Kidney International, Vol. 67 (2005), pp. 1855–1867

BSC1 inhibition complements effects of vasopressin V_2 receptor antagonist on hyponatremia in SIADH rats

Itsuro Kazama,[1] Ryo Hatano,[1] Mari Michimata, Katsuya Suzuki, Tomohiro Arata, Michiko Suzuki, Noriyuki Miyama, Akira Sato, Susumu Satomi, Yutaka Ejima, Sei Sasaki, and Mitsunobu Matsubara

Division of Molecular Medicine, Center for Translational and Advanced Animal Research, Tohoku University School of Medicine, Sendai, Japan; Division of Advanced Surgical Science and Technology, Department of Anesthesiology, Tohoku University Hospital, Sendai, Japan; and Department of Homeostasis Medicine and Nephrology, Tokyo Medical and Dental University, Tokyo, Japan

BSC1 inhibition complements effects of vasopressin V_2 receptor antagonist on hyponatremia in SIADH rats.

Background. Severe hyponatremia is most frequently caused by the syndrome of inappropriate secretion of antidiuretic hormone (SIADH). Although the expressional alteration of the kidney-specific apical water channel, aquaporin 2 (AQP2), in the collecting duct has been demonstrated to be involved in the development of hyponatremia and the subsequent physiologic reaction that is resistant to arginine vasopressin (AVP; vasopressin escape) in SIADH, the complete pathogenesis of and the appropriate medical treatment for hyponatremia have yet to be elucidated.

Methods. Hyponatremia was induced in male Sprague-Dawley rats by water loading and subcutaneous infusion of 1-deamino-8-D-arginine vasopressin (dDAVP). For the treatment, a selective AVP V_2 receptor antagonist (OPC-31260) and/or a loop diuretic (furosemide) were administered orally. Protein expression of AQP2 and rat bumetanide-sensitive cotransporter (rBSC1) was examined by Western blotting during the hyponatremia and the subsequent treatment.

Results. We noted a markedly high expression of rBSC1 during the development of hyponatremia, and a relatively low expression during vasopressin escape. OPC-31260 administration elevated serum sodium level in a dose-dependent manner. The therapeutic effect, however, declined with increasing number of treatment days, and doses higher than 15 mg/kg/day induced severe toxicity. The physiologic parameters and the alterations of AQP2 and rBSC1 expression during the treatment demonstrated reactions that were completely opposite to those of vasopressin escape. Combination of a furosemide (100 mg/kg/day) and a low dose of OPC-31260 (5 mg/kg/day) additively elevated serum sodium level and sustained the elevated serum sodium level by significantly reducing sodium accumulation in the renal medulla.

Conclusion. AVP-induced alterations of rBSC1 expression, as well as those of AQP2, are involved in the pathogenesis of SIADH. The pharmacologic blockade of AVP stimulus in SIADH limits its therapeutic efficacy by discontinuing the vasopressin escape, and the selective inhibition of rBSC1 complements this limitation.

Hyponatremia is the most common disorder of body fluid and electrolyte balance encountered in clinical practice [1]. Severe hyponatremia is most frequently caused by the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) [2]. SIADH is a condition in which plasma arginine vasopressin (AVP) level is not appropriately suppressed despite hypoosmolality [3]. Sustained water intake due to chronic exposure to AVP can result in net water retention and the development of hyponatremia [4]. Recently, the involvement of the apical collecting duct water channel, aquaporin 2 (AQP2), has been demonstrated in the pathogenesis of SIADH [5–7]. AVP stimulates the transcription of the AQP2 gene by activating adenosine 3′, 5′-cyclic monophosphate (cAMP) responsive element (CRE) upstream of the AQP2 gene [8]. For renal water reabsorption through AQP2, sodium reabsorption is required in advance to induce an osmotic gradient in the renal medulla. A kidney-specific sodium cotransporter (BSC1; bumetanide-sensitive cotransporter) in the thick ascending limb of Henle (TAL) supplies critical sodium to the medullary interstitium for concentrating urine [9, 10]. The final urinary concentration for the maintenance of body fluid has been determined by harmonious expression of these proteins and urea transporters in the collecting duct [11–16]. We have demonstrated that BSC1 expression leading to urinary concentration is regulated by at least two different mechanisms, namely, AVP-dependent and -independent mechanisms, whereas

[1]Dr. Kazama and Dr. Hatano contributed equally to this article.

Key words: arginine vasopressin, OPC-31260, furosemide, combination therapy.

Received for publication July 7, 2004
and in revised form October 1, 2004, and December 1, 2004
Accepted for publication December 14, 2004

AQP2 is highly dependent on AVP stimulus [17]. Since SIADH is a water retention disorder and is characterized by inappropriate urinary concentration [18], BSC1 expression, as well as AQP2 expression, was expected to be enhanced after 1-deamino-8-D-arginine vasopressin (dDAVP) infusion. Therefore, the first purpose of our study was to investigate whether or not changes in BSC1 expression are attributable to the development of SIADH.

The most rational therapeutic strategy for SIADH is to exclude the underlying causes of excessive AVP stimuli. In most cases, however, this is difficult, and fluid restriction for ameliorating excessive water retention is the present mainstay of the treatment of SIADH patients. Several drugs, such as ethanol, diphenylhydantoin, and opiates, have been noted to decrease AVP secretion in some cases [19], whereas demeclocycline and lithium carbonate have been advocated to reduce the renal action of AVP [20, 21]. However, because of their erratic and unpredictable responses or significant side effects, they are deemed impractical for clinical use [22, 23]. A potent peptide AVP V_2 receptor antagonist was developed by Manning and Sawyer [24]; however, its therapeutic usefulness was limited because of its low oral bioavailability and partial agonist activity. Recently, Yamamura et al developed an orally effective and highly selective nonpeptide AVP V_2 receptor antagonist, OPC-31260 [25]. It has been demonstrated to increase renal solute-free water excretion in humans [26], and early experience in SIADH patients has indicated substantial improvement of hyponatremia [27]. On the other hand, furosemide, a BSC1 inhibitor with compensatory salt intake, has been reported to be successfully used in SIADH patients [28], although its effects have not yet been demonstrated in animal models of SIADH. The second purpose of our study was to evaluate the pharmacologic therapy for SIADH based on the results of BSC1 expression in our present study. To this end, we administered OPC-31260, furosemide, or a combination of both to SIADH rats and investigated their effects on various physiologic parameters. Together with the present findings of AQP2 and BSC1 expression during the development and treatment of SIADH, our present study has provided novel insights into the pathophysiology and pharmacologic therapy of SIADH.

METHODS

Experimental animals

Male Sprague-Dawley rats weighing 200 g were used. They were housed in individual cages in a humidity- and temperature-controlled room with a 12-12-hour light-dark cycle. All experimental protocols described in the present study were approved by the Ethics Review Committee for Animal Experimentation of Tohoku University.

Animal preparation

A slightly modified version of the SIADH model originally developed by Verbalis [18] was used as described previously [5, 7, 29] (Fig. 1). Rats were acclimated for four days to a gelled-agar diet composed of 67% water, 27% finely powdered rat chow, 4.7% glucose, and 1.3% agar, in which 0.084% sodium and 0.27% potassium were contained. The agar was melted in boiling water and poured into a mold, and then the powdered chow and glucose were added and stirred to an even consistency. The diet was then chilled at 4°C to form a firm, gelatin-like state. The rats received 90 g of this preparation each day. The diet forced the rats to consume a greater volume of water to take the enough calories. After four days of the acclimatization period with water loading, the SIADH rats underwent osmotic minipump (model 2002; Alza Corporation, Palo Alto, CA, USA) implantation. Osmotic minipumps containing dDAVP (Kyowa Hakko, Tokyo, Japan) dissolved in 0.15 mol/L NaCl to make a concentration of 10 μg/mL were implanted subcutaneously on the back under ether anesthesia, and dDAVP was infused at a rate of 5 ng/h. After recovery, the animals were placed in cages with free access to the gelled-agar diet for the additional days. All the rats were given the same amount of the gelled-agar diet over the entire course of the experiment. They were placed individually in metabolic cages to facilitate urine collection and recording of individual food and water intake. Twenty-four–hour urine collection was conducted daily in order to measure urine volume, urine osmolality, and sodium and potassium levels. Body weight and water and food intake were also measured daily. Net daily sodium and potassium balance was calculated as the difference between dietary intake and urine output. Fecal electrolyte loss was assumed to be small and constant. We sampled 0.3 mL of blood from tail veins to determine serum sodium levels and plasma osmolality every two days. For the removal of kidneys, 4 mL of trunk blood was collected by bleeding the animals to death under ether anesthesia. Three series of experiments were performed (Fig. 1).

