The Metabolism of 5-Hydroxytryptamine O[³⁵S]-Sulphate in the Rat

By F. A. ROSE

Department of Biochemistry, University College, Cathays Park, Cardiff CF1 1XL, U.K.

and W. S. BLESZYNSKI

Department of Biochemistry, Mikolaj Kopernik University, Torun, Poland

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Three distinct arylsulphatase enzymes (aryl-sulphate sulphohydrolases, EC 3.1.6.1) of different specificity are ubiquitously distributed in the tissues of many mammalian species (Dodgson, Spencer & Thomas, 1955). In the liver cell, two of these enzymes, arylsulphatases A and B, are lysosomal in origin (Viala & Gianetto, 1955), and the other, arylsulphatase C, is localized in the endoplasmic reticulum (Dodgson, Spencer & Thomas, 1954), where it exists in a highly insoluble state (Dodgson, Rose & Spencer, 1957). Studies on these enzymes have generally involved the use of synthetic aryl sulphate esters as chromogenic substrates (Dodgson & Spencer, 1957), and at the present time there is no real evidence to indicate the biological role of the enzymes or the nature of their physiological substrates.

Work in progress in these laboratories is concerned with establishing the role of these enzymes and the nature of their normal substrates, and one compound that commands attention as a possible substrate is the O-sulphate ester of 5-hydroxytryptamine, although there have been reports (Kishimoto, Takahashi & Egami, 1961) that rabbit and ox arylsulphatase preparations were inactive towards it *in vitro*. Mammalian liver and brain preparations have been shown to be capable of synthesizing this ester from 5-hydroxytryptamine (Hidaka, Nagatsu & Yagi, 1966, 1969*b*) and it has been detected in the urine of mammals after the intravenous injection of the hormone (Chadwick & Wilkinson, 1960; McIsaac & Page, 1959). Two noteworthy features of the ester are that it tends to form an internal salt carrying little electrical charge at physiological pH and that, in contrast with the parent phenol, its physiological activity is very low as measured by its ability to induce contraction of the rat ileum (Kishimoto *et al.* 1961) and aorta of dog and rabbit (Hidaka, Nagatsu, Takeya, Matsumoto & Yagi, 1969*a*). A number of studies have indicated that the ability of 5-hydroxytryptamine to penetrate biological barriers (particularly the blood-brain barrier) is limited (McIsaac & Page, 1959), probably as a result of the relatively high

positive charge carried by the molecule under physiological conditions. The possibility therefore exists that penetration of such barriers is normally facilitated by a conversion into the physiologically inactive, electrically neutral, sulphate ester, which could subsequently be hydrolysed by one or other of the arylsulphatase enzymes to yield the active neurohormone.

In an attempt to establish the physiological significance of the ester and examine the activity *in vivo* of the arylsulphatases towards it the metabolism of ³⁵S-labelled 5-hydroxytryptamine O-sulphate has been studied in the rat. The labelled ester was prepared by the method of Kishimoto *et al.* (1961) by using 20 mCi of ³⁵S-labelled sulphuric acid. Male and female M.R.C. hooded rats (200 g) were lightly anaesthetized and injected intraperitoneally with 2 mg (6.6 μ Ci) of 5-hydroxytryptamine O[³⁵S]-sulphate dissolved in 2 ml of water. The rats were subsequently fed and housed and the faeces and urine collected separately as described by Dodgson & Tudball (1960). After 48 h the animals were killed and the total ³⁵S content of the carcass was determined (Dodgson & Tudball, 1960). The analytical results showed that more than 90% of the radioactivity of the dose appeared in ester sulphate-bound form in the urine within 24 h. Less than 7% of the radioactivity in the dose appeared in the urine as inorganic ³⁵SO₄²⁻ ion during the same period. Chromatography and electrophoresis of the urines by the methods described by Dodgson, Powell, Rose & Tudball (1961*b*) showed that most of the ester sulphate-bound radioactivity was associated with unchanged 5-hydroxytryptamine O[³⁵S]-sulphate, although small amounts of an additional ³⁵S-labelled sulphate ester were detected. No attempts were made to identify the latter compound, but its behaviour on chromatograms and electrophoretograms showed it to be more electronegative than 5-hydroxytryptamine O[³⁵S]-sulphate, from which it may be presumed to have arisen.

