Effect of Fluoride on the Activities of the Na^+ /Glucose Cotransporter and Na^+/K^+ -ATPase in Brush Border and Basolateral Membranes of Rat Kidney (*in Vitro* and *in Vivo*)

Yasunobu SUKETA,* Kenji SUZUKI, Toshinobu TAKI, Yasuko ITOH, Masaru YAMAGUCHI, Takashi SAKURAI, and Yukiko TANISHITA

University of Shizuoka School of Pharmaceutical Sciences, Department of Environmental Biochemistry and Toxicology, 52-1 Yada, Shizuoka, Shizuoka 422, Japan. Received August 31, 1994; accepted October 12, 1994

In this study, renal Na^+/K^+ -ATPase activity was demonstrated to be strongly suppressed prior to the glucosuria caused by a fluoride dose (NaF 35 mg/kg, i.p.), and the 50% suppression of the enzyme activity was almost at the same dose of NaF , about 30 mg/kg, i.p. to rats. In the rats, renal Na^+ /glucose cotransporter activity in brush border membranes was not affected by *in vivo* NaF , whereas the renal Na^+/K^+ -ATPase in basolateral membranes showed a dip in activity 3 h after NaF treatment of the whole animal.

Moreover, it was suggested from experiments with inhibitors of calphostin C and KT5720 that protein kinase C, but not protein kinase A, may play an important role in the suppression of Na^+/K^+ -ATPase following the administration of fluoride to rats.

Na^+ /glucose cotransporter was fairly insensitive to NaF , being competitively inhibited with a K_i of about 100 mM, whereas Na^+/K^+ -ATPase was much more sensitive, with a K_i of about 2 mM.

From these results, the elevation of urinary glucose excretion after a single dose of fluoride was deduced to be due to suppression of the renal Na^+/K^+ -ATPase activity by a direct and/or secondary action of fluoride, rather than of the corresponding Na^+ /glucose cotransporter activity.

Key words sodium ion/glucose cotransporter; sodium ion/potassium ion-ATPase; protein kinase A; protein kinase C; sodium fluoride

Taylor *et al.*¹⁾ reported increased urinary sugar excretion 1 d after the administration of near-lethal fluoride doses (20–30 mg/kg, i.v.) in rats. Moreover, we observed acute glucosuria caused by injecting fluoride (NaF 35 mg/kg, i.p.) to rats.²⁾ Thus, the glucosuria is suggested to be due to a dysfunction of renal glucose-reabsorption by an acute fluoride dose. On the other hand, glucose level is controlled by regulation of the transport and metabolism of glucose.³⁾ Reabsorption of glucose in the kidney is known to be mediated by a Na^+ /glucose cotransporter localized in the brush border membranes (BBM)⁴⁾ and by a sodium gradient. Na^+/K^+ -ATPase localized in the basolateral membranes (BLM)⁵⁾ provides the sodium gradient.

Mammalian tissues take up glucose by the passive process of facilitated diffusion, which is driven solely by the concentration gradient existing across the plasma membrane. The passive transport of glucose is catalyzed by a family of intrinsic membrane proteins. GLUT2, a specific isoform of this family, is responsible for the process in the kidney.⁶⁾ Here we focus on changes in the activities of renal Na^+ /glucose cotransporter and Na^+/K^+ -ATPase as possible mechanisms for glucosuria caused by fluoride administration.

MATERIALS AND METHODS

Animals and Treatments Five-week-old male Wistar albino rats weighing 100–120 g were purchased from Japan SLC, Ltd. (Shizuoka, Japan). The animals were maintained on basal diet MF (purchased from Oriental Yeast Ind., Tokyo, Japan) and water *ad libitum* in a room maintained at 22°C (hygrometry: 50%). To stabilize glucose metabolism and ion mobilization, all the animals

were fed the basal diet MF for 1 week until the experiments were started. The rats were anesthetized with ether and killed by cardiac puncture at various times after the administration of fluoride (NaF 35 mg/kg, i.p.) or NaCl (48.7 mg/kg, i.p.) as the control. Calphostin C (200 nmol/kg) or KT5720 (200 nmol/kg) was injected i.p. into rats at 1 h after the fluoride administration (NaF 35 mg/kg, i.p.). All experiments using animals were performed according to the Guide for Care and Use of Laboratory Animals at the University of Shizuoka.

