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ENHANCED GLUCURONIDE FORMATION IN DIFFERENT TISSUES FOLLOWING DRUG ADMINISTRATION

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Abstract—A study was made of the *o*-aminophenol glucuronide synthesis in the slices of the liver, kidney and gastrointestinal tract after the administration of 3,4-benzpyrene, cinchophen, 3-methylcholanthrene, phenobarbital and salicylic acid to elucidate whether the responses of the different tissues are similar to these drugs known to enhance drug metabolism.

A 1·6-fold increase of *o*-aminophenol glucuronide synthesis was found in liver slices 5 days after a single intraperitoneal injection of 3-methylcholanthrene whereas there was no increase in the slices from the kidney or from the various parts of the gastrointestinal tract. The i.p. administration of 3,4-benzpyrene had a similar effect. The intragastric administration of cinchophen for 3 days resulted in a two-fold increase of the synthesis in the slices from the liver, glandular stomach, caecum and colon, and a 3-fold increase was found in the kidney. In the duodenum only a slight increase of activity was observed when the analysis was carried out 24 hr after the last dose, but a 1·6-fold increase was found after 48 hr. The i.p. administration of phenobarbital for 3 days resulted in a 1·4-fold increase of the synthesis in the liver, but no change of activity was found in the kidney or in the various parts of the gastrointestinal tract. The intragastric administration of salicylic acid for 3 days resulted in a 1·9-fold increase in the kidney but only insignificant changes were found in the various parts of the gastrointestinal tract or in the liver when analysed 24 hr after the last dose. The enhancement of the glucuronide biosynthesis caused by the administration of the various drugs thus reveals marked organ specificity.

PREVIOUS studies indicate that the UDP glucuronyltransferase activity of the liver increases after the administration of carcinogens,[1] antipyretics,[2] hypnotics[3] and antimalarial drugs.[4] In addition to the liver, the kidneys, and especially the mucous membrane of the gastrointestinal tract are active in the biosynthesis of glucuronic acid conjugates.[5] Induction of UDPglucose dehydrogenase and UDP glucuronyltransferase in the gastrointestinal mucous membrane by salicylamide has previously been observed by us.[6] In the present study a comparison of the ability of various drugs, namely 3,4-benzpyrene, 3-methylcholanthrene, cinchophen, phenobarbital and salicylic acid, to enhance *o*-aminophenol glucuronide formation in the main organs of detoxication, the liver, the kidney and the gastrointestinal tract has been carried out in order to elucidate, whether the response is similar in these tissues.

METHODS

Adult male Wistar rats (140–200 g) fed *ad libitum* were used. 3,4-Benzpyrene (0·8 m-mole/kg body wt., five rats) and 3-methylcholanthrene (0·37 m-mole/kg body wt.; eleven rats) were injected i.p. as suspensions in 0·5 ml of corn oil in a single

dose. The control animals were given corn oil only. Analysis was carried out 5 days after drug injection. Cinchophen was administered intragastrically for three days in doses of 4·75 m-mole/kg body wt. Analysis was carried out either 24 (nine rats) or 48 hr (eight rats) after the last injection. Phenobarbital was injected i.p. for 3 days (0·45 m-mole/kg body wt.; seven rats) and the tissues were analysed 24 hr after the last dose. Salicylic acid was administered intragastrically at a dosage level of 5m- mole/kg for 3 days (fourteen rats) and the analysis was carried out 24 hr after the last dose.

The animals were killed by a blow on the head and bled by cutting the renal vessels. The liver and the left kidney were transferred into ice cold Ringer solution. The gastrointestinal specimens were first opened and the contents removed. Slices were prepared by punching discs, 2 mm in dia., from the border of the liver lobes, from the exmedullated kidney-halves, and from the whole wall specimens of the gastrointestinal tract. The rate of *o*-aminophenol glucuronide synthesis was determined in Warburg flasks in a 95% oxygen–5% carbon dioxide atmosphere at 37° with constant shaking as described earlier.[7]

RESULTS

The administration of 3-methylcholanthrene caused a 1·6-fold increase in *o*-aminophenol glucuronide synthesis in liver slices, but no increase was observed in the whole wall slices of the glandular stomach, duodenum, caecum and colon or in the slices from the kidney, when analysed 5 days after a single drug injection. Similar results were obtained with another carcinogen, 3,4-benzpyrene (Table 1).

