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Aquaretic and hormonal effects of a vasopressin V_2 receptor antagonist after acute and long-term treatment in rats

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Abstract

A single oral administration of 1-[4-(*N-tert*-butylcarbamoyl)-2-methoxybenzene sulfonyl]-5-ethoxy-3-spiro-[4-(2-morpholinoethoxy)cyclohexane]indol-2-one SR121463 (0.3–3 mg/kg), a vasopressin non-peptide V_2 receptor antagonist, to rats induced dose-dependent aquaresis which was accompanied by Na^+, K^+, aldosterone and arginine vasopressin excretion over 6 h after dosing. However, no solute excretion was observed over 24 h. As a result of aquaresis, hemoconcentration and increases in plasma angiotensin II and adenocorticotrophin hormone were seen with 3 mg/kg at 2 h after dosing. Chronic treatment with SR121463 (3 mg/kg/day × 28 days) induced a marked aquaresis associated with aldosterone and vasopressin excretion. After a week of treatment, urine volume and aldosterone excretion were reduced (~ 40%) and then stabilised, while urine vasopressin excretion remained almost constant throughout the study. There were no changes in arterial pressure, plasma osmolality, plasma sodium concentration, or in number and affinity of liver vasopressin V_{1A} and kidney V_2 receptors 24 h after the last treatment. These results indicate that SR121463 is a potent aquaretic agent and might be useful for the chronic management of water-retaining diseases in humans.

Keywords: Vasopressin V_2 receptor antagonist; Aquaretic effect; SR121463

1. Introduction

Arginine vasopressin plays a major role as an antidiuretic hormone through specific interaction with the renal vasopressin V_2 receptors coupled to an increase in intracellular cAMP via stimulation of adenylate cyclase (Zingg, 1996). These vasopressin V_2 receptors are present all along the collecting duct in the mammalian nephron (De Rouffignac et al., 1983; Morel et al., 1987). Vasopressin may play an important role in a number of diseases including congestive heart failure, hypertension, renal diseases, edema, hyponatremia and the syndrome of inappropriate antidiuretic hormone secretion (Laszlo et al., 1991; Sorensen et al., 1995). In this context, the development of potent vasopressin V_2 receptor antagonists could be of value for evaluating the pathophysiological role of vasopressin and thus opening the way to a new class of therapeutic agents called aquaretics.

Although several peptide vasopressin receptor antagonists have been described (Sawyer et al., 1981; Manning and Sawyer, 1989), their therapeutic usefulness has been limited by their low oral bioavailability, their short biological effect and their partial agonist activity shown in the human (Allison et al., 1988).

Some years ago, Yamamura et al., (1992) developed the first orally effective non-peptide vasopressin V_2 receptor antagonist, 5-dimethylamino-1-{4-(2-methylbenzoylamino)benzoyl}-2,3,4,5-tetrahydro-1*H*-benzazepine (OPC-31260) , and since then various other non-peptide vasopressin V_2 receptor antagonists have been described, manifesting the clinical interest in this kind of compound (Serradeil-Le Gal et al., 1996; Tahara et al., 1997; Albright et al., 1998; Yamamura et al., 1998).

1-[4-(*N-tert*-Butylcarbamoyl)-2-methoxybenzene sulfonyl]-5-ethoxy-3-spiro-[4-(2-morpholinoethoxy) cyclohexane]indol-2-one (SR121463) is a non-peptide, potent, selective, orally active vasopressin V_2 receptor antagonist showing greater V_2:V_1 selectivity in rat and human tissues (100- to 7000-fold) than does OPC-31260 (Serradeil-Le Gal et al., 1996). SR121463 increases urine volume and

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decreases urine osmolality dose-dependently in normal, hydrated rats and in Brattleboro rats without agonist activity. It has good oral bioavailability and is at least 10 times more potent than OPC-31260 in rats (Serradeil-Le Gal et al., 1996).

