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COMPARISON OF PRE-JUNCTIONAL α-ADRENOCEPTORS AT THE NEUROMUSCULAR JUNCTION WITH VASCULAR POST-JUNCTIONAL α-RECEPTORS IN CAT SKELETAL MUSCLE

E. MALTA, G.A. McPHERSON & C. RAPER

School of Pharmacology, Victorian College of Pharmacy, 381 Royal Parade, Parkville, Victoria 3052, Australia

1 Activation of pre-junctional α-adrenoceptors at the skeletal neuromuscular junction enhances acetylcholine release whereas activation of such receptors at autonomic nerve endings inhibits transmitter output. In the present study the characteristics of pre-junctional α-adrenoceptors at motor nerve terminals have been compared with post-junctional (vascular) α-adrenoceptors in the cat hind limb.

2 Reversal of partial (+)-tubocurarine blockade of contractions of the tibialis anterior muscle was used to monitor pre-junctional activity and increases in hindlimb vascular resistance to assess post-junctional actions at α-adrenoceptors.

3 Responses to intra-arterial injections of noradrenaline, adrenaline, phenylephrine, oxymetazoline, methoxamine and clonidine were monitored. Dose-response lines for all the compounds except clonidine were parallel. The latter agent produced only weak and inconsistent effects.

4 Ratios of the doses of the agents required to produce pre- and post-junctional effects indicated that oxymetazoline and adrenaline possessed some preferential activity at post-junctional sites, whereas the remaining agents were non-selective in their actions. If dose-ratios with respect to noradrenaline were compared at the two sites none of the compounds possessed a marked degree of selectivity.

5 In the presence of phentolamine or tolazoline, dose-response curves to the pre- and post-junctional effects of phenylephrine were shifted to a similar extent. Thymoxamine showed preferential activity as a pre-junctional α-receptor antagonist.

6 In comparing the results of this study with those of other authors, it is apparent that there are marked differences in the characteristics of pre-junctional α-receptors at the skeletal neuromuscular junction and at autonomic nerve endings. The pre- and post-junctional α-receptors in skeletal muscle show less divergence.

Introduction

The stimulation of prejunctional α-adrenoceptors located on sympathetic nerve terminals produces an inhibition of noradrenaline release (see Langer, 1977; Starke, 1977 for reviews). Modulation of neurotransmitter release through actions at pre-junctional α-adrenoceptors has also been demonstrated in central and autonomic cholinergic nerves (Vizi, 1968; Beani, Bianchi & Crema, 1969; Paton & Vizi, 1969; Knoll & Vizi, 1970; Kosterlitz, Lydon & Wyatt, 1970; Drew, 1977a; Dun & Karczmar, 1977; Beani, Bianchi, Giacomelli & Tamberi, 1978) and at somatic nerve terminals (Bowman & Raper, 1966; Kuba, 1970).

On the basis of the selectivity of action of a number of α-receptor agonists and antagonists it has been proposed that the presynaptic α-receptors located on autonomic nerve endings differ from the postjunctional α-receptors (Borowski, Ehrl & Starke, 1976; Drew, 1976, 1977a, b; Doxey, Smith & Walker, 1977; Starke, 1977) and Langer (1977) has suggested that the adrenoceptors may be classified as either α_1-(post-junctional) or α_2-(pre-junctional) subtypes.

In contrast to the inhibition of transmitter release observed at neuroeffector junctions in the autonomic nervous system, stimulation of prejunctional α-recep-

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tors on motor nerve terminals leads to an enhanced release of acetylcholine (Bowman & Raper, 1966; Bowman & Nott, 1969; Kuba, 1970). It was therefore of interest to compare the characteristics of the latter pre-junctional α-receptor with those of a post-junctional α-receptor.

