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SHAPE CHANGE AND UPTAKE OF 5-HYDROXYTRYPTAMINE IN HUMAN BLOOD PLATELETS: ACTION OF NEUROPSYCHOTROPIC DRUGS

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Summary

Psychotropic drugs acted as agonists, antagonists or mixed agonists-antagonists of the shape change mediated by the 5HT receptor in blood platelets of man incubated in a protein-poor medium. Regarding inhibition of 5HT uptake some of the drugs were equally potent in both reserpinized and normal platelets, whereas others showed lower or higher potencies in the former than in the latter. There was no correlation between the potencies of the drugs as shape change inhibitors and as uptake inhibitors. It is concluded that 1) human platelets may serve as models for the neuronal 5HT receptors of some areas of the central nervous system, 2) platelets can be used to determine the subcellular site of action of 5HT uptake inhibitors and 3) the receptors responsible of the shape change, and the site of the 5HT transport at the plasma membrane are different entities.

Recently a method has been described which allows the incubation of platelets in a protein-poor medium without markedly interfering with their shape change reaction or with their uptake of 5-hydroxytryptamine (5HT) (1). By using this procedure it was found that the 5HT receptor responsible for the shape change in platelets of rabbits reacted to neuropsychotropic drugs similarly to the neuronal 5HT receptors in certain areas of the central nervous system (CNS) (2). Furthermore, by comparing normal with reserpinized platelets of guinea pigs the subcellular site of action of 5HT uptake inhibitors could be determined (3).

This paper describes the reactions to neuropsychotropic drugs of human platelets incubated in protein-poor medium. The possibility of a relationship between the degrees of inhibition of 5HT-induced shape change and of 5HT uptake has also been investigated.

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Experimental

Materials:

The p-chlormetamphetamine was synthesized by Dr. H. Bruderer, F. Hoffmann-La Roche Inc. Basel. The source of all the other compounds used has been indicated earlier (2,3).

Isolation of platelets

Blood was obtained from healthy volunteers by puncturing the cubital vein and collecting into a plastic vial to which 1/10 volume of tri-Na-citrate (3.8%) had been added. Platelet rich plasma (PRP) was separated by centrifugation of the whole blood at 400 g for 10 minutes at 22°C. In the uptake experiments half of the PRP was incubated for 30 minutes at 37°C with 2 x 10^{-6}M reserpine (final concentration). Platelets were then isolated as previously described (1); PRP was layered on a dextran T-10 gradient (7 ml 10% dextran on 4 ml 20% dextran) in a Beckmann cellulose nitrate tube (8.75 x 2.5 cm). This gradient was centrifuged for 10 minutes at 7000 g at 4°C (uptake experiments) or at 2200 g at room temperature (shape change experiments). The platelets which assembled between 10% and 20% dextran were removed by puncturing the tube at the bottom with a hollow needle. Platelets were counted in a Coulter counter and their concentration was adjusted to 3 x 10^4 μl^{-1} (uptake) or 10^5 μl^{-1} (shape change) by dilution with Tris-buffer* or platelet poor plasma (shape change experiments).

Shape change experiments:

Aliquots (1 ml) of the final platelet suspension were preincubated for 60 minutes at 37°C. Then the shape change caused by the various substances was determined with a Born Mark III aggregometer, by measuring the maximal light absorption at 37°C (M, fig. 1), registered on a Rikadenki Recorder B-361 with a full-scale deflection of 20 mV. The samples were stirred with a teflon-covered magnetic stirrer at about 900 revolutions per minute. Agonists and antagonists were added in a volume of 5 - 15 µl (mostly H_2O), the antagonist 1 minute before 5HT (as creatinine sulfate; final concentration 10^{-6}M). In control experiments only the solvent was added.

