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Isolated 5-hydroxytryptamine organelles of rabbit blood platelets: physiological properties and drug-induced changes

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1. In isolated 5-hydroxytryptamine (5-HT) organelles of rabbit platelets, the concentrations of 5-HT, histamine and adenosine-triphosphate (ATP) respectively are about 200 times higher than in intact platelets. Organelles incubated in plasma at 37° C gradually lose endogenous 5-HT, histamine and ATP and take up ^{14}C-5-HT against a considerable concentration gradient. Liberation and uptake of 5-HT markedly decrease with dimishing incubation temperature.

2. Exposure to reserpine *in vitro* strongly counteracts the uptake of ^{14}C-5-HT by isolated organelles, whereas the ^{14}C-5-HT uptake of intact isolated platelets is less affected by the drug. 5-HT organelles of platelets from reserpinized rabbits also take up very little ^{14}C-5-HT.

3. Imipramine inhibits the uptake of ^{14}C-5-HT in isolated organelles less markedly than in isolated platelets.

4. It is concluded that in the organelles 5-HT and possibly histamine may be associated with ATP. Reserpine probably impairs the uptake of 5-HT at the level of the organelles (possibly by interfering with the association 5-HT/ATP), whereas imipramine seems to act preferentially on the cell membrane.

Combined electron microscopic and biochemical experiments have shown that in blood platelets of various species including man, 5-hydroxytryptamine (5-HT) is localized in highly dense osmiophilic organelles which are different from the α-granules (Tranzer, Da Prada & Pletscher, 1966, 1968; Bak, Hassler, May & Westermann, 1967). These electron dense bodies have been isolated in virtually pure form from rabbit platelets by density gradient centrifugation. Measurements in various subcellular platelet fractions confirmed that the platelet 5-HT was mostly localized in the organelles and that the histamine was equally distributed with the 5-HT (Da Prada, Pletscher, Tranzer & Knuchel, 1967a; Knuchel, 1968). In addition, after administration of reserpine *in vivo*, the isolated organelles contained only traces of 5-HT and had almost completely lost their osmiophilia, whereas their adenosine triphosphate (ATP) was decreased relatively little (to about 65%) (Da Prada, Pletscher, Tranzer & Knuchel, 1968).

In the present paper, isolated dense osmiophilic bodies and intact isolated platelets have been compared with regard to some physiological and pharmacological properties.

Methods

Rabbits weighing 2–3 kg, fasted for 16 hr, were bled under ether anaesthesia from the carotid artery through a polyethylene cannula. Some of the animals had been treated with reserpine 5 mg/kg intraperitoneally 16 hr before bleeding. The blood was supplemented with 1/10 vol. ethylenediamine-tetraacetate (EDTA) (5%), and the platelets were isolated and incubated as previously described (Bartholini, Pletscher & Gey, 1961). The isolation of the 5-HT organelles was carried out in the following way: Platelets of one animal (from about 40 ml. plasma) were washed in modified Tyrode (g/l.: NaCl 7.60, KCl 0.42, EDTA 0.80, $NaH_2PO_4.2H_2O$ 0.14, $NaHCO_3$ 2.10, glucose 2.00, sucrose 4.50), suspended in 20 ml. hypertonic citrate buffer containing 15% urografin and submitted to ultrasonication for 10 sec using a Branson Sonifier S-75, setting N3. The suspension was centrifuged at 3,090 *g* for 10 min and then at 12,350 *g* for 20 min. The sediment (still containing intact platelets) was resuspended and treated again twice in the same way. The pooled supernatants were centrifuged at 37,000 *g* for 20 min. The sediment, suspended in 1 ml. hypertonic citrate-urografin 15%, was put into a centrifuge tube on top of 4 ml. of an aqueous mixture exhibiting a continuously increasing urografin concentration of 26 to 36% from top to bottom. Thereafter, centrifugation was performed for 15 min at 122,000 *g* in a Beckmann preparative ultracentrifuge, Model L 2-65K (Titanium rotor, type SW 65 L).

The hypertonic citrate-urografin solution was prepared by mixing 450 ml. trisodium-citrate-2-hydrate (Merck; 4.5%), 300 ml. sodium chloride (Merck; 4.5%), and 150 ml. anhydrous glucose (Fluka; 27%). The mixture was adjusted to pH 7.2 with EDTA 5% and added 15% urografin (Schering).

