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CHOLESTASIS AND CHANGES IN THE MICROCIRCULATION OF PERFUSED RAT LIVER CAUSED BY THE CALCIUM IONOPHORE A23187 AND TYPE I ANTIARRHYTHMIC DRUGS

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Abstract—The calcium-ionophore A23187 causes a reversible increase of the hydrostatic pressure in the portal vein of perfused rat liver. Concomitantly, hepatic functions like the production of bile and the transhepatic movement of the bile acid taurocholate are diminished, mainly due to decreased uptake. These phenomena are partly explained by changes in the microcirculation of the liver, visualized by Trypan blue staining. Both the increase in portal pressure and the major part of the decrease of biliary excretion of taurocholate and bile flow are prevented by the addition of the vasodilator papaverine.

The type I antiarrhythmic drugs quinidine and *N*-propylajmaline bitartrate (NPA), at high concentrations, also induce a rise in portal pressure and act as a cholestatic agent. The rise in portal pressure caused by NPA requires the presence of extracellular calcium and is counteracted by papaverine. In contrast to A23187, the cholestasis caused by NPA is not influenced by papaverine. While NPA decreases the hepatic uptake and biliary excretion of taurocholate, papavarine is able to restore only the uptake and not the excretion. The concentration of taurocholate in the bile is not significantly changed by NPA.

A substantial literature exists regarding drug-induced cholestasis (see refs 1–3 for reviews). Although the mechanisms of bile formation are not completely understood [3–6], it is recognized that different targets of cholestatic chemicals exist, among these the microfilaments, cytosolic binding proteins, sinusoidal and/or canalicular membranes [3].

Recently it was observed that vasoactive compounds like α-agonists and ATP influence bile flow, and a role for intracellular calcium was proposed [7]. In the present study we have used the Ca^{2+}-ionophore A23187 and investigated whether microcirculatory events can be responsible for changes in bile formation. Also the type I antiarrhythmic drugs quinidine and *N*-propylajmaline bitartrate, which occasionally cause cholestasis in humans [8, 9], are included in this study.

MATERIALS AND METHODS

Liver perfusion. Livers from male Wistar rats, weighing 200–250 g, were perfused *in situ* with a Krebs–Henseleit bicarbonate-buffered solution at 37°, equilibrated with 95% O_2/5% CO_2 in a non-circulating (flow-through) system at a flow rate of 4 ml/min g liver [10]. The bile duct was cannulated as in ref. 11 and bile samples were collected in Eppendorf tubes at 5 min intervals. Additions were made from stock solutions in DMSO by infusion pumps entering the perfusion system directly before the portal vein. Control experiments performed with DMSO† alone showed that, at the concentration used (<0.3%), the vehicle had no effect on the measured parameters.

Portal pressure. Portal pressure was indicated by the difference in height between the liver and the fluid level in an open vertical tube shunted into the perfusion system.

Assays. Stock solutions of taurocholate were determined enzymatically with 3-hydroxysteroid dehydrogenase as described in [12]. Hepatic net uptake and biliary release of the bile acid were determined by radioactivity using the tritium labeled isotope of taurocholate, infused at a rate of 0.3 μCi/min. Hepatic uptake was calculated from the portocaval differences.

Oxygen in the effluent perfusate was monitored by a Clark type platinum electrode.

Lactate dehydrogenase activity in the perfusate was measured as in ref 12.

Biochemicals. *N*-propylajmaline bitartrate was supplied by Giulini Pharma Co. (Hannover, F.R.G.), all other chemicals and biochemicals were obtained from commercial sources at the highest purity available.

RESULTS

Cholestasis and hepatic circulatory changes by the calcium-ionophore A23187

Addition of 0.1 μM A23187 to the influent perfusate gives rise to an increase of the hydrostatic pressure in the portal vein (Fig. 1A). Simultaneously, oxygen consumption and bile flow are strongly

† Abbreviations used: NPA, *N*-propylajmaline bitartrate; DMSO, dimethylsulfoxide.

