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SHORT COMMUNICATIONS

Octan-2-sulphate degradation in the isolated perfused rat liver

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DL-Octan-2-sulphate and its individual enantiomers are extensively metabolized in the rat by ω,β-oxidation [1, 2]. Octanoate-7-sulphate, hexanoate-5-sulphate and butanoate-3-sulphate, the principal identified metabolites, are eliminated in the urine. The stereochemistry of octan-2-sulphate does not affect its metabolic fate or its biological half-life [2].

Whole-body autoradiography studies have shown that radioactivity is concentrated in the livers of rats after parenteral administration of either ^{35}S- or 2-^{14}C-labelled DL-octan-2-sulphate [1]. The finding that ω,β-oxidation is the major pathway of octan-2-sulphate degradation in rats accords with these studies for in respect of medium-chain fatty acids, the liver has the greatest ω-oxidation activity of any organ in this species [3]; the liver also possesses a highly active mitochondrial β-oxidation system [4].

The major contribution made by the liver to the metabolism of anionic surfactants has been demonstrated with two chemically disparate alkyl sulphates. For example, the isolated perfused rat liver was shown to metabolize dodecan-1-[^{35}S]sulphate by ω,β-oxidation for form butanoate-4-[^{35}S]sulphate [5]. The isolated perfused liver was also used [6] to study the fate of an anionic surfactant (10-phenyldecan-1-[^{35}S]sulphate) in which the ω-position is blocked. In this case, ω,β-oxidation was precluded and the molecule was differently metabolized. Significantly, the subsequent partitioning of the metabolites resulted in substantial biliary excretion.

The present investigation on the metabolism of the surfactant DL-octan-2-sulphate in the isolated perfused liver system was undertaken to investigate the contribution of the liver to the whole-body metabolism of a secondary alkyl sulphate. The studies were designed to establish whether the liver is the major site of metabolism of this surfactant, and to this end the capability of the isolated perfused rat liver to metabolize octan-2-sulphate was compared with the capability of the whole animal.

Materials and methods

Radiolabelled octan-2-sulphate. D(+)- and L(−)-octan-2-[^{35}S]sulphate (2.1 Ci/mole) and DL-[2-^{14}C]octan-2-sulphate (0.46 Ci/mole) were prepared as potassium salts and characterized according to Maggs *et al.* [7].

Isolated liver perfusion. The method used was that of Curtis *et al.* [8] and perfusion was carried out at a flow rate of 3–4 ml/g per min over 5 hr. The perfusate (approximately 120 ml) was freshly collected heparinized (3400 units), homologous rat blood. Radiolabelled octan-2-sulphate (in aqueous solution at pH 7.0) was added to the perfusate between 15 and 20 min after commencement of perfusion. The amounts of octan-2-sulphate added to the perfusate were as follows: D(+)- or L(−)-octan-2-[^{35}S]sulphate, 100 μmole in 5 ml; DL-[2-^{14}C]octan-2-sulphate, 40 μmole in 3 ml.

Samples of perfusate (0.5 ml) were removed at timed intervals over 5 hr and bile was collected at timed intervals. At the end of the perfusion, the liver was washed free of perfusate with isotonic saline. Samples of tissue were taken for determination of residual radioactivity.

Measurement of radioactivity. Bile containing ^{14}C or ^{35}S and plasma and tissue samples containing ^{35}S were assayed for radioactivity by liquid scintillation counting. Aliquots (5 μl–1.0 ml) in either PCS or NE 260 scintillator (Amersham/Searle, Chadwell Heath, Essex, U.K. and Nuclear Enterprises, Sighthill, Edinburgh, U.K., respectively). All measurements of radioactivity were expressed as dpm and corrections for quenching were made by either internal standardization using [^{14}C]hexadecane (10 μl per vial, equivalent to approximately 9000 dpm) obtained from the Radiochemical Centre (Amersham, Bucks, U.K.) or automatic external standardization. Counting efficiencies for aqueous solutions of sulphate esters and biological fluids ranged from 70 to 85%.

The ^{14}C contents of blood, plasma and liver were determined by combusting in a sample oxidizer (Oxymat, Intertechnique Model 1N 4101, Plaisir, France). The $^{14}CO_2$ was trapped in a mixture of ethanolamine (600 ml), methanol (1800 ml), toluene (1600 ml), 2,5-diphenyloxazole (37.5 g) and 1,4-di-2-(4-methyl-5-phenyloxazolyl)benzene (dimethyl POPOP) (2.7 g). Liquid samples (5–20 μl) were pipetted into sample capsules and combusted; liver tissue was combusted in 70–130 mg portions. The recovery of ^{14}C, measured against triplicate samples of a commercial ^{14}C standard for sample oxidizers (Radiochemical Centre, Amersham, Bucks., U.K.) ranged from 65 to 90%.

