

## LIVER DISEASE

## Effect of neonatal capsaicin treatment on haemodynamics and renal function in cirrhotic rats

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**Background:** Mechanisms underlying abnormalities of circulation and renal function in cirrhosis are not completely understood. Our previous study revealed that primary afferent denervation by neonatal capsaicin treatment prevented the development of hyperdynamic circulation in portal hypertensive and cirrhotic rats.

**Aims:** The present study aimed to clarify the role of capsaicin sensitive nerves in the development of renal dysfunction and ascites formation in cirrhosis.

**Methods:** Rat pups were injected with capsaicin (50 mg/kg) or vehicle and allowed to grow. When they reached adulthood, cirrhosis was induced by bile duct ligation while controls received sham operation. Cardiac output and regional blood flows were measured by radioactive microspheres, glomerular filtration rate by  $^3\text{H}$  inulin clearance, and urine volume, sodium excretion, and ascites formation were determined. Immunohistochemical staining for Fos in the brain stem cardiovascular regulatory nuclei, the nucleus of the solitary tract, and ventrolateral medulla was measured as an index of central neuronal activation.

**Results:** Increased cardiac output and renal blood flow, and decreased systemic vascular resistance, arterial pressure, renal vascular resistance, and glomerular filtration rate, as well as ascites, were found in vehicle treated cirrhotic rats. Neonatal capsaicin treatment completely blocked the development of hyperdynamic circulation and ascites, and improved renal function in cirrhotic rats. This was associated with complete abrogation of brain stem neuronal activation in capsaicin treated cirrhotic rats.

**Conclusions:** These results indicate that intact primary afferent innervation is necessary for the development of not only the hyperdynamic circulation but also the renal dysfunction and ascites formation characteristic of cirrhosis.

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Cirrhosis is associated with hyperdynamic circulation, consisting of peripheral vasodilatation and increased cardiac output. Regional blood flow to many vascular beds is also increased. Renal sodium and water retention eventually leads to ascites formation. Mechanisms underlying the hyperdynamic circulation and renal dysfunction remain incompletely understood. However, a theory proposed in 1988, the "peripheral vasodilation" hypothesis,<sup>1</sup> attempts to link these phenomena together. This theory suggests that peripheral arteriolar vasodilatation is the primary initiating factor that leads to effective arterial underfilling of the circulation, sensed by the kidney as an inadequate volume and thus inducing salt and water retention.

Factors responsible for arterial vasodilatation in cirrhosis remain unclear but excessive activity or levels of vasodilators such as glucagons,<sup>2-4</sup> nitric oxide,<sup>5-7</sup> prostaglandins,<sup>8-10</sup> and calcitonin gene related peptide (CGRP)<sup>11,12</sup> have been suggested. Our previous study found that the hyperdynamic circulation in portal hypertensive and cirrhotic rats is blocked by neonatal capsaicin treatment.<sup>11</sup> Capsaicin is the active ingredient of the pungent *capsicum* peppers, and in acute doses activates the primary afferent nerves whereas in higher doses given to neonatal animals it permanently ablates these nerves. It has proven remarkably useful over the past three decades as a pharmacological tool to explore the physiology of primary afferent nerves.

The aim of the present study was to investigate further the effects of capsaicin sensitive nerves on the development of hyperdynamic circulation and renal dysfunction, including ascites formation, in rats with cirrhosis induced by bile duct ligation (BDL).

## MATERIALS AND METHODS

The protocol was approved by the University of Calgary Faculty of Medicine Animal Care Committee and the experimental procedures were carried out in accordance with guidelines established by the Canadian Council on Animal Care.

### Capsaicin treatment

Newborn Sprague-Dawley rat pups (Charles River Canada, St Laurent, Canada) on the second day of life, under light halothane anaesthesia, were subcutaneously injected with 50 mg/kg capsaicin (Sigma Chemicals, St Louis, Missouri, USA) dissolved in a vehicle of 80% physiological saline, 10% Tween 80, and 10% ethanol (2% in oxygen), according to methods described previously.<sup>13,14</sup> A control group received an equivalent volume of only the vehicle. Rats were then returned to their dams and reared until a body weight of 150–200 g, under a constant photoperiod (12 hour light:dark cycles) at 20°C with free access to food and water.