Some of the isolated platelets or 5-HT organelles were analysed directly. Others were suspended in plasma which had previously been centrifuged at 314,000 *g* for 30 min. Portions (1 ml.) of the suspensions containing the amount of platelets of 1 ml. original plasma or of organelles recovered from platelets of about 13 ml. original plasma were incubated under gentle shaking. Thereafter, the platelets or the organelles were separated from the plasma by centrifugation (platelets: 2,710 *g* for 5 min; organelles: 44,000 *g* for 10 min) in order to determine the concentrations of 5-HT, histamine and ATP. The ^{14}C-3-5-HT (0.2 μg/ml. creatinine sulphate monohydrate; specific radioactivity 40 mc/mM) as well as reserpine and imipramine were added to the plasma in 1/10 vol. Tyrode. In the experiments with ^{14}C-5-HT, a blank was subtracted from the values after incubation. This blank was obtained from normal organelles which had been resuspended in plasma containing ^{14}C-5-HT and separated from the plasma immediately after resuspension. The blank amounted to 5–7% of the radioactivity found in normal organelles after incubation with ^{14}C-5-HT for 30 min at 37° C.

All the operations were performed in siliconized glassware or polyethylene tubes and (except for the incubations at elevated temperatures) in the cold (0°–4° C).

The determinations of 5-HT, ^{14}C-5-HT, histamine and ATP in platelets and organelles were carried out as previously described (Bogdanski, Pletscher, Brodie & Udenfriend, 1956; Huff, Davis & Brown, 1966; Holmsen, Holmsen & Bernhardsen, 1966; Pletscher, Burkard, Tranzer & Gey, 1967; Knuchel, 1968).

Results

The normal concentration of 5-HT, histamine and ATP per μg protein is considerably higher in the organelles than in the platelets—by factors of 234, 223 and

174 respectively (Table 1). In the organelles, the molar ratios 5-HT:ATP, histamine:ATP, and 5-HT:histamine amount to about 2.5, 1.1 and 2.3 respectively, whereas in the intact platelets these ratios are close to 1.8, 0.75 and 2.4 respectively.

TABLE 1. *Content of 5-hydroxytryptamine (5-HT), histamine and adenosine triphosphate (ATP) in m-moles/mg protein of isolated platelets and isolated 5-HT organelles of rabbits*

	Platelets (Pl) $\times 10^{-6}$	Organelles (Or) $\times 10^{-4}$	$\frac{Or}{Pl}$
5-HT	97±10	210±17	234±15*
Histamine	41±5	90±11	223±18†
ATP	55±7	85±9	174±20

P (compared with ATP): *<0·05, >0·01, †~0·05. Each value represents an average with S.E. of seventeen experiments.

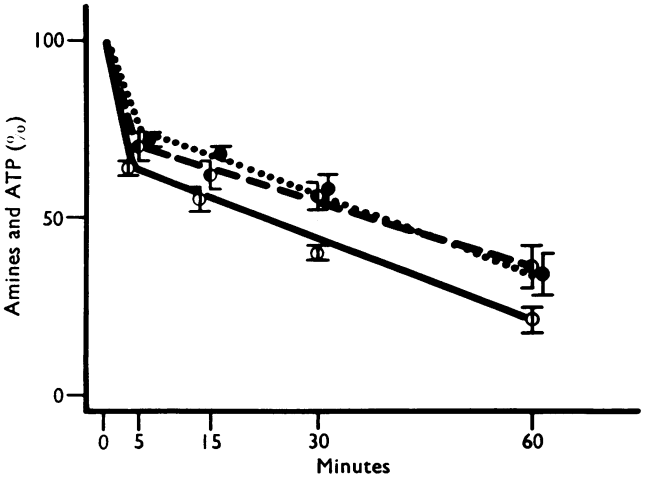

FIG. 1. Effect of the incubation time on the content of 5-hydroxytryptamine (5-HT) ○——○, histamine ◐····◐ and adenosine-triphosphate (ATP) ●- - -● of isolated organelles of rabbit platelets at 37° C. In each experiment, the organelles before incubation served as control. Each point represents an average with S.E. of four experiments.

