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A Possible Mechanism for Elevation of Glucose-6-Phosphatase Activity in Kidney and Liver of Fluoride-Treated Rats

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SUMMARY

Glucose-6-phosphatase (EC 3.1.3.9) activity in kidney and liver was found to be markedly elevated 3 hr after a single large dose of fluoride (NaF, 35 mg/kg, i.p.). The increases in renal and hepatic glucose-6-phosphatase activity were completely suppressed by adrenalectomy. Moreover, the increments were markedly suppressed by injection of dibenamine as an *alpha*-adrenergic blocker or by injection of propranolol as a *beta*-adrenergic blocker.

INTRODUCTION

Increased urinary sugar excretion in rats after administration of a near-lethal fluoride dose (NaF, 20–30 mg/kg, i.v.) has been reported (1). Fluoride administration was observed to cause numerous changes in the concentrations of glycolytic and citric acid intermediates in rat liver, indicating enzyme inhibition by fluoride, but not to change significantly the concentrations of metabolic intermediates with the exception of renal glucose (2). Glucose liberation from gluconeogenic tissues of liver and kidney has been believed to be controlled through glucose-6-phosphatase and through opposing actions of glucose phosphorylation via hexokinase and/or glucokinase (3). On the other hand, glucose-6-phosphatase activity in kidney and liver is known to be controlled by hormonal regulation of thyroxine released from the thyroid gland and of glucocorticoid hormone from the cortex of the adrenal gland (3).

The present study was designed to elucidate the biochemical and physiological mechanisms for changes in glucose-6-phosphatase activity in kidney and liver after administration of an acute fluoride dose (NaF, 35 mg/kg, i.p.) to intact and adrenalectomized rats.

MATERIALS AND METHODS

Male Wistar albino rats weighing 100–110 g were used in the study. All of the animals were conditioned for a minimum of 1 week to basal diet MF (purchased from Oriental Yeast Industries, Tokyo, Japan) and water ad libitum in a room maintained at 22°. All animals were fasted for 24 hr before the experiments to minimize the effects of glucose absorption from the bowels and to stabilize the urinary excretion of glucose. The rats were killed at various times after a single i.p. injection of NaF (35 mg/kg) or NaCl (48.7 mg/kg).

Injection of dibenamine and propranolol. Dibenamine (4) and/or propranolol (5) were injected s.c. 10 min after the fluoride administration.

Preparation of microsomes. Microsomes were prepared according to the method of Jørgensen (6). At the time of sacrifice, the rats were anesthetized with ether and killed by cardiac puncture. The kidneys and livers were removed and the tissues (1 g) were immediately homogenized in a Potter-Elvehjem Teflon-glass homogenizer with 5 ml of ice-cold 0.25 M sucrose-0.03 M histidine buffer (pH 7.2). The heavy microsomal fraction was obtained by centrifugation (25,300 × *g* for 30 min) of the supernatant after sedimentation of the mitochondria at 10,800 × *g* for 30 min. Preparations of the heavy microsomal fraction (1 mg of protein per milliliter of 0.25 M sucrose-0.03 M histidine buffer, pH 7.2) were stored in a freezer (−20°).

Membrane preparation. The plasma membrane fraction was prepared according to the method of Forte *et al.* (7). Slices of kidney or liver were homogenized in 18 mM Tris buffer (pH 8.0) containing 25 mM NaCl and 0.5 mM $CaCl_2$ by 35–40 strokes of a Dounce tight-pestle homogenizer. Phase microscopy was used to evaluate the effectiveness of cell disruption as well as all subsequent fractionation procedures. After a 5-min centrifugation at 200 × *g*, the plasma membrane fraction was obtained as the pellet from differential centrifugation of the sediment (600 × *g* for 12 min). Preparations of the plasma membrane fraction (10 ml/g of tissue) suspended in 30 mM Tris-HCl (pH 8.0) were stored in a freezer (−20°).

