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EFFECTS OF A SALT OF CHOLESTYRAMINE AND 2-[4-(*p*-CHLOROBENZOYL)PHENOXY]2-METHYL PROPIONIC ACID (α-1081) ON BILIARY LIPID SECRETION IN RATS

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1 Hypolipidaemic agents may increase biliary cholesterol in man, inducing a supersaturated bile.

2 To evaluate this possible side-effect, we have studied bile lipid secretion over a period of 8 h with intact enterohepatic circulation and 4 h with complete interruption in rats treated for two months with a salt of cholestyramine and 2-[4-(*p*-chlorobenzoyl)-phenoxy]2-methyl propionic acid (α-1081, 1.150 g/kg body wt., daily), cholestyramine (1.125 g/kg body wt. daily), procetofenic acid (25 mg/kg body wt. daily) and saline respectively (six rats for each group).

3 Cholesterol saturation index significantly ($P < 0.005$) increased (from 0.21 ± 0.01 to 0.39 ± 0.09, mean ± s.d.), in rats fed with procetofenic acid but it did not in α-1081- and cholestyramine-treated animals.

4 Procetofenic acid and, to a lesser extent, cholestyramine increased the bile flow. Procetofenic acid increased cholesterol secretion from 0.45 ± 0.17 to 0.94 ± 0.19 μmol kg^{-1} body wt. h^{-1} (mean ± s.d.).

5 Cholestyramine increased both serum cholesterol and bile acid secretion from 0.45 ± 0.17 to 0.68 ± 0.10 and 25.8 ± 9.48 to 39.96 ± 6.68 μmol kg^{-1} body wt. h^{-1} respectively; α-1081, on the contrary, had no effect on bile lipid secretion.

6 These data suggest that α-1081 may be used as a new hypolipidaemic drug without any risk of increasing cholesterol in bile.

Introduction

In recent years, hypolipidaemic drugs have been widely used to lower the concentration of serum cholesterol and triglycerides (Grundy & Mok, 1977; Rouffy & Chany, 1978; Harvengt, Heller & Desager, 1980).

Cholesterol gallstone formation has been reported as a side-effect of this therapy, probably due to a modification of biliary lipid concentration; in fact, cholesterol leaves the body by excretion either as acidic or neutral faecal steroids, after secretion by the liver into the biliary system. When more cholesterol is delivered from peripheral tissues into bile, the excess concentration of biliary cholesterol, in comparison to that of bile acids, leads to supersaturated cholesterol bile and cholesterol gallstone formation (Admirand & Small, 1963; Hofmann & Small, 1967).

In order to prevent this side-effect, some authors (Sturdevant, Pierce & Dayton, 1973; Cooper, Geizerova & Oliver, 1975; Grundy & Mok, 1977; Angelin, Einarsson & Leijd, 1978; Angelin, Einarsson & Leijd, 1979) proposed the association of hypolipidaemic drugs and bile acid binding agents, which might enhance bile acid faecal excretion, by interrupting their enterohepatic circulation (EHC). The interruption of the EHC of bile acids leads to an increased synthesis of bile acids from cholesterol in the liver and hence to an increased removal rate of cholesterol.

In the present investigation, we describe the effect of a new hypolipidaemic drug, α-1081 (a cholestyramine salt with 2-[4-(*p*-chlorobenzoyl) phenoxy-]2-methyl propionic acid) on biliary lipid secretion in the rat.

Its effect has been compared with the two drugs, procetofenic acid and cholestyramine, separately administered.

Methods

Sprague-Dawley male rats (COBS-CD-(SD)BR)

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(100–110 g body wt.), were placed in an isolated animal room, with automatic light cycling; the light phase lasted from 07 h 00 min to 19 h 00 min; the dark phase from 19 h 00 min to 07 h 00 min.

They were allowed free access to water and Altromin rat chow. The animals were divided into four groups of six rats each, according to the drug administered: procetofenic acid (PA): 25 mg/kg daily (group I); cholestyramine (CH): 1125 mg/kg daily (group II); α-1081 salt: 1150 mg/kg daily (group III) and placebo (saline solution) (group IV).

The drugs were administered orally over a 60 day period.

At the end of the study, biliary lipid secretion was studied as follows: rats were anaesthetized with ethylcarbamate. The bile duct was cannulated with PVC tubing but less than 10% of the total volume of the collected bile was retained; the remainder was continuously reinfused into the coledocus in order to maintain an intact EHC.

