Bile salts, hypotension and obstructive jaundice

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Summary

1. We have examined the effects of bile duct ligation on vascular and extravascular smooth muscle responsiveness to noradrenaline and tyramine using isolated rat hindlimb perfusion, and portal vein and vas deferens preparations.

2. Bile duct ligation reduced the contractile responses to noradrenaline of vascular and extravascular smooth muscle.

3. Exposure of smooth muscle to some bile salts caused a reduction in contractility.

4. This effect was dependent upon bile salt type and concentration.

5. These studies in vitro suggest that the reduced total peripheral resistance and hypotension seen in obstructive jaundice cannot be explained by a spasmolytic effect of some of the bile salts on smooth muscle.

Key words: bile salts, hypotension, noradrenaline, obstructive jaundice, vascular reactivity.

Introduction

Patients with obstructive jaundice are susceptible to postoperative shock and acute renal failure [1]. Moreover, these patients have deranged volume control [2]. Studies on animals show that obstructive jaundice or isolated cholaemia produced by choleductocaval anastomoses have arterial hypotension due to peripheral vasodilatation [3], are more susceptible to haemorrhagic hypotension [4] and have blunted peripheral pressor responses to noradrenaline and angiotensin [5]. Thus it has been proposed that these experimental findings on the peripheral circulation indicate important contributory factors in the development of the aforementioned clinical complications of obstructive jaundice [6]. The cause of the circulatory disturbance remains elusive, but recently Bomzon & Kew [7] postulated that the presence of bile constituents in the plasma might be responsible for these complications. In cholestasis various bile constituents accumulate in the plasma, and these include bile salts, cholesterol and bilirubin. Of these substances, bile salts have been implicated as responsible for the hypotension associated with obstructive jaundice [8]. Independent studies on extravascular smooth muscle have shown that bile salts can inhibit inward movement of sodium, chloride and potassium into cells [9]. Furthermore, bile salts also have been shown to enhance the entry of calcium into cells [10] and to modify the activity of the enzyme Na⁺,K⁺-dependent ATPase [11]. The significance of these ionic fluxes and Na⁺,K⁺-ATPase activity to vascular tone and catecholaminergic control of vascular resistance is now well documented [12]. The relationship between bile salts and both vascular and extravascular smooth muscle responsiveness to catecholaminergic stimulation is the subject of this report. Summaries of our findings have been presented previously (Annual General Meeting of the Physiological Society of South Africa, Johannesburg, October, 1982, and 52nd Meeting of the Israel Physiological and Pharmacology Society, Rehovot, April, 1983).
Materials and methods

The bile salts used in this study were sodium glycocholate, sodium taurocholate and sodium deoxycholate, all purchased from Sigma Chemical Corporation. Cholic acid as the sodium salt was obtained from Fluka AG. The noradrenaline and tyramine were purchased from Sigma Chemical Corporation. Diluted whole bile was obtained from freshly killed dogs.

Animals

For all the experiments male rats (200± g body weight) were used. The rats were divided into three groups. Group 1 was composed of control unoperated rats; group 2 was composed of sham-operated control rats (in these rats, the bile duct was exposed by laparotomy, but not ligated); group 3 was composed of rats whose bile duct had been ligated for 3-5 days. These rats were clinically jaundiced as determined by the colour of their extremities and urine and fat tissues. Upon post mortem examination, the bile duct was found to be dilated proximal to the site of ligation. Animals that did not meet these requirements were excluded from this group.

Techniques

Three techniques were used to evaluate the effect of bile salts on vascular reactivity to catecholaminergic stimulation.

Isolated rat hindlimb preparation. The right or left femoral artery of freshly killed rats was cannulated via the abdominal aorta and perfused with warmed (37°C) oxygenated Krebs' solution (composition in mmol/l: NaCl 118.05; KCl 4.69; NaH2PO4 1.33; NaHCO3 25; CaCl2 2.70; MgCl2 1.05; glucose 5.56) osmotically balanced with dextran T 70 (0.06 mmol/l) to a colloid osmotic pressure of 20 mmHg. A peristaltic constant flow pump (Watson-Marlow) was used to adjust the perfusion pressure to 60 mmHg, which corresponded to a flow rate of 7-9 ml/min. Perfusion pressure was monitored with a Bell and Howell pressure transducer, which was placed immediately proximal to the preparation and whose continuous output was recorded with a moving chart recorder (Medical Devices). Noradrenaline was injected into the perfusion system as a bolus. Perfusion of the hindlimb was done on control, sham-operated and bile duct-ligated rats as described by Naidu & Bomzon [13]. In some of the control rats, sodium glycocholate and sodium taurocholate were added to the Krebs' solution in concentrations that ranged from 0.1 to 0.3 mmol/l and non-cumulative dose-response curves to noradrenaline were constructed.

