EFFECT OF PROTHIXENE AND ALLIED COMPOUNDS ON SEROTONIN UPTAKE BY HUMAN BLOOD PLATELETS

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The order of arrangement of drugs by diminishing inhibitory effect on serotonin (5-HT) uptake by platelets corresponds to the order of their distribution by diminishing thymo-analeptic action: chlorimipramine > imipramine > amitriptyline > desipramine—nortriptyline—chlorprothixene > chlorpromazine > norprothixene > prothixene-promazine. The change in the effect of the compound on 5-HT uptake by platelets caused by introduction of a chlorine atom into its structure or by demethylation of the compound is largely determined by the chemical structure of the original compound.

KEY WORDS: *platelets; transmembrane serotonin transport; thymo-analeptics; neuroleptics; antidepressants.*

The thymo-analeptic action of drugs is associated with an increase in the activity of central serotoninergic processes [1, 6, 7]. Tricyclic antidepressants of the imipramine group block serotonin (5-HT) transport from the synaptic space through the presynaptic membrane back into the nerve ending. This leads to an increase in the quantity of functionally active 5-HT, which acts on the corresponding receptors of the postsynaptic membrane [9]. The relationship between the chemical structure of the preparations and their effect on transmembrane 5-HT transport (TTHT) was investigated: tertiary (imipramine, amitriptyline) compounds inhibit TTHT more strongly than secondary (desipramine, nortriptyline); the blocking action of chlorimipramine is stronger than that of imipramine [12]. Chlorprothixene occupies an intermediate position in its pharmacological and clinical characteristics between neuroleptics and antidepressants [2]. The writers know of only one paper which describes an investigation of its effect on TTHT; ED_{50} for chlorprothixene was found to be 5×10^{-5} M, and for imipramine 0.05×10^{-5} M [13].

The object of this investigation was to study the effect of chlorprothixene, prothixene, and norprothixene (the demethylated derivative of prothixene) on TTHT compared with that of the chlorine derivative of imipramine and promazine and the demethylated derivatives of imipramine and amitriptyline.

EXPERIMENTAL METHOD

TTHT was estimated from the 5-HT uptake by platelets, for the effect of the drugs on 5-HT transport through the membrane of neurons is similar to their action on its transport through the platelet membrane [11]. Pooled blood from 2 or 3 clinically healthy donors was used in each experiment. The 5-HT content in the platelets was determined with the Hitachi spectrofluorometer by the method described previously [3].

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TABLE 1. Inhibition of Platelet 5-HT Uptake by Compounds (M ± m; n = 6)

Substance	Concentration in incubation medium		
	10^{-5} M	10^{-6} M	10^{-7} M
	% inhibition		
Chlorimipramine	92,34±1,98*	—	82,00±2,19*
Imipramine	92,45±1,87*	72,08±3,14*	19,83±3,55†
Desipramine	57,00±2,33*	21,33±4,65†	—
Amitriptyline	88,00±2,92*	41,87±5,27†	14,16±7,05
Nortriptyline	58,33±2,34*	10,33±5,12	—
Chlorprothixene	57,00±6,29*	—	—
Norprothixene	16,00±3,99†	—	—
Prothixene	7,16±3,96†	—	—
Chlorpromazine	32,66±5,77†	—	—
Promazine	8,50±2,81†	—	—

*Compared with control (samples without preparations) $P < 0.001$.

†Compared with control $P < 0.05$.

Platelet-enriched plasma was diluted in the ratio 1:1 with a solution containing 2 mM $MgCl_2$, 20 mM KCl, 112 mM NaCl, and 20 mM Tris-HCl buffer, pH 7.4. The diluted plasma was poured in a volume of 1 ml each into polyethylene tubes and incubated with 1 μg 5-HT for 15 min. Incubation ended by cooling the tubes to 0-4°C. The concentration of endogenous 5-HT was 0.16 μg/mg protein. Protein was determined by Lowry's method [8]. The increase during incubation for 15 min was 185% (n = 6). The preparations (0.1 ml of solutions made up in 0.9% NaCl or bidistilled water from powders or ampule-packed forms) were added to the incubation medium 5 min before the 5-HT. The following therapeutic substances were used: chlorimipramine (Anabranil), imipramine (Melipramine), desipramine (Pertofran), amitriptyline (Elavil), chlorprothixene (Trural), chlorpromazine (Soviet preparation Aminazin), and promazine, and also chemical preparations generously presented by the firm of Lundbeck: nortriptyline, norprothixene, and prothixene. All the preparations, when incubated with plasma for 20 min, caused no change in the endogenous 5-HT content, demonstrating that they had no effect on the determination of 5-HT or on its metabolism in the platelets. In each experiment two parallel tests were carried out for one concentration of the preparation. The percentage inhibition of 5-HT uptake by platelets relative to its uptake in the absence of the preparations in the incubation medium was calculated. During the statistical analysis the numerical results were regarded as a population with paired variances [4].

