AFFINITY OF VARIOUS ANTIDEPRESSANTS FOR IMIPRAMINE RECEPTORS OF MOUSE BRAIN SYNAPTIC MEMBRANES

V. V. Rozhanets, D. Yu. Rusakov, and A. V. Val'dman

UDC 615.214.32.015.44:612.822.1.014.2

KEY WORDS: [^{3}H]imipramine; synaptic membranes; antidepressants.

The neurochemical mechanisms of action of substances belonging to different groups of antidepressants have been studied by no means equally. Besides substances which are basically monoamine oxidase inhibitors, it is the tricyclic antidepressants which have been studied most fully. For instance, specific high-affinity binding sites for these compounds (especially, for [^{3}H]imipramine) have been found in the brain and platelets of animals – so-called imipramine receptors [2]. Evidence has been obtained in support of the identity of receptors for tricyclic antidepressants and sites of reassimilation of serotonin and noradrenalin [3, 4]. A neurochemical correlate of the chronic administration of these antidepressants also has been found, expressed as a sharp decrease in the number of imipramine receptors in rat brain membranes [7]. All this suggests that activity directed toward imipramine receptors is in fact related to the clinical effects of tricylic antidepressants. So far as the heterogeneous group of what are called atypical antidepressants is concerned, the neurochemical mechanisms of their action have received little study.

The aim of this investigation was to compare the affinity of several typical and atypical antidepressants for the imipramine receptor and to use this model to study two new potential antidepressants.

EXPERIMENTAL METHOD

Tetrahybrid male CBWA mice weighing 20-22 g were used. After decapitation of the animals the brain was quickly removed and placed in cold isolation medium (0.32 M sucrose, 10^{-3} M EDTA in 50 mM Tris-HCl buffer, pH 7.4); a 10% homogenate of the washed tissue was prepared manually by means of a glass homogenizer with Teflon pestle. Subsequent procedures were carried out in the cold. The supernatant after sedimentation of the P_1 fraction (2000g, 10 min) was recentrifuged at 15,000g for 30 min. To produce lysis of the synaptosomes the residue of fraction P_2 was suspended in 50 mM Tris-HCl buffer, pH 7.4, and frozen overnight at −20°C. The suspension was then thawed at 10°C, centrifuged (10,000g, 10 min), and the residue was again suspended in the same buffer (2 ml to 1 g of orginal tissue), and frozen. This procedure of washing the synaptic membranes was repeated four times; the residue was then suspended in incubation medium (120 mM NaCl, 5 mM KCl in 50 mM Tris-HCl buffer, pH 7.4), frozen in small portions, and kept for two weeks. The medium for studying binding consisted of: 0.25 ml [^{3}H]imipramine (from Amersham Corporation, England) in 0.05% sodium azide solution; the concentration of [^{3}H]imipramine (23 Ci/mmole) in this solution was 8.6 nM; 0.1 ml H_2O or aqueous solution of the test substance; 0.65 ml of a suspension of washed membranes in incubation medium with a protein content of 1.5-2.5 mg. The reaction was started by the addition of the membrane suspension. After incubation for 30 min at 0° C, 4.0 ml of cold incubation medium was added to each sample and the suspension was quickly filtered in vacuo through a glass wool filter (GF/B), from Whatman, England). Filtration and the next two washings with 4.0 ml of incubation medium took no more than 15 sec. The radioactivity of the dried filters in Bray's scintillator were determined by means of a counter from Intertechnique, France. The protein concentration was determined by a modified Lowry's method [6]. Commercial preparations of chlorimipramine and demethylimipramine, pyrazidol (synthesized at the S. Ordzhonikidze All-Union Pharmaceutical Chemical Research Institute), befuraline, maclobamide, and new derivatives – morpholine (M1) and benzofuran (B1), synthesized at the Institute of Pharmacology, Academy of Medical Sciences of the USSR (V. A. Zagorevskii), were used in the investigation.

Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 10, pp. 42-43, October, 1982. Original article submitted May 23, 1982.

 0007-4888/82/9410-1358$07.50

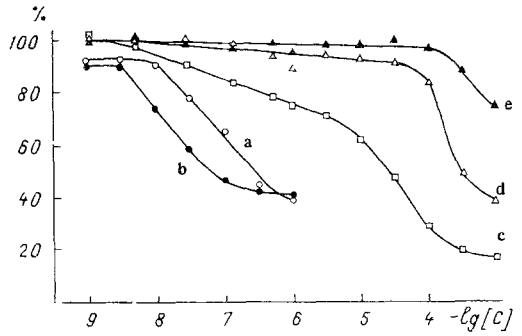

Fig. 1. Competition between various preparations and [^{3}H]imipramine for binding with mouse brain synaptic membrane. Abscissa, negative logarithm of concentration of test preparations; ordinate, total binding of [^{3}H]-imipramine (in percent). a) Chlorimipramine; b) demethylimipramine; c) pyrazidol; d) the new benzofuran derivative B1; e) the new morpholine derivative M1. Data for known benzofuran and morpholine derivatives befuraline and maclobamide respectively agree completely with data for B1 and M1 and are not given in Fig. 1. Mean results of two measurements shown. Error of measurements nowhere exceeded 5%.

