Pharmacokinetic and Pharmacodynamic Studies of Piretanide in Rabbits. III. Sodium and Potassium Excretion Under Different Hydrated Conditions

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The diuretic effect of piretanide, one of the loop diuretics, was investigated in three different hydrated conditions, namely well hydrated condition (treatment I), progressive hydropenic condition (treatment II) and complete hydropenic condition (treatment III) in rabbits. Each rabbit received intravenous administration of 1.5 or 15 mg/kg of piretanide and the urine flow rate, the excretion rates of Na and K, plasma concentrations of Na and K, urine osmolality, plasma concentration of piretanide and urinary excretion of piretanide were determined after administration. The pharmacokinetics of piretanide was not influenced by the hydration state of the body, even in treatment III. The diuretic effect of piretanide evaluated by both urine flow rate ($E_{H,O}$) and Na+K excretion rate ($E_{Na+K}$), was significantly affected by the hydration state of the body. The more the hydropenic state was developed, less amounts of urine or electrolytes were excreted. A pharmacokinetic-pharmacodynamic link model which was proposed in the previous paper was applied to the present experimental results. The result indicated that the diuretic effect, even in the complete hydropenic condition (treatment III), was reasonably described by the model, with minor modifications. The time course of the excretion rate of K ($E_K$) was not always in parallel with $E_{Na+K}$, but was dependent on treatments. We found that the K-fraction, which was known as an indicator of the Na–K exchange reaction in the distal tubule, was quantitatively related to $E_{Na+K}$, using a simple equation. Accordingly, the time course of $E_K$ was also calculated. The result also indicated that time courses of $E_K$ were described reasonably well by the model, regardless of treatments and doses.

Keywords — piretanide; diuretic; pharmacokinetics; pharmacodynamics; hydration; hydropenia; sodium excretion; potassium excretion

Introduction

Recently, extensive investigation has demonstrated that the time course of the pharmacologic effect of diuretics is closely related to the hydration state of the body. 1) The increase in urine flow or the urinary excretion rate of electrolytes after administration of loop diuretics to the normal animals, including man, rapidly decreases unless fluid replacement is made. It has also been reported that the maximum pharmacologic response intensity of the urine flow rate or excretion rates of Na and K after administration of loop diuretics was not always dependent on the dose and that the decline of each pharmacologic response did not always parallel one another. 2a) A sudden drop of urine osmolality 2b) and hypokalemia 3) as well as fluid and electrolyte imbalance after the loop diuretics administration were often reported clinically and these facts indicate that the loop diuretics should be used with caution. However, the quantitative and kinetic aspects of the physiological conditions of the body to diuretics have rarely been investigated.

In previous papers, 1b,c) we investigated the effect of piretanide, one of the loop diuretics, under three different physiological conditions, namely, well hydrated, progressive hydropenic and complete hydropenic conditions, in rabbits. The results suggested that the pharmacologic response intensity was decreased in accordance with the hydropenic state which was developed in the body during diuresis. We introduced a pharmacokinetic (PK)–pharmacodynamic (PD) link model to describe the effect of piretanide in the rabbit, and the results obtained revealed that the effect of piretanide on the well hydrated and
progressive hydropenic conditions in rabbits was reasonably described by the model. In the present study, we modified the previous PK–PD model in order to apply the effect of piretanide on rabbits even in complete hydropenic conditions. The purpose of this investigation was to verify the applicability of the modified PK–PD link model to describe piretanide action in three different hydrated conditions in rabbits.

Materials and Methods

Chemicals — Piretanide (Hoechst Japan Ltd., Tokyo), bumetanide (Sankyo Co., Tokyo) and antidiuretic hormone (ADH, [Arg₈]-vasopressin, Sigma Chemical Co., St Louis, Mo) were obtained commercially and were used without further purification. All other reagents used in the experiment were reagent grade and were also obtained commercially.

