α-Adrenoceptor binding in guinea-pig lung using [^{3}H]-prazosin

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β-Adrenoceptors in lung membranes have been characterised by radioligand studies using (−)-[^{3}H]-dihydroalprenolol ([^{3}H]-DHA) (Rugg, Barnett & Nahorski, 1978), but there has been no previous study of α-adrenoceptor binding in pulmonary tissue. We have used [^{3}H]-prazosin, a new radioligand of high specific radioactivity (33 Ci/mmol) to study α-adrenoceptors in guinea-pig lung membranes. There is considerable evidence that α-adrenoceptors in peripheral tissues may be classified as postjunctional (α_1) or prejunctional (α_2) and α-adrenoceptor agonists and antagonists vary widely in their relative pre- and postsynaptic potencies. Prazosin is an α-adrenoceptor antagonist which has a high selectivity for peripheral α_1 receptors (Cambridge, Davey & Massingham, 1977) and [^{3}H]-prazosin has recently been shown to bind to rat brain α-adrenoceptors with a high degree of specificity (Greengrass & Bremner, 1979).

Guinea-pig lungs, dissected free of major bronchi, were homogenised, centrifuged at 50,000 *g* and the final pellet resuspended at a concentration of approximately 1 mg protein/ml. Aliquots of the homogenate were incubated at 25°C for 15 min with various concentrations of [^{3}H]-prazosin from 0.05 nM to 4.0 nM in a final volume of 1.0 ml. Bound radioactivity was isolated on GF/B filters followed by 2 × 6 ml washes with incubation buffer and quantified by scintillation counting. Specific binding, defined as that displaced by phentolamine (1 μM), comprised 70–80% of the total binding at ligand concentrations of 0.05–1.0 nM.

Specific [^{3}H]-prazosin binding was rapid ($T_{\frac{1}{2}}$ association = 2 min) at a ligand concentration of 0.26 nM, remained at a steady state for more than 30 min, and was reversible ($T_{\frac{1}{2}}$ dissociation = 1 min). Specific binding was saturable reaching a plateau between 1 and 2 nM [^{3}H]-prazosin and Scatchard analysis ($n = 5$) revealed a single receptor population with K_D of 0.24 ± 0.05 nM (mean ± s.e. mean) with a maximum binding capacity (Bmax) of 54 ± 7 fmol/mg protein. A Hill plot gave a slope of 1.06 indicating absence of cooperativity.

For comparison, binding of the β-adrenoceptor antagonist [^{3}H]-DHA was measured under identical conditions and specific binding was defined as that displaced by propranolol (10 μM). This gave a K_D of 0.93 ± 0.1 nM and a B_{max} of 870 ± 112 fmol/mg protein. Thus in guinea-pig lung membranes the ratio of beta:α adrenoceptor binding sites is approximately 16:1.

Adrenoceptor agonists inhibited [^{3}H]-prazosin binding in the order: (−)adrenaline > (−)noradrenaline ≫ (−)phenylephrine > (−)isoprenaline; (+)noradrenaline was 100 × less potent than (−)noradrenaline. α-Adrenoceptor antagonists competed for binding in the order: prazosin > phentolamine ≫ piperoxan > yohimbine, indicating that [^{3}H]-prazosin binding is probably to α_1 adrenoceptors as it is inhibited by potent α_1 adrenoceptor antagonists but not by drugs more potent at α_2 sites. Propranolol, methysergide and sulpiride inhibited binding only at concentrations greater than 10 μM.

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Interaction between prazosin and benzodioxan antihypertensives (R 28935 and R 29814); a competition for central α_1-adrenoceptors

