6 Kokko, J.P., and Rector, F.C., Kidney Int. *2* (1972) 214.
7 Stephenson, J.K., Rev. Biophys. Bioeng. *7* (1978) 315.
8 Schmidt-Nielsen, B., Graves, B., and Roth, J., Am. J. Physiol. *244* (1983) F472.
9 Morel, F.F., Guinnebault, M., and Amiel, C., Helv. physiol. pharmac. Acta *18* (1960) 183.
10 Morel, F.F., and Guinnebault, M., J. Physiol., Paris *53* (1961) 75.
11 White, H.L., Rolf, D., and Tosteson, D.C., Am. J. Physiol. *200* (1961) 591.
12 Pennell, J.P., Sanjana, V., Frey, N.R., and Jamison, R.L., J. Clin. Invest. *55* (1975) 399.
13 Drost-Hansen, W., The occurrence and extent of vicinal water, in: Biophysics of Water, p. 163. Ed. F. Franks, Wiley & Sons, New York 1982.

0014-4754/86/091010-03$1.50 + 0.20/0

High potassium intake increases the plasma concentration and urinary excretion of vasopressin in the rat

D.P. Brooks, J.T. Crofton, L. Share and A. Nasjletti*

Departments of Physiology and Biophysics, and of Pharmacology, University of Tennessee Center for the Health Sciences, 894 Union Avenue, Memphis (Tennessee 38163, USA), 22 October 1985*

Summary. The effect of alterations of dietary potassium intake on the plasma concentration and the urinary excretion of vasopressin was studied in male rats. Ingestion of a high potassium diet resulted in increases in the plasma concentrations of potassium and vasopressin, systolic blood pressure, urine flow, and urinary vasopressin excretion. Ingestion of a low potassium diet had little effect on the plasma vasopressin concentration and systolic blood pressure but caused decreases in the plasma potassium concentration and urinary vasopressin excretion. The results indicate that physiological changes in the plasma potassium concentration or some other consequence of altered dietary potassium intake can affect vasopressin release and excretion.
Key words. Vasopressin; antidiuretic hormone; potassium.

Sodium and chloride ions are the major ions contributing to the osmotic pressure of plasma and, therefore, changes in their concentration are important in the osmotic control of vasopressin secretion. Changes in the plasma potassium concentration contribute little to plasma osmolality, yet these changes may alter vasopressin secretion. The results of experiments dealing with this issue, however, are inconsistent. For example, potassium depletion has been reported to increase the plasma vasopressin concentration[1] as well as attenuate the vasopressin secretion induced by hypertonicity[2]. The present study, therefore, was designed to delineate further the role of dietary potassium in the control of vasopressin secretion.

Methods. Sixty male Sprague-Dawley (Harlan) rats, weighing approximately 240 g, were placed individually in metabolism cages. They were provided with a diet containing 0.16 mmol potassium/g (designated medium-K diet) and water ad libitum. After 5 days (at day 0) they were divided into three groups. One group of 20 rats remained on the medium-K diet, the second group of 20 rats received a low potassium (low-K) diet (0.04 mmol/g), and the third group received a high potassium (high-K) diet (2.7 mmol/g). Water remained available ad libitum. The day before the change of diet (day 0) and 4, 7, and 13 days afterwards, a 24-h urine collection was made. Urine was collected into plastic bottles surrounded by dry ice and enclosed in styrofoam boxes so that the urine froze immediately after it was voided. Urine samples were stored at −40 °C for the later measurement of volume, osmolality, and sodium, potassium, and vasopressin concentrations.

On day 13, ten rats were taken from each group, lightly anesthetized with ether, and decapitated for the collection of trunk blood into chilled plastic tubes containing 0.3 ml sodium heparin (1000 U/ml). A sample of blood was taken for the determination of packed cell volume, and the remainder was centrifuged at 1400 × g at 4 °C. A sample of plasma was taken for the determination of osmolality (freezing point depression; Micro-osmette, Precision Systems) and sodium concentration (flame photometry; IL 343 Flame Photometer, Instrumentation Laboratories). Two or 3 ml of plasma were stored at −40 °C for the subsequent extraction and assay of vasopressin.

The remaining 10 rats from each group were anesthetized with ether, and systolic blood pressure was measured using the tail cuff procedure (Narco). A blood sample was then taken into a heparinized syringe from the abdominal aorta. The blood was centrifuged at 1400 × g at 4 °C, and the plasma concentration of potassium was determined by flame photometry. Aortic blood was used for this purpose because trunk blood is contaminated with potassium from the cellular fluids.