Animal Experiments — Male albino rabbits (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) weighing 3.6 to 4.1 kg were used in the experiment. Piretanide was dissolved in isotonic sodium chloride solution (JP XI, Otsuka Pharmaceuticals, Tokyo, Japan) and was administered via the right marginal auricular vein within 30 s, in a dose level of 1.5 or 15 mg/kg. The diuretic effect of piretanide was investigated under three different hydrated conditions in the rabbit.

(a) Well Hydrated Condition (Treatment I): The rabbits received a constant rate of infusion of isotonic Ringer's solution at the rate of 100 mg/h before and after piretanide administration. After reaching a steady-state urine flow, piretanide was administered. The amount of the body fluid which was lost during diuresis was replaced by infusing Ringer's solution at exactly the same rate with the urine flow using an extra infusion pump.

(b) Progressive Hydropenic Condition (Treatment II): The rabbits also received a constant rate of infusion of isotonic Ringer's solution in the same manner as in treatment I; however, no compensatory infusion was made after piretanide administration.

(c) Hydropenic Condition (Treatment III): The rabbits received a simultaneous constant infusion of hypertonic saline solution (804 mOms/l, at the rate of 50 ml/h) and ADH (33.2 mU/kg/h) under urethane anesthesia. After reaching the steady-state urine flow, piretanide was administered, and the infusion was continued until the end of the experiment. The urine samples were collected through a urinary catheter indwelling in the bladder (treatments I and II) or through the ureteral cannula (treatment III). The blood samples were withdrawn from the left marginal auricular vein. Details of the experimental procedure for the animal treatment were described previously.

Analytical Methods — Plasma and urinary concentrations of piretanide were determined by a high performance liquid chromatography (HPLC) method, and the sodium and potassium concentrations in plasma and urine were assayed by flamephotometry. The osmolality of plasma and urine was determined by a freezing-point depression method.

Data Analysis — The concentration-time or amount-time data were analyzed by a non-linear regression program based on the algorithm of Gauss-Newton and Berman using a PDP11/34 mini-computer (Digital Equipment Corp., Maynard, Mass). The weighing value and the condition of convergency were as described in previous reports.

Theoretical

(1) Pharmacokinetic Model

Plasma concentrations and urinary excretion rates of unchanged piretanide after i.v. administration in rabbits were described by a linear three compartment open model as shown in following equations.

$$C_p = \sum_{i=1}^{3} C_i e^{-\lambda_i t}$$

(1)

$$\frac{dx_u}{dt} = C_p CL_i$$

(2)

Where $C_p$ is the plasma concentration of piretanide, $C_i$ and $\lambda_i$ are the constant and the rate constant of linear compartment model, respectively, $dx_u/dt$ is the urinary excretion rate and
CL_\text{r} is the renal clearance of piretanide. The concentration of piretanide in the hypothetical effect compartment is described by Eq. 3.

\[ D = k_{\text{eo}} \sum_{i=1}^{3} \frac{(e^{-\lambda_i \phi} - e^{-k_{\text{eo}} \phi})}{k_{\text{eo}} - \lambda_i} \]  

(3)

Where \( k_{\text{eo}} \) is the elimination rate constant from the hypothetical effect compartment.  

(2) Pharmacodynamic Model on the Excretion Rate of Na + K

The relationship between the excretion rate of Na and K and the concentration of piretanide in the effect compartment was described by Hill’s equation with basal effect, as shown in Eq. 4.

\[ E_{\text{Na+K,j}} = f_j(E_0 + \frac{E_{\text{max}} D^r}{ED_{50}^r + D^r})(j = 2, \text{or } 3) \]  

(4)

Where \( E_{\text{Na+K,j}} \) is the diuretic effects of piretanide, expressed by the sum of the sodium and potassium excretion rates and \( j \) is the treatment number (1: treatment I, 2: treatment II, 3: treatment III). \( E_{\text{max}} \) is the maximum effect, \( ED_{50}^r \) is a hypothetical biophase concentration at 50% of maximum effect, \( E_0 \) is the basal value of the excretion rate of Na + K and \( r \) is the Hill’s constant. \( f_j \) is the modification factor in respect to the hydration state of the body.

