The Conjugation of Tyramine with Sulphate by Liver and Intestine of Different Animals

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Tyramine was conjugated with sulphate by extracts of monkey intestine and livers of monkey, rat, mouse, guinea pig and man. The activity measured in monkey intestine was almost three times that of monkey liver. Labelled tyramine sulphate synthesized from [^{14}C]tyramine, [^3H]tyramine or $\text{Na}_2^{35}\text{SO}_4$, on acid hydrolysis, released its radioactive precursor. Liver extracts of monkey, rat, mouse and guinea pig synthesized respectively 145, 66, 21 and 6 pmol of [^{14}C]tyramine sulphate/min per mg of protein. Except with the monkey, intestine exhibited very low activity. *trans*-2-Phenylcyclopropylamine, a monoamine oxidase inhibitor, was added as a routine to the enzyme preparation, as its omission resulted in the production of *p*-hydroxyphenylacetic acid in appreciable amounts. This oxidative deamination of tyramine, however, did not decrease the sulpho-conjugation of tyramine. The low K_m (9.1 μM) of sulphotransferase for tyramine is probably responsible.

The sulpho-conjugation of tyramine has been reported (Grimes, 1959; Segal & Mologne, 1959; Spencer, 1960; Suzuki & Strominger, 1960; Mattock & Jones, 1970). The implication of its involvement in dietary migraine (Hanington, 1967; Smith *et al.*, 1970, 1971; Mullen & Smith, 1971; Youdim *et al.*, 1971) and in Parkinsonism (Bonham-Carter *et al.*, 1974) has revived interest in this particular pathway of tyramine metabolism. As there are species differences in the metabolism of tyramine (Tacker *et al.*, 1970, 1972), the sulphation of this amine was studied by using tissues from several species of animals, including man, and a systematic study on the kinetics of the reaction was carried out with extracts of monkey liver.

Experimental

Materials

The following radioactive compounds were purchased from The Radiochemical Centre, Amersham, Bucks., U.K.: $\text{Na}_2^{35}\text{SO}_4$ (24 mCi/mmol), [$2\text{-}^{14}\text{C}$]tyramine hydrochloride (55 mCi/mmol; radiochemical purity of 98%) and [*side chain*-1,2- ^3H]tyramine hydrochloride (1.1 Ci/mmol, radiochemical purity of 98%). *trans*-2-Phenylcyclopropylamine hydrochloride, *N*-methyl-*N*-benzyl-2-propynylamine hydrochloride (pargyline), *N*-benzyl- β -(isonicotinylhydrazino)propionamide (nialamide), ATP (sodium salt, 99% pure), DL-dithiothreitol and tyramine hydrochloride were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

Methods

Preparation of enzymes. Adult male animals were used. The rats were of the Albino Wistar strain, the monkeys belonged to the *Macaca fascicularis* species and the other animals were local hybrids. Their body weights were as follows: rat, 150–200 g; mouse, 25–30 g; guinea pig, 200–300 g; monkey, 1–1.5 kg. A liver biopsy (150 mg) was obtained from a male patient (T. C. S.) aged 62, suffering from moderately differentiated adenocarcinoma of the descending colon. The biopsy was normal in appearance.

The procedure for the preparation of the high-speed supernatant fraction of liver has been described (Wong, 1975, 1976). The small intestine was removed, washed and homogenized with 2 vol. of cold 0.15 M-KCl. The suspension so obtained was subjected to the same fractionation procedure as for liver. *trans*-2-Phenylcyclopropylamine, a monoamine oxidase inhibitor, at a final concentration of 1 mM was added to the enzyme solution at the time of its preparation. Enzyme samples stored at -80°C remained active for at least 2 months. The procedure of Lowry *et al.* (1951) was used for the measurement of protein, with bovine serum albumin as standard.

Assay procedure. The assay mixture (250 μl) contained 10 mM- Na_2SO_4 , 16 mM-ATP, 10 mM- MgCl_2 , 3 mM-dithiothreitol, 45.5 μM -tyramine, 0.5 M-glycine/NaOH buffer, pH 9.4, and 50 μl of enzyme preparation containing 0.5–1.0 mg of protein. As required, [$2\text{-}^{14}\text{C}$]tyramine (0.63 μCi) or [*side chain*-1,2- ^3H]tyramine (15 μCi) was included. When 1.55 mM- $\text{Na}_2^{35}\text{SO}_4$ (9.17 μCi) was used, the concentration of

unlabelled tyramine was 1 mM. Incubation was carried out at 37°C for 10 or 15 min and the reaction was terminated with 50 μ l each of ZnSO₄ (10%, w/v) and Ba(OH)₂ (0.3 M). The precipitated protein was removed by centrifugation and 50 μ l of the supernatant was chromatographed on Whatman no. 1 paper in butan-1-ol/acetic acid/water (4:1:5, by vol., upper phase). The areas of the chromatograms that contained tyramine sulphate labelled with ¹⁴C, ³H or ³⁵S were measured for radioactivity in the liquid-scintillation counter (for details, see Wong, 1976). Standards of the radioactive precursors, namely [2-¹⁴C]- or [side-chain-1,2-³H]-tyramine or Na₂³⁵SO₄ were also applied on similar strips of paper and their radioactivity was measured.

