CVT-124, a Novel Adenosine A₁ Receptor Antagonist with Unique Diuretic Activity

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ABSTRACT

Administration of the selective adenosine A₁ receptor antagonist, CVT-124, to conscious chronically instrumented rats resulted in significant increases in urine flow rate and sodium excretion without affecting potassium excretion or renal hemodynamics. Its maximum effect was twice that of hydrochlorothiazide which was associated with a significant kaliuresis. The diuretic effect of CVT-124 was less than that observed with furosemide; however, furosemide administration was associated with a large increase in potassium excretion as well as a reduction in glomerular filtration rate. When given at equinatriuretic doses, CVT-124 enhanced the diuretic and natriuretic activity of furosemide without further increasing potassium excretion. In contrast, the combination of hydrochlorothiazide and furosemide resulted in a 3-fold increase in potassium excretion. These data suggest that CVT-124 possesses unique diuretic activity and, as such, it represents a potential new therapeutic in fluid retaining disorders. In addition, its unique mechanism of action suggests that CVT-124 would be effective in otherwise diuretic-resistant patients.

Chronic sodium retention and edema are characteristics of patients with advanced congestive heart failure, chronic renal disease and decompensated cirrhosis (Schrier, 1988). Large doses as well as combinations of diuretics such as thiazides and loop diuretics are often required in these patients, however, their effectiveness is frequently limited. This resistance to diuretic therapy is due to decreased distal delivery of fluid, a consequence of increased proximal tubular resistance to diuretic therapy is due to decreased distal delivery of fluid, a consequence of increased proximal tubular reabsorption (Balakrishnan et al., 1982, 1991; Schnermann, 1990; Schnermann et al., 1990; Schnermann, 1988).

CVT-124 is the S-enantiomer of the highly selective racemic A₁-receptor antagonist, 1,3-dipropyl-8-[2-(5,6-epoxynorbornyl)] xanthine. It displayed $K_i$ values of 0.67 and 0.45 nM for rat and cloned human A₁ receptors, respectively. In addition, it was 1800-fold more potent for rat and 2400-fold more potent for human A₁ receptors when compared to A₂A (Pfister et al., 1997). In the same study, CVT-124 was also shown to induce diuresis when administered i.v. to saline-loaded rats. In our study, we have evaluated the diuretic effects (with emphasis on electrolyte excretion) and renal hemodynamic activity of CVT-124 and compared its effects to those of FUR and HCTZ in euvolemic, conscious rats. In addition, we compared the effects of combined, equinatriuretic doses of CVT-124 + FUR and HCTZ + FUR. Renal hemodynamic studies were performed only with CVT-124 and furosemide.

The performance of reliable diuretic bioassay requires that fluid and electrolyte homeostasis be maintained throughout the procedure. In our study, therefore we used a well-estab-

ABBREVIATIONS: FUR, furosemide; HCTZ, hydrochlorothiazide; MAP, mean aortic pressure; HR, heart rate; GFR, glomerular filtration rate; RBF, renal blood flow.
lished method that involves the replacement of urinary losses via an implanted stomach catheter without any disturbance to the conscious animal, i.e., no change in blood pressure and heart rate (Gellai et al., 1986; Gellai and Edwards, 1988).

Materials and Methods

Animals

All procedures were approved by the Institutional Animal Care and Use Committee and were in accordance with NIH Guidelines for the care and use of animals.

Male Sprague-Dawley rats were obtained from Charles River Labs (Wilmington, DE). They were housed in a light-controlled room with a 12-hr light/dark cycle and were allowed ad libitum access to food and water.

Surgical Preparation

Catheters were implanted in the abdominal aorta and vena cava via the left femoral artery and vein under a mixture of ketamine hydrochloride (Parke-Davis, Morris Plains, NJ) (50 mg/kg) and acepromazine (Ayerst Labs, Rouses Point, NY) (0.2 mg/kg, i.m.) anesthesia. An additional medical grade Tygon tubing was placed in the stomach at the left extremity of the greater curvature. A silastic-covered stainless steel cannula was implanted in the bladder. Details of surgery, pre- and postsurgical care and the experimental set-ups have been described previously (Gellai and Valtin, 1979). Experiments were conducted 4 to 5 days after surgery, by which time the rats had fully recovered and were gaining weight. During the recovery, the rats were housed individually and were accustomed to a plastic retainer (model ECU, Braintree Scientific, Inc., Braintree, MA).

Experimental Procedures

Between 8:00 to 9:00 A.M., rats were weighed and placed in the restrainer. The arterial line was connected to a Gilson polygraph via a Gould pressure transducer for the recording of MAP and HR; the venous line to infusion pumps for the infusion of vehicle and test agents; and the stomach tube to a 10- or 20-ml syringe to allow for the replacement of excess urinary loss. The bladder cannula was extended with a short length of PE tubing to permit collection of urine into preweighed tubes.