### Cirrhosis induction

Cirrhosis was induced by the method of BDL, as previously described.<sup>15</sup> Briefly, under halothane anaesthesia, through a midline laparotomy, the common bile duct was doubly ligated with 4-0 silk thread and sectioned between the ligatures. Incisions were closed with silk, and the animals were given an

**Abbreviations:** BDL, bile duct ligated; NTS, nucleus of the solitary tract; VLM, ventrolateral medulla; CGRP, calcitonin gene related peptide; cpm, counts per minute; PBS, phosphate buffered saline.

intramuscular injection of benzathine penicillin G (30 000 IU) immediately after operation to prevent sepsis. Control rats (sham) underwent exactly the same surgical procedures except for ligation of the bile duct. Animals were then kept for 4–5 weeks when a body weight of 300–400 g had been attained, by which time an obvious cirrhosis had developed. Chronic cholestasis was also evidenced by marked elevations in aspartate aminotransferase, alanine aminotransferase, and bilirubin in BDL rats, as we have previously reported.<sup>16</sup> Capsaicin treatment did not change any of these parameters (data not shown). Thus there were four groups of rats: sham vehicle treated (n=7); sham capsaicin treated (n=7); BDL vehicle treated (n=11); and BDL capsaicin treated (n=14).

### Experimental procedures

Rats were housed individually in metabolic cages and food but not water was removed from the cages for 24 hours before the study. During this time urine volume of each rat was measured gravimetrically. Thereafter rats were prepared for the study as previously described.<sup>17</sup> Briefly, under halothane anaesthesia, an intravenous cannula was inserted into the left femoral vein using PE-50 tubing. The left femoral artery was cannulated with a PE-50 catheter to allow collection of blood samples and measurement of mean arterial pressure and heart rate using a pressure transducer (Gould P23XL, Oxnard, California, USA) connected to a recorder (Gould Instruments, Cleveland, Ohio, USA). The left ventricle was cannulated with PE-50 tubing via the right carotid artery and the correct position was confirmed by pressure tracings. Catheters were subcutaneously tunnelled and exteriorised at the dorsal surface of the neck.

Animals were then subjected to a 3 cm midline incision. The presence or absence of ascites was determined by gently swabbing the peritoneal cavity with preweighed cotton gauze pads, which were weighed afterwards to determine ascites volume. Pilot studies in unoperated and sham operated rats of similar body weight showed that all had <2 ml of fluid in the peritoneal cavity, so ascites was therefore defined as >2 ml of peritoneal fluid.

A PE-10 catheter was inserted into the left ureter and exteriorised through the abdominal wall. The distal end of the catheter was threaded subcutaneously and exteriorised along the leg. All incisions were closed with silk ligatures following local application of 5% lidocaine gel to minimise postoperative pain. Animals were then placed in individual metabolic cages for a recovery period of two hours. After blood pressure and heart rate had been stable for at least one hour, the studies were performed.

Glomerular filtration rate was assessed by <sup>3</sup>H inulin (Amersham Life Science, Arlington Heights, Illinois, USA) clearance.<sup>18</sup> A solution of 2  $\mu$ Ci <sup>3</sup>H inulin/ml was prepared with physiological saline and infused at 33  $\mu$ l/min through the left femoral vein for one hour. Two successive 30 minute periods were carried out for the clearance study. Urine was collected continuously in preweighed liquid scintillation tubes while blood samples were obtained at the midpoint of each period at a rate of 0.8 ml/min for one minute. Volume losses from blood sampling were replaced by physiological saline. The radioactivity of the blood sample was determined by a liquid scintillation beta counter (Wallack RackBeta, Turku, Finland). Urine volume was determined gravimetrically assuming a density of 1.0 g/ml. Urinary sodium concentration was determined by an ion selective electrode (Hitachi Instruments).

