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INACTIVATION OF 5-HYDROXYTRYPTAMINE BY MAMMALIAN BLOOD PLATELETS

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Abstract—When the platelet-rich plasma of rabbit is incubated *in vitro* with 20 μg/ml of tetrabenazine most of the 5-hydroxytryptamine released from the platelets is inactivated. This inactivation is done by the platelets themselves and not by the plasma outside. It is prevented by monoamine oxidase inhibitors and by incubation in nitrogen atmosphere instead of air. Under similar conditions, the human platelets inactivate about 30 per cent of their 5-hydroxytryptamine released and those of rat less than 10 per cent. It is possible that also when released by other mechanisms, 5-hydroxytryptamine is inactivated in blood platelets and that this inactivation has an important role in the metabolism of the amine.

INCUBATION of the platelet-rich plasma of rabbit *in vitro* with reserpine or tetrabenazine causes a decrease in 5-hydroxytryptamine (5HT) content of platelets.[1–3] However, there is no corresponding increase in the 5HT of the platelet-free plasma, which indicates that most of the amine released has been inactivated.[3] If, before collecting the blood, rabbits are pretreated with monoamine oxidase (MAO) inhibitor, isocarboxazid, all of the 5HT released from the platelets is found in the plasma.[3] The following experiments were undertaken to demonstrate, which one of the two components of rabbit blood, plasma or platelets, is responsible for this inactivation, and to study whether 5HT would be similarly inactivated when released from platelets of other species.

METHODS

Blood was taken from the carotid artery of rabbits and from the abdominal aorta of rats under ether anaesthesia. From humans the blood was withdrawn from an arm vein. The blood was immediately mixed with 1/9 volume saline containing 1% disodium ethylenediaminetetra-acetate and 0·1% heparin. Rabbits received heparin (10 mg/kg) intravenously before the anaesthesia was started.

Platelet-rich plasma was obtained by centrifugation at 200–300 **g**. Employing gentle shaking at 37 °C, 1·5–3·0 ml of the platelet-rich plasma was incubated first with β-phenylisopropylhydrazine hydrochloride (PIH, final concentration 10^{-5}, 10^{-4} or 10^{-3} M), in nitrogen atmosphere (before entering the hood of the metabolic shaker commercial nitrogen gas went through two washing flasks containing 10% solution of pyrogallol and one containing water) or saline, for 15 min and then tetrabenazine methane sulfonate [20 μg/ (base)/ml] was added and incubation continued for 3 hr. After incubation of rabbit plasma, platelets and platelet-free plasma were separated by centrifugation at about 2000 **g** for 20 min. Occasional platelet counts were made

according to Feissly[4] in the phase contrast microscope. They showed that the platelet-rich plasma was free from other formed elements and that all platelets had been sedimented by the second centrifugation. In half of the experiments with human and rat blood, the platelets and plasma were analysed separately as described above, but only the total calculated values are presented in the results.

5HT was measured by a spectrophotofluorometric method.[5] Some acetone extracts were analysed by using the rat stomach method[6] and the results agreed with the chemical method. Student's t-tests were used for statistical analysis.

RESULTS

Table 1 gives the amounts of 5HT, which were found in the platelet-free rabbit's plasma derived from the platelet-rich plasma after incubation with tetrabenazine. This treatment normally decreases the 5HT content of platelets by more than 5 μg/ml[3], but only about one tenth of the liberated amine is found in the plasma after separating it from platelets. Isoniazid has no effect but there is a significant increase in the mean amine content when the platelets are incubated in nitrogen atmosphere ($P < 0{\cdot}001$) or in 10^{-5} or 10^{-4} M of PIH ($P < 0{\cdot}01$ or $< 0{\cdot}001$, respectively). With the lower concentration of PIH more 5HT is inactivated than with the higher ($P < 0{\cdot}01$).

TABLE 1. EFFECT OF VARIOUS TREATMENTS ON THE ABILITY OF PLATELETS (AND PLASMA) OF RABBIT TO INACTIVATE 5-HYDROXYTRYPTAMINE (5HT) RELEASED BY TETRABENAZINE FROM THE PLATELETS *in vitro*

Treatment (in addition to tetrabenazine)	5HT μg/ml (mean ± s.e.m.) of platelet-free plasma*
Controls	0·70 ± 0·20 (+)†
Isoniazid 10^{-3} M	0·91 ± 0·19 (−)
Nitrogen atmosphere‡	3·80 ± 0·40 (−)
Isoniazid 10^{-3} M and nitrogen atmosphere	3·64 ± 0·10 (−)
β-Phenylisopropylhydrazine 10^{-5} M	2·62 ± 0·67 (−)
β-Phenylisopropylhydrazine 10^{-4} M	4·80 ± 0·82 (−)

* The amount of 5HT in the platelet-free plasma after incubation of the platelet-rich plasma with tetrabenazine (four to five experiments).

† Indicates that platelet-free plasma inactivates (+) or does not inactivate (−) added 5HT.[7]

‡ If the nitrogen was not washed with pyrogallol its maximum oxygen content was 0·14% and the incubation in this gas gave similar results to control experiments, i.e. incubation in air.

5HT was not inactivated during a 3-hr incubation when 10–20 μg/ml was added to the suspensions of rabbit platelets which had been broken by freezing and thawing or shaking with water. The amount of platelet-material in the final saline or pH 7·4 phosphate buffer was two to three times more than in the original platelet-rich plasma.