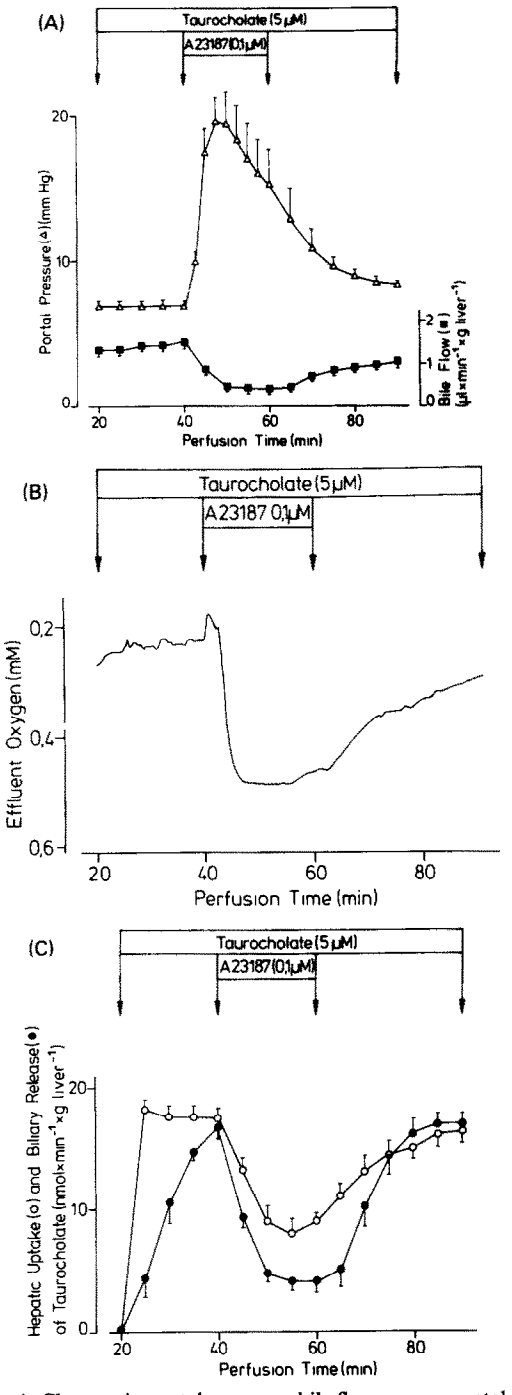

Fig. 1. Changes in portal pressure, bile flow, oxygen uptake and hepatic disposition of taurocholate during infusion of A23187. (^{3}H)-Taurocholate (5 μM) was infused from 20 min, A23187 (0.1 μM) from 40 min to 60 min. (A, C) Values are means (±SEM) from four experiments (B) One experiment representative of four. Symbols are: △, portal pressure; ■, bile flow; ○, hepatic net uptake of taurocholate; ●, biliary release of taurocholate.

depressed (Figs. 1A,B). The physical appearance of the liver, visualized by Trypan blue staining, changes from homogeneous staining to large patches on the liver surface, indicative of changes in liver microcirculation. After cessation of the A23187 addition values return to almost normal.

The addition of the vasodilator papaverine (40 μM) prevents both the rise in portal pressure and the cholestasis by the ionophore (Fig. 2). While bile flow decreases from 1.5 ± 0.1 to 0.46 ± 0.1 μl/min·g liver after 10 min addition of A23187, the same addition in the presence of papaverine leads to a value of 1.7 ± 0.2 μl/min·g liver (Fig. 2, Table 1). Papaverine alone elevates bile flow to 2.1 ± 0.1 μl/min·g liver. It seems therefore that the major part of A23187-induced cholestasis is caused by changes in liver hemodynamics.

Influence of A23187 *on hepatic disposition of taurocholate*

A further parameter for the study of liver functioning with respect to bile formation is the hepatic handling of bile acids. Infusion of 5 μM taurocholate into the influent perfusate leads to an almost complete uptake and biliary release of the bile acid (Fig. 1C, Table 1). During the addition of A23187 the hepatic uptake and its release into bile are substantially suppressed. This is consistent with bypassing of certain areas in the liver lobules, which would lead to an incomplete extraction of the bile acid from the perfusate.

