Biphasic Regulation of Renal Proximal Bicarbonate Absorption by Luminal AT_{1A} Receptor

YANAN ZHENG,* SHOKO HORITA,* CHIAKI HARA,* MOTOEI KUNIMI,* HIDEOMI YAMADA,* TAKESHI SUGAYA,† ATSUO GOTO,* TOSHIRO FUJITA,* and GEORGE SEKI*

*Department of Internal Medicine, Faculty of Medicine, Tokyo University, Tokyo, Japan; and †Discovery Research Laboratory, Tanabe Seiyaku Co, Ltd, Osaka, Japan.

Abstract. Angiotensin II (AngII) regulates renal proximal transport in a biphasic way. It has been recently shown that the basolateral type 1A receptor (AT_{1A}) mediates the biphasic regulation of Na^+–HCO_3^- cotransporter (NBC) by AngII. However, the receptor subtype(s) responsible for the luminal AngII actions remained to be established. To clarify this issue, the luminal AngII effects in isolated proximal tubules from wild-type (WT) and AT_{1A}-deficient mice (AT_{1A} KO) were compared. In WT, the rate of bicarbonate absorption (J_{HCO3^-}) was stimulated by 10^{-10} mol/L luminal AngII but was inhibited by 10^{-6} mol/L luminal AngII. Both stimulatory and inhibitory effects of AngII were completely blocked by valsartan (AT_1 antagonist) but unaffected by PD 123,319 (AT_2 antagonist). In AT_{1A} KO, in contrast, luminal AngII (10^{-10} – 10^{-6} mol/L) did not change J_{HCO3^-}. In WT, 10^{-6} mol/L luminal AngII increased cell Ca^{2+} concentrations ([Ca^{2+}]_i), which was again blocked by valsartan but not by PD 123,319. However, luminal AngII did not increase [Ca^{2+}]_i in AT_{1A} KO. On the other hand, the addition of arachidonic acid similarly inhibited J_{HCO3^-} in WT and AT_{1A} KO. Furthermore, the acute activation of protein kinase C by phorbol 12-myristate 13-acetate similarly stimulated J_{HCO3^-} in WT and AT_{1A} KO, indicating that the inhibitory and stimulatory pathways necessary for the AngII actions were preserved in AT_{1A} KO. These results indicate that the luminal AT_{1A} mediates the biphasic regulation of bicarbonate absorption by luminal AngII, while no evidence was obtained for a role of AT_2.

Angiotensin II (AngII) receptors are divided into two distinct receptor subtypes, type 1 (AT_1) and type 2 (AT_2) receptors, and AT_1 receptors are further divided into AT_{1A} and AT_{1B} in rodents (1,2). Most of the physiologic effects of AngII are thought to be mediated by AT_1, while the role of AT_2 remains unclear (1–3). In addition to the effects on renal hemodynamics, AngII has direct effects on renal tubular functions. In particular, AngII acts on sodium and bicarbonate reabsorption from proximal tubules, and this process is thought to have significant impact on body fluid and sodium homeostasis (4–7). Interestingly, AngII is known to regulate proximal transport in a biphasic way: stimulation by low (picomolar to nanomolar) concentrations of AngII, and inhibition by high (nanomolar to micromolar) concentrations of AngII (4,5). Previous studies have consistently shown that AT_1 mediates the stimulatory effect of AngII. However, conflicting results have been reported regarding the receptor subtype mediating the inhibitory effect of AngII. Thus, several studies supported a view that AT_1 mediates both stimulatory and inhibitory effects of AngII (8,9), whereas Haithcock et al. (10) presented the evidence that AT_2 mediates the inhibitory effect of AngII. Methodological differences may not fully account for these conflicting results, and the receptor subtype responsible for the inhibitory effect of AngII remained to be definitely determined. To clarify this issue, we have recently compared the effects of AngII on Na^+-HCO_3^- cotransporter (NBC) activity in isolated proximal tubules from wild-type (WT) and AT_{1A}-deficient mice (AT_{1A} KO). The results clearly showed that the basolateral AT_{1A} mediates both stimulation and inhibition of NBC by AngII (11). It could be still possible, however, that the apical AT_2 may mediate inhibition by AngII, because the study by Haithcock et al. (10) was conducted on cultured proximal tubular cells that might express the apical AT_2, whereas our study was restricted on the basolateral receptors (11). Previous studies have indeed shown that luminal AngII has also biphasic effects on proximal transport (12,13). In addition, a recent finding of remarkably high (nanomolar) proximal intratubular concentrations of AngII suggests an important role of luminal AngII actions (13). The purpose of the present study is therefore to compare the roles of luminal AT_{1A} and AT_2 in mediating the stimulatory and inhibitory effects of AngII using a combination of gene-targeted mice and specific receptor antagonists. To accomplish this task, we compared the effects of luminal AngII on the rate of bicarbonate absorption (J_{HCO3^-}) in isolated proximal tubules from WT and AT_{1A} KO. This study was facilitated by a convenient microspectrofluorometric method.
which we have recently developed for the determination of $\text{JHCO}_3^-$ in isolated rabbit proximal tubules (14,15).

