Induction and Inhibition of *In Vivo* Glucuronidation of Apomorphine in Mice

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Abstract □ The effect of various inducers and inhibitors of drug-metabolizing enzyme systems was studied on the kinetics of apomorphine disappearance and of the appearance of apomorphine glucuronides. The generally claimed inducers, phenobarbital and testosterone, and inhibitors, β-diethylaminoethyl diphenylpropylacetate and actinomycin D, were found to have no significant effect on the metabolism of apomorphine. Estradiol, however, produced a significant inhibition of the metabolism. Apomorphine pretreatment for 3 days exhibited a definite induction of its own metabolism.

Keyphrases □ Apomorphine glucuronidation—induction, inhibition □ Polyestradiol phosphate—apomorphine glucuronidation inhibition □ Metabolism, apomorphine—self-induced □ Pentobarbital sleeping time—apomorphine metabolism determination

Glucuronide conjugation is a major pathway of metabolism of numerous drugs containing phenolic hydroxyl groups (1, 2). In recent years, evidence has accumulated regarding the ability of certain compounds either to stimulate or inhibit various drug-metabolizing enzyme systems (3, 4). Induction with phenobarbital in microsomal oxidase and dealkylating systems and in glucuronidation of several compounds has been recently reviewed (3). This paper presents evidence that *O*-glucuronide conjugation, at least in the case of apomorphine, may not necessarily be induced by phenobarbital or inhibited by substances such as β-diethylaminoethyl diphenylpropyl acetate (SKF 525-A).

Apomorphine was chosen as a substrate in these studies, because this compound was shown previously to be metabolized and excreted in rabbits and rats primarily as a 7:3 mixture of 3,*O*- and 4,*O*-glucuronides (5). By using analogous criteria of characterization in the present study, mice were also found to metabolize apomorphine into 3,*O*- and 4,*O*-glucuronides in a proportion similar to that observed in rabbits.

EXPERIMENTAL

Method of Assay—The rate of glucuronide conjugation in terms of apomorphine disappearance and the appearance of conjugated apomorphine in intact Swiss mice of mixed sexes weighing 20 ± 4 g. was determined by the method described by Kaul *et al.* (6), with the following modifications: (*a*) the dose of apomorphine hydrochloride used in all kinetic studies was 40 mg./kg. i.v., and (*b*) ethyl acetate (A.R.) was used for final extraction of the colored oxidation product of apomorphine prior to spectrophotometric determination. The specificity of the assay method for apomorphine was already established (7).

Glucuronidation by Mice—The details of the procedure for isolation of apomorphine metabolites and evidence that apomorphine in rabbits is primarily metabolized into a 7:3 mixture of 3,*O*- and 4,*O*-glucuronides was presented earlier (5). Mice were also found to metabolize apomorphine into glucuronides by using the following selective ion-exchange fractionation.

Groups of Swiss mice were given 30 mg./kg. apomorphine hydrochloride intraperitoneally, and 24-hr. pooled urine samples were collected. The urine was passed through a column of ion-exchange resin[1] (H^+). The column was washed with water followed by methanol, and the conjugated apomorphine was eluted with 4 *N* ammoniacal methanol. The eluate was concentrated under reduced pressure and passed through an ion-exchange resin[2] formate column. The column was washed with water and then eluted with 1 *N* formic acid. The eluate on hydrolysis gave positive test for apomorphine. It was further purified, as described previously (5), and found to consist of two compounds in 7:3 proportion. The purified conjugated apomorphine, when hydrolyzed by mild acid conditions and tested as described before (5), gave positive tests for apomorphine as well as glucuronic acid. Based on this evidence, rabbits, like mice, also seem to metabolize apomorphine into 3,*O*- and 4,*O*-glucuronides.

Pretreatment—The inducers tested were phenobarbital sodium (50 mg./kg. i.p.), testosterone propionate in oil (2 mg./kg. s.c. in female mice only), and apomorphine hydrochloride (20 mg./kg. i.p.). SKF 525-A (50 mg./kg. i.p.) and polyestradiol phosphate[3] (4 mg./kg. s.c.) represented the inhibitors of the drug-metabolizing enzyme systems tested in this work.

Although actinomycin D was reported (3) to block only the phenobarbital-induced induction of drug-metabolizing enzymes, preliminary experiments indicated that it also directly inhibited the pentobarbital metabolism. Therefore, actinomycin D (dactinomycin, 100 mcg./kg. i.p.) was included as a possible direct inhibitor of apomorphine metabolism.

