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# REGULATION OF CANALICULAR BILE FORMATION BY α-ADRENERGIC ACTION AND BY EXTERNAL ATP IN THE ISOLATED PERFUSED RAT LIVER

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SUMMARY: In isolated perfused rat liver, addition of adrenaline induced a complex response of bile flow including rapid, reversible stimulation (1/2-2 min), reversible inhibition (2-10 min), and prolonged stimulation. Both the reversible stimulation and the inhibition were mimicked by the α-sympathomimetic agonist phenylephrine but not by the β-agonist isoproterenol. The reversible stimulation was a very early effect being terminated prior to all other α-adrenergic responses of liver. External ATP considerably lowered bile flow while inducing release of glucose and lactate, inhibition of respiration, and a reversible efflux of $Ca^{2+}$. Variations of mannitol clearance parallel to those of bile flow indicate a canalicular origin of all changes. 

The mechanisms of biliary fluid formation and the mode of its regulation are largely unknown as yet. Energy-dependent secretion of cations and anions is assumed to be the driving force leading to the secretion of isoosmotic bile (cf. 1 for a recent review).

A great number of agents has been shown to influence bile flow, among these the catecholamines. Stimulation of bile flow by the latter was mimicked by addition of dibutyryl cyclic AMP to isolated perfused liver (2). On the other hand, it has become evident in recent years that the α-adrenergic effects of catecholamines are mediated by a second messenger system distinct from cyclic AMP but associated with a liberation of calcium ions from intracellular pools (3,4).

Since liver cells are capable to respond to both α- and β-adrenergic agonists (5), we have studied the action of catecholamines on bile flow by applying the specific agonists phenylephrine and isoproterenol to isolated perfused rat livers. The catecholamine-like action of ATP (6-8) was confirmed by measuring its stimulatory effect on metabolite and $Ca^{2+}$ output into the perfusate. The different responses of bile flow to α-adrenergic effectors and to ATP, resp., are used as a tool to study the involvement of cellular $Ca^{2+}$ in the regulation of bile flow.

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## MATERIALS AND METHODS

Adrenaline (Suprarenin$^{R}$) from Hoechst (Frankfurt, F.R.G.), and phenylephrine and isoproterenol from Sigma Chemicals (St. Louis, U.S.A) were used.[$^{3}$H]mannitol was purchased from NEN Chemicals (Dreieich, F.R.G.), all other substances from Merck (Darmstadt, F.R.G.).

Livers were isolated and perfused with Krebs-Henseleit bicarbonate buffer (1.25mM $CaCl_2$, 18mM $NaHCO_3$, 122mM NaCl, 5.9mM KCl, 1.18mM $NaH_2PO_4$, 1.2mM $Na_2SO_4$, and 1.2mM $MgCl_2$), oxygenated and buffered at pH 7.3 at 37°C as described previously (9,10). In the experiments with ATP, the concentration of $CaCl_2$ was reduced to 0.2mM. This modification did not influence bile secretion in control experiments. Perfusions were performed without recirculation of the medium except when it contained radioactive mannitol. In the latter case,[$^{3}$H]mannitol (0.02 mCi) was recirculated in a volume of 150 ml of buffer. Bile was collected from a polyethylen canula (0.4 mm i.d.) and bile flow was measured by registrating the time per droplet. The concentrations of $Ca^{2+}$ and $K^{+}$ in the perfusate were determined by flame photometry. The concentrations of lactate, pyruvate, and glucose were assayed using enzymatic tests (11). Oxygen concentration in the effluent perfusate was determined with application of a Clarc type electrode. In some experiments, changes in $Ca^{2+}$ activity of the perfusate were measured with an ion specific membrane electrode (Philips, Kassel, F.R.G.). Bilirubin concentration in bile was determined by measuring its absorption at 436 nm wavelength ($\varepsilon$= 21 $mM^{-1}cm^{-1}$). To avoid bleaching, the bile samples were diluted and bilirubin was measured immediately after collection. All effects were examined at least in duplicate.

