



Research article

Impairment of natriuresis and diuresis induced by intrarenal adrenoceptor mechanisms in an experimental model of cirrhosis in rats



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ABSTRACT

The contribution of intrarenal alpha-2 adrenergic receptors in mediating the enhanced renal excretory responses evoked by the alpha-2-agonist xylazine was examined in a model of cirrhosis in rats. In sham-operated rats, xylazine (0.2 mg/kg, *i.v.*) increased diuresis and natriuresis (urine flow, control: 78 ± 12.1 , 10 min: 155 ± 17 , 20 min: 194 ± 19 , 30 min: 146 ± 16 , 40 min: 114 ± 13 , 50 min: 95 ± 10.5 $\mu\text{l}/\text{min}/\text{g}$; urinary sodium excretion, control: 6.75 ± 2.08 , 10 min: 7.12 ± 2.1 , 20 min: 13.4 ± 4.6 , 30 min: 14.6 ± 4.02 , 40 min: 12.05 ± 2.35 , 50 min: 12.7 ± 2.45 $\mu\text{eq}/\text{min}/\text{g}$), which was accompanied by a significant reduction in renal sympathetic nerve activity (RSNA) (control: 100, 10 min: 39.5 ± 5.8 , 20 min: 53 ± 8.8 , 30 min: 72 ± 7.0 , 40 min: 83 ± 5.0 , 50 min: 94 ± 6.1 AU). Xylazine (0.2 mg/kg) in cirrhotic animals, despite resulting in a significant reduction in RSNA (control: 100, 10 min: $73 \pm 4.3^*$, 20 min: $70 \pm 5.0^*$, 30 min: $76 \pm 7.0^*$, 40 min: $85 \pm 5.5^*$, 50 min: $92 \pm 4.8^*$ AU), was unable to increase natriuresis. A higher dose (20 mg/kg) of xylazine was not capable of increasing natriuresis and diuresis, even in the presence of a robust reduction in RSNA. Renal denervation did not alter the onset and time course of cirrhosis. The results indicated that during the development of cirrhosis, there is an adaptive process that disables the intrarenal alpha-2 adrenoceptor mechanisms that selectively promote water and urinary sodium excretion via a sympathetic renal nerve-independent mechanism. Thus, in cirrhotic rats, the diuresis/natriuresis induced by xylazine is independent on RSNA. Intrarenal and/or hormonal changes are probably involved in the impairment of xylazine-induced diuresis/natriuresis in cirrhosis.

1. Introduction

Renal impairment is a severe complication that occurs in cirrhotic patients. It is characterized by a marked reduction in glomerular filtration rate (GFR) and renal plasma flow (RPF), progressive azotemia, increased serum creatinine and low levels of urinary sodium excretion [1]. The mechanisms underlying renal vasoconstriction in this condition are poorly understood. The involvement of intrarenal vasoactive agents has been described; however, the participation of renal sympathetic nerve activity (RSNA) is a feature that needs to be clarified [2]. Indeed, renal nerves control the renal function, as an activation of RSNA is able to trigger sodium retention, renal vasoconstriction and a robust renin release by juxtaglomerular cells [3].

Increased RSNA in decompensated cirrhosis has been described as contributing to sodium-water retention, edema and a reduction in RPF, leading to a poor prognosis [4]. Solis-Herruzo et al. demonstrated an important role of sympathetic vasomotor activity in the pathogenesis of renal damage. They found that in cirrhotic patients with renal impairment, unilateral lumbar sympathetic blockade with an anesthetic agent induced a significant improvement in renal function, leading to an increase in GFR and RPF [5].

It has been described that sympathetic activation of the kidney produces an increase in sodium reabsorption and either an increase or a decrease in renal vascular resistance in response to activation of alpha-1 or alpha-2 adrenoceptors, respectively [6, 7]. Indeed, previous studies have shown that the antinatriuresis elicited by renal nerve stimulation

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Table 1. Body weight absolute values (on the day of the biliary duct ligation, or sham operation = “initial” and 20 days later = final) and relative weight of the liver and kidney of the control (sham operated) and cirrhotic rats. The relative weights of the organs with respect to the final weight (weight measurement just before organ removal) was also obtained. Parenthesis indicate the number of animals per group. Values represent the mean $\pm$ SEM. * $P < 0.05$.