As expected, animals receiving actinomycin D showed a considerable weight loss in the treatment period. To dissect the effects of the antibiotic on apomorphine metabolism due to: (*a*) its inhibition of protein synthesis, and (*b*) weight loss, a study was carried out wherein two groups of animals were control-fed orally with 1:1 diluted Infant Standardized Food[4] once daily but with *ad libitum* water supply. Actinomycin D (100 mcg./kg. i.p.) was given to one group; the other group served as the control.

All drugs were given once daily for 3 days before determining the kinetics of apomorphine conjugation 36 hr. later, with the exceptions of polyestradiol which was given only once 4 days prior to the kinetic study and of SKF 525-A which was given 30 min. prior to the kinetic study. Each treatment group was accompanied by a control group of mice receiving saline injections.

Pentobarbital Sleeping Time—Pentobarbital sleeping time in mice was used as a comparison control for assessing the enzyme induction and inhibition produced by the agents tested on apomorphine metabolism. Four randomly selected groups of mice were given four different treatments. One group each received daily doses of actinomycin D (100 mcg./kg. i.p.), apomorphine hydrochloride (20 mg./kg. i.p.), and saline (0.2 ml./mouse i.p.) for 3 days. These groups were subjected to pentobarbital sleeping-time determinations 36 hr. after the last treatment. The fourth group, however, received 0.25 ml./mouse saline i.p. daily for 3 days and, 35 hr. after the last saline injection, SKF 525-A (50 mg./kg. i.p.) was given. Pentobarbital sleeping time was determined 30 min. later. Pentobarbital sodium (75 mg./kg. i.p.) was given to all groups; sleeping time, according to the method of Westfall *et al.* (8), was noted as the interval from the time of injection to the time when the animal showed a righting reflex in response to tail pinching carried out every 5 min.

Student's *t* test was employed to determine the values of significance.

[1] Dowex 50-X2, Dow Chemical Co., Midland, Mich.
[2] Dowex 1-X8.
[3] Estradurin.
[4] SMA, Wyeth Laboratories, Philadelphia, Pa.