Isolated rat portal vein preparation. The portal veins of control, sham-operated and bile duct-ligated rats were excised and then placed in warmed (37°C) oxygenated Krebs' solution (see above for formula) in an organ bath of 15 ml volume at a resting tension of 300-500 mg. Longitudinal contractions of the vein were measured with a strain gauge isometric transducer (UC-2 Gould Statham) whose output was recorded on a chart recorder (Brush-Gould). The contractile response to the various doses of noradrenaline were recorded, and duplicate non-cumulative dose-response curves to noradrenaline were constructed.

In a second series of experiments, portal veins removed from normal rats were bathed in Krebs' solution to which was added various dilutions of whole dog bile and various concentrations of sodium taurocholate, cholic acid and sodium deoxycholate, over the concentration range 0.2-1.0 mmol/l. Non-cumulative dose-response curves to noradrenaline were constructed.

Isolated rat vas deferens preparation. The vasa deferentia of control, sham-operated and bile duct-ligated rats were excised and then placed in warmed (37°C) oxygenated Krebs' solution in an organ bath of 15 ml volume at a resting tension of 500 mg. Longitudinal contractions of whole vasa deferentia to noradrenaline, field stimulation or tyramine were measured with a strain gauge isometric transducer (UC-2 Gould Statham) whose output was recorded on a chart recorder (Brush-Gould). Both vasa deferentia were isolated from each rat and prepared in separate organ baths, one serving as control and the other as test.

In one series of experiments, the vasa deferentia removed from control rats were exposed to various concentrations of bile and sodium deoxycholate, sodium taurocholate and the dose-response curves to noradrenaline constructed.

In another series of experiments, ED_{50} doses for noradrenaline and tyramine were added alternately to the organ bath at 5 min intervals, at least three times. After satisfactory control responses were obtained, the tissues were exposed to bile and bile salts and the responses to the same doses of noradrenaline and tyramine recorded.

Statistical analysis

The data were analysed by using Student's t-test.

Results

Rat hindlimb perfusion studies

The responses of the hindlimb of bile duct-ligated rats to noradrenaline were not statistically
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When sodium glycocholate was added to the perfusate over the concentration range 0.1-0.3 mmol/l, no significant difference in the response to noradrenaline was seen at any of the concentrations (Fig. 2). However, when sodium taurocholate was used over the same concentration range, there were concentration-dependent reductions in the maximal responses to noradrenaline without any significant changes in the ED₅₀ (Fig. 2).

Isolated portal vein studies

The responses of the portal vein removed from bile duct-ligated rats were significantly changed from those seen in portal veins excised from control or sham-operated rats (Fig. 3). These changes are reflected by a reduction in contractile responses at higher concentrations. When dog bile was added to the buffer at dilutions greater than 1:15 000, the dose-response curve began to shift to the right and, at lower dilutions (1:7500 and 1:3750), there were significant reductions in the response to all doses of noradrenaline (Fig. 4).

When the portal veins of normal rats were exposed to various concentrations of the different bile salts, concentration-dependent reductions in the maximal response to noradrenaline were observed. Of the three bile salts used, sodium deoxycholate was more potent than either cholic acid or sodium taurocholate (Fig. 5).

Isolated rat vas deferens studies

Contractile responses to exogenous noradrenaline. Vasa deferentia removed from bile duct-ligated rats had lower maximal responses to noradrenaline without any changes in the ED₅₀ as compared with vasa deferentia from control sham-operated rats (Table 1). The exposure of the tissue by increasing concentrations of the bile salts or diluted bile caused significant reductions in the maximal responses to noradrenaline without

![Graph 1](image1.png)

**Fig. 1.** Effect of ligation of the bile duct on the vascular response to noradrenaline with the isolated rat hindlimb perfusion technique. ○, Control; △, sham-operated; □, bile duct-ligated. Values shown are means ± SEM. n = 6 for all three groups.