In the present experiments, actions at these sites were monitored following intra-arterial injections of the α-receptor agonists noradrenaline, adrenaline, phenylephrine, methoxamine, oxymetazoline and clonidine to the cat hind limb. Post-junctional activity was assessed from changes in hind limb vascular resistance and pre-junctional actions as reversal of partial neuromuscular blockade induced in the tibialis anterior muscle by infusion of (+)-tubocurarine. At autonomic neuroeffector junctions, noradrenaline and adrenaline have been classed as non-selective agonists, whereas phenylephrine and methoxamine are selective for post- and clonidine and oxymetazoline for pre-junctional α-receptor actions (Starke, 1977).

Ideally, pre- and post-junctional activity should be assessed at a single type of neuroeffector junction (Starke, 1977); however, this was not possible in the present study because of the absence of post-junctional α-adrenoceptors in skeletal muscle fibres (Bowman & Nott, 1969).

In addition to studies with the agonists, phentolamine, tolazoline and thymoxamine were compared for their ability to antagonize the pre- and post-junctional effects of phenylephrine.

Methods

Adult cats of either sex were anaesthetized by the intraperitoneal injection of a mixture of α-chloralose (80 mg/kg) and sodium pentobarbitone (6 mg/kg). The animals were bilaterally vagotomized and artificially respired at 27 breaths per min with a stroke volume of 14 ml per kg body weight.

Pulsatile arterial blood pressure was recorded from a cannulated carotid artery with a Statham P23Dc pressure transducer coupled to a Grass 7P1 preamplifier. The arterial pressure signal was fed to a further Grass 7P1F preamplifier to obtain an electronically derived trace of mean arterial pressure, and to a Grass 7P4 tachograph for the recording of heart rate.

The right hind limb was acutely denervated by sectioning the femoral and sciatic nerves and blood flow in the femoral artery recorded with either IVM model A-2 or SL-3E electromagnetic flow probes coupled to an EMI type 28 flow meter. The tendon of insertion of the right tibialis anterior muscle was dissected free and attached to a Grass FT10 force-displacement transducer for the recording of muscle contractions. These were elicited at a frequency of 1 Hz by stimulation of the distal end of the cut sciatic nerve with a bipolar platinum electrode. Square wave pulses of 100 μs duration and a voltage sufficient to induce maximal contractions were used.

Records of the above parameters were displayed on a six channel Grass model 7 polygraph.

(+)-Tubocurarine was infused into a cannulated left brachial vein by means of a Harvard 944 slow-infusion pump. The rate of infusion was initially adjusted to obtain an 80 to 90% blockade of the contractions of the tibialis anterior. In different experiments this required infusion rates of 0.1 to 0.4 mg kg^{-1} h^{-1}. If required, a steady-state blockade was maintained by small adjustments to the rate of the (+)-tubocurarine infusion.

The right brachial vein was cannulated for the administration of bethanidine (6 mg/kg). This dose was sufficient to abolish sympathetic reflexes. β-Adrenoceptor mediated effects in the cardiovascular system were eliminated by injection of either oxprenolol or bunitrolol (0.5 mg/kg initial dose followed by 0.25 mg/kg every 30 min). The use of these compounds also prevented enhancement of the (+)-tubocurarine-induced blockade through activation of post-synaptic β-adrenoceptors in the tibialis anterior muscle (Bowman & Raper, 1966).

α-Receptor agonists and antagonists

Intra-arterial injections of the α-receptor agonists to the right hind limb were made via a cannula which was advanced retrogradely up the left femoral artery until the tip was located at the bifurcation of the abdominal aorta. Doses of the drugs contained in 0.1 ml 0.9% w/v NaCl solution (saline) were injected over a period of 4 s and the cannula was then flushed with 0.3 ml of saline. Dose-response curves were established by use of a series of increasing doses of each agonist. In each experiment constant dose-response curves to noradrenaline were first established and thereafter the effects of one or more of the other agonists monitored. In the latter cases responses to noradrenaline were reassessed between each α-receptor agonist tested.

In experiments where the actions of the α-receptor antagonists were monitored, constant dose-response curves to intra-arterial phenylephrine were initially obtained. An α-receptor antagonist was then infused into a cannulated external jugular vein by means of a Palmer motor driven syringe. When a steady state α-receptor blockade had been obtained a dose-response curve to phenylephrine was re-established. The infusion rate of the α-receptor antagonist was then increased and the process repeated. At each infusion rate, constancy of α-receptor blockade was assessed by monitoring pressor responses to intravenously injected doses of phenylephrine.

