Pharmacokinetic and Pharmacodynamic Studies of Piretanide in Rabbits. II: Effects on the Proximal Tubules and the Loop of Henle

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In order to clarify the effect of piretanide in the nephrons, pharmacokinetics and pharmacodynamics of piretanide were studied under a hydropenic condition in the rabbit. The hydropenic condition was developed by a simultaneous infusion of an antidiuretic hormone and hyperosmotic saline. Lithium was used as the indicator of the proximal sodium reabsorption. Plasma concentrations and urinary excretion rates of piretanide were not influenced by the hydration state of the body. The glomerular filtration rate (GFR) showed a long lasting decrease after piretanide administration; however, the urinary excretion rates of salts and water were increased prominently just after administration. The lithium clearance ratio, which was obtained by dividing the lithium renal clearance by GFR, indicated that piretanide inhibited the proximal reabsorption of sodium. The urine osmolarity after piretanide administration showed a significant decrease, and this indicated that the osmolarity of the renal medulla was also influenced by piretanide. From these observations, a model describing the transport of water and osmotic substances in the nephrons was constructed to calculate the effect of piretanide. The results indicated that the diuretic effect of piretanide in the hydropenic rabbit was reasonably described by the model. The model parameters obtained suggested that the site of action of piretanide in the proximal tubules might be in the peritubular side rather than inside the lumen, whereas the site of action in the loop of Henle might be inside the lumen.

Keywords — piretanide; diuretic; renal clearance; proximal tubule; Henle’s loop; lithium; hydropenia; urine osmolarity; pharmacokinetics; pharmacodynamics

Introduction

It has been shown that the main site of action of piretanide is in the thick ascending limb of the loop of Henle where it exerts the diuretic effect by inhibiting chloride and sodium reabsorption.1) Winavar et al. 2) have reported that piretanide also has an effect on the proximal tubules. Although there have been many reports on the mechanism of piretanide action, 3) the relationship between the diuretic effect and the concentration of piretanide at the site of action (i.e. in the nephron) is still unclear.

In a previous paper, we reported that the relationship between pharmacokinetics (PK) and pharmacodynamics (PD) of piretanide in the rabbit was reasonably described by a PK-PD linked model. 4) We also reported that administration of piretanide induced a progressive hydropenic condition which reduced the diuretic effect significantly by a feedback mechanism. The purpose of this investigation was to elucidate the concentration effect relationship of piretanide, including the concentrating and/or diluting functions in the nephrons. All of the experiments were carried out under a hydropenic condition to minimize the feedback mechanism and the effect of the endogeneous antidiuretic hormone (ADH).

Materials and Method

Chemicals — Piretanide (Hoechst Japan Ltd., Tokyo), ADH ([Arg⁸]-vasopressin, Sigma Chemical Co., St. Louis, Mo.) and lithium chloride (Nakarai Chemical Co., Kyoto) were obtained commercially and were used without further purification. Piretanide was dissolved in isotonic sodium chloride solution (JP XI, Otsuka Pharmaceuticals Co., Tokyo) and was

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administered intravenously. Other reagents used in this study were the same as described previously.\textsuperscript{4)}

**Animal Experiments** — Adult male albino rabbits (Shizuoka Laboratory Animal Center, Hamamatsu) weighing 3.6 to 4.1 kg (mean 3.8 kg) were used. A hypodermic condition was prepared by the method of Puschett \textit{et al.} \textsuperscript{5)} with a modification as follows. The rabbit was anesthetized by i.p. administration of urethane (1.25 g/kg). Both ureters were cannulated with polyethylene tubing (Intramedic PE-50, Becton Dickinson & Co., Parsippany, N.J.) for urine sampling. The right femoral artery was also cannulated with a PE-50 tubing to take blood samples. After suturing the incision by surgical thread, a constant infusion of hyperosmotic saline solution (804 mOms/l), which consisted of NaCl (441 mM), KCl (4.0 mM), CaCl\textsubscript{2} (6.0 mM), LiCl (1.8 mM) and inulin (2%), was started at the rate of 50 ml/h, from the marginal auricular vein. ADH was also infused at the rate of 33.2 mU/kg/h. These infusions were continued until the end of the experiment. After reaching the steady-state urine flow, three samples of plasma and urine were collected every 30 min for determination of control values. Then, piretanide was administered within 30 s, at dose levels of 1.5 and/or 15 mg/kg. Plasma and urine samples were collected after the administration at the same intervals as described previously.\textsuperscript{4)} The urine samples obtained from each kidney were mixed to give one sample.