During the study, the rats were maintained at 37°C. Bile samples were collected hourly for 8 h and the total bile volume was then measured and 200 μl was retained for analysis.

At the end of the study, the EHC was re-established. Twelve hours later, a complete EHC interruption was obtained by complete bile diversion and bile lipid secretion was studied over a 4 h period. One volume of bile sample was diluted in isopropanol (4 volumes) and then centrifuged at 3500 rev/min. The supernatant was stored in glass vials at −20°C until the bile lipid analysis.

Bile lipid composition

Biliary cholesterol was determined by use of a specific cholesterol oxidase enzyme (Roda, Festi, Sama, Mazzella, Aldini, Roda & Barbara, 1975) and total bile acids were determined by use of a specific 3α-hydroxy steroid dehydrogenase (Fausa & Skalhegg, 1974) and a commercial method (3α stereognost). Phospholipid concentrations were determined by a modification of the method of Takayama, Itoh, Nagasaki & Tanimizu (1977) previously applied to serum.

Calculation of results

The cholesterol saturation index (SI) was calculated from biliary lipid composition using the solubility curves of Hegardt & Dam (1971) by means of a polynomial equation (Thomas & Hofmann, 1973). Biliary lipid secretion, calculated from the volume of the excreted bile and from biliary lipid concentration, was expressed as $\mu mol\,kg^{-1}$ body wt. h^{-1}. The relationship between the three lipids was calculated using a non-linear model (Mazer & Carey, 1980).

In this model, a phospholipid-mediated relationship between cholesterol and bile acid secretions is taken into account, i.e. cholesterol secretion is assumed to be coupled to that of phospholipids in a secretory compartment by a micellar transport mechanism, while phospholipid secretion is coupled to the flux of bile acids through the compartment by a similar mechanism.

The relationships between the three biliary lipids were fitted simultaneously, by means of a least-square procedure (Fletcher & Powell, 1963). The parameters evaluated were: the theoretical maximal outputs of cholesterol (Xol) and phospholipids (PL) related to the bile acid (BA) output (O_{MXol} and O_{MPL} respectively); the values of the BA output at the half maximal Xol (R_{MXol}) and PL (R_{MPL}) outputs and the minimal molar Xol/PL ratio related to the BA output (XPR_m).

The following equations were used to represent the relationships between Xol and BA outputs, PL and BA outputs, and between the Xol/PL ratio and the BA respectively:

$$Xol = \frac{O_{MXol} \times BA}{O_{MXol} + BA}$$

$$PL = \frac{O_{MPL} \times BA}{R_{MPL} + BA}$$

$$Xol/PL = XPR_m \times \frac{R_{MPL} + BA}{R_{MXol} + BA}$$

Results

Bile lipid composition

The mean values of bile lipid composition for each of the experimental groups are given in Table 1.

PA induced a significant increase ($P < 0.05$) of the molar percentage cholesterol in bile, while CH and α-1081 did not induce any significant modification in the bile lipid composition. SI showed a similar trend, increasing two fold ($P < 0.005$) only in rats fed with PA.

Bile secretion

PA and, to a lesser extent, CH increase the bile flow (Table 2). The increase in the bile flow was not associated with the increase of the mean bile acid output in the PA-treated group, while CH caused a parallel increase both in bile flow and bile acid output (Table 2).

Table 1 Bile lipid composition (mean value ± s.d.) and cholesterol saturation index (SI) after two-months' treatment

Group	*% cholesterol*	*% phospholipids*	*% bile acids*	*SI* (H & D)
Controls	0.92 ± 0.25	11.36 ± 2.97	87.72 ± 3.18	0.21 ± 0.04
Cholestyramine	1.08 ± 0.25	11.36 ± 2.97	87.72 ± 3.18	0.21 ± 0.04
Procetonfenic acid	1.85 ± 0.4*	13.92 ± 3.4	84.22 ± 3.4	0.39 ± 0.09**
α-1081	1.61 ± 0.95	12.86 ± 4.9	85.52 ± 5.75	0.33 ± 0.14

* $P < 0.05$; ** $P < 0.005$ vs controls

Biliary lipid secretion

Bile acid and phospholipid output in bile were not modified by PA administration (Table 2), while cholesterol output markedly increased; CH administration increased cholesterol, bile acid and phospholipid output. No modification was observed in the group treated with α-1081, compared with the control group.