TABLE 1. THE EFFECT OF 3,4-BENZPYRENE, 3-METHYLCHOLANTHRENE, PHENOBARBITAL AND SALICYLIC ACID ON THE SYNTHESIS OF *o*-AMINOPHENOL GLUCURONIDE BY SLICES FROM THE DIFFERENT TISSUES OF THE RAT. THE SYNTHESIS OF *o*-AMINOPHENOL GLUCURONIDE IS EXPRESSED IN p-moles/min · mg DRY WT. THE SIGNIFICANCE OF THE INFLUENCE OF THE DRUG IS SHOWN WHEN $P < 0{\cdot}01$, THE NUMBER OF ANIMALS IN PARENTHESES

	o-Aminophenol glucuronide synthesized p-moles/min · mg dry wt.					
	Kidney	Liver	Glandular stomach	Duodenum	Caecum	Colon
Control	14·0 ± 1·0 (27)	45·4 ± 2·7 (23)	9·6 ± 0·5 (21)	40·0 ± 2·5 (22)	12·0 ± 1·4 (16)	17·5 ± 1·4 (22)
3,4-Benzpyrene	15·3 ± 1·4 (5)	73·6 ± 8·2 $P < 0{\cdot}005$ (5)		39·0 ± 5·7 (5)		21·6 ± 1·6 (5)
3-Methylcholanthrene	14·5 ± 1·1 (11)	73·8 ± 4·9 $P < 0{\cdot}001$ (10)	11·8 ± 1·3 (7)	42·1 ± 5·5 (9)	16·1 ± 2·2 (6)	17·0 ± 2·5 (10)
Phenobarbital	13·4 ± 2·2 (6)	63·2 ± 5·8 $P < 0{\cdot}001$ (7)	10·9 ± 2·2 (5)	41·0 ± 5·4 (5)	11·2 ± 1·9 (5)	22·4 ± 2·7 (6)
Salicylic acid	25·7 ± 1·4 $P < 0{\cdot}001$ (10)	54·4 ± 7·4 (10)	13·1 ± 1·4 (10)	33·7 ± 2·7 (12)	18·9 ± 1·1 (9)	23·5 ± 2·2 (9)

The administration of phenobarbital for 3 days caused a 1·4-fold increase of glucuronide synthesis in the liver, but no effect was observed in the kidney and in the gastrointestinal tract (Table 1).

Salicylic acid, when administered intragastrically for three days, enhanced the synthesis 1·9-fold in the kidney, but no significant changes were observed in the gastrointestinal tract or in the liver (Table 1).

Another ulcerogenic drug, cinchophen caused a three-fold increase of *o*-aminophenol glucuronide synthesis in the kidney, 2-fold in the liver, glandular stomach, caecum and colon, when administered for 3 days and analysed 24 hr after the last dose. Only an insignificant increase was found in the duodenum. To elucidate more the discrepancy found in the duodenum when compared with the other tissues, a further group of animals was studied 48 hr after the last dose. In these rats the biosynthetic activity had attained a 1·6-fold level (Table 2).

TABLE 2. THE EFFECT OF CINCHOPHEN ON THE SYNTHESIS OF *o*-AMINOPHENOL GLUCURONIDE BY SLICES FROM THE DIFFERENT TISSUES OF THE RAT. THE SYNTHESIS OF *o*-AMINOPHENOL GLUCURONIDE IS EXPRESSED IN p-moles/min · mg DRY WT. THE SIGNIFICANCE OF THE INFLUENCE OF THE DRUG IS SHOWN WHEN $P < 0.05$, THE NUMBER OF ANIMALS IN PARENTHESES

	o-Aminophenol glucuronide synthesized p-moles/min · mg dry wt.					
	Kidney	Liver	Glandular stomach	Duodenum	Caecum	Colon
Control	10·2 ± 0·8 (10)	42·7 ± 1·9 (13)	9·6 ± 0·5 (11)	54·0 ± 4·3 (10)	11·5 ± 1·4 (9)	14·2 ± 1·4 (12)
Cinchophen 3 days analysed 24 hr after the last dose	32·2 ± 3·8 $P < 0.001$ (9)	114 ± 7·1 $P < 0.001$ (8)	20·8 ± 1·9 $P < 0.001$ (8)	63·4 ± 8·2 (7)	23·3 ± 3·3 $P < 0.01$ (8)	23·3 ± 3·8 $P < 0.05$ (8)
Cinchophen 3 days analysed 48 hr after the last dose	46·7 ± 3·0 $P < 0.001$ (8)	145 ± 6·6 $P < 0.001$ (7)	19·7 ± 2·7 $P < 0.001$ (8)	86·0 ± 10·2 $P < 0.01$ (8)	22·4 ± 1·9 $P < 0.001$ (7)	33·7 ± 3·3 $P < 0.001$ (7)