Series 1. Changes in the physiologic parameters of the SIADH rats were closely examined for 14 days to evaluate the appropriate rat model for SIADH (Fig. 1A). To further confirm the propriety of this model, changes in AQP2 protein expression were analyzed. Rat BSC1 (rBSC1) protein expression was also examined to investigate the involvement of altered rBSC1 expression in the development of SIADH. SIADH rats were sacrificed at different time points, specifically, on days two and seven after the dDAVP infusion, whereas control rats without

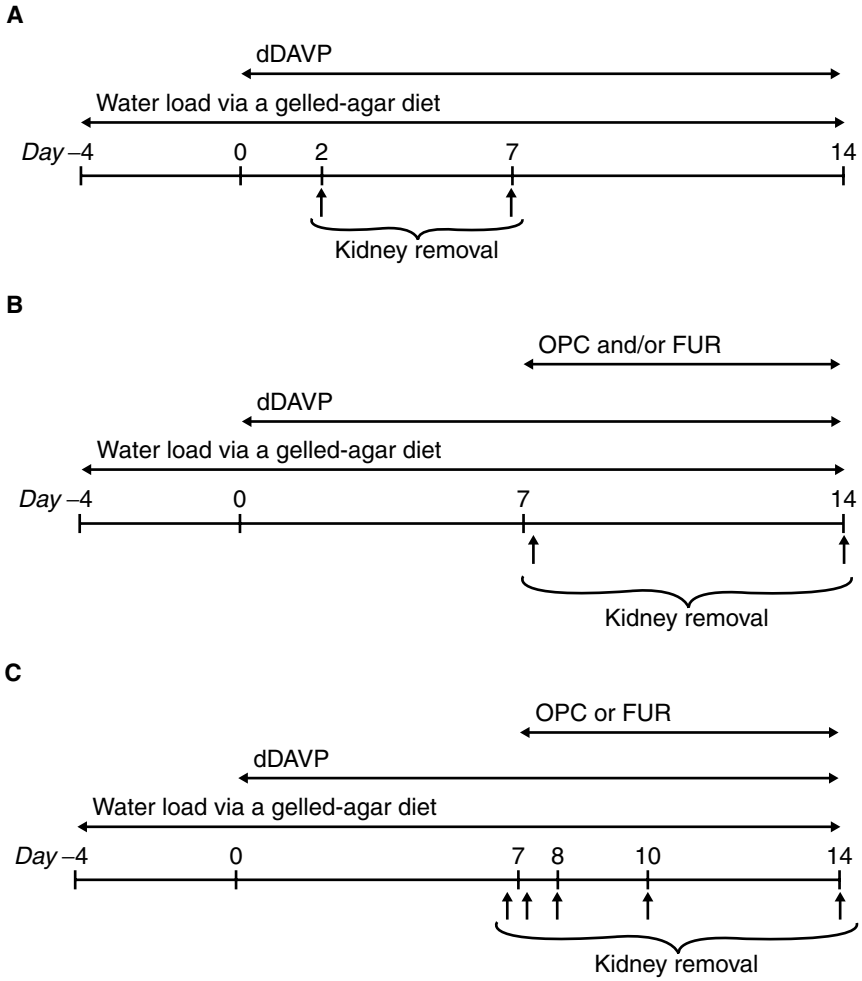

Fig. 1. Diagram of experimental format. Sprague-Dawley rats were water-loaded from day 4 and subcutaneously infused with dDAVP (infusion rate: 5 ng/hr) from day 0. (*A*) *Series 1*. Kidneys from SIADH rats were removed on days 2 and 7 after dDAVP infusion, whereas kidneys from control rats were removed after an additional 7 days of water loading. (*B*) *Series 2*. Rats were administered OPC-31260 and/or furosemide from day 7. Kidneys from rats of group 1 (5 mg/kg OPC-31260) were removed 3 hours after the initial administration and at the end of observation period, while kidneys from rats of group 7 (100 mg/kg furosemide), 8 (5 mg/kg OPC-31260 and 30 mg/kg furosemide), and 9 (5 mg/kg OPC-31260 and 100 mg/kg furosemide) were removed at the end of the observation period. (*C*) *Series 3*. Rats were administered 5 mg/kg OPC-31260 or 100 mg/kg furosemide from day 7. Kidneys from the rats were removed before the initial administration and 3 hours, 24 hours, 3 days, and 7 days after the initial administration. Six rats were used in each condition. Abbreviations are: OPC, OPC-31260; FUR, furosemide; dDAVP, 1-deamino-8-D-arginine vasopressin.

the dDAVP infusion were sacrificed after an additional seven days of water loading. The kidneys were rapidly removed and used in experiments for immunoblotting and electrolyte measurement.

Series 2. Based on the finding in *series 1* that the hyponatremic condition was stabilized from day five of the dDAVP infusion, therapeutic interventions were designed (Fig. 1B). According to the groups listed below ($N = 6$ per group), OPC-31260, furosemide, or a combination of both was administered to the SIADH rats: (*1*) Administration of OPC-31260. In group 1, the SIADH model rats were administered 5 mg/kg OPC-31260 (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan). In group 2, 10 mg/kg. In group 3, 15 mg/kg. In group 4, 20 mg/kg. In group 5, 30 mg/kg. (*2*) Administration of furosemide. In group 6, the SIADH rats were administered 30 mg/kg furosemide (Wako Pure Chemical Industries, Ltd., Osaka, Japan). In group 7, 100 mg/kg. (*3*) Combined administration of OPC-31260 and furosemide. In group 8, the SIADH rats were administered 5 mg/kg OPC-31260 and 30 mg/kg furosemide in combination. In group 9, 5 mg/kg OPC-31260 and 100 mg/kg furosemide were administered. All the SIADH rat groups were subjected to oral administration of the drugs via a gastric tube once a day from days seven to 13 at a dose of 2.0 mL/kg. The drugs were suspended in water containing 1% methylcellulose (Wako Pure Chemicals). We sampled 0.3 mL of blood from the tail veins to determine serum sodium level every day during the treatment. Serum creatinine and urea concentrations were determined before and after the entire course of treatment. Both kidneys from the rats of groups 1 (5 mg/kg OPC-31260) were rapidly removed three hours after the initial administration and at the end of observation period. Kidneys from the rats of group 7 (100 mg/kg furosemide), 8 (5 mg/kg OPC-31260 and 30 mg/kg furosemide), and 9 (5 mg/kg OPC-31260 and 100 mg/kg furosemide) were removed at the end of the observation period. These kidneys were used for measurements of electrolytes and urea in the renal medulla

Series 3. To investigate the mechanisms underlying the therapeutic limitations of OPC-31260 as demonstrated

in *series 2*, we closely examined the changes in AQP2 and rBSC1 protein expression during OPC-31260 or furosemide treatment (Fig. 1C). Kidney tissue was sampled before the initial administration and three hours, 24 hours, three days, and seven days after the initial administration of 5 mg/kg OPC-31260 or 100 mg/kg furosemide, and was used in immunoblotting experiments.

Immunoblotting

Immunoblotting using a specific antibody against AQP2 or rBSC1 was performed as previously described [17, 30, 31]. Briefly, after dividing the harvested kidneys into three parts—cortex, outer medulla, and inner medulla—the renal outer medulla and the inner medulla were separately homogenized in 2 mL of phosphate-buffered saline (PBS), 1% Triton, 1% deoxycholate, 0.1% sodium dodecyl sulfate (SDS), and 0.1 mmol/L phenylmethylsulfonyl fluoride (PMSF) for protein extraction. Twenty-five μg of protein was loaded in each lane for Laemmli's SDS-polyacrylamide gel electrophoresis (PAGE) (8 or 12.5%), and then transferred to a polyvinylidene difluoride membrane. The membrane was blocked for one hour and exposed to antibody diluted with 2.5% milk powder/TBST (10 mmol/L Tris-HCl, pH 8.5, 150 mmol/L NaCl, and 0.1% Tween 20) overnight at 4°C, and then to a second antibody (peroxidase linked antirabbit IgG) for one hour at room temperature. After washing, antigen-antibody complexes were visualized with a chemiluminescence system (ECL Plus; Amersham Bioscience, Piscataway, NJ, USA).

Measurements of electrolytes and urea in renal medulla

Electrolyte and urea concentrations in the renal medulla were determined according to previous studies in which solute accumulation in the renal medulla was measured [17, 30]. Briefly, the renal inner medulla was separated, put inside a microcentrifuge tube, and quickly weighed. The tube was then immersed in an 80°C water bath for 60 minutes to inactivate the enzymes. The tube was dried in an oven set at 80°C for 48 hours and reweighed after the tissue had attained complete dryness. Distilled water was then added and the tube was placed in an oven set at 80°C for two hours to facilitate extraction of ions and urea into the water. The tube was cooled to 18°C and reweighed to determine the volume of the added water. Then, the supernatant was used for electrolyte and chemical analysis. Sodium, potassium, and urea concentrations were expressed per gram of tissue weight.

Other measurements and statistical analyses

Serum and urinary sodium and potassium levels were measured by an autoanalyzer (M-644, Bayer Co., East Walpole, MA, USA). Serum urea and creatinine levels and tissue urea level were measured by a chemical autoanalyzer (DRI-CHEM 3500V, Fuji Film, Tokyo, Japan). Plasma and urinary osmolality was determined with an osmometer (model 3D2; Advanced Instruments, Needham Heights, MA, USA). The results are expressed as mean ± SEM. Statistical comparisons in all the physiologic and laboratory data were made among the treatment groups using analysis of variance (ANOVA) followed by Dunnett's test or Student *t* test for individual comparisons. A P value < 0.05 was considered significant.

