Long-Term Aquaretic Efficacy of a Selective Nonpeptide \( V_2 \)- Vasopressin Receptor Antagonist, SR121463, in Cirrhotic Rats

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ABSTRACT

Water retention in experimental cirrhosis can be reversed by blocking \( V_2 \)-vasopressin (AVP) receptors with the nonpeptide antagonist OPC-31260 or by using the \( \kappa \)-opioid receptor agonist niravoline, a compound inhibiting central AVP release. However, reluctance to use these drugs in human beings has emerged because the former loses aquaretic efficacy in rats after 2 days of treatment and the latter may have adverse effects in humans. Recently, a new potent and selective nonpeptide \( V_2 \)-AVP receptor antagonist, SR121463, has been developed that could be useful for the treatment of dilutional hyponatremia in human cirrhosis. The current study assessed the aquaretic efficacy of 10-day chronic oral administration of SR121463 (0.5 mg/kg/day) in cirrhotic rats with ascites and impaired water excretion after a water load (minimum urinary osmolality >160 mOsm/kg and percentage of water load excreted <60%). Urine volume (UV), osmolality (U\( \text{Osm}_{V}\)), and sodium excretion (U\( \text{Na}_{V}\)) were measured daily. At the end of the 10-day treatment, renal ability to excrete a water load and normalization in serum sodium and osmolality. During the first 6 days of treatment, SR121463 also increased UV and reduced U\( \text{Osm}_{V}\) during the 10-day treatment. This resulted in a greater renal ability to excrete a water load and normalization in serum sodium and osmolality. These data suggest that SR121463 could be of therapeutic value for chronic management of human cirrhosis.

Vasopressin (AVP) is a cyclic nonpeptide produced by and secreted from the hypothalamo-neurohypophysial system in response to increased plasma osmolality or decreased blood pressure (Arroyo et al., 1994). AVP plays a major role in the regulation of water balance and also participates in cardiovascular homeostasis. Three subtypes of AVP receptors have been cloned and characterized: \( V_1a \), \( V_1b \), and \( V_2 \) (Bichet, 1996). \( V_1 \) receptors mediate phospholipase C activation and intracellular calcium mobilization. The \( V_{1a} \) receptor subtype is considered to be the gateway for most of the known cardiovascular actions of AVP (Jard, 1988). It also is involved in liver glycogenolysis, platelet aggregation, and uterus contraction, whereas activation of \( V_{1b} \) receptors mainly induces adrenocorticotropic release from the pituitary gland. In the kidney, AVP exerts its well-known antidiuretic properties by activating a cAMP-dependent pathway, which is triggered by interaction with the \( V_2 \) receptor (Bichet, 1996).

Selective inhibition of renal AVP effects may have important therapeutic implications in the management of patients with water retention and, consequently, dilutional hyponatremia and hypo-osmolality (Martin and Schrier, 1997; Ginés et al., 1998). Impairment in water excretion is a characteristic disturbance of patients with the syndrome of inappropriate secretion of antidiuretic hormone, congestive heart failure, or advanced liver cirrhosis. In this latter group of subjects the intensity of the abnormality may vary widely from patient to patient, but in some cases it is so important that patients may show a negative free water clearance even after the administration of a water overload. Therefore, the development of aquaretic drugs is a major challenge for the treatment of these subjects. Two strategies directed toward 1) AVP activity in the kidney by using AVP-\( V_2 \) receptor

**ABBREVIATIONS:** AVP, vasopressin; mUOsm, minimum urinary osmolality; U\( \text{AVP}_{V}\), urinary excretion of AVP; ALD, aldosterone; U\( \text{ALD}_{V}\), urinary excretion of aldosterone; U\( \text{cAMP}_{V}\), urinary excretion of cAMP; U\( \text{Osm}_{V}\), urine osmolality; U\( \text{Na}_{V}\), urine sodium; U\( \text{K}_{V}\), urine potassium; MAP, mean arterial pressure.
antagonists (Sawyer et al., 1981; Yamamura et al., 1992), and 2) hypothalamic AVP production by using κ-opioid agonists (Hamon and Jouquey, 1990) have been followed. Up to now, however, these approaches have proven to be of limited value because peptide and nonpeptide AVP-V2 receptor antagonists already described displayed partial agonist, species-dependent, and/or tachyphylactic properties (Stassen et al., 1983; Liard, 1988; Bosch-Marcé et al., 1999), and in the clinical setting, reluctance to use κ-opioid agonists has emerged because of central nervous system side effects (Bellisant et al., 1996).