Groups	Control (14)		Cirrhotic (12)	
Body weight (g)	Initial	Final	Initial	Final
	209 $\pm$ 6.2	304 $\pm$ 7.4	217 $\pm$ 5.9	244 $\pm$ 8.5
Liver weight (g)	14.4 $\pm$ 1.42		17.5 $\pm$ 0.83*	
Kidney weight (g)	2.49 $\pm$ 0.08		1.85 $\pm$ 0.09*	
Liver weight/body weight	4.97 $\pm$ 0.68		7.23 $\pm$ 0.35*	
Kidney weight/Body weight	0.85 $\pm$ 0.06		0.76 $\pm$ 0.05	

was blocked by previous intrarenal administration of alpha-1 adrenoceptor antagonists but not by alpha-2 antagonists [8, 9]. Thus, it is reasonable to postulate that renal sympathetic overactivity is an important mechanism leading to sodium/water retention in cirrhosis.

Cabral et al. described an important increase in renal excretion of water in anesthetized rats when xylazine, an alpha-2 agonist, was administered intravenously [10]. However, the actions of xylazine in the brain could not be excluded, considering that injection of yohimbine, an alpha-2 antagonist, into the rostroventrolateral medulla (RVLM), a key region in the control of RSNA, significantly decreased the xylazine-induced RSNA decrease. Thus, the increase in water diuresis induced by xylazine could be secondary to the reduction of RSNA [11].

In line with this observation, studies in humans have evaluated the potential beneficial effect of alpha-2 agonists in cirrhotic patients with ascites [12, 13]. Indeed, clonidine-treated cirrhotic patients had a significant reduction in circulating levels of aldosterone, renin and norepinephrine, accompanied by increased natriuresis [13].

Therefore, all of these findings point to the beneficial effects of the reduction of RSNA in cirrhosis. Thus, in the present study, we tested the hypothesis that the improvement in renal function induced by xylazine in cirrhotic rats is mediated by a reduction in RSNA.

2. Materials and methods

Male Wistar rats weighing 250–300 g were used. The animals ($n = 36$ overall) were randomized into two independent groups: chronic ligation of the biliary duct (experimental cirrhosis) and sham operated animals (all surgical procedures, except biliary duct ligation). Both experimental groups were submitted to acute and chronic protocols as described later in this section. A subgroup of the cirrhotic animals was submitted to renal denervation. The animals were kept on a 12-h light/12-h dark cycle under artificial lighting and at a controlled temperature of 22 °C with *ad libitum* access to food and filtered water.

All procedures were conducted in accordance with the Biomedical Research Guidelines for the Care and Use of Laboratory Animals, as stated by the Federation of the Brazilian Societies of Experimental Biology (FESBE). The experimental protocol was approved by the Animal Use Committee from the Escola Superior de Ciências da Santa Casa de Misericórdia de Vitória (021/2007 – CEUA/EMESCAM, Vitória, ES, Brazil).

2.1. Experimental cirrhosis

The technique described by Franco et al. was employed to induce experimental cirrhosis [14]. Under sodium pentobarbital (50 mg/kg, *i.p.*) anesthesia, the rats were submitted to a midline laparotomy to expose the common biliary duct. The duct was tied twice using a silk line (number 3) and cut between the two knots. After closing the surgical field, the animals were treated with antibiotics (benzathine penicillin G 30,000 iu, *i.m.*). Sham rats (control group) were submitted to the same surgical procedures, except for the common biliary duct ligation.