In the presence of papaverine the hepatic uptake of taurocholate is largely uninfluenced upon addition of A23187 (Table 1), indicating that vascular changes and not transport defects at the sinusoidal plasma membrane of the hepatocyte are responsible for the A23187-induced impairment of uptake. Also the biliary excretion of the bile acid is restored to a large extent (Table 1). A substantial difference between uptake and excretion is still apparent both in the absence and presence of papaverine (Fig. 1C, Table 1), suggesting that besides the circulatory changes A23187 may have a direct effect on bile acid excretion as well. This could explain the immediate drop of taurocholate excretion into bile upon addition of the ionophore instead of a somewhat delayed decrease which would be expected when an impaired uptake is the only cause (Fig. 1C).

Effect of type I antiarrhythmic drugs

High concentrations of quinidine (Fig. 3) and *N*-propylajmaline bitartrate (Figs 4 and 5) are also able to evoke an increase in portal pressure, accompanied by impaired bile production, whereas the type II antiarrhythmic drug propranolol is without effect (not shown). A concentration dependence of NPA-induced cholestasis is shown in Fig. 4. A decrease in bile flow sets in at concentrations above 100 μM, 50% inhibition is seen at about 300 μM NPA. Under these conditions, lactate dehydrogenase leakage from the liver is not significantly increased, which is taken as evidence that the liver remains intact. Large increases in portal pressure are detected at concentrations above 100 μM. Figure 5 shows the time course of oxygen uptake, portal pressure and bile production. After 20 min of NPA infusion (400 μM) oxygen consumption is decreased from 2.0 to 0.5 μmole/min·g liver and the portal pressure is elevated from 7.5 to 17.7 mmHg. During the first 2–3 min an increase of oxygen uptake is seen, the portal

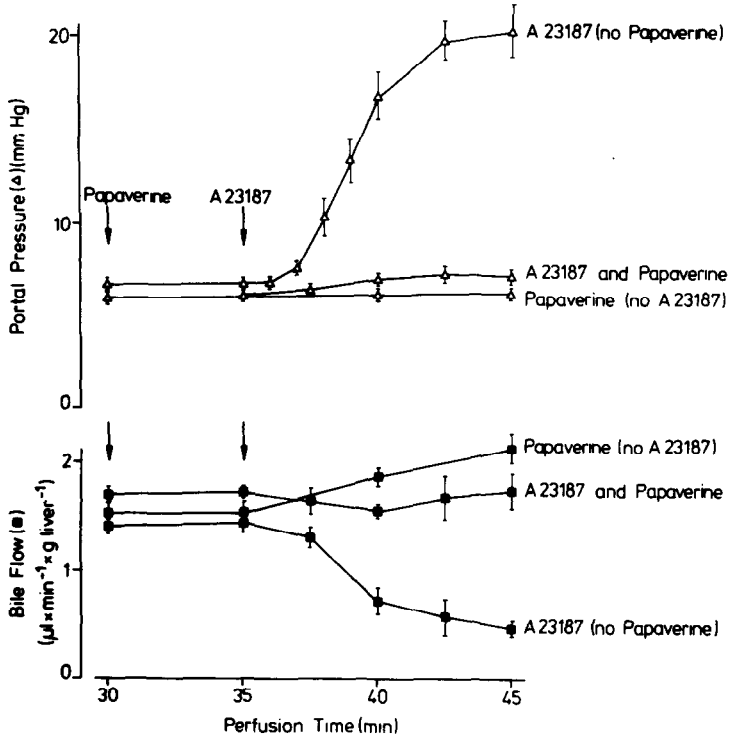

Fig. 2. Influence of papaverine on A23187-induced changes in portal pressure and bile flow. Rat livers perfused in the presence of 5 μM taurocholate. Papaverine (40 μM) was infused from 30 min and A23187 (0.1 μM) from 35 min as indicated. Values represent means ± SEM of four perfusions. Symbols: ■, bile flow; △, portal pressure.

pressure during this phase remains unaltered (Fig. 5B). It is thus tempting to conclude that the first phase displays metabolic changes and the second phase is determined by microcirculatory changes. This is supported by the finding that lower concentrations of NPA, unable to elicit changes in portal pressure, cause a monophasic increase of respiration (not shown).