TABLE 1. Comparison of Affinity of Different Antidepressants for Imipramine Receptors of Mouse Brain Membranes

Compound	Concentration giving 50% inhibition (C_{50}), nM
Chlorimipramine	11
Demethylimipramine	60
Pyrazidol*	11 000
Befuraline	>100 000
B-1	110 000
Maclobamide	>1 000 000
M-1	>1 000 000

Legend. Concentrations of antidepressants given at which specific binding (difference between total and nonspecific binding of [^{3}H]imipramine) was inhibited by 50%. Asterisk indicates that values obtained with 10^{-3} M pyrazidol were used to obtain the nonspecific binding.

EXPERIMENTAL RESULTS

Binding of ^{3}H-imipramine under the experimental conditions used was just as rapid and reversible as has been described for rat brain membranes [7]. Equilibrium was established after 10 min. The addition of 10^{-6} M chlorimipramine after this period led in the course of the next 10 min to a decrease in radioactivity down to the nonspecific binding level. Nonspecific binding of [^{3}H]imipramine, measured in the presence of 10^{-6} M chlorimipramine or demethylimipramine, amounted to about 40%, in good agreement with data obtained for rat brain membranes [5]. It was found that not less than half of the radioactivity remaining on the filters under nonspecific binding conditions was due to adsorption of the label on the material of the filters.

It follows from the data given in Fig. 1 and Table 1 that chlorimipramine and demethylimipramine were the most active competitors of [^{3}H]imipramine for binding with mouse brain synaptic membranes. Pyrazidol also was a very active competitor. It will be noted that this antidepressant, in high concentrations (0.1-1.0 mM),

was able to displace an additional amount of [^{3}H]imipramine, an effect attributable to its interaction with low-affinity (or nonspecific) binding sites.

The benzofuran and morpholine derivatives befuraline and maclobamide, like the new derivatives of these compounds B1 and M1, according to the results of these experiments, virtually do not compete with [^{3}H]-imipramine for its receptors.

Comparison of the results obtained with data in the literature on the activity of compounds studied as monoamine oxidase inhibitors [1, 8] leads to the conclusion that relations between the parameters of these substances as revealed by the two tests used were reciprocal. For instance, these compounds occupy the following order of descending ability to inhibit monoamine oxidases: morpholine derivatives > pyrazidol > tricyclic antidepressants. Pyrazidol, incidentally, had comparable values of concentrations giving inhibition by 50% (IC_{50}), namely about 10^{-5} M.

On the whole it can be concluded from these results that, unlike tricyclic compounds, the atypical antidepressants have very weak affinity for imipramine receptors of mouse brain synaptic membranes. The therapeutic effect of the atypical antidepressants is evidently linked with their action on other receptor or enzyme systems of the brain.

LITERATURE CITED

1. M. D. Mashkovskii and V. Z. Gorkin, Byull. Éksp. Biol. Med., No. 2, 169 (1981).
2. A. Biegon and D. Samuel, Biochem. Pharmacol., 28, 3361 (1979).
3. S. Z. Langer, C. Moret, R. Raisman, et al., Science, 210, 1133 (1980).
4. C. -M. Lee and S. H. Snyder, Proc. Natl. Acad. Sci. USA, 78, 5250 (1981).
5. S. M. Paul, M. Rehavi, K. C. Rice, et al., Life Sci., 28, 2753 (1981).
6. G. L. Peterson, Anal. Biochem., 83, 346 (1977).
7. R. Raisman, S. Briley, and S. Z. Langer, Eur. J. Pharmacol., 61, 373 (1980).
8. G. A. Roth and C. N. Gillis, Mol. Pharmacol., 11, 28 (1975).

EFFECT OF DIAZEPAM ON REACTIVITY OF HIPPOCAMPAL NEURONS DURING BLOCKADE OF THE GABA-ERGIC SYSTEM

P. G. Glushankov, S. L. Buldakova, V. G. Skrebitskii, and S. A. Saakyan

UDC 615.214.22:547.891.2].015.44:612.825.26

KEY WORDS: hippocampal slices; evoked potential; diazepam; bicuculline; picrotoxin.

The benzodiazepines (BD) are a group of compounds widely used in medical practice as tranquilizers and anticonvulsants. In recent years great attention has been paid to interaction between the BD and the GABA system. It has been shown, for instance, that BD potentiate the action of GABA, both liberated synaptically and extrinsically applied, on mammalian neuronal preparations [3, 6, 9].

The limbic system is evidently one of the principal structures of the CNS responsible for the clinical manifestation of the action of BD. We know that there is a high density of benzodiazepine receptors in the limbic system [11] and that GABA is the inhibitory mediator in this region of the brain [5]. Investigations conducted on formations of the mammalian limbic system have shown that BD inhibit both spontaneous and evoked unit activity [1, 4, 7]. Potentiation of GABA-inhibition is the presumed mechanism of the inhibitory effect of BD.

Laboratory of Functional Synaptology, Brain Institute, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR V. S. Rusinov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 10, pp. 44-46, October, 1982. Original article submitted May 31, 1982.

0007-4888/82/9410-1360$07.50