Temporal decrease in renal sensory responses in rats after chronic ligation of the bile duct

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Ma, Ming-Chieh, Ho-Shiang Huang, Chiang-Ting Chien, Ming-Shiou Wu, and Chau-Fong Chen. Temporal decrease in renal sensory responses in rats after chronic ligation of the bile duct. Am J Physiol Renal Physiol 283: F164–F172, 2002. First published February 19, 2002; 10.1152/ajprenal.00231.2001.—Renal responses to renal sensory receptor activation were examined in rats after 1 and 4 wk of common bile duct ligation (CBDL). Compared with sham-operated rats (Sham), urine and sodium excretion after acute saline loading was significantly reduced at both times after CBDL. The blunted excretory responses in CBDL rats, accompanied by less activation of afferent renal nerve activity (ARNA), were already apparent at 1 wk and became severe at 4 wk. The defect in ARNA activation in CBDL rats was further studied using specific stimuli to activate renal sensory receptors. Graded increases in intrapelvic pressure or renal pelvic perfusion of substance P (SP) elicited an increase in ARNA in Sham rats, these responses being temporally attenuated in CBDL rats. Despite no significant change in renal pelvic SP release, no renal sensory reflex was demonstrable in 4-wk CBDL rats. Immunoblotting showed that expression of renal pelvic neurokinin 1 (NK-1) receptors was 32 and 47% lower in 1- and 4-wk CBDL rats, respectively, than in Sham rats, this decrease correlating well with plasma SP levels. The quantitative real-time RT-PCR showed similar levels of NK-1 receptor mRNA in the renal pelvis in the Sham and 4-wk CBDL groups. We conclude that impairment of renal excretory and sensory responses increases with the duration of cirrhosis. An impaired renal sensory reflex in cirrhotic rats is involved in the defective activation of the renal sensory receptors could be due, in part, to the low expression of NK-1 receptors, which is dependent on the duration of CBDL. The decrease in NK-1 receptor protein levels is not due to a decrease in mRNA levels.

afferent renal nerve activity; renorenal reflex; substance P; neurokinin 1 receptor; cirrhosis

BOTH SUBSTANCE P (SP)-IMMUNOREACTIVE nerves (14) and the SP receptor, the neurokinin 1 (NK-1) receptor (6), are found in the subepithelial area of the renal pelvis, suggesting that all components required for renal sensory transmission are present. Activation of renal sensory neurons by SP-containing or other capsaicin-sen-

METHODS

Animal care and experimentation. Male Wistar rats weighing 200–250 g were used. All animal experiments and animal care were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

Induction of cirrhosis. Hepatic cirrhosis was induced by CBDL (27). Briefly, the abdomen was opened under anesthe-
sia, and the common bile duct was identified and cut between double ligatures (CBDL rats). Sham-operated rats (Sham) were treated similarly, but without bile duct ligation and resection. The rats were allowed to recover in individual cages and were then studied at 7–10 days (1-wk) and 28–35 days (4-wk) after surgery, at which times ongoing cholestasis and edema formation were evident in the CBDL rats. Biochemical indexes of hepatic function, such as the levels of plasma bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AP), were measured using routine clinical laboratory procedures. In addition, six rats in each group were housed in individual metabolic cages for 24-h urine collection, as previously described (5). Because no differences between 1- and 4-wk Sham rats were seen in any of the studied parameters, and the data for all Sham rats were pooled.

**General surgical procedures.** On the day of the experiment, the rats were anesthetized with urethane (1 g/kg body wt ip), and the trachea was exposed and intubated for spontaneous ventilation. Catheters (PE-50) were placed in the external jugular vein for saline infusion and in the femoral artery to measure the mean arterial blood pressure (MABP). The rat was placed on its right side, the left kidney was exposed via a left flank incision, and both ureters were cannulated for urine collection. Renal blood flow (RBF) was continuously monitored using an electromagnetic flowmeter (model FM701, Carolina Medical Electronics, King, NC) or determined using the p-aminohippurate-clearance method. All hemodynamic responses were recorded on a Grass 7P4 polygraph (Quincy, MA). To record afferent renal nerve activity (ARNA), another group of rats underwent the same surgical procedure, but, in this case, after the left-flank incision was performed, the kidney was fixed and bathed with warmed paraffin oil (38°C) to prevent drying. With the use of a stereoscopic dissecting microscope (Olympus, SZ-STU2, Tokyo, Japan), the left renal nerve at the angle between the abdominal aorta and the renal artery was carefully isolated from the renal artery to record renal nerve activity.

