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EFFECT OF MONOAMINE LIBERATORS ON THE METABOLISM OF 5-HYDROXYTRYPTAMINE IN BLOOD PLATELETS

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Abstract—DL-Amphetamine, *p*-chloro-N-methylamphetamine (Ro 4-6861), and tyramine liberate up to 95 per cent of the 5-hydroxytryptamine (5HT) of isolated, normal platelets. No deaminated metabolites such as 5-hydroxytryptophol and 5-hydroxyindoleacetic acid are formed, however, as they are with reserpine and the benzoquinolizine derivative Ro 4-1284. The sympathomimetic amines, in contrast to reserpine and Ro 4-1284, decrease the formation of 5-hydroxytryptophol and 5-hydroxyindoleacetic acid from exogenous 5HT by isolated blood platelets of reserpinized rabbits. Thereby, the sympathomimetic amines do not diminish the penetration of exogenous 5HT into the reserpinized platelets and, unlike compounds of the reserpine type, also inhibit 5-hydroxytryptophol formation by agglutinated, homogenized platelets from reserpinized animals. It is concluded that the previously described differences in the metabolic pattern of platelet 5HT after sympathomimetic amines and reserpine are probably due to an interference of the sympathomimetic amines with the oxidative deamination rather than with a localization of 5HT in two separate pools.

RESERPINE-like drugs and sympathomimetic amines have been shown to liberate the 5-hydroxytryptamine (5HT) of isolated blood platelets in two different ways. The 5HT released by reserpine (5 mg/kg) and the benzoquinolizine derivative Ro 4-1284* (50 mg/kg) is partly metabolized, whereby mainly 5-hydroxytryptophol and some 5-hydroxyindoleacetic acid are formed. Amphetamine, *p*-chloro-N-methylamphetamine (Ro 4-6861), and tyramine liberate the amine without formation of deaminated metabolites.[1-3] In order to explain the difference in the action of these drugs, the following hypotheses have been discussed:[4]

(a) The reserpine-like drugs (e.g. reserpine and Ro 4-1284) displace the 5HT from another platelet pool (or compartment) than the sympathomimetic amines. These pools might, for instance, be located differently with regard to the monoamine oxidase (MAO)-containing subcellular structures, such as mitochondria.

(b) The sympathomimetic amines, unlike the reserpine-like drugs, interefere with the metabolism of liberated 5HT by competitive inhibition of its oxidative deamination.

In the present paper, evidence for the second hypothesis is given.

METHODS

Blood platelets of rabbits with or without injection of 5 mg/kg reserpine i.p. 16 hr prior to bleeding have been isolated as previously described and incubated in a

* 2-hydroxy - 2 - ethyl - 3 - isobutyl - 9,10 - dimethoxy - 1,2,3,4,6,7, - hexahydro - 11bH - benzo [a] quinolizine

modified Tyrode solution* at 37°.[1, 5] The platelets from normal rabbits (suspension containing half the amount of platelets per cc compared with the original plasma) were supplemented with various amounts of DL-amphetamine, *p*-chloro-N-methylamphetamine (Ro 4-6861) and tyramine. The platelets from reserpinized animals were in part homogenized in a Potter-Elvehjem homogenizer following agglutination with thrombin (1–2 NIHU/ml) plus Ca^{2+} (0·2 M). Intact and homogenized platelets from reserpine-treated animals (suspensions containing twice the amount of platelets per cm^3 compared with the original plasma) were incubated with 20 γ/ml. 5HT (corresponding to the content of endogenous platelet 5HT in similar suspensions of normal rabbit platelets) and various amounts of reserpine, Ro 4-1284, or of the above-mentioned sympathomimetic amines. The 5HT of the platelets and the 5-hydroxyindoleacetic acid of the incubation medium were determined with spectrophotofluorimetric methods;[6-8] the 5-hydroxytryptophol of the incubation medium was estimated with a quantitative procedure and by paper chromatography.[9]

The quantitative procedure for the measurement of 5-hydroxytryptophol involved removal of 5HT by passage of the incubation medium (pH 6·0) through a column containing 500 mg Amberlite C.G. 50 (H-form) washed previously with 10 cm^3 potassium phosphate pH 7·4 (0·15 M). The effluent was brought to pH 10 with KOH and extracted with 2×1 aliquot of a mixture of butanol: ethylacetate (7 : 3). After evaporation of the organic layer at 60° and 70 mm Hg, the residue was dissolved in 4 N HCl and analyzed by spectrophotofluorimetry (activation wave length 310 mμ, fluorescence wave length 540 mμ). With incubation fluid from intact platelets (small amounts of proteins), this method yielded a recovery of about 70 per cent; 5HT and 5-hydroxyindoleacetic acid did not interfere to a major extent.

