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Effect of cisplatin on H^+ transport by H^+-ATPase and Na^+/H^+ exchanger in rat renal brush-border membrane

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Abstract

The effect of the potent anticancer drug cisplatin, cis-diamminedichloroplatinum (II) (CDDP), on H^+-ATPase and Na^+/H^+ exchanger in rat renal brush-border membrane was examined. To measure H^+ transport by vacuolar H^+-ATPase in renal brush-border membrane vesicles, we employed a detergent-dilution procedure, which can reorientate the catalytic domain of H^+-ATPase from an inward-facing configuration to outward-facing one. ATP-driven H^+ pump activity decreased markedly in brush-border membrane prepared from rats two days after CDDP administration (5 mg/kg, i.p.). In addition, N-ethylmaleimide and bafilomycin A_1 (inhibitors of vacuolar H^+-ATPase)-sensitive ATPase activity also decreased in these rats. The decrease in ATP-driven H^+ pump activity was observed even at day 7 after the administration of CDDP. Suppression of ATP-driven H^+ pump activity was also observed when brush-border membrane vesicles prepared from normal rats were pretreated with CDDP in vitro. In contrast with H^+-ATPase, the activity of Na^+/H^+ exchanger, which was determined by measuring acridine orange fluorescence quenching, was not affected by the administration of CDDP. These results provide new insights into CDDP-induced renal tubular dysfunctions, especially such as proximal tubular acidosis and proteinuria.

Keywords: Cis-diamminedichloroplatinum (II); Vacuolar H^+-ATPase; Na^+/H^+ exchanger; Renal brush-border membrane; H^+ secretion

Introduction

Cisplatin, cis-diamminedichloroplatinum (II) (CDDP), is the most effective drug against a variety of solid tumors, but the clinical use of the drug is limited because of its nephrotoxicity. This nephrotoxicity is characterized by dysfunction of renal proximal tubular cells. In particular, CDDP interferes with water, nutrient and electrolyte transport in renal tubular cells, such as Na^+/glucose cotransport and Na^+/phosphate cotransport (1,2).

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It has been reported that administration of CDDP caused renal tubular acidosis. Haupt et al. (3) observed that CDDP treatment developed a hyperchloremic metabolic acidosis as a result of bicarbonate loss. Furthermore, in rats exposed to CDDP, a metabolic acidosis was observed with the defect of proximal tubular H^+ secretion and reduction of bicarbonate reabsorption (4). Field et al. (5) found that sodium entry into proximal tubular cells was reduced by CDDP, which may be due to a reduction of apical Na^+/H^+ exchange. Thus, it has been pointed out that the impairment of Na^+/H^+ exchange in renal proximal brush-border membrane is possibly involved in metabolic acidosis induced by CDDP administration. However, the direct evidence showing impairment of Na^+/H^+ exchange caused by CDDP has been, until now, lacking.

H^+ extrusion into the lumen is mediated not only by a Na^+/H^+ exchanger but also by a vacuolar type proton-translocating adenosine triphosphatase (H^+-ATPase) in the brush-border membrane (6). Therefore, acidosis induced by CDDP may result from the decrease in the activity of Na^+/H^+ exchanger and/or H^+-ATPase. Vacuolar H^+-ATPase is also responsible for acidifying various intracellular compartments, including clathrin-coated vesicles, endosomes and Golgi-derived vesicles (7). This acidification is involved in a variety of important cellular functions including the dissociation of ligands and receptors after receptor-mediated endocytosis, facilitating receptor recycling (8), and the proteolytic processing of certain peptides by the activation of lysosomal hydrolases (9). Vacuolar H^+-ATPase is inhibited by N-ethylmaleimide (NEM), an alkylating agent which is relatively selective for sulfhydryl groups. Several experimental findings indicated that CDDP inhibits the activities of various proteins having sulfhydryl groups essential for their functions (2,10–12). Thus, the activity of vacuolar H^+-ATPase may also be affected by CDDP administration.

In the present study, we investigated the activity of H^+-ATPase and Na^+/H^+ exchanger in renal brush-border membrane from rats exposed to CDDP. The results suggest that CDDP inhibits vacuolar H^+-ATPase, while Na^+/H^+ exchanger is not affected. These observations may, at least in part, explain the mechanisms underlying the renal tubular dysfunctions induced by CDDP.