Analyses. Phosphodiesterase activity was measured by the liberation of *p*-nitrophenol from bis(*p*-nitrophenyl)phosphate according to the method of Sinsheimer and Koerner (8). The enzyme reaction was conducted for 30 min at 37° in an incubation mixture containing 30 mM Tris-HCl (pH 8.0), 3.7 mM $MgCl_2$, and 3 mM bis(*p*-nitro-

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phenyl)phosphate. Glucose-6-phosphatase activity was determined according to the method of Swanson (9). Cyclic AMP concentration was assayed in tissues and serum according to the radioimmunoassay method of Honma *et al.* (10), using a cyclic AMP assay kit which was purchased from Yamasa Shoyu Company (Tokyo, Japan). Cyclic AMP phosphodiesterase activity was determined according to the method of Butcher and Sutherland (11). This reaction mixture contained 0.36 μmoles of cyclic AMP, 1.8 μmoles of $MgSO_4$, and 36 μmoles of Tris-HCl buffer (pH 7.5), with 0.1 ml of renal or hepatic plasma membrane fraction (10 ml/g of tissue) as the phosphodiesterase sample being tested in a total volume of 0.9 ml. This mixture was incubated at 30° for 30 min. After the first 20 min of incubation, 0.1 ml of a *Crotalus atrox* venom solution was added containing 0.1 mg of venom in 10 mM Tris-HCl buffer (pH 7.5). The reaction mixture was terminated by the addition of 0.1 ml of cold 55% trichloroacetic acid. 17-Hydroxycorticosterone was estimated in urine and serum by the method of Porter and Silber (12). Protein was measured by the method of Lowry *et al.* (13).

Materials. C. atrox venom was a gift in the form of lyophilized crystals from Dr. Kyozo Hayashi, Department of Biochemistry, Faculty of Pharmaceutical Sciences, Kyoto University. Glucose-6-phosphate (disodium salt), bis(*p*-nitrophenyl)phosphate (sodium salt), and (−)-epinephrine were obtained from Sigma Chemical Company (St. Louis, Mo.). Dibenamine (hydrochloride) and DL-propranolol (hydrochloride) were products of Nakarai Chemicals, Ltd. (Tokyo, Japan). 17-Hydroxycorticosterone was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan).

Statistical significance of the data (means ± standard error) was analyzed by Student's *t*-test.

RESULTS

Effect of fluoride administration on renal and hepatic glucose-6-phosphatase activity. The response of glucose-6-phosphatase activity in kidney and liver was examined 3 hr after administration of various doses of fluoride (NaF, 10–35 mg/kg). Enzyme activity in both organs was not significantly changed in the dose lower range (NaF, 0–20 mg/kg, i.p.) (Fig. 1A).

Administration of an acute dose of fluoride (NaF, 35 mg/kg, i.p.) was found to cause elevation of renal and hepatic glucose-6-phosphatase activity. To determine the mechanism responsible for the elevation of renal and hepatic glucose-6-phosphatase activity caused by fluoride administration, changes in the enzyme activity in kidney with time after the injection of a single dose of fluoride were compared with those in liver. Renal and hepatic enzyme activity was maximal 3 hr after the administration of fluoride. Enzyme activity in kidney rapidly returned to control values, whereas in liver at 6 hr it was 2.6 times the control values shown in Fig. 1B.

Effects of fluoride administration on cyclic AMP and phosphodiesterase activity in kidney and liver. Cyclic AMP is known to play an important role in developmental enzyme formation. Greengard (14) has reported that glucose-6-phosphatase activity is increased by cyclic AMP. Moreover, adenylate cyclase activity is known to be stimulated by the addition of fluoride to an incubation mixture of broken mammalian cells (15). Thus, elevations of renal and hepatic glucose-6-phosphatase activity in fluoride-treated rats were possibly due to increased levels of cyclic AMP in kidney and liver. Cyclic AMP concentrations in the kidneys and livers of the fluoride-treated rats were examined. Cyclic AMP in the kidney and liver showed maximal increases of about 2 and 4 times the respective control values 1 hr after fluoride administration (Fig. 2). Moreover, cyclic AMP levels in serum were increased to about 13 times the control value at that time (Fig. 2).