The urine samples from 10 rats in each group were thawed and centrifuged at 1000 × g to remove small traces of food. The volume was determined and an aliquot was taken for the measurement of osmolality and sodium and potassium concentrations. The pH of 5 ml of urine was adjusted to 2.0 and vasopressin was extracted, using octadecysilane cartridges (Sep-Pak C_{18}, Waters). Vasopressin was assayed by radioimmunoassay using equilibrium conditions[3]. The plasma samples were thawed and acidified with 1 N HCl (0.1 ml/ml plasma) and vasopressin was extracted as described above and assayed, using disequilibrium conditions[4]. The USP Posterior Pituitary Reference Standard was used as the standard.

The recoveries of added vasopressin from rat plasma and urine, measured in the same assays in which vasopressin was measured in the experimental samples, were 74 ± 2% (means ± SE) for plasma (n = 6) and 94 ± 3% for urine (n = 12).

Statistical analysis of data was performed using one- and two-way analyses of variance for repeated measures and a subsequent Neuman-Keuls test, when appropriate, to determine differences within and between groups.

Results. The plasma vasopressin concentrations of rats on the medium- and low-K diets were similar (0.67 ± 0.03 versus 0.71 ± 0.06 μU/ml, respectively) at the end of 13 days. Rats fed the high-K diet, however, showed a small but significant ($p < 0.05$) increase in the plasma vasopressin concentration (0.94 ± 0.09 μU/ml; table 1). The urinary excretion of vasopressin (fig.) changed considerably with alteration of dietary potassium intake. Thus, after 4 and 7 days of the low-K diet, rats had a lower ($p < 0.01$) urinary vasopressin excretion (fig.). By day 13, the urinary vasopressin excretion had returned to levels not significantly different from levels observed on day 0 in the same rats or on day 13 in rats on the medium-K diet. Rats that received the high-K diet increased the urinary excretion of vasopressin substantially, such that at days 7 and 13 it was elevated between 4- and 5-fold ($p < 0.01$; fig.).

Alteration of dietary potassium intake resulted in expected changes in the plasma potassium concentration. Thus, rats fed the high-K diet had a significantly ($p < 0.01$) higher plasma potassium concentration than rats fed the medium-K diet at the

Table 1. Plasma vasopressin concentration (P_{AVP}; μU/ml), plasma potassium concentration (P_K; mmol/l), plasma sodium concentration (P_{Na}; mmol/l), plasma osmolality (P_{osm}; mOsm/kg H_2O), packed cell volume (PCV; %) and systolic blood pressure (SBP; mmHg) of rats after 13 days on a low (0.04 mmol/g), medium (0.16 mmol/g), or high (2.70 mmol/g) potassium diet. Number of rats (n)

	Low	Medium	High
P_{AVP}	0.67 ± 0.03 (10)	0.71 ± 0.06 (9)	0.94 ± 0.09* (10)
P_K	2.39 ± 0.06** (10)	4.16 ± 0.11 (10)	4.80 ± 0.24** (9)
P_{Na}	144 ± 1 (10)	142 ± 1 (10)	141 ± 1 (8)
P_{OSM}	297 ± 2* (10)	303 ± 1 (10)	302 ± 1 (8)
PCV	33.3 ± 0.5* (10)	35.6 ± 0.5 (10)	36.5 ± 0.8 (9)
SBP	109 ± 2 (10)	112 ± 3 (10)	122 ± 5* (9)

*p < 0.05, **p < 0.01 (compared to medium-K diet group).

Table 2. Urinary sodium excretion ($U_{Na}V$; mol/day), urinary potassium excretion (U_KV; mol/day), urine flow (V; ml/day), and urinary osmolality (U_{OSM}; mOsm/kg H_2O) in rats on a low (0.04 mmol/g), medium (0.16 mmol/g), or high (2.70 mmol/g) potassium diet. Ten rats in each group

