Biochemical Pharmacology, Vol. 37, No. 4, pp. 583–589, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

THE EFFECT OF METHIMAZOLE ON BILE FLOW AND BILE ACID SECRETION IN THE RAT

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(*Received* 23 *March* 1987; *accepted* 31 *July* 1987)

Abstract—The effect of methimazole on bile flow and composition was studied in male Wistar rats. Administration of methimazole as a single bolus (175–700 μmol/kg body wt) or infusion over three hours (1 μmol/100 g body wt/min) significantly increased bile flow and the biliary output of bile acids and inorganic electrolytes. Choleresis was a transient phenomenon and at the end of the experiments bile flow had returned to control values, although bile acid output was significantly lowered. When the amounts of bile acids arriving into the liver were increased by taurocholate infusion or decreased by cholestyramine pretreatment, a transitory enhancement was also found in bile flow and bile acid output, but the amounts of bile acid secreted and the choleretic effect induced were different according to the bile acid secretory rate found before methimazole administration. The bile acid content in the liver of taurocholate-infused rats was reduced during methimazole-induced choleresis. Our data indicate that the higher bile flow in methimazole-treated rats is related to an enhanced biliary secretion of bile acids probably due to a transitory stimulating effect on the transport into bile of the intrahepatocytary bile acid pool. The choleretic effect is not apparently shared by other thiocarbamide compounds.

Methimazole (1-methyl-2-mercaptoimidazole), a drug commonly used in the treatment of thyrotoxicosis, inhibits thyroid peroxidase [1, 2]. Although it is the most potent antithyroid agent in man little was known about its metabolic pathways until recently. The drug is partially metabolized in the liver by a flavin-containing monooxygenase and cytochrome P-450 with the formation of *N*-methilimidazole, 3-methyl-2-thiohydantoine and a number of metabolites [1, 3]. Following administration of methimazole most of it is recovered in urine but about 10% of the dose appears in bile [4]. The major metabolites in the bile are glucuronide and sulfate conjugates and only a small percentage corresponds to the parent compound [4, 5].

Although the development of cholestatic hepatitis following methimazole treatment at high doses has occasionally been reported [6], no information exists concerning the effects of the hepatic metabolism and biliary excretion of methimazole on the mechanisms of bile formation. The present study was undertaken to investigate the flow and composition of bile following i.v. administration of methimazole to rats.

MATERIALS AND METHODS

Animals and experimental procedures. Male Wistar rats weighing 230–260 g were housed in a room maintained at 22° with a 12 hr dark/light cycle. The animals had free access to food (standard diet from Panlab, Barcelona, Spain) and water and were not starved before experiments.

Under pentobarbital anaesthesia (Nembutal, Abbott Laboratories, Madrid, Spain; 50 mg/kg body wt, i.p.) a median laparotomy was performed and the common bile duct cannulated with polyethylene tubing. The left jugular vein and left carotid artery were catheterized. Rectal temperature was maintained at 37° by a thermostatically-controlled warming plate.

After collecting two baseline 15 min samples of bile, methimazole was injected at 175350 and 700 μmol/kg body wt (2 ml/kg body wt) or infused at 1.0 μmol/100 g body wt/min (3 ml/hr) for three hours through the jugular vein. Bile was collected over 180 min at 15 min intervals following administration of the drug. At 60 min intervals a 200 μl blood sample was obtained from the carotid artery. To further clarify the mechanisms of action of methimazole, rats were studied under two additional experimental situations, with taurocholate infusion or after cholestyramine pretreatment. Taurocholate was infused at 0.5 μmol/100 g body wt/min from the end of the basal period and 60 min later methimazole was injected at 700 μmol/kg body wt; a group of animals were sacrificed 30 min after methimazole administration to measure bile acid content in the liver. Cholestyramine was given through a stomach tube at 1.5 g/kg body wt (10 ml/kg body wt) 4 hr before the beginning of bile collection and methimazole was injected at 700 μmol/kg body wt following the baseline period. Controls receiving no injection, infused with taurocholate, or receiving cholestyramine were used in each of the three groups of experiments.