Experiments in which 5HT, 5-hydroxytryptophol or 5-hydroxyindoleacetic acid were added to the platelets or to the incubation medium before and after incubation of the platelet suspensions with the sympathomimetic amines showed that the latter did not markedly interfere with the spectrophotofluorimetric determination of the 5-hydroxyindole derivatives.

RESULTS

1. Incubation of isolated platelets from normal animals with increasing amounts of amphetamine, *p*-chloro-N-methylamphetamine (Ro 4-6861), and tyramine causes a progressive decrease in the platelet 5HT which falls to about 5–10 per cent of its original value (Fig. 1). No 5HT metabolites such as 5-hydroxytryptophol and 5-hydroxyindoleacetic acid can be detected in the incubation medium.

2. Platelets containing at the most small amounts of endogenous 5HT ($<0{\cdot}5$ γ/cm^3) (from reserpinized animals) incubated with 20 γ/cm^3 ($11{\cdot}4.10^{-5}$ M) exogenous 5HT form $1{\cdot}80\pm0{\cdot}11$ γ/cm^3 5-hydroxytryptophol and $0{\cdot}83\pm0{\cdot}16$ γ/cm^3 5-hydroxyindoleacetic acid within 2 hr. Amphetamine, Ro 4-6861, and tyramine in a dose range in which they liberate endogenous 5HT, progressively decrease the formation of 5-hydroxytryptophol with increasing doses of the drugs. Ro 4-6861 is the most and tyramine the least effective of the three sympathomimetic compounds (Fig. 2). The drugs in

* NaCl 7·60 g/l. (0·130 M), KCl 0·42 g/l. (0·006M), Versene 0·80 g/l. (0·002 M), $NaH_2PO_4{\cdot}2H_20$ 0·14 g/l. (0·001 M), $NaHCO_3$ 2·10 g/l. (0·003 M), Glucose 2·00 g/l. (0·111 M), Saccharose 4·50 g/l. (0·010 M).

concentrations of 75.10^{-5} M also considerably diminish the formation of 5-hydroxyindoleacetic acid by the platelets (Table 1). Reserpine (5 γ/cm³) and Ro 4-1284 (50 γ/cm³) do not markedly interfere with the formation of 5-hydroxytryptophol

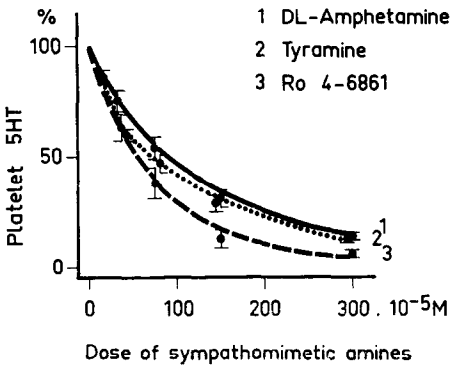

FIG. 1. Effect of sympathomimetic amines on isolated blood platelets of rabbits incubated in Tyrode solution at 37° for 2 hr. The 5-hydroxytryptamine (5HT) values of the platelets are indicated in percent of controls incubated without addition of the drugs. Each value represents an average of 3 determinations ± S.E.

(5-hydroxytryptophol formation within 1 hr=117±8 per cent and 101 ± 10 per cent respectively of controls not supplemented with the drugs) and of 5-hydroxyindoleacetic acid (Table 1).

TABLE 1. EFFECT OF SYMPATHOMIMETIC AMINES AND RESERPINE-LIKE DRUGS ON THE FORMATION OF 5-HYDROXYINDOLEACETIC ACID (5HIAA) BY ISOLATED BLOOD PLATELETS FROM RESERPINIZED RABBITS INCUBATED FOR 2 HR AT 37° IN TYRODE SOLUTION

Drugs	5-Hydroxyindoleacetic acid
DL-Amphetamine	2 ± 2
Tyramine	5 ± 3
Ro 4-6861	1 ± 1
Reserpine	89 ± 6
Ro 4-1284	89 ± 4

The 5HIAA values are indicated in per cent of the 5HIAA formed by the same platelets incubated without drugs. Each value represents an average ± S.E. from 3 experiments.
Concentration of the drugs: sympathomimetic amines: $75 . 10^{-5}$ M; reserpine: $0{\cdot}82 . 10^{-5}$ M (5 γ/cm³); Ro 4-1284 (hydrochloride): $13 . 10^{-5}$ M (50 γ/cm³).