Uptake experiments:

Aliquots (1 ml) of the final platelet suspension were preincubated at 37°C for 30 minutes under gentle shaking. Tris (0.05 ml) either alone (controls) or containing various concentrations of uptake inhibitors was added 10 minutes before the end of the preincubation period. Then the mixture was supplemented with 0.05 ml (1,2 ^{3}H)-5HT creatinine sulfate (final concentration 10^{-7}M, specific radioactivity 26 Ci/mmol) in 10^{-4}N HCl. Incubation was stopped after 5 minutes by adding 3 ml of formaldehyde 2% (4). Platelets were then separated on a Millipore filter (cellulose nitrate, diameter 2,5 cm, pore size 0.45 µ) under vacuum. Samples were washed twice with 5 ml

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* Tris-buffer (Tris): 9 volumes of a mixture containing NaCl 8.21 g/lt, KCl 0.57 g/lt, D-glucose 1.01 g/lt and tris (hydroxy-methyl-amino)-methane 0.93 g/lt plus 1 volume trisodium-citrate . $2H_2O$ 38 g/lt, pH 7.4.

Tris. Filters were dried, put into counting vials and the amount of radioactivity was measured in a scintillation counter. Proteins were determined according to Lowry et al. (5).

Calculations:
The EC_{50} (concentration causing half maximal shape change) and the IC_{50} (concentration causing 50% inhibition of either the shape change induced by 10^{-6} M 5HT or the uptake of 10^{-7} M 5HT) values of the compounds were determined graphically. Statistical analysis was performed with Student's t-test.

Results

Shape change agonists

The shape change induced by 5HT attained its maximum after about 30 seconds, remained maximal for 2 - 3 minutes and thereafter decreased, reaching close to normal values after about 1 hour (fig. 1). Various compounds known to be 5HT-agonists also caused shape change reactions (table I). The EC_{50} of D-LSD was significantly ($p < 0.05$) higher when plasma instead of protein-free buffer was used as the incubation medium of the platelets ($1.5\pm0.2 \times 10^{-7}$ versus $9.9\pm1.2 \times 10^{-9}$ M). The EC_{50} values of the other 5HT-agonists were not significantly different ($p > 0.05$) in the two media, although the values tended to be lower in plasma.

All the 5HT-agonists tested induced a smaller maximum shape change (represented by M in fig. 1) than 5HT (= 100%) both in protein-poor medium and in PRP (table I). Also, both the drugs and 5HT showed somewhat lower maximal values (on the average by about 30%) in protein-free buffer than in plasma.

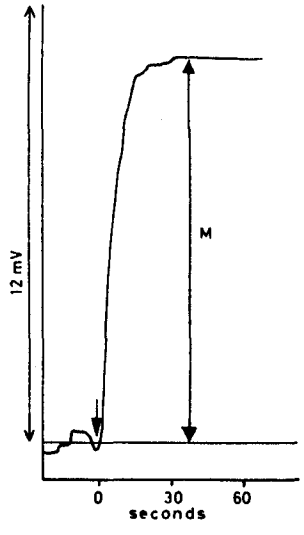

FIG. 1

Shape change induced by 10^{-6}M 5HT in human platelets. The arrow indicates addition of 5HT. M stands for maximal shape change. The ordinate indicates the light absorption expressed as deflection on the recorder scale in mV. Tris buffer.

TABLE I

Shape Change induced by 5HT-Agonists

Compounds	EC_{50} M	Maximal effect %
5HT	$1.4 \pm 0.1 \times 10^{-7}$	100
D-LSD	$9.9 \pm 1.2 \times 10^{-9}$	29.1 ± 6.0
Psilocin	$1.8 \pm 0.5 \times 10^{-7}$	11.7 ± 1.6
Tryptamine	$1.4 \pm 0.3 \times 10^{-6}$	69.6 ± 4.1
N¦N'-Dimethyltryptamine	$2.0 \pm 0.9 \times 10^{-6}$	12.2 ± 1.2
Mescaline	$2.0 \pm 0.5 \times 10^{-5}$	37.1 ± 3.6

The maximal shape change is expressed as a percentage of that induced by 10^{-6}M 5HT (= 100%). Each figure represents an average with s.e.m. of 3 - 4 experiments. Tris-buffer.

Shape change antagonists

Numerous compounds from various chemical classes inhibited the shape change caused by 10^{-6}M 5HT in protein-free medium (table II).

TABLE II

Inhibitors of the Shape Change induced by 10^{-6}M 5HT.

