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Preliminary investigations of the metabolism and pharmacological activity of β-hydroxytryptamines in mammals

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β-Hydroxytryptamine and β-hydroxy-5-hydroxytryptamine were incubated with rat liver slices and oxidative deamination was established as the main route of metabolism: in both instances the corresponding indole-3-glycollic acids and indole-3-ethane diols were the major metabolites. However, the rates of deamination of β-hydroxylated tryptamines, as measured manometrically, were found to be much slower than those of tryptamines non-hydroxylated in the side chain. The pharmacological activities of β-hydroxylated tryptamines were tested in guinea-pigs on resistance of respiratory pathways, spontaneous respiration, electrocardiogram, blood pressure and isolated ileum, using tryptamine and 5-HT as reference substances. The effects of tryptamines hydroxylated in the side chain were in general similar to those of corresponding tryptamines but of much lower intensities; only in increasing the blood pressure was β-hydroxytryptamine as active as tryptamine. The different reactions of these two groups of substances in the presence of some antagonists indicate that the receptors are probably not the same.

To study the biological importance of the hydroxylation of the side chain of indolealkylamines we synthesized β-hydroxytryptamine and β-hydroxy-5-HT (Plavšić, Kveder & Iskrić, 1974). These derivatives could be analogous to the corresponding phenethylamines, i.e. noradrenaline and β-hydroxylated octopamine.

In the present paper the metabolism of β-hydroxylated tryptamines by rat liver slices and mitochondria, and the preliminary investigations of their pharmacological activities are described.

MATERIALS AND METHODS

Compounds

Commercial compounds. Iproniazid phosphate (EGA); semicarbazide hydrochloride (Merck); methysergide bimaleate (Deseril, Sandoz); phenoxybenzamine hydrochloride (SKF); propranolol hydrochloride (Inderal, ICI); suxamethonium hydrochloride (Leptosuccin, Pliva); urethane (Fluka); 5-hydroxytryptamine creatinine sulphate (Merck); tryptamine hydrochloride (BDH); indole-3-glycollic acid sodium salt (Fluka).

Synthesized compounds. β-Hydroxytryptamine and β-hydroxy-5-HT creatinine sulphates were prepared as described previously (Plavšić & others, 1974). 5-Hydroxyindole-3-glycollic acid in solution was prepared according to Greenberg, Galston & others (1957) for indole-3-glycollic acid. Indole-3-ethane diol (indole-3-glycol) and 5-hydroxyindole-3-ethane diol (5-hydroxyindole-3-glycol) were synthesized by condensing chloromethyl-3-indolyl ketone with potassium acetate in dimethylformamide to give acetoxy-methyl-3-indolyl ketone (Preobrazhenskaya, Orlova & others, 1972) and this was hydrolysed with sodium hydroxide to hydroxymethyl-3-indolyl ketone, which on the reduction with $LiAlH_4$ gave indole-3-glycol (Suvorov, Kholodovskaya & Preobrazhenskaya, 1965); through the same course of reaction, from chloromethyl 3-(5-benzyloxy)-indolyl ketone (Plavšić & others, 1974), by modifying the experimental conditions, the so far undescribed 5-benzyloxyindole-3-ethane diol was synthesized (m.p. 98–101°; calc. for $C_{17}H_{22}NO_3$:C, 72·05; H, 6·05; N, 4·95; Found: C, 72·4; H, 6·3; N, 4·8). Since the 5-benzyloxy derivatives are more lipophilic, the products were crystallized from benzene and the hydrolysis carried out in the presence of methanol. 5-Benzyloxyindole-3-ethane diol was then catalytically debenzylated in methanol with 10% Pd/C to give semi-crystalline 5-hydroxy-3-ethane diol, which due

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to its instability was not further purified for the analysis. Thin-layer chromatography (see below) revealed only one spot (R_F 0·57 in solvent 4, red-purple with Ehrlich reagent). The compound was further characterized by preparing the diacetoxyethyl derivative (R_F 0·61 in solvent 4, visualized with potassium permanganate); infrared spectrum (film): 3450 (NH), 1750 (CO), 1590, 1540 (C = C, indole ring), 1380 (CH, CH_3), 1240 (acetate), 788 and 765 cm^{-1} (CH, 1,2,4-subst. benzene).