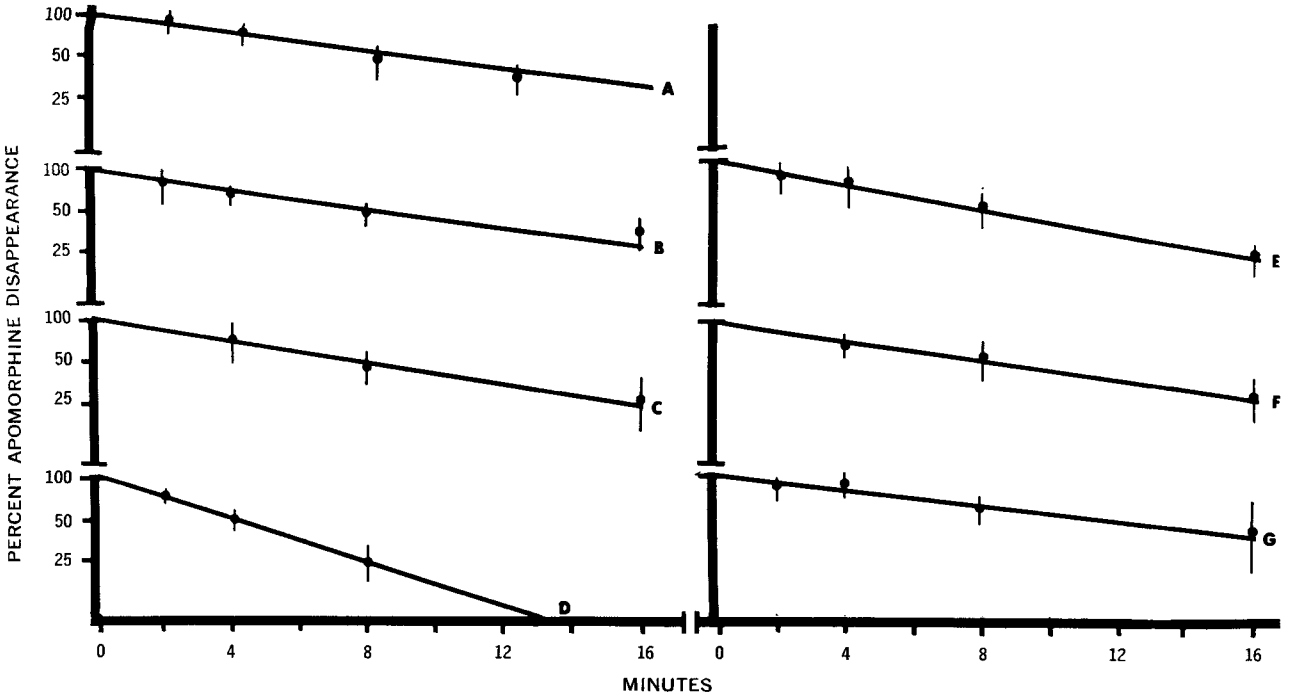

Figure 1—*Percent disappearance of apomorphine with time in whole mice given apomorphine intravenously. Each point represents the mean of 5 to 10 animals* ±SD. *Key: A, controls, no pretreatment,* $t_{1/2}$ *8 min.; B, phenobarbital treated,* $t_{1/2}$ *8 min.; C, testosterone treated,* $t_{1/2}$ *8.3 min.; D, apomorphine pretreated,* $t_{1/2}$ *5 min.; E, SKF 525-A treated,* $t_{1/2}$ *7.2 min.; F, actinomycin D treated,* $t_{1/2}$ *9 min.; and G, polyestradiol treated,* $t_{1/2}$ *10.8 min.*

RESULTS AND DISCUSSION

Determination of free and conjugated drug in the whole animal at any fixed time eliminates the need to consider influence of factors such as organ distribution, volume of distribution, and biliary and renal clearance, which all affect the drug and metabolite concentrations present in the blood. After apomorphine injection, each mouse was kept in a beaker until the time of homogenization. The washings of the beaker were added to the homogenate, including any urine or feces that may have been excreted by the animal. The assay, therefore, represents the total free as well as conjugated form (in this case, glucuronide) of apomorphine at any particular time.

Figure 1 shows the effect of various inducers and inhibitors on the rate of apomorphine disappearance in mice. Phenobarbital and testosterone did not show any induction ($p > 0.1$), but apomorphine significantly induced ($p < 0.05$) its own metabolism (Curve D). Of the inhibitors, actinomycin D and SKF 525-A did not show any reduction ($p > 0.05$) in the disappearance rate, whereas polyestradiol produced a significant inhibition ($p < 0.02$) of the kinetics (Curve G). Inhibition of apomorphine glucuronidation by polyestradiol is in line with the finding of Inscoe and Axelrod (9) that estradiol inhibits the glucuronidation of *o*-aminophenol in rats. In the case of SKF 525-A, however, no inhibition of glucuronidation was observed in the present study. This is in contrast to the finding of Ikeda *et al.* (10) that *o*-aminophenol glucuronidation in rats and guinea pigs was inhibited by this compound.

Table I includes the first-order kinetic constants of apomorphine disappearance after pretreatment of animals with the various drugs and the amounts of apomorphine glucuronides formed in the body at 8 min. (normal $t_{1/2}$) after intravenous injection of apomorphine hydrochloride. Table II gives the effect of various agents on pentobarbital sleeping time in mice, confirming the literature reports that the drugs tested on apomorphine metabolism did have definite effects on the rate of pentobarbital metabolism under the

Table I—Disappearance-Rate Constants of Apomorphine and the Amounts of Glucuronides Formed in Mice Pretreated with Various Drugs

Treatment	Apomorphine Disappearance Constants, $K \times 10^{-2}$ min.$^{-1}$	Appearance of Apomorphine Glucuronides at 8 min. Percent of total ±*SE*	Effect[a]	*n*	*p* Value
Saline	8.66	73.0 ±2.5		9	
Phenobarbital	8.66	70.6 ±3.2	0	10	>0.1
Testosterone	8.31	73.3 ±3.3	0	5	>0.1
Apomorphine	13.86	93.8 ±4.7	+	4	<0.01
Actinomycin D	7.70	63.9 ±3.5	0	5	>0.05
SKF 525-A	8.66	75.6 ±3.3	0	12	>0.1
Polyestradiol	6.49	61.2 ±3.4	−	9	<0.02
Starvation	5.54	47.6 ±9.0	−	3	<0.01

[a] Insignificant effect = 0; induction = +; and inhibition = −.

Table II—Effect of Various Drugs on Pentobarbital Sleeping Time in Mice

Treatment	n/ Sex	Sleeping Time ± SE, min.	Effect[a]	Data Source
Saline	9	67.9 ± 7.8		This study
Actinomycin D	9	202.2 ± 23.9	− ($p < 0.01$)	This study
Apomorphine	11	78.9 ± 3.0	0 ($p > 0.05$)	This study
SKF 525-A	10	>400	− ($p < 0.001$)	This study
Phenobarbital			+	(12)
Testosterone	♀		−	(8)
	♂		+	(8)
Estradiol[b]	♂		—	(13)

[a] Effect on the metabolism of pentobarbital; insignificant effect = 0; induction = +; and inhibition = −. [b] Effect on hexobarbital metabolism.

present experimental conditions.

