Expression of adenosine triphosphate-sensitive potassium channels in rats with cirrhosis: correlation with sympathetic activity and renal function

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ABSTRACT

Objective: The aim of this study was to perform a direct analysis of KATP mRNA expression by RT-PCR in kidney and isolated aorta from rats with cirrhosis (induced by carbon tetrachloride) and controls. The present study also analyses the relation between induced cirrhosis and urinary excretion of sodium and sympathetic activity in cirrhotic rats. Methods: Rats were placed in metabolic cages and allowed free access to food and water. Cirrhosis was induced by repeated doses of carbon tetrachloride by gastric gavage. After some weeks, the kidney and aorta were dissected and utilized for RNA extraction. Blood and urine were analyzed for electrolytes. Renal function was estimated by creatinine clearance and sodium urinary excretion. Serum catecholamines were measured by HPLC analysis. Results: First, RT-PCR analysis showed that KATP mRNA is expressed in liver with cirrhosis and intense fibrosis, but not with moderate fibrosis. Second, RT-PCR analysis revealed that KATP mRNA was detected only in aorta dissected from rats with cirrhosis. Finally, an enhanced reabsorption of sodium without renal failure suggests a potential mediator would increase the activity of the sympathetic system. Conclusion: These results suggest that KATP mRNA is expressed in cirrhotic rats with sympathetic activation and renal dysfunction. This channel might be involved in another route where the vascular tone can be modulated in cirrhosis.

Keywords: Potassium channels/analysis; Adenosine triphosphate; Sympathetic nervous system/pathophysiology; Liver cirrhosis, experimental; Kidney/physiology; Sodium/urine; Carbon tetrachloride; Rats

RESUMO

Objetivo: Realizar uma análise direta da expressão de RNAm de KATP por RT-PCR em rim e aorta isolados de ratos com cirrose (induzida por tetracloreto de carbono) e de controles. O presente trabalho também estudou as relações entre a cirrose induzida e a excreção urinária de sódio e a atividade simpática em ratos cirróticos. Métodos: Os ratos foram colocados em gaiolas metabólicas com acesso livre à comida e água. A cirrose foi induzida por repetidas doses de tetracloreto de carbono por gavagem gástrica. Depois de algumas semanas, o rim e a aorta foram dissecados e foi feita a extração de RNA. A dosagem de eletrólitos foi feita no sangue e na urina. A função renal foi estimada pelo “clearance” de creatinina e a excreção urinária de sódio. As catecolaminas séricas foram medidas por análise de HPLC. Resultados: Em primeiro lugar, a análise do RNAm de KATP expressou-se em fígados com cirrose e fibrose vigorosa, mas não com fibrose moderada. Posteriormente, a análise de RT-PCR revelou que a expressão de RNAm de KATP foi detectada somente na aorta dissecada de ratos com cirrose. Finalmente, uma reabsorção aumentada de sódio, sem falência renal, sugeriu que um potencial mediador aumente a atividade do sistema simpático. Conclusão: Estes resultados sugerem que o RNAm de KATP seja expresso em ratos cirróticos com ativação simpática e disfunção renal. Este canal pode estar envolvido em outra via, onde o tônus vascular pode ser modulado na cirrose.

Descritores: Canais de potássio/análise; Trifosfato de adenosina; Sistema nervoso simpático/fisiopatologia; Cirrose hepática experimental; Rim/fisiologia; Sódio/urina; Tetracloreto de carbono; Ratos
INTRODUCTION
Renal function abnormalities, such as sodium and water retention, leading to ascites, are common findings in cirrhosis\(^1\)-\(^2\). Several lines of evidence suggest that the key pathogenetic factor for sodium and water retention and functional renal failure in cirrhosis is an arterial vasodilatation that induces a hyperdynamic circulatory state. This arterial vasodilatation would cause an activation of vasoconstrictor and antinatriuretic systems (i.e., renin-angiotensin-aldosterone, sympathetic nervous systems, and the nonosmotic secretion of arginine vasopressin), eventually resulting in sodium and water retention and ascites\(^3\)-\(^5\). The mechanisms related to this vasodilatation are still only partly understood.

