The Possible Role of Endogenous Digitalis-Like Substance in the Regulation of Circadian Changes in Urinary Electrolyte Excretion in Man

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Abstract

The urinary volume (U.V.), Na excretion (UNaV) and K excretion (UKV) have been reported to show a circadian rhythm in man, but the mechanism of this rhythm has not been made clear. To investigate how atrial natriuretic peptide (ANP) and endogenous digitalis-like substance (DLS) participate in the circadian change in urinary electrolyte, the circadian changes in ANP and DLS (digoxin-like immunoactivity: DLI, Na-K-ATPase inhibitor: ATPI, ouabain binding inhibitor to Na-K-ATPase: OBI) were evaluated in 5 normal man. ANP, DLI and OBI showed no significant correlation with urinary electrolyte excretion, but there was a significant positive correlation between plasma ATPI and urinary Na excretion. From these results it is suggested that circulating Na-K-ATPase inhibitor (plasma ATPI) may be involved in the regulation of the circadian rhythm of urinary Na excretion.

It has been widely accepted that electrolyte excretion in the urine shows a circadian change. In man, the urinary volume (U.V.), Na excretion (UNaV) and K excretion (UKV) have been reported (Muratani H. et al., 1985) to be lowest at about 4–5 a.m. and highest at about 5–7 p.m. This rhythm seems to be independent of food intake (Muratani H. et al., 1985), but the mechanism responsible for the rhythm has not been made clear yet.

Recently, new humoral factors regulating Na and K excretion, such as atrial natriuretic peptide (ANP) (Miyamori I. et al., 1987) and endogenous digitalis-like substance (DLS) (Morise T. et al., 1988), have been discovered and have attracted a great deal of attention. Therefore, in the present paper, we attempted to investigate how these new natriuretic factors participate in the circadian change in urinary electrolyte excretion in man.
Subjects and Methods

Studies were performed in five healthy male volunteers, aged 20–25 years after informed consent was obtained. They commenced bed rest from 5 a.m. on the day before the experiment, and the study was performed for 24 hours from noon on the next day to noon on the day after. During this time meals were given at 6 a.m., 2 p.m. and 10 p.m. and the Na and K content of each meal was set at 60 and 20 mEq, respectively. As shown in Fig. 1, the urine was collected from noon every 4 hours, and at the midpoint of each urine collection, the blood was taken.

The blood was taken from a catheter retained in the anterior cubital vein, placed in a siliconized glass tube containing EDTA, centrifuged immediately, and stored frozen at −20°C. The urine collected every 4 hours was stored frozen at −20°C after the volume was measured.

Plasma ANP, plasma renin activity (PRA) and the plasma aldosterone concentration (PAC) were measured by radioimmunoassay (RIA), as reported previously (Takeda R. et al., 1976). Digi- talis-like immunoactivity (DLI) was measured in the urine, and Na-K-ATPase inhibiting activity (ATPI) as well as ouabain binding inhibiting activity (OBI) were measured in the urine and plasma.

DLI was measured by RIA using anti-digoxin antibody (Daiichi Radioisotope Co., Ltd), and ATPI was determined principally according to the method of Hamlyn et al. (Hamlyn J. M. et al, 1982) with minor modifications as previously reported (Morise T. et al., 1985). OBI was measured by competitive binding of 3-H ouabain with canine kidney Na-K-ATPase (Sigma Chemical Co., Ltd.). A 1.0 ml of medium containing 50 mM tris-HCl buffer (pH 7.4), 2.0 mM MgCl₂, 100 mM NaCl, 1.0 mM EDTA, and 3-H ouabain (0.892 TBq/mmol; 24.1 Ci/mmol) prepared to give a final concentration of 50 nM was incubated with or without the samples at 37°C for 60 min. The reaction was terminated by adding 3.0 ml of cold 50 mM tris-HCl. Bound 3-H ouabain was trapped with Whatman GF/B filters. The filters were washed twice with 3.0 ml of the same tris-HCl buffer, dried and assayed for radioactivity. Specific binding was calculated by comparing the binding which occurred in the presence of 10⁻³, 10⁻⁴ and 10⁻⁵ M cold ouabain.

The results are presented as the means±SE, and 0.05 probability was regarded as significant. Correlations were assessed as correlation coefficients(r).

Results

U.V., U₉₄V and U₉₅V were all low early in the morning (87.8±41.9 ml/h, 10.9±8.5 mEq/4 h, 5.5±1.2 mEq/4 h, respectively) and became high in the evening (346.3±47.8 ml/h, 62.6±0.5 mEq/4 h, 12.0±0.8 mEq/4 h, respectively), showing a circadian rhythm (Fig. 1). This rhythm was analyzed by the cosinor method (Natali G. et al., 1982), but did not give a linear
Plasma ATPI showed a rhythm which was similar to that of UNaV and UKV, being low (165.9±146.3 pmol/ml, ouabain equiv.) early in the morning and becoming high (331.7±39.0 pmol/ml, ouabain equiv.) in the evening. In contrast, plasma OBI showed a tendency to become high beginning at noon (Fig. 2). Urinary DLI was high at night, whereas urinary ATBI and urinary OBI were low at night (Fig. 3).

The correlations between DLS, ATPI and OBI on the one hand and UNaV and UKV on the other were investigated. As shown in Table 1 and Fig. 4, only plasma ATPI and UNaV showed a significant positive correlation. Plasma ANP, PRA and PAC showed no significant correlation with UNaV or UKV.

Serum sodium and potassium were 141.2±0.2, 4.2±0.1 mEq/L, respectively, and showed no circadian change. Creatinine clearance also showed no change.