In a previous study with normal hydrated rats (Lacour et al., 1997), we showed that acute blockade of vasopressin V_2 receptors with SR121463 induced aquaresis accompanied by urinary aldosterone (U_{Ald}) and vasopressin (U_{AVP}) excretion. Furthermore, a tendency to a decreased diuresis and aldosterone excretion was observed after treatment with SR121463 for 14 days (Lacour et al., 1997). Several authors (Nishikimi et al., 1996; Jonassen et al., 1997; Yamamura et al., 1998) have also reported an apparent tolerance associated with repeated administration of OPC-31260 and even, 7-chloro-5-hydroxy-1-[2-methyl-4-(2-methylbenzo-ylamino)benzoyl]-2,3,4,5-tetrahydro-1*H*-1-benzazepine (OPC-41061).

As dehydration could be a trigger for this increased U_{AVP} and U_{Ald} excretion, we investigated the kinetics over 24 h of the aquaresis during vasopressin V_2 receptor blockade after a single administration of SR121463 in normal hydrated rats. In addition, based on our preceding study (Lacour et al., 1997) and on those of others (Nishikimi et al., 1996; Jonassen et al., 1997; Yamamura et al., 1998), where a slight decrease of aquaresis was observed, we investigated the aquaretic effect and hormonal changes induced by SR121463 during a 1-month oral treatment.

2. Materials and methods

2.1. Animals

All the protocols involved in this study were approved by the Comité d'Expérimentation Animale (Animal Care and Use Committee) of Sanofi Recherche.

Male Sprague–Dawley rats (Iffa Credo Laboratories, L'Arbresle, France) weighing 280–300 g were used in this study. The animals were allowed free access to standard laboratory food and water and maintained under constant temperature (22 ± 2°C) and lighting conditions (12-h light cycle (0700 to 1900 h).

2.2. Drugs

SR121463 was synthetized at Sanofi Recherche in Toulouse. It was used as the monophosphate monohydrate salt (MW = 759.8 g).

Vasopressin was obtained from Sigma (L'Isle d'Abeau, France). The selective vasopressin V_{1A} ligand, [^{3}H] SR49059 (54 Ci/mmol) (1), and the specific vasopressin V_2 ligand, [^{3}H]SR121463 (47.5 Ci/mmol) (Serradeil-Le Gal et al., 1996), were purchased from Amersham and New England Nuclear, Life Sciences (Les Ulis, France), respectively.

2.3. Experiment 1: effects of SR121463 after an acute oral treatment

2.3.1. Urine excretion

On day 2, all the rats were randomized, on day 1, given by gavage the vehicle (0.6% methylcellulose solution, 3 ml/kg) and housed individually in metabolic cages with food and water ad libitum. Urine was collected for 24 h and water intake (W_I) was measured.

On day 1, rats were treated with SR121463 (0.3–3 mg/kg, $n = 10$) or vehicle (3 ml/kg, $n = 10$). W_I was measured from 0 to 6 h and from 6 to 24 h. Urine was also collected at the same time as W_I to measure urine volume (U_V), urine osmolality (U_{Osm}), urinary sodium, potassium (U_{Na} and U_K), vasopressin and aldosterone (U_{AVP} and U_{Ald}) excretion.

2.3.2. Plasma parameters

In a first group of rats treated according to the same protocol, under isoflurane anesthesia (2%), blood was collected from the abdominal aorta, 2 h after treatment with SR121463 (0.3–3 mg/kg) or vehicle ($n = 6$–9). Plasma osmolality (P_{Osm}) and plasma sodium and potassium (P_{Na} and P_K) concentrations were measured. Plasma angiotensin II (P_{AII}) was also measured but only after 3 mg/kg.

In a second group of rats treated with vehicle ($n = 13$) or SR121463 (0.3, 1 and 3 mg/kg, $n = 5$, 11 and 14, respectively), 2 h after administration, the rats were decapitated and trunk blood was collected for the measurement of adenocorticotrophin hormone (ACTH) plasma levels (P_{ACTH}). The kinetics of the effects of vehicle or SR121463 (3 mg/kg) were also determined in other groups of rats ($n = 7$–14/group) decapitated after 6 and 24 h following treatment.

