Low pH stimulates vasopressin V2 receptor promoter activity and enhances downregulation induced by V1a receptor stimulation

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Submitted 27 August 2008; accepted in final form 1 July 2009

MATERIALS AND METHODS

ARGinine VASOPRESSIN (AVP) plays a crucial role in the regulation of urine volume and solute excretion by the kidney. There are two types of AVP receptors in the thick ascending limb and collecting duct, V1a and V2 receptors (V1aR and V2R, respectively). AVP is involved in the modulation of urine volume and solute excretion by the kidney. Metabolic acidosis is defined in terms of a decrease in plasma bicarbonate and blood pH resulting from various clinical conditions, such as chronic renal failure, renal tubular acidosis, and uncontrolled diabetes. The acid-base balance is regulated by H⁺ excretion and HCO₃⁻ reabsorption in various nephron segments. Acidosis also has been suggested to affect water and electrolyte metabolism. Previously, we suggested (24) that intrarenal AVP could serve as an intrinsic diuretic, especially in patients with chronic renal failure. Cogan and Rector (7) reported that acidosis reduced water reabsorption in the proximal tubules. Cheval et al. (6) reported that urine volume was increased by twofold in acidic mice despite the increase of V2R expression. Musa-Aziz et al. (21) reported that V1aR and V2R were involved in bicarbonate reabsorption. These findings suggest that acidosis influences water and electrolyte metabolism regulated by V1aR and V2R.

Previously, we demonstrated (31) increase of V1aR expression and decrease of V2R expression in rat metabolic acidosis model. However, there are few reports describing the effect of pH on V1aR and V2R expression in vitro. The purpose of the present study was to investigate the regulatory mechanism of V2R and the effect of V1aR stimulation on V2R expression under acidic conditions. We examined the effect of low pH on V2R expression at the transcription, mRNA, and protein levels and inhibition of V2R promoter activity by V1aR stimulation in an LLC-PK₁ cell line stably expressing rat V1aR cells (LLC-PK₁/rV1aR cells). We also tested the effect of mitogen-activated protein kinases (MAPKs) and protein kinase A (PKA) on V2R promoter activity to analyze the regulatory mechanism of V2R expression under low-pH conditions.

Reagents. [Arg⁸]vasopressin, SP-600125, SB-203580, U-0126, H-89, 8-bromoadenosine 3′,5′-cyclic monophosphate (8-BrcAMP), and 3-isobutyl-1-methylxanthine (IBMX) were purchased from Sigma-Aldrich (St. Louis, MO). The Dual Luciferase Reporter Assay System was purchased from Promega (Madison, WI). The CAMP enzyme...
immunoassay system kit was purchased from GE Healthcare (Tokyo, Japan). The primers used for quantitative real-time RT-PCR (Sus scrofa V2R: SS 03383500, human 18S rRNA: 4319413E as internal control) were purchased from Applied Biosystems (Foster City, CA). Anti-V2R was kindly given by Dr. Robert Fenton and Dr. Søren Nielsen (University of Aarhus, Aarhus, Denmark).

**Cell culture.** LLC-PK/rV1aR cells were established as described in the previous report (15). Cells were maintained in a humidified atmosphere at 37°C with 5% CO₂-95% O₂. Cells were cultured on 10-cm plates in Dulbecco’s modified Eagle’s medium ( Gibco, Invitrogen) containing 25 mM d-glucose with 10% fetal bovine serum (FBS) and 0.8 mg/ml hygromycin B (Invitrogen).

**Reporter plasmid construction.** The 1092 bp 5'-flanking region of the rat V2R (rV2R) gene was cloned by PCR amplification from rat genomic DNA and was subcloned into a pGL3 luciferase reporter vector as described previously (15, 22). An Expand High FidelityPCR system (Roche Applied Science, Penzberg, Germany) was used for PCR amplification. The sequence of deletion mutants was synthesized by PCR using sense primers designed to downstream regions (−345, −165, −124, −62, and −52 bp of the rV2R promoter element) and antisense primers on exon 1 (+57 bp); the transcription start site was indicated as 0.

