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Microelectrode recording of the effects of agonists and antagonists on α-adrenoceptors on rat somatic nerve terminals

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1 The effects of apomorphine, catechol, clonidine, isoprenaline, (−)-and (±)-noradrenaline, phenylephrine, pyrogallol and xylazine were investigated on the frequency and amplitude of miniature endplate potentials (m.e.p.ps) and, with the exception of apomorphine, catechol and pyrogallol, on the amplitude of endplate potentials (e.p.ps) in the rat phrenic nerve diaphragm preparation.

2 Clonidine, (−)-noradrenaline, phenylephrine and xylazine (each at 1.5×10^{-5} M) increased m.e.p.p. frequency but not amplitude. The other drugs were ineffective, except isoprenaline (1.5×10^{-5} M) which enhanced m.e.p.p. amplitude but not frequency.

3 The increase in m.e.p.p. frequency was inhibited by phentolamine, prazosin and yohimbine (each 1.5×10^{-9} M). Prazosin and yohimbine alone each reduced m.e.p.p. frequency but failed to abolish m.e.p.ps even at high concentrations (10^{-3} M).

4 Clonidine, (−)-noradrenaline, phenylephrine and xylazine (each 3×10^{-6} M) enhanced e.p.p. amplitude; this enhancement was blocked by prazosin and by yohimbine (each 3×10^{-6} M).

5 In preparations fatigued by prolonged continuous nerve stimulation (5 Hz, 0.05 ms for 30 min), (−)-noradrenaline (3.3×10^{-4} M) restored m.e.p.p. frequency.

6 The results indicate that adrenoceptors on somatic nerve terminals interact with both α_1- and α_2-agonists and antagonists and show different characteristics from those at autonomic neuroeffector junctions.

7 The α-adrenoceptors on somatic nerve terminals may have an ancilliary physiological role in influencing but not controlling transmitter release.

Introduction

Somatic nerve terminals (see review by Bowman & Nott, 1969) possess adrenoceptors of the α-type as indicated by their ability to interact with α-agonists. This interaction increases the release of acetylcholine (Kuba, 1970; Kuba & Tomita, 1971) as evidenced by an increase in the frequency of spontaneously occurring miniature endplate potentials (m.e.p.ps).

The question remains as to whether these adrenoceptors on somatic nerve terminals can be accommodated within the α_1, α_2 sub-classification of α-adrenoceptors at autonomic neuroeffectors already proposed (Langer, 1977). Unlike those on somatic nerve terminals, stimulation of prejunctional adrenoceptors at autonomic neuroeffector junctions inhibits transmitter release (Langer, 1977; Starke, 1977). The availability of agonists and antagonists selective for α_1- and α_2-adrenoceptors at autonomic neuroeffector junctions (Borowski, Ehrl & Starke, 1976; Doxey, Smith & Walker, 1977) has enabled this question to be examined. The results indicate that adrenoceptors on somatic nerve terminals have less stringent structural requirements than those at autonomic neuroeffectors. High concentrations of α-agonists are required to activate these receptors and both α_1- and α_2- antagonists are equieffective. These receptors cannot be accommodated within the existing subclassification of α-adrenoceptors at autonomic neuroeffectors.

Methods

Three parameters were used to examine the pharmacological characteristics of α-adrenoceptors on

somatic nerve terminals in the rat. These were the frequency and amplitude of m.e.p.ps and the amplitude of endplate potentials (e.p.ps).

The hemidiaphragm-phrenic nerve preparation was dissected as described by Bülbring (1946) from Wistar male rats (100–200 g) killed by chloroform overdose. The preparation was pinned to the Sylgard base of a perspex bath and constantly perfused at room temperature with Krebs solution of the following composition (mM) which had been gassed with 95% O_2 and 5% CO_2:- NaCl 118.4, $NaHCO_3$ 25.0, NaH_2PO_4 1.13, KCl 4.7, $CaCl_2$ 2.7, $MgCl_2$ 1.3 and glucose 5.5

Intracellular recordings were made using glass capillary microelectrodes (10–30 megaohms resistance) filled with 3 M KCl and connected via chlorided silver wire (9 μm in diameter) to a unity gain follower and preamplifier (Neurolog NL103). Signals were displayed on a storage oscilloscope and u.v. recorder.