3. The penetration of the exogenous 5HT into the platelets of reserpinized rabbits is not decreased by amounts up to 75.10^{-5} M (140 γ/cm³) Ro 4-6861, 225.10^{-5} M (300 γ/cm³) amphetamine, and $750{,}10^{-5}$ M (1,000 γ/cm³) tyramine. Only 225.10^{-5} M and 750.10^{-5} M/l. Ro 4-6861 as well as 750.10^{-5} M amphetamine significantly diminish the penetration of exogenous 5HT. Smaller amounts of sympathomimetic amines ($7{\cdot}5$–$22{\cdot}5.10^{-5}$ M) even enhance the entry of 5HT into the platelets (Fig. 3).

4. Platelets from reserpinized animals destroyed by homogenization after agglutination with thrombin + Ca^{2+} still form some 5-hydroxytryptophol from exogenous 5HT as demonstrated by paper chromatography. Addition of reserpine (5 μ/ml) and

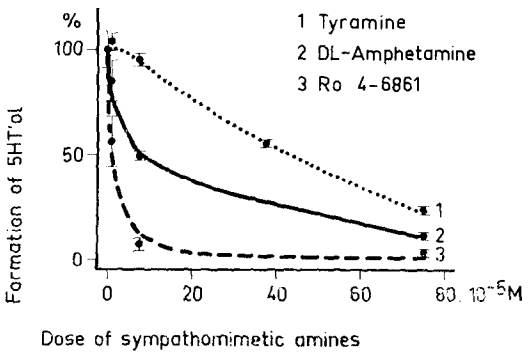

FIG. 2. Effect of sympathomimetic amines on the formation of 5-hydroxytryptophol (5HT'ol) from exogenous 5-hydroxytryptamine (5HT) by isolated platelets. Platelets from reserpinized animals were incubated at 37° for 2 hr with 20 γ/cm³ exogenous 5HT subsequent to addition of the sympathomimetic amines to the incubation fluid (Tyrode solution). The 5HT'ol of the incubation medium was expressed in percent of the 5HT'ol formed without addition of the sympathomimetic amines. Each point represents an average of 3 experiments ± S.E.

Ro 4-1284 (50–100 μ/ml) to the platelet homogenate does not suppress this formation of 5-hydroxytryptophol, whereas after 75.10^{-5} M amphetamine, Ro 4-6861, or tyramine the metabolite no longer appears (Fig. 4).

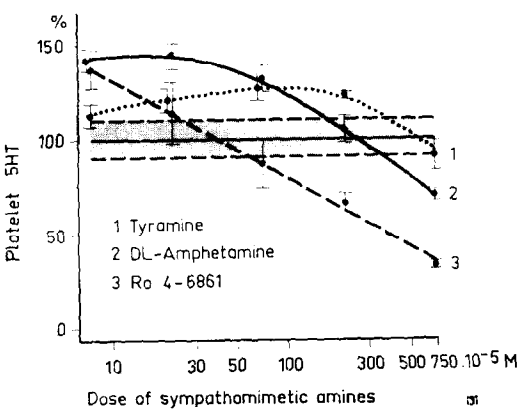

FIG. 3. Effect of sympathomimetic amines on the penetration of exogenous 5-hydroxytryptamine (5HT) into isolated platelets (semilogarithmic scale). Platelets from reserpinized animals were incubated at 37° with 20 γ/cm³ 5HT subsequent to addition of the sympathomimetic amines to the incubation fluid (Tyrode solution). The 5HT of the platelets was measured after 1 hr and expressed in per cent of the 5HT of platelets incubated without sympathomimetic amines. Each point represents an average of 3 experiments ± S.E. Absolute 5HT increase of platelets incubated with 20 γ/cm³ 5HT for 1 hr without sympathomimetic amines (controls): 1.25 ± 0.12 γ/cm³
Shaded area: controls ± S.E.