Since the Sp1 site in the V2R promoter region was speculated to be the pH-responsive site, a Sp1-mutated fragment (−124 to +57) was prepared by PCR amplification as described in a previous report (22). The Sp1 consensus sequence, 5'-GAG GCT GGG TTG CGG GGC CAA-3', was mutated to 5'-GAG GCT GGG TTG AAT ATT GGC CAA-3’ (see Fig. 5A) according to the previous report by Hwang and Ismail-Beigi (14).

**Transfection and determination of promoter activity.** Cells were seeded in 12-well plates and grown to 50–60% confluence. LLC-PK/rV1aR cells were cotransfected with 3.75 fmol of PRL-TK constructs and 0.314 pmol of pGL3-rV2R promoter constructs with FuGENE 6 transfection reagent (Roche Applied Science, Indianapolis, IN). After 18 h, medium was switched to a pH-modified solution (Table 2) was prepared instead of bicarbonate-buffered solution to examine whether pH or bicarbonate affects V2R promoter activity. The pH of the HEPES-buffered solution was adjusted by the addition of NaOH. Hypertonic solution (490 mosmol/kgH₂O) was prepared by the addition of NaCl to the pH 7.4 solution.

**RT reaction and real-time quantitative PCR.** Total RNA from LLC-PK/rV1aR cells was extracted with the RNeasy Mini Kit (Qiagen). cDNA was synthesized by the QuantiTect Reverse Transcription Kit (Qiagen). Real-time quantitative PCR was performed on a Lightcycler Roche480 (Roche Molecular System) with Taqman Universal PCR Master Kit (Roche Applied Biosystems) (1 cycle of 95°C for 10 min; 50 cycles of 95 °C for 10 s, 60 °C for 1 min, 50°C for 15 s). Threshold cycle (Ct) values for V2R (a 1494 bp amplicon spanning exon of the Sus scrofa V2R gene) were standardized against the internal ribosomal RNA (18S) control probe to calculate ΔCt values. ΔΔCt values were calculated according to a previous report (26). Fold change was calculated with the formula 2−ΔΔCt.

**Western blotting.** LLC-PK/rV1aR cells were grown in six-well plates at pH 7.4 or pH 6.7 for 24 h. Cells were washed twice with phosphate-buffered saline (PBS), and then the total protein was extracted with RIPA buffer (25 mM Tris-HCl pH 7.6, 150 mM NaCl, 1% sodium deoxycholate, 0.1% SDS; Pierce) supplemented with the protease inhibitor for each lysate at 1 μg/ml. After protein concentration was determined by bicinchoninic acid (BCA) protein assay (Pierce), 12 μg protein was used for electrophoresis in a 10% SDS polyacrylamide gel and transferred to polyvinylidene difluoride (PVDF) membrane by semidyblotting. Western blotting was performed with an ECL Advance Western Blotting detection kit (GE Healthcare). Membranes were blocked at room temperature for 1 h or overnight at 4°C in Tris-buffered saline with 0.1% Tween 20 (TBS-T) containing 2% ECL Advance Blocking agent and probed with primary antibody against the V2R protein (Bioray, Milpitas, CA).

**Measurements of cAMP generation.** LLC-PK/rV1aR cells were seeded in 96-well microplates with 6 × 10³ cells/ml and incubated in a humidified atmosphere at 37°C with 5% CO₂-95% O₂ for 24 h. To examine the effect of low pH and hypertonicity on AVP-stimulated cAMP accumulation, cells were preincubated at pH 7.4, pH 6.7, or pH 7.4 with hypertonicity (490 mosmol/kgH₂O) solution for 3 h. After preincubation, the solution was substituted with modified solution containing 0.5 mM IBMX (nonspecific phosphodiesterase inhibitor) (pH 7.4, pH 6.7, or pH 7.4 in 490 mosmol/kgH₂O). One hundred microliters of solution containing 10⁻⁸ M AVP or DMSO was added at 15 min after IBMX treatment. After 4-min incubation, 100 μl of 16.5% trichloroacetic acid (TCA) was added to stop the reaction and to extract intracellular cAMP. Samples were transferred to a 1.5-ml microcentrifuge tube and centrifuged at 3,000 rpm for 5 min at 4°C. Supernatant was stored at −20°C until the cAMP measurement. TCA was removed by diethyl ether saturated with distilled H₂O on the experimental day. cAMP was measured with a cAMP enzyme immunoassay system kit (GE Healthcare).