![Graph 2](image2.png)

**Fig. 2.** Effect of the addition of 0.3 mmol/l sodium glycocholate (▲; n = 6) and 0.3 mmol/l sodium taurocholate (●; n = 6) to the perfusion medium on the vascular response to noradrenaline with isolated rat hindlimb perfusion technique. ○, Control (n = 6). Values shown are means ± SEM. *P < 0.001.
FIG. 3. Effect of ligation of the bile duct on response to noradrenaline by the isolated rat portal vein preparation. ●, Control (n = 7); ○, sham-operated (n = 6); ▲, bile duct-ligated (n = 8). Values shown are means ± SEM; *P < 0.05, x = 1–5 (abscissa).

FIG. 4. Effect of additions of various dilutions of bile (●—●, 1:15000, n = 6; ○—○, 1:7500, n = 6; ┐—┐, 1:3750, n = 6) to the bathing medium on the contractile response of the isolated rat portal vein to noradrenaline. ●—●, Control. n, Number of observations. *P < 0.05. x = 1–5 (abscissa).

FIG. 5. Effect of the addition of 1 mmol/l cholic acid (○, n = 6), 1 mmol/l sodium taurocholate (●, n = 6) and 1 mmol/l sodium deoxycholate (□, n = 6) to the bathing medium on the contractile response of the isolated portal vein to noradrenaline. ○, Control (n = 7). At these concentrations, the contractile responses to noradrenaline were significantly reduced (P < 0.05) at all doses. n, Number of observations. x = 1–5 (abscissa).

changes in the ED50 (Fig. 6). Furthermore sodium deoxycholate was more potent than sodium taurocholate.

Contractile responses to tyramine and noradrenaline. Bile at a final concentration of 1:150 and sodium deoxycholate significantly reduced the maximal contractile response of the vas deferens to both tyramine and noradrenaline (Fig. 7). On the other hand, sodium taurocholate had no effect on tyramine- or noradrenaline-induced contractions of the vas deferens (Fig. 7).

Discussion

This investigation clearly shows that vascular and extravascular smooth muscle removed from bile duct-ligated rats has attenuated responses to noradrenaline. This attenuation can be mimicked by exposure of normal tissues to diluted bile or bile salts. The site of this loss of contractile response appears to be postjunctional, since there does not appear to be a marked effect of bile or bile salts on neuronal amine uptake. Tyramine responses were reduced in parallel to the noradrenaline responses.

It is of interest to note that this loss of responsiveness to noradrenaline could not be
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TABLE 1. Response to noradrenaline of the isolated vas deferens removed from sham-operated and bile duct-ligated rats

Values shown are means ± SEM. n, Number of observations; N.S., not significant.

<table>
<thead>
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<th>Sham-operated (n = 8)</th>
<th>Bile duct-ligated (n = 12)</th>
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<tbody>
<tr>
<td>ED₅₀ (μm)</td>
<td>2.62 ± 0.41</td>
<td>3.47 ± 0.55</td>
</tr>
<tr>
<td>Noradrenaline maximum response (mg)</td>
<td>1636 ± 56</td>
<td>1277 ± 68</td>
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<tr>
<td>Weight of vas deferens (mg)</td>
<td>37.1 ± 3.9</td>
<td>34.0 ± 2.1</td>
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adequately demonstrated with the isolated rat hindlimb perfusion technique, but is obvious in the isolated smooth muscle experiments. This difference may be explained by the various techniques used in the experiment. In the former, pressure was used to assess changes in contractility, whereas in the latter contractility was measured directly. In these experiments the tissue is isolated and removed from its natural environment and is not subjected to the processes in vivo or in situ such as uptake and endogenous vasodilator compounds. However, the results obtained with the isolated organ bath preparations are in keeping with other clinical and experimental findings. Lunzer et al. [14] showed that the forearm vasculature of cirrhotic patients has an attenuated response to noradrenaline. In an experimental study with baboons with chronic ligation of the common bile duct, Bomzon et al. [15] demonstrated, using the xenon washout technique, that the response of the skeletal vasculature to noradrenaline is reduced. In addition, Finberg et al. [5] in cholestatic dogs showed that there are blunted pressor responses to intravenous noradrenaline. Thus it would appear that there is a circulating factor or factors in plasma of patients with liver disease or animals with experimental obstructive jaundice that could account for the loss of responsiveness to noradrenaline. Altered responsiveness to noradrenaline was still detected in vasa deferentia or portal veins excised from bile duct-ligated rats. Exposure of the tissues to bile salts also resulted in a loss of contractile response to noradrenaline. Moreover, we found that this loss was bile salt type and concentration.
dependent. In this experiment, the secondary bile salt, sodium deoxycholate, was more potent than either the primary bile salt, sodium cholate, or its taurine conjugate. In obstructive jaundice of extrahepatic origin negligible amounts of bile salts gain access to the intestinal lumen to undergo bacterial dehydroxylation. Hence, the circulating concentration of sodium deoxycholate is low. However, conjugation still occurs. Thus it would be expected that there is a high circulating concentration of primary and conjugated bile salts in the plasma. In our experiments, although all three types of bile salts could induce a loss of response to noradrenaline, they did so at concentrations above the upper limits seen in obstructive jaundice. In this experiment, the secondary bile salt concentration of sodium deoxycholate is low. Hence, the circulating concentration of sodium deoxycholate is low. However, conjugation still occurs. Thus it would be expected that there is a high circulating concentration of primary and conjugated bile salts in the plasma. In our experiments, although all three types of bile salts could induce a loss of response to noradrenaline, they did so at concentrations above the upper limits seen in obstructive jaundice.