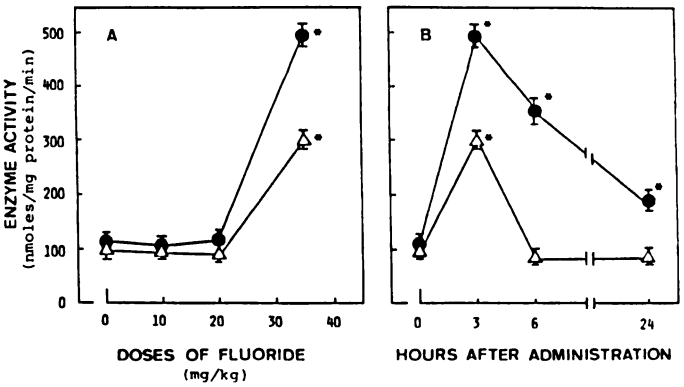

FIG. 1. *Response of hepatic and renal glucose-6-phosphatase activity to fluoride dose (A) and changes in the enzyme activity after the fluoride dose (B)*

The rats were killed 3 hr after various fluoride doses as shown in A. Changes in the enzyme activity were determined at various times after a single fluoride injection (NaF, 35 mg/kg, i.p.). Standard errors are indicated by vertical lines. *Asterisks* indicate significant difference from control ($p < 0.02$). ●, Liver; △, kidney.

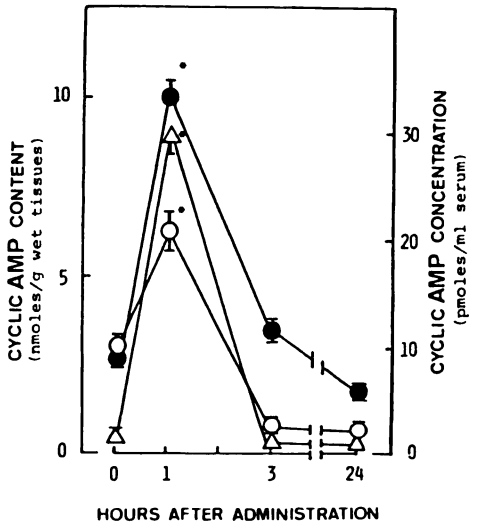

FIG. 2. *Effect of fluoride administration on cyclic AMP content of liver and kidney*

Changes in the cyclic AMP content of liver and kidney were determined and compared with serum cyclic AMP concentrations at various times after a single fluoride injection (NaF, 35 mg/kg, i.p.). Values are averages obtained from six rats. Standard errors are indicated by vertical lines. *Asterisks* indicate significant difference from control ($p < 0.02$). ●, Liver, ○ kidney; △, serum.

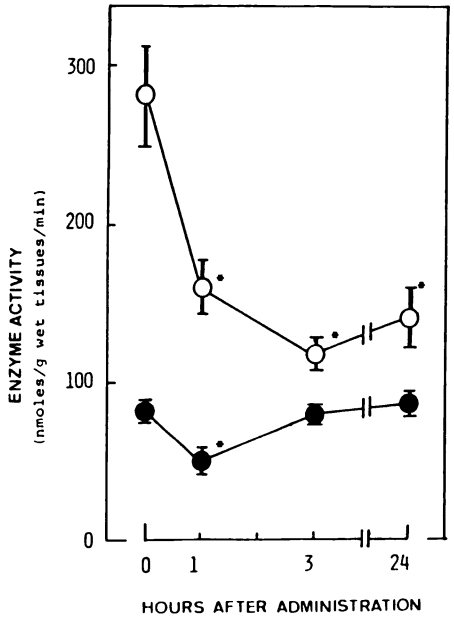

FIG. 3. *Effect of fluoride administration on hepatic and renal phosphodiesterase activity*