		Time (Days) 0	4	7	13
$U_{Na}V$	Low	2.35 ± 0.08	1.94 ± 0.06*†	1.96 ± 0.15*†	2.02 ± 0.09*†
	Medium	2.26 ± 0.07	2.21 ± 0.10	2.26 ± 0.11	2.39 ± 0.12
	High	2.18 ± 0.13	1.31 ± 0.09	3.34 ± 0.13	4.11 ± 0.12
U_KV	Low	2.01 ± 0.06	0.07 ± 0.0*†	0.05 ± 0.04*†	0.04 ± 0.0*†
	Medium	1.93 ± 0.06	1.91 ± 0.06	2.03 ± 0.08	2.07 ± 0.11
	High	1.92 ± 0.12	13.7 ± 1.2*†	34.4 ± 1.3*†	33.9 ± 1.2*†
V	Low	21.4 ± 1.7	22.4 ± 1.3	22.6 ± 2.2	23.6 ± 1.7
	Medium	24.1 ± 2.0	26.2 ± 1.8	27.2 ± 2.2	22.2 ± 1.9
	High	19.4 ± 1.7	63.5 ± 2.4*†	98.3 ± 4.2*†	97.8 ± 5.8*†
U_{OSM}	Low	1214 ± 80	970 ± 60	902 ± 63*	940 ± 46†
	Medium	1018 ± 63	992 ± 59	980 ± 58	1295 ± 80*
	High	1224 ± 83	1665 ± 98	1015 ± 38*†	1069 ± 35

*p < 0.01 (from time 0). †p < 0.01 (from medium-K diet group).

end of 13 days (table 1), whereas animals on the low-K diet had a significantly ($p < 0.01$) reduced plasma potassium concentration. The urinary excretion of potassium also reflected the changes in potassium intake. Thus, by day 13 of a low-K diet, rats were excreting negligible amounts of potassium (table 2), whereas rats fed the high-K diet were excreting 16 times more potassium than the rats on the medium-K diet (table 2).

The plasma sodium concentrations of rats fed the 3 different potassium diets were similar after 13 days (table 1), but the urinary excretion of sodium was decreased ($p < 0.01$) at day 4 and increased ($p < 0.01$) at days 7 and 13 in rats fed a high-K diet. There were no significant changes in sodium excretion in the other 2 groups.

Urine flow (table 2) increased ($p < 0.01$) substantially in rats fed the high-K diet and followed a pattern similar to the urinary vasopressin excretion (fig.). Urine flow did not change significantly in rats on the low- or medium-K diets (table 2). Over the course of the experiment, urine osmolality decreased ($p < 0.01$) in the rats on the low-K diet and varied in rats on the medium- and high-K diets (table 2). Plasma osmolality was similar in the high- and medium-K diet groups but was reduced ($p < 0.05$) in the rats on the low-K diet (table 1).

Systolic blood pressure (table 1) was significantly ($p < 0.05$) higher after 13 days in the rats on the high-K diet than in the rats on the low-K diet (122 ± 5 versus 112 ± 3 mm Hg). Rats on the low-K diet had a systolic blood pressure of 109 ± 2 mm Hg, which was not different from the medium-K diet group.

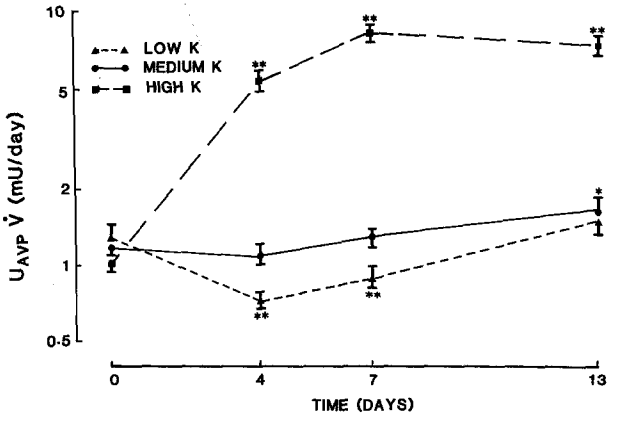
Over the course of the experiment, rats on the high-K diet did not grow at the same rate as rats on the low- and medium-K diets. Body weights in the low-K, medium-K, and high-K diet groups were 240 ± 2, 234 ± 2, and 241 ± 2 g, respectively, on day 0 and were 266 ± 3, 263 ± 3, and 248 ± 3 g, respectively, on day 13.

Discussion. This study demonstrates that large variations in dietary potassium can influence the plasma concentration and urinary excretion of vasopressin. Rats fed a high potassium diet demonstrated an increase in both the plasma concentration and urinary excretion of vasopressin. Rats fed a low potassium diet had a transient decrease in urinary vasopressin excretion. At day 13 there was little difference in either the plasma concentration or urinary excretion of vasopressin between rats fed the low and medium potassium diets.