Miniature endplate potentials

To ascertain that the rate of discharge remained constant, m.e.p.p. frequency was measured over a 1 h period in the same cell. No significant change in frequency was observed over this period. Accordingly, the following experimental protocol was adopted. M.e.p.p. frequency was recorded for 1 min, throughout the second minute following impalement in untreated preparations and during the eleventh minute following the addition of drugs (see below).

To determine the time taken for drugs to exert their maximum effect, clonidine and (−)-noradrenaline (NA) (each 1.5×10^{-5} M) were added, in separate experiments, to the bath and the frequency of m.e.p.ps recorded for 1 min periods at 5 min intervals for 25 min. The maximum effect of each drug was exerted 10 min after addition and this time was adopted subsequently for all drugs. A dose of agonist was thus added and m.e.p.p. frequency again recorded in the same cell 10 min later. To compare potency among different agonists, equimolar concentrations were chosen. Preliminary experiments confirmed the suitability of a concentration of 1.5×10^{-5} M and this was adopted routinely. The effect of adrenoceptor antagonists on m.e.p.p. frequency was investigated 10 min after their addition to the bath and compared with controls in the same cell.

Endplate potentials

Tubocurarine (1×10^{-6} M) was added to the Krebs solution bathing the preparation to prevent action potentials arising from nerve stimulation. The phrenic nerve was stimulated via Ag/AgCl electrodes (single pulse, supramaximal voltage, 0.05 ms) and the e.p.p. recorded. The effects of adrenoceptor agonists and antagonists were measured on the amplitude, time to peak and half decay time of the first e.p.p. recorded 5 min later in the same cell. This period (5 min) was that found optimal in preliminary experiments to obtain the maximum pharmacological response compatible with maintaining intracellular impalement during nerve stimulation.

Analysis of results

Since no significant variation in their day-to-day frequency or amplitude was observed, control values for m.e.p.p. frequency were pooled to provide a single value for comparison with drug-treated preparations using Student's *t* test. Endplate potential characteristics in the presence and absence of drugs were compared in the same cell using Student's *t* test. Results are given as mean ± s.d..

Drugs

The following drugs were used: apomorphine HCl (Sandoz), catechol (BDH), clonidine hydrochloride (Boehringer), isoprenaline sulphate (Wellcome), (−)-NA bitartrate (Koch-Light), (±)-NA hydrochloride (Sigma), phentolamine mesylate (Ciba), phenylephrine hydrochloride (Sigma), prazosin hydrochloride (Pfizer), pyrogallol (BDH), tubocurarine chloride (Wellcome), xylazine hydrochloride (Boehringer) and yohimbine hydrochloride (Koch-Light).

All drugs were dissolved in saline except (−)-NA, (±)-NA, isoprenaline, phenylephrine and prazosin. With the exception of prazosin, these drugs were dissolved in 0.9% saline containing disodium edetate (EDTA, 10 μg ml^{-1}) and ascorbic acid (50 μg ml^{-1}). Prazosin hydrochloride was dissolved in distilled water and then sonicated. Bath concentrations are referred to in the text.

Results

Resting membrane potential

The resting membrane potential was unaltered by any of the drugs examined with the exception of isoprenaline which significantly increased it from its resting value of −73.4 mV ± 3.0 to −79.8 mV ± 7.0.

Miniature endplate potential frequency

The mean m.e.p.p. frequency (135 min^{-1} ± 22, $n = 58$) agrees with that found by others using the same species (Liley, 1956; Kuba, 1970). Clonidine, (−)-NA, phenylephrine and xylazine each significantly increased m.e.p.p. frequency but not amplitude