**Measurement of the Osmolarity of Plasma and Urine** — The osmolarity of plasma and urine was determined by the depression of freezing-point method using a semi-micro osmometer (Type M, Knauer, Berlin, West Germany).

**Measurement of Lithium in Plasma and Urine** — After diluting the plasma and urine samples by distilled water, the concentrations of lithium were assayed by a flame photometric method (Model 139-0400, Hitachi Ltd., Tokyo) at 671 nm wavelength.

Plasma and urine concentrations of piretanide were determined by a high performance liquid chromatographic (HPLC) method. Sodium and potassium concentrations in plasma and urine were measured by flame photometry. The glomerular filtration rate (GFR) was estimated by the inulin renal clearance. Details of the experimental procedures including the method of data analysis were described in the previous paper.\textsuperscript{4)}

**Results and Discussion**

The semilogarithmic plot of the plasma concentration and the urinary excretion rate of piretanide following i.v. administration are shown in Fig. 1. Although the renal clearance was slightly higher than that in the normal (unanesthetized) condition,\textsuperscript{4)} it was evident that the hypodermic condition of the body did not affect the pharmacokinetics of piretanide. The disposition of piretanide was described by a linear three compartment open model and the pharmacokinetic parameters are shown in Table I.

The effect of piretanide on GFR is shown in Fig. 2. The premedication levels of GFR were much lower than normal condition (normal GFR value is about 1 l/h) and the basal level de-
TABLE 1. Pharmacokinetic Parameters$^a$ of Piretanide after i.v. Administration in Hydropenic Rabbits$^b$  

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>S.D.</th>
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<tbody>
<tr>
<td>$C_1$ (µg/ml)</td>
<td>11.51</td>
<td>2.19</td>
</tr>
<tr>
<td>$C_2$ (µg/ml)</td>
<td>1.154</td>
<td>0.650</td>
</tr>
<tr>
<td>$C_3$ (µg/ml)</td>
<td>0.1223</td>
<td>0.0157</td>
</tr>
<tr>
<td>$\lambda_1$ (min$^{-1}$)</td>
<td>0.1082</td>
<td>0.0204</td>
</tr>
<tr>
<td>$\lambda_2$ (min$^{-1}$)</td>
<td>0.02545</td>
<td>0.01140</td>
</tr>
<tr>
<td>$\lambda_3$ (min$^{-1}$)</td>
<td>0.006978</td>
<td>0.003951</td>
</tr>
<tr>
<td>CL (ml/min)</td>
<td>19.91</td>
<td>1.74</td>
</tr>
</tbody>
</table>

$a$) Each parameter is expressed as the mean ± S.D. ($n = 8$) of dose-normalized data.  
$b$) The pharmacokinetic parameters in the normal condition (mean ± S.D., $n = 9$) were as follows; $C_1 = 4.598 ± 1.582$, $C_2 = 0.5671 ± 0.2538$, $C_3 = 0.0314 ± 0.0194$ (µg/ml), $\lambda_1 = 0.1404 ± 0.0493$, $\lambda_2 = 0.0313 ± 0.0083$, $\lambda_3 = 0.00485 ± 0.00217$ (min$^{-1}$), CL = 14.55 ± 1.41 (ml/min). There is no significant difference between normal and hydropenic condition of rabbits.

creased gradually during the experiment. This might be the consequence of both hypotensive effect of urethane,$^6$ and the increased plasma osmotic pressure due to hyperosmotic saline infusion. In spite of the low premedication values, GFR showed a further decrease after piretanide administration, and this decrease continued even though most of the drug had been eliminated from the body. The decrease of GFR was not parallel to the body fluid balance, which was cal-

![Graph 1](image1.png)  
**Fig. 2.** Effect of Piretanide on GFR after i.v. Administration  
Upper graph is vehicle control ($n = 3$), middle graph is 1.5 mg/kg ($n = 4$) and lower graph is 15 mg/kg ($n = 4$). Each point shown is the mean ± S.E.

![Graph 2](image2.png)  
**Fig. 3.** Plasma and Urine Osmolarity in Rabbits before and after Piretanide Administration  
Upper graph is vehicle control ($n = 3$), middle graph is 1.5 mg/kg ($n = 4$) and lower graph is 15 mg/kg ($n = 4$). Each point shown is the mean ± S.E. The open symbols are plasma data and the closed symbols are urine data.
culated from the difference between input (amount of infusion) and output (amount of excretion) of water (data not shown).