Preparation of BBM and BLM The BBM and BLM were prepared according to the method of Moritoris and Simon⁷⁾ with small modifications. The procedure entailed rapid decapsulization and removal of thin cortical slices of rat kidney in a chilled medium (buffer A) composed of 18 mM Tris-HCl, 300 mM mannitol, 0.1 mM phenyl methylsulfonyl fluoride and 5 mM EGTA (pH 7.4). The cortical slices (1 g) were homogenized using a Potter-Elvehjem teflon pestle homogenizer in 9 ml of buffer A. The homogenate was centrifuged at $48000 \times g$ for 30 min. The resulting pellet (P_1) was resuspended in buffer A, and Mg^{2+} precipitation (15 mmol/l) was performed at 0°C for 20 min by adding 15 ml of 25 mM MgCl_2 . The solution was shaken vigorously at 0, 10 and 20 min and then centrifuged for 15 min at $2500 \times g$. The pellet (P_2) was saved for BLM isolation while the supernatant was centrifuged at $48000 \times g$ for 30 min to obtain the crude BBM. The crude BBM was resuspended in buffer A, taken through the Mg^{2+} precipitation process again and washed free of Mg^{2+} in buffer B (100 mM mannitol, 20 mM Tris-Hepes, pH 7.4). This solution was centrifuged at $48000 \times g$ for 30 min to obtain the final BBM fraction, which was resuspended in buffer B.

* To whom correspondence should be addressed.

The BLM was isolated from P₂. This pellet was resuspended in buffer A and adjusted to 14.8 mM of Mg²⁺ with MgCl₂ solution. After 20 min of vigorous shaking at 0, 10 and 20 min, the solution was centrifuged at 2500 × *g* for 15 min. The pellet (P₃) was suspended in buffer A and centrifuged at 755 × *g* for 15 min. The supernatant was centrifuged at 48000 × *g* for 30 min and the pellet (P₄) was suspended in 20 mM Tris-Hepes (pH 7.4). The suspension was loaded on the top of a stepwise gradient solution which was overlaid using sucrose solutions of 50, 41 and 38%. The gradient solution was centrifuged at 88000 × *g* for 30 min. The top layer was collected, washed in 20 mM Tris-Hepes (pH 7.4) and centrifuged at 48000 × *g* for 30 min. The pellet (P₅) was suspended in buffer B as the final BLM fraction. The BBM and BLM fractions were stored in a freezer (−60 °C) until used.

Assay of Na⁺/Glucose Cotransporter Activity Renal Na⁺/glucose cotransporter activity was measured by the method of Malathi *et al.*⁸⁾ using renal BBM fractions. The cotransporter activity was assayed by incubating 50 μl of BBM vesicles (0.5 mg of protein) in a reaction mixture containing 0.2 mM D-[³H]glucose, 5 mM Tris-Hepes (pH 7.4) in the presence of 100 mM NaCl or 100 mM NaF. Aliquots (50 μl) were removed at appropriate time intervals, diluted into 1 ml of 150 mM NaCl, rapidly filtered through 0.22 μm millipore filters, and washed with 5 ml of cold 150 mM NaCl.

Assay of Na⁺/K⁺-ATPase Activity The enzyme activity was measured by the method of Nakao *et al.*⁹⁾ using renal BLM. The enzymic reaction system consisted of a final volume of 200 μl containing 2 mM ATP, 25 mM imidazol-HCl at pH 7.4, 3.5 mM MgCl₂, 140 mM NaCl, 14 mM KCl, 500 μM EDTA and 25 μl of BLM (protein 1 mg/ml). The enzyme was incubated at 37 °C for 15 min in the presence or absence of 2.5 mM ouabain.

TABLE I. Total Activities, Specific Activities, Enrichment Factors and Recoveries of Marker Enzymes Obtained for BBM and BLM Fractions from Rat Kidney Cortex

Fraction	BBM	BLM	Homogenate
Na ⁺ /K ⁺ -ATPase			
Total activity ^{a)}	1.73	3.23	46.72
Specific activity ^{b)}	0.21	1.03	0.08
Recovery ^{c)}	3.70	7.00	100
Enrichment ^{d)}	2.60	13.25	1
Alkaline phosphatase			
Total activity	30.00	2.57	198.8
Specific activity	3.67	0.82	0.34
Recovery	15.10	1.30	100
Enrichment	10.70	2.40	1
Succinate cytochrome c reductase			
Total activity	0.04	0.07	7.66
Specific activity	0.005	0.02	0.01
Recovery	0.56	0.80	100
Enrichment	0.40	1.60	1
Glucose-6-phosphatase			
Total activity	0.05	0.20	N.D.
Specific activity	0.006	0.006	N.D.