Materials and Methods

Animals

Male AT1A KO mice (16) and WT mice (Discovery Research Laboratory, Tanabe Seiyaku), 5- to 8-wk-old, from the same genetic background were used in the present study. They were provided with standard food and water ad libitum. All animal procedures were in accordance with local institutional guidelines.

Microperfusion Technique

Mice were anesthetized with pentobarbital sodium, and the thin sections from the left kidney were obtained and stored in ice-cold, gas-equilibrated (5% $\text{CO}_2$/95% $\text{O}_2$) Ringer solution containing: 144 mmol/L Na+, 5 mmol/L K+, 1.5 mmol/L $\text{Ca}^{2+}$, 1 mmol/L $\text{Mg}^{2+}$, 125 mmol/L Cl−, 25 mmol/L $\text{HCO}_3^-$, 2 mmol/L $\text{H}_2\text{PO}_4^-$, 1 mmol/L $\text{SO}_4^{2-}$, and 5.5 mmol/L D-glucose. Proximal tubules (S2 segment) were microdissected without collagenase treatment and then microperfused according to the method described by Burg et al. (17) with a modified version of the perfusion and sampling capillary system (14,15). The tubular lumen was perfused with the same gas-equilibrated Ringer solution; however, D-glucose was omitted and 20 mmol/L NaCl was replaced by 40 mmol/L raffinose. We have previously shown that tissue culture media such as Dulbecco modified Eagle medium (DMEM) are essential to maintain a better functional state of isolated proximal tubules (18–20). In pilot experiments, we indeed confirmed that $\text{JHCO}_3^-$ in isolated mouse proximal tubules was kept at high rates for more than 60 min in DMEM solution, but it deteriorated within 60 min in Ringer solution. In the present study, DMEM was therefore used as the bath perfusate. The experimental chamber was continuously perfused at a rate of approximately 10 ml/min with prewarmed (38°C) and gas-equilibrated (5% $\text{CO}_2$/95% $\text{O}_2$) DMEM solution for 30 min, and the $\text{JHCO}_3^-$ measurements were started.

