Effects of KW-3902 (8-(Noradamantan-3-yl)-1,3-dipropylxanthine), an Adenosine A<sub>1</sub>-Receptor Antagonist, on Urinary Excretions of Various Electrolytes in Rats

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We examined the effects of KW-3902 (8-noradamantan-3-yl)-1,3-dipropylxanthine, an adenosine A<sub>1</sub>-receptor antagonist, on urine volume and urinary excretions of various electrolytes in saline-loaded rats, as compared with those of furosemide, trichlormethiazide (TCM), acetazolamide and amiloride. KW-3902 at doses of 0.001—1 mg/kg (p.o.) significantly increased urine volume and excretions of sodium, calcium, magnesium, chloride and bicarbonate. In addition, KW-3902 shifted urine pH to alkaline and decreased free water reabsorption. KW-3902 did not induce kaliuresis, whereas furosemide (30 mg/kg, p.o.), TCM (1 mg/kg, p.o.) and acetazolamide (25 mg/kg, p.o.) induced kaliuresis. In the KW-3902-treated group, the increases in bicarbonate excretion and urine pH were less prominent than those induced by acetazolamide, and the excretions of sodium, calcium, magnesium and chloride were similar to those induced by furosemide. The present results suggest that the adenosine A<sub>1</sub>-receptor antagonist exhibits diuresis by the inhibited reabsorption of electrolytes, not only at the proximal tubule but also at the distal tubule.

Key words adenosine; receptor antagonist; urine; kidney; rat

The physiological effects of endogenous adenosine are mediated primarily by acting on two major subtypes of adenosine receptors, high affinity A<sub>1</sub>- and lower affinity A<sub>2</sub>-receptors. Extracellular free adenosine concentration ranges from 0.1 to 1 μM, and thus, under the physiological condition, the effects of endogenous adenosine are predominantly mediated by the activation of adenosine A<sub>1</sub>-receptors. Several studies have shown that adenosine can modulate a variety of renal responses such as renal secretion, sodium and water excretion and erythropoietin production. Although the diuretic effect of alkyxanthines has been known for many years, this effect was recently found to be based on adenosine receptor antagonism.

KW-3902 (8-(noradamantan-3-yl)-1,3-dipropylxanthine) is a potent adenosine A<sub>1</sub>-receptor antagonist. In the receptor-binding study, the dissociation constant values of KW-3902 for adenosine A<sub>1</sub>-receptor and A<sub>2</sub>-receptor are 1.3 and 380 nM, respectively. In anesthetized rats, KW-3902 potently antagonizes the response mediated via adenosine A<sub>1</sub>-receptors with little influence on the response mediated via adenosine A<sub>2</sub>-receptors. KW-3902 increases urine volume and sodium excretion with little change in the potassium excretion in rats and dogs. Moreover, we found that KW-3902 causes diuresis and natriuresis with no change in renal plasma flow or creatinine clearance in anesthetized rats and dogs suggesting that the adenosine A<sub>1</sub>-receptor antagonist causes diuresis by inhibiting the reabsorption of water and sodium at tubular sites.

The purpose of the present study was to determine the effects of KW-3902 on excretions of various electrolytes, as compared with those of other diuretics: furosemide (loop diuretic), trichlormethiazide (TCM; thiazide), acetazolamide (carbonic anhydrase inhibitor) and amiloride (potassium sparing diuretic). We discuss the implication of the results in terms of the tubular site of action.

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MATERIALS AND METHODS

Animals Male Wistar rats (Japan Shizuoka Laboratory Animal Center, Inc., Hamamatsu), weighing 200—350 g, were used for the present experiments. The rats were kept at 22°C under a 12 h light–dark cycle, and they had free access to tap water and commercial chow. All animals received human care in compliance with the "Guiding Principles for the Care and Use of Laboratory Animals" formulated by the Japanese Pharmacological Society, and the protocol was approved by the bioethical committee of Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd.

Experimental Protocols Effects of drugs on urine volume and electrolyte excretion were examined with a slight modification of the previous method. In brief, rats were fasted for 18 h, and the drug suspension or vehicle was orally administered to the rat at a volume of 30 ml/kg (6 rats in each group). KW-3902 (0.0001—1 mg/kg), furosemide (30 mg/kg), TCM (1 mg/kg), acetazolamide (25 mg/kg) and amiloride (2.5 mg/kg) were suspended in saline containing 0.5% arabic gum. The control rat received an equivalent volume of saline containing 0.5% arabic gum. After the administration, the rat was individually placed in a metabolic cage without food or water. Urine was collected for 4 h and its volume (U) was determined. Urine concentrations of sodium and potassium were measured by flame photometry (775-A, Hitachi Ltd., Tokyo), and urinary excretions of sodium (U-Na) and potassium (U-K) were calculated. Urine concentrations of calcium, magnesium and phosphate were determined by an autoanalyzer (AU510, Olympus, Tokyo), and urinary excretions of calcium (U-Ca), magnesium (U-Mg) and phosphate (U-Pi) were calculated. Urine chloride concentration was determined by an assay kit (Chloride Test Wako, Wako Junyaku Co., Osaka) and urinary chloride excretion (U-Cl) was then calculated.