The relationship between cholesterol and bile acid output is shown in Figure 1. A significant correlation was observed in all the groups. Cholesterol output was close to zero at low bile acid outputs; it increased rapidly at increasing bile acid outputs and levelled off at high bile acid outputs.

The curve fitting was different in the four groups: Table 3 lists the curve fitting in terms of the evaluated parameters.

In the PA-treated group we observed a marked increase in O_{MXol} that was twice that found in the control group. No differences were found among the other groups.

A curvilinear correlation was observed also by plotting phospholipid versus bile acid outputs; curve data are listed in the same table. These data show that: (1) no bile acid independent secretion of cholesterol and phospholipid is present; (2) cholesterol and phospholipid secretion rates increase at increasing bile acid outputs up to a plateau; (3) a further increase in bile acid output is not associated with a similar increase in cholesterol and phospholipid, which level off.

In Figure 2 the cholesterol/phospholipids molar ratio is expressed as a function of the corresponding rates of bile acid outputs. At lower bile acid secretion rates the relative amount of cholesterol increased significantly in all groups, particularly in the rat fed PA. The ratio remained constant above a bile acid secretion rate of 15–20 $\mu mol\,kg^{-1}\,h^{-1}$ in all the groups. At lower bile acid secretion rates, significant differences were observed: the same ratio increased, mainly in the PA-treated group, but decreased in the α-1081 fed group compared with the controls.

This finding suggests that at low bile acid outputs cholesterol secretion continues with very low phospholipid secretion rates so that the ratio increases greatly.

α-1081 surprisingly maintains this ratio unchanged within a wide range of variations of bile acid output, thus suggesting an ideal coupling of the three lipids, even better than that of the other groups.

Discussion

In rats, chronic administration of α-1081 significantly decreases serum cholesterol and triglycerides, without any influence on bile lipid composition; in fact,

Table 2 Bile secretion and biliary lipid outputs in the groups studied

Group	*Bile secretion* ($ml\,kg^{-1}\,h^{-1}$)	*Bile acid output* ($\mu mol\,kg^{-1}\,h^{-1}$)	*Phospholipid output* ($\mu mol\,kg^{-1}\,h^{-1}$)	*Cholesterol output* ($\mu mol\,kg^{-1}\,h^{-1}$)
Controls	0.41 ± 0.14	25.80 ± 9.48	5.95 ± 1.68	0.45 ± 0.17
Cholestyramine	0.62 ± 0.10*	36.96 ± 6.68*	7.28 ± 1.78*	0.68 ± 0.09
Procetofenic acid	0.72 ± 0.09**	31.88 ± 9.37	5.55 ± 1.84	0.94 ± 0.19**
α-1081	0.49 ± 0.16	29.88 ± 8.65	5.84 ± 1.80	0.52 ± 0.19