(3) Calculation of the Modification Factor \( f_j \)

The modification factor \( f_j \) is closely related to the water balance in the body, and it is expressed by a Langmuir type equation, as follows.

\[ f_j = 1 - \frac{K_1 \cdot CE_{\text{H}_2\text{O},j}}{K_2 + CE_{\text{H}_2\text{O},j}} (j = 1, 2 \text{ or } 3) \]  

(5)

Where \( CE_{\text{H}_2\text{O},j} \) is the net loss of water in the body during the experiment and \( K_1 \) and \( K_2 \) are the constants. In the case of treatment I, there is no net loss or gain of water before and after piretanide administration, therefore \( f_1 \) is assumed to be unity. In the case of treatment II, \( CE_{\text{H}_2\text{O},2} \) is expressed by Eq. 6.

\[ CE_{\text{H}_2\text{O},2} = \int_0^\phi (E_{\text{H}_2\text{O},2} - \text{Inf}_2) \, dt \]  

(6)

Where \( \text{Inf}_2 \) is the infusion rate of Ringer’s solution, \( E_{\text{H}_2\text{O},2} \) is the excretion rate of water (urine flow rate) during piretanide administration. \( \text{Inf}_2 \) is constant during treatment II. As shown in Fig. 2, the relationship between urinary excretion rate of Na + K and urine flow rate is linear over a wide range tested, and therefore, \( E_{\text{H}_2\text{O},2} \) is expressed by Eq. 7.

\[ E_{\text{H}_2\text{O},2} = A_2 + B_2 E_{\text{Na+K},2} \]  

(7)

Where \( B_2 \) and \( A_2 \) are the slope and the intercept of the graph, respectively.

In treatment III, the \( f \) value is calculated by Eq. 8.

\[ CE_{\text{H}_2\text{O},3} = \int_0^\phi (E_{\text{H}_2\text{O},3} - \text{Inf}_3) \, dt + K_3 (T + r) \]  

(8)

Where \( K_3 \) is a proportionality constant reflecting the effect of ADH and hypertonic saline infusion and \( T \) is the duration of the infusion before piretanide administration. Accordingly \( K_3 T \) reflects the hydropenic condition of the body before medication, in treatment III. \( \text{Inf}_3 \) in Eq. 8 represents the infusion rate of hypertonic saline. Since the slope and intercept of the graph of the urinary excretion rate of Na + K versus urine flow rate relationship in treatment III was significantly different from that in treatment II, \( E_{\text{H}_2\text{O},3} \) is calculated by Eq. 9.

\[ E_{\text{H}_2\text{O},3} = A_3 + B_3 E_{\text{Na+K},3} \]  

(9)
Fig. 2. The Relationship between Urine Flow Rate and Excretion Rate of Na+K

I: treatment I, II: treatment II, III: treatment III. Each value shown is the mean ± S.E. and the solid lines in the figures are the regression lines. Each of the slope (B) and intercept (A) are as follows; I: B = 8.06 ml/meq A = -9.08 ml/h, II: B = 8.06 ml/meq A = -9.08 ml/h, III: B = 6.47 ml/meq A = -20.4 ml/h.

Where B and A are the slope and the intercept of the graph, respectively.

(4) Pharmacodynamic Model on the K-Fraction

The effect of piretanide on the K-fraction, which is defined by the fraction of potassium urinary excretion rate divided by sum of the excretion rates of sodium and potassium, is described by Eq. 10.

\[ E_{K/(Na+K),j} = f'_{j} \left( \frac{K_{4}}{E_{Na+K,j}} \right)^{K_{5}} \quad (j = 1, 2 \text{ or } 3) \quad (10) \]

Where \( K_{4} \) and \( K_{5} \) are constants and \( f'_{j} \) is another modification factor referred to the hydration state of the body, which is calculated by Eq. 11.

\[ f'_{j} = 1 - \frac{K_{6}}{K_{7} + CE_{H_{2}O,j}} \quad (j = 1, 2 \text{ or } 3) \quad (11) \]