Thin-layer chromatography (TLC). Prior to analysis by TLC the radiolabelled plasma components were extracted into methanol. Plasma samples (0.2 ml) were freeze-dried and the residues were extracted with 3 × 0.3 ml methanol at approximately 40°. The combined extracts were centrifuged and the supernatants were evaporated under nitrogen to approximately 100 μl before being subjected to TLC.

Thin-layer chromatograms of either ^{14}C- or ^{35}S-labelled material from plasma were run in two dimensions on silica gel G. Plates were developed over 15 cm with propan-2-ol–ammonia solution (sp. gravity 0.88) (7:3, v/v) and after drying at 40°, with isobutyric acid–0.5 M ammonia (5:3, v/v). Radioactive areas were located by autoradiography and the appropriate areas of silica gel were removed. The radioactive contents were determined by liquid scintillation counting.

Results and discussion

Perfusion with either D(+) or L(−)-octan-2-[^{35}S]sulphate. Isolated livers from mature MRC hooded rats were perfused with blood containing 100 μmole of either D(+)- or L(−)-octan-2-[^{35}S]sulphate. The results obtained with both isomers were substantially the same. The majority of the radioactivity was present in the plasma over the entire experimental period. Small amounts only (< 2% over 5 hr) were present in bile and at the end of the experiment *ca* 10% was associated with the liver. The relative amounts of radioactivity in plasma that were associated with the parent ester and its metabolites are shown in Fig. 1. The parent ester rapidly disappeared from the perfusate and after 1 hr only *ca* 10% of the circulating radioactivity was present in this form. After 3 hr, the parent ester was not present. All of the metabolites previously found in urine of animals receiving the ester were detected in the perfusate. However, the relative proportions of the metabolites differed from those previously recorded in urine [1]. Thus, small amounts only of the major urinary metabolites (octanoate-

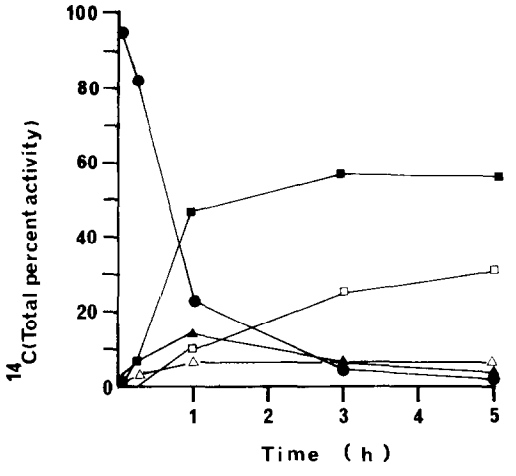

Fig. 1. Quantification of radioactivity in plasma associated with D(+)-octan-2-[^{35}S]sulphate and its metabolites during isolated liver perfusion. Octan-2-[^{35}S]sulphate (●), an aldehyde derivative of octan-2-[^{35}S]sulphate (△), octanoate-7-[^{35}S]sulphate (▲), hexanoate-5-[^{35}S]sulphate (■), butanoate-3-[^{35}S]sulphate (□). Figures represent the radioactivity associated with each component expressed as a percentage of the total radioactivity.

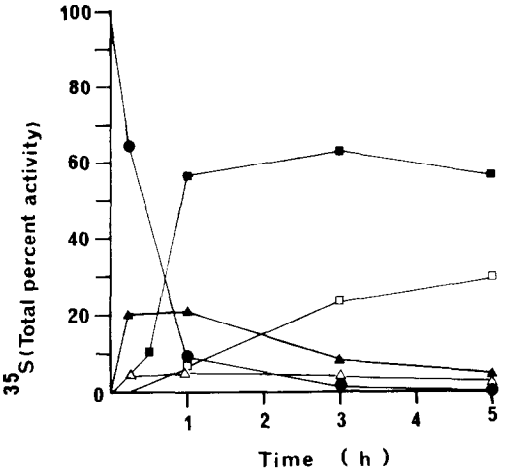

Fig. 2. Quantification of radioactivity in plasma associated with DL-[2-^{14}C]octan-2-sulphate and its metabolites during isolated liver perfusion. DL-[2-^{14}C]Octan-2-sulphate (●), an aldehyde derivative of DL-[2-^{14}C]octan-2-sulphate (△), [7-^{14}C]octanoate-7-sulphate (▲), [5-^{14}C]hexanoate-5-sulphate (■), [3-^{14}C]butanoate-3-sulphate (□). Figures represent the radioactivity associated with each component expressed as a percentage of the total radioactivity.