The plasma osmolarity ($P_{\text{osm}}$) was slightly increased by hyperosmotic saline infusion, as shown in Fig. 3; however, it was not influenced by piretanide administration. On the other hand, the urine osmolarity ($U_{\text{osm}}$) was increased by hyperosmotic saline infusion, and it was markedly decreased just after piretanide administration.

In general, $U_{\text{osm}}$ is always lower than the osmolarity of the renal medulla ($M_{\text{osm}}$). The reabsorption rate of water in the distal tubule (and also in the collecting duct) is mainly influenced by $M_{\text{osm}}$. The higher the $M_{\text{osm}}$, the greater amount of water reabsorbed. Since the present study was carried out under a hypodiscic condition, the permeability of the membranes for water in the distal tubule and in the collecting duct would be high, and therefore the difference between $U_{\text{osm}}$ and $M_{\text{osm}}$ would be minimal. It is reasonable to consider that the prominent decrease in $U_{\text{osm}}$ after piretanide administration, shown in Fig. 3, reflects the decrease of $M_{\text{osm}}$. This fact suggested that the renal concentrating and/or diluting functions were significantly influenced by piretanide administration.

In order to elucidate the effect of piretanide in the proximal tubule, the lithium clearance (CLI) and the lithium clearance ratio (CLI/GFR) were determined. It has been shown that the reabsorption of filtered lithium at the glomerulus occurs only in the proximal tubules by a common mechanism with the sodium reabsorption. Since there is no other reabsorption system or secretion system for lithium in the nephron, the urinary excretion of lithium represents the function of proximal reabsorption. Consequently, lithium has been used as an indicator of proximal reabsorption. As shown in Fig. 4A, the CLI values without piretanide administration showed a gradual decrease in accordance with GFR, and the values showed a marginal increase just after piretanide administration. On the other hand, CLI/GFR was signi-

![Graphs showing the effect of piretanide on renal clearance and clearance ratio of lithium.](image-url)
Fig. 5. The Effect of Piretanide on Urine Flow Rate and Excretion Rate of the Osmotic Substances
(A) Urine flow rate. (B) Urinary excretion rate of the osmotic substances. Upper graph is vehicle control ($n = 3$), middle graph is 1.5 mg/kg ($n = 4$) and lower graph is 15 mg/kg ($n = 4$). Each point shown is the mean ± S.E.

Significantly increased after piretanide administration (Fig. 4B). This fact indicated that the reabsorption of the glomerular filtrate in the proximal tubules was inhibited by piretanide in a dose dependent manner, and this led to a rise in the CLi/GFR. These results are consistent with the micropuncture study of Winaver et al.,) who proved that one of the active sites of piretanide was in the proximal tubule. At the lower dose, CLi/GFR decreased below the control value.

The precise mechanism of this is unknown and further investigation is required.

Figure 5 illustrates the effect of piretanide on the urine flow rate and on the excretion rate of osmotic substances in urine. Before piretanide administration, the urine flow rate was only one-half of the infusion rate (infusion rate = 0.83 ml/min), due to the existence of high concentration of ADH, and also due to the infusion of hyperosmotic saline. The excretion rate of the

### TABLE II. List of the Premedication Values

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean $^b$</th>
<th>S.E.</th>
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<tbody>
<tr>
<td>GFR$_0$ (ml/h)</td>
<td>567.5</td>
<td>78.2</td>
</tr>
<tr>
<td>$P_{osm0}$ (mOsm/l)</td>
<td>341.4</td>
<td>4.7</td>
</tr>
<tr>
<td>$U_{osm0}$ (mOsm/l)</td>
<td>711.0</td>
<td>43.2</td>
</tr>
<tr>
<td>CLi$_0$ (ml/min)</td>
<td>4.152</td>
<td>0.282</td>
</tr>
<tr>
<td>CLi/GFR$_0$</td>
<td>0.4389</td>
<td>0.0451</td>
</tr>
<tr>
<td>UF$_0$ (ml/min)</td>
<td>0.4792</td>
<td>0.1331</td>
</tr>
<tr>
<td>$dOsm/ul/lt$ (mOsm/min)</td>
<td>0.3407</td>
<td>0.0767</td>
</tr>
</tbody>
</table>

$^a$ Each subscript 0 represents the corresponding premedication level. $^b$ Mean of 8 animals.
osmotic substances before the medication was also about one-half of their infusion rate (0.67 mOsm/min). This fact indicated that the ability of the body to remove excessive salts through the urine might be limited, unless urine flow rate was increased. Both urine flow rate and excretion rate of the osmotic substances were almost constant during the experimental period without pirenidane administration. It is notable that both rates showed marked increase after pirenidane administration although the GFR showed a long lasting decrease throughout the experiment.