On the other hand, we have provided further evidence that the mechanism of peripheral vasodilatation and hypotension is associated with a plasma borne bile constituent. In our experiments, dilutions of bile as low as 1:15 000 were sufficient to cause a noticeable reduction in the response to noradrenaline of vascular smooth muscle. This result differs from that of Bloom et al. [16] using isolated rabbit femoral arteries perfused with plasma obtained from jaundiced baboons. In their experiment it was shown that response to noradrenaline was potentiated. These varied experiments in situ, in vivo and in vitro have not consistently demonstrated an inhibitory effect of bile constituents on vascular responsiveness to noradrenaline, but the various experimental conditions and vascular beds investigated may be responsible for the variability in final response. The renal vasculature, for example, behaves entirely differently from the skeletal vasculature in the presence of jaundice and bile components [7, 8]. This can also be observed in our experiments. Loss of responsiveness to noradrenaline occurred at lower concentrations of bile salts or dilution of bile in the portal vein than in either the vas deferens or hind limb. The portal circulation, however, is the only vasculature naturally exposed to high concentrations of bile constituents such as bile salts and bilirubin. Thus the increased sensitivity to bile salts of portal veins may possibly be of physiological importance in the development and maintenance of portal dilatation during digestion. On the basis of these experiments, it is difficult to establish a causative relationship between bile salts and reduced cardiovascular responsiveness to noradrenaline in jaundice. Gall bladder bile has a bile salt concentration of 115 g/l, which is equivalent to a solution of bile salts about 0.2 mol/l (molecular weight of bile salt 500). The 1:15 000 dilution is approximately equal to a 10 μmol/l bile salt solution, and was sufficient to cause a change in the response of the portal vein to noradrenaline. None of the bile salts used in this experiment caused changes at this concentration. On the other hand, the dilution of bile necessary to cause a change in the response of the vas deferens to noradrenaline is equivalent to about 1 mmol/l bile salt solution. At this concentration, all the bile salts attenuated the response to noradrenaline, but in this instance the concentration is above the upper limit seen in obstructive jaundice.

Thus it is apparent that at concentrations of bile salts between 10 μmol/l and 1 mmol/l, responsiveness to noradrenaline can be changed. These values refer to the total bile salt concentration and not to the concentrations of the individual bile salts themselves. The concentrations of individual bile salts required to induce a loss of responsiveness to noradrenaline are in excess of those seen in obstructive jaundice.

In addition to loss of end organ response to sympathetic stimulation (this study), other possible mechanisms may be involved in the causation of postoperative hypotension and increased susceptibility to shock in obstructive jaundice. For example, in one study of such patients peripheral endotoxaemia was found in 13 out of 24 patients at the time of operation, or they developed it during the 72 h after operation [17]; Intravenous infusion of endotoxin causes vasodilatation and the opening of peripheral arteriovenous shunts [18]. Another possibility is the failure of an appropriate tachycardiac response, which has been observed in bile duct-ligated dogs [19].

In conclusion, we have shown that ligation of the bile duct in rats can result in a loss of contractile response to catecholaminergic stimulation. This loss can be mimicked by exposing normal tissue to diluted bile, suggesting that high circulating levels of bile constituents are causative factors in the development of the peripheral circulatory disorders of obstructive jaundice. Of the many constituents present in bile, and this includes bile salts, bilirubin and cholesterol, we believe that bile salts are less important than the others in the mechanism of attenuated vascular response to noradrenaline and the peripheral circulatory disorders of obstructive jaundice.

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