Urine pH was determined by a pH meter (HM-30S, Toa

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Electronics Ltd., Tokyo). Blood gas pressure of carbon dioxide \( (P_{\text{CO}_2}) \) was measured by a blood gas analyzer (280 Blood Gas System, Ciba-Corning, MA, U.S.A.). Urine bicarbonate concentration \( ([\text{HCO}_3^-]) \) was determined by the following standard formula\(^1\), and the urinary bicarbonate excretion \( (U-\text{HCO}_3^-) \) was calculated:

\[
[HCO_3^-] = 0.03 \times P_{\text{CO}_2} \times 10^{pK+\text{pK}}
\]

\[
pK = 6.33 - 0.5 \times ([\text{U-Na}] + [\text{U-K}])^{1/2}
\]

\[([\text{U-Na}] + [\text{U-K}]): \text{urine sodium or potassium concentration (mol/l)}\]

Serum osmolality \( (S_{\text{osm}}) \) and urine osmolality \( (U_{\text{osm}}) \) were measured by an osmometer meter (OSM-1, Shimazu Ltd., Tokyo), and osmolality clearance \( (C_{\text{osm}}) \) was determined by the following standard formula:

\[
C_{\text{osm}} = U \times U_{\text{osm}}/S_{\text{osm}}
\]

Free water reabsorption \( (T_{\text{wH}_2O}) \) was determined by the following standard formula:

\[
T_{\text{wH}_2O} = C_{\text{osm}} - U
\]

**Drugs Used** KW-3902 was synthesized in our laboratory. Furosemide was purchased from Tokyo Kasei Co., Ltd. (Tokyo), TCM and amiloride from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and acetazolamide from Aldrich Chemical Co., Ltd. (Milwaukee, WI, U.S.A.). All other chemicals and solvents were used in their analytical pure form.

**Statistical Analyses** All the results are presented as means \( \pm \) S.E. Statistical significance was estimated using Student's t-test or the analysis of variance (ANOVA), followed by Steel's test or Dunnnett's test. p values of less than 0.05 were considered to be statistically significant.

**RESULTS**

Table 1 shows urine volume and urinary electrolyte excretions in saline-loaded rats following the oral administration of KW-3902. KW-3902 at doses higher than 0.001 mg/kg significantly increased urine volume and excretions of sodium, calcium, magnesium and chloride with little change in potassium or phosphate excretion. KW-3902 dose-dependently shifted urine pH to alkaline at doses higher than 0.01 mg/kg, and increased the excretion of bicarbonate by 6—15 times at doses higher

| Control | 17.3±1.5 | 2.22±0.15 | 0.82±0.12 | 1.04±0.10 | 1.67±0.14 | 15.8±1.3 | 2.77±0.22 |
| KW-3902 (mg/kg) | 18.8±1.0 | 2.51±0.19 | 0.84±0.08 | 1.17±0.11 | 1.82±0.10 | 18.0±1.5 | 3.16±0.26 |
| 0.0001 | 26.8±1.5** | 3.49±0.16** | 0.96±0.06 | 1.66±0.12** | 2.56±0.13** | 17.3±1.4 | 4.38±0.19** |
| 0.01 | 34.4±0.9** | 4.74±0.16** | 0.94±0.05 | 2.15±0.11** | 3.06±0.14** | 18.3±2.4 | 5.54±0.10** |
| 0.1 | 32.1±1.3** | 4.28±0.14** | 0.92±0.06 | 2.02±0.15** | 2.49±0.10** | 14.5±1.6 | 4.90±0.16** |
| 1 | 30.8±1.3** | 4.17±0.16** | 0.92±0.04 | 2.29±0.14** | 2.39±0.08** | 13.5±1.7 | 5.19±0.18** |

Each value is the mean \( \pm \) S.E. of 6 animals. **, \( p < 0.01 \) versus the control.

**Fig. 1.** Effects of KW-3902 on Urine pH and the Urinary Excretion of Bicarbonate \( (U-\text{HCO}_3^-) \) in Saline-Loaded Rats

Each value is the mean \( \pm \) S.E. of 6 animals. *, **, Significantly different from the value in the control rat (CON) at \( p < 0.05 \) and 0.01, respectively.