The effect of thiourea was tested in additional groups of rats receiving i.v. injections of the drug at doses from 0.175 to 3.5 mmol/kg body wt.

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Chemicals and preparation of solutions. Methimazole, sodium taurocholate, thiourea and cholestyramine were purchased from Sigma Chemical Co. (St Louis, MO). Methimazole and thiourea were dissolved in NaCl solutions of the suitable concentration to give a 300 mosm/kg body wt osmolality and pH was adjusted to 7.4 with NaOH 0.1 M. Sodium taurocholate was dissolved in 0.15 M NaCl with 3% (w/v) albumin and the pH adjusted to 7.4 with NaOH 0.1 M. Cholestyramine was dried under reduced pressure before use and suspended in 0.25% (w/v) methylcellulose solution.

Analytical procedures. Bile flow was determined gravimetrically assuming a bile density of 1.0 g/ml. Bile acid concentration in liver homogenates (1.9 w/v in 0.15 M NaCl) and bile was determined enzymatically with 3-α-hydroxysteroid dehydrogenase [7]. The concentration of sodium and potassium in bile was measured with a flame photometer (Nak II, Meteor, Madrid, Spain) and chloride concentration with a chloridometer (model 160, Analytical Control, Italy). The pH, pO_2 and pCO_2 in blood and bicarbonate concentration in blood and bile were measured in an automated gas analytical system (model 168, Corning Medical, Medfield, U.S.A.). Bile osmolality was determined with a vapour pressure osmometer (model 5100, Wescor, Logan, U.S.A.). Statistical analysis of the data was performed with the non-parametric Mann–Whitney U-test and linear regression analysis. Results are expressed as means ± SEM.

RESULTS

Bile flow was significantly increased following methimazole injection. Values at 30 min were, respectively, 30, 39 and 66% higher than those of the controls for the doses of 175, 350 or 700 μmol/kg body wt respectively (Fig. 1). A significant enhancement of bile acid output was caused by administration of the drug, with a peak increase of 33, 53 or 97% for each of the three doses at 45 min after methimazole injection (Fig. 1). At 180 min after administration of the drug bile acid output was significantly reduced with respect to the controls in all treated rats (Fig. 1). Values joined up again with the controls at 285 min in one group of rats receiving methimazole at 175 μmol/kg body wt (69 ± 3 nmol/100 g body wt/min vs 72 ± 8 nmol/100 g body wt/min in controls; N = 4).