The hemodynamic changes caused by A23187 or NPA are dependent on the presence of calcium in the perfusion medium. While the portal pressure remains unaltered after omission of calcium from the medium (Fig. 6A) the subsequent addition of A23187 (not shown) or NPA (Fig. 6) fails to elicit rises in the portal pressure. Due to the strict requirement of bile formation for extracellular calcium [13–15], the omission of calcium from the medium causes an immediate cholestasis. Despite the impaired biliary release of taurocholate in the absence of calcium, however, its uptake is not restricted upon addition of NPA (Fig. 6B) again illustrating that sinusoidal transport of the bile acid *per se* is not limiting during infusion with A23187 or NPA.

Influence of papaverine on NPA-induced changes

Papaverine counteracts the NPA-induced increase

Table 1. *Influence of papaverine on A23187-induced changes in portal pressure, bile flow, hepatic net uptake, biliary excretion and biliary concentration of taurocholate*

Additions				Taurocholate		
Papaverine (μM)	A23187 (μM)	Portal pressure (mm Hg)	Bile flow (μl/min·g)	Net uptake (nmol/min·g)	Biliary release (nmol/min·g)	Biliary concentration (mM)
0	0	7.5 ± 0.2	1.44 ± 0.1	16.4 ± 1.0	16.2 ± 1.3	11.3 ± 0.4
0	0.1	20.4 ± 1.6	0.46 ± 0.06	8.4 ± 0.9	4.6 ± 1.0	9.5 ± 1.6
40	0	6.2 ± 0.1	2.13 ± 0.17	18.0 ± 0.8	15.3 ± 1.8	7.5 ± 0.7
40	0.1	7.1 ± 0.4	1.72 ± 0.23	16.7 ± 0.3	11.2 ± 1.3	6.9 ± 0.4

Rat livers perfused as described under Materials and Methods. (^{3}H)-Taurocholate (5 μM) was added from 15 min, papaverine (40 μM) from 30 min and A23187 (0.1 μM) from 35 min. At 45 min the perfusion was terminated. Values were taken at this time point and represent means (±SEM) (N = 4–6).

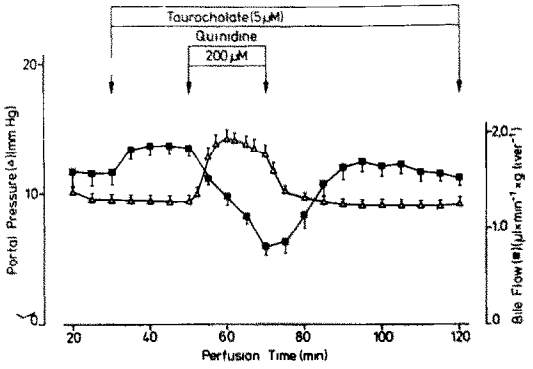

Fig. 3. Changes in portal pressure and bile flow during infusion of quinidine. Taurocholate (5 μM) infused from 30 min to 120 min, quinidine (200 μM) added from 50 min to 70 min. Values represent means ± SEM of five experiments. Symbols: ■, bile flow; △, portal pressure.

in portal pressure (Fig. 7, Table 2). In the presence of 40 μM papaverine the portal pressure rises from 6.5 to 8.1 mmHg by NPA, while in its absence an increase to a value of 17.7 mmHg is observed (Table 2). Also the hepatic uptake of taurocholate is restored by papaverine. The inhibitory effect of NPA on bile flow, however, is still existent in the presence of the vasodilator. Whereas papaverine itself is choleretic, the subsequent addition of NPA lowers bile formation and biliary excretion of taurocholate to about 50%. It seems, therefore, that the inhibition of bile flow by NPA is not simply caused by microcirculatory events and that excretory mechanisms on the canalicular part of the plasma membrane also play a role.

Papaverine alone does not significantly affect the biliary excretion rate of taurocholate, so that the choleresis caused by papaverine is responsible for a lowering of the biliary taurocholate concentration (Table 2).

