Diminution of 5-Hydroxytryptamine in Thrombocytes *in vitro* by Chlorpromazine and Related Compounds

Chlorpromazine[1], chlorprothixene[2], and imipramine[3] inhibit the uptake of labelled 5-hydroxytryptamine (5HT) by platelets *in vitro*; imipramine also decreases platelet 5HT *in vivo*[4,5]. The effect of chlorpromazine and related compounds on endogenous 5HT of blood platelets *in vitro* has, however, not been known up to now and was investigated in the present paper.

Methods. Rabbits weighing 2.5–3 kg and starved for 16 h were bled under ether anesthesia by means of a polyethylene canula inserted into a carotid artery. Some animals had been given 20 mg/kg isocarboxazid[6] i.p. 16 h before bleeding. The blood was mixed with 1/9 vol of 5% disodium ethylene diamine tetraacetate (versene) in distilled water and centrifuged at 200–300 × *g* at + 2°C for 15 min. Aliquots of platelet-rich plasma (2 ml) were supplemented with the drugs dissolved in 0.1 ml saline or with saline alone. Incubations were performed at 37°C under air or nitrogen with gentle shaking. Then the samples were centrifuged at 1700 × *g* at 2°C for 25 min. 5HT in the platelets (sediment) and in the platelet poor plasma (supernatant) was measured by a spectrophotofluorometric method[7]. All glassware was siliconized.

Platelet-rich plasma from venous blood of healthy humans was prepared and treated correspondingly.

Exclusion of possible methodical errors: *Platelet agglutination and distruction* due to *in vitro* incubation or drug effects could be excluded by counting and microscopic examination of the platelets. *pH changes* during the incubation were probably also no relevant source of error. Thus, in plasma with and without 2.8×10^{-5} *M*/l chlorpromazine a pH increase of 0.4 occurred during incubation under air; incubation under nitrogen caused a pH decrease of 0.15. The effect of chlorpromazine on platelet 5HT was, however, similar under both experimental conditions. *Interference of chlorpromazine with the spectrophotofluorometric 5HT assay* was excluded because the recovery of 5HT added to the platelet-rich plasma was not influenced by the drug.

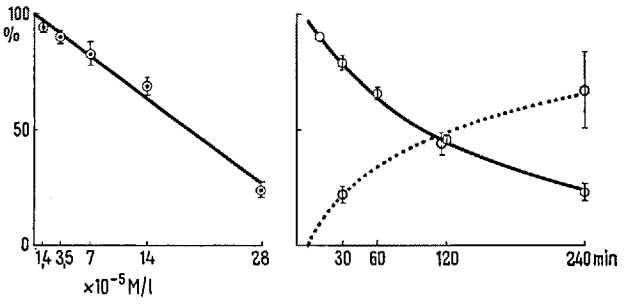
Results. (1) Chlorpromazine in amounts between 1.4×10^{-5} and 2.8×10^{-4} *M*/l (= 5 and 100 µ/ml) diminished the 5HT content of rabbit platelets significantly dependent on the concentration of the drug. With 2.8×10^{-4} *M*/l chlorpromazine a steady diminution of 5HT up to 75% occurred during the incubation period of 4 h (Figure). Platelets not supplemented with the drug showed a 5HT decrease of 3–4% under the same conditions. Pretreatment with isocarboxazid had no appreciable influence on the chlorpromazine-induced 5HT decrease.

(2) The 5HT disappearing from platelets was recovered almost completely in the plasma, if the animals were pretreated with the monoamine oxidase inhibitor isocarboxazid (Figure). In rabbits without such a pretreatment only about 30% of the 5HT disappearing from platelets could be found in the plasma.

(3) Reserpine (e.g. 5 γ/ml) decreased the platelet 5HT by a maximum of 51 ± 6% during the first 3 h of incubation and by a further 14 ± 1% during the following 3 h. A second addition of reserpine (e.g. 5 γ/ml after 3 h) had no enhancing effect, but addition of 2.8×10^{-4} *M*/l chlorpromazine 3 h after reserpine decreased the platelet 5HT by 34 ± 6%. This decrease was significantly greater than with reserpine alone (14 ± 1%) ($p < 0.01$).

(4) Various compounds such as imipramine, chlorprothixene, amitriptyline[8], tyramine also decreased the platelet 5HT. With the possible exception of chlorprothixene, however, none was more potent than chlorpromazine. Cocaine, iproniazid[9], α-methyl-3,4-dihydroxyphenylalanine (α-methyl-dopa) had only a weak effect (Table).

