The Biosynthesis of Tyramine Glucuronide by Liver Microsomal Fractions

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Labelled tyramine glucuronide was synthesized *in vitro* from UDP-[¹⁴C]glucuronic acid, [¹⁴C]tyramine or [³H]tyramine. The glucuronidation was carried out at pH 9.2 in the presence of a monoamine oxidase inhibitor, *trans*-2-phenylcyclopropylamine. The K_m values for tyramine were 69 and 125 μ M and those for UDP-glucuronic acid were 260 and 290 μ M respectively for guinea-pig and rat liver microsomal preparations. The specific activities of microsomal glucuronyltransferase measured in fresh hepatic preparations of guinea pig, mouse and rat were respectively 601, 251 and 235 pmol of [¹⁴C]tyramine glucuronide/min per mg of protein. Increase in activity ranged from 2- to 6-fold in preparations which were frozen and thawed once and 5.4- to 10-fold when the freezing and thawing was repeated. Rabbit liver has very low activity, and monkey liver and intestine were completely devoid of this conjugating capacity.

The sulphate conjugation of tyramine has been reported (Wong, 1976b). As an extension of this work, the glucuronidation of this amine was studied, as some species of animals, e.g. rat, seem to excrete it and other phenolic amines as glucuronide conjugates (Tacker *et al.*, 1970, 1972a; Axelrod *et al.*, 1958).

Experimental

Materials

The following radioactive compounds were purchased from The Radiochemical Centre, Amersham, Bucks., U.K.: [²⁻¹⁴C]tyramine hydrochloride (55 mCi/mmol, radiochemical purity 98%), [*side chain*-1,2-³H]tyramine hydrochloride (1.1 Ci/mmol, radiochemical purity 98%) and UDP-D-[U-¹⁴C]-glucuronic acid (ammonium salt; 290 mCi/mmol; radiochemical purity 98–99%). The other chemicals were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.: *trans*-2-phenylcyclopropylamine, UDP-glucuronic acid (triammonium salt, 98% pure), DL-dithiothreitol, tyramine hydrochloride and β -glucuronidase [from bovine liver, type 1-B, containing 989 000 Fishman units (Talalay *et al.*, 1946)/g].

Methods

Preparation of enzyme. All animals used, except the rabbit, were male. The rats were of the Albino Wistar strain, the monkeys were of the *Macaca fascicularis* species and the other animals were of local hybrids. Their body wts. were: rat, 150–200 g; mouse, 25–30 g; guinea pig, 200–300 g; rabbit, 1–1.2 kg; monkey, 1–1.5 kg.

For the preparation of the microsomal glucuronyl-

transferase, the procedure described by Wong & Sourkes (1967) and later modified by the introduction of a dialysis step (Wong, 1971) was used. *trans*-2-Phenylcyclopropylamine, a monoamine oxidase inhibitor, was added at a final concentration of 10 mM to the enzyme suspension.

Assay conditions. The incubation mixture (250 μ l) contained 2 mM-UDP-glucuronic acid, 45.5 μ M-[²⁻¹⁴C]tyramine (0.63 μ Ci) and/or 455 μ M-unlabelled tyramine, 5 mM-MgCl₂, 3 mM-dithiothreitol, 0.5 M-glycine/NaOH buffer, pH 9.2, and 50 μ l of enzyme preparation containing 0.6–0.8 mg of protein, measured by the procedure of Lowry *et al.* (1951), with bovine serum albumin as standard. Time of incubation was 10 min. To show the formation of tyramine [¹⁴C]glucuronide and [³H]tyramine glucuronide, UDP-[U-¹⁴C]glucuronic acid (0.63 μ Ci, 2.15 nmol) in conjunction with unlabelled tyramine and [*side chain*-1,2-³H]tyramine (15 μ Ci) respectively were used in the incubation.

The procedure used previously (Wong, 1976b) for the termination of the reaction, subsequent chromatography and measurement of radioactivity was followed except that [¹⁴C]tyramine glucuronide was determined.

Results

[¹⁴C]Tyramine glucuronide, [³H]tyramine glucuronide and tyramine [¹⁴C]glucuronide, synthesized *in vitro*, have the same R_F value (0.13) on Whatman no. 1 paper developed in the solvent system butan-1-ol/acetic acid/water (4:1:5, by vol., upper phase). On hydrolysis with β -glucuronidase (1 mg, containing 989 Fishman units), [¹⁴C]tyramine and [³H]tyramine

Table 1. *Specific activity of microsomal tyramine glucuronyltransferase of hepatic tissues*

Results are expressed as pmol of [^{14}C]tyramine glucuronide formed/min per mg of protein for a 15 min assay. Data for tyramine sulphate are reproduced from Wong (1976b) for comparison. [^{14}C]Tyramine sulphate formed was also expressed as pmol/min per mg of protein. Values in square brackets represent the number of days after its preparation when the enzyme solution was thawed and assayed. Numbers in parentheses represent the activation (fold) compared with the values obtained in fresh preparations. NM, Not measurable.

Enzyme preparations ... Animals	Activity			Tyramine sulphate
	Fresh	Frozen and thawed once [4]	Frozen and thawed twice [4, 11]	
Guinea pig	601	4206 (6)	6611 (10)	6
Rat	235	703 (2)	1511 (5.4)	66
				60* 495†
Mouse	251	1170 (3.7)	2061 (7.2)	21
Rabbit	NM	70	—	—
Monkey	NM	NM	—	145

* Experiment repeated for rat liver sulphotransferase under conditions detailed (Wong, 1976b) when the concentration of tyramine was $45.5\text{ }\mu\text{M}$.

