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STUDIES ON GLUCURONIDE SYNTHESIS IN RATS CHRONICALLY TREATED WITH MORPHINE AND PHENOL*

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Abstract—Glucuronyl transferase activity in rats chronically treated with morphine is decreased whereas that in rats chronically treated with phenol is unchanged when the enzymic activity is compared with that in control rats. The depressed enzymic activity in rats treated with morphine is apparent regardless of whether morphine or phenol is used as the substrate in assaying the activity. The activity of uridine diphosphate (UDP)-glucose dehydrogenase remains unaltered in the experimental animals. The glucuronyl transferase reaction in rats chronically treated with morphine becomes rate-limiting in conjugating foreign substances in the body.

IN STUDYING the activities of the hepatic enzymes responsible for glucuronide synthesis—UDP-glucose pyrophosphorylase, UDP-glucose dehydrogenase, glucuronyl transferase, and nucleoside diphosphate (NDP)-kinase, Takemori[1] found the activity of glucuronyl transferase decreased and that of UDP-glucose dehydrogenase increased in rats repeatedly injected with increasing doses of morphine for 5 weeks. The enzymic activity in animals chronically treated with morphine was reinvestigated in rats treated with morphine for a shorter period. We also extended the findings to see whether chronic injection of other compounds, such as phenol, which are metabolized by way of glucuronide conjugation, might alter the activities of glucuronyl transferase and UDP-glucose dehydrogenase. The activity of glucuronyl transferase was studied by employing morphine and phenol as substrates.

MATERIALS AND METHODS

Male Holtzman rats weighing 200 ± 50 g were chronically injected with an initial bi-daily dose of 15 mg of morphine sulfate/kg intraperitoneally for a week, which was increased to 30 mg/kg during the second week and to 45 mg/kg during the third week. Rats were chronically injected with phenol by the same injection schedule as above with phenol at dosages equimolar to that of morphine; *i.e.* 3·7 mg/kg (39·5 μmoles/kg) during the first week, 7·4 mg/kg (79·1 μmoles/kg) during the second week and 11·2 mg/kg (118·6 μmoles/kg) during the final week. Control animals received injections of saline solution. All rats were given food and water *ad libitum*. Eighteen to 20 hr after the last dose the rats from each of the three groups were decapitated and bled, the livers

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were removed and 10 per cent hepatic homogenates were prepared in ice-cold isotonic KCl. Microsomes and dialyzed soluble fractions of the homogenates were prepared as previously described.[1]

The activity of UDP-glucose dehydrogenase was assayed by the method of Strominger *et al.*[2] as modified by Takemori.[1] The glucuronyl transferase assay using morphine as the substrate was described previously.[1] The glucuronyl transferase assay using phenol as the substrate contained the same ingredients in the reaction mixture as the above assay except that 0·4 μmole of phenol was substituted for morphine. Phenyl glucuronide formation was estimated by difference of phenol concentrations in the reaction mixture at 0 and 20 min incubating times. Phenol was determined by employing the Folin-Ciocalteau phenol reagent. All enzymic assays in each animal were performed in duplicate and the data were statistically analyzed by Student's *t* test. Nitrogen content of the microsomal and dialyzed soluble fractions was determined by direct nesslerization after complete acid digestion.

Folin-Ciocalteau reagent was obtained from Fisher Scientific Co., UDP-glucose, UDP-glucuronic acid, and diphosphopyridine nucleotide (DPN) were procured from Sigma Chemical Co. UDP-glucuronic acid was received as the ammonium salt and converted to the sodium salt by employing a Dowex-50-Na^+ column.

RESULTS

The average increase in weight of the control rats receiving saline during the 3-week experimental period was 97 g, whereas increases in animals receiving morphine or phenol were 40 and 81 g, respectively. Aside from differences in weight, the rats of the three groups appeared healthy and active at the end of the experimental period. The animals receiving morphine seemed slightly more hyperexcitable than were the rats of the other two groups. No infections or deaths were noted in any group.

All enzymic activities are expressed on a milligram nitrogen basis to obtain uniform comparisons, since the nitrogen content of the hepatic microsomal and soluble fractions of control rats did not differ statistically from those of the experimental animals. One sees by observing the activity of glucuronyl transferase in control animals that morphine and phenol are conjugated equally well (Table 1). The enzymic activity

TABLE 1. ACTIVITY OF GLUCURONYL TRANSFERASE IN HEPATIC MICROSOMES

Chronic treatment	No. of animals	Morphine glucuronide ±s.e. (μmoles formed/ 20 min/mg N*)	*P* value	Phenyl glucuronide ±s.e. (μmoles formed/ 20 min/mg N*)	*P* value
Saline (control)	7	0·033 ± 0·002		0·034 ± 0·003	
Morphine	7	0·024 ± 0·003	>0·05	0·015 ± 0·004	>0·01
Phenol	7	0·030 ± 0·002	>0·3	0·031 ± 0·002	>0·8

* Mg of microsomal nitrogen.

assayed with morphine is statistically depressed even in rats chronically treated with morphine for 3 weeks instead of 5 as in the previous study.[1] The enzymic activity appears to be more depressed when the activity is assayed with phenol; however, this value is not statistically different from the depressed value assayed with morphine. On the other hand, the activity of glucuronyl transferase in rats chronically treated

with phenol did not differ from that in control animals regardless of the substrate used for the enzymic assay.