Materials and methods

Materials

The following reagents were used: CDDP (Bristol-Myers Squibb K.K., Tokyo, Japan), acridine orange (Kanto Chemical Co., Tokyo, Japan), NEM and Triton X-100 (Nacalai Tesque, Kyoto, Japan), and ATP, bafilomycin A_1 and valinomycin (Sigma Chemical Co., St. Louis, MO). All other chemicals used were of the highest purity available.

Animal treatment

Male Sprague-Dawley rats (200–300 g) received a bolus intraperitoneal administration of saline (normal group) or CDDP (5 mg/kg). Two to seven days after the administration, brush-border membrane vesicles were isolated from the renal cortex of the rats as stated below. The induction of acute renal failure by administration of CDDP was confirmed by the increase in blood urea nitrogen (data not shown). All animal experiments were performed in accordance

with the Guide for Animal Experimentation, Hiroshima University and the Committee of Research Facilities for Laboratory Animal Sciences, Faculty of Medicine, Hiroshima University.

Preparation of brush-border membrane vesicles

The isolation procedure of brush-border membrane vesicles was based on the Mg/EGTA precipitation method as described previously (13–15). All steps were performed on ice or at 4°C. The renal cortex was prepared by the use of a Stadie-Riggs microtome. The cortex was homogenized with a Process Homogenizer PH91 (SMT Company, Tokyo, Japan) at 18000 rpm for 2 min in an appropriate volume of 300 mM mannitol, 12 mM Tris-HCl (pH 7.1) and 5 mM EGTA, and then the same buffer was added to make a 10% homogenate. After diluting with distilled water (1:1), $MgCl_2$ was added to a final concentration of 10 mM, and the homogenate was allowed to stand for 15 min. The homogenate was centrifuged at 1900 × g for 15 min in an Avanti 30 Compact Centrifuge with rotor F0630 (Beckman Instruments, Inc., CA). The supernatant was centrifuged at 24000 × g for 30 min. The pellet was resuspended in the buffer (10 mL/g cortex) comprised of 150 mM mannitol, 6 mM Tris-HCl (pH 7.1) and 2.5 mM EGTA, and homogenized in a glass/Teflon Potter homogenizer with 10 strokes at 1000 rpm. $MgCl_2$ was added to a final concentration of 10 mM, and the suspension was allowed to stand for 15 min, then centrifuged at 1900 × g for 15 min. The supernatant was centrifuged at 24000 × g for 30 min. The pellet was resuspended in an experimental buffer [for H^+-ATPase assay, 150 mM KCl and 5 mM HEPES/Tris (pH 7.0); for Na^+/H^+ exchanger assay, 100 mM mannitol and 20 mM HEPES/Tris (pH 7.5)], and centrifuged at 24000 × g for 30 min. The final pellet (purified brush-border membrane) was resuspended in each experimental buffer 10 times through a fine needle (0.4 × 19 mm) with a plastic syringe.

Determination of marker enzyme activity and protein concentration

Alkaline phosphatase (EC 3.1.3.1), aminopeptidase (EC 3.4.11.2) and Na^+/K^+-ATPase (EC 3.6.1.3) were assayed as described previously (16,17). Protein concentration was measured by the method of Bradford (18) with bovine γ-globulin as a standard.

Reorientation of H^+-ATPase

Reorientation of the apical H^+-ATPase was carried out by the detergent-dilution procedure reported by Chambrey et al. (19) with some modifications. Briefly, the brush-border membrane vesicles were suspended with 5 mM HEPES/Tris buffer (pH 7.0) containing 150 mM KCl to a protein concentration of 1.1 mg/mL. Then, 0.23 M sodium cholate solution in water was added. The final concentration of sodium cholate was 1% and that of protein was 1.0 mg/mL. After keeping the mixture on ice for 5 min, followed by for 1 min at 37°C, the cholate-treated membrane was diluted through a fine needle (0.4 × 19 mm) with a plastic syringe into a spectrophotometer glass cuvette containing 2.5 mL of the transport medium (stated below) to allow the reformation of the vesicles.