† When tyramine concentration was increased to $500\text{ }\mu\text{M}$, which was the concentration of tyramine used in the assay of glucuronyltransferase.

were liberated; they were identified by their R_F values (0.65) on the paper chromatogram. As the R_F of glucuronic acid (0.18) was too similar to that of tyramine glucuronide, another solvent system, propan-2-ol/ NH_3 soln. (sp.gr. 0.88)/water (8:1:1, by vol.) was used. [^{14}C]Glucuronic acid was identified in the hydrolysate as a peak which migrated 11 cm from the origin and that of tyramine [^{14}C]glucuronide in the untreated control was 17 cm from the origin after descending chromatography for 24 h at room temperature (29°C); the solvent front had moved off the chromatogram under these chromatographic conditions.

Determination of K_m for tyramine and UDP-glucuronic acid

The K_m values for tyramine, determined by Lineweaver-Burk (1934) plots, were respectively 69 and $125\text{ }\mu\text{M}$ for the glucuronyltransferases of guinea-pig and rat livers, and those for UDP-glucuronic acid were 260 and $290\text{ }\mu\text{M}$. As the concentration of tyramine ($45.5\text{ }\mu\text{M}$) used in the preliminary qualitative experiments were lower than the K_m for tyramine, the assays of enzyme activity were made with an additional $455\text{ }\mu\text{M}$ of unlabelled tyramine. In this way, it was assured that the concentration of aglycone was not limiting.

Specific activity of microsomal glucuronyltransferase extracted from different animals

As shown in Table 1, the microsomal preparation of guinea-pig liver exhibited the highest specific activity, and with this enzyme preparation the formation of tyramine glucuronide increased progressively with time of incubation and enzyme concentration.

In decreasing order, the mouse, rat and rabbit showed intermediate degrees of glucuronidation, whereas the monkey liver and intestine completely lacked this conjugating ability. Data on the sulphonation of this amine (reproduced from Wong, 1976b) are included for comparison.

Effects of freezing and thawing on microsomal glucuronyltransferase activity

Freezing at -80°C and thawing of the microsomal fractions prepared from guinea-pig, mouse, rat and rabbit livers produced activation of the glucuronyltransferase activity, ranging from 2- to 6-fold in preparations subjected to this treatment once and 5.4- to 10-fold when this treatment of freezing and thawing was repeated.

Discussion

The oxidative deamination of tyramine to *p*-hydroxyphenylacetate by the high-speed supernatant of liver (Wong, 1976b) was also observed with the microsomal preparation; the latter is known to contain monoamine oxidase activity (Creasey, 1956; Jarrott & Iversen, 1968). The effective inhibition of oxidative deamination of tyramine shown previously by *trans*-2-phenylcyclopropylamine (Wong, 1976b) was also observed in this study.

Analysis of the rates of glucuronidation and sulphonation of tyramine *in vitro* by various animals (see Table 1) showed that there was a reciprocal relationship between these two modes of conjugation. Of the animals examined, only the rat has measurable activities of both glucuronyltransferase and sulphotransferase. This has also been observed in the conju-

gation of 4-hydroxy-3-methoxyphenylethanol (Wong, 1976a). The other animals conjugate tyramine with either sulphate or glucuronide, e.g. in the monkey the sulphate conjugate is produced (Wong, 1976b) but not the glucuronide (the present paper). A similar trend may exist in man, where only the sulphate conjugate has been reported (Mullen & Smith, 1971; Tacker *et al.*, 1972b; Wong, 1976b). The fact that the normal daily excretion of *p*-hydroxyphenylacetate in man is only one-third that of the rat (Horning *et al.*, 1966) suggested that oxidative deamination of tyramine is of less significance in man. Perhaps this is compensated by the sulphate-conjugating pathway, since as much as 15% of tyramine administered was excreted as tyramine sulphate by man (Mullen & Smith, 1971). Sulphate esters of other phenolic amines are also excreted by man (Goodall, 1959; LaBrosse & Mann, 1960). On the other hand, the tyramine glucuronide-conjugating system is most efficient in the guinea pig, followed by the mouse, rat and monkey. This same pattern was found with harmol as substrate (Wong, 1969). In those animals where both the sulphate- and glucuronide-conjugating activities are low, e.g. rabbit, oxidative deamination could be the major catabolic route of this amine (Ewins & Laidlaw, 1910; Bray *et al.*, 1950; Lemberger *et al.*, 1966). Even for the rat and mouse, which exhibited higher sulphate and glucuronide formation *in vitro*, oxidative deamination seemed to be the predominant pathway (Schayer, 1953; Nakijima & Sano, 1964; Horning *et al.*, 1966).

The rate of sulphation measured in the high-speed supernatant fraction of rat liver was only higher than that of glucuronidation measured in fresh microsomal preparations when the concentration of tyramine was not limiting (see Table 1). Each of the animal tissues examined appeared to possess higher inherent glucuronyltransferase activity than that measured in their fresh preparations; simple freezing and thawing could increase this activity. This phenomenon of the latency of the transferase is well established. After the administration of tyramine, the glucuronide but not the sulphate conjugate was excreted by the rat (Tacker *et al.*, 1970, 1972a). These data suggest that *in vivo*, rat liver glucuronyltransferase may be operating at a higher level, approaching perhaps those activities in the once- or twice-frozen preparations. This state of glucuronyltransferase predominance over sulphotransferase *in vivo* in the rat is augmented probably by the existence of preformed UDP-glucuronic acid in its hepatic tissue (Wong &

Sourkes, 1967; Zhivkov, 1970). In contrast, adenosine 3'-phosphate 5'-sulphatophosphate, the corresponding conjugating agent in the sulphation reaction, does not exist as such intracellularly and is presumably synthesized only when required.

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