**Recording of ARNA.** The recording technique has been previously described (8). Briefly, multifiber nerve activity was recorded by placing intact nerve fibers on a pair of thin, bipolar stainless steel electrodes. The electrical signals were amplified and filtered by a Grass model P511 AC amplifier, and the amplified signals were selected using a window discriminator (World Precision Instrument 121, Sarasota, FL) and counted on a Gould integrator amplifier (13-4615-70, Valley View, OH). Neural activity was transformed into spike counts and displayed continuously on a Gould oscilloscope (model 1604). Renal nerve activity was assessed using its pulse synchronous rhythmicity with the heartbeat. After the identification and verification of renal nerve activity, the distal parts of the nerve fibers were transected to record ipsilateral ARNA (29).

**Acute saline loading.** After 1 h of equilibration, acute saline loading was performed by intravenous infusion over 10 min of an amount of isotonic saline equal to 5% of body weight. MABP, RBF, and ARNA (in a separate group) were continuously monitored. Urine samples were collected from the left kidney at 5, 10, 20, 30, 45, 60, and 90 min after the start of infusion.

**Renal pelvic mechanoreceptor stimulation and SP perfusion.** Renal pelvic mechanostimulation was studied in the Sham (n = 12), 1-wk CBDL (n = 8), and 4-wk CBDL (n = 12) groups as described previously (26, 29). Because little is known about the chemical composition of the urine produced by cirrhotic rats, the renal pelvis in all groups was perfused throughout the experiment as described below, using saline at a rate of 20 μl/min, a perfusion rate that did not affect ureteral pressure. A PE-10 catheter with a heat-pulled tip was placed inside a PE-50 catheter extending beyond the tip of the PE-50 catheter. The tips of the two catheters were placed together in the left ureter near the renal pelvis, allowing the renal pelvis to be perfused via the PE-10 catheter, and the effluent was drained away by the PE-50 catheter. Changes in intrapelvic pressure (IPP) were recorded on a Gould polygraph with a transducer connected to the PE-50 ureteral catheter by a T tube connector. The third end of the T tube connector was connected to a 50-cm length of PE-50 tubing to increase the IPP by 2, 4, 8, 12, 16, or 20 mmHg, each value being maintained for 3 min, with 10-min intervals between changes.

The ARNA response was also tested by renal pelvic perfusion with graded concentrations of SP (1, 5, and 10 μg/ml) via the inserted PE-10 catheter, as described above. The experiment consisted of one 10-min basal period, three consecutive 10-min periods at the different SP concentrations, and one or two 10-min recovery periods.

**Renal pelvic mechanoreceptor stimulation.** Renal pelvic mechanoreceptor stimulation was studied in Sham rats (n = 14), 1-wk CBDL rats (n = 10), and 4-wk CBDL rats (n = 14). The renal pelvic mechanoreceptors of the left kidney were stimulated by increasing the IPP by ~20 mmHg, and changes in ARNA were produced, as described above. Contralateral (right kidney) urine samples were collected. To assay SP in the renal pelvic effluent, the renal pelvis was perfused at 20 μl/min with saline containing 10 μmol/l of the endopeptidase inhibitor thiorphan (Sigma, St. Louis, MO), to minimize SP degradation (20, 29). The amount of SP in the renal pelvic effluent and in the plasma was determined by enzyme-linked immunoassay (29).