Changes in the enzyme activity were determined at various times after a single fluoride injection (NaF, 35 mg/kg, i.p.). Enzyme activity is expressed as nanomoles of *p*-nitrophenol liberated per gram (wet weight) per minute. Values are averages obtained from six rats. *Asterisks* indicate significant difference from control ($p < 0.02$). ●, Liver; ○, kidney.

Fluoride is a potent inhibitor of phosphodiesterase activity (16). Cyclic AMP formation has been shown to be stimulated by inhibition of cyclic nucleotide phosphodiesterase activity (11). Thus, the elevation of cyclic AMP in fluoride-treated rats was thought to result from inhibition of cyclic nucleotide phosphodiesterase activity by fluoride administration. Changes in phosphodiesterase activity in fluoride-treated rats were examined as shown in Fig. 3. Nonspecific phosphodiesterase activity in kidney exhibited a maximal decrease 3 hr after administration of fluoride (NaF, 35 mg/kg, i.p.), whereas for liver there was a maximal decrease 1 hr after the injection. Thus, changes in renal and hepatic cyclic AMP phosphodiesterase activity were determined in fluoride-treated rats. However, cyclic AMP phosphodiesterase activity in liver was slightly elevated by fluoride, but that in the kidney was not significantly changed.

TABLE 2

Effect of fluoride administration on serum 17-hydroxycorticosterone

The data are means obtained from six rats ± standard error

	Time after administration of fluoride			
	0 Hr	1 Hr	3 Hr	24 Hr
Control (C)	<0.98			
Fluoride-treated (F)	<0.98 ±	11.64 ± 4.32	7.02 ± 2.74	<0.98 ±
Ratio F:C		>11.9	>7.2	

Relationship between elevation of renal and hepatic glucose-6-phosphatase activity and adrenal function. From the above experiment, the elevation of glucose-6-phosphatase activity in fluoride-treated rats was not correlated temporally with the increase in cyclic AMP concentration. Moreover, the data in Fig. 1 suggested that the elevation of glucose-6-phosphatase activity by fluoride might be due to stimulation of adrenal function and/or thyroid function by a secondary action of fluoride. Thus, it was thought that the elevation of glucose-6-phosphatase activity in kidney and liver would be suppressed by adrenalectomy. The comparative effect of fluoride administration on renal and hepatic glucose-6-phosphatase activity was examined using intact and adrenalectomized rats as shown in Table 1. The enhancement of glucose-6-phosphate activity in the kidney and liver by the fluoride dose was completely suppressed by adrenalectomy. From this finding, enhancement of glucose-6-phosphatase activity by fluoride was demonstrated to require the presence of adrenal function, whereas direct inhibition of cyclic AMP phosphodiesterase activity as a primary action by fluoride was eliminated from the possible mechanisms for enhancement of glucose-6-phosphatase activity in fluoride-treated rats.

In order to reconfirm stimulation of adrenal function, changes in the concentrations of glucocorticoid hormone in serum and urine were examined to assess adrenocortical function. 17-Hydroxycorticosterone as a glucocorticoid hormone in serum reached maximal levels 1 hr after fluoride administration (NaF, 35 mg/kg, i.p.), as shown in Table 2, and the elevation occurred prior to the in-

TABLE 1

Effect of adrenalectomy on elevation of renal and hepatic glucose-6-phosphatase activity induced by fluoride administration

The animals were killed 3 hr after the administration of NaF (35 mg/kg, i.p.) or NaCl (48.7 mg/kg, i.p.) (control). Values are means obtained from six rats ± standard error.