Inhibitor	IC_{50}, M	Inhibitor	IC_{50}, M
Spiroperidol	$2.2 \pm 0.4 \times 10^{-9}$	Imipramine	$7.8 \pm 1.3 \times 10^{-7}$
Methiothepin	$4.1 \pm 0.2 \times 10^{-9}$	Desmethylimipr.	$1.3 \pm 0.3 \times 10^{-6}$
D-LSD	$4.3 \pm 0.4 \times 10^{-9}$	N¦N'-dimeth.trypt.	$2.0 \pm 1.1 \times 10^{-6}$
Methysergide	$1.4 \pm 0.1 \times 10^{-8}$	L-Butaclamol	$3.2 \pm 0.4 \times 10^{-6}$
D-Butaclamol	$1.7 \pm 0.3 \times 10^{-8}$	Prenylamine	$4.0 \pm 0.5 \times 10^{-6}$
Chlorpromazine	$2.8 \pm 0.5 \times 10^{-8}$	Ro 4-4090	$4.2 \pm 0.2 \times 10^{-6}$
Cinanserin	$4.8 \pm 1.1 \times 10^{-8}$	Diazepam	$7.1 \pm 1.1 \times 10^{-6}$
Amitryptiline	$8.0 \pm 0.1 \times 10^{-8}$	L-LSD	$1.9 \pm 0.3 \times 10^{-5}$
Chlorimipramine	$1.8 \pm 0.1 \times 10^{-7}$	p-Chlormetamph.	$2.4 \pm 0.1 \times 10^{-5}$
Haloperidol	$3.4 \pm 0.0 \times 10^{-7}$	Ro 4-1284	$> 10^{-4}$
Psilocin	$4.3 \pm 2.0 \times 10^{-7}$		

The figures are averages with s.e.m. of 3 - 4 experiments. Tris-buffer.

The inhibitors with the lowest IC_{50} values included the neuroleptics spiroperidol and methiothepin, and D-LSD. Various antidepressants and the butyrophenone haloperidol were less potent ($p < 0.01$) whereas several 5HT-releasing agents (prenylamine, Ro 4-1284*, Ro 4-4090**, p-chlormethamphetamine) as well as diazepam proved to be rather weak. LSD and butaclamol exhibited marked stereospecifity, the D-isomers being respectively over 3 and 2 orders of magnitude

* 2-hydroxy-4-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzo(a)quinolizine

** bis (3,4-dichlorophenethyl)amine

more active than the L-forms. Five 5HT-antagonists were tested in platelets suspended in plasma as well as in artificial buffer: all the compounds showed a higher potency in the latter medium (table III).

TABLE III

Inhibition of 5HT-induced Shape Change of Platelets suspended in different Media

Inhibitor	IC_{50}, M PRP	Tris
Spiroperidol	$8.5\pm1.8\times10^{-8}$	$2.2\pm0.4\times10^{-9}$
Methiothepin	$3.8\pm0.9\times10^{-7}$	$4.1\pm0.2\times10^{-9}$
Chlorpromazine	$3.2\pm0.2\times10^{-6}$	$2.8\pm0.5\times10^{-8}$
Cinanserin	$1.4\pm1.3\times10^{-6}$	$4.8\pm1.1\times10^{-8}$
Desmeth.imipramin	$1.7\pm0.1\times10^{-5}$	$1.3\pm0.3\times10^{-6}$

The concentration of 5HT was 10^{-6}M. The figures are averages of 3 - 4 experiments each with s.e.m. All the values from Tris platelets were significantly ($p < 0.01$) lower than the respective values from PRP.

Mixed agonists-antagonists

D-LSD, psilocin and N¦N'-dimethyltryptamine, which by themselves caused a rather small maximal shape change (table I), induced a virtually 100% inhibition of the 5HT-induced shape change (compare also tables I and II).

Uptake inhibitors

In normal platelets the most potent inhibitors of ^{3}H-5HT uptake were the antidepressants chlorimipramine, imipramine and amitriptyline, followed by the 5HT-releasers Ro 4-1284 and 4-9040, the neuroleptics methiothepin and chlorpromazine, and the antidepressant desmethylimipramine. Spiroperidol, haloperidol, psilocin and N¦N'-dimethyltryptamine showed rather low potency (table IV). The IC_{50} values of LSD, butaclamol, diazepam and methysergide were above 10 µM. There was no stereoselectivity of one of the optical isomers of LSD or butaclamol.