**Immunoblotting of NK-1 receptors in the renal pelvis.** After the physiological studies, the rats were killed, both kidneys were exposed, and the regions of the renal pelvis and the proximal end of the ureter were sampled to prepare membrane fractions, as described previously (28, 29). Membrane proteins (80 μg) were separated on 12% polyacrylamide gels and electrophoretically transferred to nitrocellulose membranes (Amersham-Pharmacia, Buckinghamshire, UK). Positive controls of rat ileal and brain cortical membranes (10 μg of protein) were run in parallel. After being blocked, the membranes were incubated overnight at 4°C with rabbit anti-NK-1 receptor antiserum (Novus Biologicals, Littleton, CO) diluted 1,000-fold. After washes, the membrane was incubated for 1 h at room temperature with goat anti-rabbit IgG conjugated to horseradish peroxidase (Vector Labs, Burlingame, CA) and then washed, and the bound antibody was visualized using a commercial peroxidase substrate kit (Vector Labs). The density of the band with a molecular mass of ~79 kDa was determined semiquantitatively by densitometry using an image-analysis system (Alpha Innotech, San Leandro, CA).

**Quantitative real-time RT-PCR to measure NK-1 receptor mRNA levels.** The theoretical basis of the real-time quantitative PCR and the methodology of the ABI PRISM 7700 Sequence Detection System (PerkinElmer Applied Biosystems, Foster City, CA) have been described by Johnson et al. (17). Briefly, samples with a high starting copy number of the genes of interest show increased fluorescence early in the PCR process, resulting in a low-threshold cycle (C_T) number.

The primers and fluorogenic probes for the NK-1 receptor and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were designed, and total RNA extraction and the PCR procedure were performed, as previously described (28, 29). The NK-1 receptor primers and probe were 5′-GGC CAG AGG ACC AGA ACT TTG-3′ (forward primer) 5′-GCT AGC AAC ACG AC
RESULTS

**CBDL model.** The 24-h urine output (ml/day) was similar in Sham and both CBDL groups (Sham: 12.9 ± 2.1; 1-wk CBDL: 14.7 ± 3.0; 4-wk CBDL: 9.1 ± 1.8 ml/day), whereas sodium excretion (ml/day) was significantly decreased in 4-wk CBDL group (Sham: 3.8 ± 0.2; 1-wk CBDL: 4.1 ± 0.4; 4-wk CBDL: 2.5 ± 0.1 ml/day). These results are consistent with those in a previous study (12). Table 1 shows the basal data for hepatic and renal functions in Sham rats and both groups of CBDL rats. Systemic hypotension was seen in the 4-wk, but not the 1-wk, CBDL rats. Significant increases in plasma SP levels were seen in both groups of CBDL rats. These results are consistent with those from a previous study by Chu et al. (9), who suggested that the major factor resulting in the plasma SP increase seen in cirrhosis appears to be the reduced ability of the liver to degrade SP rather than increased SP release from the splanchic vascular bed. A higher liver weight-to-body weight ratio, accompanied by high

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Table 1. Summary data for hepatic and renal parameters in Sham and CBDL rats

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 12)</th>
<th>1-wk CBDL (n = 8)</th>
<th>4-wk CBDL (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP, mmHg</td>
<td>118.9 ± 4.4</td>
<td>113.8 ± 4.0</td>
<td>80.3 ± 4.2*</td>
</tr>
<tr>
<td>Plasma SP, pg/ml</td>
<td>32.0 ± 4.5</td>
<td>38.2 ± 6.17*</td>
<td>49.9 ± 5.68*</td>
</tr>
<tr>
<td>LW/BW, %</td>
<td>5.2 ± 0.10</td>
<td>5.55 ± 0.54</td>
<td>7.15 ± 0.88*</td>
</tr>
<tr>
<td>t-Bili, mmol/l</td>
<td>0.08 ± 0.01</td>
<td>1.32 ± 0.11*</td>
<td>0.46 ± 0.18*</td>
</tr>
<tr>
<td>AP, U/l</td>
<td>50.2 ± 16.8</td>
<td>187.2 ± 65.1*</td>
<td>250.8 ± 58.5*</td>
</tr>
<tr>
<td>AST, U/l</td>
<td>52.9 ± 3.1</td>
<td>254.0 ± 28.7*</td>
<td>131.9 ± 22.0*</td>
</tr>
<tr>
<td>ALT, U/l</td>
<td>4.1 ± 1.1</td>
<td>114.9 ± 6.8*</td>
<td>72.1 ± 4.6*</td>
</tr>
<tr>
<td>KW/BW, %</td>
<td>0.68 ± 0.03</td>
<td>0.64 ± 0.02</td>
<td>0.88 ± 0.12</td>
</tr>
<tr>
<td>RBF, ml min⁻¹ g⁻¹</td>
<td>4.26 ± 0.69</td>
<td>4.91 ± 0.72</td>
<td>1.76 ± 0.39*</td>
</tr>
<tr>
<td>UV, μl min⁻¹ g⁻¹</td>
<td>4.35 ± 0.34</td>
<td>4.40 ± 0.66</td>
<td>3.13 ± 0.78</td>
</tr>
<tr>
<td>U NaV, μmol min⁻¹ g⁻¹</td>
<td>0.29 ± 0.08</td>
<td>0.19 ± 0.04</td>
<td>0.11 ± 0.04*</td>
</tr>
</tbody>
</table>