2.4. Experiment 2: aquaretic and hormonal effects of SR121463 during chronic oral treatment (28 days) in rats

On day 1, all the rats were randomized and received by gavage the vehicle (0.6% methylcellulose solution, 3 ml/kg) and were housed individually in metabolic cages. Then from days 1 to 28, the rats were treated once a day with SR121463 (3 mg/kg/day, $n = 11$) or vehicle (3 ml/kg/day, $n = 11$). Body weight, food and water intake were measured weekly. Urine was collected over a 24-h period before treatment on day 1 and after treatment on days 1, 6, 13, 20 and 28 to measure U_V, U_{Osm}, U_{Na}, U_K, U_{creat} (creatinine), U_{urea}, U_{AVP} and U_{Ald} excretion.

Twenty-four hours after the last administration on day 29, the rats were decapitated, and trunk blood was col-

lected for the measurement of P_{Osm} and P_{Na}. The heart, kidneys and liver were removed, weighed (heart and kidney only) and immediately cooled (kidneys and liver for analysis of vasopressin receptors) with ice-cold saline.

Systolic arterial pressure and heart rate were measured using a photoelectric pulse detector PC 139, IITC (Woodland Hills, CA, USA) in preheated, lightly restrained rats accustomed to the procedure. Arterial pressure measurement was performed before treatment on day 2 and after treatment on days 8, 15 and 26 between 22 and 24 h after dosing.

2.5. Analytical methods

The plasma and urine samples were stored at −20°C after addition of 1 g boric acid/100 ml urine. P_{Osm} and U_{Osm} were measured with a freezing point depression osmometer (Fisk Model), U_{Na} and U_K excretion, and P_{Na} and P_K concentrations were measured with specific electrodes (Itachi 717). U_{urea} was measured using the Urease method (Itachi 717) and U_{creat} was measured using the Jaffe method (Itachi 717). U_{AVP} concentration was determined by RIA using a kit (Bülhmann Laboratories, Basel, Switzerland) without extraction. U_{Ald} concentration was determined by RIA using a Behring kit without extraction. P_{ACTH} was determined using a kit (RIA ACTH, CIS Bio International, Oris, France) and P_{AII} by RIA (ERIA-Diagnostics Pasteur kit, France).

2.6. Binding assays to rat kidney and liver membranes

Rat kidney (papilla and inner medulla) and liver membranes were prepared by the methods of Stassen and Prpic (Stassen et al., 1982; Prpic et al., 1983), and 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 using bovine serum albumin as a standard (Bradford, 1976).

Assays of [^{3}H]SR49059 binding to rat liver and of [^{3}H]SR121463 binding to reno-medullary membranes were performed as previously described (Serradeil-Le Gal et al., 1993, 1996). Non-specific binding was determined in the presence of 10 μM unlabeled vasopressin. Saturation binding experiments were conducted in the presence of [^{3}H]SR49059 (0.03–20 nM) with rat liver membranes and [^{3}H]SR121463 (0.03–20 nM) with rat kidney preparations. Of note was the absence of significant amounts of [^{3}H]SR121463 binding sites in liver which constitutively expresses the V_{1A} receptor. Similarly, no vasopressin V_{1A} receptor was detected in the papilla and inner medullary areas of the kidney, using [^{3}H]SR49059.

2.7. Statistics

2.7.1. Binding data analysis

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 using an interactive non-linear regression program (Munson and Rodbard, 1980). All binding data are expressed as the mean ± S.D. (n = 4–6 determinations).

2.7.2. In vivo data analysis

All data are expressed as the mean ± S.E.M. Statistical analyses used were: two-way analysis of variance (ANOVA) followed by a Newman–Keuls's comparison test or one-way Anova followed by Dunnett's or

