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PRELIMINARY COMMUNICATIONS

THE EFFECT OF A SERIES OF CLONIDINE ANALOGUES ON [^{3}H] CLONIDINE BINDING IN RAT CEREBRAL CORTEX

B. Jarrott, W.J. Louis and R.J. Summers[1]

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

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Many clonidine analogues have peripheral effects similar to the parent compound but are less potent central antihypertensives [1]. Factors which are important in the production of an antihypertensive effect include absorption into the circulation, metabolism, passage into and distribution within the central nervous system and potency on central α adrenoceptors. We have examined the last factor using membranes prepared from rat cerebral cortex in which [^{3}H] clonidine binding has characteristics which suggest binding to an α adrenoceptor [2].

Cerebral cortex was removed from Wistar rats [3] (175-225 g ♂ or ♀) and membranes prepared by homogenisation and centrifugation [2]. Washing and resuspension of the membranes was carried out in 1 mM $MgCl_2$. Membrane suspension (1 ml) was incubated with an equal volume of 50 mM Tris buffer pH 7.6 and [^{3}H] clonidine (5.29 Ci/mmol) in a concentration of ~2.5 nM (Scatchard & Hill analysis) or ~5 nM (3 x K_d, drug displacement experiments) for 30 min at 25°C. After filtration [2] the membranes were washed with ice cold 50 mM Tris pH 7.6 containing 1 μM clonidine. [^{3}H] clonidine remaining bound to the membranes, was eluted into toluene based scintillant and counted using standard scintillation counting techniques. Specific binding was defined as the difference between total binding and that in identical samples containing either 1 μM clonidine or phentolamine, both of which gave similar values. Specific binding represented 89.8 ± 0.7% of total binding in Scatchard and Hill analysis and 73.8 ± 1.1% in drug displacement studies.

Scatchard analysis of binding showed that in the concentration range used [^{3}H] clonidine bound to a single class of sites with a dissociation constant (K_d) of 1.7 ± 0.1 nM and maximum number of binding sites 9.4 ± 0.6 p moles/g (n = 5). This K_d is lower than that previously found [2] but may be analogous to the

1. Present Address: Department of Pharmacology, Glasgow University, Glasgow G12 8QQ, Scotland.

K_d of low affinity binding in experiments using much higher specific activity [^{3}H] clonidine (U'Prichard, personal communication). The number of binding sites was similar to that previously reported [2].

Hill plots were linear with a gradient of 0.97 ± 0.05 (n = 5) indicating the absence of cooperativity.

The drugs examined for their effect on binding fall into 3 groups. Clonidine metabolites, clonidine analogues which have peripheral effects similar to the parent drug but little antihypertensive effect, and a group of drugs said to be 'clonidine like' in their actions. The K_i (inhibition constant) for each drug was obtained from the IC_{50} derived from the inhibition curve [4].

Table 1. The effect of clonidine analogues on binding of [^{3}H] clonidine in membranes from rat cerebral cortex.

Drug	IC_{50}nM	K_inM	pK_a	Partition Coefficient Octanol/ buffer pH 7.4
clonidine, ST155,2-(2,6-dichlorophenylimino) imidazolidine HCl	8.4 ± 0.4	2.2 ± 0.2	8.05	9.3
4 hydroxyclonidine, ST666,2-(2,6-dichloro-4 hydroxyphenylimino) imidazolidine	83.4 ± 21.3	19.0 ± 4.8	7.95	0.6
HB5439-175A, 2, 6-dichlorobenzylguanidine	480 ± 154	111 ± 45	8.39	-
naphazoline, 2-(1-naphthylmethyl) 2 imidazolidine	8.5 ± 3.3	2.0 ± 0.8	10.35	0.7
ST600, 2-(2 methyl, 5-fluorophenylimino) imidazolidine	19.8 ± 4.2	4.6 ± 0.9	9.98	0.3
ST91, 2-(2,6 diethylphenylimino) imidazolidine	53.6 ± 18.8	12.4 ± 4.2	10.60	0.1
guanabenz, WY8678, 2,6 dichlorobenzylidene aminoguanidine acetate	3.3 ± 0.8	0.9 ± 0.2	8.10	18.4
Bay$_a$ 6781, 2-(2 methyl-6 ethyl-cyclohexyl-amino) 2 oxazoline	5.4 ± 1.8	1.2 ± 0.4	-	39.0
guanfacine, BS100-141, N-amidino-2-(2,6, dichlorophenyl) acetamide	8.2 ± 1.2	1.9 ± 0.5	7.10	11.0
lofexidine, 2-(α [2,6-dichlorophenoxy] ethyl) 2 imidazoline	9.9 ± 1.0	2.3 ± 0.2	9.28	4.4
xylazine, 5,6,dihydro-2-(2,6, xylidino) 4H. 1,3 thiazine	218 ± 64	48 ± 14	7.19	8.6
FLA136, 4 amino-3-(2,6, dichlorobenzyl-idenehydrazino) 1,2,4,-triazol.	>10,000	>10,000	4.20	81.6