Determination of $\text{JHCO}_3^-$

We used the recently described stop-flow microperfusion technique (14,15). In brief, isolated tubules were mounted on the stage of an inverted epifluorescence microscope (IMT-2, Olympus). After background fluorescence was measured, a pH-sensitive fluorescence dye 2',7'-bis(carboxyethyl)-5 (6)-carboxyfluorescein (BCECF) was added to the luminal perfusate. Luminal pH (pH$_L$) was monitored by a microspectrofluorometer system (OSP-10, Olympus), which alternatively illuminates the preparation with light of 440 and 490 nm and measures emission at the 530 nm wavelength. To obtain a calibration curve, lumen was perfused at high rates (>80 nl/min) with NaCl solutions buffered to pH 6.5 to 7.5 with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. To determine $\text{JHCO}_3^-$, the rapid (approximately 80 nl/min) luminal perfusion was abruptly stopped by suddenly reducing the perfusion pressure from approximately 18 to 0 cmH$_2$O. After stop-flow, pH$_L$ fell from 7.4 to values near 6.8 within 30 s, where it remained virtually constant. This decrease in pH$_L$ reflects the gradual absorption of $\text{HCO}_3^-$ and the attainment of a steady-state zero net volume flux that develops because of the presence of poorly absorbable raffinose in the luminal perfusate as described (14,15). The decay in luminal $\text{HCO}_3^-$ concentration ([$\text{HCO}_3^-$]$_L$) was calculated from the changes in pH$_L$, and [$\text{JHCO}_3^-$]$_L$ was calculated from the following equation:

$$\text{JHCO}_3^- = \pi \cdot r^2 ([\text{HCO}_3^-]_b - [\text{HCO}_3^-]_L) \cdot k$$

Where $r$ is the luminal radius before stop-flow, [$\text{HCO}_3^-]_b$, and [$\text{HCO}_3^-]_L$ are [$\text{HCO}_3^-]_b$ before stop-flow and in the steady-state, respectively, and $k$ is the rate constant of [$\text{HCO}_3^-]_L$ decline. During rapid luminal perfusion, the perfusion pressure will act to dilate the tubular lumen because the tubule itself as well as the collection pipette offers some resistance to flow. When the perfusion pressure is suddenly reduced to zero upon stop-flow, the tubular lumen tends to partially collapse. The correction for this volume loss into the pipettes was achieved by using the decaying 440 nm (pH-insensitive) fluorescence signals as a marker of the residual luminal volume. The validity of this correction strategy was confirmed by comparison with photomicrographic and video measurements as described previously (14).

Measurements of Cell Ca$^{2+}$ Concentrations ([Ca$^{2+}$_i])

[Ca$^{2+}$_i] was measured as described previously (11). Briefly, after autofluorescence was measured, the tubules were incubated with 30 mmol/L Fura-2/AM for 60 min in DMEM under 5% $\text{CO}_2$/95% $\text{O}_2$ gas at 37°C. Thereafter, the tubule was transferred into the perfusion chamber, microperfused as described above, and [Ca$^{2+}$_i] was monitored with the OSP-10 system. The calibration curves were obtained at the end of each experiment, and [Ca$^{2+}$_i] were calculated according to the method by Grynkiewicz et al. (21).

Materials and Statistics

BCECF and Fura-2/AM were obtained from Dojindo, valsartan was from Novartis, 5-ile-AngII, PD 123319, arachidonic acid, (PMA), acetazolamide, and ouabain were from Sigma, and all the other chemicals were from Wako. The data were represented as mean values ± SEM. Significant differences were determined by applying the paired or unpaired $t$ test as appropriate.