**Fig. 2.** Effects of KW-3902 on Osmolality Clearance \( (C_{\text{osm}}) \) and Free Water Reabsorption \( (T_{\text{wH}_2O}) \) in Saline-Loaded Rats

Each value is the mean \( \pm \) S.E. of 6 animals. *, **, Significantly different from the value in the control rat (CON) at \( p < 0.05 \) and 0.01, respectively.
Table 2. Urine Volume (U) and Excretions of Sodium (U-Na), Potassium (U-K), Calcium (U-Ca), Magnesium (U-Mg), Phosphate (U-Pi) and Chloride (U-Cl) after Single Oral Administration of KW-3902 (0.1 mg/kg), Furosemide (30 mg/kg), TCM (1 mg/kg), Acetazolamide (25 mg/kg) or Amiloride (2.5 mg/kg) in Saline-Loaded Rats

<table>
<thead>
<tr>
<th></th>
<th>U   (ml/kg/4h)</th>
<th>U-Na (meq/kg/4h)</th>
<th>U-K (meq/kg/4h)</th>
<th>U-Ca (mg/kg/4h)</th>
<th>U-Mg (mg/kg/4h)</th>
<th>U-Pi (mg/kg/4h)</th>
<th>U-Cl (meq/kg/4h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.1 ± 0.8</td>
<td>1.93 ± 0.06</td>
<td>0.84 ± 0.03</td>
<td>0.94 ± 0.13</td>
<td>1.27 ± 0.08</td>
<td>16.6 ± 1.4</td>
<td>2.79 ± 0.21</td>
</tr>
<tr>
<td>KW-3902</td>
<td>27.6 ± 2.1**</td>
<td>4.02 ± 0.36**</td>
<td>0.90 ± 0.13</td>
<td>2.17 ± 0.24**</td>
<td>2.44 ± 0.25**</td>
<td>17.9 ± 3.7</td>
<td>5.09 ± 0.38**</td>
</tr>
<tr>
<td>Furosemide</td>
<td>33.2 ± 2.9**</td>
<td>4.36 ± 0.37**</td>
<td>1.52 ± 0.10**</td>
<td>2.46 ± 0.27**</td>
<td>3.05 ± 0.24**</td>
<td>20.1 ± 2.2</td>
<td>6.18 ± 0.55**</td>
</tr>
<tr>
<td>TCM</td>
<td>27.6 ± 0.7**</td>
<td>4.60 ± 0.11**</td>
<td>1.28 ± 0.08**</td>
<td>0.90 ± 0.09</td>
<td>2.54 ± 0.06**</td>
<td>21.0 ± 1.6*</td>
<td>5.67 ± 0.17**</td>
</tr>
<tr>
<td>Acetazolamide</td>
<td>29.1 ± 2.3**</td>
<td>4.84 ± 0.31**</td>
<td>1.77 ± 0.15**</td>
<td>0.53 ± 0.07**</td>
<td>0.51 ± 0.10**</td>
<td>21.6 ± 2.2*</td>
<td>2.19 ± 0.17</td>
</tr>
<tr>
<td>Amiloride</td>
<td>19.7 ± 1.1**</td>
<td>3.89 ± 0.19**</td>
<td>0.16 ± 0.04**</td>
<td>1.74 ± 0.16**</td>
<td>1.77 ± 0.15*</td>
<td>18.2 ± 1.7</td>
<td>3.21 ± 0.20</td>
</tr>
</tbody>
</table>

Each value is the mean ± S.E. of 6 animals. *p < 0.05, 0.01 versus the control.

Fig. 3. Effects of KW-3902 (0.1 mg/kg), Furosemide (30 mg/kg), TCM (1 mg/kg), Acetazolamide (25 mg/kg) and Amiloride (2.5 mg/kg) on Urine pH and the Urinary Excretion of Bicarbonate (U-HCO₃⁻) in Saline-Loaded Rats

Each value is the mean ± S.E. of 6 animals. ** Significantly different from the value in the control rat (CON) at p < 0.01.

than 0.01 mg/kg (Fig. 1). KW-3902 increased osmolarity clearance by 15—25% at doses of 0.001 and 0.01 mg/kg (Fig. 2). Moreover, KW-3902 significantly and dose-dependently decreased free water reabsorption by 35—40% at doses higher than 0.1 mg/kg.

Table 2 shows the urine volume and urinary electrolyte excretions in saline-loaded rats following the oral administration of KW-3902, furosemide, TCM, acetazolamide and amiloride. All the diuretics examined significantly increased urine volume and the urinary excretion of sodium by about 2 times; however, they showed different patterns of electrolyte excretions. Furosemide (30 mg/kg, p.o.) significantly increased excretions of potassium, calcium, magnesium and chloride. TCM (1 mg/kg, p.o.) significantly increased excretions of potassium, magnesium, phosphate and chloride with little change in calcium excretion. Acetazolamide (25 mg/kg, p.o.) significantly increased excretions of potassium and phosphate, but decreased excretions of calcium and magnesium. Amiloride (2.5 mg/kg, p.o.) significantly increased excretions of sodium, calcium and magnesium with decreased potassium excretion. KW-3902, acetazolamide and amiloride shifted urine pH to alkaline with bicarbonate excretion (Fig. 3). These effects of acetazolamide and amiloride were much more prominent than that of KW-3902.