Thin-layer chromatography (t.l.c.)
Glass plates (5 × 20 cm) coated with silica gel G (Merck; 0·25 mm) were used. Solvent systems were: (1) n-propanol–25% ammonium hydroxide–water (10 : 1 : 1); (2) n-butanol–acetic acid–water (12 : 3 : 5); (3) ethyl acetate–isopropanol–water (65 : 24 : 11); (4) chloroform–ethanol (9 : 1). The plates were sprayed with either Ehrlich reagent (1 g of *p*-dimethylaminobenzaldehyde in 50 ml of 96% ethanol and 50 ml of conc. hydrochloric acid) or with 1% potassium permanganate in water.

Metabolic studies
Female Wistar rats (180–200 g) were used throughout.
Incubation with liver slices. Slices of liver (0·5 mm thick, 1 g wet weight) were cut and incubated with substrate in 5 ml of Krebs-Ringer phosphate buffer, pH 7·4 (Umbreit, Burris & Stauffer, 1959) for 2 h at 37° in an atmosphere of oxygen, with shaking. At the end of the incubation period the solution was decanted from the slices, the proteins and salts precipitated with acetone (45 ml) and filtered. After evaporation *in vacuo* to about 2 ml, the concentrate was subjected to t.l.c.
Determination of deamination rates. Mitochondria, separated from liver homogenate by the method of Schneider & Hogeboom (1950), were resuspended in 0·1 M sodium phosphate buffer, pH 7·4, so that 1 ml of suspension corresponded to 0·5 g of fresh liver. The proteins in mitochondrial suspension were determined by the biuret method (Weichselbaum, 1953).

Uptake of O_2 during deamination was measured manometrically at 37°, readings being taken every 5 min for 20 min. The composition of the reaction mixture was as follows: main compartment: mitochondrial suspension (1 ml), 0·1M semicarbazide hydrochloride (0·2 ml 30 μmol), 0·01M KCN (0·2 ml, 3 μmol), 0·1M phosphate buffer, pH 7·4 (to the final volume of 3 ml); side arm: 0·01M substrate (0·2 ml, 2 μmol); centre well: 2M KCN (0·1 ml) filter-paper strip. All solutions, except 2M KCN, were adjusted to pH 7·4 before use. The flasks were gassed with O_2 for 10 min and equilibrated in the water bath for 10 min before tipping. A control reaction mixture containing no substrate was also prepared and any O_2 absorbed in it was substracted from that absorbed in the test mixture.

Pharmacological studies
Isolated ileum. Guinea-pigs (350–450 g) were fasted for 12 h before death. Ileum was isolated according to Burn (1952). The preparation was suspended in a bath of about 16 ml of Krebs solution at 37° gassed with 5% carbon dioxide in oxygen. Contractions were recorded by an isotonic lever on a smoked drum. All test-solutions were added to the bath in a volume of 0·1–0·5 ml for 90 s at 6 min intervals.

Electrocardiogram, blood pressure, spontaneous respiration and resistance of respiratory ways. Guinea-pigs (400–550 g) were anaesthetized with urethane (1·5 g kg^{-1}, i.p.). The test compounds were injected into the jugular vein in a volume of 0·15–0·25 ml. The lead II of the electrocardiogram was recorded by means of subcutaneously inserted needle electrodes. The arterial blood pressure was recorded from the right carotid artery using a mercury Condon-type manometer. Spontaneous respiration was recorded by whole body plethysmography—using a Marey tambour and a smoked drum (Gjurič, Heicke & Westermann, 1964), as well as by pneumotachography (Varioscript 822 recorder, ET 540 amplifier, Schwarzer, Munich); (Fleisch, 1956). The resistance of the respiratory ways was also examined by a body plethysmography method, the animal being artificially ventilated at a rate of 40 min^{-1} and a constant pressure of 100 mm H_2O. Spontaneous respiratory movements were supressed by paralysing the skeletal muscles with suxamethonium (0·2 mg kg^{-1}, i.v. 90 s before the intravenous injection of each agonist) (Gjurič & Westermann, 1965).

RESULTS

Metabolic studies
After incubation of β-hydroxytryptamine and β-hydroxy-5-HT, respectively (1·25 mg of each as creatinine sulphate salt) with rat liver slices, t.l.c. of the incubation mixture revealed in each case at least two new spots, metabolites X and Y, besides smaller amounts of unmetabolized substrate. The results are summarized in Table 1.

