Heterogeneity of human vascular pre- and post-synaptic \(\alpha\)-adrenoceptors

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Summary

1. The effects of various \(\alpha\)-receptor agonists and antagonists on pre- and post-synaptic \(\alpha\)-receptors of human isolated digital arteries and metacarpal (or metatarsal) veins have been studied.

2. The relative potency of phenylephrine compared with noradrenaline on the postsynaptic receptors was significantly greater in arteries than in veins.

3. The rank order of potency of various \(\alpha\)-agonists on postsynaptic receptors was the same in arteries and veins except that in veins adrenaline was more potent than clonidine, whereas the reverse was the case in arteries.

4. Phentolamine showed no selectivity in either antagonizing postsynaptic responses or enhancing stimulation-induced transmitter release by antagonism at presynaptic \(\alpha\)-receptors.

5. Yohimbine potently, but not selectively, antagonized postsynaptic responses to noradrenaline and phenylephrine in both arteries and veins, and enhanced stimulation-induced transmitter release to a greater extent in arteries than in veins.

6. Prazosin reduced the maximum response to noradrenaline (but not phenylephrine) to a significantly greater extent in veins than in arteries, and in veins alone shifted the phenylephrine curve to a greater degree than it did the noradrenaline curve. Prazosin did not significantly increase stimulation-induced transmitter release in either arteries or veins.

7. Phenoxybenzamine produced large increases in stimulation-induced tritium outflow which were significantly greater in arteries than in veins (even in the presence of cocaine).

8. It is concluded firstly that the postsynaptic \(\alpha\)-adrenoceptors of these tissues are not a homogeneous population and that differing proportions of these receptors are present in the digital arteries and the metacarpal veins, and secondly that the sensitivities of the presynaptic \(\alpha\)-adrenoceptors in the two tissues may also be different.

Key words: \(\alpha\)-adrenoceptors, noradrenaline, phenylephrine, vessels.

Introduction

It is well accepted that pre- and post-synaptic \(\alpha\)-adrenoceptors have different sensitivities to pharmacological agents, and recent evidence suggests postsynaptic receptors from different vascular beds may also not be a homogeneous population [1]. As this has important implications in the treatment of hypertension, we have studied the effects of various \(\alpha\)-receptor agonists and antagonists on both pre- and post-synaptic \(\alpha\)-receptors of isolated human digital arteries and metacarpal (or metatarsal) veins.

Methods

Preparation

Blood vessels studied were dorsal metacarpal or metatarsal veins and palmar common digital arteries to the second and third fingers, obtained post mortem between 8 and 30 h after death.

Postsynaptic. Spiral strips from these vessels were prepared and suspended in tissue baths according to previously described methods [2].
Contractile responses were recorded by measuring isometric tension with a resting tension of 1 g in the arteries and 0.3 g in the veins.

**Presynaptic.** Cannulated segments were prepared and perfused according to previously described methods [3]. After the initial equilibration period (30 min) the vessels were removed and incubated in $[^{3}H]$noradrenaline (10 µCi/ml) for 60 min, then remounted and washed for 60 min.

Peripheral field stimulation (monophasic square-wave pulses of 1 ms duration and a supramaximal voltage of 60 V) was applied for 4 min at 30 min intervals. Total tritium content in the perfusate and superfusate was measured by continuously collecting 5 min fractions in vials, to which scintillant was added, then counting for radioactivity in a liquid scintillation spectrometer. Intra-vessel pressure was measured with a pressure transducer.

After four control stimulations were performed, the vessels were perfused with antagonist drugs 15 min before the last stimulus.

**Drugs**

Drugs used were adrenaline (L-epinephrine bitartrate, Sigma), clonidine hydrochloride (Catapres, Boehringer Ingelheim), cocaine hydrochloride (May and Baker), (+)-methoxamine hydrochloride (Vasoxyl, Burroughs Wellcome), naphazoline hydrochloride (Sigma), noradrenaline (l-arterenol bitartrate, Sigma), oxymetazoline hydrochloride (Glaxo–Allenbury), phenolamine mesylate (Regitine, Ciba), (−)-phenylephrine hydrochloride (Sterling Pharmaceuticals), phenylpropanolamine hydrochloride (Sigma), prazosin hydrochloride (Pfizer), propranolol hydrochloride (Inderal, I.C.I.) and yohimbine hydrochloride (Sigma).

Adrenaline and noradrenaline were diluted in ascorbic acid solution (10$^{-4}$ mol/l); prazosin was dissolved in glycerol and diluted in water; the other drugs were dissolved in water.