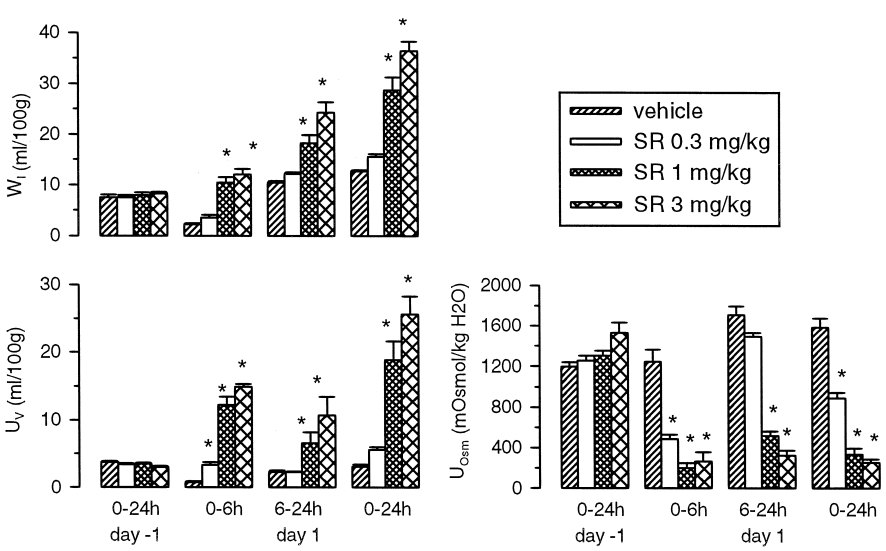

Fig. 1. Effects of a single oral administration of SR121463 (SR, n = 10) on W_I, U_V and on U_{Osm} in normal hydrated rats. The values are expressed as mean ± S.E.M. Dunnett's test: * $P < 0.05$ vs. control group.

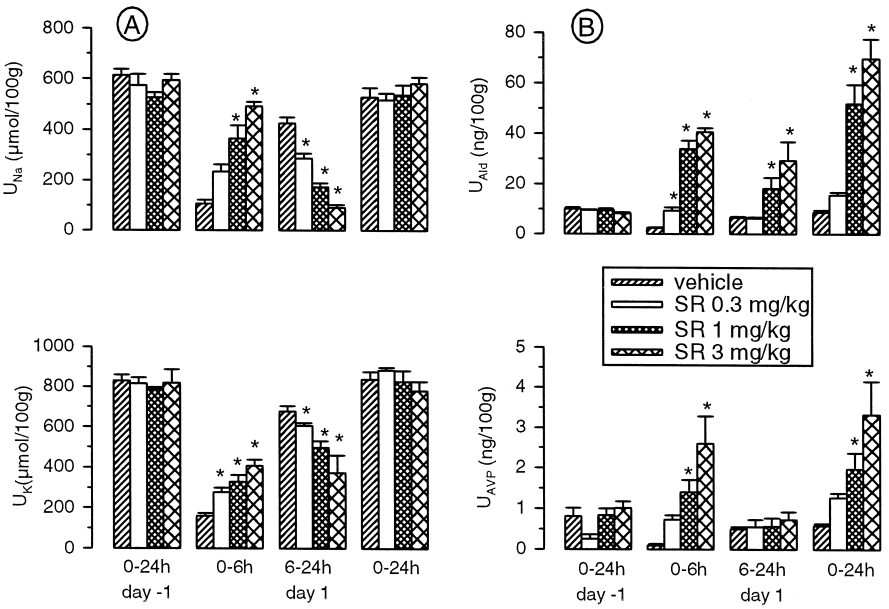

Fig. 2. Effects of a single oral administration of SR121463 (SR, $n = 10$) (A) on U_{Na} and U_K, and (B) on U_{Ald} and U_{AVP} in normal hydrated rats. The values are expressed as mean ± S.E.M. Dunnett's test: $^{*}P < 0.05$ vs. control group.

Kruskall–Wallis test. A probability value of less than 0.05 was considered statistically significant.

3. Results

3.1. Experiment 1: effects of SR121463 after acute oral treatment

3.1.1. Urine excretion

On day 1, after treatment with the vehicle, no significant differences were observed between groups, whatever the parameter (Figs. 1 and 2).

On day 1, from 0 to 6 h, SR121463 caused a dose-dependent and significant increase in urine volume and a decrease in urine osmolality (Fig. 1). This urine volume excreted was only partially offset by the water intake (Fig. 1). During this period, diuresis was accompanied by a dose-dependent increase in U_{Na} and U_K and also in U_{Ald} and U_{AVP} excretion (Fig. 2).

From 6 to 24 h, urine excretion continued to increase while water intake was strongly increased in order to compensate for the early water loss. U_{Na} and U_K decreased in a dose-dependent manner while U_{Ald} increased, as did U_V. Only U_{AVP} remained similar to the control value. Finally, over a 24-h period, the dose-dependent urine volume excreted was compensated by water intake which was increased by about 8 ml/100 g body weight per 24 h, irrespective of treatment. This difference could be attributed to extra-renal water loss. U_{AVP}, but essentially U_{Ald} excretion, increased, as did U_V, while the net solute excretion (Na^+ and K^+) remained similar to the control value (Figs. 1 and 2).