As shown in Figs. 2 to 5, some basal levels (without pirenidane administration) were slightly changed during the experimental period. In order to obtain the net pharmacologic effects of pirenidane, each datum obtained at time $t$ was subtracted by the corresponding basal value at time $t$ and then each of the corresponding premedication level was added. Subsequent data were treated by this method. The premedication levels are listed in Table II.

In order to clarify the relationship of the concentration-effect of pirenidane under a hydropenic condition in rabbits, a pharmacodynamic model was constructed using the following hypotheses. (a) Pirenidane has two different sites of action in the nephron; segments I and II. Segment I represents the proximal tubule and segment II represents the remaining part of the nephron except the glomerulus. (b) The function of segment I can be expressed by Cl$_i$/GFR, and pirenidane has a direct effect on the value of Cl$_i$/GFR. The relationship between the effect and the concentration of pirenidane in segment I can be expressed by Hill's equation. (c) Pirenidane exerts its effect on segment II by inhibiting the reabsorption of osmotic substances at the loop of Henle. The relationship of the concentration-effect is also expressed by the Hill's equation. d) The biophase compartments with respect to segments I and II, are directly connected to the central compartment by first order processes, as described previously. The schematic representation of the model is shown in Fig. 6.

From the above assumptions, the clearance ratio in the proximal tubule, the excretion rate of osmotic substances in urine, osmolarity of urine and the urine flow rate, before and after pirenidane administration, are described mathe-

![Diagram of nephron](image.png)

Fig. 6. A Schematic Representation of the Model That Describes the Effect of Pirenidane in the Nephron
matically by the following equations.

The fractional excretion rate from segment I before drug administration is described by Eq. 1.

$$\frac{CL_i}{GFR_0} = 1 - V_{01} \quad (1)$$

$GFR_0$ is the control value of GFR and $V_{01}$ is the control value of the fraction of reabsorption in the segment I. After piretanide administration, Eq. 1 becomes Eq. 2.

$$\frac{CL_i}{GFR} = \frac{CL_i}{GFR_0} + \frac{V_{m1}D_1r_1}{K_{m1}r_1 + D_1r_1} - V_{xt} \quad (2)$$

$V_{m1}$ is the maximum intensity of the effect of piretanide at segment I, and $r_1$ and $K_{m1}$ are the constants of Hill’s equation and $D_1$ is the hypothetical biophase concentration of piretanide in segment I. As shown in Fig. 4, the value of $CL_i/GFR$ was decreased even below the control value after piretanide administration. This phenomenon cannot be explained only by Hill’s equation, because the value of the second term of Eq. 2 was always positive. This fact suggested that there was an unknown factor which participated in the regulation of the proximal reabsorption. Accordingly, we introduced a correction factor for the decrease of baseline effect using a constant $V_{xt}$ and time $t$.

The urinary excretion rate of osmotic substances was expressed by Eq. 3.

$$\frac{dOsm}{dt} = CL_iP_{osm} - R_{osm} - U_{osm}Ku \quad (3)$$

$CL_iP_{osm}$ is the osmotic clearance, $R_{osm}$ is the fractional reabsorption rate of osmotic substances from the loop of Henle and $Ku$ is the reabsorption rate of the tubular fluid from the distal side of segment II. $R_{osm}$ was described by Eq. 4, using another Hill’s equation.

$$R_{osm} = CL_iP_{osm} \left( V_{02} - \frac{V_{m2}D_2r_2}{K_{m2}r_2 + D_2r_2} \right) \quad (4)$$

$V_{02}$ is the fractional reabsorption from the loop of Henle at control condition, $V_{m2}$ is the fraction of the maximum effect of piretanide at segment II, $K_{m2}$ and $r_2$ are the constants of Hill’s equation and $D_2$ is the hypothetical biophase concentration of piretanide at segment II. Because of the infusion of both hyperosmotic saline and ADH, the effect of endogeneous ADH on the reabsorption in the distal side of segment II might be negligible. This is the reason why the distal reabsorption was considered to be proportional to $U_{osm}$, in Eq. 3.