Recently, SR121463, a new potent and highly selective nonpeptide AVP-V2 receptor antagonist, has been characterized (Serradeil-Le Gal et al., 1996). This compound displays a highly selective affinity for V2 receptors in rat and human kidney preparations, without any agonistic effect. Moreover, it induces a powerful and pure aquaretic response when given orally or i.v. to rats (Serradeil-Le Gal et al., 1996). This aquaretic effect was maintained during chronic treatments in normally hydrated conscious rats (Lacour et al., 1997). The current study, therefore, aimed to investigate the therapeutic usefulness of SR121463 in a pathological situation such as cirrhosis, by examining the renal hormonal and hemodynamic effects induced by a chronic 10-day oral administration of this agent to rats with cirrhosis, ascites, and water retention.

### Experimental Procedures

#### Materials

AVP was obtained from Sigma Chemical Co. (L'Isle d'Abeau, France). The natural tritiated hormone [3H]AVP and the selective AVP-V1a ligand [125I-phenylacetyl-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH2] (Schmidt et al., 1991) were purified from New England Nuclear, Life Sciences (Les Ulis, France). The natural tritiated hormone [3H]AVP and the selective AVP-V1a ligand [125I-phenylacetyl-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH2] (Schmidt et al., 1991) were purchased from New England Nuclear, Life Sciences (Les Ulis, France). The specific AVP-V2 receptor antagonist SR121463 [1-[4-N-tetraethylcarbamoyl]-2-methoxybenzene sulfonyl]-5-ethoxy-3-spiro-[4-(2-morpho-linoethoxy)cyclohexane] indol-2-one, fumarate (Serradeil-Le Gal et al., 1996) was synthesized at Sanofi Recherche (Toulouse, France). All other chemicals were of the highest reagent grade available.

#### Pharmacological Study

The study was performed in 20 conscious adult male Wistar rats with cirrhosis, ascites, and impaired free water excretion and in 23 control Wistar rats. Both groups were fed ad libitum with standard chow and distilled water containing phenobarbital (0.3 g · l⁻¹) as drinking fluid. Cirrhosis was induced by CCl₄, which was administered twice weekly (Monday and Friday) according to a method described elsewhere (Claria et al., 1998). Cirrhotic rats were obtained from a group of 58 animals submitted to the cirrhosis induction protocol. Thirty-eight of these animals could not be included in the study for the following reasons: 29 rats died before the development of impairment of water excretion and 9 animals died before completing the experimental protocol. Two weeks after cirrhotic rats developed ascites, all animals were placed in metabolic cages and the renal ability to excrete free water was determined once weekly (Tuesday) in each rat submitted to the cirrhosis induction program as follows. Two hours after removing food and water, a water load (50 ml/kg b.wt.) was administered via a gastric tube inserted under light ether anesthesia. Immediately afterward the rats were reintroduced into their metabolic cages where each volume of spontaneously voided urine was collected separately. After 3 h, and following an abdominal massage, a final urine sample was obtained. The osmolality of each urine sample was measured. Total volume was measured gravimetrically. The renal ability to excrete free water was estimated through the minimum urinary osmolality (mUOsm) of spontaneously voided samples obtained after the water load and by calculating the percentage of the water load excreted during the 3-h urine collection period. When a significant impairment in the renal ability to excrete free water was detected (percentage of water load excreted <60% and mUOsm >160 mOsm/kg) cirrhotic animals received a 2-min CCl₄ reexposure to avoid spontaneous improvement in free water excretion and then were included in the protocol. All studies started 24 h after the administration of the water load. Because rats treated with CCl₄ and phenobarbital developed the impairment in free water excretion within 14 to 28 weeks after starting the cirrhosis induction program, control rats were investigated 14 to 27 weeks after being included in the study.

Effects of chronic administration of SR121463 or vehicle on renal sodium and water metabolism; creatinine clearance; urinary excretion of AVP (UAVPV), aldosterone (ALD) (UALDV), and cAMP (UAMVP); and systemic hemodynamics in cirrhotic rats with ascites and water retention were measured.