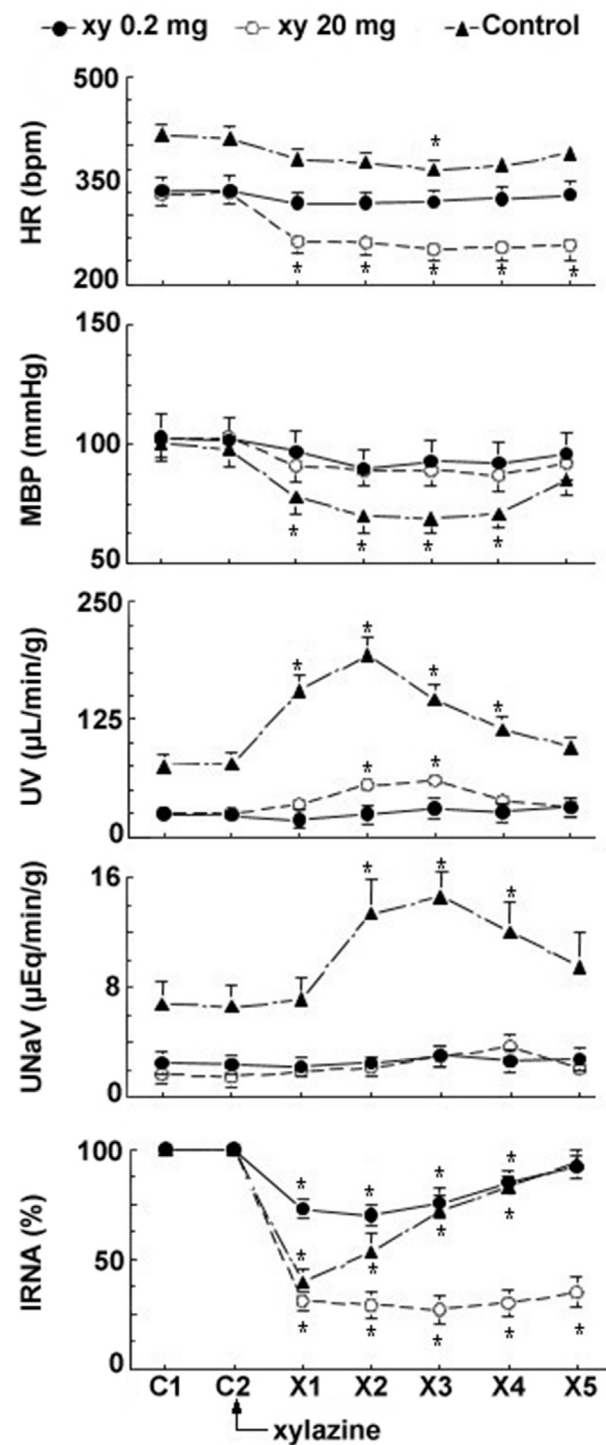


Figure 1. Cardiovascular and renal responses induced by xylazine in rats with biliary duct ligation (● 0.2 mg/kg [$n = 6$]; ○ 20 mg/kg [$n = 6$], *i.v.*) and in control rats (▲ 0.2 mg/kg [$n = 14$]). Two control urine collections (C1 and C2) were made after 2 h of saline infusion (0.9%; 55 μ L/min, *i.v.*). Following xylazine injection, 5 samples of urine were collected with 10 min. HR, heart rate; MBP, mean arterial blood pressure; UV, urinary volume; UNaV, sodium urinary volume; IRNA, integrated sympathetic activity (arbitrary units). The values represent the mean $\pm$ SEM. * $P < 0.05$ compared to baseline (before xylazine administration).

Table 2. Cardiovascular and renal responses induced by xylazine (0.2 mg/kg, i.v.) in control rats ($n = 14$). The control period is represented by the mean of two urine samples collected (10 min, C1 and C2, as in Figure 1) after 2 h of saline infusion (0.9%, 55 μ l/min, i.v.). After xylazine injection, 5 successive urine samples (X1-X5) were collected with a 10 min interval between them. MBP, mean arterial blood pressure; HR, heart rate; UV, urinary volume; UNaV, sodium urinary volume; IRNA, integrated sympathetic activity (arbitrary units). The values represent the mean $\pm$ SEM. * $P < 0.05$.

Period	Control	X1	X2	X3	X4	X5
MBP (mmHg)	100 $\pm$ 6.9	78 $\pm$ 7.2*	70 $\pm$ 7.0*	69 $\pm$ 5.7*	71 $\pm$ 5.8*	85 $\pm$ 6.3
HR (bpm)	410 $\pm$ 17	380 $\pm$ 14	376 $\pm$ 14	365 $\pm$ 14*	372 $\pm$ 13	388 $\pm$ 13
UV	78 $\pm$ 12.1	155 $\pm$ 17*	194 $\pm$ 19*	146 $\pm$ 16*	114 $\pm$ 13*	95 $\pm$ 10.5
UNaV	6.75 $\pm$ 2.08	7.12 $\pm$ 2.1	13.4 $\pm$ 4.6*	14.6 $\pm$ 4.02*	12.05 $\pm$ 2.35*	12.7 $\pm$ 2.45
IRNA	100	39.5 $\pm$ 5.8*	53 $\pm$ 8.8*	72 $\pm$ 7.0*	83 $\pm$ 5.0*	94 $\pm$ 6.1

Table 3. Cardiovascular and renal responses induced by xylazine (0.2 mg/kg, i.v.) in rats submitted to biliary duct ligation ($n = 6$). The control period is represented by the mean of two urine samples collected (10 min, C1 and C2, as in Figure 1) after 2 h of saline infusion (0.9%, 55 μ l/min, i.v.). After xylazine injection, 5 successive urine samples (X1-X5) were collected with a 10 min interval between them. MBP, mean arterial blood pressure; HR, heart rate; UV, urinary volume; UNaV, sodium urinary volume; IRNA, integrated sympathetic activity (arbitrary units). The values represent the mean $\pm$ SEM. * $P < 0.05$.