The biliary concentration of taurocholate is not significantly lowered during NPA-induced cholestasis. These findings suggest that the inhibition of the bile acid-dependent part of bile formation is

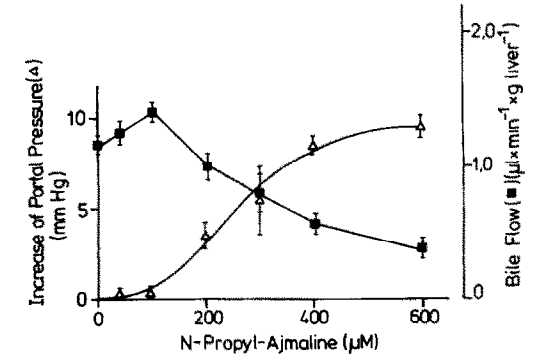

Fig. 4. Concentration dependence of NPA-induced changes in portal pressure and cholestasis. NPA infused from 30 min to 50 min. Values were taken at 50 min and represent means ± SEM of 5–6 experiments. Symbols: △, increase of portal pressure; ■, bile flow.

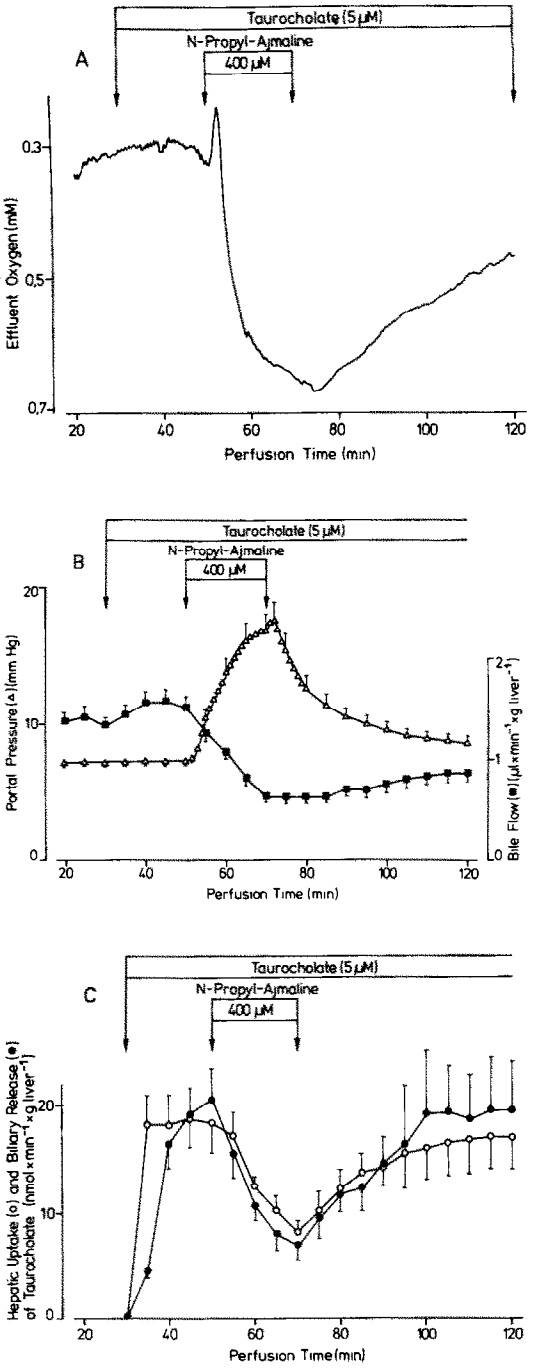

Fig. 5. Changes in oxygen uptake, portal pressure, bile flow and hepatic disposition of taurocholate during infusion of NPA. (^{3}H)-Taurocholate (5 μM) was infused from 30 min, NPA (400 μM) from 50 min to 70 min. (A) One experiment representative of six. (B) Values are means ± SEM from six experiments. Symbols: △, portal pressure; ■, bile flow. (C) Values represent means ± SEM of five experiments. Symbols are: ○, hepatic net uptake of taurocholate; ●, biliary release of taurocholate.