Determination of ATP-driven H^+ pump activity

The ATP-driven H^+ pump activity was assayed by measuring the uptake of H^+ into the vesicles by addition of ATP to the transport medium comprised of 150 mM KCl, 5 mM

$MgCl_2$, 6 μM acridine orange and 5 mM HEPES/Tris (pH 7.0). Namely, after 100 μL of cholate-treated membrane was added to 2.5 mL of the transport medium and the absorbance of the acridine orange reached to a steady-state level, 700 mM ATP solution (adjusted to pH 7.0 with Tris) was added to make a final ATP concentration of 4 mM. The H^+ uptake was estimated by the decrease in absorbance of the pH indicator acridine orange, which was measured by dual-wavelength spectrophotometry (492–540 nm) at 37°C with a Hitachi dual wavelength spectrophotometer U-3200. Initial rate of the H^+ pump activity, expressed as ΔA/mg protein/min, was calculated from the slope of the decrease in acridine orange absorbance against time. For the measurement of NEM- or bafilomycin A_1-sensitive H^+-uptake, NEM (final 10 or 100 μM) or bafilomycin A_1 (final 0.1 to 100 nM) was included in the transport medium, respectively.

Determination of NEM-sensitive ATPase

NEM-sensitive ATPase activity was defined as the difference in ATP hydrolysis activity between the presence and absence of NEM. Briefly, incubation (37°C, 15 min) was initiated by adding 25 μL of brush-border membrane vesicles to 1 mL of distilled water with or without 2 mM NEM. Incubation was terminated by the addition of 100 μL of 50% trichloroacetic acid, and then 1 mL of water was added. After centrifuged at 3000 rpm for 10 min, 1 mL of the supernatant was mixed with 3 mL of molybdate-H_2SO_4 solution and 0.1 mL of Fiske-Subarow reagent for the determination of liberated inorganic phosphate. Absorbance at 660 nm was recorded after 20 min. Similarly, bafilomycin A_1-sensitive ATPase activity was measured using brush-border membrane pretreated with 0.1% Triton X-100.

In vitro CDDP pretreatment

Brush-border membrane vesicles isolated from normal rats (2.5 mg/mL, 250 μL), suspended in the buffer comprised of 150 mM KCl and 5 mM HEPES/Tris (pH 7.0), were incubated with 10 μM CDDP (final concentration) at 37°C for 5 min. The pretreatment was stopped and CDDP was removed by adding 5 mL of the same buffer (ice-cold) and by centrifuging at 24000 × g for 30 min. The brush-border membrane pellet was then resuspended in the buffer, and the vesicles were used for the assay of H^+ uptake.

Determination of Na^+/H^+ exchanger activity

Na^+/H^+ exchanger activity was determined by measuring a concentrative uptake of H^+ into the intravesicular space coupled with Na^+ efflux (18,20). Briefly, brush-border membrane vesicles were prepared in the buffer comprised of 100 mM NaCl, 100 mM mannitol and 20 mM HEPES/Tris (pH 7.5). After incubation at 25°C for 1 h, 50 μL of the vesicles was diluted into 3 mL of 20 mM HEPES/Tris (pH 7.5) containing 100 mM KCl, 100 mM mannitol and 6 μM acridine orange. Intravesicular acidification was monitored by measuring the quenching of fluorescence of acridine orange with a Hitachi fluorescence spectrophotometer F-3000 (excitation, 493 nm; emission, 530 nm).

Results

Table 1 shows the specific activities and recoveries of marker enzymes for brush-border membrane (aminopeptidase and alkaline phosphatase) and basolateral membrane (Na^+/K^+-

Table 1
Marker enzyme activities and recoveries in the homogenate and brush-border membrane from renal cortex of normal and CDDP administered rats

	Aminopeptidase		Alkaline phosphatase		Na^+/K^+-ATPase		Protein	
	Normal	CDDP	Normal	CDDP	Normal	CDDP	Normal	CDDP
Homogenate S.A.	34 ± 6	29 ± 2	44 ± 7	59 ± 15	93 ± 16	87 ± 16		
BBM S.A.	729 ± 129	557 ± 106	732 ± 158	843 ± 266	73 ± 23	108 ± 43		
Enrichment	19.6 ± 2.1	18.7 ± 3.0	17.8 ± 3.3	21.6 ± 5.3	1.0 ± 0.3	1.1 ± 0.3		
Recovery	21.7 ± 2.3	23.2 ± 5.2	18.6 ± 2.6	21.3 ± 5.4	1.0 ± 0.3	1.1 ± 0.3	1.2 ± 0.1	1.2 ± 0.2

Enrichment indicates the ratio of the specific activities in the brush-border membrane (BBM) and the homogenate. Recovery indicates the percentage of the enzyme activity found initially in the homogenate. Each value is the mean ± S.E. of three determinations from three preparations. Specific activity (S.A.) (nmol/min per mg protein).