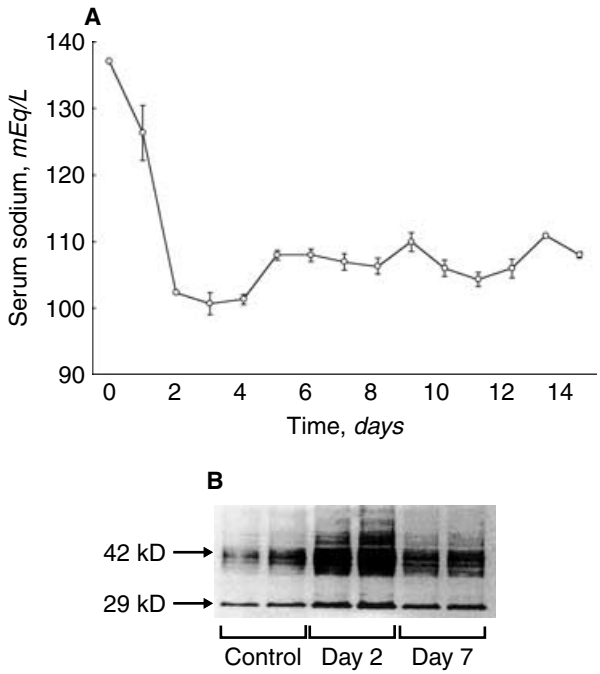

Fig. 2. Serum sodium levels and AQP2 protein expression in SIADH rats. (*A*) Serum sodium level during the 14-day subcutaneous dDAVP infusion. Data represent the mean ± SEM of 6 determinations in each condition. (*B*) AQP2 protein expression by immunoblotting. Twenty-five μg of protein extracted from the renal inner medulla was loaded in each lane. Rats without dDAVP infusion (control), and those on days 2 and 7 of dDAVP infusion, were examined.

RESULTS

Changes in serum sodium level and AQP2 protein expression

In the SIADH rats, serum sodium levels were markedly decreased to nearly 100 mmol/L on day two of the dDAVP infusion, indicating the onset of hyponatremia. Although slight elevation was noted from days four to five, continuous hyponatremia at less than 110 mmol/L was maintained thereafter (Fig. 2A). Plasma hypoosmolality was also maintained (data not shown). Then, AQP2 protein expression was examined on days two and seven, which are the onset of hyponatremia and the stabilized phase of continuous hyponatremia, respectively (Fig. 2B). Western blots of AQP2 demonstrated highly intense bands on day two, followed by a slight decline of the

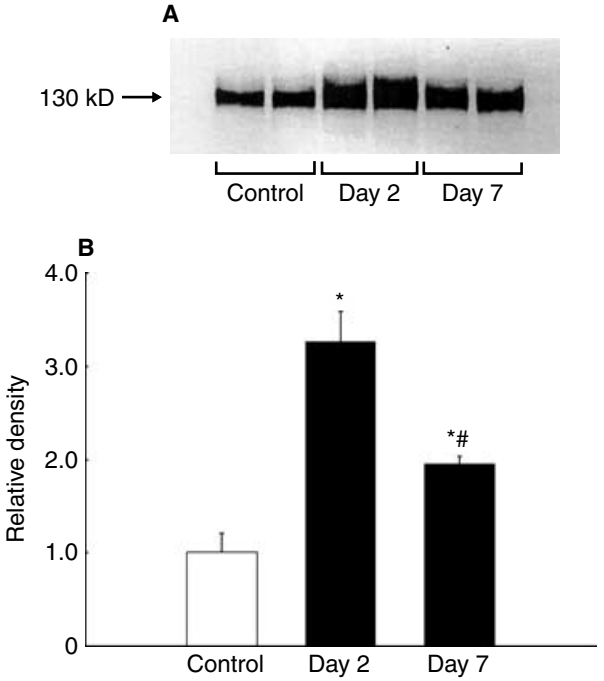

Fig. 3. rBSC1 protein expression. (*A*) Signal bands of immunoblotting. Twenty-five μg of protein extracted from the renal outer medulla was loaded in each lane. Rats without dDAVP infusion (control), and those on days 2 and 7 of dDAVP infusion, were examined. (*B*) Level of protein expression was determined from the signal density. $^{*}P < 0.05$ compared to rats without dDAVP infusion (control), $^{\#}P < 0.05$ compared to rats on day 2 of dDAVP infusion. Data are mean ± SEM of 6 determinations in each condition. Differences among the treatment groups were analyzed by ANOVA, followed by Dunnett's test or Student *t* test.

band intensity on day seven, although it was still stronger than that of control (Fig. 2B). Densitometric analysis using 42 kD bands (six determinations for each condition) confirmed a statistically significant decrease in band intensity from days two to seven and a remaining intensity on day seven that was stronger than that of the control (data not shown). These alterations in AQP2 expression are consistent with previous findings of enhanced AQP2 expression at the acute phase of SIADH, followed by the AVP escape phenomenon [5–7].

Changes in rBSC1 protein expression level

rBSC1 protein expression before and after the dDAVP infusion is demonstrated in Figure 3. A marked increase in expression was noted again on day two (Fig. 3A). In the same way as AQP2 expression, the expression was decreased on day seven, although it was still significantly higher than that of the control (Fig. 3A). Statistically significant difference between control rats and SIADH rats and between day two and day seven was confirmed by measuring signal density (Fig. 3B).

Effects of therapeutic intervention on serum sodium

Administration of OPC-31260. Based on the findings of AQP2 and rBSC1 expression, we designed the therapeutic intervention. As the expression of these two proteins is promoted by AVP through V_2 receptor mediated pathways [11, 12, 17], OPC-31260, an AVP V_2 receptor antagonist, was chosen for the treatment of the SIADH rats (Fig. 4A). Oral administration of OPC-31260 was started on day seven when the physiologic reactions to the dDAVP infusion were stabilized. Figure 4A demonstrates the OPC-31260 dose-dependent effects of serum sodium levels. OPC-31260 doses higher than 10 mg/kg abruptly elevated serum sodium levels to above 130 mEq/L in one day, followed by a gradual elevation to the maximum. All the rats administered 30 mg/kg OPC-31260 and half of

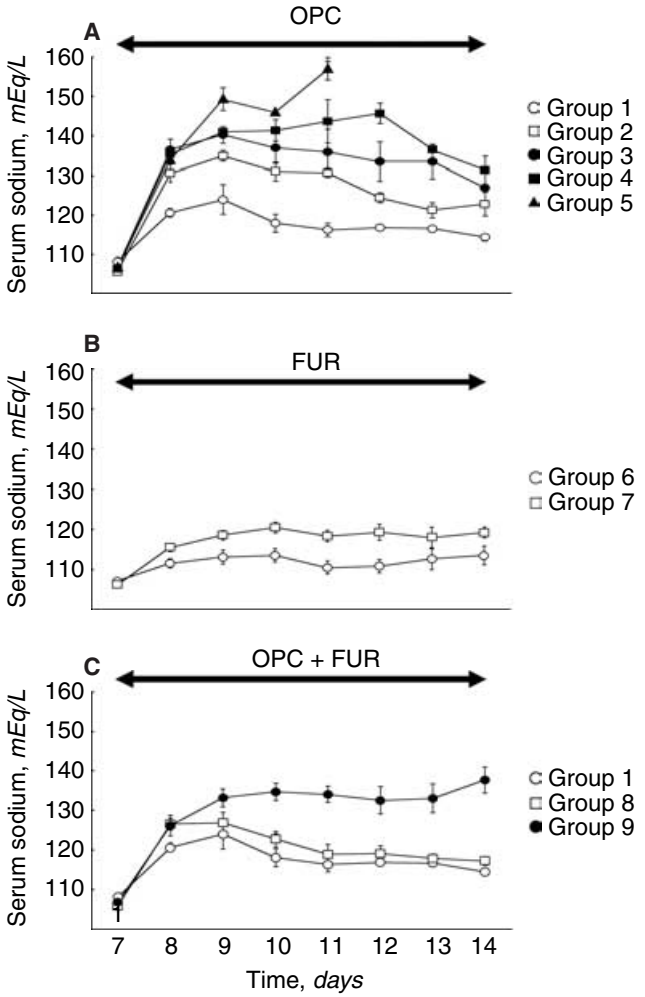

Fig. 4. Serum sodium levels in SIADH rats during treatment. (*A*) OPC-31260 alone. Open circles, group 1, OPC-31260 5 mg/kg; open squares, group 2, OPC-31260 10 mg/kg; filled circles, group 3, OPC-31260 15 mg/kg; filled squares, group 4, OPC-31260 20 mg/kg; filled triangles, group 5, OPC-31260 30 mg/kg. (*B*) Furosemide alone. Open circles, group 6, furosemide 30 mg/kg; open squares, group 7, furosemide 100 mg/kg. (*C*) Combination of OPC-31260 and furosemide. Open circles, group 1, OPC-31260 5 mg/kg; open squares, group 8, OPC-31260 5 mg/kg and furosemide 30 mg/kg; filled circles, group 9, OPC-31260 5 mg/kg and furosemide 100 mg/kg. Data represent the mean ± SEM of 6 determinations in each condition. Abbreviations are: OPC, OPC-31260; FUR, furosemide.

the rats administered 15 or 20 mg/kg OPC-31260 expired during the course of treatment. The surviving rats that were administered 15 or 20 mg/kg OPC-31260 showed such abnormal behavior as agitation, hypersensitivity, or disorientation. Although all rats that were administered 10 mg/kg survived the observation period, one of them showed the abnormal behavior described above. In rats administered 5 mg/kg OPC-31260, the elevation of serum sodium was slower and smaller than that in rats administered a higher dose of OPC-31260, and no abnormal behavior was noted.