**Preparation of pH-modified solution.** The pH-modified solution was prepared as described in Table 1. The osmolality in each solution was adjusted by the addition of NaCl. HEPES-buffered solution (Table 2) was prepared instead of bicarbonate-buffered solution to examine whether pH or bicarbonate affects V2R promoter activity. The pH of the HEPES-buffered solution was adjusted by the addition of NaOH. Hypertonic solution (490 mosmol/kgH₂O) was prepared by the addition of NaCl to the pH 7.4 solution.

**Table 1. Composition of experimental solution medium**

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Concentrations are in millimolar.
at room temperature for 1 h. Primary antibody against V2R was diluted 1:5,000 with TBS-T. Afterward, membranes were incubated with secondary antibody, anti-rabbit IgG, horseradish peroxidase-linked F(ab’)_2 fragment from donkey (GE Healthcare), at room temperature for 1 h. Secondary antibody was diluted 1:100,000 with TBS-T. All blots were re-probed with an antibody against β-actin (Santa Cruz Biotechnology) (1:1,000 dilution) and secondary antibody, donkey anti-goat IgG-horseradish peroxidase (DAKOPATTS) (1:10,000, dilution). Proteins were detected with the ECL visualized detection kit (GE Healthcare) according to the manufacturer’s instructions. Intensity of the band was determined by densitometric analysis with ATTO Densitograph 4.0 (Densitograph ATTO). Experiments were repeated three times, and the expression of V2R was normalized by the ratio to β-actin.

Data analysis. Values are shown as means ± SD. Statistical analysis was performed by analysis of variance (ANOVA) and multiple comparisons with Bonferroni’s or Scheffe’s test, or by Student’s t-test with Dr. SPSS-II software version 11 (SPSS). P < 0.05 was considered statistically significant.

RESULTS

pH-dependent regulation of V2R promoter activity. After the transfection of the 1092 bp rV2R promoter region, LLC-PK1/rV1aR cells were incubated in the pH-modified solution. rV2R promoter activity was significantly increased under acidic conditions (Fig. 1). rV2R promoter activity at pH 6.7 was twofold higher than at pH 7.4 (P < 0.01). Since the increase of V2R promoter activity was more potent under pH 6.7 than pH 7.0 as shown in Fig. 1, we defined pH 6.7 as the standard pH for low-pH conditions in subsequent experiments to show the differences between neutral and acidic conditions. The time course study under pH 7.4 or pH 6.7 conditions revealed that rV2R promoter activity was increased in a time-dependent manner under the low-pH conditions (Fig. 2). rV2R promoter activity was significantly increased at 12 h and further continued at 24 h after incubation in a low-pH solution. Since the increase of V2R promoter activity was highest at 24 h, subsequent experiments for V2R promoter analysis were performed at 24 h after the incubation at the indicated pH.

Is V2R promoter activity dependent on pH or bicarbonate? To identify whether V2R promoter activity is regulated by pH or bicarbonate concentration, cells were incubated in HEPES-buffered or bicarbonate-containing solution, in which pH was adjusted to 7.4 or 6.7 (Table 2) after the transfection of rV2R promoter vector. There was no difference in rV2R promoter activity between HEPES- and bicarbonate-buffered solution at pH 7.4. rV2R promoter activity was increased in HEPES-buffered solution as well as in bicarbonate-buffered solution at pH 6.7 (Fig. 3). This result indicated that V2R promoter activity is dependent not on bicarbonate concentration but on pH.

Is the Sp1 site a pH-responsive site in the rV2R 5′-flanking region? To address the putative sequence regulated by the Sp1 site, we performed a deletion study method. Low pH-induced stimulation of rV2R promoter activity was observed in −1092, −345, −165, −124, and −62 bp promoter-pGL3 constructs (Fig. 4). This change was not observed in the −52 bp promoter vector.
Therefore, the consensus sequence of Sp1 located at −54 to −59 in the 5′-flanking region of rV2R was speculated to be a pH-responsive site. We next examined rV2R promoter activity with Sp1-mutated, 124 bp rV2R promoter-pGL3 constructs. However, the basal rV2R promoter activity at neutral conditions was reduced by Sp1 mutation, and Sp1-mutated rV2R promoter vector did not respond to the low pH (Fig. 5B).