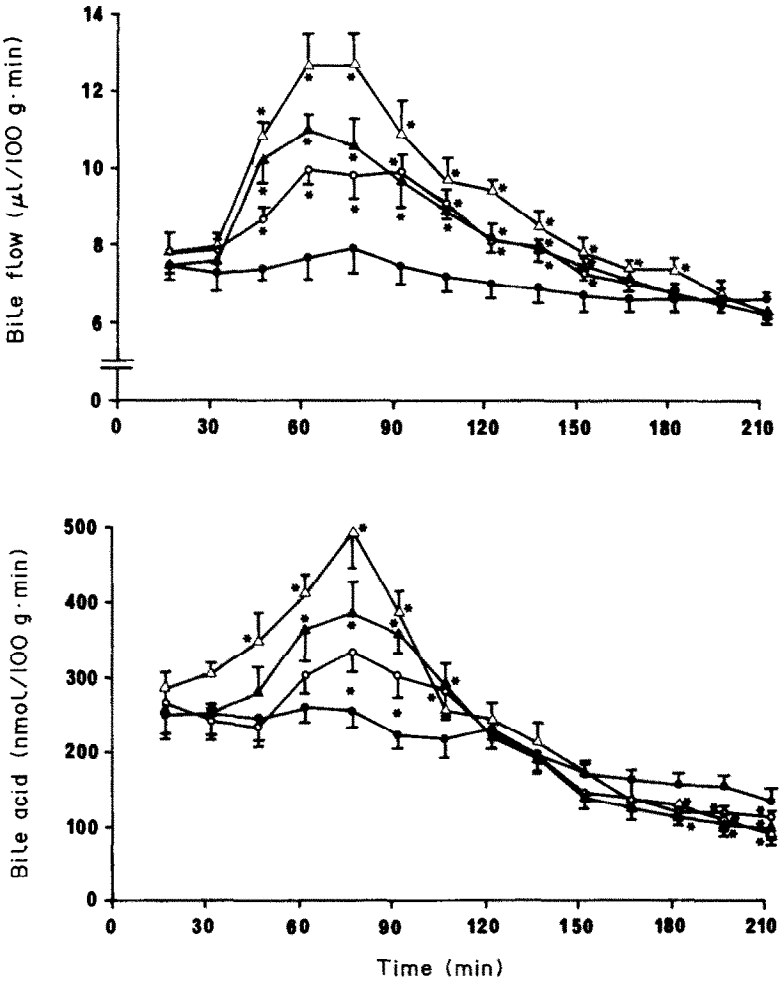

Fig. 1. Effect of i.v. methimazole injection on bile flow and bile acid output. Control (●), methimazole injection 175 μmol/kg (○), 350 μmol/kg (▲), 700 μmol/kg (△). Methimazole was injected at min 30. Mean values ± SEM from 4 to 6 rats. * $P < 0.05$ significantly different from the controls.

Table 1. Effect of methimazole injection on electrolyte composition of bile

	Control	175 μmol/kg	350 μmol/kg	700 μmol/kg
Bile acid	32.8 ± 4.7	31.9 ± 3.0	35.1 ± 4.0	35.4 ± 3.9
Sodium	151 ± 3	143 ± 3*	144 ± 3	146 ± 2
Potassium	4.5 ± 0.1	4.1 ± 0.2	4.2 ± 0.1	4.2 ± 0.2
Chloride	100 ± 3	93 ± 2	88 ± 2*	86 ± 2*
Bicarbonate	29 ± 1	25 ± 1*	25 ± 2*	24 ± 2*

Values (mEq/l) are means ± SEM from 4 to 6 rats and correspond to min 45–60 of experiments (15–30 min following methimazole injection). * $P < 0.05$ significantly different from the controls.

The output of inorganic electrolytes followed a course parallel to that of bile acids (data not shown). The composition of bile during methimazole-induced choleresis is indicated in Table 1, showing a slight but significant decrease in the bile concentration of inorganic anions. No significant differences could be documented in the plasma and bile osmolalities or in blood pH, pO_2 or pCO_2 between the control and treated rats (data not shown).

Infusion of methimazole at 1 μmol/100 g body wt/min also increased bile flow with values 37% higher than the controls at 45 min of infusion and a gradual decrease thereafter (Fig. 2). The biliary output of bile acids was significantly increased by 50% (+135 nmol/100 g body wt/min) during choleresis and lowered during the last 30 min of the experiments (Fig. 2). The output and concentrations of inorganic electrolytes were again similar to those found fol-