(5) In human platelets chlorpromazine and chlorprothixene in concentrations of 3.5×10^{-4} and 2.8×10^{-5} *M*/l diminished the 5HT significantly ($p < 0.01$). The effect was, however, less pronounced than in rabbits (Table). Cocaine did not decrease the 5HT appreciably.



Influence of chlorpromazine on the 5-hydroxytryptamine content of thrombocytes and plasma of rabbits. Ordinate: 5HT content in % of controls (platelets of the same animal incubated without chlorpromazine). Abscissa: (left) chlorpromazine concentration (incubation for 4 h under air), (right) time of incubation (chlorpromazine concentration in the plasma: 2.8×10^{-4} *M*/l). Solid lines: 5HT of platelets, dotted line: 5HT in the plasma of rabbits pretreated with 20 mg/kg isocarboxazid i.p. 16 h before bleeding. The points represent averages of 3–7 experiments with standard error. Double determinations were carried out in each experiment.

Drugs	5HT decrease at 2 different drug concentrations			
	Rabbits		Humans	
	2.8×10^{-4} *M*/l	3.5×10^{-5} *M*/l	2.8×10^{-4} *M*/l	3.5×10^{-5} *M*/l
Chlorpromazine	74 ± 4	10 ± 2	40 ± 2	11 ± 2
Chlorprothixene	93 ± 1	11 ± 1	62 ± 1	9 ± 2
Imipramine	69 ± 12	8 ± 3	42 ± 3	10 ± 1
Amitriptyline	80 ± 11	13 ± 4		
Cocaine	8 ± 2	8 ± 3	9 ± 2	5 ± 1
α-Methyl-dopa	13 ± 2	10 ± 2		
Tyramine	34 ± 7	12 ± 3		

Decrease in 5-hydroxytryptamine of thrombocytes by various drugs. Incubation of platelet-rich plasma for 4 h under air. The figures represent mean percentage values with standard error of 3 experiments with rabbit plasma and 2 to 5 experiments with human plasma. Platelets incubated for 4 h but not supplemented with the drugs served as controls. Double determinations were carried out in each experiment.

[1] 2-Chloro-10-(3-dimethylaminopropyl)-phenothiazine (Thorazine®, Largactil®).

[2] 2-Chloro-9-(ω-dimethylamino-propyliden)-thioxanthene (Taractan®).

[3] 10,11-Dihydro-5-(3-dimethylaminopropyl)-5H-dibenzo[b,f]azepine (Tofranil®).

[4] R. S. Stacey, Brit. J. Pharmacol. *16*, 284 (1961).

[5] E. F. Marshall, A. S. Stirling, A. C. Tait, and A. Todrick, Brit. J. Pharmacol. *15*, 35 (1960).

[6] N_1-Benzyl-N_2-(5-methyl-3-isoxazolylcarbonyl)-hydrazine (Marplan®).

[7] S. Udenfriend, H. Weissbach, and C. T. Clark, J. biol. Chem. *215*, 337 (1955).

[8] 1-(ω-Dimethylamino-propylidene)-2,3-6,7-dibenzocyclohepta-2,6-diene-hydrochloride.

[9] N_2-Isopropyl isonicotinic acid hydrazide (Marsilid®).

Discussion. According to the present experiments chlorpromazine and structurally related compounds cause a shift of endogenous 5HT from platelets into the plasma. This can be concluded from the observation that in animals pretreated with a monoamine oxidase inhibitor almost all the 5HT disappearing from platelets was found in the plasma. The incomplete recovery of 5HT disappearing from platelets of animals not pretreated with a monoamine oxidase inhibitor is probably due to partial oxydation of 5HT.

The chlorpromazine-induced decrease of platelet 5HT might be due to liberation of 5HT or, provided that the 5HT of platelets is in a dynamic equilibrium with the 5HT in plasma, to inhibition of 5HT uptake. From experiments with imipramine and cocaine, however, the latter possibility seems unlikely. Thus, imipramine (5×10^{-7} *M*/l) inhibits 5HT uptake in concentrations about 100 times lower than chlorpromazine (3.5×10^{-5} *M*/l)[4]; the two drugs are, however, about equally effective in decreasing endogenous 5HT in platelets. Furthermore, cocaine (2.5×10^{-5} *M*/l) decreases 5HT uptake in about the same concentration as chlorpromazine[4]; cocaine is, however, less effective in lowering platelet 5HT.