Values are averages obtained from four preparations. N.D.: not detectable.

a) Total activity is expressed as μmol/min. b) Specific activity is expressed as μmol/min/mg protein. c) Recovery is expressed as % of total homogenate activity.

d) Enrichment factor is expressed as ratio of specific activity in homogenate.

Assay of Alkaline Phosphatase Activity Alkaline phosphatase activity was measured by the method of Kramers and Robinson.¹⁰⁾

Assay of Succinate Cytochrome c Reductase Activity Succinate cytochrome c reductase activity was assayed according to the method of Tisdale.¹¹⁾

Assay of Glucose-6-phosphatase Activity Glucose-6-phosphatase activity was measured by the method of Swanson.¹²⁾

Measurements of Protein Protein was determined by the method of Schaffner and Weissman.¹³⁾

Materials Calphostin C and KT5720 were purchased from Kyowa Hakko Co. (Tokyo, Japan). ATP disodium salt was purchased from Yamasa Co. (Tokyo, Japan). Glucose-6-phosphate (disodium salt) was obtained from Sigma Chemical Co. (St. Louis, MO). D-[³H]Glucose was obtained from Dupont-New England Nuclear (Boston, MA). The other chemicals were purchased from Wako Pure Chemical Co. (Osaka, Japan).

Statistical Analysis The statistical significance of differences between the groups was determined by applying the Student's *t*-test.

RESULTS

Characterization of BBM and BLM Fractions Obtained from Rat Renal Cortical Tissues The enrichments for marker enzymes of these fractions are shown in Table I. The enrichment for BLM was evaluated by measurement of Na⁺/K⁺-ATPase activity, whereas that for BBM was estimated by the determination of alkaline phosphatase activity. To check contaminants, succinate cytochrome c reductase activity was used as a marker of mitochondria, and also, glucose-6-phosphatase activity was assayed as a marker of microsomes. The enrichment of BLM in the light layer (fraction A) was the highest as compared

Fig. 1. Suppressive Potency of Fluoride Administration (b) toward Na⁺/K⁺-ATPase Activity in Three Fractions A, B and C, Obtained by Final Purification of the Pellet (P₄) for Renal BLM as Compared to the Control (a)

The Na⁺/K⁺-ATPase activity was assayed according to Materials and Methods using these fractions prepared from kidneys 3 h after a fluoride dose (NaF 35 mg/kg, i.p.) to rats as compared to the control (NaCl 48 mg/kg, i.p.). Values are presented as averages ± S.E. of six rats. Vertical bars show S.E. Significant difference from control: a) *p* < 0.05, b) *p* < 0.01. P₄, the pellet (P₄); A, fraction A (top layer), B, fraction B (middle layer); C, fraction C (bottom layer).

to the other layers, and also, contamination of mitochondria in this fraction was the lowest (data not shown). From these results, the contamination of mitochondria and microsomes existed in a very small amount in these fractions.

In Vivo Effect of Fluoride on Renal Na^+/K^+ -ATPase Activity in BLM Changes in renal Na^+/K^+ -ATPase activity 3 h following the fluoride dose were examined as compared to the control. At first, a preliminary examination was performed using three fractions which were obtained from stepwise gradient centrifugation as the final purification step of BLM. The data are presented in Figs. 1a and b. The Na^+/K^+ -ATPase was confirmed to be concentrated in the upper layer (fraction A). Moreover, suppression of Na^+/K^+ -ATPase activity by the fluoride dose was strong in fraction A of the upper layer but was not observed in fraction B (middle layer). The inhibitory effects (*in vivo* and *in vitro*) of fluoride on the Mg^{2+} -ATPase

Fig. 2. Dose Response to Fluoride Administration of Na^+/K^+ -ATPase Activity in Renal Cortical BLM

The Na^+/K^+ -ATPase activity was measured by the method in the Materials and Methods section using renal cortical BLM 3 h after various doses of fluoride to rats. Values are presented as averages $\pm$ S.E. of six rats. Vertical bars show S.E. Significant difference from control: a) $p < 0.05$, b) $p < 0.02$.

