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CCONTRACTILE RESPONSE OF FEMORAL ARTERIES IN PIGS WITH ACUTE LIVER FILURE

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INNHOLD

Contractile response of femoral arteries in pigs with acute liver failure

Running head: Vascular function in acute liver failure

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Background: Acute liver failure (ALF) is characterized haemodynamically by a progressive hyperdynamic circulation. The pathophysiological mechanism is unknown, but impaired contractility of vascular smooth muscle might play an important role. The aim of this study was to evaluate the vascular response to stimulation with norepinephrine and angiotensin II in endothelium-denuded femoral artery rings.

Materials and methods: Norwegian Landrace pigs weighing 27.1±0.5 kg (mean±SEM) were used. ALF was induced by performing a portacaval shunt followed by ligation of the hepatic arteries (n=6). Sham operated animals served as controls (n=5). Cumulative isometric concentration contraction curves were obtained after *in vitro* stimulation of the femoral artery rings with either angiotensin II (10^{-13}-10^{-5} mol/L) or norepinephrine (10^{-13}-10^{-3} mol/L).

Results: Angiotensin II caused a concentration-dependent contraction of the arterial segments, with no significant differences in vascular responses between the two groups. Maximum force generated did not differ (55±7 vs 56±7 mN, P=0.95). Furthermore, norepinephrine did not show any differences in the cumulative concentration-response curves and the maximum contractile force was not significantly different (87 ±8 vs 93±16 mN, P= 0.55).

Conclusions: This study documents for the first time that there are no signs of endothelium-independent peripheral vascular hyporesponsiveness to angiotensin II and norepinephrine in pigs with ALF.

Key words: Acute liver failure, vascular physiology, pig.

Word count: 202

Introduction

Loss of autoregulation of vascular tone with reduction of systemic vascular resistance is a major pathophysiological derangement in acute liver failure (ALF) (1). Although the occurrence of arterial hypotension is well recognised (2), the exact pathogenesis still remains unclear. Compensatory activation of neurohumoral reflexes involving the sympathetic neurons and renin-angiotensin systems normally counteract for the loss of vascular tone (3). However, despite increased catecholamine levels in plasma, patients remain hypotensive with high cardiac outputs and low systemic vascular resistance (4). Interventions to increase blood pressure are often necessary and catecholamines (e.g. epinephrine, norepinephrine and dopamine) are frequently given intravenously (5,6).

Two human studies have demonstrated increased circulating levels of nitric oxide (NO) in ALF (7,8). Furthermore, nitric oxide synthase (NOS) seems to be upregulated in hepatic arteries of ALF patients (9). Accordingly, excessive release of NO appears to be an important pathogenetic factor for the hemodynamic derangement observed. However, impaired contractility of vascular smooth muscle tone might play a significant role as well, though there are no studies that have specifically addressed this issue. To date, neither animal nor human studies have examined the peripheral vascular reactivity to vasoconstrictors in ALF.

We hypothesised that this arterial hypotension might be caused in part by a vascular hyporesponsiveness to vasoconstrictors. Thus the aim for this study was to investigate the *in vitro* vascular response to angiotensin II and norepinephrine in denuded (to exclude interference from endothelium-derived vasoactive factors) femoral artery rings in a porcine model of ALF (10) and in sham operated controls.

Materials and methods

The present study was performed with the approval of the Norwegian Experimental Animal Board. Eleven Norwegian female Landrace pigs, weighing 27.1 ± 0.5 kg (mean ± SEM) were used. Details regarding the animal room and preparation of the animals have been published elsewhere (11). ALF was induced by performing a portacaval shunt followed by ligation of the hepatic arteries (n=6). Sham operated animals served as controls (n=5). The surgical procedure has been described elsewhere (12).