TABLE 3. THE EFFECT OF CINCHOPHEN (1·0 mM), SALICYLIC ACID (1·0 mM) AND PHENOBARBITAL (0·1 mM) ADDED *in vitro* TO NORMAL LIVER SLICES ON THE BIOSYNTHESIS OF *o*-AMINOPHENOL GLUCURONIDE BY LIVER SLICES. THE SYNTHESIS IS GIVEN IN p-moles/min · mg DRY WT.; THE S.E.M'S ARE INDICATED. THE NUMBER OF THE EXPERIMENTS IN PARENTHESES

	Control	Phenobarbital 0·1 mM	Salicylic acid 1·0 mM	Cinchophen 1·0 mM
o-Aminophenol glucuronide synthesized in p-moles/min · mg dry wt.	36·6 ± 1·8 (9)	40·2 ± 2·4 (10)	18·6 ± 1·8 (8)	19·2 ± 1·5 (9)

The addition of the above drugs to the reaction mixtures containing liver slices from the control rats had no stimulating effect on *o*-aminophenol glucuronide synthesis. In fact, cinchophen and salicylic acid caused a marked depression (Table 3).

DISCUSSION

The administration of 3,4-benzpyrene causes an induction of UDP glucuronyltransferase in the liver,[1] probably also in the skin,[8] but its effect on the enzyme activity

in the other tissues has not been clarified. The results presented here indicate, that the drug is active neither in the kidney nor in the gastrointestinal tract. On the other hand, 3,4-benzpyrene administration has been reported to enhance benzpyrene hydroxylation in the gastrointestinal mucous membrane.[9] The effect of 3-methylcholanthrene is similar to that of 3,4-benzpyrene. The time of analysis was chosen on the basis of urinary L-ascorbic acid excretion, which in the preliminary experiments had its maximum after 5 days.

The stimulatory effect of phenobarbital and salicylic acid on the *o*-aminophenol glucuronide formation was tissue specific, too. Phenobarbital caused an increase in glucuronide synthesis in the liver only. Three phenobarbital doses increased the weight of liver by 30 per cent in our rats. The drug is one of the most used model compounds in studies of the induced drug metabolism, but its inducing activity in other tissues except the liver has not received much interest. The present results support our other data, which indicate a high liver specificity. There is a slight increase in UDPglucose dehydrogenase and UDP glucuronyltransferase activity and a 4-fold increase in glucuronolactone dehydrogenase activity in the liver in contrast to the kidney and the mucous membrane of the small intestine after phenobarbital treatment in rats.[10]

Salicylic acid enhanced the synthesis in the kidney, but not in the other tissues studied. Salicylic acid administration has been found to increase urinary L-ascorbic acid excretion,[6] but its activity on enzymes participating in the glucuronide biosynthesis has not been clarified. Salicylamide administration increases the activity of UDPglucose dehydrogenase and UDP glucuronyltransferase.[6]

In contrast to the other drugs cinchophen enhanced *o*-aminophenol glucuronide synthesis in all the organs studied. The fact that the increase was lower in the duodenum than elsewhere 24 hr after the last cinchophen dose might be explained by the inhibitory action of the drug itself demonstrated *in vitro*, since its concentration in the mucosa most probably is rather high due to peroral administration. Cinchophen also blocks the protein synthesis in cell free preparations,[11] from which follows a possible inhibition of enzyme biosynthesis. The inhibitory action appears to give way, since 48 hr after the last dose the rate of glucuronide synthesis was 1·6-fold over the control level. Cinchophen also increases the activity of some enzymes in the glucuronic acid pathway, which explains the increased synthesis of *o*-aminophenol glucuronide by the tissue slices. Thus, a 2-fold increase of UDPglucose dehydrogenase and UDP glucuronyltransferase takes place in the liver and kidney, but in the duodenal mucous membrane UDP glucuronyltransferase activity remains almost at the control level, when analysed 24 hr after the last dose. The enzyme activities have reached their maximal levels in 3 or 4 days.[10]

The concentration of the drugs or their active metabolites probably varies in tissues and this could be the reason for the different responses found in the liver, kidney and gastrointestinal tract, although the doses of the drugs were very high. Two animals in the salicylic acid treated group died after the third dose.

The mechanism of the enhanced glucuronide formation by the drugs remains obscure, but they act in a tissue specific way, except cinchophen, the specificity of which is very low. These drugs had no enhancing effect when added *in vitro* to reaction mixtures. The inhibitory action of cinchophen and salicylic acid might be explained by their effects on energy metabolism and oxidative phosphorylation.[12–13]

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