	Liver			Kidney		
	Enzyme activity[a]	Relative value (%)	Suppression by adrenalectomy (%)	Enzyme activity[a]	Relative value (%)	Suppression by adrenalectomy) (%)
Control (NaCl)						
Intact	117.1 ± 5.5	100	0	100.7 ± 0.6	100	0
Adrenalectomized	113.3 ± 12.1	96.8	3.2	60.7 ± 6.7	60.3	39.7[b]
Fluoride (NaF)						
Intact	497.4 ± 16.8	425	0	300.3 ± 13.7	298	0
Adrenalectomized	115.3 ± 16.7	98.5	101[c]	46.0 ± 1.2	45.7	128[c]

[a] Enzyme activity is expressed as nanomoles of P_i liberated per milligram of protein per minute.

[b] Significant suppression by adrenalectomy ($p < 0.02$).

[c] Significant suppression by adrenalectomy ($p < 0.005$).

TABLE 3

Effect of adrenalectomy on elevation of renal and hepatic cyclic AMP content by fluoride administration

The animals were killed 1 hr after the administration of NaF (35 mg/kg, i.p.) or NaCl (48.7 mg/kg, i.p.) (control). Values are means obtained from six rats ± standard error.

	Liver			Kidney			Serum		
	Cyclic AMP level	Relative value	Suppression	Cyclic AMP level	Relative value	Suppression	Cyclic AMP level	Relative value	Suppression
	nmoles/g	%	%	*nmoles/g*	%	%	*pmoles/ml*	%	%
Control (NaCl)									
Intact	2.95 ± 0.25	100	0	2.75 ± 0.15	100	0	1.00 ± 0.33	100	0
Adrenalectomized	3.66 ± 0.10	124	−24.0	2.86 ± 0.08	104	−4.0	1.05 ± 0.13	105	−5.0
Fluoride-treated									
Intact	6.00 ± 0.35	203	0	10.0 ± 0.30	364	0	10.0 ± 0.33	1000	0
Adrenalectomized	3.66 ± 0.10	124	76.7[a]	2.33 ± 0.15	84.7	106[a]	1.84 ± 0.04	184	90.7[a]

[a] Significant suppression by adrenalectomy ($p < 0.005$).

crease in glucose-6-phosphatase activity in kidney and liver (Fig. 1; Table 2). Urinary excretion of 17-hydroxycorticosterone gradually increased after the fluoride dose. Stimulation of adrenocortical function was demonstrated in the fluoride-treated rats by the above experiment.

Since elevation of renal and hepatic cyclic AMP concentrations in fluoride-treated rats was possibly due to a stimulation of adrenal function, the effect of adrenalectomy on the elevation of cyclic AMP levels in kidney and liver was determined. The elevation of renal and hepatic cyclic AMP concentrations caused by fluoride administration was almost completely suppressed by adrenalectomy, as shown in Table 3.

Epinephrine is principally secreted from the secretory cells of adrenal medulla and also as a neurotransmitter. Renal and hepatic glucose-6-phosphatase activity has been reported to be stimulated by epinephrine (14). The effects of epinephrine can be prevented with dibenamine, an *alpha*-adrenergic blocking agent (4), and with propranolol, a *beta*-adrenergic blocking agent (5). Thus, the effect of propranolol and/or dibenamine on elevation of renal and hepatic glucose-6-phosphatase activity in fluoride-treated rats was examined to assess the adrenomedullary function. The increases in glucose-6-phosphatase activity in kidney and liver caused by the fluoride injection (NaF, 35 mg/kg, i.p.) were significantly suppressed by propranolol and/or dibenamine, as shown in Table 4. The results indicated that adrenomedullary function was stimulated by the fluoride dose.