Fig. 3. Changes in Na^+/K^+ -ATPase Activity in Renal Cortical BLM at Various Times after a Single Fluoride Dose to Rats

Rats were killed at various times after a single fluoride dose (NaF 35 mg/kg, i.p.). The Na^+/K^+ -ATPase activity was measured according to Materials and Methods. Values are presented as averages $\pm$ S.E. of six rats. Vertical bars show S.E. a) Significant difference from control: $p < 0.02$.

activity, that is basal activity of the Na^+/K^+ -ATPase, were not significant differences in the either fractions (A or B). As shown in Fig. 2, the suppression of renal Na^+/K^+ -ATPase activity was observed by increasing the doses of fluoride to rats, and half suppression of the enzyme activity was attained at about 30 mg/kg, i.p. as NaF . Changes in the renal Na^+/K^+ -ATPase activity after a single dose of fluoride (NaF 35 mg/kg, i.p.) are presented in Fig. 3. The maximal suppression of enzyme activity was revealed 3 h after the fluoride administration. These results were elucidated to be profoundly associated with glucosuria which was derived from fluoride exposure in animals.

In Vitro Effect of Fluoride on Renal Na^+/K^+ -ATPase Activity in BLM $\text{Na}^+/\text{glucose}$ cotransporter activity is operated such that it utilizes energy and the sodium movement of the sodium-pump as the driving force. Thus, the inhibitory effect of fluoride on Na^+/K^+ -ATPase activity in BLM was measured. The half inhibitory concentration of fluoride on renal Na^+/K^+ -ATPase activity was about 2 mM. The enzyme activity was markedly inhibited, by more than 80%, in the presence of 5 mM NaF (Fig. 4). This evidence is valid as a possible mechanism of glucosuria caused by acute animal fluorosis.

In Vitro Effect of Fluoride on Renal $\text{Na}^+/\text{Glucose}$ Cotransporter Activity in the BBM $\text{D-}[^3\text{H}]\text{Glucose}$ incorporation to the BBM vesicles reached a maximum 30 s to 1 min after the reaction was started (Fig. 5a). The inhibition of the $\text{Na}^+/\text{glucose}$ cotransporter activity was in a competitive manner with respect to glucose (see the insert in Fig. 5b). However, the half inhibitory concentration of D-glucose uptake by the fluoride ion was 100 mM as NaF . Accordingly, the $\text{Na}^+/\text{glucose}$ cotransporter was demonstrated to have extremely high resistance to the fluoride ion (Fig. 6). The extracellular sodium concentration of the reaction system was started at 100 mM, even when NaF was added to the incubation mixture. The glucose cotransporter activity in the BBM which was prepared in this experiment could be demonstrated to be inhibited

Fig. 4. Inhibitory Effect of Fluoride on Renal Na^+/K^+ -ATPase Activity in Cortical BLM

The Na^+/K^+ -ATPase activity was assayed at various concentrations of fluoride (0.2–10 mM) in the presence of a total concentration of 140 mM as a sodium ion. The incubation was performed according to Materials and Methods. Control value was 1.0 $\mu\text{mol}/\text{min}/\text{mg}$ protein as the initial rate of Na^+/K^+ -ATPase activity. Data present averages of triplicate experiments.

Fig. 5. Effects of Fluoride on the Overshoot Curve (a) and Glucose Dependency (b) of Na^+ /Glucose Cotransporter in Renal Cortical BBM

Uptake of D- ^3H glucose was performed as described in Materials and Methods. (a) The uptake was measured at various times in the presence of 100 mM sodium fluoride (●) or of 100 mM sodium chloride (○) as the control. (b) The initial rate of uptake with increasing concentrations (0–10 mM) of D-glucose was measured under the same conditions (100 mM NaF, ●; 100 mM NaCl, ○), and the insert is represented in a Lineweaver-Burk plot. Data present averages of duplicate experiments.

Fig. 6. Inhibitory Potency of Fluoride as Compared with Phlorizin on Na^+ /Glucose Cotransporter Activity in Renal Cortical BBM

The uptake of D- ^3H glucose was measured at the total concentration of 100 mM as sodium ion in the presence of various concentrations (0–100 mM) of fluoride as NaF. Data present the averages of duplicate experiments. ○, phlorizin; ●, fluoride.

