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INFLUENCE OF β-PHENYL*ISO*PROPYLHYDRAZINE ON THE ABILITY OF BLOOD PLATELETS TO RETAIN 5-HYDROXYTRYPTAMINE

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Abstract—Platelet-rich plasma of rabbits, rats and humans was incubated under various treatments and the 5-hydroxytryptamine (5HT) content of separated platelets was analysed per ml of original plasma volume.

More 5HT was found after the incubation when monoamine oxidase (MAO) inhibitor, β-phenyl*iso*propylhydrazine (PIH) was present in concentrations of 10^{-4} or 10^{-3}M. Incubation in nitrogen instead of air had similar effect. At higher concentrations (10^{-3} or 10^{-2}M, according to the species) PIH produced a marked 5HT depletion. Iproniazid, as well as isoniazid, which is not a MAO inhibitor, decreased the platelet 5HT when tested on rat platelet-rich plasma at 10^{-2}M.

In rabbit's platelets PIH (from 10^{-5} to 10^{-3}M) partly inhibited the release of 5HT produced by tetrabenazine. A nitrogen atmosphere had the same effect and PIH in addition to nitrogen offered no further inhibition. Isoniazid 10^{-3}M was ineffective. In suitable concentrations PIH also had an inhibitory action on the 5HT release from platelets of rats and humans. The inhibition of 5HT depletion is assumed to be connected with the inhibition of the platelets ability to inactivate their 5HT.

RAUWOLFIA alkaloids and tetrabenazine cause a decrease of 5-hydroxytryptamine (5HT) content in various tissues including the blood platelets.[1–3] After treatment with monoamine oxidase (MAO) inhibitor, iproniazid, this depletion is, at least partly, inhibited in brain tissue.[4, 5]. A further proof for this inhibition were experiments, where rabbit's platelet-rich plasma was incubated *in vitro* with reserpine. More 5HT was left in the platelets if, in addition to reserpine, iproniazid was present in the plasma.[6]

This paper describes experiments which were designed to study especially the effect of another MAO inhibitor, β-phenyl*iso*propylhydrazine,[7] on the 5HT decrease in platelets. Because platelets of rabbit inactivate their 5HT more readily than those of man and rat,[8] the platelets of all these three species have been studied. As the agent releasing 5HT, tetrabenazine methane sulphonate was used, because it is soluble in saline. The mode of action of this benzoquinolizine derivative is related to that of reserpine.[3, 9]

METHODS

Arterial blood was taken from a carotid artery of male rabbits (2·3–3·0 kg) and from the abdominal aorta of male rats (200–300 g) in ether anaesthesia. Human venous blood was collected from a cubital vein. Blood was immediately mixed with $\frac{1}{9}$ vol. of 1% disodium ethylenediaminetetra-acetate and 0·1% heparin in saline. Rabbits were heparinized (5 mg/kg intravenously) immediately before bleeding.

After centrifuging the blood at about 200 **g** for 5 min the platelet-rich plasma was withdrawn. The remaining blood was then again centrifuged at least once more, and the platelet-rich plasma was removed after each spin, and the procedure repeated once or more. This plasma (sample volume 2–3 ml) was incubated with tetrabenazine methane sulphonate (Nitoman®, F. Hoffman—La Roche & Co, Basel) under gentle shaking for 3 hr in 25 ml Erlenmeyer flasks at 37 °C in air. Some experiments were carried out in an atmosphere of nitrogen. β-Phenyl*iso*propylhydrazine hydrochloride (Catron®, Lakeside Laboratories, Inc. Milwaukee, Wisc.), iproniazid phosphate (Marsilid®, F. Hoffmann La Roche & Co. Basel) and isoniazid (Tubilysin®, Orion Oy, Helsinki) were added to the plasma 15 min before tetrabenazine. The final concentrations per ml of plasma of all substances are reported in the text. Solutions were prepared in saline and the volume of tetrabenazine solution used was 0·1 ml and that of the others 0·2 ml per ml of plasma. Solvent alone was added to the controls.

After incubation the platelets were separated by centrifugation at about 2000 **g** for 20 min at room temperature and their 5HT content was measured by a spectrophotofluorometric method.[10] Platelet counts were made in the phase contrast microscope.[11] All the experiments were carried out in siliconized glassware. The standard 5HT was used as creatinine sulphate (F. Hoffmann—La Roche & Co., Basel), but the doses are given in terms of the base. Standard error of the mean is given to indicate the distribution of values. Student's *t*-test was used to estimate the significance of the differences.

RESULTS

Incubation without tetrabenazine

The effect of incubation with PIH, iproniazid, isoniazid and atmosphere of nitrogen are reported in Table 1. The values are in per cent of the corresponding control figures obtained from the platelets of the same plasma.

TABLE 1. 5HT FOUND IN BLOOD PLATELETS PER ML OF PLATELET-RICH PLASMA AFTER INCUBATION WITH β-PHENYL*iso*PROPYLHYDRAZINE, IPRONIAZID AND ISONIAZID

P-values are given when they are less than 0·05 and it indicates that mean of the individual differences between control and experimental group is significantly different from zero.