Administration of furosemide. Based on the present findings of increased rBSC1 expression in the SIADH rats, the effects of the functional inhibitor of BSC1, furosemide, were examined, as shown in Figure 4B. The administration of furosemide also elevated serum sodium levels in a dose-dependent manner, but had none of the significant side effects observed in rats administered a high dose of OPC-31260. The maximum serum sodium level, however, was below 125 mEq/L, even when a high dose of furosemide (100 mg/kg) was administered. Administration of low-dose furosemide (3 mg/kg, 10 mg/kg) did not show any therapeutic effects.

When OPC-31260 and furosemide were compared, it was found that 5 mg/kg OPC-31260 elevated serum sodium level at a higher rate than 100 mg/kg furosemide (Fig. 4A and B). This therapeutic effect of OPC-31260, however, was gradually decreased with increasing number of treatment days (Fig. 4A), whereas furosemide maintained the serum sodium level throughout the observation period (Fig. 4B).

Combined administration of OPC-31260 and furosemide. As a high dose of OPC-31260 produces severe side effects and furosemide shows small but steady therapeutic effects, we tested the effects of combination therapy with a low dose of OPC-31260 and furosemide. As shown in Figure 4C, when 5 mg/kg OPC-31260 was used in combination with 30 mg/kg furosemide, a slightly faster and greater rise of the serum sodium level than that of the administration of 5 mg/kg OPC-31260 alone was noted. The therapeutic effects, however, were gradually decreased from day 10 as in rats administered OPC-31260 alone. In contrast, when 5 mg/kg OPC-31260 was used in combination with 100 mg/kg furosemide, the serum sodium concentration was further elevated than with that of 30 mg/kg furosemide, and the effects were well maintained throughout the observation period.

Electrolyte and urea accumulation in renal medulla of rats with OPC-31260 and/or furosemide treatment

In our therapeutic intervention, the combined treatment with OPC-31260 and furosemide showed the best therapeutic effect of increasing serum sodium level without producing any side effects. Thus, we investigated the mechanisms of the effects of furosemide by examining the accumulated levels of electrolytes and urea in the renal medulla. First, we examined the alterations of these parameters during the onset of SIADH and treatments by 5 mg/kg OPC-31260 (group 1) or 100 mg/kg furosemide (group 7), as shown in Figure 5. The infusion of dDAVP for two days markedly increased both sodium and urea accumulation (Fig. 5A and B). Although OPC-31260 significantly decreased sodium and urea accumulation transiently three hours after the initial administration, it returned at the end of observation period to the level before treatment (Fig. 5A and B). On the other hand, furosemide significantly lowered the accumulated sodium even at the end of observation period (Fig. 5A). Urea accumulation also tended to be decreased ($P = 0.07$) by this treatment (Fig. 5B). Second, these parameters were compared among the three groups, namely 5 mg/kg OPC-31260 alone, 5 mg/kg OPC-31260 and 30 mg/kg furosemide, and 5 mg/kg OPC-31260 and 100 mg/kg furosemide (Fig. 6). The accumulated level of sodium in the renal inner medulla was significantly decreased in the group administered a high dose of furosemide (Fig. 6A), in which the large additive effects of furosemide on the serum sodium level were constantly noted (Fig. 4C). Although there was no difference in potassium accumulation (Fig. 6B), sodium accumulation in the group administered a low dose of furosemide was slightly, but not significantly lower than that in the group administered OPC-31260 alone (Fig. 6A), and urea accumulation was also slightly, but not significantly decreased with increasing dose of furosemide (Fig. 6C).

Physiologic changes throughout the observation period

The therapeutic trial of the present study confirmed the therapeutic effects of a low dose of OPC-31260, which were enhanced by furosemide. However, it also revealed several problems that have to be addressed: (*1*) a high dose of OPC-31260 demonstrated severe toxicity; (*2*) the therapeutic effects of OPC-31260 were gradually decreased with increasing number of treatment days; (*3*) furosemide treatment might influence total sodium balance because of its pharmacologic effect of natriuresis. To address these problems, we closely analyzed the physiologic changes in the rats throughout the experimental period, as follows.

Changes in body weight. Figure 7 shows the changes in body weight of all the rats except those administered more than 20 mg/kg OPC-31260. Before the dDAVP infusion, constant weight gain by natural growth was noted. The weight gain was enhanced for two days after the subcutaneous infusion of dDAVP, which coincided with the onset of hyponatremia. A transient decrease was noted on days two to four, when hyponatremia was slightly

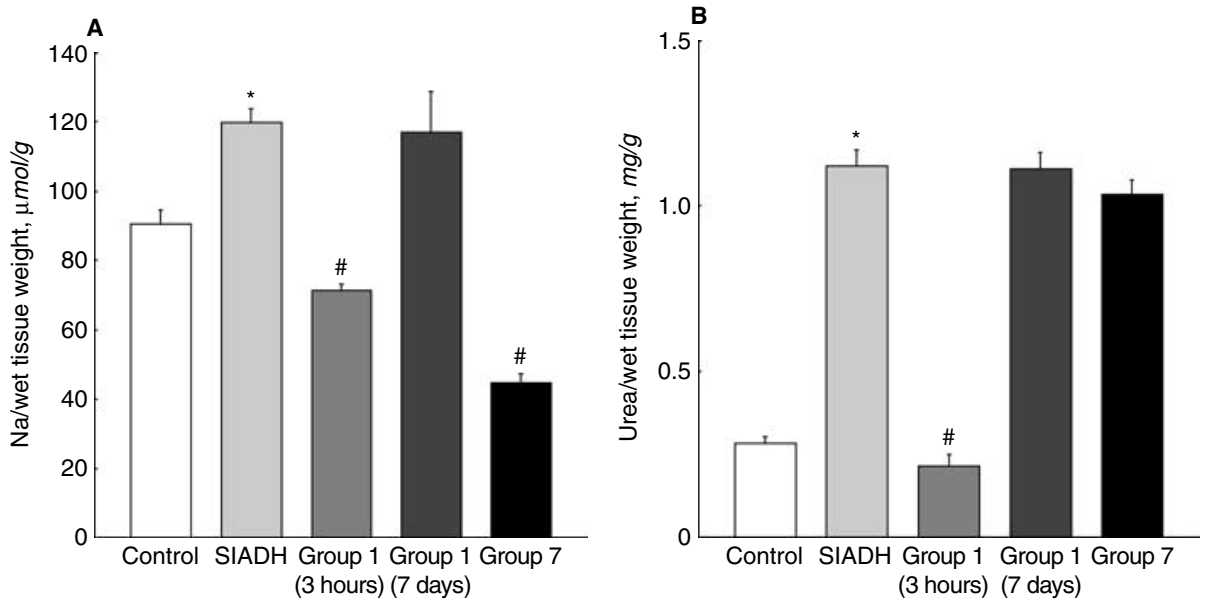

Fig. 5. Electrolyte and urea accumulation in renal medulla. Rats of control, SIADH (day 2 of dDAVP infusion, before treatment), groups 1 (5 mg/kg OPC-31260), and 7 (100 mg/kg furosemide) were examined. (*A*) Sodium. $^{*}P < 0.05$ compared to rats without dDAVP infusion (control), $^{\#}P < 0.05$ compared to rats of SIADH. (*B*) Urea. Data represent the mean ± SEM of 6 determinations in each condition. Differences among the treatment groups were analyzed by ANOVA, followed by Dunnett's test or Student *t* test.

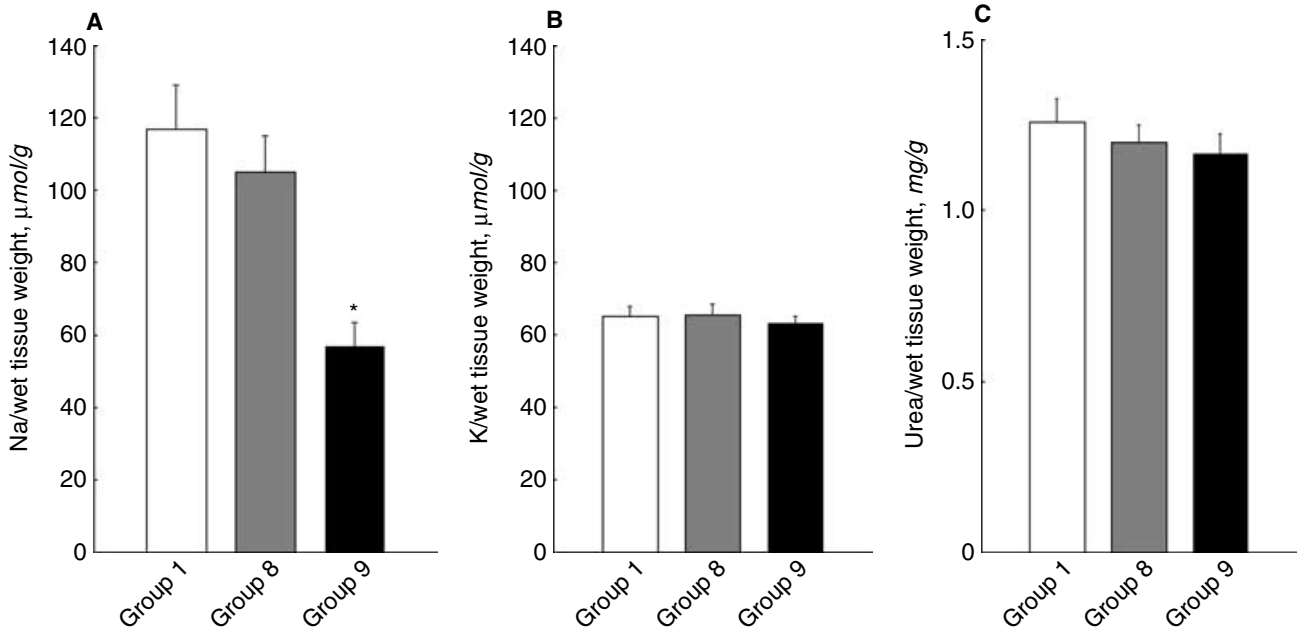

Fig. 6. Electrolyte and urea accumulation in renal medulla. Rats of groups 1 (5 mg/kg OPC-31260), 8 (5 mg/kg OPC-31260 and 30 mg/kg furosemide), and 9 (5 mg/kg OPC-31260 and 100 mg/kg furosemide) were examined. (*A*) Sodium. $^{*}P < 0.05$ compared to rats of group 1. (*B*) Potassium. (*C*) Urea. Data represent the mean ± SEM of 6 determinations in each condition. Differences among the treatment groups were analyzed by ANOVA, followed by Dunnett's test or Student *t* test.