The change of $M_{osm}$ after piretanide administration is described by Eq. 5.

$$V_{md} \frac{dM_{osm}}{dt} = P_{osm}Q_{in} + R_{osm}F_j + U_{osm}Ku - V_{md}M_{osm}Q_{out} \quad (5)$$

$V_{md}$ is the apparent volume of the medullary interstitium, $Q_{in}$ is the rate of fluid flow into the medulla, $F_j$ is the fraction of the effective nephrons among the total nephrons and $Q_{out}$ is the flow rate of the interstitium fluid from the medulla. $R_{osm}F_j$ in Eq. 5 implies that only a part of the osmotic substances reabsorbed at the loop of Henle was available to change the osmolarity of the medulla. Since $U_{osm}$ and $M_{osm}$ were considered to be identical in the present experimental condition, Eq. 5 becomes Eq. 6.

$$V_{md} \frac{dU_{osm}}{dt} = P_{osm}Q_{in} + R_{osm}F_j + U_{osm}Ku - V_{md}U_{osm}Q_{out} \quad (6)$$

If the osmotic substances are reabsorbed, obligatory reabsorption of water will occur, regardless of segments. Thus, the amount of water reabsorption at the loop of Henle with respect to $R_{osm}$ could be estimated by Eq. 7.

$$R_{fl} = \frac{R_{osm}F_j}{P_{osm}} \quad (7)$$

$R_{fl}$ is the reabsorption rate of water at the loop of Henle.

The flow rate of water from the medulla is expressed as Eq. 8.

$$Q_{out} = Q_{in} + R_{fl} + Ku \quad (8)$$

Equation 8 implies the flow rate of water into
the medulla is equal to the flow rate of water out of it. Since the volume of the medulla was constant, the marked decrease of $U_{\text{osm}}$ after piretanide administration might be explained by the lowered reabsorption of osmotic substances (and therefore water) at the loop of Henle. Because of high plasma concentration of ADH in the present experiment, the reabsorption rate of water at the distal side of segment II was considered to be constant. The urine flow rate (UF) was also described by Eq. 9.

$$\text{UF} = \frac{d\text{Osm}}{U_{\text{osm}} dt}$$

(9)

Plasma concentrations and urinary excretion rates of piretanide were described by the following linear three compartment open model, shown in Eqs. 10 and 11.

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

(10)

$$\frac{dX_u}{dt} = CL C_p$$

(11)

$C_p$ is the plasma concentration, $dX_u/dt$ is the urinary excretion rate and $CL$ is the renal clearance of piretanide. $C_i$ and $\lambda_i$ ($i = 1$ to 3) are coefficients and rate constants of the linear compartment model, respectively. The concentrations of piretanide in the hypothetical biophase compartments were calculated by Eq. 12, as reported previously.\(^4\)

$$D_n = K_{e0n} \sum_{i=1}^{3} C_i \frac{(e^{-\lambda_i t} - e^{-K_{e0n} t})}{(K_{e0n} - \lambda_i)}$$

(12)

$D_n$ is the hypothetical biophase concentration of piretanide, $K_{e0n}$ is the elimination rate constant from the biophase compartment and $n$ is the number of biophase compartments ($n = 1, 2$).

The theoretical values of CLi/GFR, $d\text{Osm}/dt$, $U_{\text{osm}}$ and UF before and after piretanide administration were obtained using Eqs. 1 through 12, and the results are shown in Figs. 7 and 8 as solid lines. It is evident that the present pharmacodynamic model well describes the experimental data. Although the dosages of piretanide used in the present study were slightly higher than the recommended dose of human study, the results can be easily extrapolated to the lower dosage. The pharmacodynamic parameters used in the calculation are listed in Table III.

