Formation of 5-Hydroxytryptophol by Blood Platelets after Thrombin and Reserpine

Thrombocytes lose most of their endogenous 5-hydroxytryptamine (5-HT) when they aggregate, e.g. under the influence of thrombin[1]. The metabolic fate of the liberated 5-HT has not been elucidated, whereas the 5-HT released from isolated platelets by drugs such as reserpine is partly transformed into 5-hydroxytryptophol and 5-hydroxyindoleacetic acid[2,3]. In previous work[2,3], the formation of 5-hydroxytryptophol had been measured by qualitative methods only. It was therefore of interest to carry out quantitative comparisons of the metabolism of the 5-HT liberated by thrombin and by reserpine.

Experimental. Platelets of rabbits were isolated as previously described[2,3], washed with physiological saline and resuspended either in a modified Tyrode solution[4] or in phosphate buffer[5], pH 7.4. After addition of 1 unit[6]/ml thrombin or 10 γ/ml reserpine (Tyrode solution) or 1 unit/ml thrombin + 0.3 g/l (0.0027 *M*) $CaCl_2$ (phosphate buffer), the 5-HT of the platelets as well as the 5-HT and the 5-hydroxyindoleacetic acid of the incubation medium were measured spectrophotofluorimetrically[7-9]. Furthermore, 5-hydroxytryptophol was assayed by a spectrophotofluorimetric method that involves extraction of this alcohol from the incubation medium with butanol-ethylacetate after removal of the 5-HT by Amberlite C.G. 50[10]. The results obtained with quantitative methods were confirmed qualitatively by paper chromatography[2,3].

Results and discussion. As described previously in a plasma medium[11], reserpine causes a slow release of platelet 5-HT which in the present experiments amounts to about 50% after 2 h. Already within 30 min of incubation, a relatively large part (about 20%) of the released 5-HT can be recovered as 5-hydroxytryptophol. This percentage does not markedly change for 2 h, although in addition some 5-hydroxyindoleacetic acid appears (Figures 1 and 2). This might indicate that 5-HT is in part metabolized during or immediately after its release.

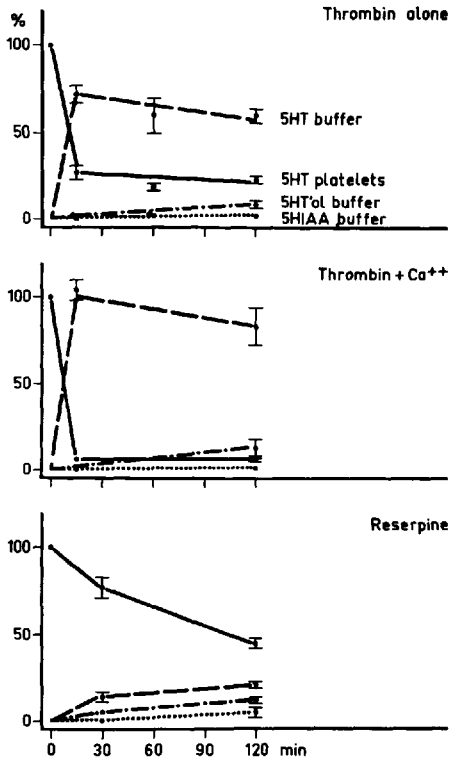

Fig. 1. Effect of thrombin, thrombin + Ca^{++} and reserpine on the 5-hydroxytryptamine metabolism of isolated blood platelets of rabbits. Ordinate: 5-hydroxytryptamine (5-HT), 5-hydroxytryptophol (5-HT'ol) and 5-hydroxyindoleacetic acid (5-HIAA) in % of the 5-HT of the platelets before incubation. Abscissa: incubation time. Averages of 4 experiments ± S.E.