Cardiac output and regional blood flows were measured by radioactive microspheres and the reference sample method.<sup>19</sup> A precounted sonicated aliquot of ~60 000–80 000 microspheres of 15 $\pm$ 3  $\mu$ m diameter labelled with <sup>113</sup>Sn (New England Nuclear, Boston, Massachusetts, USA) was injected over 15 seconds into the left ventricle. The spheres were flushed with 0.7 ml of physiological saline. Beginning 5 seconds before microsphere injection, the reference sample

was withdrawn from the femoral artery at 0.8 ml/min for one minute into a syringe connected to a motor driven withdrawal pump. Radioactivity of the sample was counted by a gamma counter (Wallack 1480 Wizard 3, Turku, Finland).

### Calculations

Cardiac index (ml/min/100 g body weight) was calculated as (total counts per minute (cpm) injected  $\times$  0.8 ml/min/reference sample cpm)/100 g body weight. Blood flow (ml/min/100 g body weight) of each organ was calculated as (organ cpm  $\times$  0.8 ml/min/reference sample cpm)/100 g body weight. Portal tributary blood flow was calculated as the sum of the flows in the stomach, spleen, small intestine, colon, and mesentery with pancreas. Hepatic arterial flow was calculated as liver cpm as described above. Systemic vascular resistance (mm Hg/ml/min/100 g body weight) was calculated as mean arterial pressure/cardiac index. Renal vascular resistance (mm Hg/ml/min/100 g body weight) was calculated as mean arterial pressure/renal blood flow.

Inulin clearance data from the two successive 30 minute periods in each study were averaged. Glomerular filtration rate (ml/min/100 body weight) was equated to clearance of <sup>3</sup>H inulin /100 g body weight.

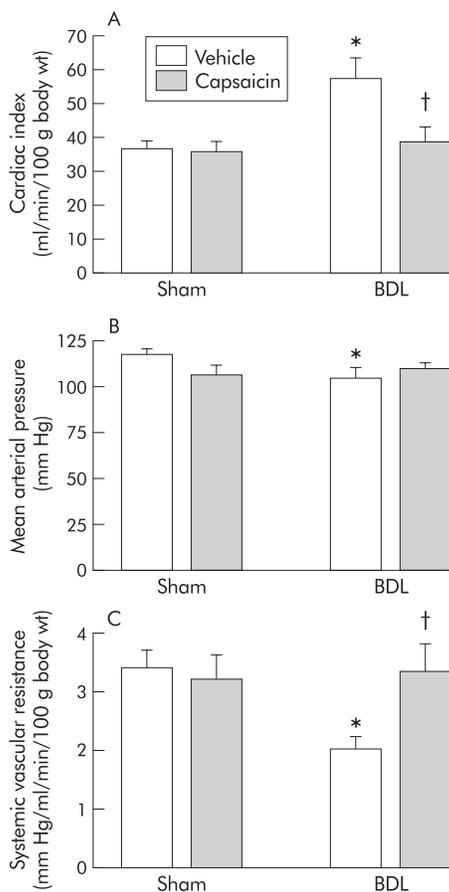
### Hepatic collagen determination

Liver tissue was immediately fixed with 10% formalin in phosphate buffered saline (PBS). Samples were later embedded in paraffin and sectioned (3  $\mu$ m). After being mounted on glass slides and deparaffinised, sections were immersed for 10 seconds in saturated aqueous picric acid containing 0.1% Fast Green FCF (Sigma) and rinsed with distilled water before staining for 15 minutes with 0.1% Sirius Red F3BA (Polysciences Inc., Warrington, Pennsylvania, USA). Sirius Red and Fast Green selectively bind to collagenous and non-collagenous proteins, respectively. Hepatic fibrosis was estimated as previously described.<sup>20</sup> Coded slides were independently assessed under light microscopy by two blinded observers. The extent of hepatic fibrosis (scored from 1 to 4) was estimated according to the following criteria: (1) collagen restricted to portal areas; (2) mild expansion of loose periportal collagen into surrounding parenchyma associated with bile ductular proliferation; (3) moderate expansion of periportal collagen into surrounding parenchyma with significant organisation of fibrous tissue and mild portoportal bridging; and (4) extensive portoportal and portoportal fibrous bridging. There was excellent agreement between the two observers; scores never differed by more than 1 point.