The figures are the means of 3-4 experiments each performed in triplicate ± S.E.M. The apparent partition coefficient was measured as described by Timmermans and van Zwieten [5].

The clonidine metabolites 4 hydroxyclonidine and HB5439-175A (2,6 dichlorophenylguanidine) were 1/10th and 1/50th the potency of the parent compound in displacing binding as shown in Table 1. Since the metabolites have similar pK_a's and hence are ionised to a similar degree to clonidine at physiological pH it is likely that they contribute little to the pharmacological actions of clonidine mainly due to their lower affinity for the central α adrenoceptor.

The imidazoline derivatives, naphazoline, ST600, and ST91 all effectively displaced [^{3}H] clonidine

binding. Naphazoline and ST91 are known to be powerful vasoconstrictors by actions on peripheral α adrenoceptors but have little central hypotensive action whereas ST600 has little peripheral vasoconstrictor action but is a centrally acting antihypertensive with about 1/5th the potency of clonidine [1]. Naphazoline was about equipotent to clonidine in terms of displacement of binding. It is likely that its lack of antihypertensive potency is due to the high pK_a, degree of ionisation and low fat solubility of the compound thus hindering passage across the blood brain barrier [1]. ST600 displaced binding but was about 2½ times less potent than clonidine. As this compound has similar physical properties to naphazoline it is difficult to explain why its in vivo antihypertensive potency should be similar to that in displacing [^{3}H] clonidine binding. It is possible that sufficient passes the blood brain barrier to produce an effect or that the antihypertensive effect is not entirely centrally mediated. The results with ST91 were very interesting in that they show that this drug was 1/6th the potency of clonidine as a displacer of binding yet it is known to be 2½ times more potent as a peripheral vasoconstrictor [1]. This might suggest that the central and peripheral α adrenoceptors differ and that one cannot use an action on peripheral α adrenoceptors to predict central hypotensive activity, even if such factors as pK_a and partition coefficient are taken into account [5].

The remaining drugs are a selection of compounds said to have 'clonidine like' actions. Both guanabenz and Bay_a 6781 were more than twice as potent as clonidine as displacers of binding and this would agree with what is known about their antihypertensive activity and ability to inhibit nerve evoked responses of the sympathetic nervous system [6, 7]. Guanfacine and lofexidine had similar potency to clonidine as displacers of binding. The results with guanfacine are worth discussion since this drug is known to be only 1/10th the potency of clonidine in vivo both in animals [8] and in man. There would appear to be no problem as regards access to the central nervous system as guanfacine has similar physical properties to clonidine. A possible explanation is that the combination of guanfacine with the receptor does not produce the same degree of response as clonidine, i.e. its efficacy is lower. Lofexidine, like guanfacine, is less effective in vivo [9] possibly for the same reason, but its higher pK_a and lower fat solubility may also hinder access to the CNS. Xylazine appears to have similar potency both as a displacer of binding and as an antihypertensive in vivo. FLA 136 is said to be clonidine-like in its ability to decrease noradrenaline turnover in the central nervous system [10]. The indication from this study is either that this effect is not mediated through central α adrenoceptors or that the binding of clonidine to rat cortical membranes is not to the same α adrenoceptors as those acted on by FLA 136.

In conclusion this type of radioligand assay provides a useful method for examining the properties of central α adrenoceptors. The main advantage of the method is that there are no access barriers to prevent interaction of drugs with the receptor. The main disadvantage would appear to be that the method may be

a good guide to the affinity with which the drug interacts with the receptor but this does not necessarily predict the effectiveness of the drug in producing a biological response.

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