Results

The Validity of Stop-Flow Microspectrofluorometric Method

To test whether the stop-flow microspectrofluorometric method, which we have recently developed to measure bicarbonate absorption from rabbit proximal tubules (14,15), can also apply to mouse proximal tubules, we performed a series of experiments on isolated proximal tubules from WT. Bicarbonate absorption from proximal tubules has been shown to largely depend on the activities of carbonic anhydrases as well as on the Na$^+$ gradient (22). To test for the role of carbonic anhydrases, we examined the effect of acetazolamide. The addition of acetazolamide (1 mmol/L) to both luminal and basolateral sides for 10 min substantially reduced $\text{JHCO}_3^-$ from 13.0 ± 1.0 to 4.1 ± 0.5 pmol/cm s (68 ± 3% inhibition; $n = 6$; $P < 0.005$). To test for Na$^+$-dependency, we examined the effect of ouabain. The addition of ouabain (1 mmol/L) to the basolateral side for 10 min markedly reduced $\text{JHCO}_3^-$ from 12.3 ± 0.8 to 3.0 ± 0.3 pmol/cm s (76 ± 2% inhibition; $n = 7$; $P < 0.005$). We also tested for Na$^+$-dependency more directly by removing Na$^+$ from the ambient solutions. Similarly to all the other experiments, tubules were first perfused for 30 min using Ringer solution and DMEM as the luminal and the basolateral perfusate, respectively. After the control $\text{JHCO}_3^-$ value was obtained, however, both perfusates were switched to Na$^+$-free solution, in which all the Na$^+$ in Ringer solution was replaced by N-methyle-D-glucamine. The bilateral Na$^+$-removal by this procedure for 10 min also markedly reduced $\text{JHCO}_3^-$ from 11.7...
Effects of Luminal AngII in WT

To examine the roles of luminal AngII receptors in the regulation of proximal bicarbonate absorption, we first examined the effects of luminal AngII in isolated proximal tubules from WT. As can be seen in Figure 1, 10-min perfusion with the luminal solution containing 10^{-10} mol/L AngII increased J_{HCO_3}^- by 42 \pm 7\% (n = 8; P < 0.005). On the other hand, 10^{-8} mol/L AngII had no effects (n = 8), and 10^{-6} mol/L AngII rather decreased J_{HCO_3}^- by 34 \pm 4\% (n = 7; P < 0.05). Time control experiments without AngII confirmed that J_{HCO_3}^- did not change during repeated stop-flow (n = 6). These results indicate that luminal AngII has biphasic effects on J_{HCO_3}^- in isolated mouse proximal tubules as previously shown in isolated rabbit proximal tubules (12).

To examine the receptor subtype(s) mediating these AngII effects, we tested an AT_1 antagonist, valsartan. The addition of 2 \times 10^{-7} mol/L valsartan into the luminal perfusate did not change J_{HCO_3}^- (11.8 \pm 2.0 \text{ versus } 12.0 \pm 1.9 \text{ pmol/cm s}; n = 6; NS), but it completely blocked the effects of luminal AngII. Thus, in the presence of valsartan, J_{HCO_3}^- was not changed by 10^{-10} mol/L AngII (12.8 \pm 1.4 \text{ versus } 12.2 \pm 1.5 \text{ pmol/cm s}; n = 7; NS) and by 10^{-6} mol/L AngII (11.3 \pm 1.2 \text{ versus } 11.3 \pm 1.1 \text{ pmol/cm s}; n = 7; NS). The addition of higher concentrations (2 \times 10^{-6} mol/L) of valsartan into the luminal perfusate also did not change J_{HCO_3}^- (n = 6). To test for a role of AT2, we examine the effects of an AT_2 antagonist PD 123,319. The addition of 10^{-5} mol/L PD 123,319 into the luminal perfusate did not change J_{HCO_3}^- (11.9 \pm 1.8 \text{ versus } 11.6 \pm 2.2 \text{ pmol/cm s}; n = 6; NS) and did not modify the effects of luminal AngII. Thus, in the presence of PD 123,319, 10^{-10} mol/L AngII increased J_{HCO_3}^- by 39 \pm 8\% (n = 7; P < 0.005), and 10^{-6} mol/L AngII decreased J_{HCO_3}^- by 32 \pm 3\% (n = 7; P < 0.005). These results indicate that the luminal AT_1 mediates both stimulation and inhibition of J_{HCO_3}^- by AngII.