Effect of V1aR stimulation on pH-induced rV2R promoter activity. Previously, we reported (15) that rV2R promoter activity is downregulated by long-term stimulation of V1aR. In the present study, we examined the effect of V1aR stimulation on V2R promoter activity under low-pH conditions. Cells were transfected with the −62 bp rV2R promoter-pGL3 constructs and were incubated with 10^{-8} M AVP for 24 h. rV2R promoter activity was significantly decreased by V1aR stimulation at pH 7.4, 7.0, and 6.7 (Fig. 6). The downregulation of V2R promoter activity by V1aR stimulation was not observed in an alkaline condition. Incubation at pH 6.7 showed the most remarkable decrease in V2R promoter activity by

![Diagram](http://ajprenal.physiology.org/)

**Fig. 3.** Effect of bicarbonate-free acidic solution on rV2R promoter activity. LLC-PK1/rV1aR cells transfected with the −1092 bp 5′-flanking region of the rV2R luciferase vector or pGL3-Basic vector were incubated 24 h with or without bicarbonate solution adjusted to pH 7.4 and 6.7. rV2R promoter activity was increased in both the presence and the absence of bicarbonate. n = 6. *P < 0.05 vs. pH 7.4.

**Fig. 4.** rV2R promoter activity in deletion series of 5′-flanking region of rV2R. Deletion series of the 5′-flanking region from −1092 bp to −52 bp of the rV2R luciferase vector or pGL3-Basic vector were transfected to LLC-PK1/rV1aR cells. The rV2R promoter activity of each fragment was measured at 24 h after incubation at pH 7.4 or pH 6.7. The pH-responsive site was limited to 10 bp including the Sp1 consensus sequence, which was located in a region from −62 to −52 bp in rV2R promoter. n = 3 for none; n = 6 for −52 to −1092 bp. *P < 0.05 vs. pH 7.4.

**Fig. 5.** Effect of Sp1 mutation on pH-induced rV2R promoter activity. A: replacement mutations of the Sp1 site in −124 to +57 bp of the 5′-flanking region of the rV2R were prepared by PCR amplification in order to identify the possible Sp1 binding site. B: LLC-PK1/rV1aR cells were transfected with the 124 bp rV2R promoter region with the original Sp1 (left) or mutated Sp1 (right) site. After transfection, cells were incubated at pH 7.4 and 6.7 for 24 h. The induction of rV2R promoter activity by low-pH solution was abolished in Sp1 mutations. n = 3. *P < 0.05 vs. pH 7.4.
V1aR stimulation, suggesting that low-pH conditions enhance the inhibition of V2R expression by V1aR stimulation.

Analysis of intracellular signal transduction. H-89 (PKA inhibitor), SP-600125 (JNK inhibitor), SB-203580 (p38 inhibitor), and U-0126 (ERK inhibitor) were used to analyze the intracellular signaling pathway to address the mechanisms of the effects of low pH on V2R promoter activity. After the transfection of the −62 bp rV2R promoter element, which is the minimal fragment responsive to acidic conditions, cells were incubated at pH 7.4 and pH 6.7 with or without these inhibitors. DMSO or H2O was used as the vehicle.

The ERK and p38 inhibitors did not affect rV2R promoter activity (Fig. 7) in both neutral and low-pH conditions. V2R promoter activity was decreased by the PKA inhibitor in both neutral and low-pH conditions (Fig. 8A). The JNK inhibitor also decreased rV2R promoter activity in both neutral and low-pH conditions (Fig. 8B). We also examined the effects of serum on these inhibitors on V2R promoter activity (Fig. 9). rV2R promoter activity was not suppressed by the PKA inhibitor in both the neutral and low-pH solutions including FBS (Fig. 9A). The JNK inhibitor decreased rV2R promoter activity not in neutral but in low-pH solution containing FBS (Fig. 9B).
These results suggest that the regulation of V2R promoter activity by low pH might be involved in the JNK pathway and serum under physiological conditions.