* $P < 0.05$; ** $P < 0.005$ vs controls

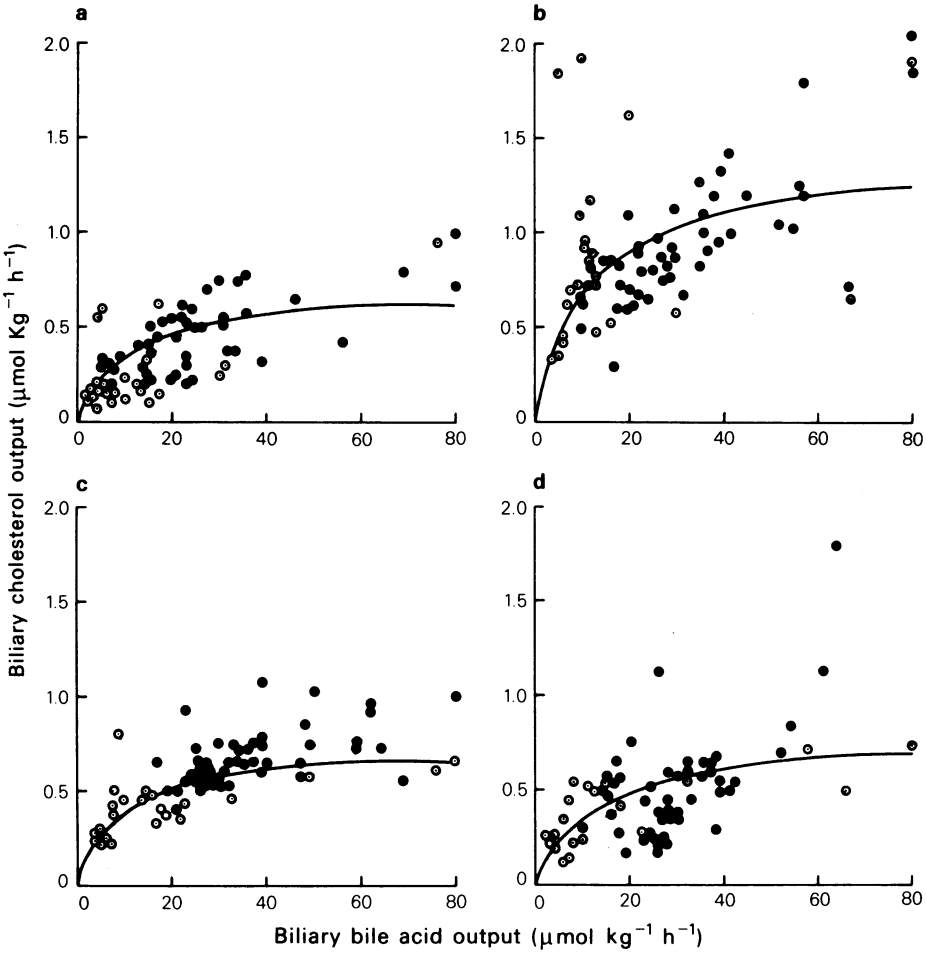

Figure 1 Relationship between biliary cholesterol and bile acid output. Each point represents the value for a single animal during intact (●) and interrupted enterohepatic circulation (○): (a) placebo; (b) procetofenic acid; (c) cholestyramine; (d) α-1081.

the biliary cholesterol saturation index of rats fed with α-1081 was similar to the controls.

Conflicting results have been reported concerning the effect of clofibrate-like derivatives on bile lipid composition and secretion in rats. However, these studies referred to a treatment period lasting no longer than two weeks and to animals with complete bile diversion.

These different models can explain the differences in bile lipid composition found by Rozé, Cuchet,

Table 3 Kinetic analysis of the biliary lipid secretion: parameters of the curve fitting the relationship between the three biliary lipid outputs in the different groups

	O_{MXol}	R_{MXol}	O_{MPL}	R_{MPL}	X_{PMR}	r	P
Controls	0.69	9.54	12.89	30.45	0.053	0.788	<0.001
Cholestyramine	0.83	7.93	13.66	36.61	0.061	0.776	<0.001
Procetofenic acid	1.41	10.40	13.34	19.12	0.079	0.418	<0.001
α-1081	0.81	13.45	9.91	17.02	0.082	0.695	<0.001

For key to abbreviations see Methods section.