The elevated plasma vasopressin concentration observed in the high potassium group may reflect an increased secretion of vasopressin from the posterior pituitary. Certainly there is evidence available to indicate that high concentrations of potassium can stimulate vasopressin release in vitro[5]. However, these data involve very high potassium levels (30 mM) and, therefore, presumably a direct depolarization of the nerve terminals in the posterior pituitary. In the present study, the increased potassium intake resulted in a small but significant increase in the plasma potassium concentration without an increase in plasma osmolality. It is possible, therefore, that physiological increases in the plasma potassium concentration may influence vasopressin secretion. The mechanism for this influence is not clear from this study, but we can rule out any indirect effects of potassium involving changes in blood pressure since, in the present study, the increased potassium intake resulted in a small elevation in systolic blood pressure. This, itself, would tend to inhibit vasopressin secretion. The data provided by the present study cannot preclude the possibility that the high potassium intake caused an increase in the plasma vasopressin concentration by a mechanism other than increased secretion from the pituitary. Thus it is conceivable that the high potassium intake resulted in a decrease in vasopressin metabolism.

In the present study, we observed no effect of 13 days of low potassium intake on the plasma vasopressin concentration. This is in contrast with the observation by Paller and Linas[1], who reported an increased plasma vasopressin concentration in rats on a low potassium diet. These investigators, however, fed their rats the low diet for a longer period (14–21 days) and also observed a decrease in blood pressure.

The small increase in the plasma vasopressin concentration observed in the rats fed the high potassium diet is unlikely to contribute significantly to the 4- to 5-fold increase in the urinary concentration of the hormone. Thus, it is possible that the increased urinary vasopressin excretion was the result of an alteration in the renal handling of vasopressin. The increased potassium intake resulted in a considerable increase in urine flow, which may be attributed to the increase in solute excretion. Increases in solute excretion have been associated with increases in vasopressin excretion[6], and, in our study, the changes in urine



Urinary vasopressin excretion ($U_{AVP}\dot{V}$; U/day) in rats fed a low-K (0.04 mmol/g), medium-K (0.16 mmol/g), or high-K (2.70 mmol/g) diet. Ten rats in each group. *p < 0.05, **p < 0.01 (compared to day 0).

flow in the high-K diet group were mirrored by the changes in urinary vasopressin excretion. Indeed, when analyzed by a regression analysis, there was a significant correlation between the two parameters ($r = 0.800$). It is possible that changes in urine flow, along with the increased solute excretion, could alter the excretion of vasopressin. It has been demonstrated that, at physiological concentrations, vasopressin is freely filterable at the glomerulus[7]. Furthermore, Kimura et al.[8] have shown that vasopressin is reabsorbed and/or metabolized in the proximal nephron and is secreted into the distal nephron. It is conceivable that the increased urine flow could result in a decrease in the removal of vasopressin (by reabsorption and/or metabolism) from the proximal nephron, thus increasing the delivery of vasopressin to the distal nephron and presumably into the urine. It is unlikely that in the present study glomerular filtration rate increased enough to account for the elevation in vasopressin excretion.

In summary, the present study indicates that an increased potassium intake can increase both the plasma vasopressin concentration and the urinary vasopressin excretion of rats. The exact mechanisms involved in these changes, however, are not clear.

Acknowledgments. The authors thank L. Mayfield, D. Ratliff, S. Jobe, J. Weakley, and M. Keller for their expert technical assistance. This work was supported by USPHS grants HL-12990 and HL-19209 from the National Heart, Lung and Blood Institute.

1 Paller, M. S., and Linas, S. L., Kidney Int. *24* (1983) 342.
2 Rutecki, G. W., Cox, J. W., Robertson, G. L., Francisco, L. L., and Ferris, T. F., J. Lab. clin. Med. *100* (1982) 53.
3 Crofton, J. T., Share, L., Shade, R. E., Allen, C., and Tarnowski, D., Am. J. Physiol. *235* (1978) H361.
4 Crofton, J. T., Share, L., Wang, B. C., and Shade, R. E., Hypertension *2* (1980) 424.
5 DeVito, W. J., Miller, M., and Sutterer, J. R., Endocrinology *111* (1982) 1958.
6 Robertson, G. L., Clin. Res. *20* (1972) 778.
7 Share, L., and Crofton, J. T., J. Endocr. *86* (1980) 501.
8 Kimura, T., and Share, L., Endocrinology *109* (1981) 2089.