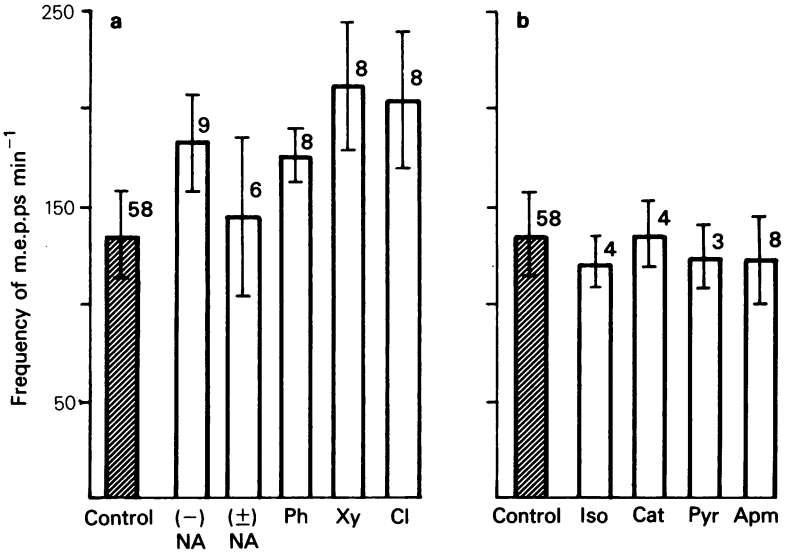

Figure 1 The effects of drugs (each 1.5×10^{-5} M) on m.e.p.p. frequency in the rat phrenic nerve diaphragm preparation. Each column represents the mean and vertical lines the s.d.; the number of observations is indicated above each. (a) Clonidine (Cl), (−)-noradrenaline (NA), phenylephrine (Ph) and xylazine (Xy) increased m.e.p.p. frequency significantly when compared with controls. (b) The β-agonist isoprenaline (Iso) and apomorphine (Apm) which interacts with dopamine receptors failed to alter m.e.p.p. frequency. Catechol (Cat) and pyrogallol (Pyr) were also ineffective in changing m.e.p.p. frequency.

(Figure 1). However the doses needed were high compared with those required for effects on α-adrenoceptors at autonomic neuroeffectors. For example, the concentrations of clonidine required to cause a 50% reduction in the height of the twitch response to field stimulation in the rat vas deferens *in vitro* was 1.76×10^{-9} M and of phenylephrine to produce a contraction in the same preparation was 5×10^{-8} M (Drew, 1977). Clonidine (10^{-11}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-5} M) was ineffective in the present experiments until a concentration of 10^{-5} M was reached. (−)-NA significantly enhanced m.e.p.p. frequency at a concentration of 10^{-6} M. Xylazine and (−)-NA each increased m.e.p.p. frequency significantly compared with controls; xylazine to 213 ± 32 min^{-1} an increase of some 58% and (−)-NA to 190 ± 25 min^{-1} an increase of some 41%. (±)-NA, apomorphine, catechol, pyrogallol and the β-agonist, isoprenaline (each 1.5×10^{-5} M), were ineffective in changing m.e.p.p. frequency (Figure 1b). To compare the ability of each compound to increase m.e.p.p. frequency, equimolar concentrations (1.5×10^{-5} M) were used. There was no statistical difference in potency between clonidine, (−)-NA and xylazine or between (−)-NA and phenylephrine. There was a significant difference between phenylephrine on the one hand and clonidine and xylazine on the other. This may be due to the small deviation in the phenyephrine mean and may not reflect a true biological difference.

The increase in m.e.p.p. frequency produced by the α-adrenoceptor agonists was reduced both by prazosin and by yohimbine (Figure 2). Three concentrations of each antagonist (1.5×10^{-9} M, 3×10^{-7} M and 1.5×10^{-5} M) were used. At each concentration, prazosin and yohimbine significantly antagonized the increase in m.e.p.p. frequency produced by clonidine, (−)-NA, phenylephrine and xylazine. As Figure 2 shows for phenylephrine, there was no significant difference in the potency of either drug at the highest concentration (1.5×10^{-5} M) used. The maximum inhibition of the phenylephrine-induced increase in m.e.p.p. frequency produced by prazosin or yohimbine at 1.5×10^{-5} M was approximately 50%. In a separate series of experiments, phentolamine (1.5×10^{-9}, 3×10^{-7}, 1.5×10^{-5} M) reduced significantly, at each concentration, the increase in m.e.p.p. frequency produced by (−)-noradrenaline (1.5×10^{-5} M). This reduction was about 53% at the highest concentration.