A previous study on isolated rabbit proximal tubules (12) suggested that the presence of physiologic concentrations of AngII in the basolateral side could counteract the inhibition by high concentrations of luminal AngII. We therefore examined the effect of luminal 10^{-6} mol/L AngII in the presence of basolateral 10^{-10} mol/L AngII. The addition of 10^{-10} mol/L AngII to the basolateral side alone for 10 min increased J_{HCO_3}^- from 10.7 \pm 0.7 to 14.1 \pm 0.8 \text{ pmol/cm s (33 \pm 5\% stimulation}; n = 6; P < 0.01), as expected. On the other hand, the simultaneous addition of basolateral 10^{-10} mol/L AngII and luminal 10^{-6} mol/L AngII for 10 min significantly reduced J_{HCO_3}^- from 11.7 \pm 0.6 to 7.8 \pm 0.5 \text{ pmol/cm s (34 \pm 2\% inhibition}; n = 7; P < 0.005). The degree of inhibition by luminal 10^{-6} mol/L AngII in the presence of basolateral 10^{-10} mol/L AngII was not statistically different from that by luminal 10^{-6} mol/L AngII alone.

Effects of AngII in AT_1A KO

We next examined the effects of luminal AngII in AT_1A KO. The control J_{HCO_3}^- in AT_1A KO (11.9 \pm 0.8 \text{ pmol/cm s}; n = 24) was very similar to that in WT (11.7 \pm 0.5 \text{ pmol/cm s}; n = 26). As shown in Figure 2, the addition of 10^{-10}, 10^{-8}, and 10^{-6} mol/L AngII all failed to change J_{HCO_3}^- in AT_1A KO. Time control experiments without AngII confirmed that J_{HCO_3}^- did not change during repeated stop-flow (n = 5). These results are consistent with a view that the biphasic regulation of J_{HCO_3}^- in WT was mediated by the luminal AT_1A.

Effects of AngII on [Ca^{2+}]_i

The increase in [Ca^{2+}]_i could be an important factor in AngII-mediated signaling pathways (8,9,23,24); we therefore
also examined the \([\text{Ca}^{2+}]_i\) responses to luminal AngII. In WT, the addition of \(10^{-10}\) mol/L AngII did not induce a significant increase in \([\text{Ca}^{2+}]_i\) \((n = 6)\), and \(10^{-8}\) mol/L induced only a marginal increase in three of seven tubules. However, \(10^{-6}\) mol/L AngII consistently induced a spikelike increase in \([\text{Ca}^{2+}]_i\), as shown in Figure 3. To examine the receptor subtype mediating the \([\text{Ca}^{2+}]_i\) response to AngII, we tested valsartan and PD 123,319. In three tubules, sequential additions of \(10^{-6}\) mol/L AngII in the same tubule, separated for more than 4 min, induced comparable \([\text{Ca}^{2+}]_i\) responses. As also shown in Figure 3, however, the addition of \(2 \times 10^{-7}\) mol/L valsartan into the luminal perfusate almost completely inhibited the \([\text{Ca}^{2+}]_i\) increase by \(10^{-6}\) mol/L AngII \((n = 4)\). On the other hand, the addition of \(10^{-5}\) mol/L PD 123,319 into the luminal perfusate did not inhibit the \([\text{Ca}^{2+}]_i\) increase by \(10^{-6}\) mol/L AngII \((n = 4)\). To test whether the luminal AngII actions are mediated solely by the apical receptor(s), we examined the effect of basolateral valsartan on the \([\text{Ca}^{2+}]_i\) response to luminal AngII. In three tubules, the \([\text{Ca}^{2+}]_i\) increase by the luminal addition of \(10^{-6}\) mol/L AngII was not inhibited by valsartan added into the basolateral perfusate. These results indicate that the \([\text{Ca}^{2+}]_i\) response to luminal AngII is solely mediated by the luminal AT\(_{1}\).

We also examined the effects of luminal AngII in AT\(_{1A}\) KO. However, up to \(10^{-5}\) mol/L AngII did not induce a significant \([\text{Ca}^{2+}]_i\) increase in AT\(_{1A}\) KO.

