Regional variations in [^{3}H]-prazosin and [^{3}H]-noradrenaline binding in the rat brain

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In the peripheral sympathetic nervous system there is considerable evidence for the presence of at least two types of α-adrenoceptor which have been classified as α_1 and α_2. A pre-junctional receptor which inhibits depolarization-induced release of noradrenaline (α_2) and a post-junctional excitatory receptor on vascular smooth muscle (α_1). In addition there is also some evidence for post-junctional α_2 receptors. The limited pharmacological and biochemical evidence available suggests that central α-adrenoceptors may also be subdivided.

Radio-ligand binding techniques are particularly suitable for direct biochemical identification of a variety of central neurotransmitter receptors and several tritiated ligands are available for identification of α-adrenoceptors. The high selectivity of prazosin for peripheral α_1 receptors (Cambridge, Davey & Massingham, 1977) suggested that it would be a suitable ligand for location and identification of these receptors in the central nervous system.

We have compared the distribution of binding of [^{3}H]-prazosin and [^{3}H]-(–) noradrenaline in the rat central nervous system. Freshly dissected tissue from male rats was homogenized in 20 vol (w/v) ice-cold Tris-HCl buffer (pH 7.7 at 25°C), centrifuged (50,000 × g, 10 mins) and washed once by resuspension and recentrifugation. The final resuspension was in 80 vol (w/v) Tris-HCl buffer (pH 8 at 25°C) for [^{3}H]-prazosin and in 40 vol (w/v) Tris-HCl buffer (pH 8 at 25°C) for [^{3}H]-noradrenaline. Aliquots of these tissue suspensions were incubated with 0.2 nM [^{3}H]-prazosin (33 Ci/mmole) at 25°C for 30 minutes. The noradrenaline assay was that described by U'Prichard & Snyder, 1977; homogenates being incubated with 2 nM [^{3}H]-noradrenaline (27.5 Ci/mmole), 1 mM pyrocatechol, 0.1 mMEDTA, 0.01 mMDTT and 0.001% ascorbate at 25°C for 40 minutes. Specific binding was defined as that binding displaced by 1000 nM phentolamine for [^{3}H[-prazosin (90% of total) and 100 nM oxymetazoline for [^{3}H]-noradrenaline (60% of total).

The specific binding was saturable and of high affinity with both ligands. The apparent dissociation constants were similar in all brain areas studied (0.29 nM, [^{3}H]-prazosin, 12 nM [^{3}H]-noradrenaline) although there were differences in the number of binding sites. The density of prazosin binding sites was frontal cortex > hypothalamic-pre-optic area > medulla > pons > striatum > cerebellum > basal hypothalamus. The highest [^{3}H]-noradrenaline binding was in the basal hypothalamus, otherwise the distribution was similar to that for [^{3}H]-prazosin. Studies with α-adrenoceptor blocking drugs suggest that central [^{3}H]-prazosin binding sites closely resemble the α_1 receptor in the peripheral vasculature. Phenoxybenzamine (Ki 1.7 nM) WB 4101 (Ki 1.1 nM) and indoramin (Ki 8 nM) which have greater affinity for α_1 than α_2 receptors, are more potent than piperoxan (Ki 340 nM) and yohimbine (Ki 1 μM) in competing for [^{3}H]-prazosin binding sites in all the brain areas studied. In the case of [^{3}H]-(–)-noradrenaline binding, piperoxan (Ki 100 nM) and yohimbine (Ki 290 nM) are considerably more potent than prazosin (Ki 6 μM) and indoramin (Ki 33 μM). Under these assay conditions [^{3}H]-noradrenaline labels an α-adrenoceptor resembling the peripheral α_2 receptor. Thus [^{3}H]-prazosin and [^{3}H]-noradrenaline appear to label different sub-classes of α-adrenoceptor in the central nervous system. In view of this it is proposed that these ligands may be of value in elucidating the precise role of the different classes of α-adrenoceptor in the central control of many physiological functions including vasomotor tone.

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References

CAMBRIDGE, D., DAVEY, M.J. & MASSINGHAM, R. (1977). The pharmacology of antihypertensive drugs with special reference to vasodilators, α-adrenergic blocking agents and prazosin. *Med. J. Aust.* Specl. Suppl., **2**, 2.

U'PRICHARD, D.C. & SNYDER, S.H. (1977). Binding of ^{3}H-catecholamines to α-noradrenergic receptor sites in calf brain. *J. Biol. Chem.*, **252**, 6450.

Some characteristics of the gastric antisecretory actions of prostaglandins in mammalian perfused whole-stomachs *in vitro*

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The recent development of viable, acid-secreting preparations of mammalian stomachs (Holton & Spencer, 1976; Bunce & Parsons, 1976; Main & Pearce, 1978; Angus, Black & Stone, 1978) has allowed study of the antisecretory actions of drugs, including prostaglandins, without such complicating factors as mucosal blood flow changes. In both the isolated stripped mucosa and lumen-perfused whole stomach of the rat, prostaglandin E_2 has been shown to inhibit histamine-stimulated acid output (Main &