EXPERIMENTAL RESULTS

It will be clear from Table 1 that all chlorine derivatives inhibited TTHT more strongly than the original preparations. The TTHT-blocking action of the chlorine derivative of imipramine was stronger than that of prothixene or promazine. The structure of the original compound can be assumed to affect the increase in inhibition of TTHT produced by introduction of the chlorine atom.

In the imipramine—desipramine and amitriptyline—nortriptyline pairs the demethylated (secondary) derivatives had a weaker inhibitory action on TTHT than the original (tertiary) compounds, in agreement with data in the literature [14]; the difference between amitriptyline and nortriptyline was not so great as that between imipramine and desipramine. In the prothixene—norprothixene pair, however, the secondary compound had a stronger inhibitory action on TTHT than prothixene. Increased inhibition of TTHT with the change from tertiary to secondary compounds was demonstrated previously for bicyclic antidepressants (Lu-3-009 and Lu-3-010) [5] and β-aminopropionyl derivatives of dibenzazepines (IPK-17 and IPK-18) [10]. The effect of demethylation on the ability of compounds to inhibit TTHT may thus also depend on the structure of the original compound. This relationship, however, does not at present

seem quite so evident as when the effect of introducing a chlorine atom into the structure of antidepressants and neuroleptics on TTHT is analyzed.

Comparison of all the substances tested with respect to the strength of their inhibition of TTHT showed that they could be arranged in the following order of decreasing effect: chlorimipramine > imipramine > amitriptyline > desipramine—nortriptyline—chlorprothixene > chlorpromazine > norprothixene > prothixene—promazine.

The most effective substances were those with a marked clinical thymo-analeptic action: chlorimipramine, imipramine, and amitriptyline; the weakest were the neuroleptics chlorpromazine, norprothixene, and promazine. It will be recalled that, in concentrations above 10^{-5} M, chlorpromazine may have a nonspecific action on TTHT, as the result of the damage it causes to the structure of the platelet membrane [11]. The intermediate position of chlorprothixene in the scheme given above corresponds to its position relative to the antidepressants and neuroleptics as regards its clinical effect. The study of the action of substances on TTHT can consequently be useful also for predicting their clinical effect.

LITERATURE CITED

1. I. P. Lapin and G. F. Oksenkrug, "The role of serotoninergic processes in pathogenesis of depressive states and in the action of antidepressants," in: Proceedings of the 5th All-Union Congress of Neuropathologists and Psychiatrists [in Russian], Vol. 1, Moscow (1969), pp. 313-315.
2. M. D. Mashkovskii, Therapeutic Substances [in Russian], Vol. 1, Moscow (1972), p. 57.
3. G. F. Oksenkrug, "A modified method of fluorometric determination of serotonin in platelets," Vopr. Med. Khim., 19, 329 (1973).
4. V. Yu. Urbakh, Statistics for Biologists and Medical Scientists [in Russian], Moscow (1963).
5. L. Ahtee and L. Saarnivaara, "The effect of drugs upon the uptake of 5-hydroxytryptamine and metaraminol by human blood platelets," J. Pharm. Pharmacol., 23, 495 (1971).
6. A. Carlsson, H. Corrodi, K. Fuxe, et al., "Effect of antidepressant drugs on the depletion of intraneuronal brain 5-hydroxytryptamine stores caused by 4-methyl-alpha-metatyramine," Europ. J. Pharmacol., 5, 357 (1969).
7. I. P. Lapin and G. F. Oksenkrug (Oxenkrug), "Intensification of the central serotoninergic processes as a possible determinant of the thymoleptic effect," Lancet, 1, 132 (1969).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., "Protein measurement by the Folin phenol reagent," J. Biol. Chem., 193, 265 (1951).
9. K. Modigh, "Effect of anafranil on the neurotransmission in brain monoamine neurons," J. Intern. Med. Res., 1, 274 (1973).
10. G. F. Oksenkrug (Oxenkrug), "Similarity between the effects of dimethyl and monomethyl tricyclic drugs on reserpine effects in the frog and 5-HT uptake by human blood platelets," J. Pharm. Pharmacol., 25, 1013 (1973).
11. M. K. Paasonen, "Release of 5-HT from blood platelets," J. Pharm. Pharmacol., 17, 681 (1965).
12. J. M. Sneddon, "Blood platelets as a model for monoamine-containing neurons," Progress in Neurology, 1, 153 (1973).
13. R. S. Stacey, "Uptake of 5-hydroxytryptamine by platelets," Brit. J. Pharmacol., 16, 284 (1961).
14. A. Todrick and A. Tait, "The inhibition of human platelet 5-HT uptake by tricyclic drugs. The relation between structure and potency," J. Pharm. Pharmacol., 21, 751 (1969).