ATPase) in the homogenate and brush-border membrane isolated from the kidneys of normal and CDDP-administered rats. The specific activities of aminopeptidase and alkaline phosphatase in the brush-border membranes were about 20-fold higher than those in the homogenate (enrichment). On the other hand, the enrichments of Na^+/K^+-ATPase were low (about 1-fold). There was no significant difference between the normal and CDDP-administered rats in specific activities, enrichments, and recoveries of these enzymes. The yield of brush-border membrane protein from the kidneys of CDDP-administered rats was also similar to that from normal rats.

Before studying the effect of CDDP on H^+ transport, we examined whether ATP-driven H^+ uptake can be detected in cholate-pretreated renal brush-border membrane vesicles. The H^+ transport was measured by monitoring the absorbance of acridine orange, an indicator of transmembrane pH gradient. As shown in Fig. 1, ATP addition caused a rapid decrease in the

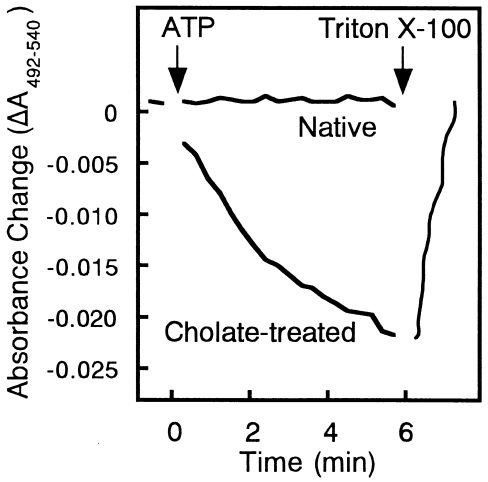

Fig. 1. ATP-driven H^+ uptake into native renal brush-border membrane vesicles and those exposed to cholate. ATP-dependent intravesicular acidification by ATP-driven H^+ uptake was estimated by measuring the absorbance change of acridine orange ($\Delta A_{492-540}$). The results are from a representative experiment performed in triplicate and the study was repeated in three different preparations.

absorbance in cholate-pretreated brush-border membrane vesicles, but not in native brush-border membrane vesicles. Addition of Triton X-100 restored the absorbance, proving H^+ uptake into an intravesicular space and creation of a H^+ gradient. In addition, NEM, an inhibitor of vacuolar type H^+-ATPase, decreased the ATP-driven H^+ uptake in a concentration-dependent manner in cholate-pretreated brush-border membrane vesicles (Fig. 2). Furthermore, the effect of bafilomycin A_1, the most specific and sensitive inhibitor of vacuolar H^+-ATPase available at present, was also examined. Bafilomycin A_1 inhibited the ATP-driven H^+ uptake dose dependently and the inhibitory effect was observed at lower concentration than that observed with NEM (Fig. 2B). Thus, H^+ uptake by vacuolar type H^+-ATPase can be detected by the method we employed.

The effect of CDDP administration on the ATP-driven H^+ uptake in renal brush-border membrane was examined. Two days after a bolus intraperitoneal administration of saline or CDDP, the ATP-driven H^+ uptake in the renal brush-border membrane vesicles was measured. Fig. 3 shows the representative tracings. CDDP administration markedly decreased the ATP-driven H^+ uptake in brush-border membrane vesicles. The decrease in the ATP-driven H^+ uptake activity was observed up to seven days after CDDP administration, although the activity tended to recover after day 2 (Fig. 3B).

