Effect of methoxamine on the outflow of [^{3}H]-noradrenaline from rat vas deferens

A.R. DEHPOUR, K. HOSSEINZADEH & M.A. KHOYI (introduced by F. MICHAL)

Department of Pharmacology, Faculty of Medicine, University of Tehran, Tehran, Iran

Methoxamine has very low affinity for neuronal uptake mechanism in the isolated rat heart (Burgen & Iversen, 1965) and cat nictitating membrane (Trendelenburg, Maxwell & Pluchino, 1970). These findings are not supported by the reports that the effect of methoxamine is potentiated by cocaine in the rabbit aorta (Kalsner & Nickerson, 1969) and guinea-pig vas deferens (Shah, Patel & Gulati, 1974). In the rat vas deferens, the mechanism of uptake seems to be different from that of other tissues; the effect of tyramine is not prevented by cocaine (Barnett, Staub & Symchowicz, 1969). In the present experiments, we used isolated rat vasa deferentia to study the mode of action of methoxamine. Pretreatment with reserpine (5 mg/kg and 2.5 mg/kg, i.p., 48 and 24 h before experiment respectively) had no significant effect on the response of the tissue to methoxamine (negative log molar ED_{50} 5.86 ± 0.11 control; 5.65 ± 0.77 reserpinized) while it potentiated significantly the effects of noradrenaline (5.34 ± 0.08 control, 6.17 ± 0.10 reserpinized) and carbamylcholine (4.19 ± 0.17 control, 5.11 ± 0.12 reserpinized). Vasa deferentia were loaded with [^{3}H]-noradrenaline and then washed at 10 min intervals for 90 minutes. Methoxamine (1.2×10^{-4} M) increased the rate of the outflow coefficient for [^{3}H]-noradrenaline by 84% (from 0.64 ± 0.09 to 1.18 ± 0.18, $n = 14$, $P < 0.05$ for paired samples). This effect of methoxamine was prevented by pretreatment of the tissue with cocaine (2.94 μM) or desipramine (1 μM). These results suggest that, in the rat vas deferens, methoxamine has a small indirect effect in addition to its main direct action.

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Localisation of [^{3}H]-clonidine binding in guinea pig kidney

B. JARROTT & R.J. SUMMERS

Clinical Pharmacology and Therapeutics Unit, University of Melbourne, Austin Hospital, Heidelberg, Victoria 3084, Australia

[^{3}H]-Clonidine binds to membranes prepared from guinea pig kidney by a high affinity, saturable process. The binding is probably to renal α-adrenoceptors since it is readily displaced by α-adrenoceptor agonists or antagonists but not by drugs acting on histamine receptors, acetylcholine receptors, β-adrenoceptors, or by prostaglandins, angiotensin II or arginine vasopressin (Summers, Jarrott & Louis, 1978a, b). The present study investigates the localisation of the binding site within the kidney.

Kidneys were removed from male guinea pigs (600–800 g) and the renal cortex, medulla and papilla separated by dissection at 4°C. Membranes were prepared for each area by a modification of the method of U'Prichard, Greenberg & Snyder (1977). After incubation with [^{3}H]-clonidine (5.29 Ci/mmol) the membranes were rapidly filtered under vacuum at 4°C onto Whatman GF/B filters and washed with 3 × 5 ml of ice cold 50 mM Tris buffer pH 7.6 containing clonidine (1 μM). [^{3}H]-Clonidine remaining bound to the membranes was estimated by liquid scintillation counting. Non specific binding was measured in identical samples containing clonidine (1 μM).

Comparison of binding in membranes prepared from the three areas showed that of the total

[^{3}H]-clonidine bound membranes prepared from the renal cortex bound 76.55 ± 1.25%, those from the medulla 18.70 ± 0.36% and those from the papilla 4.75 ± 1.37% (n = 4). More detailed analysis of binding, performed on membranes prepared from the renal cortex and medulla, showed that the association of [^{3}H]-clonidine to the binding site was rapid with a $T_{\frac{1}{2}} \ll 2$ min at 25°C and that binding had equilibrated within 20 min of the start of incubation. The binding isotherm showed that the process was saturable. Scatchard analysis of [^{3}H]-clonidine binding to membranes from renal cortex revealed that binding was to a single class of sites ($P < 0.001$ in all cases) with a dissociation constant (K_d) of 9.03 ± 0.76 (n = 4) and that the density of binding sites was 21.6 ± 1.7 p.mol/g wet wt. tissue. Hill plots of the data obtained in these experiments were linear ($P < 0.001$ in all cases) with a mean Hill coefficient of 1.02 ± 0.02 (n = 4) indicating the absence of cooperative site interactions in the binding of [^{3}H]-clonidine to its receptor site. Membranes prepared from renal medulla bound less [^{3}H]-clonidine than those from renal cortex. The K_d for [^{3}H]-clonidine binding in membranes from the medulla was 4.4 ± 0.9 nM and the density of binding sites 3.9 ± 1.0 p.moles/g wet wt. (n = 3).

The results indicate that in the guinea pig kidney [^{3}H]-clonidine binds to an α-adrenoceptor which is located primarily in the renal cortex.

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Time course of the pre- and post-junctional effects of clonidine in the pithed rat

J.R. DOCHERTY & J.C. McGRATH

Institute of Physiology, University of Glasgow, Glasgow G12 8QQ, Scotland

In the pithed rat clonidine inhibits the chronotropic response to stimulation of the cardiac sympathetic nerves by a pre-junctional α-adrenoceptor agonism and raises blood pressure by a post-junctional α-adrenoceptor agonism (Armstrong & Boura, 1975; Drew, 1976). It has been suggested that the latter is faster in onset and shorter in duration than the former (Cavero, Gomeni, Lefevre & Roach, 1977). We have compared the time courses of the pre- and post-junctional effects of clonidine in several tissues and correlated these with plasma levels of clonidine.

Male rats were pithed and respired with 100% O_2. Gallamine (20 mg/kg) was given except where cardioaccelerator responses were examined (Docherty & McGrath, 1977a). Arterial blood pressure, heart rate and, where appropriate, longitudinal isometric tension of anococcygeus and vas deferens (Gillespie & McGrath, 1973, 1974) were monitored. The optimal sympathetic outflow for each tissue was stimulated via the pithing rod (0.05 – 1 ms, supramaximal pulses) (Gillespie & McGrath, 1973, 1974; Docherty & McGrath, 1977a). Blood samples were taken at 1, 5, 15 and 40 min after clonidine injection and plasma was assayed for clonidine (Draffin, Clare, Murray, Bellward, Davies & Dollery, 1976).

The rates of onset of the effects of clonidine were tested while continuously stimulating the cardiac sympathetic nerves at 0.1 Hz. Under these conditions the heart rate is elevated to a sub-maximal plateau but no endogenous α-adrenoceptor feedback is present (Docherty & McGrath, 1977b). The onset of both cardiac inhibitory and pressor effects were as rapid as could be expected from equilibration of clonidine with the tissues. In fact the fall in heart rate caused by clonidine was as quick as could be accomplished by ceasing electrical stimulation.

The duration of clonidine's effects were studied while applying intermittent sympathetic stimulation. The inhibitory effects against responses mediated by adrenergic nerves in heart, anococcygeus and vas deferens had similar time courses e.g. the tachycardia to a single pulse was reduced to 1.7% of control in 0.5 min by clonidine (5 μg/kg) and had recovered to 50% of control by 25 minutes. The decline in plasma levels of clonidine correlated well with recovery of