In separate experiments, paper electrophoresis of urine showed that unchanged starting material

rapidly appeared in the urine whereas more than 1 h elapsed before $^{35}\text{SO}_4^{2-}$ ions appeared. The detection of small amounts of ^{35}S in faeces (Dodgson & Tudball, 1960), together with the delay in the appearance of inorganic $^{35}\text{SO}_4^{2-}$ ions in urine, raised the possibility that the latter was arising as a result of degradation of starting material by faecal micro-organisms. Support for this was obtained by studying the metabolism of 5-hydroxytryptamine $\text{O}[^{35}\text{S}]$ -sulphate in rats treated with antibiotics and sulphonamides (Dodgson *et al.* 1961b). Such treatment decreases the count of viable micro-organisms to a very low value. Each rat received 50mg of Terramycin (oxytetracycline) and 250mg of Sulphasuxidine (succinylsulphathiazole) twice a day for 3 days and was injected with 5-hydroxytryptamine $\text{O}[^{35}\text{S}]$ -sulphate on the fourth day. The distribution of ^{35}S in excreta showed that only traces of inorganic $^{35}\text{SO}_4^{2-}$ ions were produced in these treated animals, whereas the ^{35}S content of faeces rose appreciably. Electrophoretograms of the urines of these animals showed the presence of large amounts of unchanged 5-hydroxytryptamine $\text{O}[^{35}\text{S}]$ -sulphate together with small amounts of the metabolite described above. Inorganic $^{35}\text{SO}_4^{2-}$ ions could not be detected in the urines on these electrophoretograms.

Further information on the tissue distribution and metabolic fate of 5-hydroxytryptamine $\text{O}[^{35}\text{S}]$ -sulphate was obtained by employing the technique of whole-animal radioautography as described for $[^{35}\text{S}]$ sulphate esters by Powell, Curtis & Dodgson (1967). Young rats (50g) were each injected intraperitoneally with 2mg of the labelled ester and killed at various time-intervals, and whole-body radioautograms were prepared. The radioautograms showed that 10min after injection there was a general distribution of radioactivity in the blood with little or no cellular uptake. After 20min most of the radioactivity had accumulated in the kidney, whereas after 30min only the urinary bladder was noticeably labelled. After 1h the radioactivity associated with this organ had also disappeared. (No radioactivity was found in the brain.) These distribution patterns are consistent with the rapid excretion of the ester in the urine, as illustrated by the metabolic studies.

The activity of arylsulphatase enzymes towards 5-hydroxytryptamine $\text{O}[^{35}\text{S}]$ -sulphate was measured by the electrophoretic sulphatase assay method described by Dodgson, Lloyd & Tudball (1961a). Arylsulphatases A and B of rat brain, obtained by the autolysis method of Bleszynski (1967) for preparing the analogous ox brain enzymes, were inactive towards 6mM-5-hydroxytryptamine $\text{O}[^{35}\text{S}]$ -sulphate in the pH range 5.0–8.5. Arylsulphatase C of rat liver obtained by the method of Dodgson, Rose & Tudball (1959) was similarly

inactive towards the ester under these conditions. Hydrolysis could not be detected even with more concentrated (25-fold) enzyme preparations and prolonged incubation times, although the enzymes were appreciably active towards their assay substrates (see Roy, 1953; Milsom, Rose & Dodgson, 1968).

The results of this study demonstrate that, in the rat, intraperitoneally injected 5-hydroxytryptamine $\text{O}[^{35}\text{S}]$ -sulphate is rapidly eliminated from the body via the kidneys mainly in an unchanged form. The distribution of radioactivity in the carcass of animals receiving 5-hydroxytryptamine $\text{O}[^{35}\text{S}]$ -sulphate supports these findings and is comparable with that of several other sulphate esters that are metabolically inert (Powell *et al.* 1967). In view of the observation that 5-hydroxytryptamine is not easily transported across biological membranes, it is particularly noteworthy that 5-hydroxytryptamine $\text{O}[^{35}\text{S}]$ -sulphate also was found to be unable to cross the blood-brain barrier.

It appears that the arylsulphatase enzymes of rat tissues are inactive towards 5-hydroxytryptamine O -sulphate both *in vitro* and *in vivo*. However, it could be argued that in the latter case the ester is unable to penetrate to the site of the enzyme in the cell. These observations do not support the claims made by Hidaka *et al.* (1969b) that 5-hydroxytryptamine O -sulphate might represent a convenient store of the neurohormone, but suggest that it is probably an end product of 5-hydroxytryptamine metabolism that is rapidly excreted.

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