Several mechanisms have been suggested to contribute to splanchnic hyperemia, including increased levels of vasodilatators, such as adenosine, glucagons, calcitonin gene-related peptide (CGRP) and nitric oxide (NO)\(^6\)-\(^8\). It should be emphasized that these endogenous substances open vascular adenosine triphosphate (ATP)-sensitive potassium channels resulting in hyperpolarization of smooth muscle cell membrane\(^9\)-\(^10\). This hyperpolarization, in turn, closes L-type calcium channels and vascular tone decreases. Thus, vasodilator tone related to KATP channels may be abnormal in cirrhosis and contributes to vasodilatation. Recently, altered relaxation of arterial smooth muscle mediated by efflux of potassium (K) through membrane K channels has been proposed to contribute to cirrhosis-induced vasodilatation, since glibenclamide - an ATP-sensitive K channel blocker - caused significantly higher inhibition of potassium outward currents in vascular smooth muscle of cirrhotic rats than in controls\(^11\).

OBJECTIVES
The aim of this study was to perform a direct analysis of KATP mRNA expression by RT-PCR in kidney and isolated aorta from rats with cirrhosis (induced by carbon tetrachloride) and controls. The present study also analyses the relation between induced cirrhosis and urinary excretion of sodium and sympathetic activity in cirrhotic rats.

METHODS
Animals
The investigation was performed in male adult 33 Wistar rats (body weight of 170 to 285 g). The animals were placed in individual metabolic cages and fed ad libitum with normal chow and distilled water as drinking fluid. After a one-week adaptation, the first intragastric dose of carbon tetrachloride (CCl\(_4\)) was given. After 12 weeks, the CCl\(_4\) administration was discontinued in 10 animals, and the remaining (n = 13) completed an 18-week treatment. The other group (n = 10) was the control (with no CCl\(_4\)). Throughout this period, animals chosen at random from both groups were killed by gavage. The liver, kidney and aorta were removed. The groups were defined after histological analysis according to the grade of liver injury.

Cirrhosis – induction protocol
Cirrhosis was induced by the method of Proctor & Chatamra as previously described\(^12\). Carbon tetrachloride was weekly administered by gavage for 3-5 months. We used an 8% solution prepared one hour before use. The initial dose was 0.5 ml of the 8% solution. For the subsequent doses, we used a 5% variation in weight above or below the previous value as an indicator to calculate the CCl\(_4\) dose. For a weight gain of 5% or over, the dose was increased by 50%. When weight gain was lower than 5% or when there was no change in weight, the previous dose was maintained\(^13\). Segments of liver from each animal were excised after killing and were fixed in 4% paraformaldehyde for subsequent histological analysis.

Histology
Liver samples were stained with hematoxylin-eosin (HE), Masson trichrome and reticulin for standard light microscopy.

Analytical procedures
After treatment with CCl\(_4\) (and control group), rats were held in metabolic cages and urinary sodium excretion was monitored and collected for 24 hours. Glomerular filtration rate (24-hour endogenous creatinine clearance) was measured in these animals. Sodium concentration was measured by flame photometry (FC 130, CELM) and creatinine concentration by Jaffe reaction (Cobas Miras Plus, Roche). Serum norepinephrine (NE) and epinephrine (E) were analyzed by high performance liquid chromatography (HPLC) (Brownlee Lab and Shimadzu).