Period	Control	X1	X2	X3	X4	X5
MBP (mmHg)	102 $\pm$ 9.8	97.1 $\pm$ 9.0	90.7 $\pm$ 8.1	93 $\pm$ 8.9	92 $\pm$ 9.3	96 $\pm$ 9.2
HR (bpm)	336 $\pm$ 20	317 $\pm$ 17	317 $\pm$ 17	319 $\pm$ 17	324 $\pm$ 18	329 $\pm$ 19
UV	24.1 $\pm$ 9.6	18.8 $\pm$ 10	24 $\pm$ 10	29.9 $\pm$ 9.6	26.3 $\pm$ 9.0	31.4 $\pm$ 9.1
UNaV	2.5 $\pm$ 0.84	2.1 $\pm$ 0.71	2.2 $\pm$ 0.59	3.04 $\pm$ 1.2	2.6 $\pm$ 0.86	2.8 $\pm$ 0.96
IRNA	100	73 $\pm$ 4.3*	70 $\pm$ 5.0*	76 $\pm$ 7.0*	85 $\pm$ 5.5*	92 $\pm$ 4.8

Table 4. Cardiovascular and renal responses induced by Xylazine (20 mg/kg, i.v.) in rats submitted to biliary duct ligation ($n = 6$). The control period is represented by the mean of two urine samples collected (10 min, C1 and C2 as in Figure 1) after 2 h of saline infusion (0.9%, 55 μ l/min, i.v.). After Xylazine injection, 5 successive urine samples (X1-X5) were collected with a 10 min interval between them. MBP, mean arterial blood pressure; HR, heart rate; UV, urinary volume; UNaV, sodium urinary volume; IRNA, integrated sympathetic activity (arbitrary units). The values represent the mean $\pm$ SEM. * $P < 0.05$.

Period	Control	X1	X2	X3	X4	X5
MBP (mmHg)	102 $\pm$ 7	91 $\pm$ 7	89 $\pm$ 6	89 $\pm$ 6.5	87 $\pm$ 7	92 $\pm$ 7
HR (bpm)	332 $\pm$ 15	263 $\pm$ 17*	260 $\pm$ 17*	251 $\pm$ 17*	254 $\pm$ 19*	257 $\pm$ 22*
UV	24.5 $\pm$ 9	33.5 $\pm$ 9.2	54.7 $\pm$ 9.5*	60.3 $\pm$ 9.5*	38.1 $\pm$ 9.5	32 $\pm$ 10
UNaV	1.19 $\pm$ 0.25	1.93 $\pm$ 0.52	1.79 $\pm$ 0.55	2.96 $\pm$ 0.72	3.69 $\pm$ 1.15	2.1 $\pm$ 0.6
IRNA	100	31 $\pm$ 4.5*	29 $\pm$ 6.0*	27 $\pm$ 6.5*	30 $\pm$ 6.1*	35 $\pm$ 7.1*

2.2. Renal denervation

To evaluate the role of renal nerves and their capacity to induce sodium retention and to influence the evolution and morbidity of cirrhotic syndromes, a separate group of rats ($n = 5$) were submitted to bilateral renal denervation under sodium pentobarbital anesthesia (50 mg/kg, i.p.) immediately before the cirrhosis surgery procedure ($n = 5$). After exposure of the kidneys, the renal nerve fibers were carefully identified under magnification (X32), separated from the blood vessels and cut. The surrounding tissue was treated with a solution of phenol (10%) dissolved in 100% ethanol [15].

2.3. Hemodynamic recordings and urine collection

Twenty days after common biliary duct ligation, the animals were anesthetized (sodium pentobarbital, 50 mg/kg, i.p.) and operated on for hemodynamic and renal nerve recordings and urine collection in a single surgical procedure. The femoral artery was cannulated to measure blood pressure using a pressure transducer (Viggo-Spectramed, P23XL), and heart rate was calculated electronically from the blood pressure measurements using a rate meter (Biotach, Gould 13-64616-66). The left femoral vein was cannulated for drug administration. The catheters were tunneled under the skin and emerged from the neck where they were tied and left for posterior recordings. Then, the bladder was exposed and cannulated (PE240, 4 cm long catheter) using the technique described by Gellai and Valtin [16]. The catheter was tied to adjacent muscle and the skin to allow continuous collection of urine by gravity. Urine volume (μ l/min/g of the kidney) and urinary sodium and potassium concentrations (μ eq/min/g of the kidney) were measured with a photometer (Instrumentation Laboratories, model 943).