TABLE II. *In Vitro* and *In Vivo* Effects of Fluoride on Alkaline Phosphatase Activity in Renal Cortical BBM

Treatment		Enzyme activity (units/mg protein)	Relative value (% of control)
<i>In vivo</i> ^{a)}	<i>In vitro</i>		
Control	None	3.68 ± 0.32	100
	100 mM NaCl	5.01 ± 0.12	136 ^{b)}
	100 mM NaF	4.91 ± 0.14	133 ^{b,c)}
Fluoride-treated	None	3.75 ± 0.31	102

a) Rats were killed 3 h after a single dose of fluoride (NaF 35 mg/kg, i.p.) or of chloride (NaCl 48 mg/kg, i.p.) as the control. Values are averages obtained from four rats or preparations ± S.E. b) Significant difference from control: $p < 0.02$. c) Not a significant difference from the incubation in the presence of 100 mM sodium chloride: $p < 0.05$.

by the fluoride ion in the presence of a high sodium ion concentration, and it was also strongly sensitive to phlorizin (IC_{50} : 1 μM) under the same conditions.

Moreover, in order to search for any damage of BBM by

Fig. 7. Changes in Renal Na^+ /Glucose Cotransporter Activity after a Single Dose of Fluoride to Rats

The uptake of D- ^3H glucose was measured using BBM vesicles obtained from the renal cortex 3 h after a fluoride dose (NaF 35 mg/kg, i.p.) to rats or a chloride dose (NaCl 48 mg/kg, i.p.) as the control. Values are presented as averages ± S.E. of six rats. Vertical bars show S.E. a) Not a significant difference from control: $p > 0.05$. □, control; ■, fluoride-treated.

the fluoride ion, the *in vitro* and *in vivo* effects of fluoride on alkaline phosphatase, which is localized in BBM, were examined, and the results are shown in Table II. The enzyme activity was not significantly changed by the fluoride treatments (*in vitro* and *in vivo*).

***In Vivo* Effect of Fluoride on Renal Na^+ /Glucose Cotransporter Activity in the BBM** Subsequently, changes in Na^+ /glucose cotransporter activity in the kidney were investigated by the fluoride dose to rats. The Na^+ /glucose cotransporter activity using BBM vesicles was measured at various times after the incubations were started. At the same time, measurements were performed in the control rats. The pattern of glucose uptake in the fluoride-administered rats was similar to that in the control and not significantly different (Fig. 7).

TABLE III. Role of PKC and/or PKA on the Suppression of Renal Na^+/K^+ -ATPase Activity Caused by Fluoride Administration

Treatment	Enzyme activity (units/mg protein)	Relative activity (% of control)	Retardation by inhibitor (%)
Control	1.03 ± 0.04	100	—
Fluoride (F)	0.42 ± 0.10	40.8 ^{a)}	—
F + calphostin C	0.63 ± 0.08	61.2 ^{a)}	34.5 ^{b)}
F + KT5720	0.44 ± 0.11	42.7 ^{a)}	3.2

Calphostin C (200 nmol/kg, i.p.) or KT5720 (200 nmol/kg, i.p.) was administered to rats 1 h after a single dose of fluoride (NaF 35 mg/kg, i.p.). Values are averages obtained from four rats ± S.E. a) Significant difference from control: $p < 0.05$. b) Significant difference from fluoride treatment alone: $p < 0.05$.

The Contribution of Protein Kinases to the Suppression of Renal Na^+/K^+ -ATPase Activity by Fluoride Administration Protein kinase A (PKA) and/or protein kinase C (PKC) were suggested to contribute to the suppression of the renal Na^+/K^+ -ATPase activity as a secondary action of the fluoride dose.

Thus, the *in vivo* effects of calphostin C and KT5720 on renal Na^+/K^+ -ATPase were examined as shown in Table III. Calphostin C treatment significantly alleviated the suppression of enzyme activity by the fluoride dose, whereas KT5720 treatment did not. This finding demonstrates that PKC is involved in the suppression of renal Na^+/K^+ -ATPase activity by fluoride administration.

