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SHORT COMMUNICATIONS

On the existence of arylamine sulphotransferase activity

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In 1957 Boyland *et al.* [1] isolated potassium 2-naphthyl sulphamate from the urine of rabbits dosed with 2-naphthylamine and in the following year the synthesis of this and other aryl sulphamates by extracts of mammalian livers was reported by Roy [2]. The characterisation of the 2-naphthyl sulphamate formed *in vitro* seemed adequate, although incomplete, and the sulphotransferase, arylamine sulphotransferase, presumed to be involved in its synthesis in rat and guinea-pig liver was studied in some detail [3–6]. The relation of arylamine sulphotransferase to other sulphotransferases was queried by Banerjee and Roy [7] and the situation remains unresolved [8]. There have been no recent studies of the enzyme, which does not feature in a current review of the sulphotransferases [9], and doubts have been expressed about both the existence of the enzyme and the original characterisation [2] of its presumed product, 2-naphthyl sulphamate. The present communication describes the isolation and characterisation of potassium 2-naphthyl sulphamate from reaction mixtures containing crude sulphotransferases from guinea-pig and rat livers.

A preparation containing both the sulphate-activating system and arylamine sulphotransferase was obtained from liver cytosol by precipitation between 1.5 and 2.3 M $(NH_4)_2SO_4$ as previously described [3]. The reaction mixture had the following composition: phosphate buffer, pH 7.5, 0.02 M; K_2SO_4, 6 mM; $MgSO_4$, 4 mM; ATP, 5 mM; 2-naphthylamine hydrochloride, 2 mM; and the enzyme prepared from approximately 50 g of guinea-pig or rat liver in a total volume of 1 litre. With the rat enzyme, 10 μM 3β-methoxyandrost-5-en-17-one was present as an activator of arylamine sulphotransferase [4]. The reaction mixtures were incubated for 5 hr at 37° and the presumed 2-naphthyl sulphamate isolated as described below. At each stage the amount of sulphamate present was estimated by the methylene blue method [4]: this is not specific for sulphamates but will also detect other types of sulphate ester, for many of which it is more sensitive than for 2-naphthyl sulphamate. The purification of the latter was also followed by infrared spectrometry, using KBr discs in a Unicam SP1050 spectrophotometer.

The reaction mixtures were heated to 80°, cooled, coagulated proteins removed by centrifugation, and the supernatants concentrated *in vacuo* at 40° to about 100 ml before precipitating inorganic salts by adding 500 ml of 95% ethanol. After filtration, the filtrates were concentrated as above to about 50 ml and the pH adjusted to 9 with KOH. 2-Naphthylamine was removed by extracting six times with 20 ml portions of diethyl ether and then the sulphamate with six 20 ml portions of butan-1-ol. After drying over Na_2SO_4, the butanol extracts were taken to dryness *in vacuo* at 40° to give 70 mg and 25 mg of crude 2-naphthyl sulphamate from the guinea-pig and rat liver preparations, respectively. Table 1 shows the amounts of 2-naphthyl sulphamate, determined by the methylene blue method, apparently present at each stage.

The crude 2-naphthyl sulphamate from guinea-pig liver was dissolved in 1.5 ml of boiling 75% ethanol and the solution cooled to 0°: the precipitated material was filtered off to give 13 mg of buff powder (fraction 1, see below) and the mother liquors were taken to dryness to give 55 mg of brown solid. The latter was crystallised from 0.8 ml of 75% ethanol containing 15% of potassium acetate to give 21.5 mg of glistening, slightly brownish platelets of potassium 2-naphthyl sulphamate. (Calc. for $C_{10}H_8KNO_3S$: C, 46.0; H, 3.09; K, 15.0; N, 5.36; S, 12,3. Found: C, 44.3; H, 2.98; K, 16.1; N, 5.06; S, 12.4.) The high values for K and S and low values for the other elements were consistent with a 4% contamination by K_2SO_4: determination of SO_4^{2-} by the rhodizonate method [10] showed a 5% contamination by K_2SO_4. The infrared spectrum of the crystals was identical with that of authentic potassium 2-naphthyl sulphamate.

A sample (5.4 mg) of the crystals was heated with 0.5 ml of 1 M HCl in a boiling water bath for 30 min, cooled, made alkaline with NaOH and extracted five times with 1 ml portions of diethyl ether. After drying over Na_2SO_4, the extract was taken to dryness to give 2.1 mg of brown solid (expected yield, 3.0 mg of 2-naphthylamine), the infrared spectrum of which was identical with that of 2-naphthylamine.

