EFFECT OF VARIOUS ANTIDEPRESSANTS ON BRAIN MONOAMINE OXIDASE ACTIVITY

E. M. Gankina, T. A. Moskvitina,
V. Z. Gorkin, and A. V. Val'dman

UDC 615.214.32.015.4:612.822.1.015.11:
577.152.14

KEY WORDS: monoamine oxidase; antidepressants; imipramine; iproniazid.

According to the present classification antidepressants can be subdivided, depending on their mechanism of action, into two classes: monoamine oxidase inhibitors (MAO; acetylene, hydrazine, and cyclopropinylamine derivatives) and inhibitors of reassimilation of monoamines from the synaptic space (tricyclic antidepressants). However, contrary to the traditional classification there is evidence that many tricyclic antidepressants inhibit brain MAO activity [8], and that typical hydrazine MAO inhibitors depress monoamine uptake [10]. New antidepressant drugs, similar in structure neither with the tricyclic antidepressants nor the classical MAO inhibitors, but capable of exhibiting some degree of antimonoamine-deaminase activity or of inhibiting reassimilation of neurotransmitters in the brain, have recently appeared [5, 6]. Not only antidepressants, but also neuroleptics and psychostimulants possess the property of inhibiting brain MAO, as the results of many investigations have shown [7, 8].

To explain the mechanism of action of certain antidepressants their antimonoamine-oxidase activity was investigated. Among the compounds studied were some familar preparations — imipramine, pyrazidol, inkazan, viloxazine, zymelidine, iproniazid, and moclobamide, and also some potential therapeutic preparations, namely the hydrazine derivatives Nos. 33 and 34.

EXPERIMENTAL METHOD

Fragments of mitochondrial membranes from bovine brain were used as the source of enzyme [1]. The velocity of the enzyme reaction was judge by the quantity of ammonia removed from the substrate, and determined by isothermic diffusion followd by nesslerization. Fragments of mitochondrial membranes in a dose equivalent to 3 mg protein were added to the samples together with one of the following substrates: 4.4 mM serotonin, 0.8 mM 2-phenylethylamine, and 0.1 M K,Na-phosphate buffer, pH 7.4, and MAO activity was determined after incubation of the samples for 30 min in an atmosphere of oxygen at 37°C with shaking. The reaction was stopped by addition of 50% TCA. Treatment with the inhibitor was carried out without preliminary incubation. The inhibitors were dissolved in water or in 96% ethanol, and in the latter case they were diluted 100 times with 0.1 M phosphate buffer, pH 7.4, and added to the samples. The alcohol concentration was accounted for in the control. Protein was determined by Lowry's method.

EXPERIMENTAL RESULTS

Various compounds possessing antidepressant properties were studied. The "specific" substrate of type A MAO (serotonin) and the specific substrate of type B MAO (2-phenylethylamine) were used [9]. Inhibitors were used in concentrations of 10^{-4}-10^{-5} M. As Table 1 shows, imipramine and iproniazid — inhibitors of different classes — possessed the highest antimonoamine-oxidase activity of all the compounds tested, but imipramine inhibited deamination of 2-phenylethylamine more selectively, whereas iproniazid had the same property with respect to serotonin deamination. These results agree with those obtained by other workers [3, 8]. Thus not only do antidepressants which are MAO inhibitors actively affect the velocity of the deaminase reaction, but antidepressants which are inhibitors of monoamine uptake also possess this same property in full measure.

Two tetracyclic antidepressants, pyrazidol and inkazan, as was shown previously, inhibit rat brain MAO activity considerably [2, 4]; the results of the present experiments confirm

Institute of Pharmacology, Academy of Medical Sciences of the USSR. Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 11, pp. 29-31, November, 1982. Original article submitted June 1, 1982.

0007-4888/82/9411-1499$07.50

TABLE 1. Effect of Different Antidepressants on Bovine Brain MAO Activity (M ± m, n = 4)

Drug	Concentration, M	Inhibition of MAO, %	Type of MAO
Control	—	0	A, B
Iproniazid	10^{-5}	87±13	A
		40±4	B
Imipramine	10^{-5}	55±5	A
		62±6	B
Pyrazidol	10^{-5}	77±7	A
		50±5	B
Inkazan	10^{-5}	70±5	A
		50±7	B
Viloxazine	10^{-4}	1±0,7	A
		16±1	B
Zymelidine	10^{-4}	5±0,1	A
		30±2	B
Norzymelidine	10^{-4}	30±3	A
		39±4	B
Moclobamide	10^{-5}	52±5	A
		11±1	B
No. 33	10^{-4}	31±3	A
		20±2	B
No. 34	10^{-4}	21±2	A
		38±2	B

Legend. Activities taken as 100%: 1.95 ± 0.05 nmoles serotonin/mg protein/min and 2.78 ± 0.04 nmoles 2-phenylethylamine/mg protein/min.

this fact for bovine brain MAO. These preparations exhibit some degree of selectivity relative to serotonin deamination but they actively reduce the velocity of the 2-phenylethylamine-deaminase reaction also.

The morpholine derivatives studied, despite their structural similarity, differed in their effects on bovine brain MAO activity. Viloxazine, for instance, virtually did not inhibit the deamination of either substrate, whereas moclobamide proved to be an active and highly selective inhibitor of type A MAO. In its antiserotonin-deaminase activity moclobamide was no better than imipramine and was inferior to iproniazid, pyrazidol, and inkazan; however, unlike the latter, it did not affect deamination of 2-phenylethylamine.