Preparation of the vessels

The pigs were allocated to ALF or sham by the sealed enveloped system before induction of hepatic failure at t=0hrs. After six hours an incision was made medially on the right leg and the right femoral artery dissected free. The artery was clamped and immediately transferred into oxygenised (95% O_2, 5% CO_2) modified ice-cold Krebs-Henseleit solution (in mmol/L: NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.17, KH_2PO_2 1.18, $NaHCO_3$ 25.0, EDTA 0.026, glucose 11.1). The vessels were cleaned of connective tissue under a magnifying glass and the endothelium was removed mechanically by gently rubbing with a rough steel rod. The vessels were cut into 2-mm-wide rings and mounted in an organ bath filled with modified Krebs-Henseleit solution, which was continuously exchanged with oxygenised (95% O_2, 5% CO_2) modified Krebs-Henseleit buffer via a syringe pump. Temperature was maintained at 37°C with an outer water jacket. The vasoconstriction was measured with an isometric force transducer (Fort 10; World Precision Instruments, Berlin, Germany). Transducer outputs were amplified (Gould Inc. Recording System, Cleveland, OH) and recorded at a pc by an in-house developed LabVIEW® program (Danielsen, Arild, arildd@fagmed.uit.no). During an equilibration period of 1 hour, a baseline tension of 20 mN was adjusted. Acetylcholine (10^{-5} mol/ L) was added to prove the absence of intact endothelium. In order to ensure viability of the vessels 40 mmol/L KCl was perfused through the organ bath at the end of the experimental period.

Vascular response to stimulation of Angiotensin II and norepinephrine

The contractile response to angiotensin II and norepinephrine was assessed in the denuded femoral artery rings obtained from 11 pigs (5 Sham, 6 ALF). The drugs were dissolved in oxygenised modified Krebs-Henseleit buffer and added via a syringe pump to the organ bath at a constant infusion rate of 120 mL h^{-1}. Cumulative concentration-response curves for angiotensin II (10^{-13} to 10^{-5} mol/L) and norepinephrine (10^{-13} to 10^{-3} mol/L) were plotted. The concentration ranges were identified as appropriate from prior pilot experiments and in accordance to concentrations applied to human hepatic arteries (14). Contraction force was monitored continuously and maximum force was noted within 10 minutes. These readings were used to plot a cumulative dose-response curve.

Materials

All substances were purchased from Sigma-Aldrich, Oslo, Norway.

Statistical analysis

Statistical analyses were performed by using the SPSS 11.0 software package (SPSS, Chicago, IL) and Excel 5.0 (Microsoft). Data are presented as mean ± SEM. Vasoconstriction is expressed in milli Newton (mN). The unpaired Student's t-test was applied to test for differences in maximum force generated by the vessels and to test for differences in haemodynamic variables at t=6hrs. P value ≤ 0.05 was taken to determine statistical significance.

Results

The pigs with ALF developed evidence of progressive increase in cardiac index (CI) ($P< .001$) and a reduction in mean arterial pressure (MAP) ($P=0.016$) and systemic vascular resistance index (SVRI) ($P=0.008$), while the sham animals did not.

Stimulation with angiotensin II caused a concentration-dependent contraction of the arterial segments. There were no differences in vascular responses between the two vessel groups (Figure 1). The contractile response to 10^{-5} mol/L angiotensin II was less than for 10^{-7} mol/L, which obeys a plateau phase in the contractile response. Maximum force generated after stimulation with angiotensin II did not differ between the ALF and control groups (55±7 and 56±7 respectively, $p=0.95$). (Figure 2). A plateau effect seemed to develop at concentrations $\geq 10^{-5}$ mol/L. There was no significant difference between the groups for norepinephrine 10^{-3} mol/L. Maximum force generated after stimulation with norepinephrine did not differ between the two vessel groups (87 ±8 and 93±16 respectively, $p= 0.55$). Endothelial denudation was confirmed by adding acetylcholine to the organ bath after a stabilisation period of one hour. No vascular relaxation was observed. Intact vascular viability was tested before termination of the experiments by adding 40 mmol/L KCl to the organ bath. All vessels were viable with a significant increase in the contraction force after stimulation (data not shown).

Discussion

The present study investigated the vascular response to angiotensin II and norepinephrine in denuded femoral arteries from pigs with ALF. Our results show that neither maximal contraction forces nor the dose-response curves differed between pigs with ALF and sham operated controls. Accordingly, we found no evidence for a peripheral vascular hyporesponsiveness to these vasoconstrictor neurohormones in ALF.