Atmosphere during incubation	5HT μg/ml	5HT % of the corresponding controls					
	Controls	β-phenyl*iso*propylhydrazine M				Iproniazid M	Isoniazid M
		10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-2}	10^{-2}
				Rabbit			
Air	10·1(7)* ± 0·8†	98(4) ± 1	101(4) ± 1	106(7) ± 2 $P<0·05$	26(4) ± 5 $P<0·02$		
Nitrogen	16·2(4) ± 1·5			96(4) ± 8			
				Rat			
Air	3·3(12) ± 0·3	100(3) ± 6	109(6) ± 3 $P<0·05$	77(8) ± 3 $P<0·01$	16(3) ± 3 $P<0·02$	70(5) ± 6 $P<0·01$	53(4) ± 13 $P<0·001$
				Human			
Air	0·17(8) ± 0·06		123(5) ± 13	92(6) ± 18	36(5) ± 8 $P<0·05$		

* Number of animals
† Standard error of the mean

In *rabbit* platelets PIH in concentrations of 10^{-5} and 10^{-4}M did not alter the 5HT content of platelets but at 10^{-3}M the amount of 5HT was always higher when compared to the control value. Ten times higher concentration of PIH had a marked opposite effect and only one-quarter of the amine was left in the platelets during 3 hr incubation. This 5HT releasing effect of PIH was time-dependent as illustrated by the following example: 5HT left after incubation with PIH 10^{-2}M for $\frac{1}{2}$ hr, 90 per cent; $1\frac{1}{2}$ hr, 60 per cent; 3 hr, 36 per cent.

In four experiments the platelet-rich plasma was incubated in nitrogen atmosphere. After the nitrogen incubation the mean control value was significantly ($P<0{\cdot}02$) more than the control value obtained after incubation in air. In nitrogen, PIH did not further increase the amount of 5HT. Isoniazid at 10^{-3}M in four experiments had no effect on the platelet 5HT.

When *rat* platelet-rich plasma was incubated with PIH at 10^{-4}M there was more 5HT in the platelets than in the controls and in those which were treated with 10^{-5}M of PIH. Also in the rat platelets a decrease of 5HT was demonstrated but a concentration only of 10^{-3}M was required to produce it.

Iproniazid and isoniazid were also tested at 10^{-2}M and they both produced a decrease of 5HT of about equal degree but their effect was weaker than that of PIH. 5HT released from platelets by PIH or iproniazid could be found in the platelet-free plasma both in rats and rabbits.

In *human* platelets PIH produced changes which were more similar to those found in rat than those found in rabbit platelets. PIH 10^{-4}M increased the 5HT yield but 10^{-2}M lowered it.

Incubation with tetrabenazine

The results are shown in Fig. 1, which gives decrease in per cent from the corresponding normal or PIH-treated control value.

In *rabbit* platelets all PIH concentrations tested significantly inhibited the release of 5HT by tetrabenazine. Incubation in nitrogen had a marked inhibitory effect on the 5HT decreasing action of tetrabenazine. Addition of PIH did not further reduce the effect of tetrabenazine under these conditions.

In four experiments with 10^{-3}M of isoniazid tetrabenazine released $50{\cdot}6 \pm 5{\cdot}5$ per cent of 5HT. In the same plasmas without isoniazid the value was $52{\cdot}4 \pm 7{\cdot}0$ per cent.

At the concentration of 10^{-4}M PIH had no inhibitory effect on the 5HT release in *rat* platelets. However, also here a significant inhibition was demonstrated at ten times higher concentration of PIH.

In *human* platelets, 10^{-3}M of PIH had a significant inhibitory effect and there was an indication that 10^{-4}M also had some effect on the 5HT release.

Biological method: pH of the platelet-rich plasma; platelet counts

Before employing the spectrophotofluorometric method, a number of experiments with rabbit platelets and some with rat platelets were made by using the rat stomach method[12] for 5HT estimation. On the whole the results were in agreement with those obtained by chemical means and reported above.

During the incubation of platelet-rich plasma in air the pH rose to about 8·0. The saline solutions of PIH, isoniazid and iproniazid are acid but the buffering capacity of plasma is high and only with the concentrations of 10^{-2}M some lowering in plasma

pH was regularly seen. This was most marked with iproniazid and least with PIH. Further experiments with rabbit and rat platelet-rich plasma not included in Table 1 have shown that the results are similar if the MAO inhibitor solutions are neutralized with sodium hydroxide before use.