DISCUSSION

In the previous study, we showed, by using the clearance study and the stop-flow technique, that KW-3902 causes diuretic and natriuretic effects via the inhibited reabsorption of water and sodium, mainly at the proximal tubule. Other adenosine A₁-receptor antagonists, such as 8-cyclopentyl-1,3-dipropyl-xanthine (DPCPX) or (R)-1-[(E)-3-(2-phenylpyrazolo[1,5-α]pyridin-3-yl)acryloyl]-2-piperidine ethanol (FK-453), also exhibit diuretic and natriuretic action with little or no change in renal hemodynamics. The present study demonstrated that KW-3902 increased excretions of calcium, magnesium, chloride and bicarbonate with little change in phosphate excretion, and that this drug shifted urine pH to alkaline and decreased free water reabsorption. These results suggest that KW-3902 acts on, in addition to the proximal tubule, the distal tubule, resulting in the excretions of various electrolytes.

In this study, the diuretic effect of KW-3902 was qualitatively similar to that of acetazolamide in terms of changes in urine pH and bicarbonate excretion. Urine pH is mainly regulated by the bicarbonate system. For this reason, the increased urine pH reflects the urinary excretion of bicarbonate. Most bicarbonate reabsorption is executed in the proximal tubule. In fact, the inhibition of Na⁺/H⁺ transport via the increased intracellular cAMP, or the removal of sodium from luminal fluid, results in about an 80% decrease in proximal bicarbonate reabsorption. FK-453 is reported to increase the urinary excretion of bicarbonate by inhibiting the basolateral Na-HCO₃⁻ co-transporter. In the present study, we demonstrated that KW-3902 caused significant and dose-dependent increases in sodium and bicarbonate excretions, in addition to exerting a diuretic effect. This observation suggests that KW-3902, like FK-453, inhibits the Na-HCO₃⁻ co-transporter, resulting in inhibited bicarbonate reabsorption at the proximal tubule.

KW-3902 showed diuretic and natriuretic effects, with little change in potassium excretion, as has been reported
previously. Other potassium-sparing diuretics include spironolactone and amiloride, which have in common the properties of inhibiting sodium reabsorption in the collecting duct and diminishing potassium and hydrogen excretions. Spironolactone exerts its action by the competitive inhibition of mineralocorticoids, acting on the distal tubule, while amiloride inhibits the sodium-specific ion channel localized on the luminal side of the distal tubule and the collecting duct. In the present study, amiloride was used as a potassium-sparing diuretic because spironolactone is reported to show minimal diuretic effects in rats. Although the implication is unclear, adenosine receptors are observed to be rich in the medullary collecting duct cells. This observation suggests that the adenosine receptors in the distal tubule may play a regulatory role in renal function, e.g., potassium reabsorption. KW-3902 might have stimulated potassium reabsorption at the distal tubule. Further studies are necessary to clarify the exact mechanism for the potassium sparing effect of KW-3902.

In the present study, KW-3902 increased excretions of sodium, calcium, magnesium and chloride, as was the case with furosemide. Moreover, KW-3902 (0.1 mg/kg) and furosemide (30 mg/kg) decreased free water reabsorption to a similar extent (K. Nagashima et al., unpublished observation). The major portion of the filtered magnesium is reabsorbed in the loop of Henle, and the evidence indicates that the thick ascending limb is the principal segment involved in the reabsorption. A significant proportion of filtered chloride is reabsorbed through the Na-K-2Cl transporter in the thick ascending limb of the loop of Henle. Furosemide, an inhibitor of the Na-K-2Cl transporter, decreases free water reabsorption and increases excretions of water, sodium, chloride, potassium, calcium and magnesium, as was confirmed in the present study. Perfusion studies using the isolated rectal grand tubule, which resembles the thick ascending limb in the characteristics of water and electrolyte transport, demonstrated that adenosine stimulated electrogenic chloride transport in the tubule, and that the effect was inhibited by theophylline and reversed by furosemide. These results suggest that KW-3902 might have inhibited the reabsorption of water and the transport of chloride and magnesium in the thick ascending limb.

In summary, the present study demonstrates that KW-3902, like acetazolamide, increases bicarbonate excretion and urine pH, and that this drug, like furosemide, increases excretions of calcium, magnesium and chloride. The present results suggest that the adenosine A₁-receptor antagonist exhibits diuretic effects by the inhibition of the reabsorption of various electrolytes, not only at the proximal tubule, but also, though to a lesser extent, at the distal tubule.

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