Calculations

In preliminary experiments central venous pressure was measured from a cannula advanced towards the right atrium from an external jugular vein. Changes in central venous pressure produced by doses of the α-receptor agonists were negligible in comparison with those obtained for arterial blood pressure. Hind limb vascular resistance (HLVR) were therefore calculated by dividing mean arterial pressure by blood flow and expressing changes produced by the agonists as a percentage of control values (=100%). During the course of an experiment, basal hind limb vascular resistance did not alter appreciably.

Reversal of the (+)-tubocurarine blockade was measured by dividing the height of the contractions at the peak response to a drug by the height of contractions during steady-state neuromuscular blockade and expressing this as a percentage reversal (resting = 0%).

Drugs

The following drugs were used: α-chloralose (BDH); pentobarbitone sodium (Nembutal; Abbott); bethanidine sulphate, (±)-methoxamine hydrochloride and (+)-tubocurarine hydrochloride (Burroughs Wellcome); oxprenolol hydrochloride, phentolamine hydrochloride, tolazoline hydrochloride and angiotensin amide (Ciba-Geigy); (−)-noradrenaline bitartrate, (−)-adrenaline bitartrate, (−)-phenylephrine hydrochloride (Sigma Co.); bunitrolol hydrochloride, clonidine hydrochloride (Boehringer-Ingelheim); oxymetazoline hydrochloride (Glaxo-Allenburys) and thymoxamine hydrochloride (Warner).

Stock solutions of the drugs were prepared daily. The α-receptor agonists were dissolved in 0.01 M HCl and suitable dilutions made in saline. Doses of the α-receptor agonists refer to the base and other drugs to their respective salts.

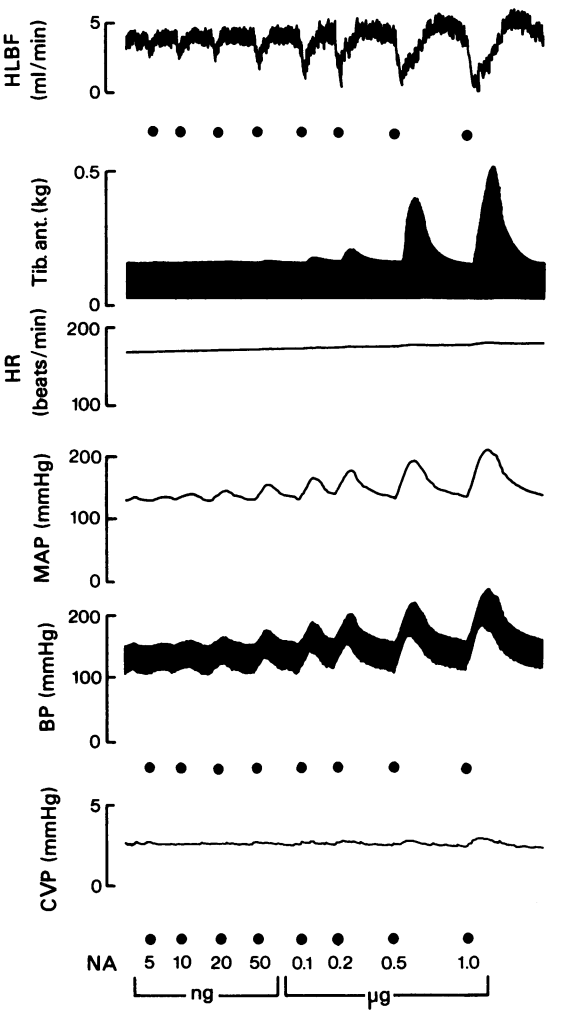

Figure 1 Records from an experiment showing the effects of intra-arterial injections of noradrenaline (NA) on hind limb blood flow (HLBF), contractions of the tibialis anterior muscle (Tib. ant.), heart rate (HR), mean arterial blood pressure (MAP), pulsatile blood pressure (BP), and central venous pressure (CVP).