Where \( K_{6} \) and \( K_{7} \) are the constants. In the case of treatment I, \( f'_{1} \) value is unity because \( CE_{H_{2}O,1} \) is considered to be zero. By definition, urinary excretion rate of potassium is calculated by Eq. 12.

| TABLE I. Comparison of Pharmacological Response Intensity of Piretanide after i.v. Administration in Rabbits with Different Hydrated Conditions \( a \) |
|---|---|---|
| | 1.5 mg/kg | 15 mg/kg |
| | I | II | III | I | II | III |
| \( I_{H_{2}O} \) | 5.92 ± 1.10 | 0.454 ± 0.645 | 0.458 ± 1.08 | 17.5 ± 4.46 | 3.10 ± 0.93 | 7.45 ± 2.54 |
| \( I_{Osm} \) | 5.90 ± 0.33 | 1.20 ± 0.20 | -1.20 ± 0.45 | 15.8 ± 2.07 | 3.25 ± 0.68 | 1.46 ± 0.77 |
| \( I_{Na} \) | 4.65 ± 0.47 | 1.24 ± 0.81 | -0.430 ± 0.525 | 14.0 ± 2.3 | 4.53 ± 0.95 | 1.86 ± 0.53 |
| \( I_{K} \) | 3.75 ± 1.99 | 0.435 ± 0.560 | 0.616 ± 0.763 | 7.54 ± 0.86 | 2.52 ± 0.62 | 3.27 ± 0.54 |

\( a \) Each value of the data is shown as the mean ± S.D. and the number of animals used in each treatment is indicated as shown in the legend of Fig. 3.
Table II. List of Basal Values in Three Different Hydrated Conditions in Rabbits

<table>
<thead>
<tr>
<th></th>
<th>Treatment I and II $^a$</th>
<th>Treatment III $^b$</th>
</tr>
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<tbody>
<tr>
<td>$E_{\text{H}_2\text{O}}$ (ml/h)</td>
<td>82.8 ± 14.4</td>
<td>31.8 ± 19.2 $^c$</td>
</tr>
<tr>
<td>$E_{\text{Osm}}$ (mOsm/h)</td>
<td>24.1 ± 3.5</td>
<td>20.2 ± 10.3</td>
</tr>
<tr>
<td>$E_{\text{Na}}$ (meq/h)</td>
<td>9.31 ± 1.24</td>
<td>8.37 ± 4.93</td>
</tr>
<tr>
<td>$E_{\text{K}}$ (meq/h)</td>
<td>1.08 ± 0.20</td>
<td>0.378 ± 0.162 $^c$</td>
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</tbody>
</table>

$^a$ Each value of the data is shown as the mean ± S.D. ($n=16$).  
$^b$ Each value of the data is shown as the mean ± S.D. ($n=8$).  
$^c$ $p < 0.001$.

\[ E_{\text{K},j} = E_{\text{K}/(\text{Na}+\text{K}),j} E_{\text{Na}+\text{K},j} \quad (j = 1, 2 \text{ or } 3) \quad (12) \]

Results and Discussion

Table I shows the comparison of the pharmacologic response intensities among three different experimental conditions after i.v. administration of piretanide in rabbits. The pharmacologic response intensity up to 6 h after the administration ($I$) was calculated by Eqs. 13 through 16, as the area under the relative pharmacologic effect-time curve.

\[ I_{\text{H}_2\text{O}} = \int_0^6 \frac{E_{\text{H}_2\text{O}} - E_{\text{H}_2\text{O}}^0}{E_{\text{H}_2\text{O}}^0} \, dt \quad (13) \]

\[ I_{\text{Osm}} = \int_0^6 \frac{E_{\text{Osm}} - E_{\text{Osm}}^0}{E_{\text{Osm}}^0} \, dt \quad (14) \]

\[ I_{\text{Na}} = \int_0^6 \frac{E_{\text{Na}} - E_{\text{Na}}^0}{E_{\text{Na}}^0} \, dt \quad (15) \]

\[ I_{\text{K}} = \int_0^6 \frac{E_{\text{K}} - E_{\text{K}}^0}{E_{\text{K}}^0} \, dt \quad (16) \]