The arterial hyporesponsiveness observed in other studies in liver failure may result either from the presence of excessive vasorelaxing factors or an impairment of the contractile apparatus of the vascular smooth muscle cells. However, a combination of these mechanisms is the most likely. Smith et al. found impaired vascular response to phenylephrine in hepatic arteries from ALF patients (9), but this was not confirmed in a recently published human study of omental arteries (13). Since both phenylephrine and norepinephrine act via the α_1 -receptors, we assume that our results are comparable and a difference (if any) would have been detectable over the concentration and time range investigated. Smith et al. performed their study on intact hepatic artery rings, but they were unable to demonstrate any relaxation to stimulation with acetylcholine, which implies a lack of endothelium. The difference in result of our study and that of Smith et al is likely to reflect differences in the responsiveness of vessels obtained from different territories. We studied femoral arterial rings whereas Smith et al studied hepatic arterial rings. Peripheral vascular response to angiotensin II has not previously been described either in experimental or in human ALF, but Schepke et al. demonstrated vascular hyporesponsiveness to angiotensin II in hepatic arteries in humans with cirrhosis (14). This finding has recently been confirmed in a study in patients with cirrhosis, where hyporesponsiveness to angiotensin II was demonstrated in the forearm, but this hyporesponsiveness was corrected following inhibition of NOS, suggesting that this hyporesponsiveness is principally due to enhanced NO generation (15). Our porcine ALF model exhibited a hyperdynamic circulation with high cardiac output and decreased systemic vascular resistance during the first 6 hours after induction of ALF (Table 1) (10). Accordingly, our data support the suggestion that it is unlikely that the peripheral vasodilation in our model is due to hyporesponsiveness to vasoconstrictors, but is likely to result from increased vasodilatory factors. However, our results are based on *in vitro* experiments on femoral arteries and it is not possible from these experiments to comment upon vascular responses in the splanchnic bed. Whether smaller resistance arterioles from the hind leg or other vascular beds such as the splanchnic circulation exhibit a similar pattern upon stimulation with angiotensin and norepinephrine remains unknown.

On the other hand, nitric oxide synthase (NOS) is upregulated in hepatic arteries and increased levels of nitric oxide (NO) have been demonstrated in humans with ALF (7,8). Moreover, prostaglandins are another group of endothelium-derived vasodilators, which play an important role in the autoregulation of vasogenic tone. Inducible cyclooxygenase (COX-2) was recently demonstrated to be upregulated in omental arteries harvested from ALF patients and selective inhibition of COX-2 had a significant influence on both norepinephrine and thromboxane A_2 elicited vasoconstriction *in vitro* (13). Taken together, we believe these results support our hypothesis that the pathophysiological basis for the hyperdynamic circulation in ALF is caused by increased amounts of vasodilators rather than a primary hyporesponsiveness to endogenous vasoconstrictors.

In summary, this study documents for the first time that there are no signs of peripheral vascular hyporesponsiveness to angiotensin II and norepinephrine in pigs with ALF. Accordingly, our data give further support to the suggestion that the primary vascular pathogenesis in ALF is associated with excessive amounts of vasodilators rather than impaired response to circulating vasopressors.

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Legends to table and figures

Table 1

Systemic hemodynamic characteristics of pigs with ALF and sham operated controls.

Figure 1

Cumulative concentration-response curves to angiotensin II in isolated, endothelium-denuded femoral arteries rings of pigs with ALF and sham-operated controls. Contraction forces are expressed in milli Newton as mean±SEM.