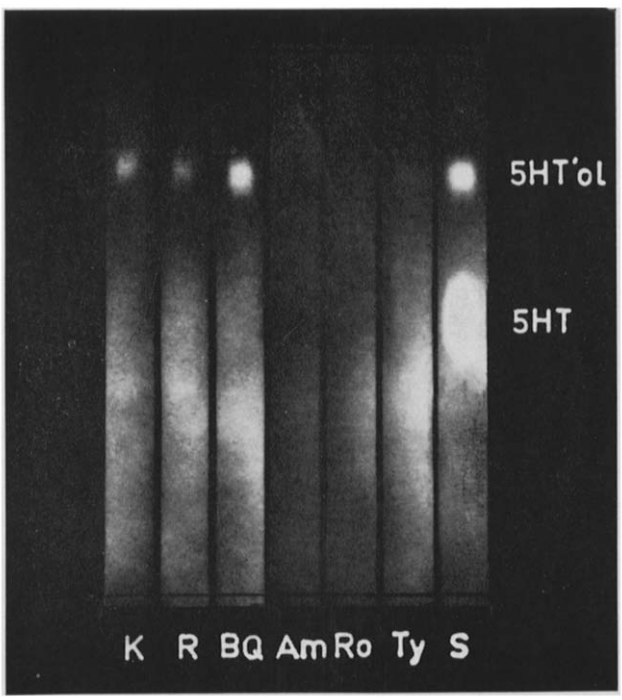

FIG. 4. Paper chromatography of homogenized platelets from reserpinized rabbits 2 hr after incubation with 20 γ/cm^3 exogenous 5-hydroxytryptamine (5HT) at 37° (Tyrode solution). The homogenates were passed through columns of Amberlite (see text) in order to remove the proteins and the 5HT. The effluent was extracted with butanol at pH 10. Schleicher & Schuell No. 2043; solvent system: *n*-propanol/ammonia 1 N (5 : 1). K=control not incubated with sympathomimetic amines; R=reserpine; BQ=benzoquinolizine derivative Ro 4-1284; Am=DL-amphetamine; Ro=para-chloro-N-methylamphetamine (Ro 4-6861); Ty=tyramine; S=standards: 5HT=5-hydroxytryptamine (4 γ); 5HT'ol=5-hydroxytryptophol (4 γ).

DISCUSSION

According to the above experiments, the differences in the metabolism of the 5HT liberated by reserpine-like drugs and by sympathomimetic amines are probably not due to displacement of the amine from two separate pools. They may rather be related to the fact that sympathomimetic amines, in contrast to reserpine-like drugs, cause competitive inhibition of the oxidative deamination of 5HT. This hypothesis is supported by the following findings:

1. Up to 90–95 per cent of the platelet 5HT can be liberated by the sympathomimetic amines without 5HT metabolites being formed (Fig. 1).

2. The formation of 5-hydroxytryptophol and 5-hydroxyindoleacetic acid from exogenous 5HT by platelets containing virtually no endogenous 5HT (reserpinized animals) is decreased by the sympathomimetic amines, but not by reserpine and Ro-4-1284. This diminution occurs in a dose range in which the sympathomimetic amines cause liberation of 5HT and in which they do not diminish but rather increase the penetration of exogenous 5HT into the platelets (Figs. 1–3). The mechanism of the enhancing effect of small doses of sympathomimetic amines on the 5HT penetration remains to be elucidated.

3. In agglutinated, homogenized platelets from reserpinized animals, the formation of 5-hydroxytryptophol from exogenous 5HT is also inhibited by the sympathomimetic amines but not by the reserpine-like drugs (Fig. 4).

Previous findings also support the hypothesis that the pattern of metabolism of platelet 5HT might be connected with the interference of the drugs with oxidative deamination. Thus, reserpine and benzoquinolizine derivatives in the doses used for the present experiments* do not inhibit MAO *in vitro*.[10] In contrast, α-alkylated aralkylamines like amphetamine have been shown by numerous investigators to cause competitive, reversible inhibition of mitochondrial MAO. These amines seem to have a relatively high affinity for the enzyme but cannot be deaminated like the natural substrates (for references see 11, 12). Tyramine in most species is a somewhat better substrate for MAO than 5HT.[11, 12] The sympathomimetic amines in the concentrations used in the present experiments ($8–80.10^{-8}$ M/cm^3 platelet suspension) are thus likely to compete with the endogenous 5HT present in the platelet suspensions ($\sim 10.10^{-8}$ M/cm^3). Competitive inhibition of oxidative deamination by Ro 4-6861 might also explain the finding that cerebral 5HT liberated by this drug *in vivo* is not metabolized to 5-hydroxyindoleacetic acid.[13]

In conclusion, the different metabolic pattern of platelet 5HT liberated by sympathomimetic amines and by reserpine-like drugs is probably due to the fact that the sympathomimetic amines cause competitive inhibition of the oxidative deamination of 5HT, whereas substances of the reserpine type do not.

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* Experiments carried out by Dr. W. P. Burkard in our laboratories.

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