In the present study, the relationship between the concentration and the effect of piretanide was described by two different Hill’s equations, namely Eqs. 2 and 4. At first, we calculated the CLi/GFR and UF using the identical piretanide concentration of the same effect compartment; however, no successful results were obtained. The discrepancy between the observed data and the calculated values could not be explained only by changing the values of $V_m$, $r$ and/or $K_m$ of the Hill’s equation. This fact indicated that the time course of the biophase concentration with respect to the proximal segment (segment I) and the loop of Henle (segment II) might be different. It has been shown that the active site of piretanide is in the lumen side of the loop of Henle.\(^1\) If the active site of piretanide at the proximal tubule (segment I) is also inside the

Fig. 7. Comparison of Calculated Values with Observed Data

The solid lines are calculated values according to Eq. 2 in the text and the plots are clearance ratio of lithium. Upper graph is 1.5 mg/kg and lower graph is 15 mg/kg.
Fig. 8. Comparison of Calculated Values with Observed Data
(A) Urinary excretion rate of osmotic substances. (B) Osmolarity of urine. (C) Urine flow rate. The solid lines are calculated values and the plots are observed data. Upper graph is 1.5 mg/kg and lower graph is 15 mg/kg.

lumen, the time course of the biphase concentrations of two sites should be similar. However, this was not the case. As shown in Fig. 4, there was a prominent time lag between the maximum excretion rate of piretanide and C1i/GFR. If the site of action in segment I was outside of the proximal tubule (for example, at the peritubular space), all of the contradictions could be explained. Accordingly, two distinct concentrations of piretanide, \( D_1 \) and \( D_2 \) were used in this study. As listed in Table III, \( K_\text{e01} \), the elimination constant from the effect compartment concerning segment I was much smaller than \( K_\text{e02} \), the elimination constant in segment II. This implies that the time required to reach the maximum concentration of piretanide in segment I is

<table>
<thead>
<tr>
<th>TABLE III. Pharmacodynamic Parameters of Piretanide</th>
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<tr>
<td><strong>Segment I</strong></td>
</tr>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>( K_\text{e01} ) (min(^{-1}))</td>
</tr>
<tr>
<td>( V_{01} )</td>
</tr>
<tr>
<td>( V_{m1} )</td>
</tr>
<tr>
<td>( r_1 )</td>
</tr>
<tr>
<td>( K_{m1} ) (µg/ml)</td>
</tr>
<tr>
<td>( V_X ) (min(^{-1}))</td>
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</table>
Fig. 9. Calculated Values for Plasma Concentrations, Urinary Excretion Rates and the Hypothetical Biphase Concentrations of Piretanide
(A) 1.5 mg/kg i.v. (B) 15 mg/kg i.v. 1, urinary excretion rate; 2, plasma concentration; 3, biophase concentration with respect to segment I; 4, biophase concentration with respect to segment II.

longer than that in segment II. The time course of piretanide in these effect compartments was shown in Fig. 9. The time course of piretanide in the effect compartment concerning segment II was almost parallel to that of the urinary excretion rate. This result was consistent with the fact that the active site of piretanide was in the lumen side of the loop of Henle. Although we speculated that the active site of piretanide at the segment I was outside the lumen, the precise mechanism of action was still unclear and, for clarification further investigation is required.

In the present study, lithium was used as an indicator of the proximal reabsorption. It has been reported that lithium competed with sodium for proximal absorption.\textsuperscript{12} In order to avoid the competitive inhibition of sodium reabsorption by lithium, the two ions were infused simultaneously and the lithium content in the infusion medium was much lower than the sodium content, in the present study. It is well known that about 70% of sodium in the glomerular filtrate is reabsorbed by the proximal tubules. Thomsen et al.\textsuperscript{7} reported that the fractional clearance of lithium had a similar value. In the case of excessive sodium intake, the fraction of proximal reabsorption was decreased.\textsuperscript{13} The present study indicated that the infusion of hyperosmotic saline inhibited the fractional reabsorption significantly, and that only 55% of the filtrate was found to be reabsorbed.

In the calculation of the change of osmolarity of the medulla, the $F_j$ value was used in Eqs. 5 to 7. The estimated $F_j$ value was 0.17, as shown in Table III, and this value was very close to the value of the fraction of juxta-medullary nephrons in total nephrons (0.14 in humans, and 0.28 in rats\textsuperscript{14}). This fact suggests clearly that the osmolarity of $M_{\text{osm}}$ was influenced by the nephrons, whose loop of Henle existed inside the medulla.

**Conclusion**

By utilizing lithium as an indicator of proximal reabsorption characteristics, it was possible to distinguish the effect of piretanide on the proximal tubules from that on the loop of Henle
and/or on the distal tubules. A pharmacodynamic model concerning the effect of piretanide in the nephrons adequately described the experimental data. The site of action of piretanide on the proximal part (segment I) might exist in the peritubular side rather than inside the tubule, whereas the site of action on the loop of Henle might exist inside the tubule.

References