To ensure whether these effects of CDDP administration were due to the decrease in H^+-ATPase activity itself or failure in the reorientation of H^+-ATPase molecule during the detergent-dilution procedure, NEM-sensitive ATPase activity was estimated under a hypotonic condition. Under such condition, the vesicles become leaky and ATP can freely access to the enzyme. As shown in Fig. 4, the specific activity of the NEM-sensitive ATPase, as well as total ATPase activity, in brush-border membrane from rats two days after CDDP administration

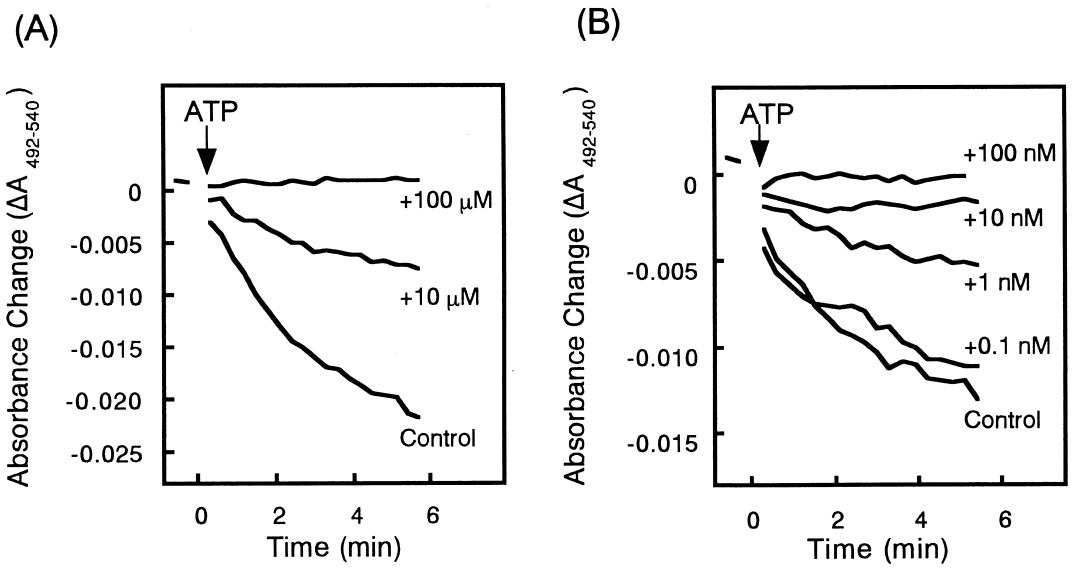

Fig. 2. Effect of N-ethylmaleimide (A) or bafilomycin A_1 (B) on ATP-driven H^+ uptake into renal brush-border membrane vesicles exposed to cholate. The results are from a representative experiment performed in triplicate and the study was repeated in three different preparations.

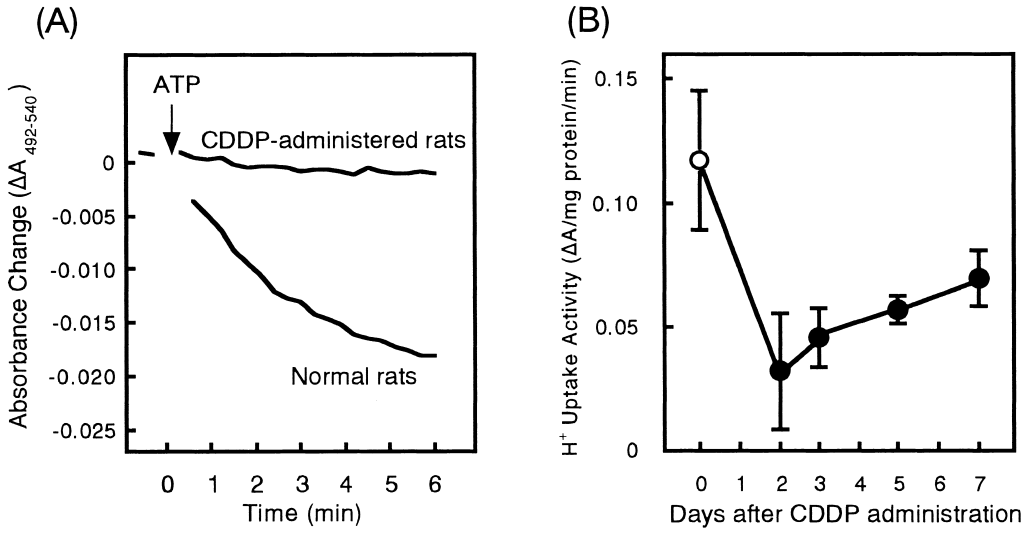

Fig. 3. ATP-driven H^+ uptake into brush-border membrane vesicles from rats after intraperitoneal administration of saline (Normal) or CDDP (5 mg/kg). (A) The ATP-driven H^+ uptake two days after the administration of saline or CDDP. The results are from a representative experiment performed in triplicate and the study was repeated in three different preparations. (B) ATP-driven H^+ uptake in brush-border membrane vesicles prepared from rats at various days after intraperitoneal administration of CDDP. The activity of ATP-driven H^+ uptake was shown as the initial rate of intravesicular acidification. Open circle represents the results of normal rats. Each point is the mean ± S.E. of three determinations.