Values are means ± SE, n, No. of rats; Sham, Sham-operated rats; CBDL, common bile duct ligation; MABP, mean arterial blood pressure; SP, substance P; LW/BW, liver weight-to-body weight ratio; t-Bili, total bilirubin; KW/BW, kidney weight-to-body weight ratio; AP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; RBF, renal blood flow; UV and U NaV, urinary flow rate and urinary Na⁺ excretion rate, respectively. *P < 0.05, compared with Sham rats.

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**A** Fig. 1. Responses to saline loading in the left kidney. A: changes in mean arterial blood pressure (MABP), renal blood flow (RBF), urinary flow rate (UV), and urinary sodium excretion rate (U NaV) in sham-operated (Sham) and rats after 1 and 4 wk of common bile duct ligation (CBDL). B: a separate experiment in Sham (n = 12), 1-wk CBDL (n = 8), and 4-wk CBDL rats (n = 12) showing the percent change (%Δ) in afferent renal nerve activity (ARNA). VE, time of saline infusion. *Significantly different (P < 0.05) from time 0.
biochemical indexes of hepatic function (bilirubin, AP, AST, and ALT) was also noted in both CBDL groups. The kidney weight-to-body weight ratio and baseline UV values for both CBDL groups were similar to those in Sham rats. The baseline RBF and \( U_{NaV} \) values in 1-wk CBDL rats did not differ from those in Sham rats but were significantly lower in 4-wk CBDL rats.

**Acute saline loading.** Figure 1 shows the response to acute saline loading. As shown in Fig. 1A (panel 1 and 2, respectively), the systemic MABP was significantly increased by saline loading in all groups, whereas the RBF was not significantly increased. The MABP and RBF values in the Sham and 1-wk CBDL groups were not significantly different but were lower in the 4-wk CBDL rats at all time points. The increase in blood pressure after saline loading in the 4-wk CBDL rats (36 ± 5%) was significantly greater than that in Sham rats (15 ± 4%). The UV (panel 3) and \( U_{NaV} \) (panel 4) increased in all groups in response to saline loading. However, diuresis and natriuresis were both attenuated in the CBDL rats, and severe impairment was seen in 4-wk CBDL rats. In the 1-wk CBDL rats, the cumulative urine output and sodium excretion values were, respectively, 63 ± 5 and 78 ± 7% of those in Sham rats (\( P < 0.05 \)) and fell to 46 ± 2 and 67 ± 8% of the Sham values in the 4-wk CBDL rats (\( P < 0.05 \)). Cumulative urine and sodium excretion was also significantly different between the CBDL groups (\( P < 0.05 \)). This difference in response was paralleled by a similar difference in ARNA activation. As shown in Fig. 1B, ARNA activation of the left kidney in all groups was significantly increased at 5 and 10 min after the start of acute saline loading and at 10 and 20 min after the end of loading; however, both groups of CBDL rats showed blunted ARNA responses.