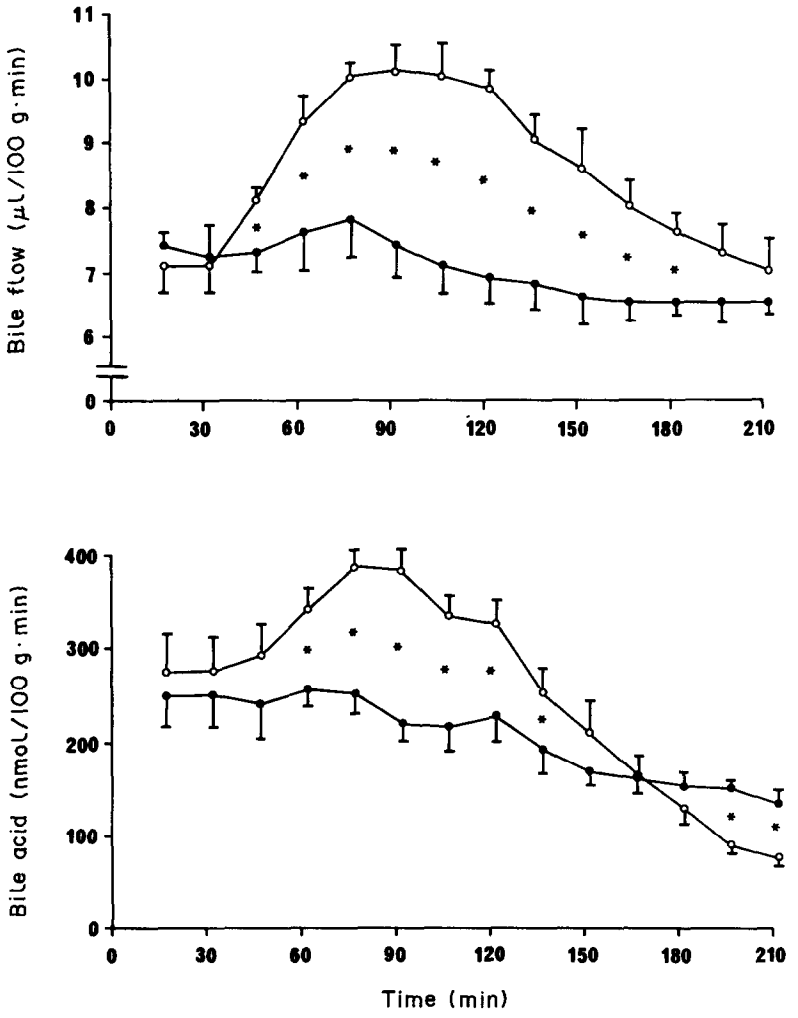

Fig. 2. Effect of i.v. methimazole infusion on bile flow and bile acid output. Control (●), methimazole infusion at 1.0 μmol/100 g/min. (○) Methimazole was infused from 30 to 210 min. Mean values ± SEM from 4 to 6 rats. * $P < 0.05$ significantly different from the controls.

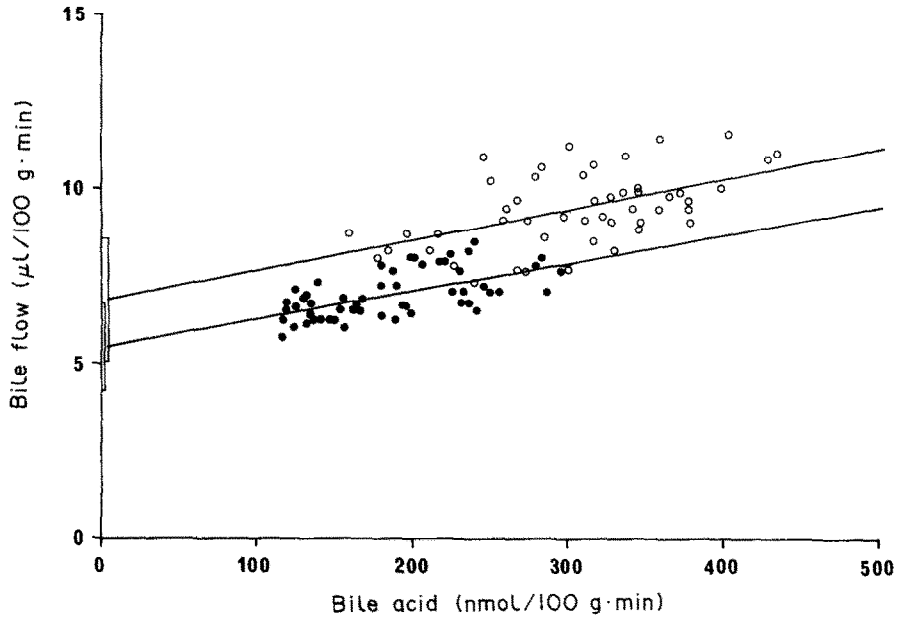

Fig. 3. Relationship between bile flow and bile acid output in control and methimazole-infused rats. Regression lines with 95% confidence limits are represented. Control (●) $y = 0.008x + 5.33$; $r = 0.4838$; $P < 0.001$. Methimazole infusion at 1.0 μmol/100 g/min (○) $y = 0.009x + 6.61$; $r = 0.5200$; $P < 0.001$.