Results

The sulphate conjugate synthesized from Na₂³⁵SO₄, [¹⁴C]- or [³H]-tyramine has the same chromatographic mobility (R_F 0.32) in the solvent system used and it could be separated from its labelled precursor, which was Na₂³⁵SO₄ (R_F 0.05) or [¹⁴C]- or [³H]-tyramine (R_F 0.56). The conjugates, on acid hydrolysis, released their respective radioactive precursors. In the absence of a monoamine oxidase inhibitor in the incubation medium, another radioactive area (R_F 0.90) was consistently observed. It was apparently a product of enzyme action on tyramine, as its formation was greater with increasing enzyme concentration and length of incubation. Addition of pargyline or *trans*-2-phenylcyclopropylamine at 1 mM decreased this radioactive area considerably. These results indicated that this radioactivity was associated with a product of tyramine oxidative deamination. The material contained in this area was isolated, rechromatographed in three solvent systems [B, C and D of Tacker *et al.* (1970)] and its mobility (R_F values 0.88, 0.42 and 0.86 respectively) was the same as that of authentic *p*-hydroxyphenylacetic acid chromatographed at the same time.

Effects of different monoamine oxidase inhibitors on the production of p-hydroxyphenylacetate

In the presence of nialamide (0.33 mM), pargyline (1 mM) or *trans*-2-phenylcyclopropylamine (1 mM), the amount of tyramine sulphate synthesized was the same as that in the control (without inhibitor), but the production of *p*-hydroxyphenylacetate was decreased by 86, 90 and 93% respectively. The effect of *trans*-2-phenylcyclopropylamine, over the concentration range 62.5 μ M to 1 mM, was also tested. Again, the amount of tyramine sulphate formed remained the same, whereas that of *p*-hydroxyphenylacetate was decreased by 91–96%. There seemed to be a residual conversion of tyramine into *p*-hydroxy-

phenylacetate (3–5%) which could not be controlled by monoamine oxidase inhibition.

Effects of pH, time of incubation and enzyme concentration on the sulphation of tyramine by monkey liver

The pH optimum of the reaction was 9.2–9.4 in 0.5 M-glycine/NaOH buffer. Progress curves were linear with time up to 20 min of incubation and the rate of tyramine sulphate formation was proportional to enzyme concentration.

Measurement of K_m of tyramine sulphotransferase

The K_m of tyramine for the sulphotransferase extracted from monkey liver was measured by the procedure described (Wong, 1975, 1976). With [¹⁴C]tyramine as the substrate, the K_m value, determined by the Lineweaver–Burk (1934) plot was 9.1 μ M.

Measurement of sulpho-conjugation with [¹⁴C]- or [³H]-tyramine

Under identical conditions of assay, the amount of [¹⁴C]- and [³H]-tyramine sulphate formed by monkey liver was 185 and 184 pmol/min per mg of protein respectively, for a 10 min assay.

Sulphation of tyramine by liver, intestine and brain of different animals

The amounts of [¹⁴C]tyramine sulphate formed by the livers of monkey, rat, mouse and guinea pig were 145, 66, 21 and 6 pmol/min per mg of protein respectively, for a 15 min assay. Monkey intestine had 2.8 times the activity of monkey liver. In contrast, the intestines of rat, mouse and guinea pig exhibited very low activity. In one preliminary qualitative experiment, the production of tyramine sulphate was also observed with a 10% (w/v) homogenate of human liver. Monkey and rat brains did not form tyramine sulphate.

Discussion

The sulpho-conjugation of tyramine was catalysed *in vitro* especially well by monkey liver and intestine. Although the high-speed supernatant preparation was used, monoamine oxidase, a predominantly mitochondrial enzyme, was also present, as manifested by the formation of *p*-hydroxyphenylacetate. Presumably a small degree of solubilization of monoamine oxidase had occurred during the process of homogenization. Though this may represent a small fraction of the total monoamine oxidase activity within a cell, the oxidative deamination of tyramine that it catalyses may be substantial in some preparations. Nevertheless, the amount of tyramine sulphate synthesized in the presence or absence of different concentrations of various monoamine oxidase inhibitors was the same, perhaps because of the very high

affinity of sulphotransferase for tyramine (K_m 9.1 μM). This value is much lower than the K_m of 2 mM obtained by Mattock & Jones (1970) at pH 7.5 for rat liver sulphotransferase, which was the same as the K_m of rat liver monoamine oxidase for tyramine (Tipton, 1975). If the same order of magnitude of K_m is assumed for monkey liver monoamine oxidase, then the very low K_m for the sulpho-conjugation of tyramine would suggest that this process could be a significant pathway for its metabolic disposal, although studies *in vivo* showed that oxidative deamination predominates in some animals (Schayer, 1953; Lemberger *et al.*, 1966). Perhaps a limiting availability of inorganic sulphate imposes a restriction on sulpho-conjugation *in vivo*; indeed, an increase in excretion of tyramine sulphate in normal subjects was observed after sulphate supplementation (Smith & Mitchell, 1974). Especially at the gut wall, there is evidence that different substrates compete for this sulphate (Bennett *et al.*, 1975). The present study suggests that the small intestine of monkey is more important than liver in the sulphation of this amine. In this connexion, it would be interesting to find out if this is due to the high tyramine content of the diet of this animal (e.g. bananas and other fruits). If the same situation occurs in man, it would explain the greater excretion of tyramine sulphate after oral (Mullen & Smith, 1971) as compared with intravenous (Tacker *et al.*, 1972) administration of tyramine. There seems no doubt that, in man, tyramine is excreted as a sulphate, and, consistent with that observation, present work shows the ability of the human liver to conjugate it with inorganic sulphate *in vitro*.

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