In the same surgical approach, the left kidney was exposed via a retroperitoneal approach, and a renal nerve bundle was dissected carefully and freed from the surrounding tissue under magnification (32x). The nerve was placed on a bipolar electrode made of platinum wire and covered with a dental resin (Presidente®). The microelectrodes were connected to a fine wire (A-M Systems, Inc) that was tunneled under the skin and emerged from the neck where they were tied and left for posterior recordings. Nerve activity was displayed on an oscilloscope (Tektronix TDS 210) and monitored by means of an audio amplifier (Neurolog NL120). The signal was amplified (5–10k; Neurolog NL104, Digitimer) and filtered (low frequency: 10 Hz, high frequency: 1 kHz, with a 60 Hz notch; Neurolog NL126, Digitimer), passed through a window discriminator (NL 201, to eliminate movement artifacts) and integrated (NL 601). Renal nerve activity was reported as the difference (Δ) from each basal value. All cardiovascular and nerve activity data were digitalized (Biopac MP 100) and stored on a PC hard disk for processing. All recordings were made from awake animals. The animals were kept in cages that allowed urine sample collection and hemodynamic and renal nerve recordings.

2.4. Experimental protocol

Immediately after the surgical procedures, a saline infusion (55 μ l/min, i.v.) was started (Harvard model 600–900 V infusion pump) and maintained during the entire experimental procedure.

Two hours later, the hemodynamic and renal nerve activity recordings started, with all the data being digitalized for later analysis. The protocol started with the collection of two successive urine samples (10 min duration each, control). Following these sample collections, xylazine (bolus injection, i.v.) at different doses was injected (0.2 mg/kg [$n = 14$ control and 6 cirrhotic rats] or 20 mg/kg [$n = 6$ cirrhotic rats]), and five sample collections were performed. Throughout the protocol, hemodynamic and renal nerve activity recordings were made. At the end of the experiment, the animals were killed with an overdose of anesthetic (sodium pentobarbital 120 mg/kg, i.v.), and nerve recordings were continued for 15 min to evaluate the background noise level (shown in Figure 2).

2.5. Histological procedures

For light microscopic analysis, liver tissues from each group were fixed with 10% buffered formalin and embedded with paraffin. After routine processing, paraffin sections of each tissue were cut at 10 μ m thickness and stained with hematoxylin and eosin or Picro-sirius red.

2.6. Statistical analysis

Data are expressed as the mean $\pm$ SEM. Comparisons of the mean differences (Δ) of the parameters were analyzed using a two-way

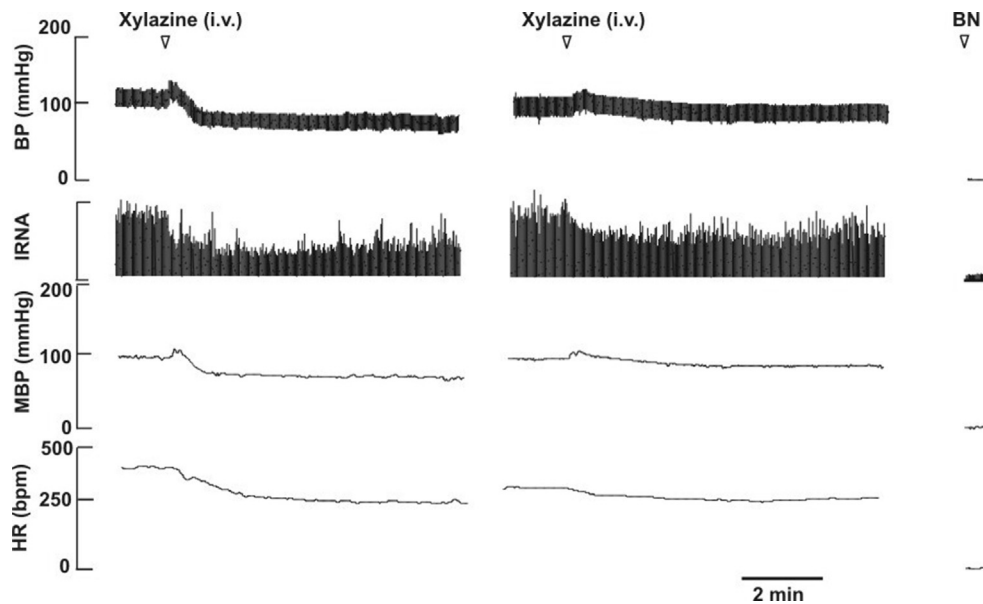


Figure 2. Typical recordings obtained from a control animal (left panel) and a rat submitted to biliary duct ligature (20 days after, right panel). Xylazine (0.2 mg/kg, *i.v.*) effects on BP (pulsatile arterial blood pressure), IRNA (integrated renal sympathetic activity; arbitrary units), MBP (mean arterial blood pressure) and HR (heart rate). BN (basal noise level) was obtained after the death of the animal.