**Radiochemicals**

In all experiments either L-[7-$^{3}$H]noradrenaline hydrochloride (The Radiochemical Centre, Amersham, Bucks, U.K.), specific radioactivity 15 Ci/mmol, or L-[7,8-$^{3}$H]noradrenaline hydrochloride, 15 or 34.5 Ci/mmol, in a radioactive concentration of 1.0 mCi/ml, was used.

**Calculation of results**

**Postsynaptic.** Contractile responses to agonists were characterized by cumulative concentration–effect curves, each response being expressed as a percentage of the standard supramaximal reference contracture to KCl (80 mmol/l). Maximum response ($E_{max}$) values and pD$_2$ (taken as the negative logarithm of the median effective concentration) values were obtained from each agonist concentration–effect curve. Effects of antagonists were assessed by the repetition of agonist concentration–effect curves (at 30 min intervals) in the presence of increasing concentrations of the antagonist (15 min contact time), and were corrected by taking into account shifts obtained in paired (non-antagonist treated) control strips. pA$_2$ values were calculated by the method of Arunlakshana & Schild [4]. Results for prazosin, which produced a shift of the concentration–effect curves to the right, together with a depression of the $E_{max}$, were calculated in two ways. Firstly, the amount by which prazosin reduced the $E_{max}$ was expressed as a percentage of the original $E_{max}$, after correction for any reduction of the $E_{max}$ in the control. Secondly, dose ratios were calculated as the ratio of the EC$_{50}$ in the presence of prazosin to the EC$_{50}$ of the agonist alone, again corrected by dividing by the dose ratio of the control.

**Presynaptic.** In a train of stimuli, total tritium outflow collected over 30 min during and after each period of stimulation (constant frequency and time interval) decreases in an exponential manner [3], so the results were calculated as the ratio (expressed as a percentage) of the total tritium outflow produced by each period of stimulation to the outflow produced by the preceding period of stimulation.

**Statistics**

Means and SEM were calculated for the grouped data. Data were statistically analysed by either a paired or an unpaired two-tailed Student's t-test. P values less than or equal to 0.05 were designated as being statistically significant.

**Results**

**Postsynaptic**

α-Adrenoceptor agonists. Contractile responses were obtained in both arterial and venous preparations with the directly acting α-adrenoceptor agonists and the indirectly acting sympathomimetic phenylpropanolamine.

The mean pD$_2$ values for each of the agonists are shown in Table 1. It can be seen that the order of activity of the agonists in arteries is oxymetazoline > clonidine > adrenaline > naph-
The order of potency is the same, except that methoxamine for which the pD₂ values were significantly lower and noradrenaline were the only agonists tested. Phenylephrine, with respect to noradrenaline, was more sensitive.

Different in veins and arteries, the veins being the more sensitive.

The mean value for the relative potency of phenylephrine, with respect to noradrenaline, was found to be significantly greater in arteries (0.54 ± 0.10, n = 9) than in veins (0.24 ± 0.05, n = 7).

No significant differences in Eₘₐₓ. values were found between arteries and veins for any of the agonists tested.

α-Adrenoceptor antagonists. (a) Phentolamine and yohimbine. Both of these antagonists produced a parallel shift of the concentration–effect curves to both noradrenaline and phenylephrine without a significant reduction of the E_max.

True competitive antagonism (as defined by slopes of the Arunlakshana & Schild plots not being significantly different from 1) did not occur with yohimbine in some cases. However, at all times where competitive antagonism did occur there were no significant differences between the values obtained with the two agonists.

[pA₂ yohimbine against noradrenaline = 8.20 ± 0.17 (n = 7) arteries; 8.41 ± 0.09 (7) veins; against phenylephrine = 8.27 ± 0.10 (5) arteries; 8.59 ± 0.04 (5) veins. pA₂ phentolamine against noradrenaline = 7.59 ± 0.07 (n = 5) arteries; 7.68 ± 0.05 (5) veins; against phenylephrine = 7.66 ± 0.10 (5) arteries; 7.73 ± 0.05 (5) veins.]

(b) Prazosin. In contrast to phentolamine and yohimbine, prazosin produced a concentration-related depression in E_max., together with a slight shift of the concentration–effect curves to the right.