By themselves, prazosin and yohimbine each decreased frequency significantly but failed to abolish m.e.p.ps at the maximum concentration (10^{-3} M) used. Combined, each in a concentration of 10^{-3} M, they reduced (but failed to abolish) m.e.p.p. frequency by 37% (from 135 ± 22 min^{-1} to 85 ± 11 min^{-1}).

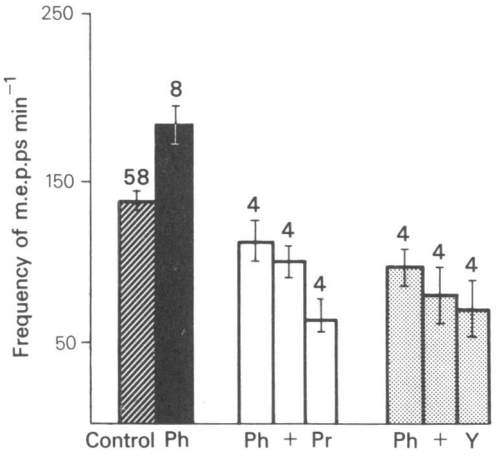

Figure 2 The effect of prazosin (Pr, open columns) and of yohimbine (y, stippled columns) each 1.5×10^{-9}, 3.0×10^{-7} and 1.5×10^{-5} M (left to right) on the increase in m.e.p.p. frequency produced by phenylephrine (Ph 1.5×10^{-5} M, solid column) in the rat phrenic nerve diaphragm preparation. M.e.p.p. frequency in untreated preparations (control) is shown in the hatched column. Prazosin and yohimbine each decreased the enhancement produced by phenylephrine significantly. In equimolar concentrations prazosin and yohimbine were equieffective in this respect. Each column represents the mean of the number of experiments indicated above each column; vertical lines indicate s.d.

Following prolonged electrical stimulation (30 min, 5 Hz, 0.05 ms supramaximal voltage) of the phrenic nerve to fatigue the preparation, m.e.p.p. frequency was reduced significantly (from 135 $\text{min}^{-1} \pm 22$, $n = 58$ to $76 \pm 18\ \text{min}^{-1}$, $n = 4$). Under these circumstances (−)-NA (3.3×10^{-4} M) restored and even enhanced m.e.p.p. frequency to 164 $\text{min}^{-1} \pm 8$ ($n = 4$).

Miniature endplate potential amplitude

Isoprenaline (1.5×10^{-5} M) increased m.e.p.p. amplitude significantly from $0.51\ \text{mV} \pm 0.1$ to $0.8\ \text{mV} \pm 0.1$ without changing frequency. (−)-NA, phenylephrine, xylazine, clonidine, catechol, pyrogallol and apomorphine, each at a concentration of 1.5×10^{-5} M, were ineffective.

Endplate potentials

Clonidine, (−)-NA, phenylephrine and xylazine (each 3×10^{-6} M) enhanced, significantly, the amplitude of endplate potentials compared with controls in the same cell (Table 1). (Catechol and pyrogallol were not investigated.) Neither the time to peak nor the half decay time was affected significantly. Expressed as a % of the control value in individual experiments, (−)-NA and phenylephrine were the most potent and were indistinguishable from each other. Clonidine and xylazine, though comparable with each other, were less potent than (−)-NA and phenylephrine. The membrane potential was not affected significantly. On the other hand isoprenaline (3×10^{-6} M) hyperpolarized the membrane and enhanced e.p.p. amplitude. Both prazosin and yohimbine (each 3×10^{-6} M) inhibited the enhancement of the endplate potential amplitude produced by phenylephrine (Figure 3).