**Effects of Arachidonic Acid and PMA on J\(_{\text{HCO}_3^-}\)**

Previous studies have shown that the activation of phospholipase A\(_2\) (PLA\(_2\)) and the subsequent release of arachidonic acid could be responsible for the inhibition by high concentrations of AngII (9,25,26). We therefore compared the effects of arachidonic acid on J\(_{\text{HCO}_3^-}\) in WT and AT\(_{1A}\) KO. As shown in Figure 4, the addition of \(10^{-8}\) mol/L arachidonic acid to the bath perfusate similarly reduced J\(_{\text{HCO}_3^-}\) in WT \((-27 \pm 2\%; n = 7)\) and AT\(_{1A}\) KO \((-32 \pm 1\%; n = 7)\).

On the other hand, activation of protein kinase C (PKC) could be involved in the stimulation by low concentrations of AngII (24,27). Therefore we finally compared the effects of acute PKC activation in WT and AT\(_{1A}\) KO. As shown in Figure 5, the addition of \(5 \times 10^{-7}\) mol/L PMA similarly increased J\(_{\text{HCO}_3^-}\) in WT \((33 \pm 4\%; n = 5)\) and AT\(_{1A}\) KO \((36 \pm 6\%; n = 5)\).

**Discussion**

In 1977, Harris and Young (4) reported that AngII added into the peritubular fluid regulates volume absorption from rat proximal tubules in the biphasic way. This finding has been confirmed in various experimental conditions (8,9,23,28,29), and other investigators have shown that AngII added into the luminal fluid can also exert the similar biphasic effects (12,13). Regarding the signal transduction pathways, the stimulation by AngII is generally thought to be mediated by the activation of PKC and/or the decrease in the level of cAMP in the cell (7,23,24,27), though a recent study suggests that the activation...
of tyrosine kinases could be also involved (30). On the other hand, the activation of PLA2 and the subsequent release of arachidonic acid seem to be involved in the inhibition by AngII. In particular, a P-450-dependent arachidonic acid metabolite, 5,6-epoxyeicosatrienoic acid, could be the final mediator of the inhibition by AngII (9,25,26). Although the stimulation by AngII is certainly mediated by AT1, conflicting results have been reported as to the receptor subtype responsible for the inhibition by AngII (8,9,10). By comparing the effects of AngII in WT and AT1A KO, we have recently shown that the biphasic regulation of NBC by AngII is mediated by AT1A in the basolateral membranes (11). The study was, however, limited on the basolateral receptors; we could not therefore exclude a possibility that the luminal AT2 might mediate the inhibition by AngII. To clarify this issue, we compared the effects of luminal AngII in WT and AT1A KO in the present study. To measure bicarbonate absorption, we used the stop-flow microspectrofluorometric method, which we have recently developed for isolated rabbit proximal tubules (14,15). The validity of this method was confirmed by series of experiments with acetazolamide, ouabain, and Na+-free solution on isolated proximal tubules from WT, which reproduced the previously characterized properties of proximal bicarbonate transport (22).

In WT, the biphasic regulation of JHCO3− by AngII was completely inhibited by the highly selective AT1 antagonist, valsartan (31). However, the AT2 antagonist PD 123,319 did not modify the effects of AngII. In AT1A KO, the effects of luminal AngII on JHCO3− were completely lost. On the other hand, the stimulation by PMA as well as the inhibition by arachidonic acid was very similar in WT and AT1A KO, indicating that the stimulatory and inhibitory signaling pathways required for AngII actions are intact in AT1A KO as we previously reported (11). These results are consistent with a view that the luminal AT1A mediates the biphasic regulation of bicarbonate absorption from proximal tubules.