3.1.2. Plasma parameters

Two hours after dosing, the two highest doses of SR121463 (1 and 3 mg/kg) caused slight but significant increase in P_{Na} ($P < 0.05$) while P_{Osm} increased at 3 mg/kg only ($P < 0.05$) and P_K never changed significantly. At this higher dose, P_{AII} and P_{ACTH} ($P < 0.05$) were significantly increased ($P < 0.05$) (Table 1). However, the increase in P_{ACTH} was only significant ($P < 0.05$) at 2 h after dosing (Table 2).

Table 1
Effects of SR121463 (SR) on plasma parameters at 2 h after dosing
Data are mean ± S.E.M. $n = 6$–9 in control and SR groups except for P_{ACTH} (nm) = not measured.

Group (mg/kg)	P_{Osm} (mosm/kg)	P_{Na} (mmol/l)	P_K (mmol/l)	P_{AII} (pg/ml)	P_{ACTH} (pg/ml)
Control	305 ± 2	139 ± 1.6	4.1 ± 0.1	5.4 ± 0.8	90 ± 17 ($n = 14$)
SR (0.3)	305 ± 2	142 ± 0.4	4.4 ± 0.1	nm	130 ± 37 ($n = 5$)
SR (1)	308 ± 2	145 ± 1[a]	3.7 ± 0.2	nm	139 ± 22 ($n = 11$)
SR (3)	315 ± 2[a]	147 ± 1.1[a]	3.6 ± 0.2	18.4 ± 2.4[a]	281 ± 48[a] ($n = 14$)

[a] Dunnett's test or Kruskall–Wallis' test: $P < 0.05$ vs. control group.

Table 2
Kinetics of effects of SR121463 (SR) on P_{ACTH}
Data are mean ± S.E.M. $n = 14$ at 2 h and $n = 7$ at 6 and 24 h in control and SR groups, respectively.

Group (mg/kg)	P_{ACTH} (pg/ml)		
	2 h	6 h	24 h
Control	90 ± 17	83 ± 15	107 ± 37
SR(3)	281 ± 48[a]	72 ± 17	100 ± 20

[a] Kruskall–Wallis' test: $P < 0.05$ vs. control group.

Table 3
Effects of SR121463 (SR) on U_{Na} and U_K excretion at 24 h after dosing in normally hydrated rats
Data are mean ± S.E.M. ($n = 11$)

Days	U_{Na} (μmol/100/24 h)		U_K (μmol/100/24 h)	
	Control	SR (3 mg/kg)	Control	SR (3 mg/kg)
−1	675 ± 41	687 ± 25	754 ± 36	802 ± 23
1	690 ± 23	907 ± 24[a,b]	967 ± 28[b]	1170 ± 26[a,b]
6	725 ± 33	732 ± 26	801 ± 30	881 ± 20[b]
13	622 ± 37	681 ± 27	751 ± 26	757 ± 27
20	554 ± 26[b]	665 ± 22	670 ± 28[b]	755 ± 16
28	511 ± 19[b]	569 ± 32	592 ± 19[b]	653 ± 22[b]

[a] Dunnett's: $P < 0.05$ vs. control group.
[b] Dunnett's: $P <$ vs. day 1 before treatment.

3.2. Experiment 2: aquaretic and hormonal effects of SR121463 during chronic oral administration (28 days) to rats.

Body weight was similar throughout the study in the control and the SR121463-treated groups.

Before the start of the treatment on day 1, no differences were observed between the control and the treated groups whatever the parameter (Fig. 3 and Tables 3 and 4).

From the first day of treatment, on day 1, SR121463 induced a strong increase in U_V excretion ($P < 0.05$) and a decrease in U_{Osm} ($P < 0.05$). On day 6, in comparison to day 1, attenuation of the diuresis by about 40% and consequently of W_I (not shown) was observed, showing however, significant differences from the control group. In spite of the decrease in diuresis, U_{Osm} remained approximately at the same level as on day 1. These parameters then remained constant up to the end of the treatment (Fig. 3).