Experiments using adrenalectomized rats were then performed to determine whether the elevations of renal and hepatic glucose-6-phosphatase activity caused by fluoride would be enhanced with epinephrine and/or hydrocortisone. Fluoride-induced elevations of hepatic and renal glucose-6-phosphatase activity were enhanced by injections of epinephrine and/or hydrocortisone, but these effects were very slight. Moreover, the response to epinephrine and/or hydrocortisone of renal and hepatic glucose-6-phosphatase activity in fluoride-treated rats was suppressed by adrenalectomy.

Renal and hepatic cyclic AMP phosphodiesterase activity in fluoride-treated rats was significantly increased by epinephrine and/or hydrocortisone.

DISCUSSION

In these experiments, elevation of renal and hepatic glucose-6-phosphatase activity after a near-lethal fluoride dose (NaF, 35 mg/kg, i.p.) was demonstrated and correlated with stimulation of adrenal function.

Augmentation of cyclic AMP content and a decrease

TABLE 4

Effects of propranolol and dibenamine on elevation of renal and hepatic glucose-6-phosphatase activity by fluoride administration

Propranolol (P) (1.0 mg/kg) or dibenamine (D) (2.0 mg/kg) was injected s.c. two times, immediately after and 30 min after the administration of NaF (35 mg/kg, i.p.) or NaCl (48.7 mg/kg, i.p.) (control). The rats were killed 3 hr after the fluoride dose. Values are means obtained from four rats ± standard error.

	Liver			Kidney		
	Enzyme activity[a]	Relative value (%)	Suppression by antagonist (%)	Enzyme activity[a]	Relative value (%)	Suppression by antagonist (%)
Control	139.0 ± 17.4	100	0	114.0 ± 8.0	100	0
NaCl + P	128.5 ± 7.9	92.4	7.6	91.4 ± 3.6	80.2	19.8[b]
NaCl + D	104.0 ± 3.2	74.8	25.2[c]	85.5 ± 5.0	74.1	25.9[c]
NaCl + P + D	90.2 ± 4.6	64.9	35.1[c]	75.2 ± 4.2	66.0	34.0[c]
Fluoride-treated	475.2 ± 42.3	342	0	300.4 ± 18.2	264	0
NaF + P	257.6 ± 4.0	185	64.7[c]	171.3 ± 9.2	150	69.3[c]
NaF + D	218.4 ± 15.5	157	76.4[c]	189.9 ± 5.9	167	59.3[c]
NaF + P + D	125.4 ± 6.8	90.2	104[c]	121.2 ± 9.9	106	96.1[c]

[a] Enzyme activity is expressed as nanomoles of P_i liberated per milligram of protein per minute.

[b] Significant suppression by propranolol and/or dibenamine ($p < 0.05$).

[c] Significant suppression by propranolol or dibenamine ($p < 0.02$).

in phosphodiesterase activity were initially thought to occur prior to the elevation of glucose-6-phosphatase activity in kidney and liver of fluoride-treated rats. However, this possibility was eliminated from the possible mechanisms for the elevation of glucose-6-phosphatase activity by the findings that the elevation was completely suppressed by adrenalectomy and that hepatic cyclic AMP phosphodiesterase activity is only slightly elevated and renal cyclic AMP phosphodiesterase activity not at all, by fluoride treatment. Accordingly, the augmentation of cyclic AMP in kidney and liver and fluoride-treated rats may be due to a secondary reaction (e.g., elevations of adrenal hormones) rather than to inhibition of phosphodiesterase activity.

In contrast, cyclic AMP, epinephrine, and glucocorticoid hormones are known to stimulate glucose-6-phosphatase activity (3, 14). In this experiment, elevation of glucose-6-phosphatase activity in kidney and liver of fluoride-treated rats was found to be markedly suppressed by dibenamine, an *alpha*-blocker, and by propranolol, a *beta*-blocker of epinephrine. Moreover, elevation of renal and hepatic glucose-6-phosphatase activity was completely suppressed by concomitant dose of dibenamine and propranolol. From these results, increased renal and hepatic glucose-6-phosphatase activity after fluoride administration is attributed to stimulation of adrenal function.

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