Where $E_{\text{Osm}}, E_{\text{Na}}$ and $E_{\text{K}}$ are the urinary excre-

Fig. 3. Effect of Piretanide on the Concentrations of Plasma Na and K

○, plasma Na concentration (meq/l); □, plasma K concentration (meq/l). Upper figures represent 1.5 mg/kg dose studies and the lower figures represent 15 mg/kg dose studies. Each value shown is the mean ± S.E. and the number of animals used in each treatment is indicated as follows; treatment I: 1.5 mg/kg ($n=3$), 15 mg/kg ($n=3$) treatment II: 1.5 mg/kg ($n=5$), 15 mg/kg ($n=6$), treatment III: 1.5 mg/kg ($n=4$), 15 mg/kg ($n=4$).
tion rates of osmotic substances, sodium and potassium, respectively. The superscript 0 represents the basal value of each response, which was obtained experimentally as the premedication level (before piretanide administration). The basal values of each response are listed in Table II. The \( I_{H_2O} \) value in treatment I at 15 mg/kg dose study was the largest and the values in treatments II and III decreased significantly in that order. Similar results were observed in the other pharmacologic response intensities, regardless of dose. These results suggest that the pharmacologic effects after piretanide administration were closely related to the hydration state of the body and that the more the hypodermic state progressed in the body, the more the pharmacologic response intensities decreased.

As shown in the previous papers, prominent but temporal increases in \( E_\text{H}_2\text{O} \), \( E_{\text{O}_{3\text{am}}} \) and \( E_{\text{Na}^+\text{K}^-} \) were observed just after piretanide i.v. administration in treatments II and III; however, the pharmacologic effects rapidly decreased thereafter. This phenomenon was reasonably expressed by the effect of the feedback system which was activated by the loss of water and electrolytes from the body during diuresis. \(^{b,c}\)

Figure 3 shows the effect of piretanide on the plasma Na and K concentrations in the three different experimental conditions. Plasma Na concentrations after piretanide administration in treatments I and II showed a marginal increase compared to premedication levels; however, they were significantly increased by piretanide administration in treatment III. Plasma K concentrations were not influenced by piretanide administration, regardless of treatments. These results indicate that the concentrations of plasma electrolytes were strictly controlled by the body fluid regulation system in the normal condition (treatments I or II), and that small changes in water or electrolyte contents in the body which were caused by administration of diuretics did not affect plasma concentrations. In treatment III, the basal level of Na was significantly greater than the normal (pre-infusion) level, and the basal level of K was significantly lower than normal level. This fact indicates that the hypertonic saline infusion in treatment III exceeded the controllable range of plasma concentrations of electrolytes, and this might be the reason for the high basal value of plasma Na. The low basal level of plasma K in treatment III might be attributable to the Na–K exchange reac-

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Fig. 4. Effect of Piretanide on the Excretion Rate of Na+ K in Different Hydration State in Rabbits
Upper figures represent 1.5 mg/kg dose studies and the lower figures represent 15 mg/kg does studies. Each value shown is the mean ± S.E. and the number of animals used in each treatment is indicated in the legend of Fig. 3. The solid lines in the figures are the calculated values according to the Eqs. 1 to 9 in the text.
tion in the distal tubule under high Na concentration; however, its precise mechanism is unknown and further investigation is required.

Figure 4 shows the time courses of $E_{Na+K}$ before and after piretanide administration in treatments I, II and III. In each hydration condition, the maximum excretion rate was similar to the two doses used, but the duration of the effect in the 15 mg/kg dose study was much longer than that of the 1.5 mg/kg dose study. The hydration state of the body affected the $E_{Na+K}$ distinctly. The more the hydropenic condition progressed, the less Na + K was excreted.