ameliorated (Fig. 2A). The weight gain recovered thereafter under the constant hyponatremic condition. The body weights of the rats administered OPC-31260 are shown in Figure 7A. Whereas the rats administered 5 mg/kg OPC-31260 continued to gain weight, those administered 10 mg/kg OPC-31260 showed a transient decrease. On the other hand, the rats administered 15 mg/kg OPC-31260 showed progressive weight loss, and half of them died during the course of treatment. The rats administered 20 or 30 mg/kg OPC-31260 showed further weight loss (data not shown), and most of them died. The body weight of the rats administered furosemide (Fig. 7B) or

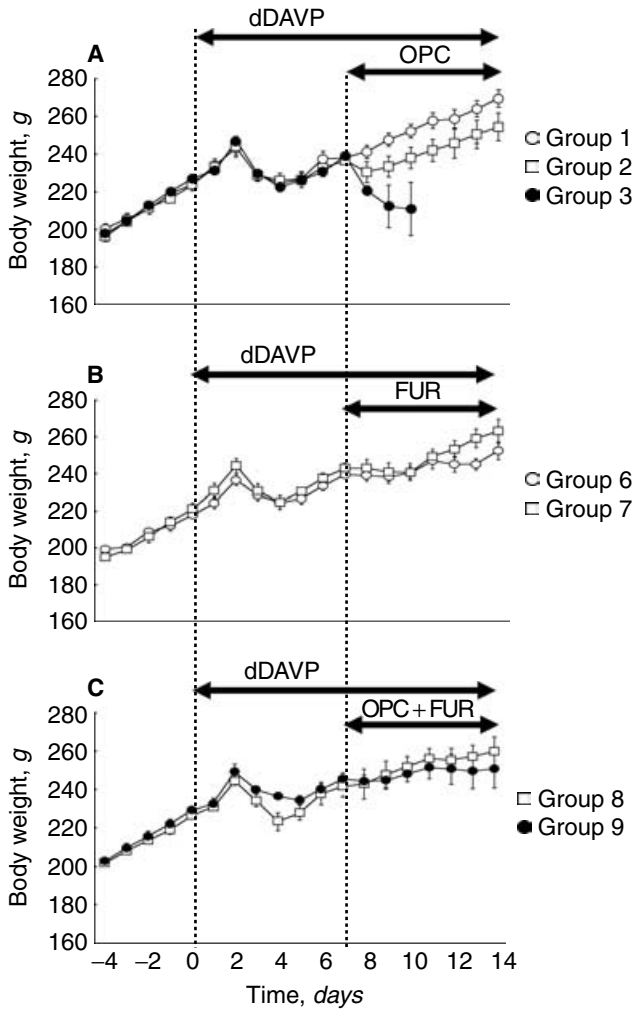

Fig. 7. Body weight of SIADH rats before and during treatment. (*A*) OPC-31260 alone. Open circles, group 1, OPC-31260 5 mg/kg; open squares, group 2, OPC-31260 10 mg/kg; filled circles, group 3, OPC-31260 15 mg/kg. (*B*) Furosemide alone. Open circles, group 6, furosemide 30 mg/kg; open squares, group 7, furosemide 100 mg/kg. (*C*) Combination of OPC-31260 and furosemide. Open squares, group 8, OPC-31260 5 mg/kg and furosemide 30 mg/kg; filled circles, group 9, OPC-31260 5 mg/kg and furosemide 100 mg/kg. Data represent the mean ± SEM of 6 determinations in each condition. Abbreviations are: OPC, OPC-31260; FUR, furosemide; dDAVP, 1-deamino-8-D-arginine vasopressin.

subjected to combination therapy (Fig. 7C) did not show any abnormal weight loss.

Changes in urine volume, urine osmolality, and water balance. Figures 8A and B show the time courses of urine volume and urine osmolality, respectively. To further analyze body fluid condition, water balance was determined by subtracting urine volume from water intake, as shown in Figure 8C. Urine volume was markedly reduced and urine osmolality was markedly increased on day two (Fig. 8A and B), when both AQP2 and rBSC1 were strongly expressed (Figs. 2B and 3). The fact that urine volume and urine osmolality gradually recovered thereafter (Fig. 8A and B) is physiologic evidence of vasopressin escape; this was also supported by the decline in AQP2 and rBSC1 protein expression (Figs. 2B and 3). The transient increase in water balance on day one or two (Fig. 8C) coincided with the increase in weight gain (Fig. 7), producing dilutional hyponatremia (Fig. 2A). These changes in urine volume, urine osmolality, and water balance are consistent with the results of previous studies [5, 7, 18, 29], and confirm the physiologic propriety of the rats as models of SIADH.

To determine the influence of therapeutic intervention on the above parameters, we continued to measure the parameters after administration of OPC-31260 and/or furosemide. In rats subjected to OPC-31260 administration or combination therapy, a rapid increase in urine volume (Fig. 8A) and a corresponding decrease in urine osmolality (Fig. 8B) were noted one day after OPC-31260 administration. These changes, however, were transient and absent in rats administered furosemide alone. These differences in the results of urinalysis may be related to the different patterns of elevating serum sodium (Fig. 4) between OPC-31260 and furosemide. In rats administered more than 10 mg/kg OPC-31260, water balance was decreased significantly within two days after the administration (Fig. 8C). Although no data are shown, all rats administered more than 20 mg/kg OPC-31260, and half of the rats administered 15 mg/kg OPC-31260, showed severe decreases in water balance and expired during the observation period, indicating that a high dose of OPC-31260 may induce marked reduction of intake, resulting in severe dehydration and malnutrition. In contrast, in rats subjected to furosemide administration or combination therapy with 5 mg/kg OPC-31260, no changes in any of these parameters were observed.

Changes in net sodium and potassium balance. Furosemide causes natriuresis in normal rats with euhydration [25]. This natriuretic effect of furosemide is considered to counter the therapeutic effects in the SIADH rats. Thus, we investigated the presence or absence of inappropriate natriuresis induced by furosemide administration in SIADH rats by analyzing sodium and potassium balance (Fig. 9). Net daily sodium and potassium balance was determined by subtracting urine output from dietary intake. As shown in Figure 9A, a positive net sodium balance was maintained during the course of treatment in all the treatment groups. A transient increase in the positive net sodium balance was noted in rats administered OPC-31260 alone or subjected to combination therapy one day after drug administration, which might have contributed to a rapid and large elevation of serum sodium and its subsequent decline (Fig. 4A and C). On the other hand, there were no significant changes in kaliuresis in all the treatment groups (Fig. 9B). These indicate that furosemide, as well as OPC-31260, does not have any specific natriuretic effects on SIADH rats. The