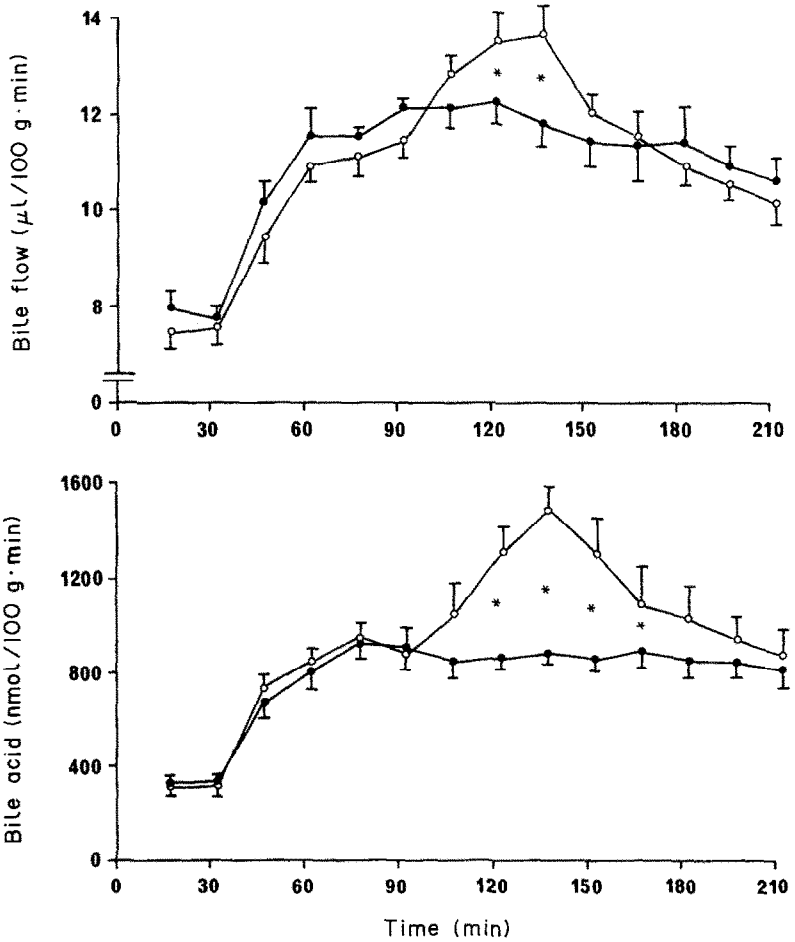

Fig. 4. Effect of i.v. methimazole injection on bile flow and bile acid output in taurocholate-infused rats. Taurocholate infusion at 0.5 μmol/100 g/min (●), taurocholate infusion plus methimazole injection at 700 μmol/kg (○). Taurocholate was infused from 30 to 210 min and methimazole was injected at min 90. Mean values ± SEM from 4 to 6 rats. * $P < 0.05$ significantly different from the controls.

lowing methimazole injection (data not shown). Figure 3 plots the bile flow from controls and methimazole-infused rats as a function of bile acid output. No significant effect was caused by administration of the drug on the choleretic capacity of bile acids. A slight but non-significant increase was found in the bile acid independent fraction of bile flow. Similar relationships between bile flow and bile acid output were obtained in the groups of rats injected with the drug.

When taurocholate was infused bile acid output started to increase, reaching steady state within the first 30 min and this was accompanied by a marked choleresis (Fig. 4). Injection of methimazole caused a small additional increase in bile flow with a bile acid output 71% higher (+600 nmol/100 g body wt/min) than that of the animals receiving only taurocholate (Fig. 4). The bile acid concentration in the liver of the methimazole-treated rats was lowered with respect to the controls (17.8 ± 2.8 μmol/g liver vs 22.2 ± 0.6 μmol/g liver; N = 4) 90 min after beginning of taurocholate infusion.

Cholestyramine-pretreated rats showed bile flow values 18% lower and bile acid outputs 70% lower than those of normal rats. Additional injection of methimazole caused a significant increase in bile flow accompanied by a 23% enhancement (+15 nmol/100 g body wt/min) in bile acid output (Fig. 5). As occurred in the rats that were not treated with cholestyramine, bile acid output was significantly lowered at the end of the assays (Fig. 5).