Results

α-Adrenoceptor agonists

Intra-arterial injections of noradrenaline, adrenaline, phenylephrine, oxymetazoline and methoxamine produced dose-related decreases in hind-limb blood flow and increases in systemic arterial blood pressure and twitch tension in the partially curarized tibialis anterior muscle. These effects were antagonized by phentolamine (1 mg/kg i.v.).

Figure 1 shows a trace from an experiment in which the effects of increasing doses of noradrenaline were assessed. In general, noradrenaline (Figure 1), adrenaline and oxymetazoline produced small changes in blood flow at dose-levels which were subthreshold for effects in the tibialis anterior muscle. With phenylephrine and methoxamine, similar threshold doses were required for both effects. The duration of the drug-induced changes in hind limb blood flow, skeletal muscle contractility and systemic arterial blood pressure were similar with noradrenaline (Figure 1) and adrenaline, whereas effects on skeletal muscle and arterial blood pressure outlasted the flow changes with phenylephrine, oxymetazoline and methoxamine.

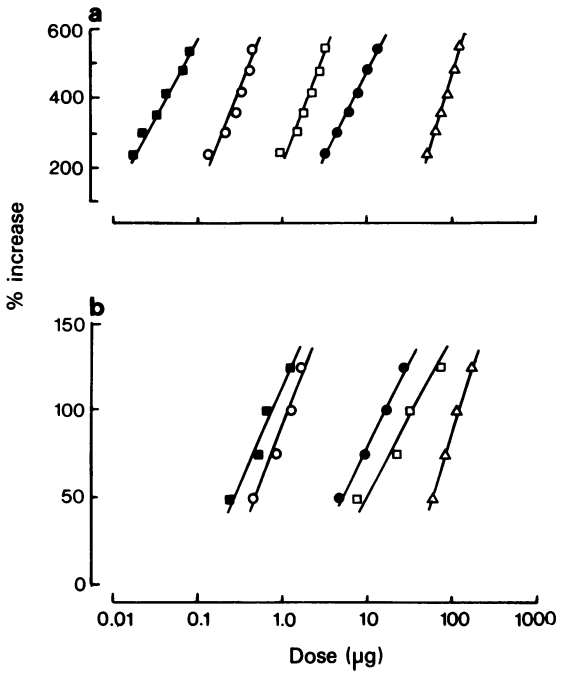

Figure 2 Mean dose-response lines ($n \geqslant 4$) for the effects of adrenaline (■), noradrenaline (○), oxymetazoline (□), phenylephrine (●) and methoxamine (△) on hind limb vascular resistance (a) and contractions of the tibialis anterior muscle (b). Each point represents the mean dose required to produce a given percentage increase in the two parameters.

Theoretically it is possible that intense α-receptor-mediated vasoconstriction could reduce the rate of delivery of (+)-tubocurarine to the muscle and hence contribute to the reversal of the neuromuscular block. However, this possibility was discounted, since intra-arterial injections of angiotensin (10 to 100 ng) which produced similar degrees of vasoconstriction to the α-receptor agonists, or mechanical occlusion of the femoral artery, produced a slowly developing decrease rather than an increase in twitch tension. These findings confirm and extend those of Bowman & Raper (1966) who showed that the anticurare effect of adrenaline occurred independently of vascular changes produced by the amine.

In constructing dose-response curves to the various agonists, the maximum doses used were restricted to those producing a 7 to 10 fold increase in hind limb vascular resistance and a 2 to 3 fold reversal of neuromuscular blockade. This allowed dose-response curves to be constructed with 3 to 4 points on the linear portion of the plot. The use of higher doses was deleterious to the stability of the preparation and reproducibility of responses was difficult to achieve.