Studies on actinomycin D generally have supported that it only blocks the enzyme induction initiated by inducers such as phenobarbital (3). In the present experiments, however, this compound showed a significant prolongation of the pentobarbital sleeping time, suggesting a direct inhibitory effect on the metabolism of pentobarbital. However, on apomorphine metabolism, actinomycin D showed no significant effect (Fig. 1).

In the experiments involving restricted oral feeding, the actinomycin D group showed nearly the same degree of inhibition of apomorphine metabolism as did the control group (Fig. 2), suggesting that the inhibition ($p < 0.01$) was a result of partial starvation due to restricted feeding. Dixon *et al.* (11) also showed that weight loss associated with starvation generally causes a decrease in the animal's ability to metabolize drugs.

Actinomycin D appears to bind with deoxyribonucleic acid (DNA) and thus blocks the DNA-dependent synthesis of nuclear ribonucleic acid (RNA) which is required for protein synthesis (14). This indirect inhibition of protein synthesis was postulated to be the mechanism by which actinomycin D inhibits or blocks the enzyme induction produced by phenobarbital (3). However, enhancement of protein synthesis or its inhibition need not necessarily reflect an induction or inhibition of a particular enzyme. For example, Wada *et al.* (15) showed that phenobarbital may increase the cytochromes P-450 and b_5, and also *N*-demethylase, but it had no effect on the delta-aminolaevulinic acid (ALA) synthetase. Likewise, another compound, 3,5-dicarbethoxy-1,4-dehydrocollidine (DDC), decreased the cytochromes P-450 and b_5 whereas it enormously induced the ALA synthetase.

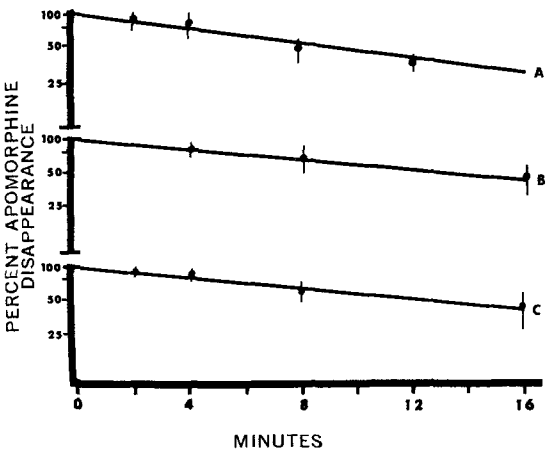

Figure 2—*Rate profiles of apomorphine disappearance with time in intact mice control-fed orally, showing the effect of partial starvation on apomorphine metabolism. Each point represents the average of 5 to 10 animals* ±SD. *Key: A, control,* ad lib. *feed,* $t_{1/2}$ *8 min.; B, control-fed, saline treated,* $t_{1/2}$ *12.5 min.; and C, control-fed, actinomycin D treated,* $t_{1/2}$ *12 min.*

Species such as horse, rabbit, and rat metabolize apomorphine into a mixture of two *O*-monoglucuronides (5, 16). The present studies show that the mouse also excretes apomorphine predominately as a mixture of 3,*O*- and 4,*O*-glucuronides. The uridine 5′-diphosphate (UDP)-glucuronyl transferase, which catalyzes the glucuronidation of most foreign and endogenous compounds, was partially purified by Isselbacher *et al.* (17). In fact, there is some evidence that more than one glucuronyl transferase may be present in the body (17, 18), but their localization in respect to the smooth and rough surfaced endoplasmic reticulum has yet to be demonstrated. Phenobarbital causes proliferation of the smooth endoplasmic reticulum but has little effect on the tough surfaced component of the membrane (19). It was suggested that the glucuronyl transferase for the synthesis of ester and ethereal glucuronides may be localized in this rough component (17) and, subsequently, it was shown to be so, at least in the case of *o*-nitrophenol (20). The apomorphine case apparently would seem to substantiate this belief, because phenobarbital did not have any effect on its glucuronidation. However, induction of ethereal glucuronidation by phenobarbital reported in the rat (21) and man (22, 23) raises a question of whether or not there is any species variation in regard to this suggested glucuronyl transferase distribution in the microsomal surfaces.

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