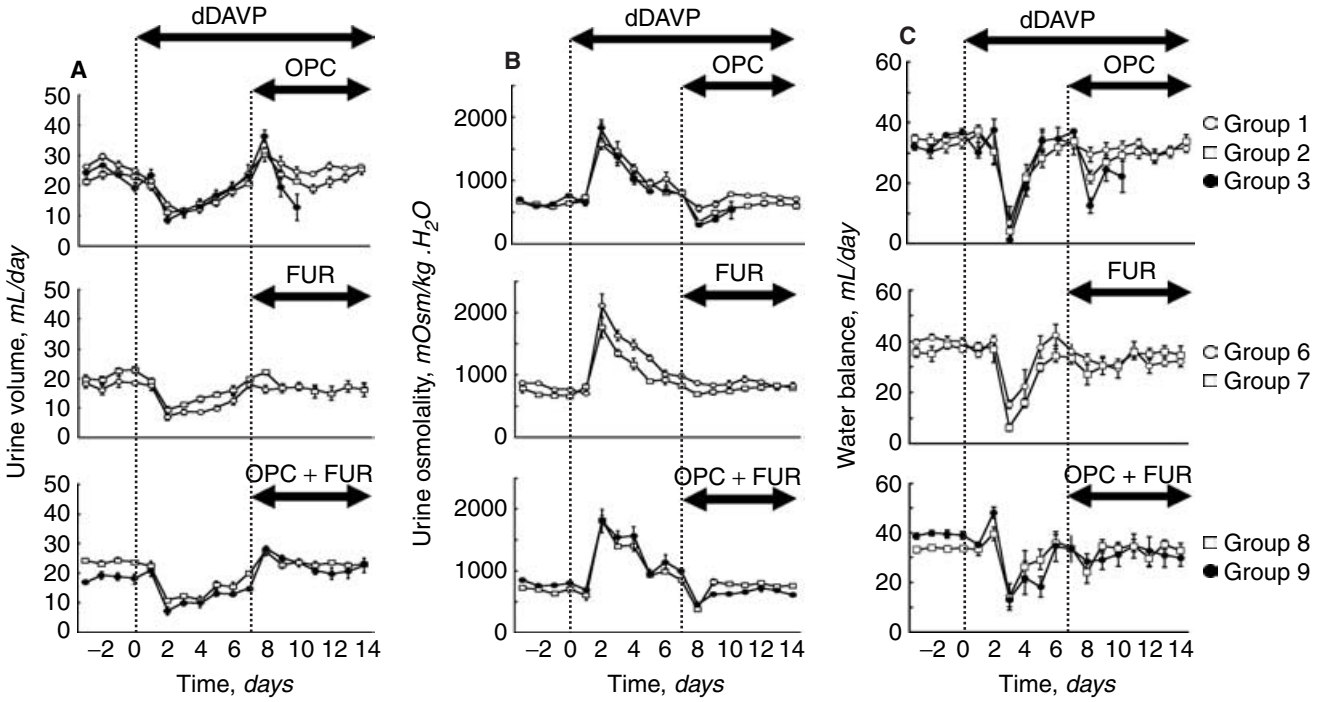

Fig. 8. Urinalysis before and during treatment. (*A*) Urine volume. (*B*) Urine osmolality. (*C*) Water balance. Top figure represents OPC-31260 treatment: open circles, group 1, OPC-31260 5 mg/kg; open squares, group 2, OPC-31260 10 mg/kg; filled circles, group 3, OPC-31260 15 mg/kg. Middle figure represents furosemide treatment: open circles, group 6, furosemide 30 mg/kg; open squares, group 7, furosemide 100 mg/kg. Bottom figure represents combination therapy: open squares, group 8, OPC-31260 5 mg/kg and furosemide 30 mg/kg; filled circles, group 9, OPC-31260 5 mg/kg and furosemide 100 mg/kg. Data represent the mean ± SEM of 6 determinations in each condition. Abbreviations are: OPC, OPC-31260; FUR, furosemide; dDAVP, 1-deamino-8-D-arginine vasopressin.

combination of those two drugs does not have any natriuretic effects either. In contrast, OPC-31260 showed transient sodium sparing effect one day after the initial drug administration.

Serum levels of urea, creatinine, and potassium. Serum levels of urea, creatinine, and potassium were measured before and after drug administration in rats that were alive at the end of the observation period. Neither OPC-31260 nor furosemide altered the serum levels of creatinine and potassium. Even in rats administered 15 mg/kg OPC-31260, the three surviving rats showed no alteration of these parameters. Serum urea levels, however, were slightly but significantly ($P < 0.05$) elevated in rats administered OPC-31260 (from 11.1 ± 0.4 to 14.2 ± 0.7 mg/dL and from 11.4 ± 0.5 to 14.6 ± 0.9 mg/dL in rats administered 5 mg/kg and 10 mg/kg OPC-31260, respectively), whereas it remained unchanged in rats administered furosemide alone (from 11.2 ± 0.6 to 12.5 ± 0.6 mg/dL in rats administered 100 mg/kg furosemide). This difference may also be induced by the transient decrease in water balance (Fig. 8C) and the increase in urine volume (Fig. 8A) one day after OPC-31260 administration.

Changes in protein expression after OPC-31260 administration

All the physiologic parameters examined indicated the rapid appearance of the effects of OPC-31260, which supported the rapid and strong therapeutic effects on hyponatremia. Such physiologic alterations, however, were transient, and the therapeutic effects were also decreased with increasing number of treatment days. To further investigate the mechanisms underlying these therapeutic limitations of OPC-31260, we closely examined AQP2 and BSC1 protein expression during OPC-31260 administration (Fig. 10). Western blots demonstrated a rapid and marked reduction of the expression of both AQP2 and rBSC1 three hours after the initial administration of 5 mg/kg OPC-31260. The expression, however, was recovered 24 hours after the initial administration, followed by further enhancement of the expression of both AQP2 and rBSC1 three and seven days after the initial administration. We confirmed the lack of alterations in these proteins' expression by furosemide treatment (data not shown).

DISCUSSION

In the present study, we investigated rBSC1 expression in rat with hyponatremia, which has been well established as a disease model for SIADH in previous studies [5, 7, 18, 29]. To confirm the presence of reported physiologic reactions during the development of the disease, we closely examined serum sodium level, AQP2 protein expression, and the results of urinalysis. Our examinations revealed

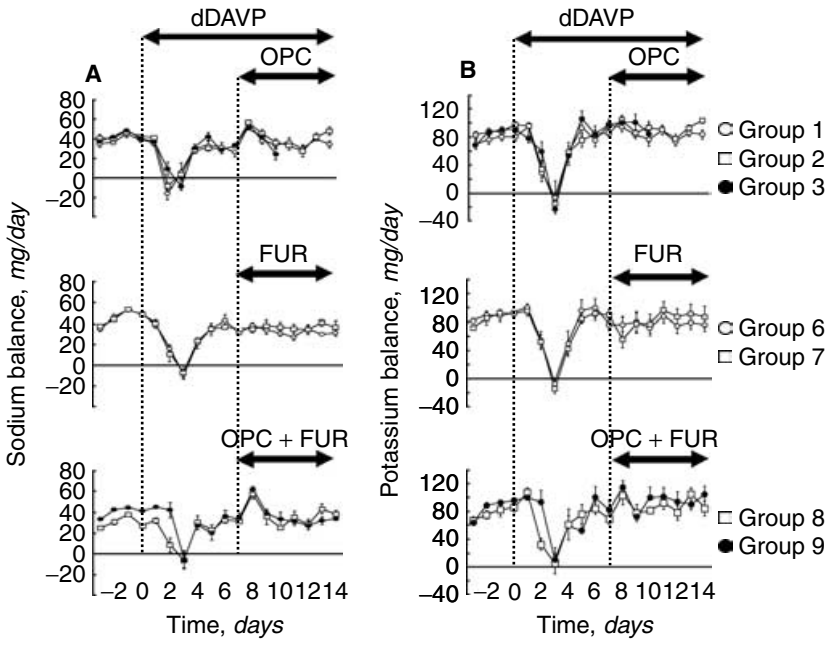

Fig. 9. Electrolyte balance before and during treatment. (*A*) Sodium. (*B*) Potassium. Top figure represents OPC-31260 treatment: open circles, group 1, OPC-31260 5 mg/kg; open squares, group 2, OPC-31260 10 mg/kg; filled circles, group 3, OPC-31260 15 mg/kg. Middle figure represents furosemide treatment: open circles, group 6, furosemide 30 mg/kg; open squares, group 7, furosemide 100 mg/kg. Bottom figure represents combination therapy: open squares, group 8, OPC-31260 5 mg/kg and furosemide 30 mg/kg; filled circles, group 9, OPC-31260 5 mg/kg and furosemide 100 mg/kg. Data represent the mean ± SEM of 6 determinations in each condition. Abbreviations are: OPC, OPC-31260; FUR, furosemide; dDAVP, 1-deamino-8-D-arginine vasopressin.

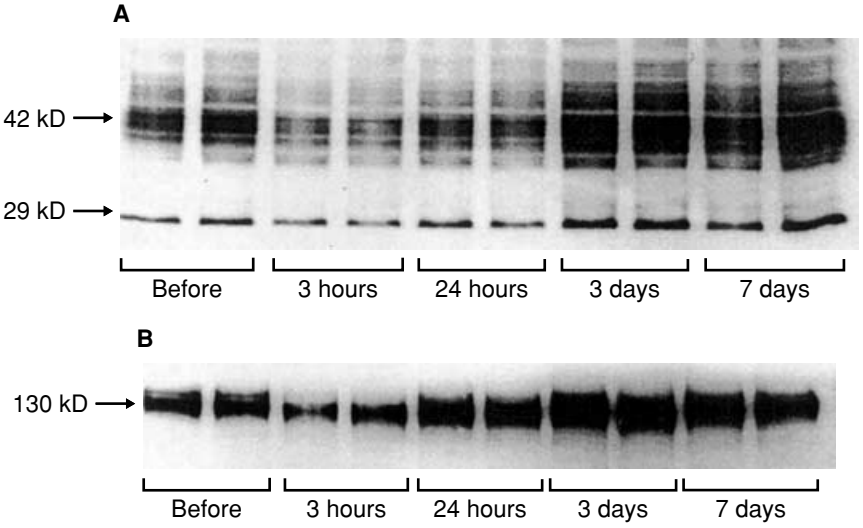

Fig. 10. Protein expression during OPC-31260 (5 mg/kg) treatment. (*A*) AQP2 protein expression. Twenty-five μg of protein extracted from the renal inner medulla was loaded in each lane. (*B*) rBSC1 protein expression. Twenty-five μg of protein extracted from the renal outer medulla was loaded in each lane. Rats before the initial administration and 3 hours, 24 hours, 3 days, and 7 days after the initial administration were examined.

that the onset of SIADH involves a combination of excessive water intake and exogenous dDAVP infusion, as well as the presence of vasopressin escape, in response to continuous exposure to high levels of plasma AVP. Using this rat model for SIADH, we observed a high rBSC1 protein expression after dDAVP infusion. Although rBSC1 expression is promoted by multiple mechanisms [17, 32, 33], AVP is one of the strong stimulators of its expression [17], and specific conditions leading to high rBSC1 expression, such as dehydration [15, 17], heart disease [34], and liver disease [35] were absent in the rats of the present study. In addition, rBSC1 expression was altered consistently with the alteration of AQP2 expression: (*1*) rBSC1 expression was markedly enhanced by dDAVP infusion, and (*2*) it was markedly reduced by the administration of an AVP V_2 receptor antagonist. These results indicate that rBSC1 expression is regulated by an AVP-mediated mechanism in the rats of the present study. As we also demonstrated the increased sodium and urea accumulation in renal medulla leading to water reabsorption, the high rBSC1 expression by AVP stimulus may also participate in the development of hyponatremia in the SIADH rats.