**Stimulation of afferent renal nerve activity by mechanostimulation and SP.** Raising the ureteral catheter to various levels above the left kidney increased the IPP and stimulated mechanosensitive neurons in the renal pelvis. Original tracings are shown in Fig. 2. Increases in the IPP resulted in a dose-dependent increase in ARNA in Sham rats (Fig. 2A), but this was reduced in 1-wk CBDL rats (Fig. 2B) and greatly attenuated in 4-wk CBDL rats (Fig. 2C). The RNA increase was reversed when the IPP returned to the basal level. The grouped data are shown in Fig. 2D. The threshold IPP for a significant ARNA increase in the test kidney was 4.7 ± 0.1 mmHg in Sham rats, 12.4 ± 0.3 mmHg in 1-wk CBDL rats, and 19.2 ± 0.3 mmHg in 4-wk CBDL rats. At pressures below 16 mmHg, the IPP-induced ARNA increase in 1-wk CBDL rats was significantly lower than that in Sham rats. At the highest pressure, the increase in the ARNA in 4-wk CBDL rats was significantly reduced compared with that in Sham rats and 1-wk CBDL rats.

Administration of exogenous SP into the renal pelvis was used to directly test whether the impaired ARNA activation in CBDL rats might be related to SP receptor function. Figure 3A shows typical traces of the effect of intrapelvic SP perfusion on ARNA. SP caused an increase in ARNA in Sham rats (top trace); this response was attenuated in both 1-wk (middle trace) and 4-wk (bottom trace) CBDL rats. As with the mechanoreceptors, the increase in ARNA was reversible when SP perfusion was stopped. The grouped data are shown in Fig. 3B. In both Sham and 1-wk CBDL rats, graded increases in SP resulted in a concentration-dependent increase in the ARNA, whereas a severely blunted response was seen in 4-wk CBDL rats. At the SP dose of 1 \( \mu \)g/ml, the 1-wk and 4-wk CBDL rats showed a significantly attenuated ARNA increase compared with Sham rats. At 5 and 10 \( \mu \)g/ml of SP, although the 1-wk CBDL rats displayed a lower ARNA increase than Sham rats, the difference was not statistically significant, whereas 4-wk CBDL rats showed a very low ARNA increase.

![Image](http://ajprenal.physiology.org/DownloadedFrom/biweeklyسورس/10.2203.3.2.on.April.25.2017)
Renorenal reflex and SP release. Figure 4 shows that stimulation of renal mechanoreceptors elicited an inhibitory renorenal reflex. The MABP in all groups was unchanged during the increased IPP and recovery periods (data not shown). In Sham rats (left), an increase in IPP in the ipsilateral kidney to 20 mmHg resulted in significant increases in the contralateral kidney of the UV and the UNaV, which were accompanied by an increase in ipsilateral ARNA. The 1-wk CBDL rats also displayed a reflex response (middle) with a similar IPP increase, the contralateral UV and UNaV and the ipsilateral ARNA being significantly increased. In contrast, contralateral diuretic and natriuretic responses were not seen in 4-wk CBDL rats (right), despite a moderate increase of 40 ± 8% in ARNA.

As shown in Fig. 4 (bottom), the basal values for renal pelvic ipsilateral (left side) SP release did not differ significantly between the groups. An increase in IPP resulted in significant increases in SP release in all three groups, with no significant difference between the groups. SP release returned to the basal level after the IPP was reduced.

Immunoblotting of NK-1 receptors in the renal pelvis. As shown in Fig. 5A, in rat ileal and brain cortical membranes, the anti-NK-1 receptor antiserum recognized a protein band with a molecular mass of 79 kDa (lanes 1 and 2). Renal pelvic tissue membranes from six animals from each group showed a band with the same molecular mass as that in the brain cortical and ileal membranes, this band being weaker in the CBDL rat membranes than in those from Sham rats. Figure 5B shows the semiquantitative data; the band density in Sham rats was significantly higher than the values in 1-wk CBDL rats and in 4-wk CBDL rats. Figure 5C shows that there was a strong negative correlation between plasma SP levels and renal pelvic NK-1 receptor expression in the three groups (P < 0.05).