Intravenous injection of thiourea at different doses ranging from 0.175 to 3.5 mmol/kg body wt did not significantly modify bile flow as compared to the control animals (data not shown).

DISCUSSION

In the present study methimazole induced a pronounced and promptly reversible choleresis in Wistar rats. These experiments indicate that the drug increases both bile acid output and that of inorganic electrolytes into bile.

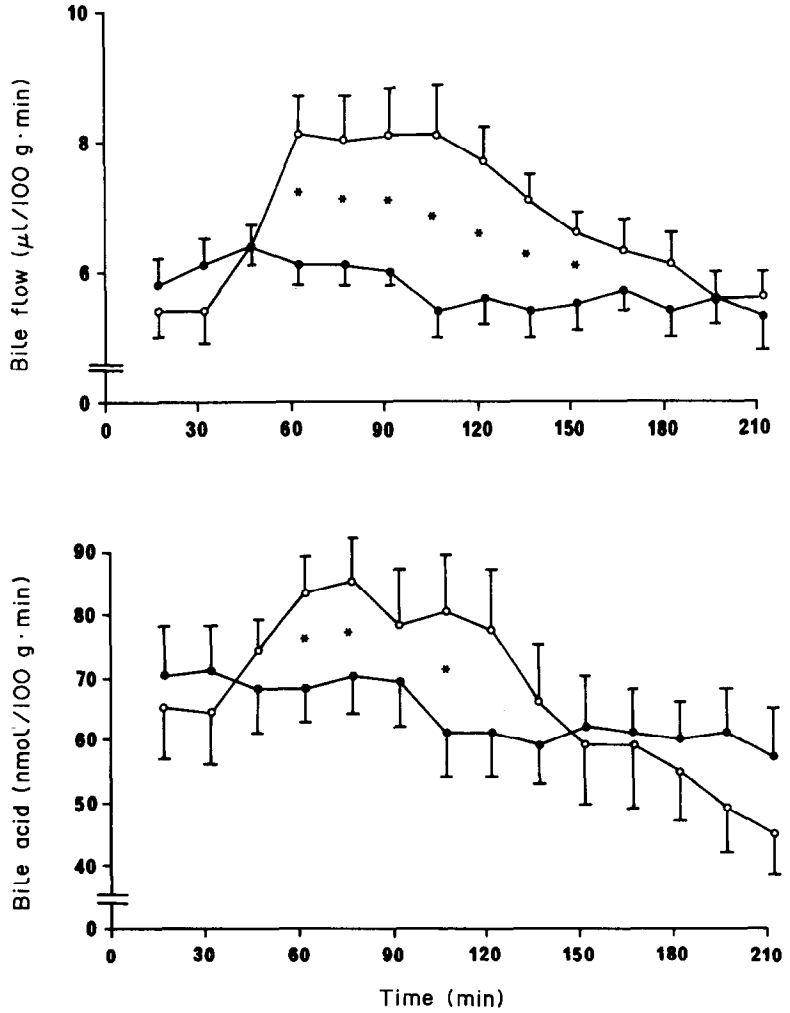

Fig. 5. Effect of i.v. methimazole injection on bile flow and bile acid output in cholestyramine pretreated rats. Cholestyramine administration at 1.5 g/kg (●), cholestyramine administration plus methimazole injection at 700 μmol/kg (○). Cholestyramine was given intragastrically 4 hr before beginning the collection of bile and methimazole was injected at min 30. Mean values ± SEM from 4 to 6 rats. * $P < 0.05$ significantly different from the controls.