Animals included in the study were randomly assigned to one of the following groups: 1) intragastric administration of SR121463 (0.5 mg/kg, dissolved in 0.6% methyl cellulose in water) administered daily for 10 days (10 cirrhotic and 12 control rats), and 2) intragastric administration of 0.6% methyl cellulose in water (4.2 ml/kg) administered daily for 10 days (10 cirrhotic and 11 control rats). The dose used in the study was based on preliminary experiments performed with SR121463 in cirrhotic rats in which animals received 5, 1, or 0.5 mg/kg b.wt. daily. The two highest doses induced a massive diuretic effect in cirrhotic rats with ascites that eventually led to animal death due to dehydration (J. Ros, unpublished observations).

Measurements of the 24-h urine volume and osmolality (U Osm) and sodium excretion (U NaV) were made 1 day before the water overload and for 9 consecutive days after the animals were included in the protocol. An aliquot of each 24-h urine collection was frozen at −30°C until analyzed to determine urea, creatinine, UAVPV, UALDV, and UAMVP.

On the 10th day, animals were submitted to a second water overload, as previously described, and the mUOsm and percentage of water excreted were determined in the 3-h urine collection. Thereafter, animals were anesthetized with ketamine (50 mg · kg⁻¹) and prepared with a polyethylene-50 polyvinyl catheter in the left femoral artery for blood pressure recording and blood sampling. The catheter was tunneled s.c., exteriorized in the nape of the neck, and ran through a flexible stainless steel sheath that was attached to a harness made of polyestrene worn by the animal. The femoral artery catheter was connected to a highly sensitive transducer (Hewlett Packard, Avondale, PA). Mean arterial pressure (MAP) and heart rate were recorded in a multichannel system (MX4P and MT4, Lec- tram Ltd., Jersey Channel Islands, UK). Animals were placed in rectangular cages with no restriction of movement and allowed to recover from surgery and anesthesia for 3 h. Eight hours after the last administration of SR121463 or the vehicle a blood sample (0.5 ml) was obtained to measure serum Na⁺, K⁺, osmolality, urea, and creatinine, and in those rats receiving the aquaretic agent, the serum levels of SR121463 also were measured. Thereafter, packed cells were reconstituted to an equal volume with Ringer’s solution, reinfused over 3 min, and measurements of MAP and heart rate were made. Then, cirrhotic and control rats were sacrificed by decapitation and the liver and kidneys dissected, quickly frozen in dry ice, and stored in liquid nitrogen until further analysis. The study was performed according to the criteria of the Investigation and Ethics Committee of the Hospital Clinic Universitari.

#### Biochemical Measurements

**Binding Assays.** With whole rat kidney and liver samples, membranes were prepared by the method of Stassen et al. (1982) and Pripie et al. (1983), respectively, and then stored as aliquots in liquid nitrogen until used at a final concentration of about 10 mg/ml.
Protein concentration was determined by the method of Bradford (1976) with BSA as a standard. Binding assays of $^{125}$I-AVP Antag to rat liver and $[^{3}H]$AVP to rat kidney preparations were performed as previously described (Schmidt et al., 1991; Serradeil-Le Gal et al., 1996). The selective, high-affinity $^{125}$I-AVP Antag ligand (Schmidt et al., 1991) was used for the characterization of AVP binding sites in liver that constitutively express $V_{1a}$ receptors. This radioiodinated, high-affinity ligand enabled the binding study to be carried out by using a minimal quantity of membranes compared with tritiated ligands. Of note was the absence of significant amounts of $^{125}$I-AVP Antag binding sites in rat kidney preparations. Therefore, renal AVP binding sites ($V_{2}$) were further characterized by using $[^{3}H]$AVP as a ligand. Saturation binding experiments were conducted in the presence of $^{125}$I-AVP Antag (0.001–0.5 nM) on rat liver membranes (0.5 mg/ml) and $[^{3}H]$AVP (0.03–20 nM) on rat kidney preparations (0.7 mg/ml). Nonspecific binding was determined in both cases in the presence of 10 µM unlabeled AVP.