decreased compared with that from normal rats. The decrease in total ATPase activity after CDDP administration was comparable to that in NEM-sensitive ATPase activity in normal rats, suggesting that the effect of CDDP administration is relatively specific against H^+-

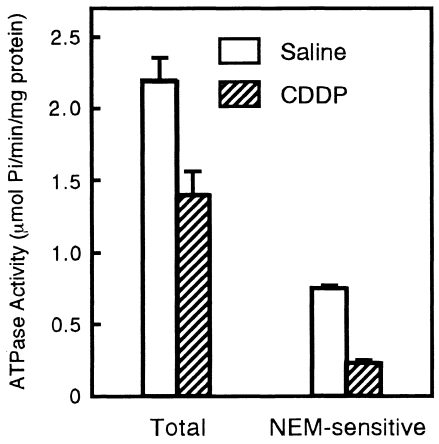

Fig. 4. Total and NEM-sensitive ATPase activity (ATP hydrolysis activity) in brush-border membranes. The membranes were prepared from rats two days after administration of saline (open column) or 5 mg/kg CDDP (hatched column). The activity of NEM-sensitive ATPase was estimated by subtracting ATPase activity in the presence of 2 mM NEM from that in the absence of NEM (Total). Each column is the mean ± S.E. of three determinations.

ATPase among others. In addition, we performed measurements of bafilomycin A_1-sensitive ATPase in the brush-border membrane pretreated with 0.1% Triton X-100, as an alternative method with which to open the membrane vesicles. The result also showed that CDDP administration inhibited the bafilomycin A_1-sensitive ATPase (Normal rats, 0.30 ± 0.01 mmol Pi/min/mg protein; CDDP-administered rats, 0.14 ± 0.03 mmol Pi/min/mg protein, mean ± S.E. of three determinations). Therefore, the inhibitory effect of CDDP would be due to the decrease in H^+-ATPase activity, and not due to the reorientation failure.

Since active H^+ transport by a vacuolar type H^+-ATPase is an electrogenic process, it is also possible to explain that the CDDP-induced decrease in ATP-driven H^+ uptake is due to the inhibition of the current movement via chloride channel, which is necessary for charge compensation inside the vesicles (21). We therefore examined whether the ATP-driven H^+ uptake in brush-border membrane vesicles prepared from CDDP-administered rats is changed in the presence of valinomycin and potassium (short circuit condition). However, the CDDP-induced inhibition of the ATP-driven H^+ uptake did not recover at all even under such a condition (data not shown). Thus, the decrease in the ATP-driven H^+ uptake in brush-border membrane vesicles from CDDP-administered rats would not be due to the disturbance of the charge compensation system.

We then examined whether in vitro pretreatment with CDDP of renal brush-border membrane vesicles from normal rats affects the ATP-driven H^+ uptake. The in vitro CDDP pretreatment decreased the ATP-driven H^+ uptake in brush-border membrane vesicles (Fig. 5), suggesting the involvement of a direct interaction between CDDP and H^+-ATPase under in vitro as well as in vivo condition.

Effect of CDDP administration on Na^+/H^+ exchanger in renal brush-border membrane was also examined using fluorescence quenching of acridine orange (Fig. 6). In contrast with

