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SHORT COMMUNICATIONS

The effect of the route of administration on the metabolism of 5-hydroxytryptamine

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EARLIER studies showed that 5-hydroxytryptamine (5HT) caused a more marked excretion of 5-hydroxyindoleacetic acid (5HIAA) when injected subcutaneously than when rapidly administered intravenously.[1] Since it appeared possible that the difference was due to dissimilarity in the metabolism, indole derivatives excreted in rat and rabbit urine after the administration of 5HT in different manners were studied by paper chromatography, and the isolated spots were further analysed by other methods.

Following a large i.v. or s.c. dose of 5HT (5–10 mg/kg of 5HT creatinine sulphate calculated as base) the 8-hr urine sample of rat showed, as the clearest spot in addition to 5HIAA and its O-sulphate ester,[2] one that stained purple with *p*-dimethylaminobenzaldehyde. It had low R_f values in *iso*propanol–ammonia–water (10:1:1), butanol–acetic acid–water (4:1:5), and 80% tertiary amyl alcohol solvents (0·03, 0·14, and 0·04 respectively). The spot was always more distinct in the urine of rats given 5HT by rapid intravenous injection than in those receiving it subcutaneously, as was already reported.[3] It was most distinct when 5HT was given *per os*. This spot was also clear in chromatograms made from the urine collected from catheterized unanesthetized rabbits during 6 hr after a dose of 10 mg/kg of 5HT. In these urines it was more distinct after rapid i.v. injection than after slow i.v. infusion (1–2 hr) or s.c. administration. The substance was purified from the rabbit urine after a rapid i.v. administration of 5HT as follows.

The substance was precipitated with 40 vols. of acetone. The sediment was washed first with chloroform and then with diethyl ether, ethyl acetate, and ethanol, in which the substance was not found to be soluble. It was purified in preparatory chromatogram paper (Schleicher and Schüll 2230) by allowing it to run four times indifferent solvents: *iso*propanol–ammonia, butanol–acetic acid, 20% KCl (R_f 0·67), and butanol–pyridine–water (1:1:1) (R_f 0·38). The eluations were made with water, last time, however, with pyridine, which was evaporated into dryness *in vacuo*.

In the spectrophotofluorometer the activation maximum at pH 1, pH 7, and pH 11 lay at 305 mμ, and the fluorescence maximum at 365 mμ; at pH 14 a fluorescence peak was seen at 410 mμ. These and the infrared spectrum taken as a KCl tablet suggested a substance containing the indole nucleus. The failure to stain with α-nitroso-β-naphthol suggested a derivative, in which the phenolic OH-group is not free. The infrared spectrum suggested a glucuronide or sulphate ester. Boiling the substance in N-HCl for 5–20 min and running the hydrolysed product on paper gave a spot corresponding to 5HT. Treatment with β-glucuronidase (Fluka AG, Buchs SG) with deoxy-ribonucleinic acid at pH 5 gave the same spot. At the same time the biologic activity on the rat stomach preparation[4] was increased 100-fold or more. This activity was completely inhibited with yohimbine (0·1 μg/ml) and 1-methyl-D-lysergic acid butanolamide-2-bimaleinate ("Deseril", generously supplied by Sandoz AG, Basle) (2 ng/ml). The contraction of rat stomach by the unhydrolysed material was only partly inhibited by the above mentioned substances. After hydrolysis the material also stained with naphthoresorcinol. On the other hand it was not found to contain sulphur.

The spot thus seems to be due to the O-glucuronide of 5HT, which was found in the urine of mice by Weissbach *et al.*[5] and in the urine of rats by Keglević *et al.*[6] and McIsaac and Page.[7] after the intraperitoneal administration of ^{14}C-labelled 5HT. We have now found that the intensity of this spot following the various methods of administration in rat and rabbit is inversely proportional to the 5HIAA excretion determined by spectrophotometer.[8] The spot was also found after the administration of D,L-5-hydroxytryptophan (5HTP), but there was no difference between the i.v. and s.c. administration. After the injection of 5HTP, the spot of 5HT was also seen. 5HT excretion determined biologically[4] has been found greater after 5HTP than after 5HT itself.[9]

There was often another spot which also seemed to be more intense after a rapid i.v. injection than after s.c. administration or slow i.v. infusion of 5HT in rabbit. This spot also showed more activity in rat stomach preparation after HCl hydrolysis than before it. This spot probably was due to the 5HT O-sulphate ester giving R_f-values corresponding to those of Chadwick and Wilkinson.[10]

A spot corresponding to the O-glucuronide of 5HT was obtained also from the urine collected from a carcinoid patient during and after severe flush attacks. Before paper chromatography the urine was precipitated by 40 vols of acetone, and the active material dissolved from the sediment into pyridine. The spot was not present in the urine of a healthy human subject after ingestion of 2 mg/kg of 5HT or 1/2 kg of peeled bananas.

The results give support to the opinion that the phenolic conjugation with sulphonic and sulphuric acids is an emergency route for the elimination of 5HT. The faster the amine enters the circulation the more of the conjugate is formed. On the other hand, there is an indication that the most intense ester formation occurs after oral administration. It is also probable that a part of the 5HT O-glucuronide and O-sulphate esters is further oxidised to the corresponding 5HIAA conjugates.

The results will be published in detail elsewhere.

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Actions of cocaine and tyramine on the uptake and release of H^3-norepinephrine in the heart

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PREVIOUS work in this laboratory has shown that circulating H^3-catecholamines are taken up and bound in the heart, spleen and adrenal gland.[1, 2] We have also observed that pre-treatment of cats with cocaine[3] or tyramine[4] markedly reduced the concentration of H^3-norepinephrine in heart and other tissues. This effect might be a consequence of an inhibition of the uptake of the circulating hormone or a release of the bound H^3-catecholamine, or both. To enable us to distinguish between these actions, these compounds were given before or after the administration of H^3-norepinephrine. If the drug interfered with the uptake, it would decrease the H^3-norepinephrine only if given before the administration of the catecholamine. If, after the injection of H^3-norepinephrine, when the catecholamine is bound in the tissue, the level of amine in the tissue is lowered by the drug, the latter releases the bound hormone.

Male rats (Sprague–Dawley) weighing from 160–180 g were given 10 μc of DL-7-H^3-norepinephrine (20 mc/mg) per 100 g by injection into the tail vein and the animals were killed 2 hr later by decapitation. The hearts were homogenized with 12 ml of perchloric acid (0·4 N). After centrifugation, the supernatant solution was assayed for H^3-norepinephrine.[2] Cocaine HCl, 10 mg/kg, was given