Glucuronide Formation of Narcotic Drugs *in vitro* and *in vivo*. (25631)

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A major pathway for metabolism of the narcotic drugs morphine(1,2), nalorphine(3), *l*-dromoran(4,5) and codeine(6) involves conjugation. Morphine(2) and *l*-dromoran(4) have been shown to be excreted as glucuronides, but the nature of "bound" codeine and nalorphine has not been established. The enzymatic mechanism whereby glucuronides are formed has been recently clarified. Dutton and Storey(7) found that o-aminophenol is conjugated with glucuronic acid by an enzyme in the microsomes of the liver that transfers glucuronic acid from UDP-glucuronic acid to the phenolic acceptor. This process has been shown to occur with many normally occurring and foreign phenols(8,9, 10), carboxylic acids(11) and amines(12) as acceptors. The formation of UDP-glucuronic acid is catalyzed by an enzyme that oxidizes UDP-glucose and requires diphosphopyridine nucleotide as the hydrogen acceptor(13). This paper describes *in vitro* formation of glucuronides of the narcotic drugs morphine, nalorphine, *l*-dromoran and codeine by an enzyme in the microsomes of guinea pig liver requiring UDP-glucuronic acid. In addition, excretion of glucuronides of morphine, nalorphine, *l*-dromoran and codeine by the guinea pig is shown.

Methods and materials. Preparation and assay of glucuronide forming enzyme. Adult male guinea pigs were killed, exsanguinated and the livers immediately removed and chilled in cracked ice. All subsequent steps were carried out at 0-5°C. The liver was homogenized with 4 volumes of isotonic KCl and the resulting homogenate centrifuged at 8000 x g to remove mitochondria, nuclei and unbroken cells. After separating the microsomes by centrifugation at 78,000 x g, they were washed with isotonic KCl and resuspended in this medium. Incubations were carried out at 37°C, using microsomes obtained from 1 g of guinea pig liver, 20 μmoles $MgCl_2$, 200 μmoles tris (hydroxymethyl) aminomethane buffer pH 8.0, 0.8 μmole UDP-glucuronic acid, 0.5 μmole substrate and water to make a final volume of 4 ml. To determine extent of enzyme activity aliquots of the incubation mixture were taken at 0 time and after 30 minutes of incubation, and amount of substrate remaining was measured. *Estimation of narcotic drugs.* Morphine and nalorphine were isolated from biological material by extraction into chloroform containing 3% isoamyl alcohol at pH 9.0 after saturating the aqueous phase with sodium chloride. The narcotic drugs were returned to 0.1 N HCl and measured using silicomolybdate reagent (14). Codeine(14) and *l*-dromoran were determined by procedures described previously (5). *Materials.* UDP-glucuronic acid was prepared enzymatically from UDP-glucose (Sigma Co.) and diphosphopyridine nucleotide by a procedure described previously(13). Bacterial β-glucuronidase was obtained from Sigma Chemical Co.

Results. Glucuronide conjugation of narcotic drugs in vitro. Incubation of morphine, nalorphine, *l*-dromoran and codeine with microsomes obtained from guinea pig liver and UDP-glucuronic acid resulted in disappearance of these compounds (Table I). In the absence of UDP-glucuronic acid, disappearance of these substrates was negligible. After 30 minutes incubation with UDP-glucuronic acid, 1 ml of pH 6.0 acetate buffer 0.5 N containing 500 units of β-glucuronidase was added to an aliquot of the reaction mixture and incubation was continued for 18 hours. Almost all of the narcotic drug that disappeared when incubated with UDP-glucuronic acid reappeared after treatment with β-glucuronidase. From these results it can be concluded that an enzyme present in guinea pig microsomes can transfer the glucuronic acid of UDP-glucuronic acid to narcotic drugs. The conjugation presumably occurs on the hydroxyl group.

Glucuronide formation of narcotic drugs in vivo. Guinea pigs were given morphine, nalorphine, *l*-dromoran and codeine intraperi-

TABLE I. Glucuronide Formation of Narcotic Drugs *In Vitro*.

Substrate	Substrate disappearing, μmole	Substrate reappearing after β-glucuronidase, μmole
Morphine + UDP-glucuronic acid	.31	.29
Morphine, UDP-glucuronide omitted	.06	.06
Nalorphine + UDP-glucuronic acid	.20	.19
Nalorphine, UDP-glucuronide omitted	.05	.05
Codeine + UDP-glucuronic acid	.18	.16
Codeine, UDP-glucuronide omitted	.03	.03
l-Dromoran + UDP-glucuronic acid	.36	.29
l-Dromoran, UDP-glucuronide omitted	.07	.07

Microsomes obtained from 1 g of guinea pig liver were incubated at 37°C with cofactors described under *Methods*. After 30 min. an aliquot of reaction mixture was removed for assay of substrate and 1 ml of pH 6.0 acetate buffer containing 500 units β-glucuronidase was added to another portion of reaction mixture. Incubation was continued for 18 hr and the mixture again assayed for substrate.

TABLE II. Excretion of Narcotic Drugs as Glucuronides in Guinea Pig.

Drug admin.*	Free drug excreted	Total conjugated drug excreted†	Drug excreted as glucuronide‡
	%		
Morphine sulfate, 90 mg/kg	7	39	38
Nalorphine sulfate, 90 mg/kg	5	12	12
l-Dromoran, 30 mg/kg	16	53	53
Codeine, 90 mg/kg	8	18	10

* Drugs given in 3 divided portions intraper. every 3 hr.

† Urine was autoclaved with one-tenth vol of concentrated hydrochloric acid at 115°C for 30 min.

‡ Urine (3 ml) was incubated with 25,000 units of bacterial β-glucuronidase, pH 6.0 for 24 hr.

toneally and the urine collected for 24 hours. Urine was examined for unchanged compound, drug liberated after acid hydrolysis and after treatment with β-glucuronidase (Table II). All the administered drugs were excreted as glucuronides. With the exception of codeine, all conjugated material was present in the urine as glucuronide. Codeine was excreted partly as a glucuronide and partly in another conjugated form.

Summary. Glucuronides of morphine, nalorphine, *l*-dromoran and codeine are formed by an enzyme in liver microsomes that can transfer glucuronic acid from UDP-glucuronic acid to the narcotic drug. Morphine, nalorphine, *l*-dromoran and codeine are excreted as glucuronic acid conjugates in guinea pigs.

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