Quantification of NK-1 receptor mRNA expression. The raw data for the CT values for NK-1 receptor and GAPDH mRNAs, the calculated ΔΔCT values, and the degree of induction of NK-1 receptor mRNA in the Sham and 4-wk CBDL groups are shown in Table 2. The CT results for the housekeeper GAPDH mRNA from a variety of samples were similar, indicating the high precision of the quantitative real-time RT-PCR. The CT values showed that NK-1 receptor mRNA was expressed in the renal pelvis in both groups, and the values for the degree of induction of renal pelvic NK-1 receptor mRNA were

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**Fig. 3.** Effects of graded SP concentrations on the ipsilateral ARNA in Sham and 1-wk and 4-wk CBDL rats. A: original traces showing the effect of renal chemoreceptor stimulation by intrapelvic perfusion with graded SP concentrations (horizontal bars) on integrated multifer SP release. B: grouped data for the effect of SP on the ipsilateral ARNA. *Significantly different (P < 0.05) from the initial value.

**Fig. 4.** Renorenal reflex and renal pelvic substance P (SP) release from the left kidney in Sham and 1-wk and 4-wk CBDL rats. UV and UNaV values are from the contralateral right kidney (A); ΔARNA, change in the afferent renal nerve activity recorded in the left kidney (B); IPP, increased intrapelvic pressure in the left kidney; Rec, recovery period. *Significantly different (P < 0.05) from the basal state. †Significantly different (P < 0.05) from the Sham group.
receptor mRNA, relative to the ileum, showed no significant difference between the groups.

DISCUSSION

Temporal decrease in renal excretion and sensory nerve activity in CBDL rats. In response to saline loading, the CBDL rats had an impaired renal sodium and urine excretory capacity. These alterations were very similar to those seen in various rat models of cirrhosis (2, 12, 33). Plausible mechanisms include enhanced renal sympathetic nerve activity (10, 12, 39), atrial natriuretic peptide (ANP) resistance (33), and a reduced sensitivity of the pressure-diuretic response (1). Our present results agree with these mechanisms. First, when we simultaneously recorded efferent renal nerve activity and ARNA in 4-wk CBDL rats (not shown), we found that the ARNA activation defect seen during acute saline loading was accompanied by increased efferent renal nerve activity as a result of less reflex inhibition. Second, acute saline loading is known to stimulate endogenous ANP release, and ANP resistance in CBDL rats is suggested to result from increased cGMP-dependent phosphodiesterase activity in the cirrhotic kidney (33). Interestingly, we also noted that, during acute saline loading, the increase in the blood pressure in 4-wk CBDL rats was greater than that in Sham rats (Fig. 1A). However, because of insufficient pressure-diuresis, the cirrhotic kidney could not excrete sodium and water efficiently in response to the increased arterial pressure; this may be due to intrarenal factors, rather than to the effect of reduced systemic blood pressure (1).

Atucha et al. (2) also showed that the blunted volume expansion response in cirrhotic rats might result from an attenuated increase in renal interstitial hydrostatic pressure. This raises the possibility that the lower excretory response and the attenuated increase in ARNA after saline loading in our cirrhotic rats may be related, at least in part, to impaired transmission of intrarenal pressure, caused by hypoperfusion of the kidney. Despite the lower MABP in 4-wk CBDL rats than in the Sham group throughout the experiment, we can rule out an effect of renal hypoperfusion on the ARNA response, because, although the 1 wk-CBDL rats (i.e., early stage of cirrhosis) showed a similar change in MABP to that in Sham rats, their renal excretion and ARNA activation by saline loading were already affected (Fig. 1A). This suggested that some intrarenal factors, rather than systemic hypotension, might contribute to impaired renal excretory response in cirrhotic kidneys.

Normally, body fluid expansion activates the renal afferent nerves, which trigger a diuretic signal for reflex inhibition of the renal sympathetic nerve, resulting in diuresis and natriuresis, thereby overcoming the fluid imbalance. Because we found that acute saline loading resulted in reduced excretory ability and lower activation of renal sensory nerves and these effects increased with the duration of CBDL (Fig. 1B), these changes seem to be temporal events during the pathogenesis of cirrhosis. Taking these results together, we conclude that attenuated renal sensory activation contributes to impaired renal excretory function and worsens fluid retention in cirrhosis, especially under fluid-overloading conditions.