To replace normal fluid and electrolytes loss, heparinized (50 U/ml) Ringer’s solution was infused throughout the experiment via the arterial line and 5% dextrose during the equilibration and control period via the venous line, each at 10 μl/min. When excess fluid loss reached 2 ml, it was replaced slowly via the gastric tube with room temperature Ringer’s solution. A period of 45 to 60 min was allowed for equilibration in all studies, at the end of which a 0.5-ml blood sample was taken for measurement of hematocrit, plasma electrolytes and creatinine. This was followed by the various experimental procedures described below.

Group 1. Sustained i.v. infusion of diuretic agents. Urine was collected in 5-min periods throughout the experimental period. After equilibration, three collections were made to establish baseline. This was followed by the infusion of vehicle [20% ethyl alcohol (ETOH), 30% polyethylene glycol (PEG) and 50% distilled water] and one of the diuretic agents in increasing doses, each dose for a 30-min duration. The infusion rates were determined in preliminary experiments and were (μg/kg/min): 0.3, 1, 3 and 10 for CVT-124 (n = 5); 10, 30, 100 and 300 for FUR (n = 5), and 3, 10, 30 and 100 for HCTZ (n = 7). At the end of the experiments, the rats were weighed again and returned to their home cages.

Group 2. Sustained i.v. infusion of combination diuretics. After equilibration, control collections and infusion of vehicle, a combination of FUR and CVT-124 (10 and 1 μg/kg/min, respectively), or FUR and HCTZ (10 and 30 μg/kg/min, respectively) was infused for 30 min at a rate of 10 μl/min. The doses were selected on the basis of their equinatriuretic potency, selected from the results of the first study (fig. 2B). A 10-min period, allowed for the washout of dead space, was followed by a 20-min urine collection. After the termination of infusion, urine flow was allowed to return to baseline, approximately 15 to 20 min. The rats were then weighed and returned to their home cages.

Group 3. Renal clearance studies with CVT-124 and furosemide. Inulin (10%) and PAH (2%) were infused i.v. at 20 μl/min from the start. To establish baseline values, two 20-min urine collections were performed after equilibration. One blood sample (500 μl) was taken in the middle of the second collection. Subsequently, vehicle, CVT-124 or FUR was infused for 110 min. A 20-min period was allowed for the diuresis to reach steady state after which three 30-min urine collections were performed, and a further blood sample taken between the second and third period. The same equinatriuretic doses of the two compounds were selected that were used in the combination study (group 2), 1 and 10 μg/kg/min for CVT-124 and FUR, respectively.

Analytical and Data Analysis

Urinary and plasma concentrations of inulin and aminohippurate sodium (PAH) were determined by spectrophotometry, electrolyte concentrations were measured using the Synchro AS8 Clinical System (Beckman Instr. Inc., Brea, CA) and osmolality by an automatic osmometer (model 2430, Precision Systems, Inc., Natick, MA). Renal clearance values were calculated using standard clearance formulae and expressed per 100 g body weight.

All results are expressed in absolute values, and are reported throughout as group means ± S.E.M. Urinary excretion and renal hemodynamic values are corrected for body weight. Data from the dose-response studies (group 1) were analyzed using an analysis of variance for repeated measures; post hoc comparisons were made with the Scheffe f test. Analysis of variance was used to analyze differences in the results between the combination of compounds (group 2) and the renal clearance data (group 3). A value of P < .05 was considered statistically significant.

Drugs and Solutions

CVT-124 (CV Therapeutics, Palo Alto, CA), furosemide and hydrochlorothiazide (Sigma Chemical Co., St. Louis, MO) were prepared daily, all in a mixture containing 20% ethanol, 30% PEG-200 and 50% sterile water.

Results

Careful replacement of fluid and electrolyte loss ensured the maintenance of fluid and electrolyte homeostasis, with the exception of a small decrease in plasma potassium level in one group of animals (table 1). Fluid replacement prevented severe volume contraction, thus permitting evaluation of sustained effects of various doses of diuretic agents. Mean basal blood pressure (112 ± 4 mmHg) and heart rate (368 ± 14 beats/min) were not different between groups, and there was no change in either parameter during any of the studies. Infusion of vehicle for 30 min (groups 1 and 2) or 90 min (group 3) had no effect on any of the measured parameters.