Figure 2 shows the mean dose-response curves for the effects of noradrenaline, adrenaline, phenylephrine, oxymetazoline and methoxamine on hind limb vascular resistance and reversal of (+)-tubocurarine-induced blockade. The dose-response lines were close to parallel for these agents. Although the individual doses required to produce a given effect varied in different experiments the relative potencies of the drugs with respect to noradrenaline were much less variable. Table 1 shows the mean doses of the drugs required to produce a 75% increase in twitch tension (ED_{75}) and 420% increase in hind limb vascular resistance (ED_{420}), together with their relative potencies with respect to noradrenaline (=1). The ED_{75} and ED_{420} values were interpolated from dose-response curves in each experiment. These values were chosen because they invariably fell on the straight line portions of the dose-response curves.

In characterizing drugs as being selective agonists at different but closely related receptor sites, two general methods have been used. In the first, the relative doses or concentrations of a compound required to produce a given response at an individual receptor site are compared, and in the second, a comparison is made between the relative potency of a drug and a standard compound at the two receptors.

Table 1 shows the pre- to post-junctional selectivities of the compounds using individual dose-ratios (ED_{75}/ED_{420}) and ratios with respect to noradrenaline (=1) at the two receptor sites. On the basis of individual dose-ratios noradrenaline, phenylephrine and methoxamine would appear to show little selectivity in their actions at the two α-receptor sites, while adrenaline and oxymetazoline are selective for post-junctional actions. However, if selectivity is based on relative potencies with respect to noradrenaline, little selectivity is apparent with any of the compounds studied.

The effects produced by clonidine differed from those of the other agonists. In five experiments doses of 1 to 200 μg intra-arterially produced only small changes in blood flow. These were maximal with doses of 5 or 10 μg, however, systemic arterial pressure continued to increase in a dose-related fashion with higher doses of the drug. Thus, the dose-response curves for clonidine were less steep than those obtained to noradrenaline. Only a doubling in hind limb vascular resistance was obtained with maximal doses of the imidazolidine. Within the dose-range used, clonidine produced a reversal of the (+)-tubocurarine blockade in only 2 of the 5 experiments. In both cases the dose-response curves were parallel to those of noradrenaline, and were therefore reminiscent of a full agonist. However, the relative potencies of the two drugs differed to a considerable extent. In one case clonidine was approximately 20 times and in the other 200 times less potent than noradrenaline.

α-Adrenoceptor antagonists

In these studies phenylephrine was used as an α-receptor agonist since it was short acting and effects on both hind limb vascular resistance and skeletal muscle contractility occurred over a similar dose range.

Steady state α-receptor blockade with phentolamine, tolazoline and thymoxamine occurred within 30 min of the start of infusions. At this time the lowest infusion rates of the compounds produced a 20 to 30% reduction in arterial blood pressure and a similar increase in hind limb blood flow. Higher infusion rates had little further effect on these parameters. At the infusion rates used the α-receptor antagonists were without effect on skeletal muscle contractility.

The three antagonists produced dose-related parallel shifts in dose-response curves to phenylephrine. Mean dose-ratios for effects on hind limb vascular resistance and (+)-tubocurarine-reversal are shown in Table 2. With phentolamine, there was no significant difference in the dose-ratios for effects at pre- or post-junctional α-receptors (Student's paired t test 0.47 mg kg^{-1} h^{-1}, $0.30 < P < 0.35$ and 2.97 mg kg^{-1} h^{-1}, $0.20 < P < 0.25$). A similar situation was also apparent with tolazoline (1.62 mg kg^{-1} h^{-1}, $0.40 < P < 0.45$ and 3.24 mg kg^{-1} h^{-1}, $0.25 < P < 0.30$). Thus both phentolamine and tolazoline are non-selective in their α-receptor antagonistic actions.