The time courses of $E_K$ after piretanide administration are shown in Fig. 5. Although the excretion pattern of K in each treatment appeared to be similar to that of Na + K, they were different from each other. In order to clarify the difference, K-fraction in each dose and in each treatment were calculated and were plotted against the respective $E_{Na+K}$. As shown in Fig. 6 the relationship between $E_{Na+K}$ and K-fraction before (open symbols) and after (closed symbols) piretanide administration showed a hyperbola-like curve, regardless of treatments. The K-fraction values after piretanide administration decreased as the excretion rates of Na + K increased and their values finally returned to the respective premedication levels. This result indicates that K-fraction was dependent on both $E_{Na+K}$ and the hydration state in the body, and that the Na–K exchange reaction in the distal tubule could not be explained by a simple linear relationship.

In the previous paper,\textsuperscript{1,2} we demonstrated that the diuretic effect of piretanide in both well hydrated (treatment I) and progressive hydropenic (treatment II) conditions in rabbits was reasonably described by a pharmacokinetic-pharmacodynamic link model, including a feedback mechanism in respect to the body fluid depletion. In the present study, the model was modified in order to respond to the effect of piretanide even in the entirely hydropenic condition (treatment III). The solid lines in Fig. 4 through Fig. 6 represent the calculated values according to the model described in the theoretical section, and the parameter values used in the calculation are listed in Table III. Since plasma concentrations of piretanide were not influenced by the hydration state of the body even in the complete hydropenic condition (treatment III),\textsuperscript{1,2} all of the pharmacokinetic parameters were taken from the previous report.\textsuperscript{1,2} The pharma-

![Fig. 5. Effect of Piretanide on the Excretion Rate of K in Different Hydration State in Rabbits](https://example.com/image.png)

Upper figures represent 1.5 mg/kg dose studies and the lower figures represent 15 mg/kg dose studies. Each value shown is the mean ± S.E. and the number of animals used in each treatment is indicated in the legend of Fig. 3. The solid lines in the figures are the theoretical values calculated by Eq. 12 in the text.
Fig. 6. The Relationship between K-Fraction and $E_{\text{Na+K}}$ in Different Hydrated Conditions in Rabbits

- ○, before piretanide administration; ●, after piretanide administration. Upper figures represent 1.5 mg/kg dose studies and the lower figures represent 15 mg/kg dose studies. Each value shown is the mean ± S.E. and the number of animals used in each treatment is indicated in the legend of Fig. 3. The solid lines in the figure are the regression lines according to Eq. 10 in the text.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate ± S.D.</th>
<th>Parameter</th>
<th>Estimate ± S.D.</th>
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<tr>
<td>Pharmacokinetic parameters $a)^1$</td>
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<td></td>
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<tr>
<td>$C_1$ (μg/ml)</td>
<td>11.3 ± 3.1</td>
<td>$\lambda_1$ (h$^{-1}$)</td>
<td>10.4 ± 2.23</td>
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<tr>
<td>$C_2$ (μg/ml)</td>
<td>0.490 ± 0.30</td>
<td>$\lambda_2$ (h$^{-1}$)</td>
<td>2.25 ± 0.95</td>
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<tr>
<td>$C_3$ (μg/ml)</td>
<td>0.017 ± 0.0165</td>
<td>$\lambda_3$ (h$^{-1}$)</td>
<td>0.319 ± 0.152</td>
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<tr>
<td>CL$_f$ (ml/h)</td>
<td>1090 ± 108</td>
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<tr>
<td>$k_{eo}$ (h$^{-1}$)</td>
<td>13.2 ± 4.9</td>
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<tr>
<td>$E_0$ (meq/h)</td>
<td>7.80 ± 2.21</td>
<td>$E_{\text{max}}$ (meq/h)</td>
<td>49.2 ± 3.9</td>
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<tr>
<td>$r$</td>
<td>0.660 ± 0.107</td>
<td>ED$_{50}$ (μg/ml)</td>
<td>0.170 ± 0.036</td>
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<td>$K_1$</td>
<td>0.580 ± 0.022</td>
<td>$K_5$ (ml)</td>
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<td>$B_4$ (ml/meq)</td>
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<td>$K_4$ (meq/h)</td>
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<td>$K_5$</td>
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<td>$K_5$</td>
<td>0.569 ± 0.025</td>
<td>$K_7$ (ml)</td>
<td>1110 ± 300</td>
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<td>Parameters in respect to the fluid infusion</td>
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<tr>
<td>$\text{Inf}_2$ (ml/h)</td>
<td>100.0</td>
<td>$\text{Inf}_5$ (ml/h)</td>
<td>50.0</td>
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<td>$K_5$ (ml/h)</td>
<td>907 ± 676</td>
<td>$T$ (h)</td>
<td>3.50</td>
</tr>
</tbody>
</table>