## RESULTS

Infusion of adrenaline ($10^{-7}$M) into the portal vein of isolated perfused rat liver resulted in a triphasic response of bile flow (fig. 1): An instantaneous stimulation (phase 1) was terminated within less than 2 min and followed by a reversible inhibition (phase 2). Ten min after beginning of adrenaline infusion bile flow was restored and subsequently further increased to values above the original rate. All changes in bile

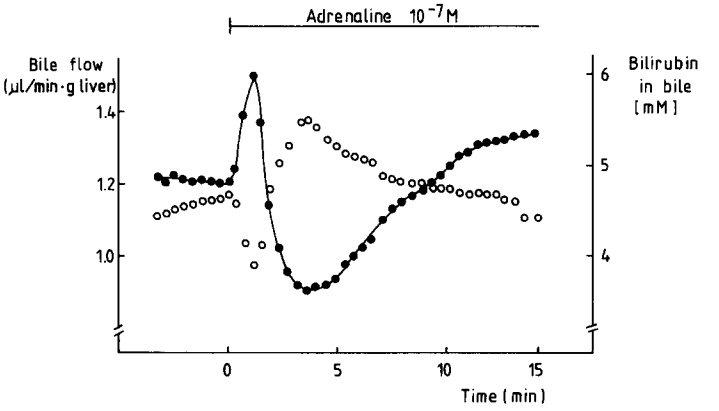


Fig. 1 Effects of adrenaline on bile flow and on bilirubin concentration in bile. After a preperfusion time of 30 min, adrenaline was continuously infused into the influent medium to obtain a concentration of $10^{-7}$M. The time of adrenaline addition was defined as zero time. A liver wet weight of 8.3 g was determined at the end of the perfusion. The liver was perfused at a constant rate of 30 ml/min.

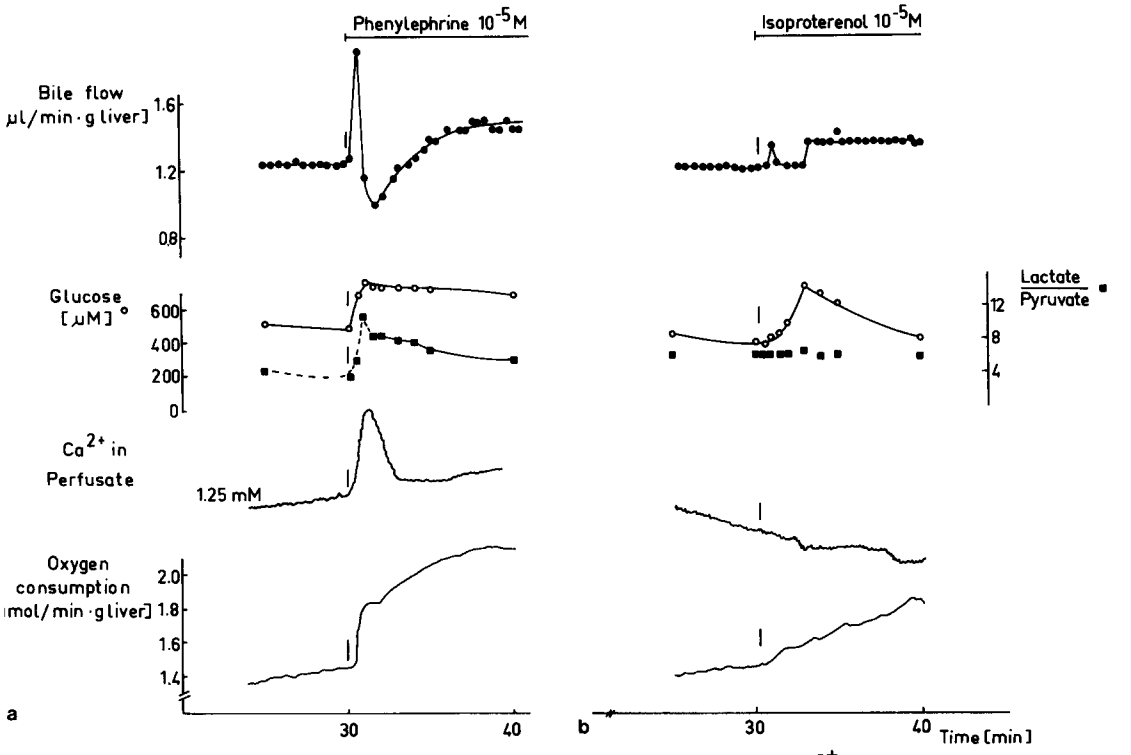


Fig. 2 Effects of α- and β-sympathomimetics on bile flow, $Ca^{2+}$ distribution, metabolite output, and oxygen consumption. Livers (a: 8g, b: 7.5g) were perfused at a rate of 30 ml/min. 30 min after canulation of the portal vein, either phenylephrine (a) or isoproterenol (b) was continuously infused into the influent medium to obtain a concentration of $10^{-5}$M. $Ca^{2+}$ in perfusate was measured semi-quantitatively by using a $Ca^{2+}$-selective electrode. Total $Ca^{2+}$ release was in the range of 1 µmole.

flow were accompanied by inverse responses of the bilirubin concentration in bile, e.g. when bile flow was stimulated, a respective decrease in bilirubin concentration was observed. Due to these inverse responses, the secretion rate of bilirubin remained essentially constant at 4 nmol/min/g liver during all variations of bile flow.