It will be clear from Table 1 that zymelidine had virtually no effect on the deamination of serotonin, but its metabolite, norzymelidine, exhibited some degree of antiserotonin-deaminase activity; deamination of 2-phenylethylamine was inhibited about equally by both compounds. It may be that zymelidine acquires the property of inhibiting MAO in the course of its metabolism in the body in mammals.

According to the results of this investigation hydrazine derivatives Nos. 33 and 34 inhibited bovine brain MAO moderately. The action of these compounds was practically identical as regards selectivity toward the reaction substrate.

It can be concluded from analysis of the results that ability to inhibit brain MAO is a property of antidepressants of different structures, but in most cases selectivity in the inhibition of different types of MAO is not observed. The morpholine derivative moclobamide, on the other hand, inhibits type A MAO activity highly selectively. This property does not extend to the other morpholine derivative viloxazine, which has no effect in general on MAO activity. On the basis of the facts described above it can be postulated that a definite role in the mechanism of action of certain antidepressants (imipramine, iproniazid, pyrazidol, inkazan, moclobamide) is played by the antimonoamine-oxidase activity which they exhibit. As regards the other compounds tested, a more important role in the mechanism of their specific effect is evidently played by their influence on other stages of monoaminergic transmission. However, taking all the foregoing facts into consideration, it will be evident that the traditional classification of the antidepressants is not entirely in harmony with the results of recent research.

LITERATURE CITED

1. É. Ya. Baumanis, I. É. Kalninya, T. A. Moskvitina, et al., Biokhimiya, 43, 1496 (1978).
2. L. G. Vasil'evykh, R. G. Glushkov, V. Z. Gorkin, et al., Khim.-farm. Zh., No. 7, 20 (1979).
3. V. Z. Gorkin, Amine Oxidases and Their Importance in Medicine [in Russian], Moscow (1981).
4. M. D. Mashkovskii, V. Z. Gorkin, I. V. Verevkina, et al., Byull. Éksp. Biol. Med., No. 2, 169 (1981).
5. T. P. Blackburn, G. A. Foster, D. T. Greenwood, et al., Eur. J. Pharmacol., 52, 357 (1978).
6. A. Coppen and V. A. Rama Rao, Psychopharmacology, 63, 125 (1979).
7. A. L. Green and M. A. S. Hait, J. Pharm. Pharmacol., 30, 262 (1978).
8. J. A. Roth and C. N. Gillis, Mol. Pharmacol., 11, 28 (1975).
9. R. F. Squires, Adv. Biochem. Pharmacol., 5, 355 (1972).
10. R. J. Ziance, K. Moxley, M. Mullis, et al., Arch. Int. Pharmacodyn., 228, 30 (1977).

SELECTIVE LOSS OF HISTONE H1 — A NEW CHROMATIN RESPONSE TO ADDED TOTAL HISTONE IN A MEDIUM OF PHYSIOLOGICAL IONIC STRENGTH

V. D. Paponov, P. S. Gromov, and D. M. Spitkovskii

UDC 612.014.22:576.315.42

KEY WORDS: chromatin; histones.

Analysis of the chemical composition of chromatin in most eukaryote cells gives a histone/DNA ratio close to 1 [6]. However, there is some evidence that this ratio in the nuclei of some cells may reach 3.3 [5], or even 2000 [9]. Moreover, in all proliferating cells a local rise in the histone/DNA ratio can be expected, if only temporarily, in the case of addition of extra histone octamers to individual nucleosomes in the S phase [14].

To analyze the consequences of such situations, when the cell chromatin comes into contact with an extra quantity of histones, the effect of total histone of calf thymocyte chromatin on chromatin isolated from these same cells in a medium of physiological ionic strength was studied. It was shown previously [14] that under these conditions chromatin can bind a limited number of extra histones. A new type of response of chromatin to added histone was discovered, namely selective loss of its own histone H1.

EXPERIMENTAL METHOD

Chromatin was obtained [15] by washing tissue homogenate in a medium consisting of 0.025 M EDTA–NO_2 + 0.075 M NaCl (pH 8.0). After five washings the chromatin suspension was dispersed in a solution of physiological ionic strength consisting of 0.15 M NaCl + 0.7 mM Na-phosphate buffer (pH 7.0) + 1 mM PMSF. The total histone of the chromatin was extracted with 0.4 N HCl from the same chromatin preparation obtained previously (30 min at 0°C), after which the extract was dialyzed against 0.9 N CH_3COOH and lyophilized.

Preparations of chromatin (C_{DNA} = 25 μg/ml) were mixed with equal volumes of solutions of total histone of different concentrations in an appropriate solvent. The mixtures were incubated for 48 h at 4°C, the stand with the tubes being slowly rotated to prevent the suspension from being thrown down. The mixtures were then centrifuged for 30 min on an L2-65B centrifuge (Beckman, USA; 40.3 rotor) at 40,000 rpm. The residues and walls of the tubes were washed with distilled water. The residues were then dispersed in water by means of an ultrasonic disintegrator and used for electrophoretic analysis of the protein composition of the nucleoprotein complexes formed in mixtures of chromatin and total histone. Electrophoresis was carried out by Laemmli's method [7]. Densitometry of the polyacrylamide gels, stained with Coomassie blue R-250, was carried out on a spectrophotometer (Gilford, England) at 580 nm.

Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 11, pp. 31-33, November, 1982. Original article submitted May 4, 1982.

0007-4888/82/9411-1501$07.50