By cochromatography with authentic compounds, X_A was identified as indole-3-glycollic acid

Table 1. *Thin-layer chromatography after incubation of β-hydroxytryptamine (A) and β-hydroxy-5-HT (B) (for details see Materials & Methods).*

Compound	A R_F in solvent: 1	2	3	4	B R_F in solvent: 1	2	3	4
Substrate	0·44	0·51	0·38	0·15	0·40	0·52	0·18	0·12
Metabolite X	0·29	0·62	0·10	0·00	0·27	0·52	0·52	0·00
Metabolite Y	0·65	0·82	0·82	0·65	0·57	0·63	0·67	0·57

and X_B as the analogous 5-hydroxy derivative. In the same way, metabolites Y_A and Y_B were identified as the corresponding idole-3-ethane diols. In some experiments with β-hydroxytryptamine, an additional spot with high R_F values in all solvents was found, which was identified as indole-3-aldehyde. Since the spot always appeared after standing of the incubation mixture, it was concluded to be a decomposition product.

When liver slices were pretreated with iproniazid (0·5 mg) the amount of metabolites X and Y decreased considerably, while substantial amounts of non-metabolized substrates remained.

The deamination rates of both β-hydroxylated tryptamines, compared with the deamination rates of tryptamine and 5-HT are presented in Table 2.

Table 2. *Deamination rates of β-hydroxytryptamine and β-hydroxy-5-HT in comparison with tryptamine and 5-HT measured by the uptake of O_2 by rat liver mitochondria.*

Substrate	μl O_2 min^{-1} g^{-1} tissue	μl O_2 min^{-1} mg^{-1} protein
β-Hydroxy-tryptamine	1·04 ± 0·11 (11)[a]	0·058 ± 0·003
Tryptamine	1·76 ± 0·06 (14)	0·095 ± 0·004
β-Hydroxy-5-HT	0·68 ± 0·08 (4)	0·030 ± 0·003
5-HT	1·87 ± 0·05 (13)	0·096 ± 0·004

The final concentration of substrates was $0·66 \times 10^{-3}$M
[a] Number of experiments.

Pharmacological studies

The amounts of all four tested agonists refer to the free base.

Isolated ileum

On the isolated ileum preparation β-hydroxy-5-HT showed a similar effect to 5-HT. Concentrations of the β-hydroxy compound 6–10 times higher were necessary to produce equal contractions. Tryptamine and β-hydroxytryptamine in a concentration up to 1250 times higher than that of 5-HT were inactive.

Resistance of respiratory ways

In our experiments a reduction of the plethysmographic amplitude, i.e. a reduction of the inflation volume, indicates an increase of respiratory pathway resistance (Fig. 1). 5-HT, β-hydroxy-5-HT and tryptamine increased, dose-dependently, the resistance to artificial respiration. Tryptamine and

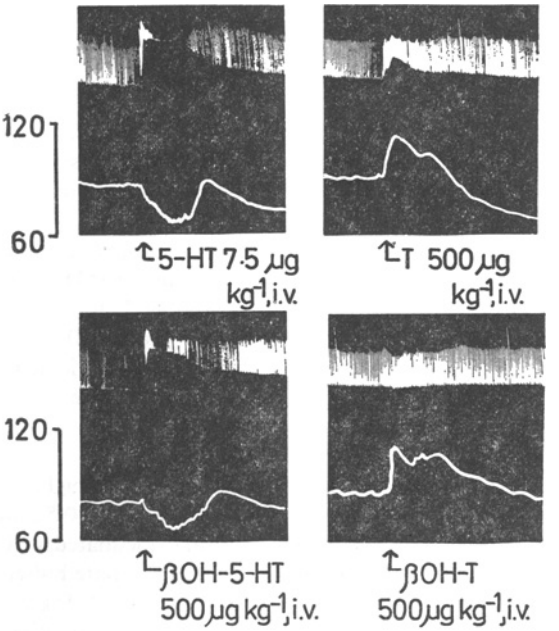

FIG. 1. Effects of 5-HT, (β-OH–5-HT, tryptamine (T) and β-OH-tryptamine on the respiratory pathway resistance in urethane-anaesthetized guinea-pig. Whole body plethysmography, suxamethonium (0·2 mg kg^{-1}, i.v., 90 s before the injection of each agonist), artificially ventilated (40 min^{-1}) at constant pressure (100 mm H_2O) Doses in μg kg^{-1}.

β-hydroxy-5-HT showed an effect almost equal to that of a fifty times lower concentration of 5-HT. Methysergide (20–40 μg kg^{-1},i.v.) completely abolished these effects. β-Hydroxytryptamine had no influence on the resistance of respiratory pathways.