Serum sodium level was progressively lowered with the elevation of urinary osmolality and the reduction of urine volume for a period of two to three days after the start of dDAVP infusion. Then, the decrease of serum sodium level stopped at the lowest hyponatremia of nearly 100 mmol/L on day three, and serum sodium level was settled a little higher than that from day five by reducing the

physiologic responses to AVP stimulus. This reduction is recognized as vasopressin escape. Recently, Saito et al presented direct evidence of the reduced response to AVP stimulus by showing diminished AQP2 expression during vasopressin escape [5]. In the present study, we found that rBSC1 expression at this stage of SIADH is also less than that of the lowest hyponatremia on day two. As rBSC1 expression influences the corticomedullary osmotic gradient leading to urinary concentration [17], this alteration of rBSC1 expression is thought to explain the previous finding of dissipation of the osmotic gradient during vasopressin escape [36]. Vasopressin escape is therefore considered to be induced by the decreased expression of both AQP2 and rBSC1.

We investigated the therapeutic intervention based on the present finding that the expressional alteration of rBSC1 is involved in the pathogenesis of SIADH. We started by reexamining the therapeutic effects of OPC-31260, an AVP V_2 receptor antagonist. OPC-31260 is the first nonpeptide V_2 receptor antagonist that possesses high selectivity for the V_2 receptor [25]. In the SIADH rats, the high expression of both AQP2 and rBSC1 is thought to be induced by AVP stimulus, as mentioned above. Thus, OPC-31260, which blocks the effects of AVP, may reduce the expression of these cell surface proteins, theoretically leading to the remission of the disease. As expected, this drug elevated serum sodium level in a dose-dependent manner. High doses of OPC-31260 exceeding 15 mg/kg, however, showed severe toxicity. Fujisawa et al used the same drug and reported therapeutic effects at 5 mg/kg OPC-31260 and severe toxicity at 30 mg/kg, which was attributable to brain myelinolysis as a result of rapid correction of hyponatremia [29], as was histopathologically demonstrated by Ayus et al [37]. However, they did not describe the effects of doses between 5 and 30 mg/kg. Our present study revealed that OPC-31260 at as low a dose as 10 mg/kg led to the development of neurologic disorder in one of six rats. Thus, the low dose of 5 mg/kg was thought to be the upper limit for the therapeutic use of this drug. The effects of a low dose of OPC-31260, on the other hand, were slow and weak, and a gradual decrease of serum sodium level was noted after it reached a maximum (on day two of the treatment). The significant effects on urinary osmolality and urine volume were also transient. In addition, although electrolytes and urea accumulation in renal medulla decreased transiently three hours after the initial administration, it returned at the end of observation period to the level before treatment. These results indicate the therapeutic limitations of the treatment with OPC-31260 alone for SIADH. Although the therapeutic efficacy and the safety of OPC-31260 have been reported in SIADH patients [27, 38], there are some conditional differences between clinical trials and our present experimental models. First, the clinical trials showed the effects of a single administration of this drug within a short observation period, and thus, the influence of long-term OPC-31260 treatment remains to be determined, as was demonstrated with conivaptan, a nonpeptide V_{1A} and V_2 receptor antagonist [39]. Second, our experimental models underwent extremely low-serum sodium, whereas SIADH patients in the clinical trials demonstrated higher serum sodium. Thus, large alteration in serum sodium might lead to the potential brain myelinolysis in our experimental models.

To assess the mechanisms underlying the therapeutic limitations of OPC-31260, we examined AQP2 and rBSC1 expression before and after OPC-31260 treatment (Fig. 10). OPC-31260 markedly reduced both AQP2 and rBSC1 protein expression three hours after the initial administration, similar to the finding of Fujita et al that AQP2 expression was reduced three hours after OPC-31260 administration [7]. The expression, however, recovered within 24 hours to almost the same extent as that before the treatment, and was further intensified on days three and seven in spite of continuous treatment with OPC-31260. These changes were consistent with the transient increase of hypotonic urine during the 24-hour period after the initial administration. These results suggest that OPC-31260 shows therapeutic effects soon after administration, but the blockade of the AVP V_2 receptor may discontinue the physiologic reactions to continuous AVP stimulus, resulting in the dissipation of vasopressin escape. The reelevation of rBSC1 and AQP2 expression is therefore thought to be induced by the competitive effects of excess dDAVP. Therefore, AVP V_2 receptor antagonists, such as OPC-31260, might limit their own therapeutic effects in the SIADH rats by diminishing the physical adaptation by vasopressin escape. On the other hand, the administration of a higher dose that completely blocks AVP stimulus may induce severe systemic toxicity.

After confirming the therapeutic limitations of the AVP V_2 receptor blocker for the treatment of SIADH, we investigated other possible treatment regimens based on the expressional alteration of rBSC1. Although the increased level of rBSC1 expression by dDAVP infusion was decreased due to vasopressin escape, its expression under the vasopressin escape condition was still higher than that under the basal condition, indicating that the enhanced sodium transport through this transporter resulted in excessive water reabsorption, leading to the maintenance of hyponatremia in the SIADH rats. Thus, we attempted to reduce this sodium transport by administering furosemide, a loop diuretic. The clinical usefulness of furosemide in SIADH patients has been demonstrated by the amelioration of hyponatremic symptoms without causing any significant side effects [28]. In most cases, however, possibly due to the negative sodium balance during the treatment [28], the patients had compensatory salt intake. In the present study, the treatment with furosemide without changing dietary salt

intake elevated serum sodium level in a dose-dependent manner, although the elevation was slower and smaller than that of a low dose of OPC-31260. Neither toxic effects nor disruption of sodium balance was noted even when a high dose of furosemide (100 mg/kg) was administered, indicating the beneficial effects of this treatment on the SIADH rats. As the functional inhibition of rBSC1 by furosemide is considered to have increased sodium delivery to the distal convoluted tubules, compensatory sodium reabsorption through the thiazide-sensitive cotransporter (TSC) and the epithelial sodium channel (ENaC) is thought to be induced in the distal convoluted tubules and the cortical collecting ducts [40, 41]. This adaptation may work adequately in furosemide treatment of SIADH.

Finally, we designed a combination therapy using a low dose of OPC-31260 and furosemide, expecting the compensational effects of furosemide on the possible discontinuation of vasopressin escape by OPC-31260 as well as the additive therapeutic effects. The results demonstrated the presence of the additive effect on serum sodium elevation at both doses of furosemide, 30 and 100 mg/kg, although they were extremely high doses. Furthermore, the highly elevated serum sodium level mainly by OPC-31260 treatment was well maintained thereafter by administering a high dose of furosemide (100 mg/kg). We measured the accumulated levels of sodium and urea in the renal medulla of rats subjected to the combination therapy (Fig. 6), and demonstrated that medullary sodium accumulation was significantly reduced by 100 mg/kg furosemide. Therefore, in the combination therapy, the acute reduction of both AQP2 and rBSC1 protein expression by a low dose of OPC-31260 leads to a rapid and marked elevation of serum sodium levels in the short term, after which furosemide may work to conserve the elevated serum sodium level by inhibiting the countercurrent multiplier enhanced by the reappearance of rBSC1 and AQP2.

Since the original report of SIADH by Bartter and Schwartz [4], SIADH has been described in an increasing number of clinical settings, such as malignancies, pulmonary diseases, central nervous system disorders, and administration of certain drugs. Excessive AVP stimuli underlie the development of SIADH in those diseases in specific conditions, and therefore, the treatment involves the exclusion of such AVP stimuli by inducing the remission of primary diseases or the cessation of medication. In most cases, however, such complete treatment is difficult, and fluid restriction is the mainstay of the treatment for SIADH patients. However, the clinical effects of fluid restriction have been limited, in part, by the poor compliance of the patients. Therefore, therapeutic medication, if any, may help SIADH patients improve their quality of life. The present study suggests that the combination of a low dose of an AVP V_2 receptor antagonist and furosemide ameliorates hyponatremia in SIADH patients.