**Biochemical and Hormonal Assays.** Serum concentration of SR121463 was measured as follows. To each 50-µl plasma sample 100 µl of a 25-ng/ml internal standard solution in 80% aqueous methanol was added plus 50 µl of 50% aqueous methanol. After a 12,000g x 5-min centrifugation, SR121463 concentration was measured on the supernatant by liquid chromatography-mass spectrometry/mass spectrometry chromatography (Burton et al., 1997). Calibration samples (0.25, 0.5, 1, 2, 5, 10, 25, 35, 50, 60, 75, and 100 ng/ml) were prepared and assayed according to the same protocol. This validated method provided a limit of quantification of 0.25 ng/ml (within run precision and accuracy of ±20%). Serum and urinary osmolality were determined from osmometric depression of the freezing point (osmometer 3300; Advanced Instruments, Needham, MA) and sodium concentration by flame photometry (IL 943; Instrumentation Laboratory, Lexington, MA). Urinary AVP was determined by radioimmunoassay (Bühlman Laboratories AG, Basel, Switzerland) of unextracted samples as previously described (Camps et al., 1987). The urinary concentration of ALD was measured with the use of a commercial kit (Coat-A-Count Aldosterone; Diagnostic and Products Corporation, Los Angeles, CA), in urine samples (0.5 ml) adjusted to pH 1.0 with 1 ml of 0.2 N HCl and kept during 20 h at 30°C. With this procedure most ALD-18-glucuronide is transformed into ALD (Jiménez et al., 1985). CAMP concentration in urine was assessed by radioimmunoassay (Amersham Pharmacia Biotech UK, Buckinghamshire, England). Creatinine and urea were measured by the Ektachem Clinical Chemistry Slide method (Johnson & Johnson Clinical Diagnostic Inc., Rochester, NY). A liver specimen was obtained from the middle lobe of each animal. Liver specimens were fixed in 10% buffered Formalin and stained with H&E, reticulum, and Masson’s trichrome for histological examination.

**Statistical Analysis**

Statistical analysis of results was performed by one-way ANOVA, the Newman-Keuls test, and the paired and unpaired Student’s t tests when appropriate. Results are given as mean ± S.E. Data from saturation binding experiments were analyzed and equilibrium binding data (i.e., $K_{D}$, apparent equilibrium dissociation constant and $B_{max}$, maximum binding density) were determined by using an interactive nonlinear regression program (Munson and Rodbard, 1980).

**Results**

The liver histology of all animals treated with CCl4 included in the study had a finely granulated surface and histological examination showed marked architectural distortion leading to fully developed cirrhosis (Claria et al., 1998), with no significant differences between rats receiving SR121463 or the vehicle. Control rats showed no appreciable alterations in liver histology.

Table 1 shows that cirrhotic rats included in the protocol were investigated after they had developed marked sodium retention and severely impaired renal ability to excrete free water after the water load test (93% versus only 20 to 40% water excretion for cirrhotic animals). The impairment of water excretion occurred within the range of 14 to 28 weeks after starting the cirrhosis induction program. Ascites preceded the impairment in free water excretion by at least 2 weeks. Before starting the 10-day treatment regime, cirrhotic rats receiving the $V_{2}$-AVP receptor antagonist did not differ in the renal response to the water load and $U_{Na}$ from cirrhotic rats receiving vehicle. Moreover, no significant differences were found in any of these parameters between the two groups of control rats.

The effect of SR121463 or the vehicle on urine flow and osmolality is shown in Figs. 1 and 2, respectively. No significant changes in any of these parameters were observed throughout the study in cirrhotic and control rats receiving the vehicle. The urinary flow rate was not modified after the oral administration of SR121463 (0.5 mg/kg/day) to control rats; however, these animals displayed a significant decrease in $U_{Osm}$ during the first days of treatment (ANOVA: $F = 2.18$, $P < .05$). In contrast, the same dose of SR121463 markedly increased urinary flow (ANOVA: $F = 10.62$, $P < .0001$) and decreased $U_{Osm}$ (ANOVA: $F = 14.66$, $P < .0001$) in cirrhotic rats throughout the 10-day study. The aquaretic effect of SR121463 was, however, more intense during the first 4 days of treatment and then reached a stabilized steady-state value. More importantly, SR121463 also induced a natriuretic effect in cirrhotic rats (ANOVA: $F = 2.46$, $P < .05$) that was significant during the first days of the treatment and disappeared thereafter (Fig. 3).