DISCUSSION

In this study, we have investigated a possible mechanism for glucosuria as the result of experimental fluorosis.^{1,2)} Renal cortical D-glucose transporter is responsible for the reabsorption of the bulk of the filtered load of D-glucose from the urine. The renal cortical transporter has been demonstrated to exhibit a mutually competitive interaction between phlorizin and glucose, usually associated with renal glucose reabsorption.¹⁴⁾ It is known that the transporter is associated with the high-affinity sodium-dependent phlorizin binding site commonly found in renal BBM preparation.¹⁵⁾ Accordingly, the renal $\text{Na}^+/\text{glucose}$ cotransporter activity is observed as an overshoot of sodium-dependent glucose uptake using BBM vesicles. In intact renal tubular epithelial cells, sodium ion movement to the blood stream is performed by Na^+/K^+ -ATPase as a Na^+ -pump localized in the BLM site. On the other hand, the enrichment of BLM and BBM fractions prepared according to Molitoris and Simon,⁷⁾ respectively, was satisfactory.

Thus, in order to understand the mechanism of glucosuria in acute fluorosis, we studied the *in vivo* and *in vitro* effects of fluoride on the activities of sodium-dependent D-glucose cotransporter ($\text{Na}^+/\text{glucose}$ cotransporter) and Na^+/K^+ -ATPase. To determine the effect of fluoride on the driving force for the $\text{Na}^+/\text{glucose}$ cotransporter, the BLM Na^+/K^+ -ATPase activity was measured using fluoride-treated rats (*in vivo*) and in the presence or absence of fluoride using control rats (*in vitro*). The renal BBM $\text{Na}^+/\text{glucose}$ cotransporter activity of rats was found to be insensitive (IC_{50} : 100 mM) for fluoride ions despite

the competitive inhibitory mode, whereas the BLM Na^+/K^+ -ATPase was 50-times more sensitive (IC_{50} : 2 mM) to the BBM $\text{Na}^+/\text{glucose}$ cotransporter. Moreover, the activity of the $\text{Na}^+/\text{glucose}$ cotransporter in our BBM preparation was strongly inhibited by a very low concentration of phlorizin. From the results, this BBM glucose cotransporter was demonstrated to be dependent on sodium.

On the other hand, since we observed glucosuria after fluoride administration (NaF 35 mg/kg, i.p.) to rats,²⁾ the $\text{Na}^+/\text{glucose}$ cotransporter activity and/or Na^+/K^+ -ATPase activity as driving force of the transporter were suggested to be remarkably suppressed after the fluoride dose. The Na^+/K^+ -ATPase activity was strongly suppressed by the fluoride dose, but the corresponding $\text{Na}^+/\text{glucose}$ cotransporter activity was unchanged. The renal cortical tissues from the pre-treated animals underwent extensive fractionation with multiple washings, and even if the transporter were inhibited *in vivo*, the inhibition would be relieved by the preparative steps. The inhibition was a $K_{1/2}$ of 100 mM and the amount of NaF injected, if evenly distributed in the animal, is suggested to be about 1 mM. Moreover, the result (as shown in Fig. 4) demonstrated that the regulation system of the transporter (its balance of the synthesis and the cleavage) was not affected or only slightly affected by the fluoride administration.

Interestingly, it was indicated that some isozymes of the renal Na^+/K^+ -ATPase occurred from the finding that the inhibitory effect of fluoride on the Na^+/K^+ -ATPase in fraction A was much stronger than in fraction B (Figs. 1a and b). On the other hand, we described in previous papers that renal PKA contributes to the elevation of renal glucose-6-phosphatase activity by fluoride administration¹⁶⁾ and that PKC operates as a down regulator at the restoration period of the elevation.^{17,18)} In this experiment, the stimulation of PKC¹⁹⁾ was implicated in the suppressive mechanism of the renal Na^+/K^+ -ATPase activity due to a secondary action of fluoride administration using calphostin C²⁰⁾ as a strongly specific antagonist of PKC. However, no implication of PKA could be demonstrated by the treatment of KT5720²¹⁾ as a specific antagonist of PKA after a fluoride dose to rats.

In conclusion, the glucosuria caused by fluorosis is shown to be due to the suppression of renal Na^+/K^+ -ATPase activity as the driving force by the fluoride administration rather than to a suppression of the corresponding $\text{Na}^+/\text{glucose}$ cotransporter activity. However, other reducing mechanisms for the driving force are as follows: A diminished Na^+ gradient across the BBM might result in reduced driving force for reclaiming glucose from the glomerular filtrate. In addition, fluoride is known to stimulate GTP-binding protein,^{15,22)} which regulates the opening of K^+ -channels in various animal cells.²³⁾ Thus, driving force might be reduced by K^+ leaks if the K^+ -channels were opened by the fluoride dose.