not the only cause of the cholestasis but that the cholestatic action of NPA is also located in the bile acid-independent part of bile formation. This is in line with the observation that the hepatic uptake and

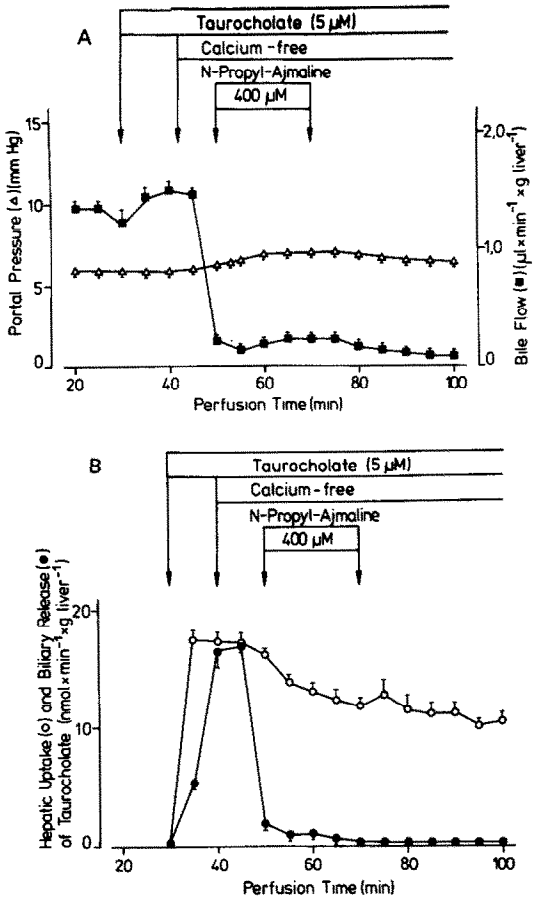

Fig. 6. Calcium dependence of NPA-induced changes in portal pressure, bile flow and hepatic disposition of taurocholate. (^{3}H)-Taurocholate (5 μM) was infused from 30 min, from 41 min a perfusion medium without calcium was used, infusion of NPA (400 μM) from 50 min to 70 min. Values plotted represent means ± SEM from five experiments. Symbols: A: △, portal pressure; ■, bile flow; (B) ○, hepatic net uptake of taurocholate; ●, biliary release of taurocholate.

biliary release of taurocholate (Fig. 5C) show more complete recovery than the bile flow (Fig. 5B), in the observed time interval until 120 min.

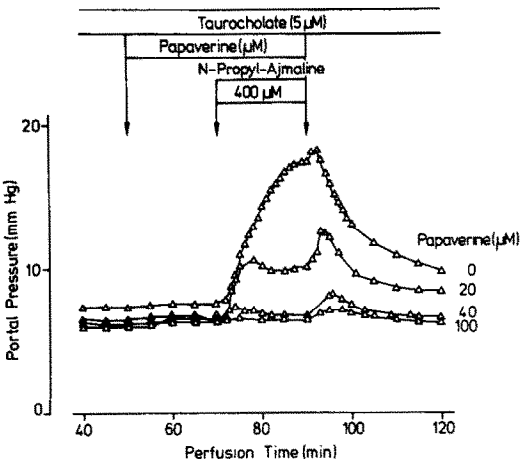

Fig. 7. Concentration-dependent suppression by papaverine of NPA-induced rises in portal pressure. Taurocholate (5 μM) was infused from 30 min, papaverine from 50 min to 90 min and NPA from 70 min to 90 min. Papaverine concentrations used were 20, 40 and 100 μM as indicated in the figure.

DISCUSSION

Responses to A23187

This study shows that in perfused rat liver A23187 leads to changes in liver microcirculation. Large parts of the liver are deprived of sufficient supply of oxygen and liver functions like the production of bile are substantially depressed.

Although we observed large pressure responses at higher concentrations of A23187 up to 2 μM, we have chosen a suboptimal concentration to avoid deleterious effects by the ionophore. At a concentration of 0.1 μM, the effects of A23187 are reversible, and no significant increase of lactate dehydrogenase leakage from the liver is found.