CONCLUSION

We have demonstrated the involvement of the expressional alteration of rBSC1 in both the onset of SIADH and the subsequent vasopressin escape. We have also demonstrated the therapeutic limitations of the AVP V_2 receptor antagonist, as the V_2 receptor antagonist limits its own therapeutic effects possibly by discontinuing the physiologic reactions of vasopressin escape. Our present study on therapeutic intervention suggests that the combination of a low dose of a V_2 receptor antagonist and a loop diuretic most effectively ameliorates hyponatremia in SIADH patients.

ACKNOWLEDGMENTS

This work was supported by research grants for scientific research (12877163) from the Ministry of Science and Education.

Reprint requests to Mitsunobu Matsubara, M.D., Ph.D., Division of Molecular Medicine, Center for Translational and Advanced Research, Tohoku University School of Medicine, 2-1 Seiryo-cho, Aoba-ku, Sendai 980-8575, Japan.
E-mail: mmitsu2i@mail.tains.tohoku.ac.jp

REFERENCES

1. Anderson RJ, Chung H, Kluge R, Schrier RW: Hyponatremia: A prospective analysis of its epidemiology and the pathogenetic role of vasopressin. *Ann Intern Med* 102:164–168, 1985
2. Gross P, Reimann D, Neidel J, *et al*: The treatment of severe hyponatremia. *Kidney Int* (Suppl 64):S6–11, 1988
3. Zerbe R, Stropez L, Robertson G.: Vasopressin function in the syndrome of inappropriate antidiuresis. *Annu Rev Med* 31:315–327, 1980
4. Bartter FC, Schwartz WB: The syndrome of inappropriate secretion of antidiuretic hormone. *Am J Med* 42:790–806, 1967
5. Saito T, Higashiyama M, Nagasaka S, *et al*: Role of aquaporin-2 gene expression in hyponatremic rats with chronic vasopressin-induced antidiuresis. *Kidney Int* 60:1266–1276, 2001
6. Ecelbarger CA, Nielsen S, Olson BR, *et al*: Role of renal aquaporins in escape from vasopressin-induced antidiuresis in rat. *J Clin Invest* 99:1852–1863, 1977
7. Fujita N, Ishikawa S, Sasaki S, *et al*: Role of water channel AQP-CD in water retention in SIADH and cirrhotic rats. *Am J Physiol* 269:F926–931, 1995
8. Hozawa S, Holtzman EJ, Ausiello DA: cAMP motifs regulating transcription in the aquaporin 2 gene. *Am J Physiol* 270:C1695–1702, 1996
9. Jamison RL, Maffly RH: The urinary concentrating mechanism. *N Engl J Med* 295:1059–1067, 1976
10. Molony DA, Reeves WB, Andreoli TE: Na^+:K^+:2Cl- cotransporter and the thick ascending limb. *Kidney Int* 36:418–426, 1989
11. Hayashi M, Sasaki S, Tsuganezawa H, *et al*: Expression and distribution of aquaporin of collecting duct are regulated by vasopressin V_2 receptor in rat kidney. *J Clin Invest* 94:1778–1783, 1994
12. Yamamoto T, Sasaki S, Fushimi K, *et al*: Vasopressin increases AQP-CD water channel in apical membrane of collecting duct cells in Brattleboro rats. *Am J Physiol* 269:C655–C664, 1995
13. Shyayakul C, Smith CP, Mackenzie HS, *et al*: Long-term regulation of urea transporter expression by vasopressin in Brattleboro rats. *Am J Physiol* 278:F620–F627, 2000

14. Michimata M, Nogae S, Ohta M, *et al*: Topographic distribution of aquaporin 2 mRNA in the kidney of dehydrated rats. *Exp Nephrol* 8:28–36, 2000
15. Marumo R., Kaizuma S, Nogae S, *et al*: Differential upregulation of rat Na-K-Cl cotransporter, rBSC1, mRNA in the thick ascending limb of Henle in different pathophysiological conditions. *Kidney Int* 54:877–888, 1998
16. Michimata M, Wang W, Fujita S, *et al*: Limited urinary concentration and damaged tubules in rats with a syngeneic kidney graft. *Kidney Int* 60:672–679, 2001
17. Michimata M, Mizukami H, Kazama I, *et al*: Vasopressin independent urinary concentration: Increased rBSC1 expression and enhanced countercurrent multiplier. *Kidney Int* 64:933–938, 2003
18. Verbalis JG: An experimental model of syndrome of inappropriate antidiuretic hormone secretion in the rat. *Am J Physiol* 247:E540–553, 1984
19. Robertson GL, Aycinema P, Zerbe RL: Neurogenic disorders of osmoregulation. *Am J Med* 72:339–353, 1982
20. de Troyer A: Demeclocycline: Treatment for syndrome of inappropriate antidiuretic hormone secretion. *JAMA* 237:2723–2726, 1977
21. Cogan E, Abramow M: Inhibition by lithium of the hydroosmotic action of vasopressin in the isolated perfused cortical collecting tubule of the rabbit. *J Clin Invest* 77:1507–1514, 1986
22. Forrest JN, Cox M, Hong C, *et al*: Superiority of demeclocycline over lithium in the treatment of chronic syndrome of inappropriate secretion of antidiuretic hormone. *N Engl J Med* 298:173–177, 1978
23. Miller PD, Linas SL, Schrier RW: Plasma demeclocycline levels and nephrotoxicity: Correlation in hyponatremic cirrhotic patients. *JAMA* 243:2513–2515, 1980
24. Maning M, Sawyer WH: Discovery, development, and some uses of vasopressin and oxytocin antagonists. *J Lab Clin Med* 114:617–632, 1989
25. Yamamura Y, Ogawa H, Yamashita H, *et al*: Characterization of a novel aquaretic agent, OPC-31260, as an orally effective, nonpeptide vasopressin V_2 receptor antagonist. *Br J Pharmacol* 105:787–791, 1992
26. Ohnishi A, Orita Y, Okahara R, *et al*: Potent aquaretic agent. A novel nonpeptide selective vasopressin 2 antagonist (OPC-31260) in men. *J Clin Invest* 92:2653–2659, 1993
27. Saito T, Ishikawa S, Abe K, *et al*: Acute aquaresis by the nonpeptide arginine vasopressin (AVP) antagonist OPC-31260 improves hyponatremia in patients with syndrome of inappropriate secretion of antidiuretic hormone (SIADH). *J Clin Endocrinol Metab* 82:1054–1057, 1997
28. Decaux G.: Treatment of the syndrome of inappropriate secretion of antidiuretic hormone by long loop diuretics. *Nephron* 35:82–88, 1983
29. Fujisawa G, Ishikawa S, Tsuboi Y, *et al*: Therapeutic efficacy of non-peptide ADH antagonist OPC-31260 in SIADH rats. *Kidney Int* 44:19–23, 1993
30. Michimata M, Fujita S, Araki T, *et al*: Reverse pharmacological effect of loop diuretics and altered rBSC1 expression in rats with lithium nephropathy. *Kidney Int* 63:165–171, 2003
31. Michimata M, Kazama I, Mizukami K, *et al*: Urinary concentration defect and limited expression of sodium cotransporter, rBSC1, in a rat model of chronic renal failure. *Nephron Physiol* 93:34–41, 2003
32. Fernandez-Llama P, Ecelbarger CA, Ware JA, *et al*: Cyclooxygenase inhibitors increase Na-K-2Cl cotransporter abundance in thick ascending limb of Henle's loop. *Am J Physiol* 277:F219–226, 1999
33. Attmane-Elakeb A, Sibella V, Vernimmen C, *et al*: Regulation by glucocorticoids of expression and activity of rBSC1, the Na+-K+-2Cl-cotransporter of medullary thick ascending limb. *J Biol Chem* 275:33548–33553, 2000
34. Nogae S, Michimata M, Kanazawa M, *et al*: Cardiac infarcts increase sodium transporter transcripts (rBSC1) in the thick ascending limb of Henle. *Kidney Int* 57:2055–2063, 2000
35. Jonassen TE, Brond L, Torp M, *et al*: Effects of renal denervation on tubular sodium handling in rats with CBL-induced liver cirrhosis. *Am J Physiol Renal Physiol* 284:F555–563, 2003
36. Chan WY: A study of the mechanism of vasopressin escape: Effects of chronic vasopressin and overhydration on renal tissue osmolality and electrolytes in dogs. *J Pharmacol Exp Ther* 184:244–252, 1973
37. Ayus JC, Krothapalli RK, Armstrong DL: Rapid correction of severe hyponatremia in the rat: Histopathological changes in the brain. *Am J Physiol* 248:F711–719, 1985
38. Kamoi K: Syndrome of inappropriate antidiuresis without involving inappropriate secretion of vasopressin in an elderly woman: Effect of intravenous administration of the non-peptide vasopressin V_2 receptor antagonist OPC-31260. *Nephron* 76:111–115, 1997
39. Decaux G: Long-term treatment of patients with inappropriate secretion of antidiuretic hormone by the vasopressin receptor antagonist conivaptan, urea, or furosemide. *Am J Med* 110:582–584, 2001
40. Santon BA, Kaissling B: Adaptation of distal tubule and collecting duct to increased Na delivery. Na+ and K+ transport. *Am J Physiol* 255:F1269–F1275, 1988
41. Kaissling B, Santon BA: Adaptation of distal tubule and collecting duct to increased Na delivery. Ultrastructure. *Am J Physiol* 255:F1256–F1268, 1988