In the rat kidney, the majority of renal sensory neurons are found in the renal pelvis (14), and their
RENAL SENSORY RESPONSES IN CIRRHOTIC RATS

Table 2. Analysis of NK-1 receptor mRNA expression in Sham and 4-wk CBDL rats using the comparative Ct method

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>NK-1 Receptor Ct</th>
<th>GAPDH Ct</th>
<th>ΔCt: (NK-1 Receptor Ct – GAPDH Ct)</th>
<th>ΔΔCt (ΔCt – ΔCt Calibrator)*</th>
<th>NK-1 ReceptormRNA Relative to Ileum†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sham</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>29.98</td>
<td>18.43</td>
<td>11.55</td>
<td>1.51</td>
<td>0.35</td>
</tr>
<tr>
<td>2</td>
<td>30.24</td>
<td>19.57</td>
<td>10.67</td>
<td>0.63</td>
<td>0.65</td>
</tr>
<tr>
<td>3</td>
<td>30.42</td>
<td>18.20</td>
<td>12.22</td>
<td>2.18</td>
<td>0.22</td>
</tr>
<tr>
<td>4</td>
<td>31.15</td>
<td>19.11</td>
<td>12.04</td>
<td>2.00</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>32.42</td>
<td>17.14</td>
<td>15.28</td>
<td>0.87</td>
<td>0.55</td>
</tr>
<tr>
<td>6</td>
<td>33.59</td>
<td>17.11</td>
<td>16.48</td>
<td>1.07</td>
<td>0.48</td>
</tr>
<tr>
<td>7</td>
<td>36.60</td>
<td>17.33</td>
<td>19.27</td>
<td>1.54</td>
<td>0.34</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>32.06 ± 0.90</td>
<td>18.13 ± 0.37</td>
<td>13.93 ± 1.19</td>
<td>1.40 ± 0.22</td>
<td>0.41 ± 0.06</td>
</tr>
</tbody>
</table>

| **4-Wk CBDL** |                       |                 |                                   |                          |                                     |
| 1       | 28.91           | 17.94    | 10.96                             | 0.91                         | 0.53                                |
| 2       | 30.26           | 17.82    | 12.32                             | 2.28                         | 0.21                                |
| 3       | 30.41           | 17.61    | 12.59                             | 2.55                         | 0.17                                |
| 4       | 28.81           | 17.73    | 11.20                             | 1.16                         | 0.45                                |
| 5       | 36.53           | 17.73    | 18.80                             | 1.46                         | 0.36                                |
| 6       | 42.75           | 17.40    | 25.35                             | 2.55                         | 0.17                                |
| 7       | 35.96           | 17.29    | 18.67                             | 1.44                         | 0.37                                |
| Mean ± SE | 33.38 ± 1.97 | 17.7 ± 0.11 | 15.70 ± 2.04 | 1.76 ± 0.26 | 0.32 ± 0.05 |

NK-1, neurokinin 1; Ct, low-threshold cycle. *The ΔΔCt value is calculated by subtracting the ΔCt value for the ileum sample from that for each renal pelvis test sample. †NK-1 receptormRNA relative to the ileum, the degree of induction of NK-1 receptor mRNA expression in the renal pelvic sample relative to that in the ileum (calibrator sample).

Responses can be evaluated using specific stimuli, namely, graded increases in IPP (26). As previously reported (20, 26), mechanostimuli resulted in dose-dependent ARNA responses in the Sham rats, whereas CBDL rats displayed blunted responses, the extent of which was dependent on the duration of CBDL, with a shift of the threshold for ARNA activation to a higher pressure (Fig. 2B). These results directly show a temporal deficiency in mechanosensitive response and also support the observation of defective ARNA responses on saline loading.