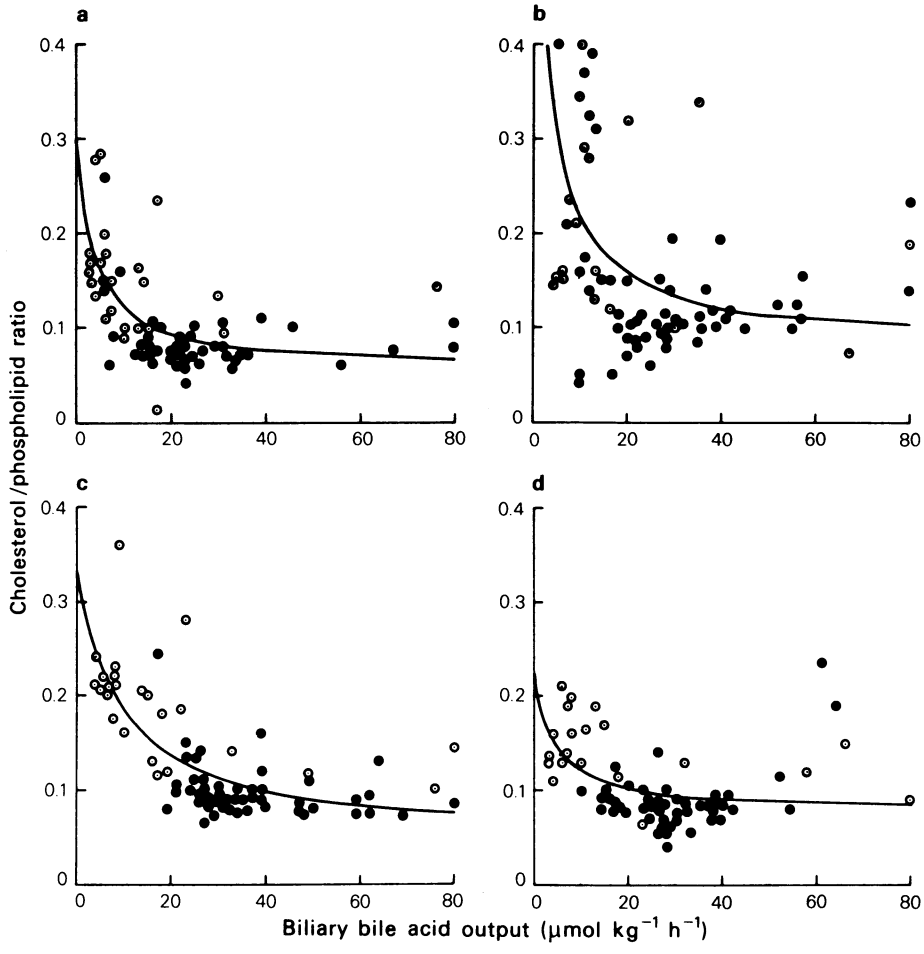

Figure 2 Relationship between the molar ratio of cholesterol to phospholipids and the output of bile acids. For symbols see Figure 1.

Souchard, Vaille & Debray (1977). They observed that clofibrate (500 mg/day, seven days' treatment) has no effect on bile lipid composition, whilst Cohen, Raicht, Scheffer & Mosbach (1974) showed that this drug (300 mg/day, 14 days' treatment) reduces the biliary cholesterol.

The conclusions drawn from these experiments are valid provided the rate of biliary lipid output is similar both in these conditions and in those observed in the intact animal (Dowling, Mack & Small, 1971). This is true in the first 2 h of the secretion study but drastic differences were observed after this period (unpublished data). Phospholipid and mainly bile acid secretion rates fall after 4 h while cholesterol output remains unchanged.

PA alone markedly increases the percentage of cholesterol in bile via a selective increase in cholesterol secretion rates. At low bile acid outputs, the bile becomes enriched in cholesterol and more saturated. In addition, a significant increase in water secretion was observed; this may be due to a preferential excretion of both PA and its main metabolite (reduction compounds) in bile.

CH feeding increases the secretion of bile acids and, to a lesser extent, of cholesterol and phospholipids. Previous studies in man (Vand den Linden & Nakayama, 1969) have shown that CH feeding increases several-fold the faecal excretion (i.e. synthesis) of bile acids, whereas the faecal excretion of cholesterol is almost unchanged. Turley & Dietschy (1979) recently reported that rats fed *ad libitum* with a diet containing 2% cholesterol for 1 week, doubled the rate of hepatic cholesterogenesis, despite normal biliary lipid outputs and composition.

Two main differences can be found between the above study and our investigation: the authors did

not standardize the daily dose of the drug, which was about twice the one we used (1 g/day, administered by gavage) and they operated a complete interruption of the EHC of the animals. In addition, the treatment period was relatively shorter than in our study. As far as the α-1081 is concerned, the data so far reported suggest that the side-effect of increasing cholesterol in bile is lacking.

As to whether the drug may inhibit or enhance the hepatic cholesterol synthesis, by modulation of β-hydroxy-β-methyl-glutaryl CoA reductase activity, recent studies showed that, at least in the rat, newly synthesized cholesterol provides only a small proportion of the total biliary cholesterol, and a wide variation in the rate of the hepatic cholesterol synthesis does not significantly change total biliary cholesterol output (Turley & Dietschy, 1979). As biliary cholesterol secretion into bile is mainly regulated by the amount delivered to the liver by chylomicrons and serum lipoproteins, the effect of this hypolipidaemic drug (α-1081) on biliary secretion must be due to an optimal coupling of the three bile lipids.

Chronic administration of α-1081 maintains a biliary lipid composition similar to that of the control group; the hourly secretion into bile of the three lipids is also similar.

Cholesterol output into bile is significantly lower in α-1081 fed animals than in CH or PA fed rats; similarly, the drug reduces the three lipids, which show the opposite trend after CH administration.

From the present investigation, α-1081 does not seem to produce side-effects on bile lipid composition; its possible mechanism of action seems to combine the effects of the two drugs from which it is derived. Therefore this drug may be more safely used as a hypolipidaemic drug than the two above drugs alone.

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