0014-4754/86/091012-03$1.50 + 0.20/0

Effects of kallidinogenase on urinary kallikrein excretion and plasma prostanoid concentrations in patients with essential hypertension

K. Ogawa, T. Ito, M. Ban, M. Motizuki and T. Satake

The 2nd Department of Internal Medicine, Nagoya University School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya 466 (Japan), 24 April 1984

Summary. The effects of kallidinogenase on urinary kallikrein excretion, plasma immunoreactive prostanoids and platelet aggregation were investigated in patients with essential hypertension. Urinary kallikrein excretion and plasma 6-keto $PGF_{1\alpha}$ concentration were significantly decreased in these patients. Significant decreases in blood pressure, as well as significant increases of urinary kallikrein excretion and plasma 6-keto $PGF_{1\alpha}$ concentration after kallidinogenase administration were also observed.
Key words. Kallidinogenase; kallikrein; 6-keto $PGF_{1\alpha}$; thromboxane B_2; platelet aggregation; essential hypertension.

Many reports have suggested that the kallikrein-kinin system is of pathogenic significance in human hypertensive disease[1,2,3]. The authors studied, in patients over an 8-week period, the role of kallikrein, prostacyclin and thromboxane A_2 in essential hypertension, and the effects of kallidinogenase on these parameters as well as blood pressure.

Materials and methods. Twelve patients (10 males, 2 females, mean age 43.2) with essential hypertension but not on medication were selected for this study after their informed consent was obtained. The average, casual blood pressure on sitting during outpatient visits on more than 2 occasions was greater than 160/90 mmHg in all patients. Each patient was given a sufficient work-up to exclude any known cause of hypertension. All patients were considered to be mildly or moderately hypertensive and corresponded to stage I or II of the WHO classification (1962). After a 2-week control period, each patient was given, orally and over an 8-week-period, 450 kallikrein units (KU)/day of kallidinogenase prepared from hog pancreas by Sanwa Chem. Lab. Twenty-four hour urine and venous blood were collected on the morning previous to, and at 2 and 4 weeks after kallidinogenase administration, respectively. Urinary kallikrein was measured by radioimmunoassay[4]. Plasma 6-keto $PGF_{1\alpha}$ and thromboxane B_2 were measured by radioimmunoassay according to Jaffe and Granström et al.[5,6], using antiserum prepared by New England Nuclear. Platelet aggregation was measured with the addition of ADP, collagen or ristocetin by aggregometer. Statistical significance of difference was determined by Wilcoxon's signed ranks test for matched pairs and Wilcoxon's rank sum U test for unpaired data, respectively. The correlation was determined by Spearman rank correlation coefficient test. Statistical significance was taken as $p < 0.05$.

Results. As shown in table 1, mean urinary kallikrein excretion was 62.8 ± 9.8 µg/day in patients with essential hypertension, while that in normal subjects was 122.0 ± 6.9 µg/day. There was a significant difference between them ($p < 0.001$). The mean concentration of plasma 6-keto $PGF_{1\alpha}$ in patients with essential hypertension was 145.2 ± 17.8 pg/ml, against 304 ± 24.7 pg/ml in normal subjects. There was a significant decrease of 6-keto $PGF_{1\alpha}$ in patients with essential hypertension with $p < 0.001$. On the other hand, there was no significant difference of plasma thromboxane B_2 concentration between patients with essential hypertension and normal subjects.

The mean systolic and diastolic blood pressure before the administration of kallidinogenase was 172.6 ± 3.2 mmHg and 94 ± 4.6 mmHg, respectively. These decreased to 160.6 ± 2.9 mmHg (6.9%, $p < 0.01$) and 88.6 ± 3.3 mmHg (5.7%, $p < 0.23$) after 4 weeks and to 155.9 ± 3.4 mmHg (9.7%, $p < 0.01$) and 81.5 ± 3.8 mmHg (13.3%, $p < 0.01$) after 8 weeks of kallidinogenase administration, respectively. The mean blood pressure was 120.2 ± 3.5 mmHg before administration and significantly decreased to 113.6 ± 2.4 mmHg (5.5%, $p < 0.05$) after 4 weeks and 106.3 ± 3.3 mmHg (11.6%, $p < 0.01$) after 8 weeks.

The mean urinary kallikrein excretion significantly increased from 62.8 ± 9.8 µg/day to 105.1 ± 14.9 µg/day after 4 weeks and to 119.3 ± 16.0 µg/day after 8 weeks of kallidinogenase in patients with essential hypertension (both, $p < 0.01$); it reached the same excretion level in normal subjects as shown in the table.

There was a significant negative correlation between mean blood pressure and urinary kallikrein excretion in patients with essential hypertension ($r = -0.514$, $p < 0.01$).

The mean plasma concentration of 6-keto $PGF_{1\alpha}$ in patients with essential hypertension was 145.2 ± 17.8 pg/ml before kallidino-