After completing the study, no significant differences were observed in the renal response to the water load, serum sodium, and serum osmolality between the two groups of control rats receiving the vehicle or SR121463 (Table 2). In contrast, cirrhotic rats with ascites receiving the aquaretic agent showed a significantly higher percentage of water load excreted and lower $mU_{Osm}$ than cirrhotic animals receiving

**TABLE 1**

<table>
<thead>
<tr>
<th>Body weight, renal response to the water load, and urinary excretion of sodium before starting the treatment in cirrhotic and control rats receiving SR121463 or vehicle</th>
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<tr>
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<tr>
<td>Body wt. (g)</td>
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<tr>
<td>Percentage of water load excreted mUOsm (mOsm/kg)</td>
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<tr>
<td>$U_{Na}$ (µEq/min)</td>
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<td>U$_{Osm}$ (µOsm/kg)</td>
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With respect to control rats receiving the same treatment (unpaired Student’s t test), $^a P < .001$ and $^b P < .01$. 

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the vehicle. This improvement in renal water metabolism was associated with a normalization in serum sodium and osmolality.

Figure 4 shows the individual values of the percentage of water load excreted after the water challenge in control and cirrhotic rats under baseline conditions and after 10 days of oral SR121463 or vehicle treatment. Because two cirrhotic rats treated with SR121463 and one control animal receiving the vehicle died during the administration of the second water overload, this figure includes individual values of 18 cirrhotic and 22 control rats. The mean values are given in Tables 1 and 2. The administration of the vehicle did not produce any significant change in percentage of water load excreted in either cirrhotic or control rats. Treatment with SR121463 was associated with a slight, albeit significant, increase in water excretion in control animals (Table 2). However, the amelioration in the renal water handling in cirrhotic rats was so remarkable that, at the end of the treatment, only two cirrhotic animals still showed values of percentage of water load excreted lower than 60% (Fig. 4).
The average values of $U_{Na}V$, $U_{K}V$, $U_{Urea}V$, $U_{ALD}V$, $U_{AVP}V$, and $U_{AMP}V$ during the entire period of treatment and creatinine clearance at the end of the study in control and cirrhotic rats receiving the vehicle or SR121463 are shown in Table 3. Control rats receiving the V$_2$ receptor antagonist showed similar values of $U_{Na}V$, $U_{K}V$, $U_{AMP}V$, and creatinine clearance, significantly decreased $U_{Urea}V$, and increased $U_{ALD}V$ and $U_{AVP}V$ compared with those receiving the vehicle. As expected, cirrhotic rats receiving the vehicle showed marked sodium retention that occurred in the setting of decreased potassium excretion, pronounced hyperaldosteronism, a significant increase in $U_{AVP}V$, and no change in creatinine clearance. Compared with control rats receiving vehicle, nontreated cirrhotic animals also showed a significant diminution in the urinary excretion of urea and cAMP (59 and 61%, respectively). Chronic administration of SR121463 to cirrhotic rats was associated with increased sodium, potassium, urea, and AVP excretion (184, 22, 13, and 55%, respectively) and no changes in $U_{ALD}V$, $U_{AMP}V$, and creatinine clearance compared with cirrhotic rats receiving the vehicle.

Interestingly, cirrhotic rats treated with the aquaretic agent showed markedly higher serum levels of SR121463 (2.54 ± 0.55 ng/ml) than control animals under the same treatment (lower than 0.25 ng/ml in eight animals and between 0.92 and 0.51 pg/ml in the remaining animals), supporting the higher aquaretic effect of SR121463 observed in cirrhotic versus control rats.

As shown in Fig. 5, chronic SR121463 administration did not induce any significant change in MAP in control rats. As previously observed, cirrhotic rats showed marked arterial hypotension (Claria et al., 1998). This characteristic circulatory dysfunction of cirrhotic animals, however, did not experience any significant change after chronic SR121463 administration (Fig. 5).

