SEROTONIN UPTAKE BY HUMAN PLATELETS AS A METHOD OF SCREENING ANTIDEPRESSANTS

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UDC 615.214.32 .015.45:612.111.7.018.577.175.823

Antidepressants of the imipramine group considerably inhibit serotonin uptake by human platelets in concentrations of 10^{-7} to 10^{-5} M. Of the neuroleptics, only haloperidol (10^{-5} M) significantly inhibits serotonin uptake by platelets. Amphetamine and cholinolytics do not affect serotonin uptake by platelets even in a concentration of 10^{-4} M. The specificity of the effect of the antidepressants confirms the view that their thymoanaleptic action is an indirect effect brought about through activation of serotoninergic processes.

KEY WORDS: screening; antidepressants; neuroletpics; serotonin transport through membranes; platelets.

Manifestations of the action of antidepressants are associated with their central adrenergic and serotoninergic effects [3, 5]. Many methods of screening the adrenergic action of drugs have been developed [1]. However, only a few of these methods can reveal the serotoninergic properties of psychotropic drugs [4]. These methods record changes in the behavior of animals that are considered to be connected with the inhibition of serotonin transport from the synaptic space into the presynaptic nerve ending, leading to an increase in the number of serotinin molecules acting on postsynaptic receptors [7]. Direct measurement of serotinin-H^3 transport through presynaptic membranes (synaptosomes of rat brain tissue) has been suggested as a test for antidepressant screening [9].

Considering the species differences in the action of drugs on transmembrane serotonin transport (TST) and also the similarity of the processes of serotinin transport through presynaptic membranes of neurons and membranes of platelets [11], the possibility of using measurement of the rate of serotinin uptake by human platelets for antidepressant screening was investigated. For this purpose the effects of standard antidepressants of the imipramine group were compared with the effects of neuroleptics, cholinolytics, and amphetamine, similar to them in pharmacological action and chemical structure.

EXPERIMENTAL METHOD

In each experiment pooled samples of blood from 2 or 3 healthy blood donors were used. The method of taking the blood, obtaining platelet-rich plasma, and determining the serotonin concentration fluorometrically in the platelets was described previously [6]. Platelet-rich plasma was diluted (1 : 1) with a solution containing 2 mM $MgCl_2$, 20 mM KCl, 112 mM NaCl, and 20 mM tris-buffer (pH 7.4). The diluted plasma was distributed in samples of 1 ml among polyethylene tubes and incubated for 15 min with 1 μg serotonin. The preparations (0.1 ml of solutions made up in 0.9% NaCl from powders or ampule-packed forms) were added to the incubation medium 5 min before the serotonin. Two parallel tests were carried out with each concentration of the substance used. Incubation was stopped by cooling the samples to 1-4°C. The rate of TST was judged from the increase in the serotinin concentration during incubation (in % of its initial concentration, which was 0.17 ± 0.01 g μg serotinin/mg protein; n = 16). The protein concentration in the platelet residue was determined by Lowry's method [10]. Preliminary tests showed that none of the drugs, when incubated with plasma for 20 min, altered the serotonin concentration. The names and doses of the drugs

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 1, pp. 51-53, January, 1976. Original article submitted May 16, 1975.

TABLE 1. Effect of Psychotropic Drugs on Serotonin Uptake by Human Platelets (M ± m)

Drug	Concentration (in M)	Serotonin uptake by platelets (in % of initial concentration, Zn = 6	P
0,9% NaCl		180,77 ± 15,03 (26)	
Chlorimipramine	10^{-7}	34,17 ± 5,64	
"	10^{-5}	19,17 ± 3,94	
Imipramine	10^{-7}	144,65 ± 14,23	NS
"	10^{-6}	59,02 ± 2,28	
"	10^{-5}	16,50 ± 3,27	
Amitriptyline	10^{-7}	156,21 ± 12,45	NS
"	10^{-6}	106,66 ± 11,54	
"	10^{-5}	26,50 ± 4,52	
Dimethylimipramine	10^{-6}	141,33 ± 11,39	<0,05
"	10^{-5}	80,33 ± 8,76	
Nortriptyline	10^{-6}	151,50 ± 8,85	NS
"	10^{-5}	75,33 ± 12,03	
Stelazine	10^{-5}	144,50 ± 22,41	NS
"	10^{-4}	102,83 ± 10,92	
Chlorpromazine	10^{-5}	125,17 ± 14,82	<0,02
"	10^{-4}	40,83 ± 16,92	NS
Promazine	10^{-5}	166,17 ± 10,91	
"	10^{-4}	55,50 ± 5,96	
Levomepromazine	10^{-5}	151,33 ± 16,25	NS
"	10^{-4}	74,67 ± 10,52	
Haloperidol	10^{-5}	83,33 ± 8,83	
"	10^{-4}	25,33 ± 4,09	
Amphetamine	10^{-5}	155,00 ± 19,31	NS
"	10^{-4}	147,00 ± 18,11	NS
Benactyzine	10^{-5}	172,33 ± 14,42	NS
"	10^{-4}	191,83 ± 17,83	NS
Atropine	10^{-5}	187,33 ± 24,55	NS
"	10^{-4}	178,33 ± 20,35	NS
Scopolamine	10^{-5}	216,50 ± 27,05	NS
	10^{-4}	195,00 ± 21,78	NS

Legend. In every case unless specified to the contrary, $P < 0.01$; NS) differences not significant ($P > 0.05$).

are given in Table 1. The numerical results were subjected to statistical analysis [2].

EXPERIMENTAL RESULTS

During incubation of plasma with 1 μg serotinin for 15 min the serotonin content in the platelets increased amost threefold. Antidepressants with a marked thymoanaleptic (improving the mood) action, namely chlorimipramine, imipramine, and amitriptyline, considerably reduced this increase in the serotinin concentration even in low concentrations (10^{-7}–10^{-6} M). Their secondary derivatives, the clinical action of which is to abolish motor inhibition in depressive patients [3], were effective only in a higher concentration (10^{-5} M). Neither neuroleptics (except haloperidol) nor amphetamine or cholinolytics had any effect on TST in this concentration. Amphetamine and cholinolytics likewise had no effect in a higher concentration (10^{-4} M). The action of neuroleptics in this concentration, however, could be considered to be nonspecific, for they may have injured the structure of the platelets [13]. Inhibition of TST by haloperidol could perhaps depend on its ability to block α-adrenergic receptors, which are similar in structure to the serotonin receptors of platelets [12]. However, in the overall effect of haloperidol, its effect on postsynaptic receptors was predominant [8].

The results show that inhibition of TST is specific for the antidepressants. This result confirms the view that their thymoanaleptic effect is mediated through activation of central serotoninergic processes [3]. Estimation of serotonin uptake by platelets can be used to differentiate drugs belonging to the antidepressant group and also to distinguish between antidepressants and other classes of psychotropic drugs.

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