The IC_{50} values of the drugs in reserpinized platelets ($IC_{50}R$) were either similar to, or higher or lower than those in normal platelets ($IC_{50}N$). Accordingly the quotient $IC_{50}R/IC_{50}N$ was close to one (e.g. antidepressants and chlorpromazine), above one (Ro 4-1284, Ro 4-9040, prenylamine and butyrophenones), or below one (N¦N'-dimethyltryptamine and p-chlormetamphetamine) (table IV).

Correlation between inhibition of shape change and uptake

No correlation could be found between the IC_{50} values of the various compounds for the 5HT-induced shape change and those for the ^{3}H-5HT uptake (corr.coeff. 0.235 and 0.150 with normal and reserpinized platelets respectively).

TABLE IV

Effect of Inhibitors of 5HT-Uptake in Normal and Reserpinized Platelets

Inhibitor	Normal (N)	Reserpinized (R)	R / N
Ro 4-1284	21.3± 1.4	> 10^5	> 100
Ro 4-9040	51.2± 15.9	536.4±119.1**	11.5±1.7
Prenylamine	258.3± 41.4	1570.0±165.0**	6.5±1.5
Spiroperidol	3133.3±328.3	7833.3±796.5**	2.5±0.2
Haloperidol	1508.7±154.9	3370.0±149.7**	2.3±0.3
5HT	290.0± 34.6	400.0± 50.0	1.4±0.1
Chlorpromazine	65.3± 10.6	66.3± 4.2	1.1±0.2
Methiothepin	59.7± 10.3	64.7± 10.7	1.1±0.0
Imipramine	4.2± 0.2	4.6± 0.3	1.1±0.0
Amitryptiline	9.7± 1.2	11.1± 2.0	1.1±0.1
Desmethylimipr.	52.0± 2.1	59.0± 1.5	1.1±0.0
Chlorimipramine	0.5± 0.1	0.5± 0.1	1.0±0.1
Psilocin	2415.7±245.5	2100.0±219.1	0.9±0.1
p-Cl-metamphet.	620.0± 12.2	484.0± 45.7*	0.8±0.1
N,N'-dimeth.trypt.	1400.0±108.0	892.5±104.4**	0.6±0.0

The IC_{50} of the drugs is indicated in nM. Tris-buffer. R/N means quotient of IC_{50} in reserpinized platelets versus IC_{50} in normal platelets. The values are averages with s.e.m. from 3 - 4 experiments, each performed with platelets from 1 individual. Significance (values in reserpinized versus normal platelets): * $p < 0.05$ ** $p < 0.01$

Discussion

The present experiments show that the shape change reactions of human platelets isolated by polysaccharide gradients and incubated in a protein-poor medium are comparable to those of rabbit platelets treated in a similar way (2). In fact, various drugs known to be specific 5HT agonists, antagonists or mixed agonists-antagonists in rabbit platelets showed the same properties in human platelets, the order of potencies of the compounds being similar in both species. Therefore, platelets of man, like those of rabbits (2), can be considered as models for the neuronal 5HT receptor in some areas of the CNS (e.g. cortex, spinal cord, possibly reticular formation). A comparison of recent findings (6,7) with the present results confirms this view; the order of potencies of various neuroleptic drugs and other 5HT antagonists in platelets and in cerebral cortex are reasonably parallel, an exception being the inhibition of 5HT-stimulated adenylate cyclase by haloperidol (table V). Shape change experiments in protein-poor buffer seem to give more accurate data on the potencies of drugs than those in plasma. In fact, in PRP the drugs may be partly inactivated by protein binding, as indicated by the higher EC_{50} and IC_{50} values of drugs compared with the corresponding values in protein-poor medium.

