Blunted pressor response to angiotensin and sympathomimetic amines in bile-duct ligated dogs

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Summary

1. The pressor and heart rate response to angiotensin II, noradrenaline, isoprenaline, tyramine and β-phenylethylamine were examined before and after bile-duct ligation in conscious trained dogs.

2. The pressor response to angiotensin II was markedly suppressed after bile-duct ligation, although responses to noradrenaline and the indirectly acting sympathomimetic amines were only slightly reduced.

3. Bradycardia, in response to the pressor drugs, and tachycardia, in response to isoprenaline, were unchanged after bile-duct ligation.

4. Refractoriness to the pressor action of angiotensin after chronic bile-duct ligation in the present study is similar to that reported previously by others in patients with liver cirrhosis.

5. No evidence was found in the bile-duct ligated dog to support a role of false neurotransmitters as mediators of the circulatory disturbances associated with liver injury.

Key words: angiotensin, blood pressure, indomethacin, isoprenaline, noradrenaline, obstructive jaundice, β-phenylethylamine, tyramine.

Introduction

Patients with cirrhosis and ascites suffer from a complex circulatory disturbance including increased cardiac output, decreased total peripheral resistance [1] and elevated renal vascular resistance [2, 3]. They also suffer from enhanced susceptibility to haemorrhagic hypotension [4]. This abnormality is accompanied by a reduced pressor response to angiotensin II [5-7], reduced peripheral vascular response to noradrenaline and reflex adrenergic stimulation [8, 9] and reduced pressor response to tyramine in the hypotensive oliguric phase of the disease [10]. One explanation for the reduced effectiveness of the adrenergic nervous system in cirrhosis is that false neurotransmitters accumulate in sympathetic nerve endings [11] but altered function of prostaglandins may also play a role, since treatment with indomethacin corrected the angiotensin pressor response in patients with cirrhosis [7].

Dogs with chronic bile-duct ligation show certain similarities to patients with cirrhosis of the liver, including elevated cardiac output and reduced peripheral resistance [12] with renal sodium and water retention [13, 14]. The purpose of the present study was to investigate whether such dogs also resemble patients with cirrhosis of the liver in their systemic pressor insensitivity to angiotensin II and indirectly acting sympathomimetic agents. If this was the case, then the dog model could be used to study the aetiology of the altered vascular responsiveness associated with liver injury. Two indirectly acting sympathomimetic agents, tyramine and β-phenylethylamine, were used, since the former, but not the latter, is a substrate for the high-affinity amine uptake system of adrenergic nerves [15, 16], whereas noradrenaline and isoprenaline were used to assess the functions of postsynaptic α- and β-adrenoceptors.
Methods

Experimental

Female mongrel dogs weighing 16–28 kg were maintained on standard dog food (Bonzo) supplemented with meat scraps and tap water. The animals were trained to stand quietly in a restraining harness. A branch of a femoral artery was cannulated under local anaesthesia and blood pressure monitored with a Statham P23Dd pressure transducer coupled to a Brush-Gould recorder. Integrated beat-to-beat heart rate was recorded from an ECG ratemeter channel with input from front and rear-limb needle electrodes. Drug infusions were made into a saphenous vein with a Harvard syringe pump. Cumulative dose–response curves for angiotensin II, noradrenaline and isoprenaline were established by stepwise increase in infusion rate every 5 min, to a maximum infusion rate of 4 ml/min. Increasing doses of tyramine and β-phenylethylamine were injected as boluses.

Control pressor and heart rate responses were determined in each dog, on three separate pre-operative days, and then the common bile duct was ligated with two silk sutures (six dogs) or sham operated (two dogs) under pentobarbitone anaesthesia. Cardiovascular responses to the various pressor agents were redetermined at 1, 3, 5 and 7 weeks after bile-duct ligation. A small (200 mg) sample of marginal liver tissue was removed from each dog at the time of bile-duct ligation and post mortem, for determination of hepatic monoamine oxidase with [14C]-tyramine (1-0 mmol/l) as substrate. Liver function tests (serum bilirubin, alkaline phosphatase, glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase activities) were determined in venous blood before and after bile-duct ligation at the time of blood pressure measurements. All experimental dogs were examined post mortem for absence of bile leakage into the duodenum and for gross pathology. Statistical significance was determined by Student’s paired t-test.