To distinguish between the effects mediated via different adrenergic receptors, phenylephrine and isoproterenol were applied. To elicit the temporal relationships to other adrenergic responses, the time courses of ionic and metabolic changes were followed in parallel (fig. 2). A rather high concentration of $10^{-5}$M of each drug was applied in order to achieve maximal effects (12). The α-agonist phenylephrine rapidly stimulated bile flow. The maximal deviation was higher than in the case of adrenaline and was reached within the first min. It is not clear, at the resolution obtained here, whether it preceeded the maximum of $Ca^{2+}$ efflux and the maximal ratio of lactate over pyruvate. At least, all these responses occured very early, and both $Ca^{2+}$ efflux and a high degree of reduction of

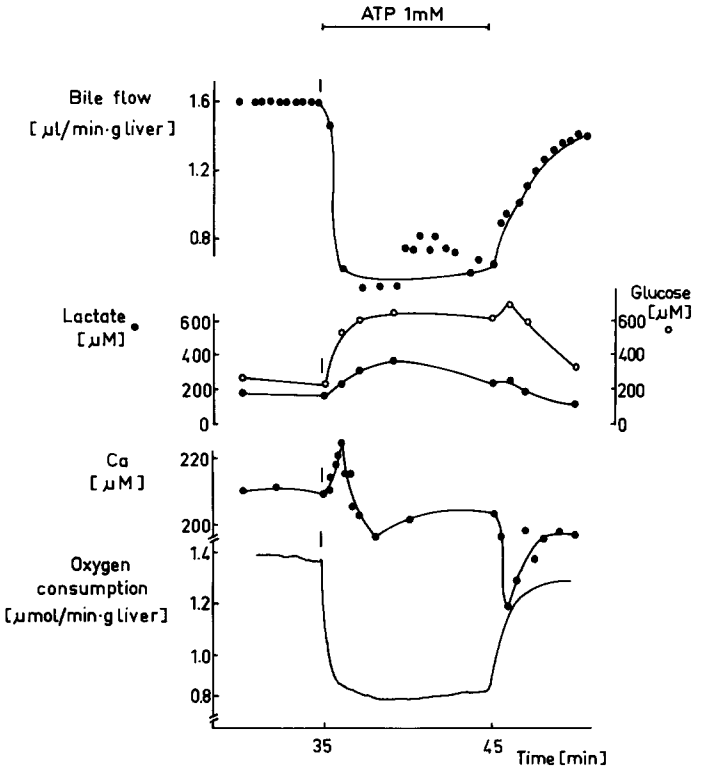


Fig. 3 Changes in bile flow, $Ca^{2+}$ distribution, and cellular metabolism induced by external ATP. A liver (7.5 g) was perfused at a rate of 30 ml/min with Krebs-Henseleit bicarbonate buffer containing 0.2 mM $Ca^{2+}$. 35 min after canulation of the potal vein, the medium was replaced by one containing additionally 1 mM ATP and 1 mM $Mg^{2+}$. Ten min later, the perfusion was continued with the original medium.

the redox couple prevailed when the stimulation of bile flow was already followed by the phase of inhibition. The same holds for $K^+$ efflux (results not shown) which has previously been shown to parallel $Ca^{2+}$ efflux during α-adrenergic stimulation (3).

Isoproterenol, a β-agonist, did not show the phase of rapid reversible stimulation of bile flow. Accordingly, neither $Ca^{2+}$ release nor an increase of lactate over pyruvate ratio was observed. Only a slight, delayed increase of bile flow occured. Both oxygen consumption and glucose release were moderately stimulated, also with a time lag compared to the effects of phenylephrine.