**Different effects of low pH and hypertonicity on cAMP and PKA pathway.**

AVP-stimulated cAMP accumulation was significantly decreased under low-pH conditions compared with neutral conditions \((P < 0.05)\) (Fig. 10). On the other hand, AVP-stimulated cAMP accumulation was increased under hypertonic conditions compared with isotonic conditions at pH 7.4 \((P < 0.05)\). These results indicate that the expression of V2R could be differently altered by the low pH and the hypertonicity.

To identify the role of PKA in mediating the hypertonicity- or low-pH-induced V2R promoter activity, LLC-PK1/rV1aR cells were treated with 8-BrcAMP (a membrane-permeant cAMP analog) for 12 h in the presence or absence of a PKA inhibitor (H-89). H-89 decreased V2R promoter activity only under hypertonic conditions in the presence of 8-BrcAMP, but under neutral and low-pH conditions with the presence of 8-BrcAMP V2R promoter activity was not inhibited by the PKA inhibitor (Fig. 11).

**Effects of low pH on V2R mRNA and protein expressions in LLC-PK1/rV1aR cells.** To investigate the effect of low pH on V2R expression, real-time PCR and Western blot analysis were performed with LLC-PK1/rV1aR cells. V2R mRNA was increased by incubation under low-pH conditions for 24 h \((P < 0.01)\). The level of V2R mRNA in low-pH conditions was threefold higher than that in neutral conditions (Fig. 12A). As shown in Fig. 12B, 24-h treatment with low-pH conditions caused a significant increase of V2R protein expression \((P < 0.05)\). These results indicate that the expression of V2R is increased under low-pH conditions at the mRNA and protein levels as well as at the transcriptional level.

**DISCUSSION**

Metabolic acidosis, characterized by a decrease in blood pH and bicarbonate concentration, is a common clinical symptom that is caused by overproduction of acid or reduction of bicarbonate reabsorption. Acidosis is also known to decrease...
cated that the induction of V2R promoter activity could be increased even in the bicarbonate-free low-pH solution as well with or without bicarbonate after transfection with rV2R promoter. The expression of V2R mRNA in neutral conditions (pH = 7.4) is expressed as 1, and relative expression of V2R mRNA in acidic conditions is presented. n = 12. *P < 0.01 vs. pH 7.4. B: Western blot analysis of V2R in LLC-PK1/rV1aR cells after incubation under neutral and low-pH conditions for 24 h. 12 μg of cell proteins was loaded for each lane. Top: protein bands for V2R and β-actin as an internal control. Bottom: densitometry analysis showing an increase in V2R protein abundance under low-pH conditions (n = 6). Densitometry data were obtained from 2 individual experiments. *P < 0.05 vs. pH 7.4.

Previously, we reported (15) that AVP increased rV2R promoter activity through V2R stimulation at the early phase and decreased rV2R promoter activity by V1aR stimulation at the late phase in LLC-PK1/rV1aR cells. It has been suggested that V1aR affects V2R expression under the condition of high levels of urinary AVP that is observed in chronic renal failure and chronic dehydrated state (16, 24). In the present study, we demonstrated that rV2R promoter activity was upregulated under low-pH conditions in LLC-PK1/rV1aR cells. rV2R promoter activity was significantly increased at 12 h and further continued up to 24 h under low-pH conditions (Fig. 2). LLC-PK1/rV1aR cells exhibited a threefold increase in V2R mRNA when cells were incubated under low-pH conditions for 24 h (Fig. 12A). V2R proteins were also significantly increased under low-pH conditions (Fig. 12B). Since V2R promoter activity is downregulated through the V1aR pathway, we also examined the effect of low-pH conditions on the decrease of V2R promoter activity by V1aR stimulation. Downregulation of V2R promoter activity by V1aR stimulation was observed not at alkaline conditions but at neutral and low-pH conditions. The decrease of V2R promoter activity was larger at low-pH than neutral conditions. This result indicated that low-pH-dependent regulation could be induced for enhancement of the downregulation of V2R promoter activity via V1aR stimulation in low-pH conditions. Further investigation will be required to elucidate the relationship between pH and V1aR stimulation in the regulation of V2R expression.