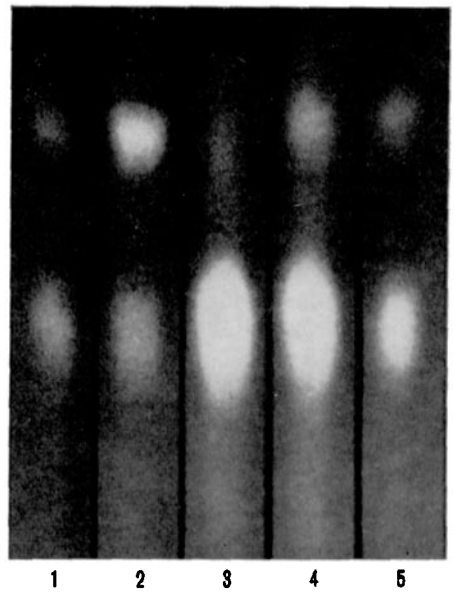

Fig. 2. Paper chromatogram of basic extracts of the incubation medium after incubating isolated blood platelets of rabbits with thrombin and reserpine. Paper: Schleicher & Schüll, No. 2043. Solvent system: *n*-propanol-NH_3 1 *N* (5:1 v/v). 1 = reserpine 1/4 h; 2 = reserpine 2 h; 3 = thrombin 1/4 h; 4 = thrombin 2 h; 5 = standards: (A) 5-hydroxytryptamine 3 γ, (B) 5-hydroxytryptophol 3 γ. In each experiment with reserpine, 5 cm^3 of the incubation medium were extracted, whereas the experiments with thrombin were carried out with 2.5 cm^3.

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[2] G. Bartholini and A. Pletscher, Exper. *20*, 376 (1964).
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[4] NaCl 7.60 g/l (0.130 *M*); KCl 0.42 g/l (0.006 *M*); Versene 0.80 g/l (0.002 *M*); $NaH_2PO_4 \cdot 2H_2O$ 0.14 g/l (0.001 *M*); $NaHCO_3$ 2.10 g/l (0.003 *M*); Glucose 2.00 g/l (0.011 *M*); Saccharose 4.50 g/l (0.010 *M*)
[5] NaCl 6.87 g/l (0.120 *M*); KH_2PO_4 0.86 g/l (0.006 *M*); $Na_2HPO_4 \cdot 2H_2O$ 4.94 g/l (0.027 *M*); Glucose 2.00 g/l (0.011 *M*); Saccharose 4.50 g/l (0.010 *M*)
[6] N.I.H. units.
[7] D. F. Bogdanski, A. Pletscher, B. B. Brodie, and S. Udenfriend, J. Pharmacol. exp. Therap. *117*, 82 (1956).
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Thrombin exhibits a pattern of release and metabolism of 5-HT different from that of reserpine (Figures 1 and 2)[12]. In the presence as well as in the absence of Ca^{++}, thrombin causes a rapid loss of 5-HT from isolated blood platelets. The liberation of the amine is much more rapid than with reserpine, being maximal within 15 min (according to preliminary experiments already after 2 min). As found by previous authors with another buffer (NaCl-Tris)[13], the thrombin-induced 5-HT liberation is more marked in the presence of Ca^{++}. Thus with thrombin + $CaCl_2$ the platelets lose about 95% of the original 5-HT and simultaneously coarse platelet aggregates are formed. On incubation with thrombin alone, about 75% of the 5-HT leaves the platelets; aggregation is in general very fine and often cannot be seen macroscopically. After 15 min, practically all the 5-HT liberated from the platelets is identified in the incubation fluid and no appreciable amounts of metabolites are detectable with the spectrophotofluorimetric method. After 1 and 2 h, about 2 and 10–15% respectively of the liberated 5-HT can be recovered as 5-hydroxytryptophol and 1–2% as 5-hydroxyindoleacetic acid. Both these metabolites have been identified by paper chromatography[2,3]. Their formation explains the slight decrease in 5-HT in the incubation medium between 15 min and 2 h.