The mean percentage reduction of the E_max. for noradrenaline produced by prazosin (8 × 10⁻⁹ mol/l) was significantly greater in veins (42.6 ± 4.0, n = 9) than in arteries (19.8 ± 4.1, n = 11), whereas the reduction of the E_max. by phenylephrine produced by prazosin (8 × 10⁻⁹ mol/l) was not significantly different in arteries (22.9 ± 7.0, n = 10) and veins (28.8 ± 4.1, n = 10). In veins, this same concentration of prazosin had a significantly greater effect on the E_max. for noradrenaline than on the E_max. for phenylephrine. The mean log dose ratios obtained with prazosin (8 × 10⁻⁹ mol/l) were significantly higher for phenylephrine (0.600 ± 0.097, n = 10) than for noradrenaline (0.230 ± 0.139, n = 9) in veins, but not in arteries (0.889 ± 0.238, n = 9) for phenylephrine; 0.632 ± 0.144, n = 11 for noradrenaline). At this concentration of prazosin the difference between arteries and veins for the dose ratios obtained with either agonist was not significant.

Presynaptic

(a) Phentolamine. Phentolamine (10⁻⁶ mol/l) significantly increased stimulation-induced tritium outflow in both arteries and veins. This increase in tritium outflow (expressed as a percentage of the preceding stimulus) was not significantly different in arteries (120 ± 10, n = 6; control 78 ± 5) and veins (144 ± 42, n = 3; control 77 ± 11).

In all experiments, phentolamine reduced the postsynaptic pressure response to stimulation.

(b) Yohimbine. Yohimbine also significantly increased stimulation-induced tritium outflow in both arteries and veins. This increase was significantly greater in arteries (142 ± 14, n = 5) than in veins (103 ± 2, n = 5) for yohimbine at 10⁻⁴ mol/l but not for the higher concentration of 10⁻³ mol/l (156 ± 19, n = 5 for arteries; 147 ± 15, n = 5 for veins).

(c) Prazosin. Prazosin (10⁻⁸ and 10⁻⁷ mol/l) had very little presynaptic effect and did not signifi-
cantly increase stimulation-induced tritium outflow from controls in either arteries or veins. Values obtained in the presence of this antagonist were also not significantly different in arteries (97 ± 9, n = 5) and veins (94 ± 17, n = 3) for 10^{-8} mol/l and 10^{-7} mol/l (78 ± 8, n = 3 for arteries; 94 ± 13, n = 3 for veins).

(d) Phenoxybenzamine. Phenoxybenzamine (10^{-6} and 10^{-5} mol/l) produced very large increases in stimulation-induced tritium outflow. These increases were significantly greater in arteries (480 ± 92, n = 10) than in veins (189 ± 7, n = 12) for the 10^{-5} mol/l concentration but not for 10^{-6} mol/l (190 ± 32, n = 11 for arteries; 121 ± 15, n = 10 for veins). When the experiments were performed in the presence of cocaine (3 × 10^{-5} mol/l; to inhibit neuronal uptake of transmitter), the difference between arteries (350 ± 75, n = 7) and veins (152 ± 19, n = 3) was still significant for phenoxybenzamine at 10^{-5} mol/l.

Discussion

In this study a number of findings have indicated that there are differences between human arteries and veins in both the pre- and post-synaptic α-adrenoceptors. At the postsynaptic receptor veins were more sensitive than arteries to noradrenaline; prazosin had a greater effect on the maximum response to noradrenaline in veins than in arteries and produced a greater shift of the phenylephrine than of the noradrenaline concentration-effect curves in veins; low slopes of the Arunlakshana & Schild plots [4] were obtained with yohimbine, thus suggesting that not all the receptors were being antagonized.

At the presynaptic receptor, phenoxybenzamine had a greater effect in arteries than in veins, even when neuronal uptake was blocked by cocaine; yohimbine also had a greater effect in arteries than in veins. Phentolamine was a non-selective antagonist at both the pre- and postsynaptic receptor, whereas prazosin, as expected, was only an antagonist at the postsynaptic receptor.

The possibility must be considered of modification of responses by uptake processes (either neuronal or extraneuronal) or the presence of β-adrenoceptors. However, we have shown previously that the neuronal uptake blocking drug cocaine alters responses to noradrenaline by a mechanism different from the blockade of neuronal uptake, and that hydrocortisone (which blocks extraneuronal uptake) and propranolol (which blocks β-adrenoceptors) have no effect on responses of either arteries or veins to noradrenaline [5].

It is thus concluded that these differences between α-receptors in human blood vessels may be important in understanding the pathogenesis of hypertension in man, and may be able to be exploited in the therapy of hypertension.

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References