On the first day of treatment, U_{AVP} and U_{Ald} were markedly increased ($P < 0.05$). Then, while U_{AVP} remained almost constant throughout the study, U_{Ald} decreased slightly, then evolved parallel to U_V until the end of the treatment period. However, both hormonal parameters always remained significantly different ($P < 0.05$) from their respective control group (Fig. 4). Slight but significant increases ($P < 0.05$) in U_{Na}, U_K, U_{creat} and U_{urea} excretion were observed only on day 1 (Tables 3 and 4).

Twenty-four hours after the 28th administration of SR121463, P_{Osm} and P_{Na} remained unchanged (not shown). On the same day, no significant differences were observed relatively in heart and kidney weights (not shown).

No significant changes in binding parameters (K_d, B_{max}) were observed after chronic treatment with SR121463. In the liver, which constitutively expresses vasopressin V_{1A} receptors, [^{3}H]SR49059 detected high-affinity binding sites which were not significantly modified in terms of density

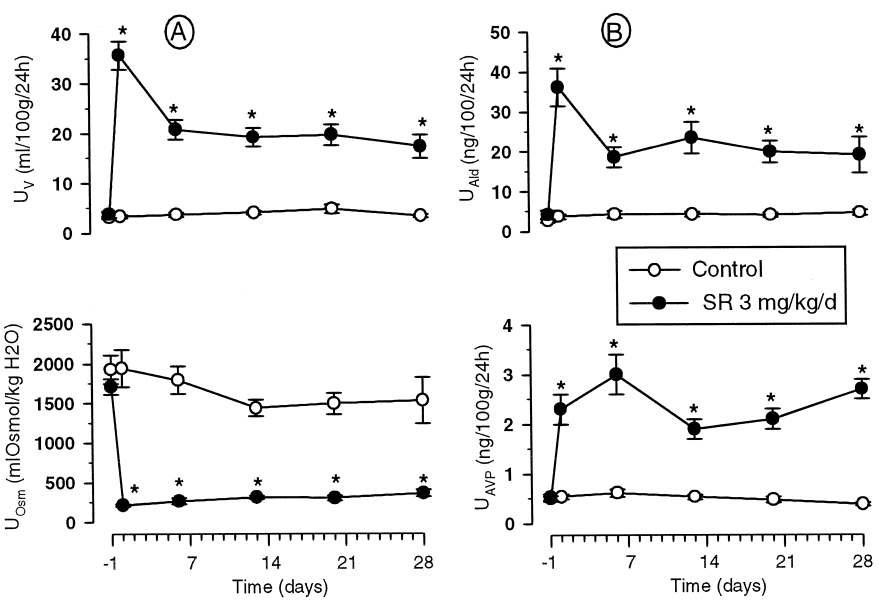

Fig. 3. Effects of repeated oral administration of SR121463 (SR), 3 mg/kg/day ($n = 11$) or vehicle ($n = 11$) (A) on U_V, U_{Osm}, and (B) on U_{Ald} and U_{AVP} excretion throughout the treatment period, in normal hydrated rats. The values are expressed as mean ± S.E.M. Newman–Keuls' test: $^*P < 0.05$ vs. control group.

Table 4
Effects of SR121463 (SR) on U_{urea} and U_{creat} excretion at 24 h after dosing in normally hydrated rats
Data are mean ± S.E.M. ($n = 11$)

Days	U_{urea} (μmol/100/24 h)		U_{creat} (μmol/100/24 h)	
	Control	SR (3 mg/kg)	Control	SR (3 mg/kg)
−1	2652 ± 103	2736 ± 133	27.8 ± 0.9	28 ± 0.8
1	2614 ± 107	3397 ± 143[a,b]	30 ± 0.9	33 ± 0.7[a]
6	3010 ± 147	3008 ± 144	30 ± 0.8	26.7 ± 0.6
13	3131 ± 152	3089 ± 144	30 ± 0.7	26.9 ± 0.6
20	2945 ± 120	2894 ± 69	31 ± 0.8[b]	29.6 ± 0.6
28	2886 ± 123	3143 ± 109[a]	29.6 ± 0.6	28.6 ± 1.1

[a] Dunnett's test: $P < 0.05$ vs. control group.
[b] Dunnett's test: $P < 0.05$ vs. day 1 before treatment.