### Brain stem medullary immunohistochemical staining for Fos

For the Fos studies, separate groups of animals were prepared as described previously.<sup>21, 22</sup> In brief, rats were anaesthetised with 50 mg/kg intraperitoneal sodium pentobarbitol (MTC Pharmaceuticals, Mississauga, Ontario, Canada). To avoid Fos expression due to stress, rats that struggled or squealed during injection were removed from further study. The right femoral artery and vein were cannulated with PE 50 tubing (Becton-Dickenson, Parsippany, New Jersey, USA). A few minutes before any incision, topical lidocaine 2% ointment (Astra Pharma, Mississauga, Ontario, Canada) was applied locally to avoid Fos expression due to pain. Haemorrhage was induced by manually withdrawing 12 ml blood/kg body weight (estimated 20% of blood volume) at a rate of 2 ml/min. The right femoral arteries and veins were also cannulated in unchallenged controls, but no blood withdrawn.

After 90 minutes, rats were intravenously infused with sodium pentobarbital (50 mg/kg), perfused with 1 l/kg body weight of cold PBS at pH 7.4, followed by 1 l/kg of ice cold 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4. Brains were carefully removed from the rats and post fixed overnight



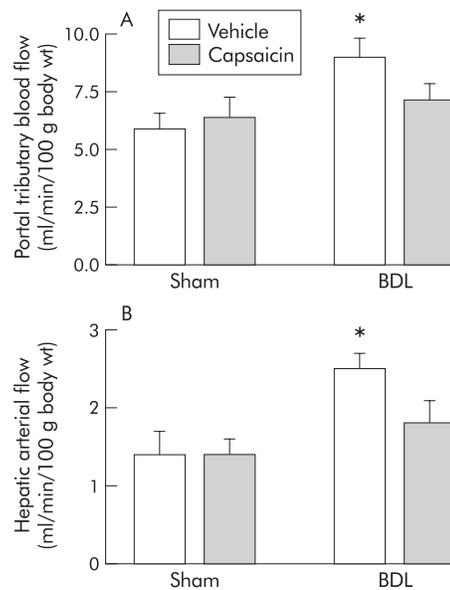
**Figure 1** Systemic haemodynamics in the four groups of rats. Cardiac index (A), mean arterial pressure (B), and systemic vascular resistance (C) in bile duct ligated (BDL) or sham operated rats treated with capsaicin or vehicle. Data are mean (SEM). Significant difference: \*compared with the sham vehicle group; †compared with BDL vehicle rats.

in 4% paraformaldehyde at 4°C and then cryoprotected in 30% sucrose in 0.1 M phosphate buffer for 1–3 days at 4°C. Serial 40 µm sections of brains were cut using a cryostat, and sections were analysed for Fos immunoreactivity. Adjacent sections were stained with cresyl violet to reveal the nuclear boundaries.

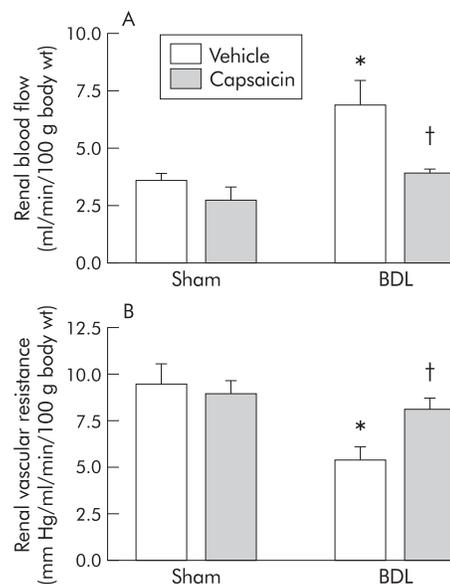
Fos was detected using rabbit anti-Fos polyclonal antisera (Oncogene Science, Manhasset, New York, USA). In brief, sections were incubated in a blocking serum consisting of 1.5% normal goat serum (Vector Labs, Burlingame, California, USA) diluted in PBS containing 0.4% Triton X-100 for one hour at room temperature. The blocking serum was removed and sections were incubated with the primary antibody, rabbit anti-Fos polyclonal antisera diluted 1:20 000 with blocking serum, for 48–72 hours at 4°C. Sections were incubated with secondary antibody, biotinylated goat antirabbit IgG (1:200, Vector), washed, incubated in Vectastain Elite ABC Reagent (Vector) and diaminobenzidine-nickel peroxidase substrate (Vector) for colour development, and mounted on chrome-alum coated slides.