7-sulphate and the putative aldehyde intermediate) were found in the perfusate. The major perfusate metabolite was hexanoate-5-sulphate, representing *ca* 50% of the perfusate radioactivity.

Presumably, in the intact animal, the precursors of hexanoate-5-sulphate are rapidly cleared from the blood by the kidneys. However, in the 'closed' perfused system, the precursors are available for further degradation by the liver and the increased extent of hexanoate-5-sulphate formation in the perfused system is explicable in these terms. The perfusion medium also contained greater relative amounts of butanoate-3-sulphate than the urines of animals receiving octan-2-sulphate.

The results suggested, however, that even under the closed perfusion system conditions there are constraints on the formation of butanoate-3-sulphate from its immediate precursor hexanoate-5-sulphate. Thus, although hexanoate-5-sulphate is the major metabolite after 1 hr, its relative concentration then remains about the same. It is possible that the constant levels are explained by similar rates of formation and degradation. However, it is also possible that the further metabolism of hexanoate-5-sulphate is limited either because it does not readily re-enter the liver or, alternatively, that further chain shortening is limited by the proximity of the sulphate moiety. In the light of experience with other alkyl sulphates [5, 9, 10], the latter explanation seems the more likely.

Perfusion with DL-[2-^{14}C]*octan-2-sulphate.* Isolated livers from mature male and female MRC hooded rats were perfused with blood containing 40 μmole DL-[2-^{14}C]octan-2-sulphate. As in perfusions with ^{35}S-labelled octan-2-sulphate, the majority of the radioactivity was present in the perfusate at all times during the experiments. Very little radioactivity was present in bile (< 4% over 5 hr) and at the end of the experiments approximately 10% was present in the livers. The parent ester rapidly disappeared from the perfusate and when the relative amounts of the metabolites of octan-2-sulphate in the perfusate were determined, substantially the same results were obtained as those recorded with the individual enantiomers (Fig. 2). Thus after 1 hr and until the end of the experiment, the predominating metabolite was hexanoate-5-sulphate. There were no discernible differences between male and female livers.

In summary, the results obtained in this investigation demonstrate unequivocally that the rat liver is capable of metabolizing rapidly the secondary alkyl sulphate octan-2-sulphate. The metabolic profiles were substantially the same regardless of the nature of the enantiomeric form of the ester, administered either as a racemic mixture or as the individual enantiomers. The results were not affected by the sex of the donor animal. The range of metabolites formed strongly supports the hypothesis that the liver is the major site of metabolism in the whole animal. Further, the findings demonstrate that the ω,β-oxidation degradative pathway for secondary alkyl sulphates is operational in the rat liver and the liver may be solely responsible for the detoxication of these molecules *in vivo*.

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REFERENCES

1. J. L. Maggs, G. M. Powell, K. S. Dodgson, D. Howes, J G. Black and A. H. Olavesen, *Xenobiotica* **12**, 101 (1982).
2. J. L. Maggs, G. M. Powell, K. S. Dodgson, D. Howes, J. G. Black and A. H. Olavesen, *Xenobiotica* **12**, 611 (1982).

3. K. Ichihara, E. Kusunose and M. Kusunose, *Biochim. biophys. Acta* **176**, 704 (1969).
4. K. K. Stanley and P. K. Tubbs, *Biochem. J.* **150**, 77 (1975).
5. W. H. B. Denner, A. H. Olavesen, G. M. Powell and K. S. Dodgson, *Biochem. J.* **111**, 43 (1969).
6. B. Burke, A. H. Olavesen, C. G. Curtis and G. M. Powell, *Xenobiotica* **8**, 145 (1978).
7. J. L. Maggs, A. H. Olavesen and C. T. James, *J. labelled Compds Radiopharmaceuticals* **17**, 793 (1980).
8. C. G. Curtis, G. M. Powell and S. L. Stone, *J. Physiol., Lond.* **213**, 14P (1970).
9. B. Burke, A. H. Olavesen, C. G. Curtis and G. M. Powell, *Xenobiotica* **5**, 573 (1975).
10. B. Burke, A. H. Olavesen, C. G. Curtis and G. M. Powell, *Xenobiotica* **6**, 667 (1976).