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Previous studies have established that the erythro-isomer of 1-{1-(2-(1,4-benzodioxan-2-yl)-2-hydroxyethyl)-4-piperidyl}-2-benzimidazolinone (R 28935) lowers arterial pressure via an action within the central nervous system. Although appreciably less active than R 28935, the threo-isomer (R 29814) also displays a central hypotensive effect. However, the exact mechanism is still unknown. Central α-adrenoceptors have been excluded to play a substantial role, since central α-adrenoceptor antagonism accomplished by yohimbine, piperoxan, tolazoline and phentolamine did not affect the responses (Finch, 1975; Wellens *et al.*, 1975; van Zwieten, 1975; Taylor & Antonaccio, 1978). The present study reports on the impairment of

the hypotensive activity of both R 28935 and R 29814 by the α-sympatholytic drug prazosin. Following the administration of prazosin (3 μg/kg) via the vertebral artery (v.a.) of the chloralose-anaesthetized cat, the depressor effect of 3 μg/kg of R 28935 (41.9 ± 4.3%; $n = 6$) subsequently applied via this artery was reduced to 13.2 ± 0.6% ($P < 0.001$). Similarly, the central hypotensive action of R 29814 (30 μg/kg) was abolished by prazosin (3 μg/kg; v.a.). I.v. pretreatment with prazosin (3 μg/kg) did not diminish the centrally initiated hypotensive responses to R 28935 and R 29814. The quantitative aspects of this antagonism at a central level resulted from a parallel shift to the right of the dose-response curve for the central hypotensive effect of R 28935, induced by prazosin (3 μg/kg) applied previously to the v.a.

I.p. treatment of pentobarbitone-anaesthetized normotensive rats with prazosin (100 μg/kg) caused parallel shifts of the dose-response characteristics of the hypotensive effects of i.v. R 28935 and R 29814 in anaesthetized animals (1 h later). R 28935 and R 29814 showed no increase in arterial pressure in pithed rats, indicating the absence of α-sympathomimetic properties. As compared with prazosin both compounds were only moderately effective in antagonizing the pressor responses of i.v. L-phenylephrine. Finally, at low concentrations R 28935 and R 29814 displaced [^{3}H]-prazosin from its specific binding sites in membranes from rat cerebral cortex. The results suggest that the interaction between prazosin and benzodioxan antihypertensives occurs within the central nervous system. In contrast to earlier reports α-adrenoceptors seem to play a part. In view of the predominant blocking activity of prazosin at postsynaptically located (α_1) adrenoceptors, the receptive sites involved in this mutual interference may be characterized accordingly. It remains uncertain, however, whether R 28935 as well as R 29814 have to be considered either as agonists or antagonists.

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Effects of α-adrenoceptor agonists and antagonists on adrenergic neurotransmitter overflow from dog isolated saphenous veins

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Recent reports suggest that the presynaptic α-adrenoceptors in different tissues may have different characteristics (Doxey & Everitt, 1977; Dubocovich, 1979). Presynaptic α-adrenoceptors have been identified, but not subclassified, in dog saphenous vein (McGrath, 1977); we have therefore examined their sensitivity to α-adrenoceptor agonists and antagonists in an attempt to subclassify them.

The neuronal noradrenaline stores in spirally cut pieces of saphenous vein (10–15 mm long) were labelled with (−)-[^{3}H]-noradrenaline as previously described (Drew, Levy & Sullivan, 1979). After loosely bound noradrenaline had been washed from the tissues, each strip was mounted between platinum electrodes in a 1.5 ml organ bath and superfused with Krebs solution at 37°C, gassed with 95% O_2 and 5% CO_2 and containing cocaine (3×10^{-5} M), corticosterone (4×10^{-5} M), propranolol (10^{-6} M) and indomethacin (3×10^{-6} M). Adrenergic nerves were stimulated at 2 Hz (supramaximal voltage, 0.5 ms pulse duration) for periods of 3 min at intervals of 18 min; as many as six periods of stimulation were applied during an experiment. The tritium overflowing into the superfusate before, during and after nerve stimulation was measured by liquid scintillation counting.

The overflow of radioactivity caused by nerve stimulation was enhanced 2–4 fold by yohimbine (10^{-8}–10^{-6} M), phentolamine (10^{-7}–10^{-5} M) or prazosin (10^{-6} M); phentolamine and prazosin were respectively about 10 and 100 times less potent than yohimbine at doubling the overflow, which suggests that adrenergic neurotransmission in the dog saphenous vein is regulated by presynaptic α_2-adrenoceptors. This view is supported by the finding that tritium overflow was reduced 20–80% by (−)-adrenaline (10^{-7} and 10^{-6} M), (−)-α-methylnoradrenaline