Biochemical Pharmacology, Vol. 29, pp. 1869-1870.
Pergamon Press Ltd. 1980. Printed in Great Britain.

MULTIPLE BINDING SITES IN HUMAN BRAIN FOR [^{3}H]-CLONIDINE AND [^{3}H]-WB-4101

Pnina Weinreich,* John Deck† and Philip Seeman*

Departments of *Pharmacology and †Pathology,
University of Toronto,
Toronto, Canada M5S 1A8

(Received 12 March 1980; accepted 10 April 1980)

Although rat brain alpha-adrenergic receptors can be labeled by the agonist ^{3}H-clonidine (for α_2 receptors) and by the antagonist ^{3}H-WB-4101 (for α_1 sites; WB-4101 is 2-([2', 6'-dimethoxy]-phenoxyethylamino)methylbenzodioxan) (1-5), the present study was done to determine whether these ^{3}H-ligands could successfully identify alpha-adrenergic receptors in post-mortem brain.

[^{3}H]-Clonidine (22.2 Ci/mmole) and [^{3}H]-WB-4101 (23 Ci/mmole) were obtained from the New England Nuclear Corp. (Boston, MA). Post-mortem neurologically normal brains were from individuals 22 to 91-years-old. The interval between death and freezing of the brains was 3-25 hr. The causes of death were car accidents, pneumonia, myocardial infarction, bronchogenic carcinoma or exposure. Brain areas were dissected from frozen slices; non-frontal cerebral cortex was defined as all cerebral cortex other than frontal cortex.

The binding sites for [^{3}H]-WB-4101 and [^{3}H]-clonidine in the human frontal cortex were tested for their rank order of sensitivity to various drugs. The drug concentrations that inhibited the binding of these ^{3}H-ligands by 50 percent (IC_{50} values) were averaged from triplicate determinations on at least three separate human brains. The binding of [^{3}H]-WB-4101 or [^{3}H]-clonidine was defined as that which could be inhibited by 1 μM WB-4101 or clonidine, respectively (see general methods in Ref. 4).

As shown in Table 1, the rank order of drug action on the [^{3}H]-WB-4101 binding site indicated that this ^{3}H-ligand bound to an α_1 type of adrenoceptor in the human frontal cortex (cf. Refs. 1-3). It was found, moreover, that the concentration-inhibition graphs for prazosin, phentolamine and WB-4101 were all biphasic in inhibiting the binding of [^{3}H]-WB-4101. The fact that these drugs yielded two distinct IC_{50} values (see Table 1) for [^{3}H]-WB-4101 suggested that this ^{3}H-ligand bound to at least two sub-types of α_1 adrenoceptors, an observation not previously reported.

The rank order of drugs which inhibited the binding of [^{3}H]-clonidine to the human frontal cortex homogenates revealed a pattern of the α_2 type of adrenoceptor (cf. Refs. 1-3). In addition, it was observed that phentolamine inhibited the binding of [^{3}H]-clonidine in two phases, one with an IC_{50} of 2.8 nM and the other having a very high IC_{50} value (see Table 1); these data suggested that [^{3}H]-clonidine might bind to more than one type of site. Also, since the amount of [^{3}H]-clonidine inhibited by 1 μM clonidine was only 20 percent of the total binding, while phentolamine, epinephrine and norepinephrine inhibited about 70 percent of the total [^{3}H]-clonidine binding, this further indicated two types of binding sites for [^{3}H]-clonidine. Finally, the inhibition of [^{3}H]-clonidine by clonidine itself revealed a Hill coefficient of 0.58, a very low value compatible with the ^{3}H-ligand having more than one binding site.

Table 1. Drug inhibition of the binding of [^{3}H]-WB-4101 and [^{3}H]-clonidine to human frontal cortex homogenates

	IC_{50} (nM)	
	[^{3}H]-WB-4101	[^{3}H]-Clonidine
Prazosin	1.5 7,500	>1,000
Phentolamine	7.5 >10,000	2.8 >10,000
WB-4101	9.8 38,000	50
Phenoxybenzamine	50	-
Epinephrine	660	6.6
Clonidine	3,600	13
Norepinephrine	4,500	5.6

It appears that the high-affinity binding site for [^{3}H]-clonidine may be the functional α_2 receptor, since epinephrine and norepinephrine only inhibited the binding to that site.

Scatchard analysis of [^{3}H]-clonidine binding revealed biphasic curves with the largest amount of high- and low-affinity binding in the hypothalamus, followed by frontal cortex, hippocampus and parieto-occipital cortex (Table 2).

Table 2. Scatchard analysis of [^{3}H]-clonidine binding in different areas of the human brain

Region	Affinity type	K_D (nM)	B_{max} (fmoles/mg protein)
Hypothalamus	High	1.9	51
	Low	12.3	158
Frontal cortex	High	0.98	32
	Low	8.4	118
Hippocampus	High	0.77	26
	Low	8.3	86
Non-frontal cerebral cortex	High	0.5	10
	Low	4.6	59

The present [^{3}H]-WB-4101 and [^{3}H]-clonidine IC_{50} values in the human brain are consistent with those found in the calf and rat brain (2).

This study indicates the existence of at least two types of binding sites for [^{3}H]-clonidine as well as for [^{3}H]-WB-4101 in the human brain. Future research must elucidate the functional roles of these multiple sites. For example, some brain diseases (e.g. essential hypertension) may possibly be associated with abnormal properties of either the post-synaptic α_1 or the autoreceptor α_2 sites.

Acknowledgements: We thank Anna Banaszuk for technical assistance. This work was supported by the Ontario Mental Health Foundation and the Medical Research Council of Canada.

REFERENCES

1. S.J. Peroutka, D.A. Greenberg, D.C. U'Prichard and S.H. Snyder, Molec. Pharmac. 14, 403 (1977).
2. D.C. U'Prichard, W.D. Bechtel, B.M. Rouot and S.H. Snyder, Molec. Pharmac. 16, 47 (1979).
3. D.C. U'Prichard, D.A. Greenberg and S.H. Snyder, Molec. Pharmac. 13, 454 (1977).
4. M. Titeler and P. Seeman, Proc. Natl. Acad. Sci. U.S.A. 75, 2249 (1978).
5. J. Vetulani, M. Nielsen, A. Pilc and K. Golembiowska-Nikitin, Eur. J. Pharmac. 58, 95 (1979).