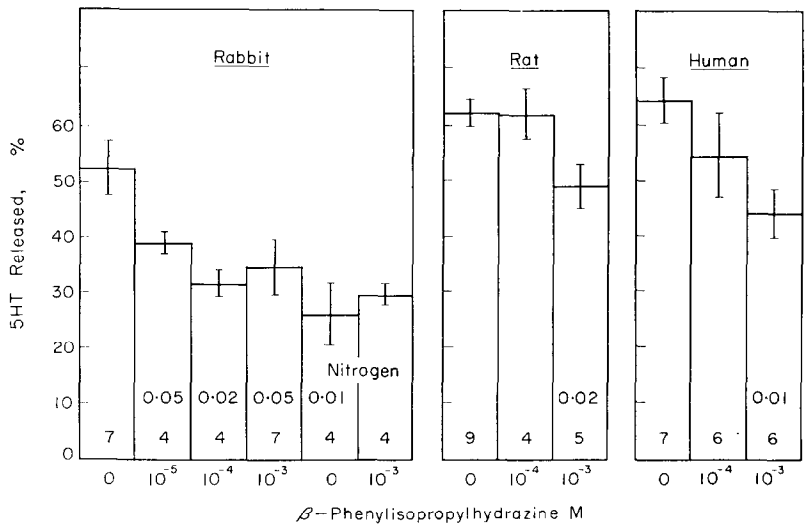

FIG. 1. Effect of β-phenylisopropylhydrazine on the amount of 5-hydroxytryptamine (5HT) in per cent (± s.e.m.) released from rabbit's blood platelets during 3-hr incubation of platelet-rich plasma with 20μg/ml of tetrabenazine. The incubations were done in air unless otherwise indicated. The significance of the differences between the means of the β-phenylhydrazine and corresponding control group are given only when $P<0{\cdot}05$. P is less than the over value in columns. The control group in nitrogen is compared to the control group in air. The lower value in columns indicates the number of experiments.

When PIH significantly increased the platelet 5HT (see Table 1) there was generally a roughly proportional increase in the number of platelets per ml of plasma, although this was not a consistent finding. The inhibition of tetrabenazine-induced 5HT decrease cannot be explained by higher numbers of platelets in the samples containing PIH in addition to tetrabenazine. Also, the low 5HT values obtained after incubation with high concentrations of the hydrazine derivatives were not due to the destruction of platelets.

DISCUSSION

In suitable concentrations MAO inhibitor β-phenyl*iso*propylhydrazine (PIH) inhibits the slight decrease in 5HT of the platelets (when estimated per ml of platelet-rich plasma), which normally occurs during incubation of platelet-rich plasma *in vitro*. The results indicate that fewer of the platelets are destroyed when incubated with PIH, but the reason, or an additional reason, may be MAO inhibition through mechanisms as discussed below in connection of tetrabenazine experiments. The role of MAO inhibition is emphasized by the findings that incubation in nitrogen instead of air had a similar effect to PIH, and a hydrazine lacking MAO inhibitory action, isoniazid,[13] had no effect.

In concentrations of 10^{-3}M or higher, PIH itself lowered the 5HT content of platelets. At least on rat's platelets another MAO inhibitor, iproniazid, had similar effect. This action, however, was not bound to MAO inhibition, because isoniazid was more effective than iproniazid. The concentrations necessary for this release *in vitro* are beyond the therapeutic *in vivo* levels. The possibility of this action, however, has to be kept in mind at least while using relatively high doses of hydrazine derivatives in animal tests.

In addition to iproniazid, previously shown to inhibit reserpine induced depletion of 5HT from the platelets[6], PIH in various concentrations has inhibitory action on the tetrabenazine-induced 5HT release in platelets. PIH had an inhibitory effect in rabbit's platelets also in low concentrations which were ineffective in the platelets of the two other species. At least intact platelets of rabbits destroy their own 5HT released by tetrabenazine while platelets of man inactivate about one-third of their 5HT and those of rats less than 10 per cent of the amine released by this treatment.[8] The destruction of 5HT by platelets is inhibited if PIH is present in the medium, or if the incubation is carried out in an atmosphere of nitrogen.[8] Previous results also indicate that other MAO inhibitors inhibit this inactivation.[3, 14] PIH did not further increase the inhibition of tetrabenazine action produced by nitrogen. The inhibition mechanisms of PIH and nitrogen are probably similar, and involve the inhibition of 5HT oxidation.

In many respects the platelets are autonomous living cells with measurable metabolic functions.[15, 16] It is also probable that 5HT in the platelets is localized in subcellular particles[17] from which it is released by tetrabenazine to the cytoplasma. Therefore the mechanism for the inhibition produced by MAO inhibition for the 5HT depletion could be the same in platelets as in other tissues. Because the platelets have no 5-hydroxytryptophan decarboxylase activity[18] the picture is here less complex.

Unlike 5HT itself, its breakdown products can probably easily penetrate cell membranes. If MAO and 5HT are stored in different granules, as is suggested by studies on mastocytoma cells,[19] the amine has to penetrate the intracellular membrane before being metabolized, even when no MAO inhibitor is present. However, MAO inhibitor, with or without 5HT releasing agent, increases the free 5HT in the cytoplasm and this may, through the operation of the principle of mass action, prevent 5HT from coming out from the storage particle. The depletion of 5HT from the platelet could also be inhibited by the difficulty the free amine has in penetrating the cell membrane. Histological findings on intestinal mucosa[20] as well as those obtained by centrifuge from the brain homogenates[21] have indicated that iproniazid prevents to some degree the decrease of granular 5HT produced by reserpine. It is quite possible, however, that MAO inhibitors do not inhibit the fundamental action of substances like reserpine in preventing the cells from storing their 5HT normally, i.e. producing the change from bound to free amine as emphasized by Brodie *et al.*[22]

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