(B_{max}) or affinity (K_d) after a 28-day 3 mg/kg SR121463 oral treatment (B_{max} values of 87 ± 47 vs. 63 ± 34 fmol/mg protein and K_d values of 2.10 ± 0.41 vs. 3.20 ± 1.80 nM for the control and the treated groups, respectively). As expected, medullo-papillary membranes from the rat kidney expressed a high density of vasopressin V_2 labeled by [^{3}H]SR121463 whose number (B_{max}) and dissociation constant (K_d) remained unchanged after the SR121463 chronic treatment (B_{max} values of 193 ± 41 vs. 243 ± 36 fmol/mg protein and K_d value of 0.95 ± 0.47 and 0.89 ± 0.40 nM for control and treated rats, respectively).

Throughout the treatment period, there were no significant differences in systolic arterial pressure and heart rate (not shown) between the SR121463 group and the control group.

4. Discussion

This study further investigated the kinetics of the aquaretic and hormonal effects of SR121463 after acute and long-term oral treatment.

The acute blockade of the V_2 receptors with SR121463 induced early and marked dose-dependent water aquaresis, up to 6 h at doses from 1 mg/kg. Over the 24-h following administration, the water loss was fully offset by the water intake; however, it is to be noted that this water loss was mainly compensated for in the 6–24 h period rather than in the earlier period. This delay in the rehydration of the animals was probably the cause of significant and positive early increases in U_{Na} and U_K excretion, while excretion was significantly and dose-dependently below the control values with any dose during the 6–24-h; electrolyte excretion unchanged over the 24 h. This finding is consistent with previous data reported for SR121463 in rats (Serradeil-Le Gal et al., 1996; Lacour et al., 1997), or in monkeys (Marchionni et al., 1998). However, in monkeys, only slight increases in U_K excretion were observed in the early period (Marchionni et al., 1998). It has also been reported that OPC-31260 and OPC-41061, two other new peptide vasopressin V_2 receptor antagonists, induced slight increases during the early period for urine electrolyte excretion (Na^+ and K^+) in rats as well as in humans (Yamamura et al., 1992, 1998; Ohnishi et al., 1993, 1995; Nakamura et al., 1994). However, it may be considered that these effects observed with vasopressin V_2 receptor blockade were slight when compared to those induced by loop diuretic agents such as furosemide which induce high urinary Na^+ and K^+ excretion, associated to the diuresis (Yamamura et al., 1992; Serradeil-Le Gal et al., 1996). In the same way, slight increases in P_{Osm} and P_{Na} observed 2 h after treatment with SR121463B, especially at the higher dose, were a consequence of excessive hypotonic urine excretion. Therefore, the increase in U_{AVP} excretion probably reflects the positive change in P_{Osm} caused by early water loss. Previous findings indicated that a change in P_{Osm} of only 1% could be expected to change P_{AVP} concentration by ~1 pg/ml in healthy subjects (Robertson et al., 1976). Additionally, as a P_{AII} increase was found in the present study, there is also the possibility that activation of the renin–angiotensin system and osmotic stimuli work through a common pathway to elicit vasopressin release (Qadri et al., 1993; Hogarty et al., 1994). Slight U_{AVP} increases have also been reported for monkeys in the early period after treatment with SR121463 (Marchionni et al., 1998) in spite of immediate rehydration, unlike in rats. Vasopressin release has also been reported with OPC-31260 in humans and dogs (Ohnishi et al., 1993, 1995; Naitoh et al., 1994; Shimizu, 1995) and with OPC-41061 in rats (Yamamura et al., 1998). These authors hypothesized that P_{AVP} increases are due to rapid water loss as a result of vasopressin V_2 receptor blockade. In addition, the present data showed a dose- and time-dependent increase in U_{Ald} which was closely correlated to aquaresis. In the same way, P_{AII} and P_{ACTH} increased in the early period after dosing. Some authors have also reported an early increase in plasma renin activity after OPC-31260 (Naitoh et al., 1994; Ohnishi et al., 1995) whereas Yamamura et al., (1998) did not observe any stimulation of the renin–angiotensin system in rats 24 h after treatment. These last findings were not totally the opposite of our results since P_{ACTH} was also increased at 2 h after dosing but not after 6 h. Thus, as opposed to the urinary hormonal excretion, which reflects the net excretion over 24 h, the circulating hormonal level represents secretion at a given time. Finally, according to our previous data, the release of U_{Ald} might be the result of complex mechanisms, such as the possibility that vasopressin stimulates aldosterone secretion via the adrenal V_{1A} receptor subtype. Several studies in vitro with rat and human adrenal glomerulosa cells (Hinson et al., 1987; Guillon et al., 1995) have confirmed this. The early sodium excretion, the result of free water excretion, might also be partly responsible for aldosterone release. It is well-known that aldosterone secretion is controlled indirectly by the juxtaglomerular apparatus which is sensitive to the composition