Table 1 Effects of α- and β-adrenoceptor agonists, (each at 3×10^{-6} M) on endplate potentials in the rat phrenic nerve-diaphragm preparation

Drugs	n	*Amplitude* (mV)	*Time to Peak* (ms)	*Half-decay time* (ms)
Control	7	11.08 ± 1.97	2.86 ± 0.35	2.82 ± 0.41
(−)-NA	7	38.31 ± 3.0	2.86 ± 0.45	2.70 ± 0.30
Control	4	10.00 ± 2.88	3.34 ± 0.48	3.15 ± 0.48
Phenylephrine	4	41.84 ± 5.19	4.53 ± 1.13	3.34 ± 0.78
Control	3	15.76 ± 4.48	3.81 ± 0.66	3.56 ± 0.96
Xylazine	3	31.82 ± 8.33	4.06 ± 0.79	3.43 ± 0.76
Control	4	13.64 ± 3.23	3.62 ± 0.68	3.57 ± 0.72
Clonidine	4	32.27 ± 6.57	3.62 ± 0.79	3.15 ± 0.84
Control	3	14.85 ± 1.38	3.37 ± 0.77	3.43 ± 0.66
Isoprenaline	3	27.58 ± 4.1	4.32 ± 0.79	3.93 ± 0.22

Values represent mean ± s.d. before (control) and 5 min after application of drugs. Each value was obtained from an experiment in the same cell. n = number of observations. Each of the drugs investigated significantly ($P < 0.02$ or better) increased the amplitude but not the time to peak or half-decay time of the endplate potentials.

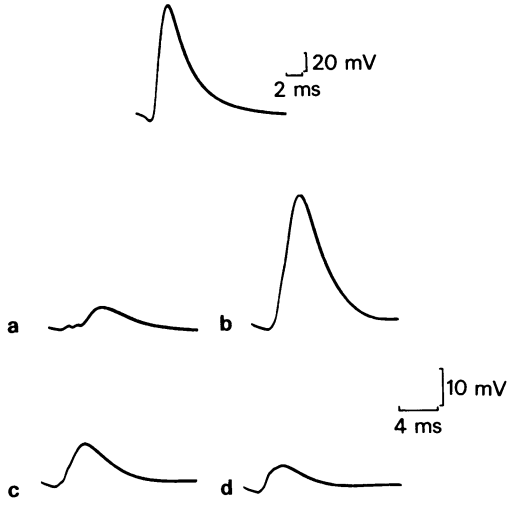

Figure 3 The enhancing effect of (b) phenylephrine (3×10^{-6} M) on the endplate potential elicited in response to stimulation (supramaximal voltage, 0.05 ms) of the phrenic nerve (a) in the tubocurarine-treated (1.5×10^{-6} M) rat phrenic nerve-diaphragm preparation. The enhanced endplate potential (b) was virtually abolished by yohimbine (3×10^{-6} M added at (c) and present throughout (c) and (d)); time between each panel was 5 min. Top trace shows an action potential from the same cell in the absence of drugs.

Discussion

With the exception of (±)-NA which affected neither parameter, each of the α-adrenoceptor stimulants examined increased m.e.p.p. frequency but not amplitude. M.e.p.p. frequency is itself a measure of transmitter release and the site of drug-induced increases in m.e.p.p. frequency is presumably presynaptic. The absence of α-adrenoceptors postsynaptically (Kuba, 1970; Malta, McPherson & Raper, 1979) supports an exclusive presynaptic site of action for drugs interacting with adrenoceptors at the skeletal neuromuscular junction. Analysis of e.p.p. characteristics allows the effects of drugs in the release of acetylcholine to be made during activation of the nerve terminal by the action potential. Changes in e.p.p. amplitude may arise from a prejunctional and/or postjunctional effect. In the present experiments, however, enhancement of e.p.p. amplitude by adrenoceptor agonists is likely to be mediated presynaptically due (a) to the lack of postsynaptic α-adrenoceptors (Malta *et al.*, 1979) and (b) to the lack of effect of drugs on m.e.p.p. amplitude. However the rank order of enhancement of e.p.p. amplitude and m.e.p.p. frequency differed. On the basis of the comparison of equimolar concentrations, (−)-NA and phenylephrine were the most potent in enhancing the e.p.p. amplitude; clonidine, (−)-NA, phenylephrine and xylazine were equipotent on m.e.p.p. frequency. This difference in the order of potency may reflect the extent of the interaction between the α-agonist and the tubocurarine used to block the action potential. Alternatively, it might represent real differences in the processes of generating m.e.p.ps and e.p.ps. Tubocurarine reduces transmitter output in rat diaphragm (Lilleheil & Naess, 1961; Hubbard, Wilson & Miyamoto, 1969). In the present results, in the absence of α-agonists, the size of the e.p.p. progressively diminished in the presence of tubocurarine. Variation in the extent of the antagonism with tubocurarine could account for the different order of potency among the α-adrenoceptor agonists when measured against m.e.p.p. frequency and e.p.p. amplitude. The ability of isoprenaline to enhance the amplitude of both m.e.p.ps and e.p.ps and to increase membrane potential confirms previous evidence (Kuba, 1970) of its postsynaptic activity mediated via β-adrenoceptors.