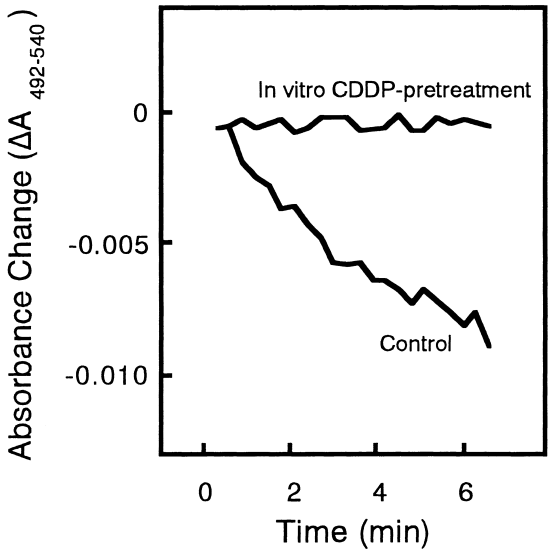

Fig. 5. Effect of in vitro pretreatment of brush-border membrane vesicles with CDDP on ATP-driven H^+ uptake. After 5 min exposure of the membrane vesicles to 10 μM CDDP at 37°C, ATP-driven H^+-uptake was measured. The results are from a representative experiment performed in triplicate.

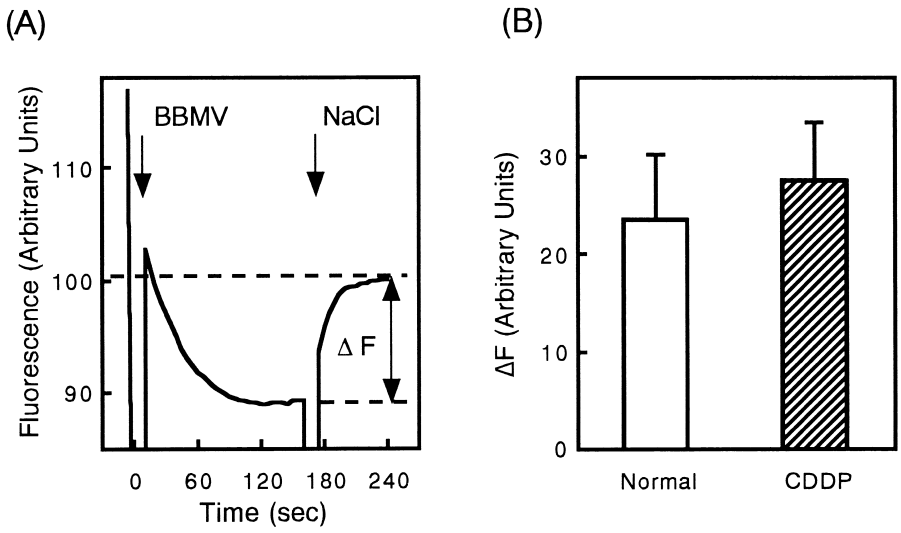

Fig. 6. Effect of CDDP administration on Na^+/H^+ exchanger activity in renal brush-border membrane vesicles. (A) Acridine orange fluorescence was continuously recorded as described in the text. The first and second arrows indicate the addition of brush-border membrane vesicles (BBMV, 375 μg) and 1 M NaCl (77 μL; final concentration 25 mM), respectively. ΔF was calculated as the difference between a final steady-state level and quenching of acridine orange fluorescence. (B) ΔF was measured in the brush-border membrane vesicles obtained from rats two days after administration of saline (Normal) or 5 mg/kg CDDP. Each column is the mean ± S.E. of five preparations.

H^+ ATPase, there was no difference of Na^+/H^+ exchanger activity in brush-border membrane vesicles from saline-administered normal rats and from rats two days after CDDP administration (Fig. 6B). Moreover, Na^+/H^+ exchanger activity was not altered in the vesicles prepared from rats seven days after CDDP administration when compared with that from normal rats (data not shown).

Discussion

The aim of the present study was to investigate whether CDDP administration has an influence on H^+ transport by H^+-ATPase and/or Na^+/H^+ exchanger in rat renal brush-border membrane.

Preisig et al. (22) suggested that an amiloride-sensitive Na^+/H^+ exchanger mediates about 65% of apical H^+ secretion, whereas the remaining H^+ secretion is mediated by a Na^+-independent, amiloride-insensitive system, probably by a vacuolar type H^+-ATPase. In addition, biochemical and functional evidences for H^+ secretion by a vacuolar H^+-ATPase in the apical membrane of rat proximal tubular cells have been reported (23,24). These reports strongly indicate that, in addition to Na^+/H^+ exchanger, an apical H^+-ATPase plays an important role in H^+ secretion and bicarbonate reabsorption in the renal proximal tubules. Lacchini et al. (4) reported that a moderate metabolic acidosis was observed in rats given a single dose of CDDP (5.5 mg/kg) intraperitoneally. They suggested that the defect of proximal H^+ secretion, reflected by reduced bicarbonate reabsorption, might be due to a decrease