### FOS quantification

The diaminobenzidine-nickel stained Fos cells were identified by their size, shape, and colour. The relevant brain nuclei were identified using the cresyl violet stained sections. The main parts of the nucleus tractus solitarius (NTS) and ventrolateral medulla (VLM) were chosen from three representative sections of each area. Counting was done visually at 200×



**Figure 2** Splanchnic haemodynamics in the four groups of rats. Portal tributary blood flow (A) and hepatic arterial flow (B) in bile duct ligated (BDL) or sham operated rats treated with capsaicin or vehicle. Data are mean (SEM). \*Significant difference compared with the sham vehicle group.

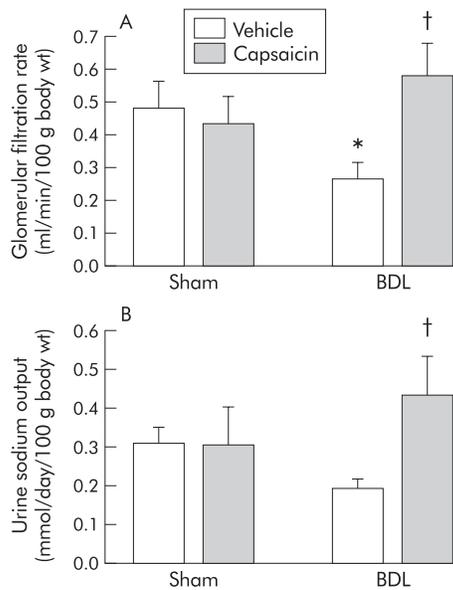


**Figure 3** Renal haemodynamics in the four groups of rats. Renal blood flow (A) and renal vascular resistance (B) in bile duct ligated (BDL) or sham operated rats treated with capsaicin or vehicle. Data are mean (SEM). Significant difference: \*compared with sham vehicle group; †compared with BDL vehicle rats.

magnification. The final value used was the mean of the three sections. All slides were analysed together in order to reduce variability in counting.

### Statistical analysis

Results are expressed as mean (SEM). The ascites data were analysed by Fisher's exact test and other data by one way analysis of variance with a Newman-Keuls post hoc test for multiple comparisons. The significance level was set at  $p < 0.05$ .



**Figure 4** Renal function in the four groups of rats. Glomerular filtration rate (A) and urine sodium concentration (B) in bile duct ligated (BDL) or sham operated rats treated with capsaicin or vehicle. Data are mean (SEM). Significant difference: \*compared with sham vehicle group; †compared with BDL vehicle rats.

## RESULTS

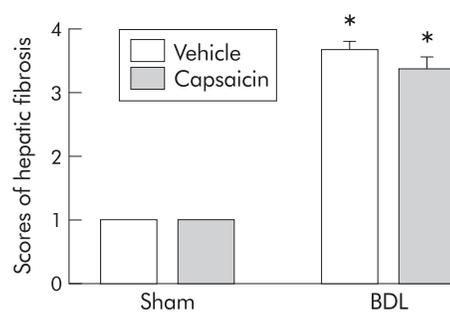
### Systemic and splanchnic haemodynamics

Vehicle treated BDL rats exhibited the expected hyperdynamic circulation, with increased cardiac index, hepatic arterial flow, and portal tributary blood flow, and decreased mean arterial pressure and systemic vascular resistance, compared with sham operated vehicle rats (figs 1, 2). Capsaicin treated BDL rats displayed similar systemic haemodynamics as sham operated controls, confirming our previous observations that hyperkinetic circulation is absent in this group (figs 1, 2).