In eight experiments with *human* platelet-rich plasma (Table 2) the total 5HT content of platelets and plasma was reduced by 22·5 per cent. The mean of individual differences (0·038 μg/ml ± 0·13 μg/ml) was significantly different from zero ($P < 0{\cdot}05$). About 73 per cent of 5HT was released in four of these experiments and, therefore, two-thirds of the liberated amine escaped inactivation. Addition of 10^{-3} M of PIH

before tetrabenazine to the platelet-rich plasma seemed to inhibit or reduce the inactivation of 5HT in four experiments.

In six experiments with *rat* platelet-rich plasma (Table 2), the total 5HT was reduced during the incubation by only 4·4 per cent. The mean of differences (0·11 μg/ml ± 0·03 μg/ml) is also here significantly different from zero ($P < 0·02$). About 60 per cent of 5HT was depleted from rat's platelets by this treatment and, therefore, less than 10 per cent of the released amine was metabolized.

TABLE 2. THE ABILITY OF PLATELET-RICH PLASMA OF HUMAN AND RAT TO INACTIVATE 5-HYDROXYTRYPTAMINE (5HT) RELEASED BY TETRABENAZINE FROM THE PLATELETS *in vitro**

Species	5HT μg/ml incubation without tetrabenazine	Reduction of 5HT during incubation with tetrabenazine	
		(μg/ml)	(%)
Human	0·145 ± 0·03	0·038 ± 0·13	22·5 ± 5·0
Rat	2·62 ± 0·29	0·11 ± 0·03	4·4 ± 1·3

* The values refer to mean ± s.e.m.

DISCUSSION

The platelet-free plasma of rabbit, but not that of men and rat, slowly inactivates added 5HT *in vitro*.[7] From 20 μg of 5HT added per ml about 1·5 μg/ml per hr is inactivated. This inactivation is prevented by isoniazid, MAO inhibitors, like iproniazid and PIH, but not, or only slightly prevented by nitrogen atmosphere.[7] It is likely that this is not an important pathway of 5HT inactivation even in the rabbit's blood, because during the incubation of platelet-rich plasma with 5HT releasing substances more of the amine disappears than what can be ascribed to the inactivation potency of platelet-free plasma.

That 5HT released by tetrabenazine from platelets is destroyed in the platelets before it enters the plasma is further emphasized by the following points.

(1) Isoniazid does not significantly increase the mean amount of 5HT present in the platelet-free plasma after the incubation of the platelet-rich plasma. The same concentration of isoniazid, however, completely inhibits the inactivation of 5HT by platelet-free plasma.

(2) Unlike isoniazid, nitrogen atmosphere inhibits only slightly the ability of platelet-free plasma to inactivate 5HT. In nitrogen, nevertheless, much more 5HT is present in the plasma. In addition, when isoniazid is present in the nitrogen atmosphere the amount of non-inactivated 5HT is not increased.

(3) PIH in concentration of 10^{-5} M inhibits 5HT inactivation by platelet-free plasma. When platelet-rich plasma is incubated with tetrabenazine in the presence of 10^{-5} and 10^{-4} M of PIH more 5HT is found in the plasma with the higher concentration. This indicates that the mechanism which inactivates 5HT inside the platelets is not inhibited completely by the lower PIH concentration which already makes the platelet-free plasma inactive. More 5HT is released by tetrabenazine with PIH 10^{-5} M than 10^{-4} M[8] and, therefore, relatively even more amine is destroyed by platelets at 10^{-5} M of inhibitor than what appears from the figures. Also iproniazid and nitrogen

atmosphere, but not isoniazid, inhibit partly the release of 5HT by tetrabenazine from the platelets.[8, 9] From the limited information received from the present and previous experiments[3] it seems possible that the inactivation in platelets is due to amine oxidase.[10, 11] This is further emphasized by the finding that incubation of rabbit's whole blood with reserpine results in an increase in the plasma 5-hydroxyindoleacetic acid, but not if the rabbit is pretreated with iproniazid.[12]

Because platelet-free plasma of human and rat does not inactivate 5HT, the results indicate that the platelets of these species are able to destroy part of the amine released from them by tetrabenazine. The concentration of tetrabenazine used releases percentwise more 5HT from the platelets of man and rat than from those of rabbit. The relatively weaker 5HT metabolizing activity of the platelets of man and rat may be partly due to this.

It is possible that platelets inactivate 5HT also when it is liberated by other mechanisms. The rate of depletion in these experiments is probably much higher than that which occurs in any physiological condition. Therefore it would not be improbable to assume that under normal conditions the platelets in any of these species are able to inactivate *in vivo* all their 5HT. Experiments on the blood of a thrombocytemic patient have shown that the blood platelets in this pathological case were able to inactivate all the 5HT released during incubation with tetrabenazine, and no 5HT inactivation was found in the plasma.[13]

The failure to demonstrate inactivation of 5HT by broken platelet suspensions may be due to a too great dilution of the active principle. It is possible that the very high concentration of 5HT in the platelet has to be located close to the oxidase in order to be inactivated after being "released" from its binding sites. Further experiments are expected to show whether the activity is connected with the intact platelet structure or not. The fate of other platelet-bound amines will also be studied under the same conditions.

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