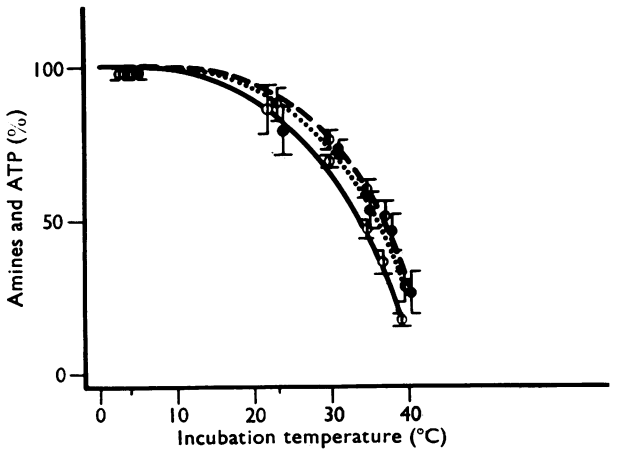

FIG. 2. Effect of temperature on the content of 5-hydroxytryptamine (5-HT) ○——○, histamine ◐····◐ and adenosine-triphosphate (ATP) ●- - -● of isolated organelles of rabbit platelets incubated for 30 min. In each experiment, the organelles before incubation served as controls. Each point represents an average with S.E. of two to four experiments.

TABLE 2. *Effect of reserpine on the uptake of ^{14}C-5-hydroxytryptamine (5-HT) as well as on the total 5-HT content in isolated platelets and isolated 5-HT organelles of rabbits*

Reserpine (μg/ml.)	^{14}C-5-HT		Total 5-HT	
	Platelets	Organelles	Platelets	Organelles
1·0	61±5*	14±2*	90±3*	99±3
0·1	92±3	81±4*	101±0	101±3

*P (compared with controls): <0·01. The incubation was carried out at 37° C for 30 min. The values are expressed in % of controls incubated for 30 min without reserpine. They represent averages with S.E. of three experiments each.

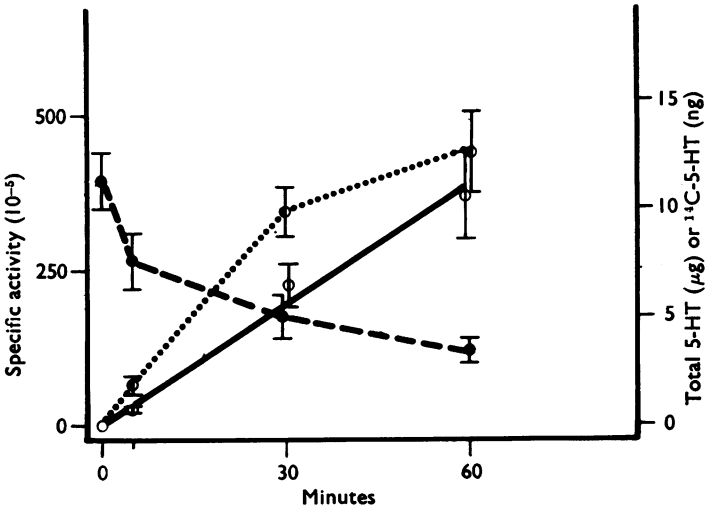

FIG. 3. Uptake of ^{14}C-5-hydroxytryptamine (5-HT) of isolated organelles of rabbit platelets incubated with 0.2 μg/cm^3 ^{14}C-5-HT (creatinine sulphate monohydrate) at 37° C. The values of total 5-HT ●- - -● and ^{14}C-5-HT ◐···◐ represent the content of one incubation sample. Each point indicates an average with S.E. of four to five experiments. ○——○, Specific activity.