repeated measures ANOVA followed by a multiple comparison test (Tukey). Differences were considered statistically significant at $P < 0.05$.

3. Results

Table 1 shows values of body, liver and kidney weight, and the relative weights of these organs, 20 days after the animals were submitted to biliary duct ligature or sham operation. Cirrhotic rats presented a significant increase in liver weight compared to the sham group. A significant reduction in kidney weight was found; nevertheless, when normalized by body weight, this reduction was not statistically significant. Cirrhotic rats did not have the same body weight gain as control animals, even though this group presented with ascites.

Figure 1 shows the changes in mean blood pressure, heart rate, urinary flow, sodium excretion and renal sympathetic activity induced by xylazine in rats with biliary ligatures (20 days after) and their respective controls. The heart rate and blood pressure of the control animals were significantly reduced by xylazine administration (0.2 mg/kg, *i.v.*). The reduction of blood pressure was more intense and significant in the first minutes after xylazine administration, while the reduction of heart rate was significant after 30 min (Table 2). In the cirrhotic animals, xylazine (0.2 mg/kg, *i.v.*) did not induce any significant changes in blood pressure or heart rate (Table 3). Following administration of a larger dose of xylazine in the cirrhotic group (20 mg/kg, *i.v.*), a significant reduction of heart rate was observed while the blood pressure was unchanged (Table 4). Figure 2 shows a typical recording of these parameters.

In Figure 1, the following parameters are also shown: urinary flow, sodium excretion and renal sympathetic nerve activity. In the control animals (Table 2), acute administration of xylazine (0.2 mg/kg, *i.v.*) significantly reduced RSNA (Figure 2), with a maximal reduction occurring at 10 min. This was accompanied by a simultaneous and significant increase in urinary flow and sodium excretion that reached its peak at 20 and 30 min, respectively. These three parameters returned to control values 50 min after xylazine administration. However, in the cirrhotic animals (Table 3), xylazine administration (0.2 mg/kg, *i.v.*) provoked a significant reduction in RSNA (Figure 2), without any significant change in urinary flow or sodium excretion. After administration of the higher dose (20 mg/kg, *i.v.*), a more intense, immediate and persevering reduction of RSNA was found, without any changes in urinary flow and sodium excretion. Figure 1 shows the reduction in RSNA

after xylazine administration that was maintained during the entire recording period.

In an independent group of rats, bilateral renal denervation was performed prior to the biliary duct ligature procedure. Cirrhotic rats submitted to renal denervation followed by ductal ligation died at 30 ± 4 days after the ligation of the biliary duct, which was not significantly different from animals who suffered sham denervation, whose survival time was 27 ± 3 days.

Histological sections of the liver of a control rat and an animal submitted to ligation and biliary duct section are shown in Figure 3. The slides display an intense proliferation of the biliary ducts in the external part of the portal space. Proliferation of biliary ducts was also observed, forming a septum that delimited hepatic lobules and discrete fibrosis. In panel E, an intense proliferation of biliary ducts is shown in the lobules (asterisks), isolating groups of hepatocytes (arrows).

4. Discussion

The present study evaluated the influence of RSNA on natriuresis and diuresis induced by xylazine in an experimental model of cirrhosis in rats. Three weeks after ligation of the biliary duct, a marked level of disorganization of the hepatic lobular architecture was found. In parallel, these animals present jaundice and ascites. Furthermore, cirrhosis completely blocked the potent natriuretic and diuretic effects induced by xylazine, even at a higher dose of xylazine (20 mg/kg) and in the presence of a robust decrease in RSNA.