Figure 2

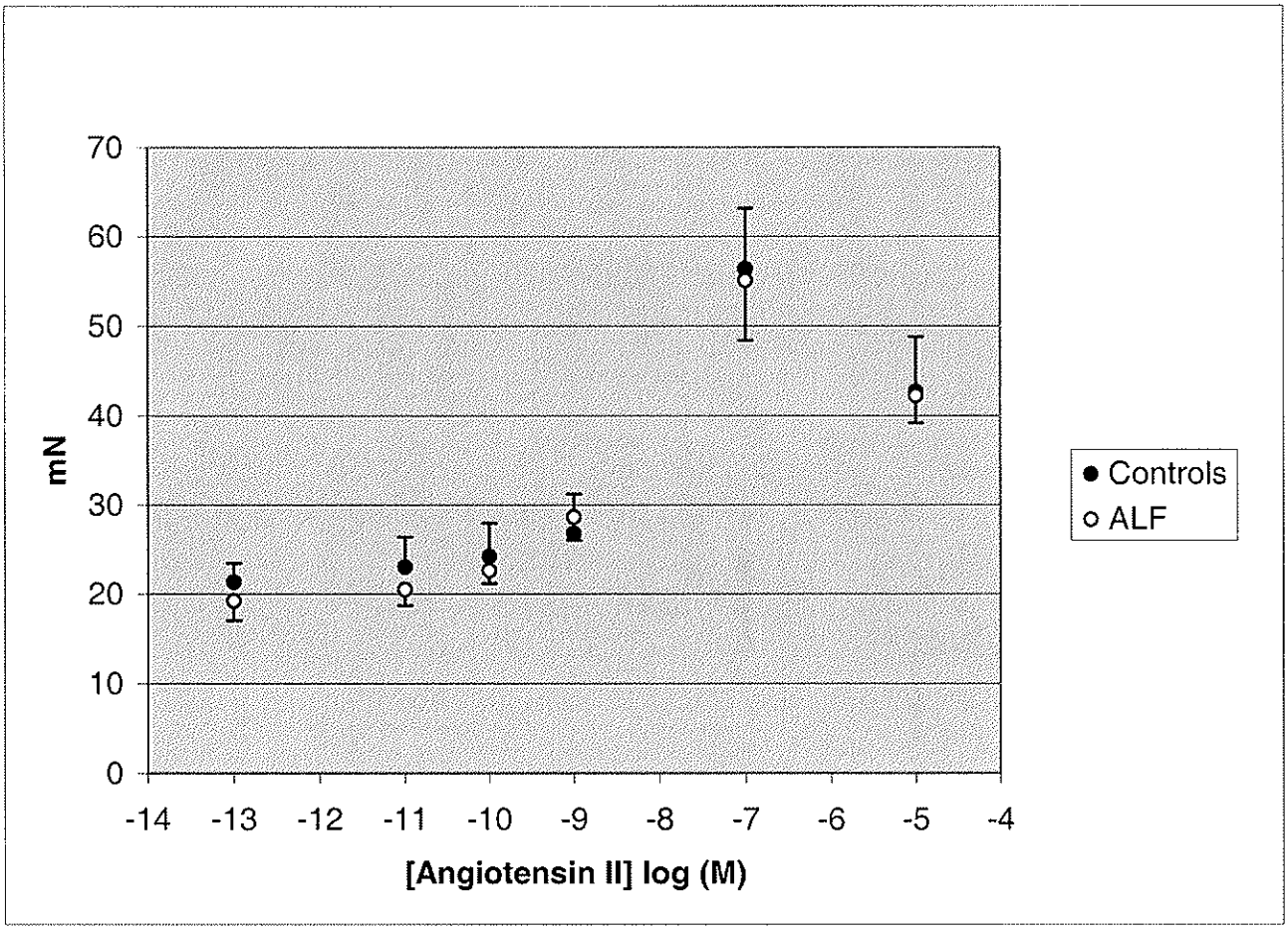
Cumulative concentration-response curves to norepinephrine in isolated, endothelium-denuded femoral arteries rings of pigs with fulminant hepatic failure and sham-operated controls. Contraction forces are expressed in milli Newton as mean±SEM.