Metabolic acidosis is caused by a reduction of bicarbonate concentration along with a subsequent low blood pH level. To elucidate whether the induction of V2R promoter activity was caused by the decrease in bicarbonate or low pH itself, we incubated LLC-PK1/rV1aR cells in neutral or low-pH solution with or without bicarbonate after transfection with rV2R promoter vector. As shown in Fig. 3, rV2R promoter activity was increased even in the bicarbonate-free low-pH solution as well as in the low-pH solution with bicarbonate. This result indicated that the induction of V2R promoter activity could be dependent not on bicarbonate but on plasma pH level. It is speculated that an upregulation of V2R promoter activity might also be observed in respiratory acidosis.

Sp1 is a ubiquitous transcription factor that is essential for the expression of many genes (17). As shown in Fig. 5A, mutating the Sp1 site, which is located at −59 to −54 bp in the rV2R promoter region, repressed the low pH-induced rV2R promoter activity, even though basal rV2R promoter activity was also suppressed (Fig. 5B). Torigoe et al. (33) reported that low pH increased Sp1 DNA binding activity. They investigated Sp1 DNA binding activity under various pH conditions. The DNA binding activity of Sp1 was increased only at a low pH. It is speculated that V2R promoter activity is upregulated by low pH, probably through the activation of Sp1 transcription factor. Our present study suggested that the Sp1 site is required for the V2R promoter activity control, which is dependent on pH. We have demonstrated that V2R promoter activity was regulated by V2R, V1aR stimulation, and hypertonicity through the Sp1 site in previous studies (15, 16). Sp1 consensus sequence might unite the stimulations of V2R, V1aR, tonicity, and pH for the appropriate expression of V2R. However, analysis of the 5′-flanking region of rV2R is still insufficient. As shown in Fig. 4, V2R promoter activity showed the largest value in the 345 bp deletion construct. There are several consensus sequences such as AP1, AP3, and CAAT in the rV2R promoter region that were expected to be involved in basal rV2R promoter activity. Further examination to investigate the relationship between Sp1 and V2R transcription in detail is required.

The MAPK signaling cascades are activated by various forms of extracellular stimulation including hyperosmolality (25). We examined the effects of MAPK pathways on low pH-induced increase of V2R promoter activity. ERK and p38 inhibitors did not affect rV2R promoter activity under both neutral and low-pH conditions (Fig. 7). The JNK inhibitor decreased rV2R promoter activity under low-pH conditions as well as under neutral conditions (Fig. 8B). It appeared that the inhibition of rV2R promoter activity by JNK inhibitor was independent of change in pH. However, the JNK inhibitor decreased rV2R promoter activity only in low-pH conditions.
when the cells were incubated in low-pH solution containing FBS (Fig. 9B), suggesting the involvement of the JNK pathway in pH-dependent increase of V2R promoter activity in vivo. Although the PKA inhibitor also decreased rV2R promoter activity under both neutral and acidic conditions (Fig. 8A), it did not affect rV2R promoter activity in the solution containing FBS (Fig. 9A). The PKA pathway might be unrelated to pH-induced V2R promoter activity.

A number of studies have described that low extracellular pH is able to activate ERK, JNK, and p38 MAPK in a variety of cell lines (37, 38). JNK signaling is activated in many diseases involving chronic inflammation (18, 19), cancer (40), and insulin-resistant diabetes (13). Yamaji et al. (39) reported the increase of c-Jun mRNA in cultured kidney cells in acidic medium. Although V2R promoter activity was increased in low-pH solution containing FBS as well as in FBS-free low-pH solution, the JNK inhibitor demonstrated pH-dependent decrease of rV2R promoter activity only in the presence of FBS. We showed the inhibition of rV2R promoter activity by PKA and JNK inhibitors in hypertonic medium containing FBS in a previous study (16). Serum could be crucial for the accurate regulation of water and electrolyte homeostasis through V2R stimulation under physiological conditions.