Reverse transcription-polymerase chain reaction (RT-PCR) analysis for KATP
The tissues (kidney and thoracic aorta) were harvested from all animals and RNA extracted by TRIzol reagent (Gibco BRL). The cDNAs synthesized from RNAs extracted from tissues with oligo(dT) primers were used as templates for PCR amplification. Oligonucleotides directed to the consensus pore sequence of cloned rat heart potassium channel were used\(^14\). The primer sequences were as follows: (F) 5’TCCCAGGACCACAAGAAGAT 3’ and (R) 5’GAAAGCAGACACAAAGCACC3’.
The cDNA templates were PCR-amplified with Taq polymerase (Gibco BRL) under the following conditions: 35 cycles at 94°C x 45 s, 50°C x 45 s, and 72°C x 45 s, and the products were submitted to electrophoresis on 2% agarose gel and visualized by ultraviolet (UV) fluorescence. The mRNA of β-actin was measured as internal control (15).

**STATISTICAL ANALYSIS**

Data are presented as mean ± standard deviation (SD). Statistical analysis was performed by one-way analysis of variance (ANOVA) and Kruskal-Wallis test with Dunn’s correction for multiple comparisons. Correlations were tested by Pearson regression analysis. P < 0.05 was considered significant.

**RESULTS**

Table 1 shows the effect of CCl₄ treatment in animals after 12 and 18 weeks. After a 12-week treatment with CCl₄, most animals showed early fibrosis (n = 6). Most animals who survived from CCl₄ treatment for over than 18 weeks, developed advanced hepatic injury, characterized by gross distortion of liver architecture (fibrosis ++ and cirrhosis, n = 10). Table 2 shows absolute sodium excretion (UNaV), fractional sodium excretion (FENa) and creatinine clearance in CCl₄-induced rats and controls. These parameters remained steady in control animals throughout the study. No significant changes in FENa and sodium urinary excretion (UNaV) were observed in animals with minimum fibrosis (fibrosis +) and controls. All CCl₄-induced rats with severe liver injury (fibrosis ++ and cirrhosis) developed sodium retention, which was observed in the absence of changes in creatinine clearance in all groups. There was a close relation between sodium retention and sympathetic activity in the animals with more severe liver injury. Figure 1 illustrates a significant inverse correlation between UNaV and NE in CCl₄-induced cirrhosis rats with severe injury (r = -0.53, p < 0.0018).

Using PCR primers to amplify the KATP-sensitive channels of rat heart, a 366-bp product was obtained from aorta of rats with severe liver injury (fibrosis ++ and cirrhosis) (figure 2). We obtained the same product in kidneys of all animals (CCl₄ and controls) (figure 2).

**Table 1.** Histologic analysis at 12 weeks and 18 weeks in control rats and rats with CCl₄-induced cirrhosis

<table>
<thead>
<tr>
<th>Histology</th>
<th>Control group (n = 10)</th>
<th>Groups (n = 10)</th>
<th>Groups 1 - CCl₄ (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fibrosis +</td>
<td>–</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Fibrosis ++</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>–</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

**Table 2.** Baseline measurements in rats with cirrhosis, fibrosis and control

<table>
<thead>
<tr>
<th></th>
<th>Normal (n = 10)</th>
<th>Fibrosis + (n = 9)</th>
<th>Fibrosis ++ (n = 9)</th>
<th>Cirrhosis (n = 5)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mEq/L)</td>
<td>139 ± 2</td>
<td>139 ± 2</td>
<td>139 ± 2</td>
<td>140 ± 1</td>
<td>0.55</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.58 ± 0.07</td>
<td>0.62 ± 0.12</td>
<td>0.62 ± 0.08</td>
<td>0.68 ± 0.05</td>
<td>0.18</td>
</tr>
<tr>
<td>Norepinephrine (pmol/mL)</td>
<td>127.9 ± 34.4</td>
<td>250.6 ± 122.3</td>
<td>308.0 ± 139.0*</td>
<td>358.0 ± 136.2*</td>
<td>0.001</td>
</tr>
<tr>
<td>Epinephrine (pmol/mL)</td>
<td>149.5 ± 87.3</td>
<td>137.4 ± 116.7</td>
<td>206.0 ± 219.2</td>
<td>163.0 ± 58.0</td>
<td>0.71</td>
</tr>
<tr>
<td>FENa (%)</td>
<td>0.60 ± 0.13</td>
<td>0.45 ± 0.11</td>
<td>0.37 ± 0.11*</td>
<td>0.30 ± 0.10*</td>
<td>0.0007</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.4</td>
<td>1.1 ± 0.5</td>
<td>0.63</td>
</tr>
<tr>
<td>Urinary excretion of sodium (mEq/24h)</td>
<td>1.44 ± 0.21</td>
<td>1.00 ± 0.33</td>
<td>0.77 ± 0.28*</td>
<td>0.74 ± 0.66*</td>
<td>0.0027</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. control.
DISCUSSION