Bile duct ligation is a well-established experimental model for the study of cirrhosis. The ligation evokes an accumulation of hepatic stellate cells in the perisinusoidal space of the liver, leading to an excess of extracellular matrix components and fibrosis [17, 18]; this mechanism may reflect the increase in liver weight found in the present study, however, although it is a common feature in cirrhotic rats, liver volume can be found reduced in some stages of cirrhosis in humans [19]. Considering that the morphological changes in the liver could induce a reduction of albumin synthesis, urea, renin substrate, bilirubin conjugation and metabolism of various hormones, including aldosterone, these factors could contribute to renal function alterations described in the present study [20]. Such dysfunctions could decrease the glomerular filtration rate, leading to a reduction in water and electrolyte excretion. Thus, we propose that the cirrhotic model utilized in the present study

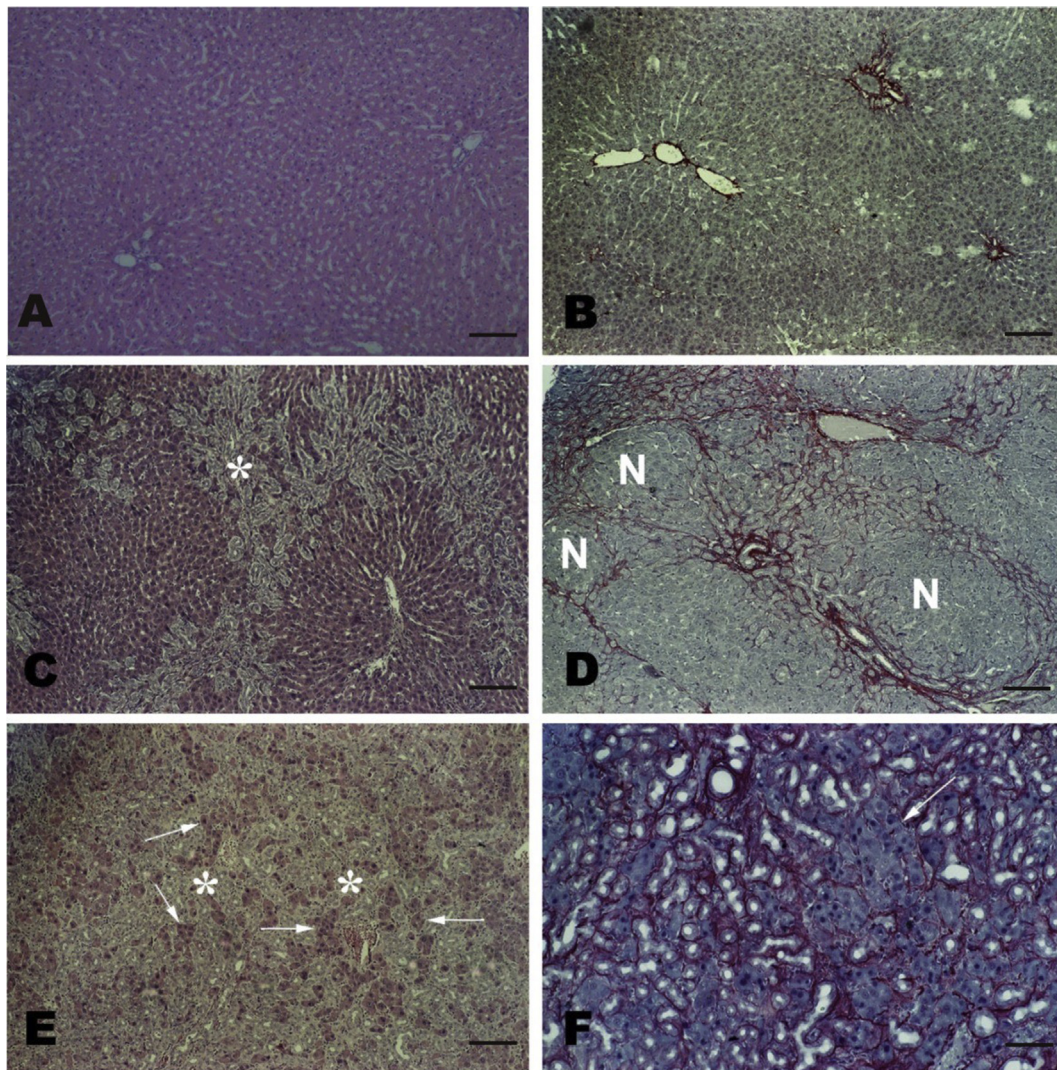


Figure 3. Left slides were stained with H&E, and right slides were stained with Picro-sirius. Panels A and B show histological liver sections obtained from a control rat. Panels C, D, E and F, obtained from a cirrhotic rat, show liver changes in rats 20 days after ligature and bile duct section. In panel C, intense proliferation of biliary ducts is observed in the vicinity of the portal spaces (asterisks), forming bands to other portal spaces. In panel D, biliary duct proliferation with septa that delimit hepatic lobules (N) and thin fibroses around the biliary ducts is observed. Panel E shows the intense presence of biliary ducts penetrating irregularly into the liver lobules (asterisks), isolating groups of hepatocytes (arrows). Panel F shows biliary duct proliferation with moderate fibrosis neighboring the ducts, isolating groups of hepatocytes (arrows), and ducts presenting with mild dilatation. Bars represent 100 μ m.