SP-mediated renal mechanoreceptor activation can be blocked by a SP receptor antagonist (22), suggesting a role for the SP receptor in the renal sensory response. Our results showing that SP increased ARNA in control rats are similar to those of a previous study (20), and the fact that these responses were attenuated in both CBDL groups (Fig. 4B) suggests a deficiency in the action of SP on NK-1 receptors involved in renal pelvic sensory function. Although the ARNA increase seen in 1 wk-CBDL rats at the SP dose of 10 μg/ml was not significantly different from that in Sham rats (Fig. 4B), which can be explained by an excess of SP, at SP doses of 1 and 5 μg/ml the temporal defect in ARNA responses in both CBDL groups was apparent.

Renorenal reflex: role of SP. The existence of a contralateral inhibitory renorenal reflex in rats was initially demonstrated by activating renal mechanoreceptors (21). This mechanoreceptor-induced renorenal reflex is mediated by SP and abolished by capsaicin pretreatment (24). SP is found at high concentrations in the spinal cord, where it plays a role as a transmitter (34), and the presence of SP in the area of the renal pelvis also suggests a role in renal sensory transmission (14). Our results showed that 1-wk CBDL rats displayed a reflex function after an ipsilateral ARNA increase, but the percent changes in the increase in contralateral urinary (1-wk CBDL vs. Sham: 49 ± 7% vs. 20 ± 5%) and sodium (1-wk CBDL vs. Sham: 98 ± 11 vs. 60 ± 6%) excretion were still significantly diminished. The temporal changes in renorenal reflex function in CBDL rats led us to suspect that SP secretion in the renal pelvis would be decreased, but, in fact, a higher, although statistically insignificant, amount of SP was released in CBDL rats on mechanoreceptor stimulation compared with in Sham rats. Therefore, it seems that the extent of renal pelvic SP release is not related to the impaired renorenal reflex response in cirrhotic rats.

NK-1 receptors in the renal pelvis. Vigna et al. (37) have demonstrated the specificity of the antiserum used in the present study and shown that, in Western blot analysis, the antiserum recognizes rat NK-1 receptors with molecular masses ranging from 80 to 90 kDa. Our results show that the NK-1 receptor is expressed in the renal pelvis, the pattern of expression being similar to that seen for NK-1 receptors in the ileum and brain cortex. NK-1 receptor expression was lower in the 1-wk CBDL group (~68% of that in Sham rats) and was again reduced in the 4-wk CBDL group (~53% of that in the Sham group).

Although we did not study in detail the mechanism responsible for the reduced expression of NK-1 receptors in cirrhosis, the higher plasma SP concentration seems to indicate downregulation of NK-1 receptors in CBDL rats (Fig. 5C). The quantitative RT-PCR analysis showed the expression of renal pelvic NK-1 receptor mRNA to be similar in the Sham and 4-wk CBDL groups, ruling out the possibility of an effect of NK-1 receptor mRNA levels on receptor protein expression in...
the cirrhotic renal pelvis. The reduction in NK-1 receptors could be due to an increase in plasma SP levels in CBDL rats. Previous studies have examined the effect of SP-evoked desensitization or internalization of NK-1 receptors in vitro in rat kidney epithelial cells (36) or other cell lines (15, 16) and in vivo in tracheal endothelial cells (3), ileal myenteric neurons (30), spinal neurons (32), and the striatum (31). Further studies are needed to evaluate the roles of SP-mediated regulation in renal pelvic NK-1 receptor expression and in the renal sensory response in the cirrhotic kidney.

In summary, the present study shows that renal sensory impairment in CBDL rats is a time-dependent event, the defect being most severe at the cirrhotic stage. The renal mechanoreceptor-mediated inhibitory renorenal reflex is attenuated in cirrhotic rats, in which a temporal reduction in renal pelvic NK-1 receptors, but no change in renal pelvic SP release, is seen. This reduction is strongly correlated with increased plasma SP levels, but it is not due to altered mRNA expression. Taking these results together, we conclude that the physiological role of decreased renorenal reflex in CBDL rats may contribute to the overall dysfunction of sodium and water handling present in cirrhosis.

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