In platelets of guinea pigs, inhibitors of 5HT uptake with a quotient $IC_{50}R/IC_{50}N$ of about 1 (e.g. imipramine) have been shown to act preferentially at the plasma membrane, whereas those with a

quotient of over 100 (e.g. benzoquinolizine derivative Ro 4-1284) mainly influence the intracellular 5HT-storage organelles. There is also evidence that compounds with a quotient above 1 but below 100 exert a mixed effect, i.e. both on the plasma membrane and on intracellular storage (3). Platelets of man behaved like those of guinea pigs. It is of special interest that the various antidepressants seemed to act primarily at the plasma membrane (quotient of about 1). On the other hand, prenylamine and Ro 4-9040 (compounds previously thought to preferentially influence granular storage) (8,9) probably had a mixed action (quotients of 6.5 and 11.5 respectively). In addition, there was a difference between the butyrophenones (quotient > 2) and tricyclic neuroleptics such as chlorpromazine (quotient about 1); therefore, the former, unlike the latter, probably act not only on the plasma membrane, but also on the intragranular storage. An interaction of butyrophenones with amine storage granules e.g. in DA neurons of the nigrostriatum might possibly explain why they impair extrapyramidal motricity more markedly than tricyclic neuroleptics like chlorpromazine.

TABLE V

Potencies of Various Drugs as Antagonists of 5HT Receptors in Platelets and Cerebral Cortex

Compounds	Potencies			
	Platelets		Cerebral cortex	
	Man[1]	Rabbit[2]	Monkey[3]	Rat[4]
Spiroperidol	$2.2\pm0.4\times10^{-9}$	1.2×10^{-9} (0.9-1.2)	8	8.45±0.05
Methiothepin	$4.1\pm0.2\times10^{-9}$	1.2×10^{-9} (1.1-1.2)	11±1	8.23±0.07
Cyproheptadine		2.1×10^{-9} (1.9-2.2)	29±1	
Methysergide	$1.4\pm0.1\times10^{-8}$	6.2×10^{-9} (5.2-5.7)	42±1	
Chlorpromazine	$2.9\pm0.5\times10^{-8}$	6.8×10^{-8} (6.0-7.6)	64±1	7.21±0.07
(+)-Butaclamol	$1.7\pm0.3\times10^{-7}$	1.3×10^{-7} (1.1-1.6)		7.0 ±0.1
Haloperidol	$3.4\pm0.0\times10^{-7}$	1.8×10^{-7} (1.3-2.6)	40±4	6.83±0.07

[1] IC_{50} with standard error (see table II). [2] IC_{50} values (M) with 95% confidence limits (2). [3] Antagonism against 5HT-stimulated adenylate cyclase in anterior limbic cortex: Ki values (nM) (6). [4] Inhibition of ^{3}H-spiroperidol binding in frontal cortex: -log IC_{50} (M) (7).

In the present experiments with human platelets compounds (e.g. N¦N'-dimethyltryptamine and p-chlormetamphetamine) were also found with quotients $IC_{50}R/IC_{50}N$ of less than 1. It is possible that these substances exert an effect on that part of the 5HT which is stored intracellularly but extragranularly. The proportion of 5HT stored in the extragranular compartment (in relation to that stored intragranularly) is probably higher in reserpinized platelets than in normal ones; therefore, the former may be more sensitive than the latter to compounds interfering with extragranular storage.

Inhibitors of 5HT uptake showing a specific action on the plasma membrane or the storage organelles may lose their specifity if they are present in high concentrations i.e. far above the IC_{50}. For

example, in human and rabbit platelets high concentrations (10^{-4}M) of tricyclic antidepressants and chlorpromazine have been shown to impair granular storage (10). It should also be borne in mind that the quotient $IC_{50}R/IC_{50}N$ gives only a semiquantitative estimation of the granular and membrane components of an inhibitor, not an exact quantitative one (3).

The absence of any correlation between the IC_{50} values for the ^{3}H-5HT uptake (in normal and reserpinized platelets) and those for the shape change indicates that the transport system for 5HT at the plasma membrane and the shape change receptors are probably independent of each other. The high stereospecificity of inhibitors such as D-LSD and D-butaclamol in the shape change reaction, but not in the 5HT uptake confirms this view. A lack of correlation between the IC_{50} values for 5HT uptake and shape change has been previously observed for some antidepressants and 5HT-analogues, whereas there seemed to be a negative correlation for tryptamine derivatives (11).

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