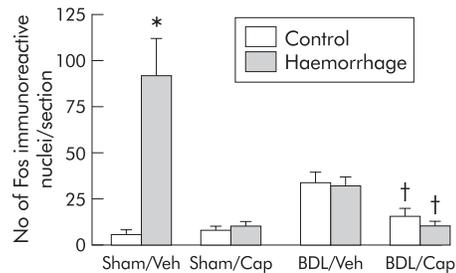
### Renal function and haemodynamics

In vehicle treated cirrhotic rats compared with the corresponding vehicle treated sham controls, renal vascular resistance was decreased and renal blood flow increased (fig 3). In contrast, glomerular filtration rate was significantly decreased in BDL vehicle treated rats compared with sham vehicle treated rats (fig 4). Capsaicin treatment completely eliminated the abnormalities of renal function observed in the BDL vehicle group. These parameters were similar between BDL capsaicin treated and sham vehicle treated groups. Renal function in sham capsaicin treated rats remained unchanged compared with sham vehicle treated rats (figs 3, 4).

Urine sodium output was lower in vehicle treated BDL rats compared with sham vehicle treated animals but this was not significant (fig 4). However, capsaicin treatment significantly increased urine sodium excretion in BDL rats compared with the vehicle BDL group (fig 4). Urine volumes showed marked



**Figure 5** Hepatic collagen deposition in the four groups of rats. Hepatic fibrosis scores in bile duct ligated (BDL) or sham operated rats treated with capsaicin or vehicle. Data are mean (SEM). \*Significant difference compared with the corresponding sham group.



**Figure 6** Number of Fos immunoreactive neurones in the nucleus of the solitary tract. Bile duct ligated (BDL) or sham operated rats treated with capsaicin (Cap) or vehicle (Veh) with (haemorrhage) or without haemorrhage challenge (control). Data are mean (SEM) of eight animals in each group. Significant difference: \*compared with sham vehicle control group; †compared with BDL vehicle rats.

individual variability but the means did not differ significantly between the four groups (in ml/day): sham 50 (9); sham capsaicin 19 (4); BDL 57 (8); BDL capsaicin 48 (7).

Seven of 11 BDL vehicle treated rats showed ascites of 3–19 ml whereas none of 14 BDL capsaicin treated animals had ascites ( $p < 0.05$ ; Fisher's exact test). No ascites was detected in the two sham operated groups (table 1).

### Hepatic fibrosis

In both vehicle and capsaicin treated sham rats, normal amounts of hepatic collagen were seen in the portal areas (fig 5). In contrast, both the BDL vehicle and BDL capsaicin rat livers showed fibrous expansion and parenchymal loss. Bridging (portocentral) fibrosis was frequently observed. There were no significant differences in fibrosis scores between BDL vehicle and BDL capsaicin groups (fig 5).

### Medullary neuronal Fos immunoreactivity

Compared with sham vehicle treated rats, BDL vehicle rats showed increased Fos expression in the NTS (fig 6), similar to previous studies.<sup>21,22</sup> In contrast, capsaicin treated BDL rats

**Table 1** Effect of capsaicin treatment on ascites formation

	Sham vehicle	BDL vehicle	Sham capsaicin	BDL capsaicin
Ascites present	0	7*	0	0†
Ascites absent	7	4	7	14
Total (n)	7	11	7	14

BDL, bile duct ligated.

Ascites was defined as <2 ml of peritoneal fluid.

Significantly different ( $p < 0.05$ ) from: \*sham vehicle group; †BDL vehicle group (Fisher's exact test).

had very low levels of Fos expression, comparable with both vehicle and capsaicin treated sham operated rats. The expected reactivity of the NTS to haemorrhage was also completely eliminated in the BDL and sham operated capsaicin treated rats, as well as the BDL vehicle group (fig 6). Fos immunoreactivity in the VLM showed a similar pattern in all groups (data not shown).

## DISCUSSION

The results of the present study indicated that capsaicin treated cirrhotic rats failed to demonstrate the expected derangement of the circulation and renal function. Even formation of ascites was blocked in these animals. This suggests that primary afferent nerves can modulate not only haemodynamics but also renal function in cirrhosis.