**Discussion**

It has been known that urinary electrolyte
Table 1. The correlation between DLI, ATPI and OBI on the one hand and UNa and U\textsubscript{K}V.

<table>
<thead>
<tr>
<th></th>
<th>PAC</th>
<th>P-ANP</th>
<th>P-ATPI</th>
<th>P-OBI</th>
<th>U-DLI</th>
<th>U-ATPI</th>
<th>U-OBI</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNaV</td>
<td>N.S.</td>
<td>N.S.</td>
<td>p&lt;0.01</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>UNaV</td>
<td>0.07</td>
<td>0.01</td>
<td>0.514</td>
<td>0.02</td>
<td>0.08</td>
<td>-0.32</td>
<td>-0.2</td>
</tr>
<tr>
<td>UKV</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>UKV</td>
<td>0.11</td>
<td>0.04</td>
<td>-0.35</td>
<td>0.15</td>
<td>-0.01</td>
<td>-0.31</td>
<td>-0.3</td>
</tr>
</tbody>
</table>

Fig. 4. Correlation between plasma Na-K-ATPase inhibitor (P-ATPI) and urinary Na excretion (U\textsubscript{Na}V).

\[ Y = 34 + 6.2X \]
\[ r = 0.514 \quad (p < 1\%) \]
\[ N = 30 \]

excretion in man has a circadian rhythm (Muratani H. et al., 1985). However, it has not been made clear what factors are responsible for the formation of this rhythm. UN\textsubscript{Na}V and UN\textsubscript{K}V is mainly regulated by the glomerular filtration rate (GRF) and secretory activity of aldosterone. However, in this study GFR (evaluated with creatinine clearance) showed no circadian rhythm, being always constant, and aldosterone showed no significant correlation with UN\textsubscript{Na}V and UN\textsubscript{K}V. From these results it is suggested that neither GFR nor aldosterone is a major factor in the regulation of the circadian rhythm. The influence of food intake may also be an important factor. Cohn et al., (Cohn C. et al., 1970) have reported that the circadian rhythm of urinary electrolyte excretion in the rat is altered by changing the feeding habits. However, Muratani et al., have reported that there is no change in the circadian rhythm when intravenous hyperalimentation for 24 hours is performed in man, suggesting no influence of food intake. In our experiments, meals containing 60 mEq of Na and 20 mEq of K in each were given every 8 hours and the circadian rhythm of UN\textsubscript{Na}V and UN\textsubscript{K}V was found to be independent of the feeding time. In view of this, the food intake pattern is not an important factor.

Recently, as a new humoral regulator for UN\textsubscript{V}, UN\textsubscript{Na}V and UN\textsubscript{K}V, ANP (Kangawa K. et al., 1985, Sonnenberg H., 1987) and DLS (Clarkson E.M. et al., 1970, de Wardener H. E. et al., 1981) has been discovered as the third factor following GFR and aldosterone. Since the chemical characteristics of DLS have not been made clear yet, we used the parameters of digitoxis like immunoactivity (DLI), Na-K-ATPase inhibiting activity (ATPI) and ouabain binding inhibiting activity (OBI). Among these indices, plasma ATPI showed nearly
the same circadian rhythm as UNaV, and a significant positive correlation was noted between plasma ATPI and UNaV. This suggests that plasma ATPI is concerned somehow in the regulation of the circadian rhythm of UNaV. To support this hypothesis, it is necessary to clarify the chemical characteristics of ATPI and confirm that an increase in ATPI can actually increase UNaV. Concerning the chemical characteristics of ATPI, Mir M. A. et al., suggested ATPI was of peptidal origin (Air M. A. et al., 1988.), Cloix J. F. et al., suggested steroidal substance and its molecular weight was 431 (Cloix J. F. et al., 1985) and Tamura et al. reported that ATPI was a non peptidal substance and its molecular weight was 336 (Tamura M. et al., 1988), however, no common view of chemical characteristics of this substance has been obtained. Since the chemical characteristics of ATPI (DLS) have not been determined, it is difficult to prove directly. When DLS purified from human urine was administered to rats for 1 week with an osmotic mini-pump or as a bolus dose, a significant increase in UNaV was observed (Morise T. et al., 1988). This result is considered to support the above-mentioned hypothesis, though the origin of semi-purified ATPI was urine (not plasma) and was indirect.

The mechanism of increase in ATPI from the evening to the night is unknown at present. However, a report (Moore-Ede M. C. et al., 1975) describing an increase in intracellular potassium from the evening to the night when U_KaV and U_KV increase, is of interest in considering the mechanism of the circadian rhythm of ATPI. The increase in intracellular potassium is related to increased reabsorption of potassium by the kidney. This change can be understood as a reaction to maintain the level of the potassium constant in the blood against increased U_KV from the evening. In the adjustment of intra-cellular potassium, Na-K-ATPase plays an important role as pH (Malnic G. et al., 1971) and insulin (Zierler K. L., 1986). Considering that the increase in ATPI, which has an inhibitory action on this enzyme, occurs towards the evening, it is possible that the increase in intracellular potassium is brought about by the inhibition of Na-K-ATPase by ATPI. It is also possible that U_KV increases first due to some unknown mechanism and plasma ATPI then increase to maintain the homeostasis of potassium (to decrease intracellular potassium). This increase in ATPI may simultaneously inhibit the reabsorption of Na, resulting in the increase in UNaV.

In conclusion, increased ATPI which was conducted by the change in potassium may be partly involved in the regulation of the circadian change in UNaV. But the above is only speculative and the mechanism which causes the change in U_KV is unknown. To confirm these points it will be necessary to clarify the nature of DLS, and to develop effective agonists and antagonists.

References