Finally, as shown in Table 4, the 10-day treatment with SR121463 did not modify binding parameters ($K_d$, $B_{max}$) for V$_{1a}$ receptors in rat liver and for V$_2$ receptors in rat kidney, in terms of receptor affinity and number. An important finding in this study is the dramatically (50%) lower number of AVP V$_{1a}$ binding sites observed in cirrhotic rat liver, a target injury organ in this disease, whereas renal [H$^3$]AVP V$_2$ receptors were not affected by the cirrhotic condition.

**Discussion**

The search for selective inhibitors of renal AVP activity in human beings has been the subject of intense activity over the past years (Robertson and Berl, 1996). Inhibiting AVP binding to renal V$_2$ receptors seems a useful therapeutic strategy for edematous disorder with dilutional hyponatremia. Initially, several peptide AVP-V$_2$ receptor antagonists were designed. However, their peptide nature hampered their clinical use because of poor oral bioavailability. In addition, partial agonistic action and species-specific properties have been reported with this class of drugs (Allison et al., 1988). Manning et al. (1987) suggested that small molecules also could interact with AVP receptors. Currently, a number of nonpeptide, selective (more or less), orally active AVP-V$_2$ receptor antagonists have been described. Among the most extensively characterized are those of the OPC family (31260 and 41061) (Yamamura et al., 1992, 1998) and 5-fluoro-2-methyl-N-[5H-pyrrolo[2,1-c]-[1,4]benzodiazepin-10(11H)-ylcarbonyl]-3-chlorophenyl]benzamide (Albright et al., 1998), and SR121463 (Serradeil-Le Gal et al., 1996), the latter compound belonging to a new chemical series. Single oral administration of OPC-31260 has been shown to induce a potent aquaretic effect in human, dogs, and rats under normal or pathological conditions (Yamamura et al., 1992; Ohnishi et al., 1993; Tauboi et al., 1994). This compound, however, failed to show continuous aquareasis in cirrhotic rats in a 10-day treatment regime at 5 mg/kg p.o. (Bosch-Marcé et al., 1999) and also displayed significant antagonist V$_{1a}$ activity ($V_{1a}/V_2$ selectivity ratio of 1:10) in human tissue (Serradeil-Le Gal et al., 1996). More recently, OPC-41061 has shown an improved effect...
selectivity for V₂ receptors (V₃/V₂ selectivity ratio of 1:29) and produces aquarexia after multiple dosing in normal rats (Yamamura et al., 1998). 5-Fluoro-2-methyl-N-[5H-pyrrolo[2,1-c]-[1,4]benzodiazepin-10(11H)-ylcarbonyl]-3-chlorophenylbenzamide is a highly selective V₂ antagonist that increases water excretion, serum sodium, and osmolality (Albright et al., 1998). Its aquaretic efficacy has been tested in cirrhotic patients who experienced a dose-dependent augmentation in urinary flow and osmolality at oral single doses up to 300 mg (Guyader et al., 1998). Finally, the pharmacological and aquaretic properties of SR121463 have been characterized in several in vivo and in vitro models. This agent is devoid of any agonistic effect and shows a highly competitive affinity for V₂ receptors in rat and human kidney (Serradeil-Le Gal et al., 1996). Binding studies have shown a relative V₂/V₁ selectivity of SR121463 of more than 7000-fold in rat and 100-fold in human tissue, and in euvolemic rats, oral administration of this agent induces a pure dose-dependent aquaretic effect between the range of 0.03 and 10 mg/kg b.wt. (Serradeil-Le Gal et al., 1996). Chronic oral treatment with SR121463 at 3 mg/kg induced a powerful and maintained aquaretic effect without modifying Na⁺ and K⁺ urinary excretion (Lacour et al., 1997). Very few data, however, exist on the suitability of SR121463 for the treatment of edematous states with hyponatremia and hypo-osmolality.