Because of remarkably high proximal intratubular concentrations of AngII and the presence of angiotensinogen and its mRNA in proximal tubules, AngII is thought to be directly secreted into proximal tubular lumen by the epithelial cells (13). Baum et al. (32) reported that this endogenously secreted AngII was able to modify bicarbonate absorption from isolated proximal tubules. In the present study, however, the addition of sufficient concentrations of valsartan did not change JHCO3− in WT, which did not support a role of endogenous AngII. One possible explanation for these apparently conflicting results could be that we used the very high luminal perfusion rates (>80 nl/min) to perform stop-flow measurements, whereas Baum et al. (32) used the much lower (approximately 10 nl/min) perfusion rates to measure JHCO3− by the conventional picapnotherm method. On the other hand, a previous study on isolated rabbit proximal tubules (12) provided data suggesting that the inhibition by high concentration of luminal AngII could be blunted by the presence of physiologic concentrations of basolateral AngII. In contrast, we showed that the inhibition by 10−6 mol/L luminal AngII in WT was preserved even in the presence of basolateral 10−10 mol/L AngII. The reason for these discrepant results is not apparent, but species difference could be involved.

In the previous study, we observed that 10−6 mol/L basolateral AngII slightly stimulated the NBC activity in AT1A KO, which was completely inhibited by valsartan (11). In the present study, however, 10−6 mol/L luminal AngII had no effects on JHCO3− in AT1A KO. The pharmacologic properties of AT1A and AT1B are known to be very similar (1,2); therefore, the most likely explanation for these observations would be that AT1B is expressed at a low level in the basolateral membranes but not in the apical membranes of proximal tubules. A previous study has confirmed the low-level expression of AT1B in proximal tubules (33), but whether it is expressed in the basolateral or the apical membranes has not been determined. The future studies on AT1B-deficient mice would help clarify this issue.

When high concentrations of AngII were added into the luminal fluid, some of the peptide could diffuse out of the lumen through the paracellular pathways and might act also on the basolateral receptors. However, the [Ca2+]i increase by 10−6 mol/L luminal AngII was completely inhibited by the luminal valsartan but unaffected at all by the basolateral valsartan in WT. In addition, up to 10−5 mol/L luminal AngII did not increase [Ca2+]i in AT1A KO. These observations indicate that the effects of luminal AngII are really mediated by the luminal AT1A, but not by the basolateral receptor. Regarding the concentration dependency of [Ca2+]i responses to AngII in WT, 10−10 mol/L luminal AngII did not increase [Ca2+]i, and only 10−6 mol/L luminal AngII induced a typical spike-like [Ca2+]i increase. Previously Nagami (34) reported, however, that much lower concentrations of luminal AngII can increase [Ca2+]i in isolated mouse proximal tubules. At present the reason for these discrepant results is unknown, but the difference in metabolic status of isolated tubules could be responsible.

Taken together with the results of our previous study about the NBC regulation by AngII (11), we can now conclude that AT1A, whether expressed in the basolateral or the apical membranes, mediates the biphasic regulation of proximal transport. In contrast to our conclusion, Haithcock et al. (10), using cultured proximal tubular cells, presented the evidence that AT2 mediates the inhibition by high concentrations of AngII. Other studies on cultured proximal tubular cells also suggested a role of AT2 in the acute inhibitory effect of AngII (35,36). While the reason for the discrepant results is not apparent, it has been shown that the expression of AT2 in kidney is very high in the developing fetus but declines soon after birth (37,38). It could be possible that cultured proximal tubular cells, depending on the culture conditions, might express a significant amount of AT2. On the other hand, we cannot exclude a possibility that AT2 may play some physiologic roles in adult kidney. For example, AngII has been shown to activate NF-κB in proximal tubules through both AT1 and AT2 (39), and AT2 could mediate long-term effects of AngII in vivo through such a mechanism. Nevertheless, the results from our studies on isolated proximal tubules strongly argue against a
significant role of AT₂ in the acute regulation of proximal transport by AngII.

Acknowledgments

This study was in part supported by grant 14571013 from the Ministry of Education, Science and Culture of Japan.

References