clearly it is necessary to examine a wide range of frequencies of preganglionic nerve stimulation, in order to obtain some further information regarding the conditions under which presynaptic receptor mechanisms may exert more substantial effects on ganglionic transmission; the results of the present study merely indicate the existence of such mechanisms.

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