Table 1

		Time			
Variable	**Groups**	**0 hrs**	**2 hrs**	**4 hrs**	**6 hrs**
MAP (mmHg)	ALF	83±6	84±4	67±3	57±3
	Controls	104±8	102±6	88±5	88±4
Cardiac index (mL min^{-1} kg^{-1})	ALF	161±15	156±10	171±14	182±8
	Controls	166±7	137±13	140±8	139±5
Systemic vascular resistance index, (dyne sec cm^{-5} kg^{-1})	ALF	508±54	522±46	375±28	287±14
	Controls	596±45	740±91	606±65	595±38

Mean ± SEM

Figure 1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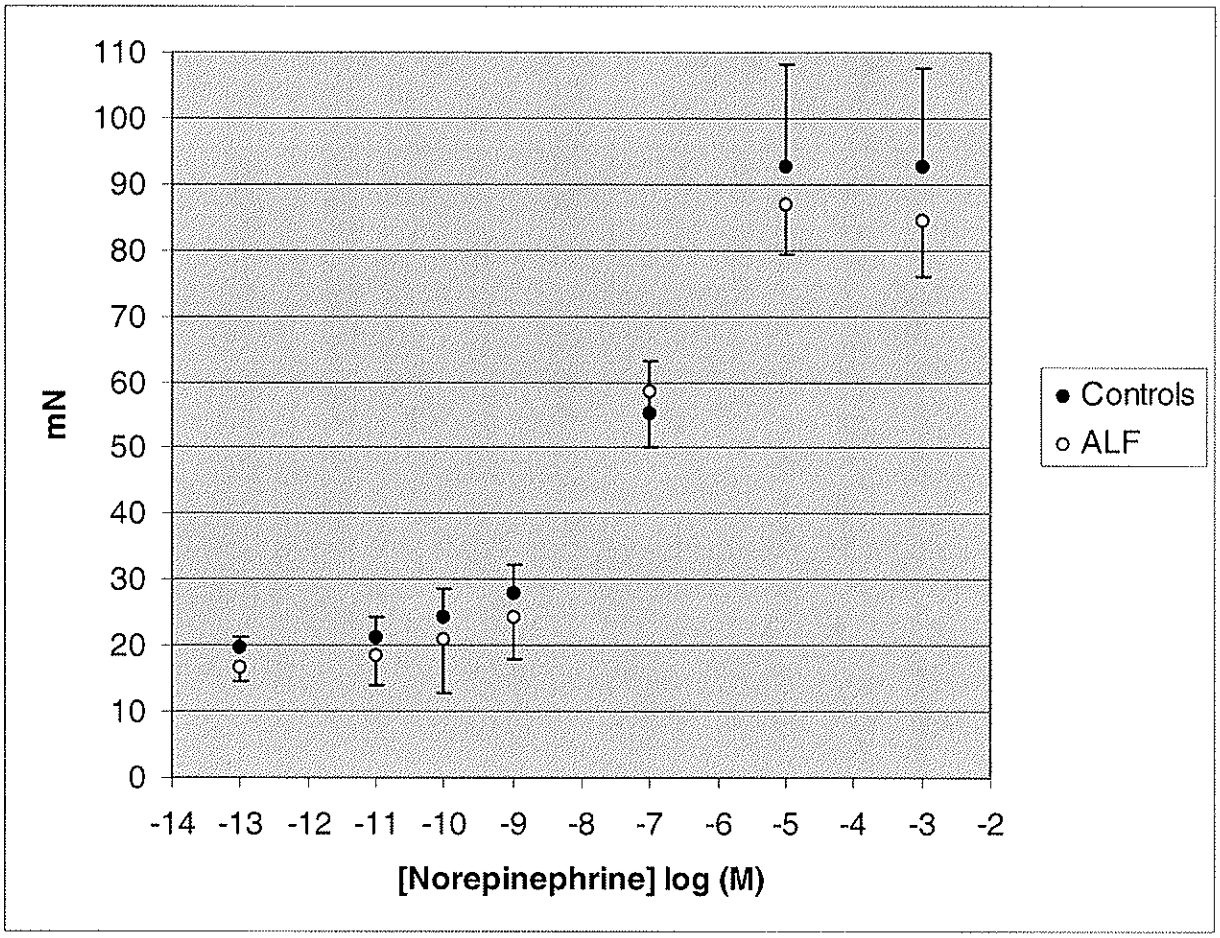
-5
-4
[Angiotensin II] log (M)
Controls
ALF

Figure 2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-14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4 -3 -2
[Norepinephrine] log (M)
Controls
ALF