During the past few years several studies have characterized the systemic and renal function abnormalities in rats with CCl₄-induced cirrhosis(16-17). In addition to a histological lesion characterized by severe fibrosis or cirrhosis, these animals develop abnormalities of systemic (sympathetic activity) and renal function (impaired renal ability to excrete sodium).

The present study in rats with CCl₄-induced cirrhosis demonstrated sodium retention, which was considered secondary to increased tubular reabsorption of sodium, since the animals did not present lower glomerular filtration rate. Our animals with severe fibrosis and cirrhosis started retaining sodium with no decrease in endogenous creatinine clearance. Several mechanisms, including activation of renal sympathetic nervous activity, may explain this relation. A role for the sympathetic nervous system in renal dysfunction in cirrhosis was proposed(18-20). The sympathetic activity in cirrhosis can be quantified by plasmatic NE(21). Most data reported for our cirrhotic animals showed increased NE plasma levels, which may be correlated with renal function abnormalities, such as impaired ability to excrete sodium.

Studies in patients and experimental animals without ascites corroborate the idea that circulatory abnormalities leading to arterial vasodilatation in cirrhosis occur before changes in glomerular filtration rate.

The kidneys are richly innervated by sympathetic nerve fibers that supply vessels, glomeruli, and tubular cells(22). Sodium retention in this situation could be caused by an increase in sodium reabsorption in the proximal tubules, owing to a direct tubular effect (alpha-adrenoreceptor)(23). The results of this study showing the relation between plasma NE and urinary sodium excretion may suggest this. In addition, our findings demonstrated a direct relation between histological findings of cirrhosis (and severe fibrosis) and plasmatic NE levels. A minor rise in epinephrine (E) concentration detected in all CCl₄-induced cirrhosis rats might be related to decreased hepatic metabolism. Previous studies reported that the splanchic area extracted E more efficiently than NE(24-25). This could be due to differences in enzymatic activity of the metabolic pathways of epinephrine and norepinephrine.

The results of the present study do not explain the mechanism of circulatory abnormalities. However, experimental models showed that arterial vasodilatation in cirrhosis can be induced by activation of adenosine triphosphate (ATP)-sensitive K channels on vascular tone(11,26-27). In our study, RT-PCR analysis demonstrated that transcripts encoding K-ATP channel were expressed in all intact aortae of CCl₄-induced rats (moderate fibrosis, severe fibrosis, and cirrhosis), but not in controls. Recent data demonstrated that glibenclamide (a KATP channel blocker) increased vascular tone in cirrhotic rats, indicating that inhibition of a baseline vasodilator tone in these animals can occur due to opening of KATP channels(11). In addition, Atucha et al.(28) stated that the mechanism underlying the mesenteric hyporesponsiveness of portal hypertensive rats is based on activation of potassium channels. We also describe KATP mRNA in kidneys from cirrhotic (and fibrosis) and normal rats. The expression of the variant KATP channel (ROMK) was reported at high levels in the renal microtubular epithelium(29-30). Since the primers used in this study could not discriminate between these variants, we cannot conclude whether this transcript was from epithelium or smooth muscle.

CONCLUSION

Our results suggest that KATP mRNA is expressed in cirrhotic rats with sympathetic activation and renal dysfunction. This channel might be involved in another route in which vascular tone can be modulated in cirrhosis.

REFERENCES

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