In the current study, 10-day oral administration of 0.5 mg/kg SR121463 to control rats induced a moderate effect of renal excretory function that was only evidenced when analyzing \( U_{\text{Osm}} \), or the renal ability to excrete a water overload. In contrast, the same dose of AVP-V₂ receptor antagonist administered to cirrhotic rats with ascites and water retention resulted in a marked increase in the urinary flow rate and in an acute reduction in \( U_{\text{Osm}} \). These effects lasted for the entire period of treatment and, at the end of the investigation, cirrhotic rats receiving SR121463 did not show hyponatremia or hypo-osmolality, and most of the animals exhibited a renal water handling within the normal range. Of note, the aquaretic effect of SR121463 was more intense during the first 5 days of treatment than in subsequent days, whereas the effect on \( U_{\text{Osm}} \) remained rather constant (Fig. 2). A similar pattern in urine flow rate has been observed in previous chronic studies (Lacour et al., 1997). The rise in endogenous AVP secretion subsequent to important water loss after the exposure to V₂ antagonism could explain this profile of urine flow rate. As to whether this phenomenon could ultimately result in a lack of therapeutic efficacy in cirrhotic rats cannot be ascertained from the results of the current investigation. Chronic studies for extended periods of time would be of major interest to define this point.

Compared with control animals receiving the aquaretic agent, cirrhotic rats treated with SR121463 also showed markedly increased circulating levels of this compound (from 2.5- to more than 10-fold higher) in agreement with a reduced metabolism of SR121463 in cirrhotic rats. This finding, which results from a decrease in hepatic enzyme activity in CCl₄-treated rats (data not shown), explains the higher pharmacological diuretic effect of the aquaretic agent in cirrhotic rats than in SR121463-treated control animals. It is impor-

### TABLE 3

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<tr>
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<th>SR121463</th>
<th>Vehicle</th>
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<tr>
<td>( U_{\text{Na}} V ) (mEq/day)</td>
<td>1.11 ± 0.03</td>
<td>1.10 ± 0.05</td>
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<tr>
<td>( U_{\text{K}} V ) (mEq/day)</td>
<td>3.07 ± 0.08</td>
<td>2.92 ± 0.07</td>
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<tr>
<td>( U_{\text{Urea}} V ) (mg/h)</td>
<td>23.0 ± 0.56</td>
<td>21 ± 1</td>
</tr>
<tr>
<td>( U_{\text{ALD}} V ) (ng/day)</td>
<td>8.44 ± 0.22*</td>
<td>3.0 ± 0.18</td>
</tr>
<tr>
<td>( U_{\text{AVP}} V ) (ng/day)</td>
<td>2.99 ± 0.18*</td>
<td>1.52 ± 0.12</td>
</tr>
<tr>
<td>( U_{\text{UAMP}} V ) (mmol/day)</td>
<td>140 ± 8</td>
<td>126 ± 9</td>
</tr>
<tr>
<td>( C_{\text{Osm}} ) (mOsm/kg)</td>
<td>1.2 ± 0.1</td>
<td>0.9 ± 0.1</td>
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</table>

With respect to control rats receiving the same treatment (unpaired Student’s t test), *P < .001; **P < .005, and ***P < .05 vs. vehicle and ****P < .001.

### FIG. 5

**MEAN ARTERIAL PRESSURE**

**CONTROL**

**CIRRHOSIS**

**Fig. 5.** MAP after completing the entire period of treatment in control rats and in cirrhotic rats with ascites and water retention receiving SR121463 (0.5 mg/kg b.wt.) or vehicle. *P < .001 and **P < .005 with respect to control rats receiving the same treatment (unpaired Student’s t test). □, vehicle; ★, SR121463.

### TABLE 4

Characterization of rat liver and kidney AVP binding sites in normal and cirrhotic rats

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<tr>
<th></th>
<th>Liver</th>
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<th>Kidney</th>
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<tr>
<td></td>
<td>( K_d )</td>
<td>( B_{\text{max}} )</td>
<td>( K_d )</td>
</tr>
<tr>
<td></td>
<td>nM</td>
<td>fmol/mg protein</td>
<td>nM</td>
</tr>
<tr>
<td>Control rats</td>
<td></td>
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</tr>
<tr>
<td>SR121463</td>
<td>0.17 ± 0.002</td>
<td>17 ± 2</td>
<td>0.56 ± 0.06</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.021 ± 0.002</td>
<td>21 ± 2</td>
<td>0.93 ± 0.12</td>
</tr>
<tr>
<td>SR121463</td>
<td>0.017 ± 0.008</td>
<td>12 ± 4</td>
<td>3.22 ± 0.33</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.037 ± 0.010</td>
<td>9 ± 2</td>
<td>1.66 ± 0.20</td>
</tr>
</tbody>
</table>
tant to note that in control rats, SR121463 induced a significant decrease in U_{\text{Osm}} during the first 4 days of the treatment (Fig. 2), indicating that U_{\text{Osm}} is a more sensitive parameter and also confirming an adaptation phase after exposure to SR121463 treatment.