The mechanism by which α-adrenoceptors enhance acetylcholine release at the skeletal neuromuscular junction was not investigated in the present experiments. It has been proposed (Kuba & Tomita, 1972; Bowman, 1976) that noradrenaline increases the probability of release of transmitter quanta by facilitating Ca^{2+} binding to a specific binding site on the nerve terminal. The effect of noradrenaline in this respect resembles that of excess Ca^{2+}.

Differences in the pharmacological characteristics of α-adrenoceptors on somatic nerve terminals and at autonomic neuroeffectors clearly exist; (a) in the concentration of drug required to produce an agonist effect as measured by an increase in m.e.p.p. frequency, (b) in the apparent lack of discrimination among α-agonists and antagonists. Each factor contributes to make difficult any attempt to incorporate the adrenoceptors on somatic nerve endings into the existing α_1/α_2-adrenoceptor subclassification. Thus while the α_2-agonists clonidine and xylazine each enhanced m.e.p.p. frequency significantly, they did so only at concentrations at which, at least in the case of clonidine, an acknowledged α_1-agonism (Starke, Montel, Gayk & Merker, 1974) would be exerted. These results suggest that the adrenoceptors in somatic nerve terminals are more α_1-than α_2- in character. Nor is evidence from the use of α-adrenoceptors helpful in this respect. Prazosin which is selective for α_1-adrenoceptors and yohimbine which antagonizes α_2-adrenoceptors were approximately equipotent at doses (1.5×10^{-9} M) at which their selectivity might have been expected to have been apparent. Significantly, phentolamine which antagonizes both α_1- and α_2-adrenoceptors (Docherty & McGrath, 1980) was comparable in effectiveness with prazosin and yohimbine at equimolar concentrations. These results suggest that the α-adrenoceptors involved in the

enhancement of transmitter release belong neither to the α_1- nor the α_2-subclassification but show characteristics of both in their response to adrenoceptor agonists and antagonists. Their structural requirements appear less stringent than those at pre- or postsynaptic autonomic neuroeffector sites. Whether such differences are due to alterations in accessory binding areas which control drug selectivity as suggested by Malta *et al.* (1979) or arise from changes in the ionic environment in which the receptors exist (Stone, 1974) cannot be ascertained at present. The present results do not imply however that the adrenoceptors on somatic nerve terminals are totally unselective; they are highly stereospecific. They have a much higher affinity for the (−)-isomer than for the (±)-racemate noradrenaline, which did not enhance m.e.p.p. frequency significantly. The receptor has no affinity for dopamine agonists or for catechol or pyrogallol which are devoid of the ethylamine side chain found in noradrenaline. The inactivity of the latter two compounds contrasts with the results of Blaber & Gallacher (1971, 1973) who found, in the cat tenuissimus muscle, that catechol enhanced m.e.p.p. frequency but not amplitude or time course and that these effects were not blocked by α-adrenoceptor antagonists. These authors suggested that the prejunctional α-adrenoceptor comprised two sites, one occupied by the catechol moiety and the other sensitive to α-antagonists and occupied by the amine side chain. There is nothing in the present results in the rat to confirm the dual nature of the α-site. Each of the drugs examined which enhanced m.e.p.p. frequency was susceptible to α-antagonists.

Some indication of the possible physiological function of the prejunctional receptors at the skeletal neuromuscular junction may be obtained from the present results. Prazosin and yohimbine each in high concentrations (10^{-3} M), failed to abolish either the spontaneous release of transmitter or the enhancement of m.e.p.p. frequency produced by α-adrenoceptor agonists. This suggests that α-adrenoceptors are at best ancillary at this site, influencing but not controlling transmitter release.

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