of the fluid in the distal tubule. A decrease in the sodium concentration of the filtrate in this part of the kidney will stimulate the renin–angiotensin system, which in turn will stimulate the release of aldosterone by the adrenal cortex. Therefore, we could speculate that strong dehydration, following high doses of SR121463, leads to high vasopressin elevation which in turn, through vasopressin interaction with adenohypophyseal V_{1B} receptors (Jard et al., 1986; Bernardini et al., 1994) induces adenocorticotrophin secretion and consequently aldosterone release (Gallo-Payet, 1993).

In the chronic study, after 6 days of treatment with SR121463, we observed attenuation of the diuresis without a return to the control value throughout the study. Nevertheless, the decrease in U_{Osm} and the increase in U_{AVP} remained more or less constant throughout the study. The attenuation of the diuresis after chronic treatment has already been described with OP-31260 (Nishikimi et al., 1996; Jonassen et al., 1997), SR121463 (Lacour et al., 1997) and also with OPC-41061 (Yamamura et al., 1998). However, for this latter author and some others (Hofbauer et al., 1986), since U_{Osm} and U_{AVP} remained constant during the study, the slight decrease in U_V could be considered the result of activated compensatory mechanisms. Unlike vasopressin release, U_{Ald} excretion decreased slightly after the first day of treatment and finally followed the same kinetics as did U_V excretion. In addition, slight increases in urinary electrolyte and urea excretion were observed on the first day of treatment, in this chronic study. These results were unusual (Serradeil-Le Gal et al., 1996; Lacour et al., 1997; first study) and might be attributable to the strong diuresis observed particularly on this day (36 ± 3, chronic study vs. 26 ± 3 ml/100 g/24 h, acute administration). Furthermore, as it might be expected, P_{Osm} and P_{Na} had returned to their control level 24 h after dosing even after 28 days of treatment. There were also no differences in the number and dissociation constant of vasopressin V_{1A} and V_2 receptors in the liver and kidney between the control and treated groups at equivalent times. Similar results for liver vasopressin V_{1A} and kidney V_2 binding were recently reported for another non-peptide vasopressin V_2 receptor antagonist after repeated oral dosing in rats (Yamamura et al., 1998).

Finally, we can speculate that the neurohormonal activation observed after a single dose of SR121463 can take place during the long-term treatment in spite of the attenuation of the diuresis observed in the latter study. It is likely that the strong aquaresis observed in the early period was associated with an increase in P_{Osm} and release of vasopressin and aldosterone to compensate for the reduction in the circulating blood volume following V_2 receptor blockade.

The results of this study also showed that the hemodynamic parameters, arterial blood pressure and heart rate, were not affected by this long-term treatment with the vasopressin V_2 receptor antagonist. Similar results have already been obtained in rats during chronic treatment (Nishikimi et al., 1996), or after a single administration in dogs (Naitoh et al., 1994) and humans (Ohnishi et al., 1993) with OPC-31260 and in monkeys with SR121463 (Marchionni et al., 1998).

5. Summary

This study showed that, after a single oral dose, the aquaretic effect of SR121463 was evidenced by an increase in diuresis and a decrease in urine osmolality accompanied by an increase in plasma concentration. Long-term treatment also induced a sustained, potent, aquaresis although the results showed that multiple dosing was associated with attenuation of the diuretic effect. Therefore, we may speculate that this drug could be useful for the treatment of human diseases characterized by water retention, provided that water consumption intake is well supervised.

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