Dose-response studies. The experimental protocol and values for 5-min changes in urine flow from the CVT-124 dose-response study are illustrated in figure 1. The increase in urine flow was gradual during the first 10 to 15 min after changes in infusion rates of CVT-124. A similar diuretic profile was observed with FUR and HCTZ, including slight variations between 5-min values at the highest doses. Thus, we decided to calculate the maximum effect for each dose by averaging the values of the last three 5-min collections. Such
averaged values for urine flow, sodium and potassium excretion for the three compounds are shown in figure 2. The maximum diuretic, natriuretic and kaliuretic effects of CVT-124, FUR and HCTZ were reached with doses of 3, 100 and 30 μg/kg/min, respectively (fig. 2A). Furosemide was twice as potent diuretic as CVT-124, whereas HCTZ had only minor, albeit significant effects (fig. 2A; table 2). Maximum values for changes in sodium excretion were: 10.2 ± 1.1, 17.3 ± 1.2 and 5.7 ± 1 mEq/min/100 g with CVT-124, FUR and HCTZ, respectively (fig. 2B). Potassium excretion was significantly increased with FUR and HCTZ, but not with CVT-124 (fig. 2C). Excretion of urea slightly increased with the lowest doses (data not shown), but returned to baseline during the infusion of higher doses (table 2). In these, as well as the following studies (groups 2 and 3), the increase in urine flow was solely due to the increase in solute excretion (Cosm). Clearance of free water, CH₂O, was not altered (table 2).

Combination studies. Combinations of FUR + CVT-124 or FUR + HCTZ both elicited an additive diuretic effect as illustrated by the change in urine flow in figure 3A. However, CVT-124 and HCTZ affected FUR’s action on electrolyte excretion differently. CVT-124 significantly potentiated the natriuretic effect of FUR in an additive manner (fig. 3B) without further increasing its kaliuretic effect (fig. 3C). In contrast, HCTZ significantly increased the kaliuretic action of furosemide and increased its natriuretic effect to a lesser degree (fig. 3B and C).

Effect of CVT-124 and furosemide on renal hemodynamics. Sustained infusions of vehicle had no effect on GFR and RBF, or any of the renal excretory functions (figs 4–7), nor did the 90-min infusion of CVT-124 (1 μg/kg/min) altered renal hemodynamics. In contrast, FUR infusion (10 μg/kg/min) significantly lowered GFR and RBF (fig. 4A and B). The onset of the urine flow increase was faster with FUR, and the change was significantly higher at 30 min than with CVT-124 (fig. 5A), however, the difference between the diuretic effects of the two compounds lost significance with time. The changes in urine osmolality mirrored those in urine flow (fig. 5B), confirming that the diuresis resulted from the increase in Cosm alone. Even though the diuretic effects of the two

### TABLE 1

Indices of fluid balance during severe diuresis (group 1)

<table>
<thead>
<tr>
<th></th>
<th>CVT-124 (n = 5)</th>
<th>FUR (n = 5)</th>
<th>HCTZ (n = 5)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>345 ± 4</td>
<td>346 ± 5</td>
<td>342 ± 11</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>43.5 ± 1.5</td>
<td>41.9 ± 1</td>
<td>39 ± 0.6</td>
</tr>
<tr>
<td>Posm (mOsm/kg H₂O)</td>
<td>296 ± 1.2</td>
<td>296 ± 1.6</td>
<td>298 ± 0.6</td>
</tr>
<tr>
<td>P_Na (mEq/liter)</td>
<td>139 ± 0.5</td>
<td>140 ± 1.1</td>
<td>142 ± 3.5</td>
</tr>
<tr>
<td>P_K (mEq/liter)</td>
<td>4.3 ± 0.4</td>
<td>4.1 ± 0.6</td>
<td>4.4 ± 0.1</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. Posm, plasma osmolality; P_Na, plasma concentration of sodium; P_K, plasma concentration of potassium. 
\*a Significantly different from initial values.
compounds were different, their effects on total sodium excretion were identical (fig. 6A). However, when the values were expressed as percent of filtered load (FE), the natriuretic effect of FUR was significantly higher (fig. 6B). As observed with the dose-response protocol (group 1), FUR, but not CVT-124, induced sustained increase in total potassium excretion during the prolonged infusion (fig. 7A). The fractional excretion of potassium increased from 28.5 ± 3 to 46.6 ± 5%. Again, the slight transient increase in urea excretion was not significant (fig. 7B).

### Discussion

In our study, we have demonstrated that the selective adenosine A1 receptor antagonist, CVT-124, possesses remarkable diuretic activity. Administered alone, CVT-124 increased urine flow and sodium excretion without affecting either potassium excretion or renal hemodynamics. The maximum changes in urine flow and sodium excretion induced by CVT-124 were 47.5 and 60.3%, respectively, when compared with those elicited by furosemide infusion. The differences in the ratios of diuretic and natriuretic effects of the two com-