Unlike phentolamine and tolazoline, thymoxamine produced a significantly greater shift in the dose-re-

Table 1 Pre- and post-junctional α-adrenoceptor activity of noradrenaline, adrenaline, phenylephrine, methoxamine and oxymetazoline

	Tib. ant. ED_{75}		*HLVR* ED_{420}		$ED_{75}:ED_{420}$	$DR_{Tib}:DR_{HLVR}$	n
Noradrenaline	0.89	(0.20)	0.38	(0.09)	2.34		14
Dose-ratio	1.00		1.00			1.00	
Adrenaline	0.56	(0.23)	0.055	(0.018)	10.18		4
Dose-ratio	0.38	(0.07)	0.48	(0.19)		0.79	
Phenylephrine	9.93	(3.95)	8.43	(3.37)	1.18		4
Dose-ratio	10.6	(3.2)	29.3	(7.9)		0.36	
Methoxamine	83.3	(42.7)	96.3	(32.0)	0.87		4
Dose-ratio	164	(43)	368	(75)		0.45	
Oxymetazoline	20.8	(5.8)	1.93	(0.70)	10.77		5
Dose-ratio	17.3	(5.6)	16.3	(7.0)		1.06	

For each compound the mean dose (± s.e. mean) required to produce a 75% increase in tension development of the tibialis anterior muscle (Tib. ant. ED_{75}) and a 420% increase in hind limb vascular resistance (HLVR ED_{420}) are shown, together with the mean dose-ratios (± s.e. mean) of the drugs with respect to noradrenaline [$ED_{(drug)}:ED_{(noradrenaline)}$]. Indications of selectivity are given by ratios of mean ED values at the two sites ($ED_{75}:ED_{420}$) and by mean dose-ratios with respect to noradrenaline ($DR_{Tib}:DR_{HLVR}$).

Table 2 Effects of the α-adrenoceptor antagonists phentolamine, tolazoline and thymoxamine on responses to phenylephrine

	Infusion (*mg* kg^{-1} h^{-1})	*Dose-ratios* *Tib. ant.*	*HLVR*	n
Phentolamine	0.47	6.0 (1.5)	5.1 (1.0)	4
	2.97	76.2 (21.2)	52.2 (5.7)	4
Tolazoline	1.62	3.1 (0.7)	2.9 (0.5)	3
	3.24	7.2 (2.2)	9.4 (3.1)	3
Thymoxamine	1.60	20.9 (3.8)*	*3.1 (1.2)	3
	3.20	67.7 (21.9)†	†8.8 (1.9)	3

Mean dose-ratios (± s.e. mean) for the effects of phenylephrine in the tibialis anterior muscle (Tib. ant.) and on hind limb vascular resistance (HLVR) obtained in the presence of the indicated infusion rates of the three antagonists.
Significant difference, paired t test, *$0.005 < P < 0.01$, †$0.025 < P < 0.05$.

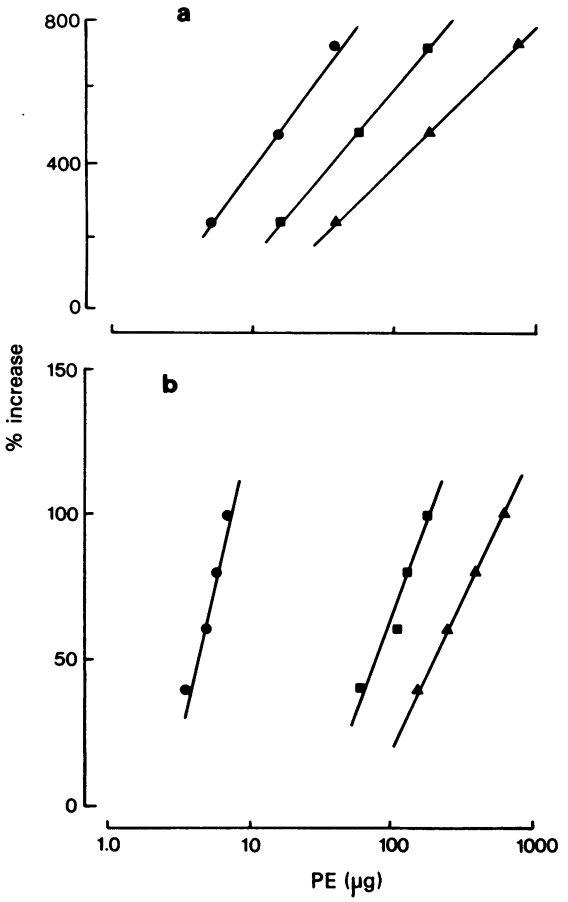

Figure 3 Mean dose-response lines ($n = 3$) for the effects of phenylephrine (PE) on hind limb vascular resistance (a) and contractions of the tibialis anterior muscle (b) in the absence (●) and in the presence of infusions of thymoxamine; (■) 1.6 mg kg^{-1} h^{-1}; (▲) 3.2 mg kg^{-1} h^{-1}. Each point represents the mean dose required to produce a given percentage increase in the two parameters.