It has been reported that the amount of bile produced is dependent on the flow rate through the liver beneath a critical value [16]. A logical consequence of this observation seems to be that if microcirculatory changes in the liver lobules cause local impairment of flow rate, total bile production would be decreased. Up till now little is known on the influence of microcirculation on hepatic bile for-

Table 2. Influence of papaverine on NPA-induced changes in portal pressure, bile flow, hepatic net uptake, biliary excretion and biliary concentration of taurocholate

Additions Papaverine (μM)	NPA (μM)	Portal pressure (mm Hg)	Bile flow (μl/min·g)	Taurocholate		
				Net uptake (nmol/min·g)	Biliary release (nmol/min·g)	Biliary concentration (mM)
0	0	7.5 ± 0.2	1.44 ± 0.1	16.7 ± 0.9	16.7 ± 0.7	11.4 ± 0.5
0	400	17.7 ± 1.3	0.63 ± 0.06	8.2 ± 1.1	6.9 ± 1.3	13.0 ± 2.3
40	0	6.5 ± 0.2	2.09 ± 0.09	17.7 ± 0.6	16.8 ± 1.3	8.1 ± 0.7
40	400	8.1 ± 0.7	0.83 ± 0.12	18.2 ± 1.1	8.9 ± 1.6	10.7 ± 1.5

Rat livers perfused as described under Materials and Methods. (^{3}H)-Taurocholate (5 μM) was added from 15 min, papaverine (40 μM) from 35 min and NPA (400 μM) from 50 min. At 70 min the perfusion was terminated. Values were taken at this time point, and represent means (±SEM) (N = 4–5).

mation as induced by nerve stimulation or by vasoconstrictory agonists. Recently, a multiple effect of adrenaline on bile flow and an inhibition by ATP were described by Krell *et al.* [7]. They concluded that the cholestatic action of both compounds is of canalicular origin and suggested that intracellular deprivation of calcium is responsible for the inhibition of oxidative metabolism and bile flow. The observation that papaverine prevents both the changes in liver hemodynamics and the cholestasis points to the possibility that the block in bile formation by A23187 is mainly vascular in nature. This is in line with the close inverse relationship observed between the changes in portal pressure and inhibition of bile flow and biliary excretion of taurocholate during and after the addition of the ionophore (Fig. 1). At present, it is not known whether hepatocyte calcium release is involved in these vascular effects of A23187.

Two observations indicate that changes in microcirculation may not be the only cause of cholestasis by A23187. Firstly, biliary excretion of taurocholate is significantly lower than hepatic uptake, both in the absence and presence of papaverine (Fig. 1). Secondly, the inhibition of bile flow is not completely prevented by papaverine, if one takes into account the choleretic effect of the vasodilator (Fig. 2).

Antiarrhythmic drug effects

With the antiarrhythmic drugs quinidine and *N*-propylajmaline bitartrate significant hemodynamic effects are also observed (Figs 3 and 4). In a more detailed analysis with NPA it is found that in contrast to A23187 there is almost no recovery in bile flow and taurocholate excretion by papaverine (Table 2).

Recently, the existence of two sinusoidal multifunctional translocators has been postulated, both of which translocate taurocholate and NPA [17]. These studies with isolated hepatocytes using photoaffinity labelling techniques, showed mutual interference of taurocholate and NPA for binding to the transport proteins as well as inhibition of NPA uptake by taurocholate. In perfused rat liver, the addition of papaverine completely restores the suppression of taurocholate uptake by NPA (Table 2), and no inhibition of taurocholate uptake is observed, leading to the conclusion that there is no net competition for transport. This could be explained by the high capacity for taurocholate uptake [18]. At the low doses of taurocholate used in the perfused liver, giving uptake rates far below the T_m-value, significant restriction of net uptake by the whole organ might not be expected upon the addition of the drug.