The activity of UDP-glucose dehydrogenase in dialyzed soluble fractions of livers of rats treated with morphine or phenol is not statistically different from that of control rats (Table 2). Thus, glucuronide synthesis is not limited by the availability of UDP-glucuronic acid in these animals.

TABLE 2. ACTIVITY OF URIDINE DIPHOSPHOGLUCOSE DEHYDROGENASE IN DIALYZED SOLUBLE FRACTIONS OF RAT LIVER

Treatment	No. of animals	Absorbance ± s.e. (340 mu/min/mg N*)	*P* value
Saline (control)	7	0·027 ± 0·002	
Morphine	7	0·025 ± 0·003	0·5
Phenol	7	0·031 ± 0·002	0·3

* Mg nitrogen of dialyzed soluble fraction.

DISCUSSION

Way *et al.*[3] have shown in slices of rat liver that the ability to conjugate morphine is not altered when the rats become tolerant to morphine. Zauder[4] has shown an increased ability to conjugate morphine in hepatic slices of tolerant rats, whereas Deneau *et al.*[5] have shown a decreased capacity to biotransform morphine in hepatic minces of tolerant rats when the hepatic preparations are compared with those of non-tolerant or control rats. Although the results *in vitro* are equivocal, both Zauder and Way *et al.* have shown that the urinary excretion of conjugated morphine decreases as the rats receive repeated injections of morphine.

Our data support the view that the ability to form glucuronides decreases when the rats are chronically injected with morphine. Additionally, since the activities of UDP-glucose pyrophosphorylase and NDP-kinase are unaltered,[1] and the activity of UDP-glucose dehydrogenase is either increased[1] or unaltered, we can pinpoint glucuronyl transferase of the conjugating process as the enzymic system that becomes rate-limiting in rats chronically treated with morphine. The activity of glucuronyl transferase in these animals becomes limiting not only for conjugating morphine but for other foreign substances as well. The decreased enzymic activity would presumably interfere with the glucuronide synthesis of physiological substances also, but we have not investigated this point.

The decreased activity of glucuronyl transferase may explain the decreased urinary excretion of conjugated morphine observed by the other investigators[3,4] but cannot explain the results obtained in hepatic slices by Zauder.[4] The dosage of morphine used to produce tolerance in rats may be a factor in explaining the difference. The dosages used in this study were much higher than those used by either Zauder[4] or Way *et al.*[3] and were much lower than those used by Deneau *et al.*[5]

The property of decreasing the activity of glucuronyl transferase appears inherent to narcotic analgesics, since chronic injections of phenol did not alter the enzymic activity. However, chronic injection of morphine also depresses the enzymic system that N-demethylates the narcotic agent in hepatic microsomes of rats.[6, 7] In either case, tolerance due to chronic injections of the narcotic agent cannot be related to

altered biotransformation of the analgesic unless other undescribed pathways are involved.

The decrease in glucuronyl transferase activity may possibly be attributed to stunting of the rats upon injection of morphine. This possibility does not seem likely, since the other enzymes involved in glucuronide synthesis and the nitrogen content of the microsomal preparations were unaffected. Mannering and Takemori[7] have also shown that the N-methylating system in microsomes is unaltered in rats that are put on a restricted food intake to simulate the rate of weight gain of the rats injected with morphine.

The unaltered activity of UDP-glucose dehydrogenase in morphinized rats is in contrast to the earlier observation in which an increased enzymic activity was noted.[1] We cannot attribute this difference to anything except a shorter morphinization schedule and the younger rats employed in this than in the previous study. In the earlier study the activity of UDP-glucose dehydrogenase increased even after a large, acute dose of morphine (150 mg/kg). We have been able to confirm the acute effect with doses of 90 mg morphine/kg in the younger rats. Thus, the enzymic activity may have increased during the initial stages of morphinization. However, the important and rate-limiting step is still the transferase reaction, whether the activity of UDP-glucose dehydrogenase is increased or unchanged.

REFERENCES

1. A. E. TAKEMORI, *J. Pharmacol. Exp. Ther.* **130**, 370 (1960).
2. J. L. STROMINGER, E. S. MAXWELL, J. AXELROD and H. M. KALCKAR, *J. biol. Chem.* **224**, 79 (1957).
3. E L. WAY, C. Y. SUNG and J. M. FUJIMOTO, *J. Pharmacol. Exp. Ther.* **110**, 51 (1954).
4. H. L. ZAUDER, *J. Pharmacol. Exp. Ther.* **104**, 11 (1952).
5. G. A. DENEAU, L. A. WOODS and M. H. SEEVERS, *Fed. Proc.* **12**, 315 (1953).
6. J. AXELROD, *Science* **124**, 263 (1956).
7. G. J. MANNERING and A. E. TAKEMORI, *J. Pharmacol. Exp. Ther.* **127**, 187 (1959).