The experiments show that after thrombin the 5-HT leaves the platelets very rapidly without being metabolized initially. If the liberated 5-HT remains in contact with the thrombocytes, however, part of it is secondarily transformed into 5-hydroxytryptophol and to a minor extent into 5-hydroxyindoleacetic acid. Both these metabolites derive from 5-hydroxyindoleacetaldehyde, the product of oxidative deamination of 5-HT by monoamine oxidase. In the absence of major amounts of erythrocytes, which probably contain aldehyde oxidase, the 5-hydroxyindoleacetaldehyde is mainly converted to 5-hydroxytryptophol[3]. These findings demonstrate therefore that monoamine oxidase is probably still active in platelets aggregated by thrombin. They furthermore indicate the possibility that 5-hydroxytryptophol formation might also occur in vivo, e.g. in white thrombi which contain only few erythrocytes.

Zusammenfassung. In isolierten Blutplättchen von Kaninchen bewirkt Thrombin rasche und hochgradige Freisetzung von unverändertem 5-Hydroxytryptamin, welches durch weitere Inkubation sekundär teilweise zu 5-Hydroxytryptophol und 5-Hydroxyindolessigsäure umgewandelt wird. Nach Reserpin scheint hingegen ein Teil des 5-Hydroxytryptamins bereits bei seiner Freisetzung zu diesen Metaboliten abgebaut zu werden.

M. DA PRADA, G. BARTHOLINI, and A. PLETSCHER

Medizinische Forschungsabteilung, F. Hoffmann-La Roche & Co. AG, Basel (Switzerland), December 14, 1964.

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Inhibition of Acute Effects of Hydralazine by an Adrenergic β-Receptor Blocking Agent

Some of the acute effects of hydralazine such as reduction of peripheral resistance, tachycardia and coronary dilatation, resemble the effects that would result from stimulation of adrenergic β-receptors. We, therefore, attempted to inhibit these effects of hydralazine by administration of an adrenergic β-receptor blocking agent, pronethalol.

Blood pressure and heart rate were measured in the unanaesthetized trained dog, in the pentobarbital-anaesthetized[1] dog and in the allobarbital-urethane anaesthetized[2] cat. Blood pressure, heart rate, total coronary flow and oxygen saturation in arterial and coronary venous blood were measured in the allobarbital-urethane-anaesthetized[2] cat. For these experiments the open-chest, right heart bypass preparation with fixed cardiac output was used[3]. Oxygen saturation was measured by a photometric method. Doses of the substances used are given in Tables I and II.

In the unanaesthetized dog hydralazine reduced blood pressure and increased heart rate. Pronethalol, in a dose which has no pronounced influence on blood pressure or heart rate, inhibited these effects (Table I). In the anaesthetized dog and cat, hydralazine produced no tachycardia. The blood pressure fall was more pronounced in the anaesthetized than in the unanaesthetized dog. Administration of the β-blocker in the anaesthetized dog, did not inhibit, but enhanced the hypotensive effect of hydralazine.

Coronary flow was markedly increased by hydralazine and, as oxygen consumption remained unchanged, oxygen extraction decreased correspondingly. In the doses used, pronethalol had no pronounced effect on the parameters measured. Pronethalol abolished the effect of hydralazine on coronary flow. In addition, oxygen consumption, which was not influenced by either compound given singly, was markedly reduced. Therefore, oxygen extraction remained low in spite of the return of coronary flow towards normal levels.

The effect of pronethalol is apparently different in the anaesthetized and in the unanaesthetized dog. An increase in heart rate in the unanaesthetized and a decrease in heart rate in the anaesthetized dog were seen. The blood pressure and heart rate effects of hydralazine were inhibited in the unanaesthetized dog by pronethalol, while the hypotensive effect was enhanced in the anaesthetized dog and cat.

The effect of hydralazine on coronary flow was antagonized by pronethalol. The effect on oxygen consumption may have been antagonized, if we assume that the

[1] Nembutal, Abbott; 25 mg/kg i.v.
[2] Dial, CIBA; 35 mg/kg i.p. + 35 mg/kg s.c.
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