Drugs

Angiotensin II amide (Hypertensin, Ciba) was dissolved in sodium chloride solution (150 mmol/l: saline), stored at −20°C and diluted in saline immediately before use.

Noradrenaline acid tartrate (Sigma) and isoprenaline hydrochloride (Sigma) were dissolved in HCl (0-01 mol/l; 1 mg/ml) and stored at −20°C. Dilute solutions were made up just before use in saline containing ascorbic acid (10 mmol/l). Tyramine hydrochloride and β-phenylethylamine hydrochloride (Sigma) were dissolved in saline (1 mg/ml) and stored at −20°C. Solutions were discarded after 1 week.

Results

The general reaction of the dogs to chronic bile-duct ligation was as described previously [12, 14]. The animals survived for periods ranging from 2 to 14 weeks after bile-duct ligation, with loss of body weight and appearance of jaundice. Body weight decreased by an average of 0.5 kg at 1 week and 3.6 kg at 3 weeks postoperatively. Serum bilirubin levels increased to 7.6 ± 1.5 (SEM) mg/100 ml at 1 week and 12.2 ± 1.0 mg/100 ml at 3 weeks postoperatively. Serum glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase and alkaline phosphatase activities were markedly increased by 1 week postoperatively and these enzyme activities remained elevated until the end of the experiment. No significant changes in packed cell volume, plasma sodium or plasma potassium concentrations were seen. Resting systemic arterial blood pressure decreased slightly but insignificantly from 111-8 ± 2.6 mmHg at the start of the experiment to 104 ± 5.8 at 1 week and 97-6 ± 5.7 mmHg at 3 weeks postoperatively.

There was close agreement among the three control pressor-infusion tests in each dog and no significant change in response to any of the pressor agents was seen 1 week after sham operation. After bile-duct ligation, however, there was a powerful suppression of the pressor response to angiotensin II (Fig. 1). Suppression of the angiotensin response was evident 7 days

![Graph showing increase in mean arterial pressure (mmHg) in response to intravenous infusion of angiotensin II (ANG II) before (●) and 1 week (○) and 3 weeks (□) after ligation of the common bile duct in conscious dogs. Results shown are means ± SEM. For control and 1 week data, n = 6; at 3 weeks, n = 5.](image)
Pressor responses in obstructive jaundice

Tyramine

Fig. 1 shows the pressor responses to angiotensin II at 1 and 3 weeks postoperatively. The response at 5 weeks postoperatively was similar to that seen at 1 week. Both at 1 and at 3 weeks, pressor responses to dose levels of angiotensin between 10 and 80 pmol min$^{-1}$ kg$^{-1}$ were significantly different from control values ($P$ values 0.01–0.001).

Alterations in pressor responses to the sympathomimetic amines were of much smaller degree than those seen with angiotensin. Noradrenaline pressor responses were slightly but insignificantly reduced at 1 week postoperatively. At 3 weeks postoperatively responses to low doses of noradrenaline (0.6–2.4 nmol min$^{-1}$ kg$^{-1}$) were significantly reduced with respect to control values ($P < 0.05$), but responses to higher doses were normal (Fig. 2).

Slight but significant reductions in pressor responses to tyramine and $\beta$-phenylethylamine were seen at 1 and 3 weeks postoperatively (Fig. 3). The reductions in pressor responses to the indirectly acting sympathomimetic amines were of a similar extent to the reduction in the noradrenaline pressor. Intravenous injection of all the pressor substances produced bradycardia in conjunction with the increase in arterial blood pressure. Bradycardia after angiotensin infusion was less marked after bile-duct ligation in association with the smaller pressor response. Thus, an infusion rate of 40 pmol of angiotensin II min$^{-1}$ kg$^{-1}$ produced a fall in heart rate of 34.6 ± 8.2 (SEM) beats/min in the control period, but only 5 ± 5.8 beats/min 3 weeks after bile-duct ligation ($P < 0.05$).