in Na^+/H^+ exchanger activity. However, as far as we know, direct evidence showing that CDDP administration affects Na^+/H^+ exchanger activity in the proximal brush-border membrane has not been provided. In the present study, we found a decrease in H^+-ATPase activity, but not in Na^+/H^+ exchange activity, in isolated brush-border membrane from CDDP-administered rats. Therefore, it seems likely that the CDDP-induced metabolic acidosis is, at least in part, due to a reduction in H^+-ATPase activity in the brush-border membrane. This result may also explain the CDDP-induced metabolic acidosis in human (3).

The detailed studies on H^+-ATPase in intact renal brush-border membrane vesicles have been difficult because the brush-border membrane vesicles are oriented right side out (25), in which the catalytic domain of H^+-ATPase is located inside the vesicles. Recently, methods allowing rapid reorientation of the H^+-ATPase were reported in the renal brush-border membrane vesicles from porcine (26), dog (27) and rat (19), which make it possible to estimate H^+ transporting activity by measuring H^+ uptake into the vesicles. In the present study, we employed a detergent-dilution procedure. By using this procedure, we could measure NEM- and bafilomycin A_1-sensitive H^+ uptake driven by ATP, and found a decrease in ATP-driven H^+ uptake in the brush-border membrane vesicles from rats administered CDDP. However, there is a possibility that the inhibitory effect of CDDP on H^+-ATPase was not due to change in the transport activity itself but due to a failure of the reorientation of H^+-ATPase. Therefore, we evaluated the effect of CDDP administration on NEM- or bafilomycin A_1-sensitive ATPase activity in the brush-border membrane under a hypotonic condition or using membrane pretreated with Triton X-100, respectively. The results showed that the ATPase activity in the brush-border membrane was inhibited by CDDP administration, suggesting that CDDP-induced decrease in H^+ transport is not due to the reorientation failure. We also examined another possibility that the inhibition of the ATP-driven H^+ uptake could be due to the decrease in a chloride conductance which provides the anion for charge compensation (21). However, we found it to be unlikely, by measuring the H^+ uptake under a short circuit condition. Taken together, the decrease in ATP-driven H^+ transport by CDDP administration would be due to its effect on the H^+-ATPase activity.

As observed in Na^+-dependent transport of glucose and phosphate (1), the present result showed that H^+-ATPase was also inhibited in the brush-border membrane vesicles exposed to CDDP in vitro as well as in vivo. Therefore, it is likely that the CDDP-induced inhibition of H^+-ATPase results from a direct interaction between CDDP and the enzyme. Several reports suggest that covalent binding of platinum to sulfhydryl groups is involved in CDDP-induced inhibition of the activity of various functional proteins (11,12). Moreover, a vacuolar H^+-ATPase in the brush-border membrane is inhibited by a thioalkylating agent NEM (28), suggesting that a sulfhydryl residue plays an important role in the activity of this enzyme. Therefore, the decrease in H^+-ATPase activity by CDDP may result from direct chemical binding of CDDP to the protein. However, further investigation is necessary to clarify the mechanism of the CDDP-induced inhibition of H^+-ATPase in the brush-border membrane.

Impairment of reabsorption in renal tubule after CDDP administration leads to increase in urinary excretion of proteins such as β_2-microglobulin and albumin, as well as small organic and inorganic molecules such as sodium and glucose (29). Most of plasma proteins and several hormones filtered in the glomerulus are reabsorbed in the proximal tubule by receptor-mediated endocytosis (30). Vacuolar H^+-ATPase is responsible for lowering pH inside endo-

somes, which leads to dissociation of ligands and receptors after receptor-mediated endocytosis. Therefore, proteinuria induced by CDDP administration may partly be explained by the decrease in receptor-mediated endocytosis of proteins due to the inhibition of vacuolar H^+-ATPase activity.

In conclusion, CDDP inhibited H^+-ATPase in the brush-border membrane in vivo and in vitro, while Na^+/H^+ exchanger was not affected. The inhibition of H^+-ATPase activity in the renal brush-border membrane may be related to the CDDP-induced metabolic acidosis and proteinuria.

Acknowledgments

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