Upon addition of high concentrations of NPA a biphasic response of oxygen consumption is seen (Fig. 5A), of which the second inhibitory phase is correlated with changed hemodynamics. A similar response has been reported for A23187 by Reinhart *et al.* [19] and was also observed in our experiments (Fig. 1). This means that changes in the liver microcirculation could provide an explanation for the inhibition of respiration by the calcium-ionophore and NPA. Also electrical stimulation of liver nerves is thought to decrease hepatic oxygen consumption via such mechanism [20–22].

Regarding cholestasis observed in humans upon treatment with NPA, it is not known whether it is accompanied by impaired hepatic blood flow and/or portal hypertension. The concentration dependence determined here in the perfused rat liver shows that cholestasis sets in at concentrations above 100 μM (Fig. 3). Venous plasma concentrations measured in humans after application of therapeutical doses are in the range of 1 μM [23, 24]. However, the concentration of NPA in the portal blood would be needed for comparison. Portal NPA concentrations in the rat are about 2 μM when the concentration in the systematic blood is 0.4 μM (Dr. G. Achtert, unpublished observations). Thus, the difference between the values estimated from pharmacokinetics for humans and rats and the values causing cholestasis in rats is large.

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REFERENCES

1. G. L. Plaa and B. G. Priestly, *Pharmac. Rev.* **28**, 207 (1977).
2. C. D. Klaassen and J. B. Watkins, *Pharmac. Rev.* **36**, 1 (1984).
3. R. Coleman, *Biochem. J.* **244**, 249 (1987).
4. J. L. Boyer, *Physiol. Rev.* **60**, 303 (1980).
5. R. C. Strange, *Physiol. Rev.* **64**, 1055 (1984).
6. H. Popper, *Hepatology* **1**, 187 (1981).
7. H. Krell, H. Jaeschke and E. Pfaff, *Biochem. biophys. Res. Commun.* **131**, 139 (1985).
8. H. P. Buscher, H. Talke and H. P. Radermacher, *Dtsch. Med. Wochenschr.* **101**, 699 (1976).
9. A. Deisseroth, J. Morganroth and S. Winokur, *Ann. Int. Med.* **77**, 595 (1972).
10. H. Sies, *Meth. Enzymol.* **52**, 48 (1978).
11. T. P. M. Akerboom, M. Bilzer and H. Sies, *J. biol. Chem.* **267**, 4248 (1982).
12. H. U. Bergmeyer, *Methoden der Enzymatischen Analysen* Verlag Chemie, Weinheim (1974).
13. J. Graf, in *Stimulus-Secretion Coupling in the Gastrointestinal Tracts* (Eds. R. M. Case and H. Goebell). University Park Baltimore, MD (1976).
14. A. Wahllländer and H. Sies, *Eur. J. Biochem.* **96**, 441 (1979).
15. J. Reichen, F. Berr, M. Le and G. H. Warren, *Am. J. Physiol.* **249**, G48 (1985).
16. R. W. Brauer, G. F. Leong and R. J. Holloway, *Am. J. Physiol.* **177**, 103 (1954).
17. H.-P. Buscher, G. Fricker, W. Gerok, G. Kurz, M. Müller, S. Schneider, U. Schramm and A. Schreyer, in *Bile Acids and the Liver*, MTP Press, Lancaster, in press (1987).
18. G. Paumgartner, R. Herz, K. Sauter and H. P. Schwarz, *Naunyn-Schmiedebergs Archs Pharmac.* **285**, 165 (1974).
19. P. H. Reinhart, W. M. Taylor and F. L. Bygrave, *Biochem. J.* **214**, 405 (1983).
20. W. W. Lautt, *Can. J. Physiol. Pharmac.* **58**, 105 (1980).
21. S. Ji, K. Beckh and K. Jungermann, *FEBS Lett.* **167**, 117 (1984).
22. D. Häussinger, T. Stehle, W. Gerok and H. Sies, *Eur. J. Biochem.* **163**, 197 (1987).
23. E. Schaumlöffel, *Med. Welt* **25**, 2008 (1974).
24. H. J. Hausleiter, G. Achtert, M. A. Khan, W. R. Kukovetz and E. Beubler, *Eur. J. Drug Metab. Pharmacokin.* **7**, 329 (1982).