In the first part of the study, we decided to reconfirm that in our laboratory neonatal capsaicin treatment successfully abrogates the hyperdynamic circulation of cirrhosis. This was because following our initial observation of this capsaicin effect in both BDL cirrhotic and portal hypertensive rats,<sup>11</sup> Fernandez *et al* failed to demonstrate this finding in portal hypertensive rats.<sup>23</sup> Thus the current results again showing absence of hyperdynamic circulation in capsaicin treated BDL rats were reassuring. We believe that significant differences in our experimental protocols and those of Fernandez *et al* explain the discrepancy, in particular our conscious animal protocols versus their use of anaesthetic. Moreover, Fernandez *et al* did not study the BDL rat model. Finally, several other studies indirectly support our position that primary afferent nervous tone is altered in this BDL model. Specifically, Ferraz *et al* found that gastric microcirculatory responses to acute capsaicin application were depressed<sup>24</sup> in the same BDL model, and Casini *et al* reported that neonatal capsaicin treatment significantly reduced hepatic fibrosis in the eight week BDL rat.<sup>25</sup> A preliminary study has reported significant effects of an ablative dose of capsaicin on hepatic blood flow in cirrhotic rats.<sup>26</sup>

We chose to study the chronic BDL rat model because in most centres, and certainly in our hands, the majority of these animals develop easily detectable ascites. The haemodynamics and renal functional changes in the BDL rat have been extensively characterised previously. In those respects, our results in the vehicle treated BDL rats generally agree with the literature.<sup>27–29</sup> However, because the four week BDL rat has dramatically increased renal blood and plasma flows, unlike the cirrhotic patient, direct extrapolation of the present results to the human condition would be speculative.

There are four possible explanations for normalisation of renal function in our capsaicin treated BDL rats. Firstly, could the results be due to a direct effect of capsaicin itself, independent of its effect on the primary afferent nerves? The available evidence concerning the renal effects of neonatal capsaicin treatment suggests in fact that the opposite result might occur. Previous studies indicated that neonatal capsaicin administration to normal rat pups results in an antinatriuretic and antidiuretic effect in adult life.<sup>30–32</sup> The mechanism underlying this possible renal inhibitory effect of capsaicin remains unclear. However, acute capsaicin dosing appears to have no effect on renal function,<sup>30–32</sup> so the effect of neonatal treatment is likely mediated through primary afferent denervation. In any case, we did not observe any renal or haemodynamic effect of capsaicin in our sham operated control rats, and the effects on renal function in our BDL rats were opposite to the effects described in normal rats. Accordingly, we believe that capsaicin per se did not cause the changes in renal function in the present study.

Secondly, as Casini *et al* noted decreased hepatic fibrosis in their eight week BDL rats, the question arises whether the present results are simply due to a decrease in portal pressure. However, based on our data and other results, this possibility

seems extremely unlikely. Chronic bile duct ligation induces a progressive cirrhosis/liver failure,<sup>33–34</sup> and the eight week BDL rat is probably very different from the four week BDL model used in our study. Moreover, in a previous study, we found no difference in portal pressure between capsaicin and vehicle treated four week BDL rats.<sup>11</sup> Finally, the present results showed a similar extent of hepatic fibrosis in the two groups.

Thirdly, could the results be directly due to denervation of the primary afferent innervation? It is clear from previous studies that the neonatal dose of capsaicin used in the present study was sufficient to permanently ablate the primary afferent neurones and induce lifelong denervation.<sup>13–14</sup> Previous studies provide circumstantial evidence in favour of the notion that the renal effects noted in the present study were due to afferent denervation. Over the past decade, DiBona *et al* have elegantly demonstrated that in the BDL rat, renal sympathetic nervous tone is increased, and this leads to salt and water retention.<sup>35</sup> Ablation of the renal efferent nerves corrects the renal functional abnormalities, including sodium retention.<sup>36</sup> In general, the neural pathway controlling the cardiovascular system is comprised of the primary afferent innervation, the brain stem medullary cardiovascular regulatory nuclei, predominantly the NTS and VLM, and the effector arm composed of sympathetic and parasympathetic efferent nerves.