In any event, renal Na^+/K^+ -ATPase activity was demonstrated in this study to be strongly suppressed, prior to the glucosuria as the result of a fluoride dose (NaF 35 mg/kg, i.p.).²⁴⁾

REFERENCES

- 1) J. M. Taylor, J. K. Scott, E. A. Maynard, F. A. Smith, H. C. Hodge, *Toxicol. Appl. Pharmacol.*, **3**, 278 (1961).
- 2) Y. Suketa, M. Sato, *Toxicol. Appl. Pharmacol.*, **52**, 386 (1980).
- 3) R. C. Nordlie, "Control of Glycogen Metabolism," ed. by W. J. Whelan, Academic Press, New York, 1968, pp. 153—159.
- 4) M. E. Blank, F. Bode, K. Bauman, D. F. Diedrich, *Am. J. Physiol.*, **257**, C385 (1989).
- 5) K. R. H. Repke, R. Schön, *Biochim. Biophys. Acta*, **1154**, 1 (1993).
- 6) S. T. Baldwin, *Biochim. Biophys. Acta*, **1154**, 17 (1993).
- 7) B. A. Molitoris, F. G. Simon, *J. Memb. Biol.*, **83**, 207 (1985).
- 8) P. Malathi, H. Preiser, P. Fairclough, P. Mallett, R. K. Crane, *Biochim. Biophys. Acta*, **554**, 259 (1979).
- 9) T. Nakao, M. Nakao, N. Mizuno, Y. Komatsu, M. Fujita, *J. Biochem. (Tokyo)*, **72**, 609 (1973).
- 10) M. T. C. Kramer, G. B. Robinson, *Eur. J. Biochem.*, **99**, 345 (1979).
- 11) H. D. Tisdale, *Methods Enzymol.*, **10**, 213 (1967).
- 12) M. A. Swanson, *Methods Enzymol.*, **2**, 541 (1955).
- 13) W. Schaffner, C. Weissmann, *Anal. Biochem.*, **56**, 502 (1973).
- 14) R. Kinne, "Renal Biochemistry—Cell, Membranes, Molecules," ed. by R. K. H. Kinne, Elsevier, Amsterdam, 1985, pp. 99—141.
- 15) J. T. Lin, K. Szwarc, R. Kinne, C. Y. Jung, *Biochim. Biophys. Acta*, **777**, 201 (1984).
- 16) Y. Suketa, M. Yamada, J. Hasegawa, Y. Asao, *Mol. Pharmacol.*, **22**, 116 (1982).
- 17) Y. Suketa, Y. Sakai, "Calcium Protein Signaling," ed. by H. Hidaka, E. Carafoli, A. R. Means, T. Tanaka, Plenum Publ. Corp., New York, 1989, pp. 515—524.
- 18) Y. Suketa, K. Suzuki, T. Taki, M. Yamamoto, Y. Ibuki, T. Imagawa, T. Sakurai, Abstract of Papers, Eighth International Symposium on Calcium-Binding Protein and Calcium Function in Health and Disease, Davos, Switzerland, August 1992, Py 16.
- 19) A. Bertorello, A. Aperia, *Am. J. Physiol.*, **256**, F370 (1989).
- 20) E. Kobayashi, H. Nakano, M. Morimoto, T. Tamaoki, *Biochem. Biophys. Res. Commun.*, **159**, 548 (1989).
- 21) H. Kase, K. Iwasaki, S. Nakanishi, Y. Matsuda, K. Yamada, M. Takahashi, C. Murakata, A. Sato, M. Kaneko, *Biochem. Biophys. Res. Commun.*, **142**, 436 (1987).
- 22) P. C. Sternwise, A. G. Gilman, *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 4888 (1982).
- 23) H. Kume, M. P. Graziano, M. I. Kotlikoff, *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 11051 (1992).
- 24) Y. Suketa, Y. Asao, Y. Kanamoto, T. Sakashita, S. Okada, *Toxicol. Appl. Pharmacol.*, **80**, 199 (1985).