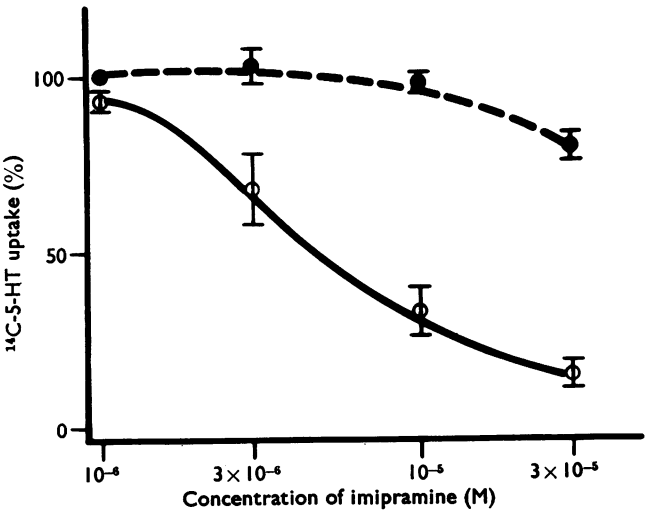

FIG. 4. Influence of imipramine *in vitro* on the uptake of ^{14}C-5-hydroxytryptamine (5-HT) by isolated platelets ○——○ and isolated 5-HT organelles of platelets ●- - -● of rabbits. The incubation was carried out at 37° C for 30 min. In each experiment, organelles and platelets incubated for 30 min without imipramine served as controls. Each point represents an average with S.E. of three experiments. ^{14}C-5-HT uptake of controls: platelets (contained in 1 ml. suspension): 0.0571±0.0067 μg; organelles: see Fig. 3.

Isolated organelles resuspended in plasma and incubated at 37° C spontaneously lose 5-HT virtually all of which appears in the incubation medium. The diminution of 5-HT in the organelles shows a maximal rate during the first 5 min ; thereafter it is less marked and almost linear with time. Histamine and ATP of the organelles disappear with a similar time course as 5-HT, though more slowly ($P<0.01$ at 30 min) (Fig. 1). The spontaneous liberation of 5-HT, histamine and ATP diminishes with decreasing incubation temperatures. This temperature dependence is most marked between 30° and 40° C. At 0°–4° C practically no liberation occurs (Fig. 2). Intact platelets incubated in plasma at 37° C for 1 hr lose practically no 5-HT, histamine or ATP.

Isolated 5-HT organelles incubated in plasma at 37° C with ^{14}C-5-HT take up the radioactive amine. The rate of uptake seems to be higher between 0 and 30 min than between 30 and 60 min. The specific radioactivity of the 5-HT of the organelles increases almost linearly with the incubation time (Fig. 3).

The uptake of ^{14}C-5-HT by isolated organelles also depends on the incubation temperature. At 20° C it amounts to only $41 \pm 5\%$ of that at 37° C, and at 4° C no significant quantities of ^{14}C-5-HT enter the organelles.

Reserpine and imipramine *in vitro* influence the 5-HT transfer in different ways. Reserpine (1.0 μg/ml.) strongly inhibits the uptake of ^{14}C-5-HT, but does not decrease the endogenous 5-HT in isolated organelles incubated for 30 min at 37° C. Lower concentrations of reserpine (0.1 μg/ml.) still diminish the ^{14}C-5-HT uptake by the organelles, but less markedly. In intact platelets, the inhibition of the ^{14}C-5-HT uptake is considerably less pronounced than in isolated organelles, and the higher dose of the drug (1 μg/ml.) causes a small loss of the endogenous 5-HT (Table 2).

Imipramine inhibits the uptake of ^{14}C-5-HT more markedly in the intact platelets than in the organelles (Fig. 4), whereas the endogenous 5-HT is not significantly influenced in either organelles or platelets.

After reserpine *in vivo*, the isolated 5-HT organelles take up only about 8% of the ^{14}C-5-HT compared with normal organelles (Table 3). In these experiments, the ^{14}C-5-HT content of the organelles has been related to the endogenous ATP, because this, as previously shown (Da Prada *et al.*, 1968), is diminished relatively little by reserpine.

Discussion

According to these experiments, ATP, like 5-HT and histamine, is concentrated to a much greater extent in the organelles as compared with intact platelets (for preliminary results see Da Prada, Pletscher & Bartholini, 1965). The presence of

TABLE 3. *Influence of reserpine in vivo on the uptake of ^{14}C-5-hydroxytryptamine (5-HT) by isolated organelles of rabbit platelets*

	Endogenous 5-HT	^{14}C-5-HT
Controls	10·62±0·79	2·14±0·64
Reserpine	0·13±0·03*	0·16±0·09†

P (compared with controls): *<0·01. †~0·01.
The organelles were incubated in plasma for 30 min at 37° C with ^{14}C-5-HT 0·2 μg/ml. (creatinine sulphate monohydrate). Reserpine (5 mg/kg) was administered intraperitoneally 16 hr before exsanguination. The endogenous 5-HT was determined in platelets of 1 ml. plasma before ultrasonic treatment. The endogenous 5-HT is indicated in μg/ml. plasma, the ^{14}C-5-HT in mμg/μg adenosine triphosphate. The figures represent averages with S.E. of seven experiments each.

large amounts of the nucleotide and of 5-HT in the same subcellular structures supports the previously expressed hypothesis of a possible physico-chemical association between the two constituents in platelets (see Pletscher, 1968). A similar association, the nature of which is still unknown, might also exist between ATP and histamine. The fact that the concentration factor of ATP seems to be somewhat inferior to that of 5-HT and histamine may be due to a partial destruction of the nucleotide during the isolation of the organelles or to a partial localization of ATP in other subcellular structures, for example, the mitochondria. The lower concentration factor of ATP might also explain why the molar ratios 5-HT/ATP and histamine/ATP seem to be somewhat higher in the organelles than in the intact platelets.