Table 1. The apparent amounts of 2-naphthyl sulphamate, determined by the methylene blue method, at different stages of the isolation of the sulphamate from reaction mixtures of the composition given in the text.

Stage of preparation	2-Naphthyl sulphamate (mg) from reaction mixtures containing the enzymes from livers of: Guinea-pig	Rat
Reaction mixture	70	9.3
Deproteinised mixture	82	11
Desalted mixture	81	9.0
Extracted with diethyl ether	71	8.7
Extracted with butan-1-ol	3	0.9
Butanol extract	62	7.9

Infrared spectroscopy showed that fraction 1 also contained considerable amounts of 2-naphthyl sulphamate and this was confirmed by thin-layer chromatography on silica gel (Kieselgel 60: Merck, Darmstadt, F.R.G.) in the solvent system butan-1-ol: propan-1-ol: 0.1 M NH_4OH (2:1:1, v/v) [1]. The area containing the sulphamate was located by its fluorescence and eluted with 75% ethanol: the resulting solution was taken to dryness to give 4 mg of brownish powder, the infrared spectrum of which was indistinguishable from that of authentic 2-naphthyl sulphamate. Hydrolysis of 3 mg of the powder in HCl, as described above, gave 1.4 mg of 2-naphthylamine, the infrared spectrum of which was indistinguishable from that of the authentic compound.

The crude preparation of 2-naphthyl sulphamate from rat liver was extracted with 1 ml of cold ethanol to leave 7.3 mg of light brown solid which was crystallised from 0.8 ml of 95% ethanol containing 15% of potassium acetate to give 2.4 mg of glistening white platelets. The infrared spectrum of this was identical with that of authentic 2-naphthyl sulphamate.

These results confirm the existence in rat and guinea-pig livers of an enzyme or enzymes capable of converting 2-naphthylamine into 2-naphthyl sulphamate, and so confirm the existence of the activity ascribed to arylamine sulphotransferase. They do not resolve the doubts about the nature of this activity which could be due to a separate enzyme or simply a facet of the activities of other sulphotransferases, as was suggested by Banerjee and Roy [7]. This does not detract from a possible role for this activity in the metabolism of xenobiotics containing arylamine groups and more attention should therefore be given to the possible formation of sulphamates *in vivo*: it has already been shown that they can be formed from sulphonamides [11] and from metoclopramide [12].

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Direct action of D-lysergic acid diethylamide on dispersed mucosal cells from guinea pig stomach*

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Histamine receptors are classified as H_1- or H_2-receptors according to their ability to interact with specific agonists and antagonists. Those on intestinal and gastric smooth muscles are H_1-receptors, whereas the ones on gastric mucosa and atrial or uterine smooth muscles are H_2-receptors [1, 2]. In certain tissues, however (e.g. heart and brain), both H_1- and H_2-antagonists interact with histamine H_2-receptors linked to adenylate cyclase [3–5]. We reported recently that the action of histamine on guinea pig gastric mucosal cells could be blocked by H_1- and H_2-antagonists [6]. In all these preparations, the potencies of H_1-antagonists in inhibiting the histamine-induced responses were approximately 1000 times less than their potencies in inhibiting the action of histamine on functions which are mediated by H_1-receptors [1, 7]. Since D-lysergic acid diethylamide (LSD) was reported to be a competitive antagonist of histamine on H_2-receptors in the brain [3] and a partial agonist in guinea pig right atrium [8], we explored the histamine receptors in isolated gastric cells by examining the effects of LSD on [^{3}H]histamine binding, cellular cyclic AMP, and acid formation as reflected by the uptake of the weak base [^{14}C]aminopyrine.

Methods

Dispersed mucosal cells from guinea pig stomach were prepared and suspended in standard solution (Hanks' buffer, GIBCO, Grand Island, NY) as previously described [9]. Cyclic AMP was determined by radioimmunoassay [10], and uptake of [^{14}C]aminopyrine (13 mCi/mmole) and binding of [^{3}H]histamine (10 Ci/mmole) (both from the New England Nuclear Corp. Boston, MA) by centrifugation [11, 12].

Results and discussion

In dispersed mucosal cells, histamine and LSD increased cellular cyclic AMP in a dose-related fashion with half-

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