The value of the bile acid independent fraction of secretion obtained by extrapolation to the *Y*-axis of the regression line relating bile flow and bile acid output was slightly and non-significantly modified by methimazole administration. The validity of the extrapolation method to calculate the independent fraction has been questioned because of the changes in slope and elevation of the regression line that occur at different bile acid secretion rates [8, 9]. Nevertheless bile acid-independent flow is a real entity and the possibility of its enhancement induced by biliary secretion of the drug cannot be ruled out. In fact, both the parent compound and its glucuronide and sulfate-conjugated metabolites are excreted into bile following administration of methimazole in rats [4, 5] and could induce an osmotic effect similar to that found for other xenobiotics [10]. However, the transient effect on bile flow even during methimazole infusion, as well as the non-significantly different choleretic capacity of the secreted bile acids (8 μl/μmol and 9 μl/μmol in the control and methimazole-treated rats respectively) are events that support the hypothesis that methimazole-induced choleresis is not related to the biliary secretion of itself and its metabolites but mainly to an increased bile acid-dependent flow.

The increase in bile acid secretion induced by substances other than natural or synthetic bile acids is an uncommon phenomenon. Although some years ago it was suggested that both insulin and glucagon increase bile acid output in dogs [11, 12], later works have not been able to demonstrate such an effect [13].

The exact mechanism responsible for the effect of methimazole on bile acid secretion is difficult to elucidate. Following phenobarbital administration at doses large enough to induce enzymes of the endoplasmatic reticulum, increased bile acid outputs have been found in rhesus monkeys [14] and humans [15], although the effect in rats apparently varies from one laboratory to another [16, 17]. The possibility of a similar stimulating effect of methimazole on bile acid synthesis cannot be ruled out, though it seems remote. A direct stimulation of cholesterol 7α-hydroxylase, an enzyme catalyzing the limiting step in the synthesis of bile acids, is not supported by our results because we found the lowest increase in bile acid output induced by methimazole when the enzyme was presumably activated by pretreatment with the bile acid sequestrant cholestyramine. Increases in bile acid synthesis [18] and cholesterol 7α-hydroxylase activity [19, 20] have been demonstrated in livers of rats with their enterohepatic circulation of bile acids interrupted by bile diversion or cholestyramine treatment. A stimulating effect of methimazole is even less probable if one considers that the rise in the enzyme activity induced by interruption of the enterohepatic circulation of bile acids is delayed by at least 24 hr [21].

The difference in the electrical potential between the hepatocyte and the canalicular lumen is now considered to be a driving force for the secretion of bile acids and other anions across the canalicular membrane [22]. If cationic metabolites of methimazole were present in bile, this could account for the increase in bile acid secretion. Nevertheless, stimulating electric effects on anion transport are not observed in the case of bicarbonate and chloride in our experiments. On the contrary, the biliary concentrations of these two anions are decreased in animals given methimazole.

The transient character of choleresis, even after infusion of the drug, together with the lowered output of bile acids at the end of the assays, suggest the possibility of stimulated canalicular exit of the intracellular pool of bile acids rather than a stimulated uptake. This is also favoured by the fact that bile acid secretion in methimazole-treated rats join up again with the controls when experiments are carried out for longer periods of time and by the lowered bile acid content of the liver in parallel to the enhancement of bile acid output in taurocholate-infused rats. Moreover, the increase induced in bile acid output is related to its hepatic availability prior to the administration of methimazole, being higher in the taurocholate-infused animals than in the control or cholestyramine-treated rats.

Little is known about the molecular processes involved in the intracellular transport and canalicular secretion of bile acids. In the hepatocyte cytoplasm bile acids are bound to proteins [23]. It has been found that the rat liver is unable to excrete a load of taurocholate into bile after colchicine pretreatment [24] and additional studies with labelled cholylglycinetyrosine and autoradiography have shown an association of the label with intracellular vesicles [25]. One model postulated for bile acid transport includes binding to proteins, translocation to intracellular vesicles, possibly derived from the endoplasmatic reticulum, and canalicular extrusion of the vesicular content by membrane fusion [26]. Some of the above steps could be modified by methimazole administration.

One point of pharmacological interest is the absence of choleresis found in the animals receiving thiourea. Thus, the effect induced by methimazole on bile flow and bile acid secretion could be assumed to be specific for the compound and not shared by other thiocarbamides.

In summary, the present experiments show that in the rat the administration of methimazole enhances bile acid secretion, most probably by stimulating the transport into bile of the intracellular pool of bile acids. The exact mechanism of this remains to be elucidated.

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