sponse curves to phenylephrine in the tibialis anterior muscle than on hind limb vascular resistance (Figure 3). This significant difference was apparent at both of the infusion rates used (1.60 mg kg^{-1} h^{-1}, $0.005 < P < 0.01$ and 3.20 mg kg^{-1} h^{-1}, $0.025 < P < 0.05$). Thymoxamine therefore appears to possess selectivity as an antagonist at pre- as opposed to post-junctional α-receptors.

Discussion

The results of the present experiments confirm and extend those of other workers who have shown that catecholamines possessing α-adrenoceptor stimulant properties reverse (+)-tubocurarine-induced blockade through actions at pre-junctional sites in skeletal muscle (see Bowman & Nott, 1969 for references). β-Receptor-mediated effects on skeletal neuromuscular transmission result in an enhancement of (+)-tubocurarine blockade through a post-junctional hyperpolarizing action (Bowman & Raper, 1966; Kuba, 1970). In the present work the latter complication was obviated by the use of β-receptor antagonists. These drugs also prevented β-receptor-mediated effects which could affect changes in hind limb vascular resistance.

In comparing pre- and post-junctional activities at α-receptor sites it is obviously advantageous to assess effects occurring at a single type of neuroeffector junction. However, the absence of post-junctional α-receptors at the skeletal neuromuscular junction precludes such measurements in the present experiments. In an attempt to resolve this difficulty, effects on hind limb vascular resistance and on reversal of (+)-tubocurarine blockade were assessed concurrently, following intra-arterial injections of the agonists to the cat hind limb.

Assessment of possible differences in α-receptor characteristics on the basis of the doses of compounds required to produce specified pre- and post-junctional effects is complicated in the *in vivo* situation, particularly when different tissues are used for comparisons. Thus apart from difficulties in obtaining steady state biophase concentrations of drugs in an *in vivo* situation, differences in the access of drugs to the receptor sites could play a major role in determining their apparent selectivity of action. Following intra-arterial injections, it might be expected that vascular actions of compounds would be favoured. However, the fact that noradrenaline, phenylephrine and methoxamine are structurally related, possess similar lipid solubilities, and produce both pre- and post-junctional effects over a similar dose-range suggests but does not prove, that access of drugs to the somatic nerve terminals is relatively unhindered.

A further complication in monitoring selectivity by comparison of the doses required for pre- and post-junctional actions, involves the response levels at which these doses are assessed. This is particularly difficult when determining pre-junctional effects since true maximal effects are difficult to monitor and the frequency of nerve stimulation can also influence the results obtained. Previous results (Bowman & Raper, 1966) indicate that the 'anticurare' effect of adrenaline is less marked at low (0.1 Hz) than at higher (1.0 Hz) frequencies of stimulation in skeletal muscle. Although precise quantitative frequency-response relationships were not studied, the results indicate that adrenaline would produce anticurare effects at lower doses when the muscle was stimulated at 1 Hz.

The use of higher frequencies (2 and 3 Hz) is precluded by the onset of muscle fatigue (Raper, unpublished observations).

In the present experiments the selection of ED_{75} and ED_{420} levels for dose-comparisons (Table 1) of effects on skeletal muscle contractility and hind limb vascular resistance respectively was based on the observation that these values invariably fell on the straight-line portions of the parallel dose-response lines. While use of other dose-levels for comparisons would certainly result in small quantitative changes in selectivity indices, these changes would not substantially affect interpretations regarding the selectivity or otherwise of the compounds.