External ATP (1 mM) was added to the perfusion medium together with additional 1 mM $MgCl_2$ to exclude effects of $Ca^{2+}$ complexation (fig. 3). Without additional $Mg^{2+}$, the results obtained were the same (not shown). The concentration of $Ca^{2+}$ in the medium was lowered to 0.2 mM in these experiments to simplify the measurements of calcium by atomic absorption photometry. It was known that reduction of the $Ca^{2+}$ concentration to this

Table I [$^3$H]mannitol clearances upon perfusion with adrenaline and ATP, resp.

| | Bile flow (µl/min/g liver) | Mannitol clearance |
|---|---|---|
| Adrenaline ($10^{-7}$M) | | |
| phase 0 | 1.16 | 1.03 |
| phase 1 | 1.70 | 1.67 |
| phase 2 | 0.78 | 0.73 |
| phase 3 | 1.30 | 1.26 |
| ATP (1mM) | | |
| prior to ATP | 1.21 | 1.14 |
| during ATP | 0.62 | 0.56 |
| after ATP | 1.12 | 1.05 |

The conditions were as in figures 1 and 3, resp., but after 20 min of preperfusion, [$^3$H]mannitol (0.02mCi) was added in 150ml of recirculating medium. After 15 min of recirculation, $^3$H-radioactivity in bile had reached a constant level and adrenaline was infused at a concentration of 10 M. In the experiments with adrenaline addition, phase 0 is equivalent to the condition prior to hormone addition, phase 1 to that of maximal stimulation after 70 sec of infusion, phase 2 to that of maximal inhibition after 4 min, and phase 3, the state of prolonged stimulation, was reached after 10 min of hormone addition in these experiments. The concentrations of radioactive material in bile were corrected for the delay of appearance at the end of the bile canula. The data given are mean values from two experiments, resp..

extent did not influence bile secretion (unpublished results). ATP markedly reduced bile flow which remained at a low level until ATP was omitted. The catecholamine-like action is demonstrated by the stimulation of glucose and lactate release as well as by a temporary $Ca^{2+}$ efflux. In contrast to the other agents, however, oxygen consumption decreased on ATP addition. Subsequent omission of ATP restored all parameters and temporarily stimulated $Ca^{2+}$ uptake.

The clearance of mannitol was used to estimate whether or not the changes of bile flow were of canalicular origin. Table 1 shows that [$^3$H]mannitol clearance paralleled all variations of bile flow induced by both adrenaline and ATP indicating canalicular origin of all changes observed (13).

## DISCUSSION

This report describes a triphasic response of hepatic bile flow to catecholamines in hemoglobin-free perfused rat liver. The rapidly reversible stimulation of bile flow (phase 1) was a very early event preceeding or at least paralleling the metabolic and ionic responses of the cells. It was clearly α-adrenergic in nature since it was mimicked by the

α-agonist phenylephrine but not by the β-agonist isoproterenol. The reversibility of stimulation is as yet unexplained. The second phase, a reversible reduction of bile flow, was also obtained with phenylephrine, but it was more pronounced with adrenaline. This suggests an involvement of a β-adrenergic function. The interrelationships between α- and β-adrenergic stimuli with respect to bile formation are currently studied. A prolonged stimulation of bile flow (phase 3) was beginning after the maximum of $Ca^{2+}$ efflux, but no clear-cut relationship with the other adrenergic effects could be detected during this phase, either.

The inversely related changes of bile flow and biliary bilirubin concentration exclude the possibility that the bile duct could contract (14) and thereby extrude pre-formed bile. Taking into account the parallel variations of mannitol clearance it can be concluded that canalicular fluid formation was the site of action of the α-adrenergic agonists.

The triphasic behavior of bile flow resembles that of the $Ca^{2+}$ transport characteristics upon α-adrenergic stimulation of isolated liver cells (4). First, a rapid release of calcium from intracellular stores occurs. In a second step, calcium is extruded from the cells by an activated transport system. In a third phase the $Ca^{2+}$ permeability of the plasma membrane is increased possibly allowing $Ca^{2+}$ reentry. According to this model, an increase in cytosolic $Ca^{2+}$ activity would cause the stimulation of bile flow in phase 1 as well as in phase 3. In phase 2, on the other hand, the $Ca^{2+}$ activity in the cytosol would be reduced due to its extrusion across the plasma membrane.

ATP was used as an independent effector known to exert a catecholamine-like action in isolated liver cells (6-8). In contrast to the latter model, ATP did not promote an uptake of $Ca^{2+}$ in the perfused liver, but a release was observed instead. The metabolite output and the $Ca^{2+}$ efflux were accompanied by a prolonged decrease in bile flow and by respiratory inhibition. All effects were reversed on omission of ATP. With the concept described above, it is tentative to speculate that $Ca^{2+}$ efflux that was activated by ATP deprived the cells of $Ca^{2+}$, thereby inactivating oxidative metabolism and bile flow. Such an inactivation of pyruvate oxidation has been reported for the action of vasopressin, α-adrenergic agonists, and the $Ca^{2+}$ ionophore A 23187 (15). All these substances activate $Ca^{2+}$ efflux from isolated perfused liver (15).

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