Inhibition of AVP activity with nonpeptide AVP-V\(_2\) receptor antagonists has been described to increase the circulating levels of this hormone (Ohnishi et al., 1998). Moreover this may affect endogenous vasoactive systems, other than AVP, also implicated in the regulation of blood volume and electrolyte homeostasis. In the current investigation, 10-day SR121463 administration to normally hydrated rats resulted in a significant increase in the urinary excretion of ALD and AVP. Because these parameters likely reflect the circulating levels of these substances, our findings indicate that long-term SR121463 treatment activates the renin-angiotensin-ALD and AVP systems in normal animals. This probably constitutes a compensatory mechanism to counteract the aquaretic effect induced by SR121463 in control rats. As anticipated, cirrhotic rats receiving vehicle showed an 18-fold increase in U_{\text{ALD}}V and a 2-fold increase in U_{\text{AVP}}V compared with nontreated control rats. Long-term SR121463 administration to cirrhotic animals did not modify the degree of activation of the renin-angiotensin-ALD system, which is already highly activated, but induced a further 2-fold increase in U_{\text{AVP}}V. The pronounced overproduction of AVP in cirrhotic SR121463-treated rats had no major consequences because the compound maintained its aquaretic effect under the multiple-dosage regime. However, this AVP increase could explain the decrease in the aquaretic activity of SR121463 observed after the first days of the treatment.

Chronic SR121463 treatment in cirrhotic rats did not result in major modifications in the density and the affinity of V\(_2\) receptors. However, we observed a dramatic decrease (50%) in liver AVP-V\(_2\) receptor number. This could be explained by a down-regulation of these receptors or could reflect a general injury in this organ because we also observed a diminution in cAMP and urea production, synthesized in large amounts by the liver.

An unexpected result of the current investigation was the improvement in sodium excretion induced by SR121463 in cirrhotic rats, which was significant during the 6 first days of treatment. In fact, during the entire period of treatment, the average U_{\text{Na}}V in cirrhotic animals chronically treated with the AVP-V\(_2\) receptor antagonist was similar to that found in noncirrhotic control animals. A possible effect of SR121463 on the renin-angiotensin-ALD system is unlikely because no differences in U_{\text{ALD}}V were observed between treated and nontreated cirrhotic rats. The mechanisms by which long-term administration of SR121463 increases natriuresis in experimental cirrhosis were not specifically addressed in the current study. However, besides the fact that AVP augments Na\(^+\) transport in the collecting duct, recent data showed that AVP increased the expression and activity of the luminal epithelium sodium channel in the rat kidney, suggesting that high AVP levels could participate in Na\(^+\) retention (Nicco et al., 1999).

Aquaretic effector in cirrhosis is generally considered to be a compensatory mechanism to the arterial vasodilation occurring in advanced liver disease (Arroyo et al., 1994). Thus, it is theoretically possible that any modification in the circulating levels of AVP and/or its biological activity may have hemodynamic consequences. Chronic administration of SR121463 actually increased AVP levels because the urinary excretion of this hormone in rats treated with this agent was approximately twice that of those receiving vehicle, regardless of whether they were cirrhotic or controls. However, the increase in AVP levels did not result in any effect on MAP because chronic SR121463 administration did not modify this parameter in either cirrhotic or control rats.

In summary, the results of the current investigation indicate that long-term administration of SR121463 has an aquaretic effect in rats with cirrhosis, ascites, water retention, and hypo-osmolality. In fact this agent increased urine volume and reduced U_{\text{Osm}} during the entire period of treatment. This resulted in a greater renal ability to excrete a water load and normalization in serum sodium and osmolality. This nonpeptide AVP-V\(_2\) receptor antagonist also increased U_{\text{Na}}V without affecting creatinine clearance and MAP. These data suggest that SR121463 may be therapeutically useful for the chronic treatment of water retention in human cirrhosis.

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