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>CVT-124 (n = 5)</th>
<th>Furosemide (n = 5)</th>
<th>HCZT (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine flow (ml/min. 100 g body weight)</td>
<td>21.3 ± 5</td>
<td>13.5 ± 4</td>
<td>16.3 ± 4</td>
</tr>
<tr>
<td>Urine osmolality (mOsM/kg H2O)</td>
<td>596 ± 40</td>
<td>697 ± 53</td>
<td>723 ± 80</td>
</tr>
<tr>
<td>Sodium excretion (µEq/min. 100 g body weight)</td>
<td>1.3 ± 0.3</td>
<td>0.98 ± 0.2</td>
<td>1.01 ± 0.2</td>
</tr>
<tr>
<td>Potassium excretion (µEq/min. 100 g body weight)</td>
<td>1.9 ± 0.5</td>
<td>1.5 ± 0.2</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>Urea excretion (µmol/min. 100 g body weight)</td>
<td>4.1 ± 0.3</td>
<td>3.5 ± 0.2</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>Free water clearance (µl/min. 100 g body weight)</td>
<td>−9.8 ± 3</td>
<td>−15.5 ± 4</td>
<td>−15.8 ± 5</td>
</tr>
<tr>
<td>Clearance of osmol (µl/min. 100 g body weight)</td>
<td>31.0 ± 6</td>
<td>26.1 ± 5</td>
<td>29.7 ± 4</td>
</tr>
</tbody>
</table>

The values listed under baseline are the means ± S.E.M. of three 5-min periods; those under the diuretics are the means ± S.E.M. of six 5-min periods. Compared with baseline: * P < .05.
pounds can be explained by the additional effect of furosemide on potassium excretion. One of the major differences between CVT-124 and FUR is related to their effects on renal hemodynamics; only FUR infusion lowered GFR and RBF. When combined in moderate, equi-natriuretic doses, CVT-124 was also able to enhance the natriuretic activity of FUR without further increasing potassium excretion. This was in contrast to HCTZ, a weak diuretic in the euvolemic rat, which, when given in combination with FUR, resulted in a 3-fold increase in potassium excretion. This was in contrast to HCTZ, a weak diuretic in the euvolemic rat, which, when given in combination with FUR, resulted in a 3-fold increase in potassium excretion. The ability of CVT-124 to enhance the effect of FUR without further increasing potassium excretion is an important observation because there is an abundance of data demonstrating that the combined use of loop and thiazide diuretics results in hypokalemia and potentially serious side effects (Knauf and Mutshler, 1995; Dormans and Gerlag, 1996).

These data suggest that CVT-124, when given alone or in combination with a loop diuretic, will provide an important therapeutic tool in patients with congestive heart failure, chronic renal disease or cirrhosis. In addition, its unique mechanism of action suggests that CVT-124 will be effective in otherwise diuretic-resistant patients. In addition to stimulating sodium reabsorption in the proximal tubule (Balakrishnan et al., 1996; Takeda et al., 1993), adenosine activates the tubuloglomerular feedback mechanism mostly by vasoconstriction of afferent arteriole mediated by A1 receptor (Osswald et al., 1982; Schnermann et al., 1990; Schnermann, 1988), and inhibits chloride transport in the collecting duct (Arend et al., 1989; Spielman and Arend, 1991). Thus, blockade of the tubular glomerular feedback mechanism as well as distal sodium chloride reabsorption would not negate the proximal tubular effects of CVT-124. Micropuncture studies demonstrating increased flow rates in both the proximal and distal tubules after administration of CVT-124 (Welch et al., 1996) support this. In addition, blockade of adenosine-induced inhibition of Na/K exchange in the distal tubule could provide the mechanism for the observed potassium-sparing activity of CVT-124.

The diuretic and natriuretic effects of adenosine A1 receptor blockade have been reported previously. Indeed, adenosine antagonists have been shown to increase sodium excretion in animals as well as hypertensive patients and normal volunteers (Knight et al., 1993; Kuan et al., 1993; Balakrishnan et al., 1993; van Buren et al., 1993; Wolff et al., 1996). The successful development of adenosine receptor antagonists, however, has been complicated by receptor selectivity, for many adenosine A1 receptor antagonists also possess some degree of A2A receptor antagonist activity. Any such activity would lead to cardiac liabilities because antagonism of cardiac A2A receptors would oppose adenosine-mediated coronary vasodilation (Belardinelli et al., 1995). As stated in
the introduction, CVT-124 is one of the more potent and selective adenosine A1 receptor antagonists yet identified. The selectivity profile of CVT-124 is superior to that of other adenosine A1 receptor antagonists, like KW 3902 and CPX, which demonstrate A1 vs. A2A selectivity of 150- and 60-fold, respectively, compared to the 2400-fold human A1 receptor selectivity for CVT-124 (Pfister et al., 1997).

In summary, we have demonstrated that CVT-124 possesses unique diuretic properties being able to result in potassium-sparing diuretic activity as well as enhancing the proximal and distal tubular sites of action.

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References


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