Bradydardia after noradrenaline, tyramine and $\beta$-phenylethylamine injection was of a similar degree before and after bile-duct ligation (heart rate changes after injection of the indirectly acting amines are shown in Fig. 3). Infusion of isoprenaline caused tachycardia and a slight fall in mean arterial blood pressure. These responses were unchanged after bile-duct ligation (Fig. 3).

The administration of indomethacin to two dogs with a suppressed angiotensin response did not result in any changes in the angiotensin response (Fig. 4).

Hepatic monoamine oxidase activity was slightly reduced after bile-duct ligation [16.8 ± 3.32 (SEM) nmol min$^{-1}$ mg$^{-1}$ of protein as opposed to control values of 20.7 ± 2.67]. The difference was just significant ($P < 0.05$) by Student’s paired $t$-test.

Discussion

The present study shows a selective reduction in pressor responsiveness to angiotensin II after bile-duct ligation and was observed throughout the period in which the dogs remained alive. Fig. 1 shows the pressor responses to angiotensin II at 1 and 3 weeks postoperatively. The response at 5 weeks postoperatively was similar to that seen at 1 week. Both at 1 and at 3 weeks, pressor responses to dose levels of angiotensin between 10 and 80 pmol min$^{-1}$ kg$^{-1}$ were significantly different from control values ($P$ values 0.01–0.001).

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Discussion

The present study shows a selective reduction in pressor responsiveness to angiotensin II after
chronic bile-duct ligation in the dog. This observation provides a further point of similarity between this experimental model and human liver cirrhosis. As in the bile-duct ligated model, the pressor response to noradrenaline is much less affected in cirrhosis [6].

Pressor resistance to angiotensin in states such as sodium depletion or cirrhosis has commonly been related to an increased circulating level of endogenous angiotensin II, which has been suggested to down-regulate the number of vascular angiotensin receptors [18] or to reduce receptor availability by continuous occupancy [19]. No information exists at present on circulating angiotensin II levels in bile-duct ligated animals. Since these animals may show sodium retention, measurements of activity of the renin-angiotensin system should be related to sodium status. In bile-duct ligated dogs in sodium balance plasma renin activity was elevated [20], but, in similar animals showing sodium retention, normal values for plasma renin activity were found [21].

Enhanced vasodilator prostaglandin secretion opposes the vasoconstrictor effect of both angiotensin and noradrenaline [22] and administration of indomethacin corrected the selective suppression of the angiotensin response in cirrhotic patients [7]. Stimulation of prostaglandin synthesis did not appear to be the factor involved in angiotensin suppression in bile-duct ligated dogs, since indomethacin failed to correct the angiotensin response. The selective reduction in angiotensin response in bile-duct ligation may, therefore, be related to elevated circulating levels of endogenous hormone or to changes in the angiotensin receptors, although the presence of a specific circulating factor antagonistic to angiotensin is an additional possibility.

The present work does not provide evidence in favour of false neurotransmitter accumulation during liver injury, since pressor responses to the indirectly acting amines were reduced only slightly and in parallel with the reduction in noradrenaline responses. The experimental situation of chronic bile-duct ligation, however, is essentially different from cirrhosis in that extensive shunting of venous portal blood probably does not occur, and so the initial degradative function of the liver on dietary amines is not bypassed. Production of experimental hyperbilirubinaemia by bile-duct ligation also was not associated with any marked change in hepatic monoamine oxidase activity; the small change found is unlikely to be of physiological significance, although a reversible inhibition of monoamine oxidase could have occurred, which would not have been detected by the test in vitro. In the study of Mashford et al. [10] tyramine pressor responses were decreased only in the hypotensive oliguric phase of cirrhosis and normal responses were seen in the milder form of the disease.

The normal isoprenaline, and nearly normal noradrenaline, responses show that hyperbilirubinaemia did not markedly affect α- and β-adrenoceptor sensitivity. Reductions in sympathetic nervous function seen in cirrhosis [8], therefore, may be mainly the result of reduced neuronal release of neurotransmitter, although the reduction in response to low doses of noradrenaline may be physiologically important. The normal bradycardia in response to sympathomimetic amine injections shows that baroreceptor function is not grossly altered in liver injury, although the sensitivity of the baroreceptor mechanism was not measured by the present technique.

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References

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