The results obtained with clonidine in the present study fail to clarify issues relating to receptor differentiation. The effects found with this drug when measuring hind limb vascular resistance are in accord with those of Hepburn, Reynoldson, Li & Bentley (1976). These authors showed that clonidine produced only weak vasoconstrictor effects in the cat hind limb, and that dose-response curves were less steep than those for noradrenaline. Although results from *in vitro* experiments suggest that clonidine displays selective actions at pre-synaptic α-receptors (see Starke, 1977 for references) evidence for selectivity *in vivo* is far less convincing (Drew, 1976; Haeusler, 1976; Andén, Grabowska & Strömbom, 1976; Doxey & Everitt, 1977). The fact that clonidine can display effects that are reminiscent of a partial agonist in some tissues may complicate interpretations of receptor differentiation with this agent.

When ED_{75} and ED_{420} values are used to assess differential activity it would appear that phenylephrine, noradrenaline and methoxamine are non-selective, and adrenaline and oxymetazoline selective for post-junctional α-receptors. However, if selectivity is based on potencies relative to noradrenaline at both sites, none of the agonists used display pre- or post-junctional selectivity. It is of interest that selectivity based on dose-ratios with respect to noradrenaline at the two sites minimizes the apparent selectivities obtained using the effective dose (ED) values. This applies not only to the present results but also to those reported by Starke (1977) for autonomic neuroeffector actions. The use of noradrenaline as a standard for comparison can be justified on the basis of its lack of selectivity at the two receptor sites. In addition, at many neuroeffector junctions this catecholamine would be the natural agonist responsible for both pre- and post-junctional actions. However, dose-ratios with respect to noradrenaline must be interpreted with caution when uptake processes are not inhibited.

From the results obtained with agonists it might be inferred that post-junctional vascular α-receptors and pre-junctional α-receptors on somatic nerve terminals possess very similar characteristics. A similar conclusion could also be reached when considering the results obtained with the α-receptor antagonists, phentolamine and tolazoline. From the results of Cubeddu, Barnes, Langer & Weiner (1974); Borowski *et al.* (1976) and Drew (1977b) it could be argued that one or both of these antagonists are inadequate for receptor differentiation. However this does not appear to be so in all investigations (Andén *et al.*, 1976; Borowski *et al.*, 1976; Drew, 1976; Doxey *et al.*, 1976).

The results obtained with thymoxamine in the present experiments indicate that the antagonist possesses marked selectivity for pre-junctional receptors at the skeletal neuromuscular junction, whereas it is generally regarded as a selective post-junctional antagonist at autonomic neuroeffectors (Drew, 1976; 1977a, b). While this, in conjunction with previously described results, may suggest that the α-receptors at the two sites differ to a small degree, one may also postulate that thymoxamine possesses its selective activity through a binding process which involves differing accessory receptor areas around the α-receptors at the two sites.

In 1967, when Lands and his coworkers (Lands, Arnold, McAuliff, Luduena & Brown, 1967) delineated β_1- and β_2-adrenoceptors, they arrived at their conclusion by comparing the relative potencies of a number of agonists with isoprenaline in different tissues from various species. Using this method of analysis for α-receptors, it can be shown that the relative potencies of agonists for post-junctional α-receptor activity in the cat hind limb are very similar to those found for post-junctional actions by Drew (1976) and Starke (1977) who studied effects at autonomic neuroeffector sites. However, relative potencies for pre-junctional actions at somatic nerve terminals are very different from those reported by these authors for actions at sympathetic nerve endings.

In conclusion, the results of the present experiments indicate that the pre-junctional α-receptors responsible for enhancing transmitter release at somatic nerve terminals possess differing characteristics from those which decrease transmitter release in autonomic nerves. The pre-junctional α-receptors at the skeletal neuromuscular junction would appear to possess a closer similarity to the adrenoceptors responsible for post-junctional activity. Comparison of the results of the present study with previously published material suggests that pre-junctional α-receptors may differ in different tissues, a point which has previously been raised by Doxey & Everitt (1977) who studied the selective actions of some imidazolines in a variety of tissues. It would therefore appear that Langer's (1977) division of post- and pre-synaptic α-receptors as α_1- and α_2-receptors respectively may be oversimple.

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