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Phosphorylation of brain synaptosomal proteins in lithium-treated rats*

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It has been suggested that the state of phosphorylation of proteins in the synaptic membrane could influence the ease of passage of the nerve impulse [1, 2] and that phosphorylation may play a key role in nervous tissue function [1, 3–5]. It has also been proposed that synaptic phosphorproteins may influence neurotransmitter release [6, 7] and/or ion conductance and membrane polarization [3]. Lithium is known to alter certain behaviours such as aggression [8], and to cause impairment of learning and memory. Acute lithium treatment has been reported to be directly depressant to the central neurons [9, 10], altering the neuronal discharge frequency [10]. In the present study, the state of phosphorylation of the synaptic membranes in lithium-treated rats has been examined.

Materials and methods

Chemicals. The various chemicals used were obtained as follows: acrylamide and methylene-bis acrylamide from BDH Chemicals Ltd., Poole, England; glycine, sodium dodecyl sulfate and mercaptoethanol from the Sigma Chemical Co., St. Louis, MO, U.S.A.; and TEMED from Koch Light Ltd., Buckinghamshire, England. Tissue solubiliser was obtained from the Eastman Kodak Co., Rochester, NY, U.S.A. The rest of the chemicals were of "Analar" grade, obtained from the Chemicals Division, Glaxo Laboratories, Bombay, India. ^{32}Pi and [γ-^{32}P]ATP were obtained from the Bhabha Atomic Research Centre, Bombay, India.

Animals and diet regimen. Male Wistar rats, weighing between 150 and 200 g, were fed nutritionally adequate standard laboratory diet in which Li_2CO_3 (40 m-equiv/kg diet) was incorporated for 20 days [11].

In vivo *phosphorylation.* For the *in vivo* phosphorylation studies, ^{32}P was injected intracranially into the frontal cerebral cortex of rats maintained under mild ether anaesthesia [12]. Each animal was injected with 40 μl (2 × 20 μl bilaterally) of carrier-free ^{32}P orthophosphate (total 40 μCi) in an aqueous solution.

The rats were killed 1 hr after ^{32}P injection, and the whole brain was removed. The synaptosomes were separated [13], and the membranes were isolated therefrom by published procedures [14]. Phosphorylated protein from the membrane was isolated after fractionation with $HClO_4$ [15] and dissolved in tissue solubilizer, and an aliquot was taken for counting of radioactivity by liquid scintillation spectrometry. The protein concentration was determined by the method of Lowry *et al.* [16].

In vitro *phosphorylation.* Phosphorylation of the synaptic membranes was carried out with 100 μl of synaptosomal membrane fraction (4 mg/ml), and the incubation medium was made up to contain 5 μM [γ-^{32}P]ATP [15]. The reaction was terminated after 2 min by addition of an equal volume of 0.5 N perchloric acid and was purified, as described earlier, and counted [15].

Polyacrylamide gel electrophoresis and autoradiography. Acrylamide gel electrophoresis of the synaptosomal fraction obtained from rats injected with 1000 μCi ^{32}P was carried out at room temperature, using a constant current of 25–30 mA/slab. The protein concentration that loaded in each track was 100 μg. After electrophoresis, the slab was fixed with propanol–acetic acid (25%:10%) and stained with Coomassie Blue. The stained gels were dried on a filter paper under vacuum and placed in contact with an X-ray film for 8 days. The autoradiogram was scanned in a densitometer.

Results

The *in vivo* incorporation of ^{32}P into the total rat brain synaptosomal fraction was found to increase significantly after 20 days of feeding Li_2CO_3 at the level of 40 m-equiv/kg diet. This increase in the labelling of the total synaptosomal fraction was also reflected in an increase in the radioactivity associated with the purified protein (Table 1). However, when the phosphorylation of the synaptosomal proteins was examined *in vitro*, a small but insignificant decrease was observed in the lithium-fed rats.

Polyacrylamide slab gel electrophoresis of the synaptosomal membrane protein from control and lithium-treated rats did not show an alteration in either the number of bands or the protein content per band (Fig. 1A). The *in vivo* incorporation of ^{32}P was, in general, higher in the synaptosomal membranes from the lithium-treated animals as compared to controls. However, there were large differences in the extent of lithium-induced changes in the various bands (Fig. 1C; Table 2). Whereas the four bands with the highest mobilities (Nos 11–14) showed lower radioactivity in the lithium-treated rats compared to the control rats, all the others showed an increase in radioactivity as a result of lithium treatment. The maximum increase was shown by bands 6 and 7, while 1 and 2 showed a marginal increase.

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