The mechanism by which chlorpromazine causes 5HT decrease in platelets is probably different from that of reserpine. Thus, within 4 h reserpine reduced the 5HT of platelets by a maximum of 50% only[10], whereas chlorpromazine decreased the amine by 76%. Furthermore, chlorpromazine was able to cause an additional decrease of the 5HT after reserpine had exerted its maximal effect. It may be assumed that reserpine impairs active transport or storage of 5HT and that therefore the amine content of platelets decreases with the velocity of passive diffusion of 5HT into the plasma[11]. In consequence, an enhancement of the reserpine-induced 5HT decrease by chlorpromazine might indicate accelerated diffusion of 5HT possibly due to increased permeability of the membrane of platelets.

It remains to be elucidated whether chlorpromazine and related compounds have a similar action *in vivo* and whether this effect is related to the chlorpromazine-induced changes of monoamine metabolism observed in the brain *in vivo*[12,13].

Riassunto. Cloropromazina, imipramina, cloroprotixene e amitriptilina *in vitro* diminiscono la 5-idrossitriptamina (5HT) endogena nei trombociti di coniglio e, per quanto si è visto, anche in quelli umani. Nel plasma di coniglio pretrattato con isocarbossazide, inibitore della mono-amino-ossidasi, si osserva un corrispondente aumento di 5HT. Cocaina, α-metil-dopa e triptamina hanno un effetto meno pronunciato sulla 5HT delle piastrine.

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[14] Guest worker from the Istituto di Patologia sp. Medica dell'Università di Bologna (Italy).

Photosynthesis in *Cuscuta*

The genus *Cuscuta* is characterised by its comparative lack of chlorophyll and its complete dependence, after the seedling stage, on a host plant. Some species such as *C. gronovii* are greenish, but do not survive independently though Loo[1] succeeded in growing excised tips of *C. campestris* for a period of five months in an organic culture medium. The presence of chlorophyll in *Cuscuta* has been noted by many workers notably THOMSON[2], MACKINNEY[3], and WALZEL[4]. However, in the literature, no reference to conclusive evidence that dodders fix carbon dioxide has been found.

In the present work two species were tested for CO_2 fixation, *C. gronovii* which is green mottled with red, and *C. campestris* Yuncker which is orange-yellow. Into each of six 250 ml flasks was placed 1 g of freshly harvested *C. gronovii* filament, 1 g of *C. campestris* filament, and one leaf of *Pelargonium* sp. respectively. (The Pelargonium leaves used varied between 0.3 and 0.35 g fresh weight.) In the bottom of each flask was placed a small dish containing 4 μ C ^{14}C sodium bicarbonate solution together with sufficient $NaHCO_3$ carrier to give a final atmosphere of 2% CO_2 within the flask. The volume of solution in the dish was 0.3 ml. To release the CO_2, 0.1 ml saturated citric acid solution was added by pipette to the dishes, and the flasks stoppered immediately. The flasks were left in bright sunlight for periods ranging from 15 min to 4 h. Two controls were set up; one flask similar to the above was left in the dark for 4 h, while another flask containing plant material previously boiled for 5 min was exposed to sunlight for 4 h.

At the end of the experimental periods, the *Cuscuta* and leaf samples were separately placed in 25 ml of 90% ethanol, macerated in a mortar, and the extracts filtered. A few drops of $NaHCO_3$ solution, followed by a similar amount of saturated citric acid solution were added to the filtrate to remove any traces of radioactive bicarbonate contamination. Each extract was evaporated to dryness at 100°C and then taken up in 1 ml distilled water. 0.5 ml of this extract was evaporated to dryness on a planchette and analysed for radioactivity on a Geiger-Müller end-window counter. The results are shown in the Table.

Exposure time to $^{14}CO_2$	*C. gronovii*	*C. campestris*	*Pelargonium* leaf
15 min	124	160	340
30 min	200	263	508
1 h	280	379	528
2 h	567	468	1009
3 h	811	604	2044
4 h	1065	938	4218
Control in dark	121	113	207
Control (boiled material)	68	71	71

Results given as counts per 5 min. Background: 63 counts/5 min. Efficiency of counter: 1% approximately.

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