Furthermore, we examined AVP-stimulated cAMP accumulation under neutral, low-pH, and hypertonic conditions in LLC-PK1/rV1aR cells (Fig. 10). AVP-induced cAMP accumulation was lower under low-pH than neutral conditions. On the contrary, AVP-induced cAMP accumulation was increased in hypertonic conditions. This result is consistent with previous reports that demonstrated the increase of AVP-induced cAMP accumulation by hyperosmolarity (28, 29). We also reported (16) that V2R promoter activity is increased by hyperosmolarity via a PKA pathway. Taken together with these observations, this suggests that there would be different pathways between acid- and hyperosmolarity-induced V2R stimulation. The activation of V2R promoter activity in LLC-PK1/rV1aR cells showed that cAMP is responsible for the activation of V2R promoter activity in both hyperosmolarity and low-pH conditions (Fig. 10). Interestingly, inhibition of PKA did not reduce the 8-Br-cAMP-induced V2R promoter activity under low pH but did reduce the activity under hypertonic conditions (Fig. 11). Our results suggest a possibility that a cAMP-mediated but PKA-independent signaling pathway could be involved in low-pH-induced V2R promoter activity. Further study needs to be done to clarify this hypothesis.

Although PKA and JNK pathways were suggested to be involved in hyperosmolarity-induced V2R expression (16), only the JNK pathway might be a candidate for low-pH-induced V2R expression. There could be other possible pathways that modulate V1aR and V2R functions and their interactions at acidic conditions. Goldberg et al. (12) demonstrated that low pH markedly elevated the cellular cAMP/PKA cascade. It is known that extracellular pH reduction increases endothelin-1 (ET-1) secretion in human renal microvascular endothelial cells (35). ET-1 is also known to inhibit AVP-induced cAMP accumulation in the collecting ducts (8, 34). In ET-1-deficient mice, AVP-induced cAMP levels were increased (30). It is suggested that AVP-induced cAMP generation may be inhibited at low pH through the ET-1 pathway. In addition, hypoxia-induced pathways, which are activated by acidosis (4, 9, 20), could be involved in V1aR and V2R functions.

AVP is known to modulate tubular function and inhibits diuresis by increasing intracellular cAMP via the V2R. There is evidence that luminal AVP inhibits the V2R-stimulated water and urea permeability in the collecting ducts, probably through the V1aR (23). Our previous report (31) showed that chronic metabolic acidosis increased V1aR mRNA expression and decreased V2R mRNA in rat collecting ducts. We also demonstrated (15) that rV2R promoter activity was downregulated by V1aR stimulation. In the present study, we showed the larger downregulation of V2R promoter activity by V1aR stimulation under low-pH conditions compared with neutral conditions. The effect of low pH on V2R mRNA expression was different between LLC-PK1/rV1aR cells in the present study and the collecting ducts in the previous study (31). Since exposure of LLC-PK1/rV1aR cells to low pH was performed in the absence of AVP in the present study, the downregulation of V2R promoter activity and subsequent mRNA expression by V1aR stimulation could not be observed. However, low-pH conditions stimulated V1aR mRNA and decreased V2R mRNA expression in microdissected outer medullary collecting duct (OMCD) in the presence of AVP (31). These findings suggest that the V1aR function and the suppression of V2R function could be enhanced by acidosis.

Valeria et al. (27) reported that V1aR stimulation induced an alkaline intracellular pH (pHi). They incubated cortical collecting duct cells in the presence of 10^-8 M AVP with V1aR or V2R antagonist. In that study, they demonstrated that treatment with AVP and V1aR antagonist elicited an alkalization of pHi. On the contrary, treatment with AVP and V2R antagonist elicited an acidification of pHi. These results are consistent with our findings that low pH induced V2R promoter activity and V1aR-induced downregulation of V2R promoter activity. There is also another report demonstrating that not the V2R but the V1aR is involved in bicarbonate reabsorption (21). Metabolic acidosis arises in complicated symptoms in patients with critical conditions such as sepsis, in which plasma and urinary AVP exhibit large increases (11, 36). The close interaction between V1aR and V2R stimulations could be required in metabolic acidosis to maintain body fluid and electrolyte homeostasis.

Our results can be summarized as follows. 1) Low pH enhances the expression of V2R promoter mRNA and protein in LLC-PK1/rV1aR cells. 2) Low pH enhances the suppression of V2R promoter activity by the V1aR pathway. 3) The close interaction between the V1aR and V2R functions in acidosis might be required to maintain water, electrolyte, and acid-base homeostasis.

ACKNOWLEDGMENTS

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GRANTS

This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (21591064, 21591034, and 19590955, 19590957, 18590895, 17590833, 167904660, 16590791, 16590792, 16390246, 15590852, 14370321, 13877166, 13671121, and 13671119).
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