has a correlation with the impairment of renal function in the early stage of cirrhosis in humans [21, 22, 23]. These authors demonstrated the existence of a deficit in sodium transport following volume expansion in cirrhotic rats. Such alterations are probably due to tubular function deficits, while cirrhotic patients with ascites also present with glomerular filtration rate changes that interfere with sodium excretion [24]. As a whole, experimental evidence suggests that intrahepatic hypertension with impairment of venous hepatic flow is the primary causal agent responsible for the initiation of sodium retention in cirrhosis in humans and animals [25, 26, 27].

As in other pathological states where edema is present, in cirrhosis, there are changes in the circulatory control system that impact renal function and therefore induce water and sodium retention. Some of the more important mechanisms are mediated by the sympathetic nervous system [28, 29, 30], suggesting that increased RSNA in cirrhotic patients contributes to water and sodium retention. Pharmacological sympathectomy with bilateral local anesthetic lumbar blockade resulted in diuresis and natriuresis in cirrhotic patients with ascites, sodium retention, renal function reduction and a lack of a natriuretic response to volume expansion [28, 30, 31]. Nevertheless, in our experiments, the administration of xylazine (20 mg/kg) significantly reduced RSNA but

did not induce any increases in natriuresis in the cirrhotic rats. Xylazine was previously shown to induce an increase in natriuresis and diuresis in control animals [11, 32, 33]. Thus, maneuvers that decrease renal sympathetic activity and induce natriuresis and diuresis can have a beneficial effect on the renal function of edematous patients. However, in the present study, xylazine was unable to increase sodium and water excretion in cirrhotic rats; apparently, the diuretic/natriuretic effects of xylazine are independent of RSNA reduction, considering that at the higher dose of xylazine, no changes in sodium/water excretion were found even in the presence of an intense decrease in RSNA.

The results suggest an impairment of the tubular mechanisms of α -2 intrarenal receptors leading to diuresis. On the other hand, the extrarenal α -2 mechanisms are preserved as seen by the reduction of sympathetic renal nerve activity and blood pressure (Figure 1), probably due to central effects [32]. Xylazine at a higher dose (20 mg/kg) did not induce hypotension (Figure 1, top panel), most likely as a consequence of activation of the α -2 vascular receptor. Indeed, Cabral et al. demonstrated that the increase in renal excretion of water and electrolytes in rats anesthetized by a combination of ketamine and xylazine [10] is partially mediated by central α -2 adrenergic mechanisms involved in the release of vasopressin [32] simultaneously with the decrease of RSNA [11, 33].

The present study indicates an important deactivation of intrarenal alpha-2 receptors in cirrhotic rats, which was probably not reversed by renal denervation. Renal denervation did not impair the evolution of all signs and manifestations of cirrhosis or the time of survival of these animals. In normal conditions, the alpha-2-dependent mechanisms increase urinary rate by increasing osmolar clearance and free water clearance [34, 35] and are partially regulated by renal sympathetic activity. These mechanisms are dependent on two subtypes of alpha-2 receptors, alpha-2a, which increases osmolar clearance, and alpha-2b, which modulates free water clearance, with the latter being particularly sensitive to clonidine, a more specific alpha-2b agonist [36]. However, the underlying molecular mechanisms involved in the attenuated diuretic and natriuretic responses triggered by xylazine in cirrhosis are unknown. Whether the impairment of responses is due to a change in the number and/or sensitivity of the intrarenal alpha-2 receptors requires further investigation.

We therefore conclude that in the cirrhotic model induced by biliary duct ligation, the actions of xylazine regulating sodium and water excretion are impaired, apparently by the marked inactivation of intrarenal alpha-2 adrenoceptor mechanisms. Xylazine is not capable of increasing diuresis/natriuresis in cirrhotic rats despite the robust reduction in RSNA.

Declarations

Author contribution statement

Maycon I. O. Milanez: Analyzed and interpreted the data; Wrote the paper.

Antônio M. Cabral, José G. P. Pires, Henrique A. Futuro Neto, Nyam F. Silva: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Cássia T. Bergamaschi, Ruy R. Campos: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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