With regard to the liberation of endogenous 5-HT, the organelles are different from the intact platelets. In the latter the endogenous 5-HT does not decrease during incubation, so the loss of 5-HT from the isolated organelles, especially that occurring during the first 5 min, is possibly an artefact—due to partial damage during the isolation procedure, for example. The 5-HT storage of the organelles may also depend on the nature of their environment (for example, its chemical composition) which is certainly different within the platelets and in the plasma. The temperature sensitivity of the 5-HT liberation does not necessarily indicate the participation of an active mechanism dependent on metabolic energy. Thus, up to now there is no evidence that isolated organelles contain major amounts of metabolic enzymes. As previously discussed on the basis of experiments with intact platelets, the temperature dependence might rather be explained by physico-chemical changes, for example, in the membrane of the organelles, leading to alterations in the size of its pores (Da Prada *et al.*, 1965).

The marked uptake of ^{14}C-5-HT indicates that at least part of the isolated organelles remain functionally intact during the incubation procedure. The 5-HT uptake which, according to preliminary experiments, is also seen in synthetic media such as Tyrode, occurs against a considerable concentration gradient. Thus, at the beginning of incubation, the 5-HT concentration of the organelles is at least 1,000 times higher than that of the medium. As in the case of the 5-HT liberation, it cannot be concluded that an active mechanism participates in the uptake of ^{14}C-5-HT by the organelles. A physico-chemical binding of the ^{14}C-5-HT to the ATP might, for instance, be involved, whereby the radioactive amine possibly replaces part of the endogenous 5-HT which has been liberated. Preliminary experiments indicate that 5-HT may be bound to ATP because addition of ATP to the incubation medium seems to diminish the entry of ^{14}C-5-HT into the isolated organelles.

The temperature dependence of the ^{14}C-5-HT uptake might be due to a mechanism similar to that discussed above for the liberation of endogenous 5-HT.

The experiments with reserpine show that the drug primarily interferes with the 5-HT storage at the level of the dense osmiophilic bodies. Thus reserpine *in vitro* inhibits the uptake of ^{14}C-5-HT more markedly in the isolated organelles than in the isolated platelets. Furthermore, isolated organelles from reserpinized rabbits take up much less ^{14}C-5-HT than organelles from normal animals. These findings are therefore a direct confirmation of the previously expressed hypothesis that reserpine acts at the site of the intracellular 5-HT organelles (Pletscher *et al.*, 1967). The drug does not markedly diminish the ATP content of the dense osmiophilic bodies (Da Prada *et al.*, 1968), so it might interfere with the binding of 5-HT to

ATP. The missing or weak effect *in vitro* of reserpine on the endogenous 5-HT of organelles or platelets respectively is probably due to the shortness of the incubation period (30 min).

Imipramine inhibits the ^{14}C-5-HT uptake of platelets at a different site from reserpine. Thus, in contrast to reserpine, the intact platelets are more sensitive to imipramine than the isolated organelles. Only in relatively high concentrations does imipramine interfere with the ^{14}C-5-HT uptake of the isolated organelles. This confirms the hypothesis that the drug primarily acts at the platelet membrane (Pletscher *et al.*, 1967). According to previous findings with platelets of guinea-pigs, the 5-HT uptake at the membrane seems to be an active process (Pletscher *et al.*, 1967; Pletscher, 1968). Imipramine might therefore interfere with this transport system.

In conclusion, these experiments demonstrate rather directly that there are at least two different uptake mechanisms for 5-HT in platelets. The one operates at the intracellular 5-HT organelles, which also store histamine, and might involve binding of the amines to ATP. The other mechanism which, according to previous results, seems to depend on metabolic energy, probably acts at the level of the platelet membrane. The uptake mechanisms for 5-HT in platelets thus seem to be similar to those suggested for noradrenaline in sympathetic nerve endings (see Carlsson, 1966).

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