$a)^1$ These parameter values were taken from the previous report. $b)^1$
codynamic parameters in respect to Hill’s equation were fundamentally identical with that in
the previous report. 1b) As shown in each graph, the calculated values were coincided reasonably
well with the experimental data.

The relationship between the $CE_{H_2O,j}$ and
the $f$ value or the $CE_{H_2O,j}$ and the $f'$ value
was expressed by the Langmuir equation, as shown
in Eqs. 5 and 11 in the theoretical section, and
this model is fundamentally the same as that
used in the previous report. 2b) In order to de-
scribe the relationship between the $CE_{H_2O,j}$ and
the $f$ value quantitatively, we examined several
equations, such as linear, log-linear and Hill’s.
The results of curve fitting indicated that Hill’s
equation gave the smallest value of the sum of
the squares, however, the value of Hill’s con-
stant tended to be unity. Thus, we used the
Langmuir equation instead of Hill’s equation, in
the series of the investigation. We, therefore, do
not claim that this equation is a unique way to
describe the relationship between $CE_{H_2O,j}$ and $f$
but this equation can describe the relationship
in the simplest manner. Although $CE_{H_2O}$ values
instead of $CE_{Na+K}$ values were used in the pre-
sent study, the time course of $f_2$ value was simi-
lar to that in the previous report. 1b)

On the other hand, the time course of the $f_0$
value was different from that of the previous
report, because the hyperosmotic saline infusion
itself induced a hydropenic state in rabbits.
Although a linear relationship between $E_{H_2O}$
and $E_{Na+K}$ still existed during treatment III (as
shown in Fig. 2), the relationship between the
cumulative amount of Na+K in the body and
the development of the hydropenic state during
hyperosmotic saline infusion was too complex
to explain mathematically. Therefore, in the pre-
sent study, $CE_{H_2O}$ was approximated by a linear
function, shown in Eq. 8. The second term of
Eq. 8 reflects the effect of ADH and hyper-
osmotic saline infusion on the balance of the
body water, as a function of time. The calculated
curves shown in Fig. 4 described the time
courses of $E_{Na+K}$ regardless of treatments and
doses. These results indicated that the develop-
ment of the hydropenic condition caused by
ADH and hyperosmotic saline infusion could be
approximated by a simple linear equation and
that the effect of the hydropenic condition on
the diuretic effect of piretanide in treatment III
was fundamentally the same as in treatment II.

The relationship between K-fraction and
$E_{Na+K}$ was approximated by a hyperbolic equa-
tion, as shown in Eqs. 10 and 11 in the theoreti-
cal section. We reported previously that K-
fraction could be related to $E_{Na+K}$ using a log-
linear function in the furosemide study. 4b) We
also applied this log-linear function to the pireta-
nide study; however, the application was not
successful. This result indicates that the mode of
action of piretanide on the Na–K exchange reac-
tion in the distal tubule might be different from
that of furosemide. The precise mechanism of
this is unknown and thus requires further in-
vestigation. In the calculation of K-fraction, we
assumed another modification factor $f'$ (Eq. 11)
as shown in the theoretical section. Since the
time courses of the effect of hydropenic condi-
tions on $E_{Na+K}$ and $E_{K/(Na+K)}$ were slightly
different from each other, this assumption might
be appropriate.

In spite of the simplicity of the model em-
ployed, the diuretic effect of piretanide in dif-
ferent physiological conditions could be reason-
ably described by a single PK-PD link model. We
trust that the present results using piretanide
will be of assistance in the preparation of a better
dosage schedule.

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