Spontaneous respiration

5-HT β-hydroxy-5-HT and tryptamine induced similar alterations of respiration, but of different intensity (Fig. 2). These alterations are characteristic of bronchospasm, i.e. there was a dose-dependent reduction of the tidal volume, tachypnoea of short duration. It was also evident by the pneumotachographic experiments that the expiratory air-flow speed could be reduced by β-hydroxy-5-HT as it was by 5-HT and tryptamine. Tryptamine and

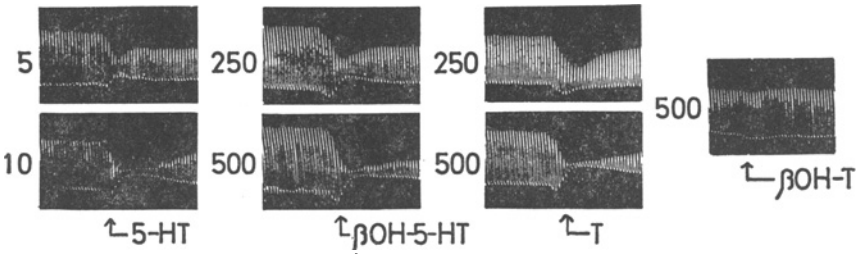

FIG. 2. Effects of 5-HT, β-OH-5-HT, tryptamine (T) and β-OH-tryptamine (β-OH-T) in the urethane-anaesthetized guinea-pig on the spontaneous respiration by body-plethysmography, inspiration upwards (upper traces) and on arterial pressure (bottom traces). Each trace represents 3·5 min.

β-hydroxy-5-HT produce an effect similar to that of 5-HT but require a dose 50–100 times higher to achieve this. Methysergide (20–40 μg kg^{-1}, i.v.) abolished almost completely the effects of 5-HT and tryptamine, but had little action on the effect of β-hydroxy-5-HT. Again, β-hydroxytryptamine (up to 750, μg kg^{-1}, i.v.) did not show any effect.

Electrocardiogram

There were no alterations in electrocardiogram pattern for all four compounds tested. Only β-hydroxy-5-HT caused a marked bradycardia during the first minute after injection, e.g. 125 μg kg^{-1} after 15 s diminished the heart rate from 265 to 112 min^{-1}.

Blood pressure

All agonists tested caused characteristic effects on blood pressure immediately after intravenous injection (Fig. 2). 5-HT and its β-hydroxy derivative induced a biphasic blood pressure effect with a decrease during the first minute. For equal effects, doses of β-hydroxylated compounds 50–100 times higher were necessary. Phenoxybenzamine (2·5 mg kg^{-1}, i.v.) completely antagonized the fall in blood pressure induced by 5-HT and β-hydroxy-5-HT. β-Hydroxytryptamine altered the blood pressure in a manner similar to tryptamine: immediately after intravenous injection, a sudden blood pressure rise, followed by a stepwise return to normal was observed. These two substances were equally active in altering the blood pressure, in contrast to their action on respiration. Phenoxybenzamine (2·5 mg kg^{-1}, i.v.) which antagonized pressure effects caused by β-hydroxytryptamine, was ineffective against tryptamine. Methysergide (40 μg kg^{-1}, i.v.) and propranolol (5 mg kg^{-1}, i.v.) were inactive against the blood pressure effects of all four agents.

DISCUSSION

β-Hydroxylated tryptamines in rat liver slices follow the same metabolic route as other arylethylamines: through the action of monoamine oxidase they are deaminated to intermediate indole-3-glycoladehydes. These are further oxidized to the corresponding indole-3-glycollic acids or reduced to indole-3-glycols. This chain of reactions can be considerably supressed by inhibiting monoamine oxidase with iproniazid. However, the rates of oxidative deamination of β-hydroxylated tryptamines are much slower than those of tryptamines. This was visible on chromatograms in experiments with liver slices and quantitatively verified in Warburg's respirometer. Similar differences in deamination rates were found between dopamine and noradrenaline (Erwin & Hellerman, 1967) and octopamine and tyramine (Snyder & Hendley, 1968; McEwen, Sasaki & Jones, 1969).

Pharmacological studies showed both β-hydroxylated tryptamines to cause definite pharmacological effects. In guinea-pigs their actions are in general similar to the corresponding tryptamines but usually 50–100 times higher amount are required to produce the same effects. Only in increasing the blood pressure were β-hydroxytryptamine and tryptamine equally active.

However, β-hydroxytryptamines react differently from β-non-hydroxylated derivatives to some of the antagonists used suggesting a different mechanism of action.

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