In the last part of our study, we aimed to test the hypothesis that capsaicin induced interruption of the afferent loop would disrupt the normal functioning of this pathway by examining Fos immunohistochemical staining in the brain stem medullary nuclei. It has been clearly established that neuronal activation is associated with induction of the *c-fos* gene and its protein product Fos.<sup>37–39</sup> *c-fos* is an immediate early gene that has important roles in cellular signal transduction and transcriptional regulation.<sup>40</sup> Immunohistochemical detection of Fos has been used as a metabolic marker to identify neuronal groups and trace neuronal pathways that have been activated by physiological and pathophysiological stimuli.<sup>41–42</sup> In a previous study, we demonstrated a baseline increase in Fos staining in the NTS and VLM of BDL cirrhotic rats.<sup>22</sup> Moreover, an intense stimulus such as hypotensive haemorrhage did not affect the level of neuronal activation in BDL animals whereas controls showed the expected increase.<sup>22</sup>

In addition to a role as a marker of neuronal activation, there is increasing evidence to suggest that *c-fos* mediated signal transduction and transcription can directly activate neurones.<sup>43–44</sup> We have recently demonstrated that *c-fos* mediated signalling is a sine qua non for the development of hyperdynamic circulation in the prehepatic portal hypertensive rat.<sup>45</sup> In that study, microinjections of *c-fos* antisense oligonucleotides into the NTS eliminated the hyperdynamic circulation in portal hypertensive rats.<sup>45</sup> All of these findings indicate that the *c-fos* pathway is a key mediator of the hyperdynamic circulation of portal hypertension and cirrhosis.

In the present study, neonatal capsaicin treatment of BDL rats markedly diminished baseline Fos staining in BDL rats through to the scant levels seen in normal controls. This result was not unexpected, as our previous study with subacute (seven days prior to the study) administration of capsaicin to the adult rat had shown similar blunting of Fos staining in the NTS and VLM.<sup>21</sup> The present results indicated that neonatal capsaicin treatment also abolished the central neural response to cardiovascular challenge in BDL rats. However, because the hyperdynamic circulation was also eliminated in these capsaicin treated animals, it is impossible to completely rule out a major or permissive effect of such elimination as a cause of the observed changes in renal function.

Thus the fourth possibility is that elimination of the hyperdynamic circulation induced the beneficial effects on renal function. This is consistent with the “peripheral vasodilatation” hypothesis.<sup>1</sup> According to this hypothesis, peripheral vasodilatation leads to decreased effective circulating volume, thus inducing the cirrhotic kidney to retain salt and water, by the pressure diuresis mechanism.<sup>46–47</sup>

Exactly how neonatal capsaicin denervation of primary afferent nerves blocks the development of systemic vasodilatation in cirrhotic rats remains unclear. As central neural activation is necessary for the hyperdynamic circulation to develop, the current finding of abrogated central activation in capsaicin treated BDL rats strongly suggests that the peripheral to central signal that leads to central activation and thus increased efferent cardiovascular nervous activity is carried by primary afferent nerves. The exact nature of the peripheral to central signal remains speculative; we have previously suggested that portal or mesenteric venous hypertension or congestion might be the initial peripheral signal.<sup>45</sup> Primary afferent nerves also contain vasodilatory peptides such as substance P and CGRP. These substances might exert a local effect, particularly in the gut circulation.

In the present study, because capsaicin treatment induced the dual and probably intertwined phenomena of afferent denervation and abrogation of hyperdynamic circulation, we cannot definitively ascertain which mechanism (vasodilatation versus denervation) is operative. Indeed, it is possible that both mechanisms may contribute: we speculate that primary afferent denervation interrupts the neural control pathway, leading to decreased activity in the brain stem cardiovascular regulatory centres, thereby eliminating systemic and renal vasodilatation and removing the major stimulus for renal sodium retention.

In conclusion, neonatal capsaicin treatment prevented the development of hyperdynamic circulation and renal dysfunction as well as ascites formation in cirrhosis. The beneficial renal effect might be mediated through denervation of capsaicin sensitive primary afferent nerves or by elimination of systemic peripheral vasodilatation.

## ACKNOWLEDGEMENTS

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