Protein kinase G activates inwardly rectifying K⁺ channel in cultured human proximal tubule cells

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Nakamura, Kazuyoshi, Junko Hirano, Shun-ichi Itazawa, and Manabu Kubokawa. Protein kinase G activates inwardly rectifying K⁺ channel in cultured human proximal tubule cells. Am J Physiol Renal Physiol 283: F784–F791, 2002.—An ATP-regulated inwardly rectifying K⁺ channel, whose activity is enhanced by PKA, is present in the plasma membrane of cultured human proximal tubule cells. In this study, we investigated the effects of PKG on this K⁺ channel, using the patch-clamp technique. In cell-attached patches, bath application of a membrane-permeant cGMP analog, 8-bromoguanosine 3',5'-monophosphate (8-BrcGMP; 100 μM), stimulated channel activity, whereas application of a PKG-specific inhibitor, KT-5823 (1 μM), reduced the activity. Channel activation induced by 8-BrcGMP was observed even in the presence of a PKA-specific inhibitor, KT-5720 (500 nM), which was abolished by KT-5823. Direct effects of cGMP and PKG were examined with inside-out patches in the presence of 1 mM MgATP. Although cytoplasmic cGMP (100 μM) alone had little effect on channel activity, subsequent addition of PKG (500 U/ml) enhanced it. Furthermore, bath application of atrial natriuretic peptide (ANP; 20 nM) in cell-attached patches stimulated channel activity, which was blocked by KT-5823. In conclusion, cGMP/PKG-dependent processes participate in activating the ATP-regulated K⁺ channel and producing the stimulatory effect of ANP on channel activity.

patch-clamp; human kidney; guanosine 3',5'-cyclic monophosphate; atrial natriuretic peptide; KT-5823

IT IS GENERALLY ACCEPTED THAT THE BASOLATERAL ATP-REGULATED K⁺ CHANNELS IN THE PROXIMAL TUBULE ARE IMPORTANT FOR FORMATION OF THE MEMBRANE POTENTIAL THAT PROVIDES THE DRIVING FORCE FOR ELECTROGENIC PASSIVE TRANSPORT ACROSS THE APICAL AND BASOLATERAL MEMBRANES (2). INTRACELLULAR ATP IS CONSIDERED TO ACT AS A MODULATOR COUPLING THE FUNCTION OF THESE K⁺ CHANNELS TO BASOLATERAL Na⁺-K⁺ PUMP ACTIVITY (1, 8, 29, 33, 34). IN ADDITION TO ATP, SEVERAL FACTORS AFFECTING CHANNEL ACTIVITY HAVE BEEN REPORTED. PROTEIN PHOSPHORYLATION IS ONE OF THE KEY MODIFIERS FOR CHANNEL FUNCTION. IT HAS BEEN DEMONSTRATED THAT ACTIVITY OF THE BASOLATERAL K⁺ CHANNEL IN AMBYSTOMA PROXIMAL TUBULE CELLS IS SUBJECT TO PHOSPHORYLATION BY PKA AND PKC (21). FURTHERMORE, THE ATP-REGULATED K⁺ CHANNEL IN OPOSSUM PROXIMAL TUBULE CELLS IS MODULATED BY PKA (16), PKG (17), PKC, AND Ca²⁺/CALMODULIN-DEPENDENT PROTEIN KINASE II (CaMKII) (23).

THE IMPORTANCE OF PROTEIN PHOSPHORYLATION IN THE REGULATION OF CHANNEL ACTIVITY IS ALSO OBSERVED IN K⁺ CHANNELS OF OTHER NEPHRON SEGMENTS OR CLONED RENAL K⁺ CHANNELS. FOR EXAMPLE, THE APICAL SECRETORY K⁺ CHANNEL IN RAT CORTEX COLLECTING DUCT (CCD), WHICH WOULD BE IDENTICAL TO THE CLONED RENAL K⁺ CHANNEL, ROMK (15, 22, 26), IS MODULATED BY PKA-, PKC- (32), AND CaMKII-MEDIATED PHOSPHORYLATION PROCESSES (18). HOWEVER, THE BASOLATERAL K⁺ CHANNELS IN CCD ARE REGULATED BY PKG (13, 31) AND PKC-MEDIATED PROCESSES (19).

DESPITE THE ABUNDANT REPORTS ABOUT THE ATP-REGULATED K⁺ CHANNELS ALONG THE NEPHRON AS MENTIONED ABOVE, THESE DATA WERE BASED ON THE EXPERIMENTS USING ANIMAL SPECIES, AND PROPERTIES OF THE K⁺ CHANNELS IN HUMAN RENAL TUBULE CELLS HAVE NOT BEEN SUFFICIENTLY ELUCIDATED. RECENTLY, WE IDENTIFIED AN ATP-REGULATED INWARDLY RECTIFYING K⁺ CHANNEL WITH AN INWARD SLOPE CONDUCTANCE OF ~42 pS IN CULTURED HUMAN PROXIMAL TUBULE CELLS OF NORMAL KIDNEY ORIGIN AND DEMONSTRATED THAT ACTIVITY OF THIS K⁺ CHANNEL WAS MAINTAINED, AT LEAST IN PART, BY PKA-MEDIATED PHOSPHORYLATION (24). HOWEVER, THERE REMAINED THE POSSIBILITY THAT SOME MECHANISMS OTHER THAN PKA-MEDIATED PROCESSES MIGHT BE INVOLVED IN REGULATION OF THIS CHANNEL, BECAUSE A PKA-SPECIFIC INHIBITOR ONLY REDUCED CHANNEL ACTIVITY TO ~50% OF CONTROL (24). IN THIS STUDY, WE TESTED THE EFFECT OF ANOTHER PROTEIN KINASE, PKG, ON ACTIVITY OF THIS K⁺ CHANNEL, USING THE PATCH-CLAMP TECHNIQUE. FURTHERMORE, WE EXAMINED THE EFFECTS OF A GUANYLATE CYCLASE-ACTIVATING PEPTIDE, ATRIAL NARIURETIC PEPTIDE (ANP), ON CHANNEL ACTIVITY. ALTHOUGH THE MAJOR SITES OF ANP ACTION IN THE KIDNEY WERE SHOWN TO BE LOCATED IN THE GLOMERULI AND THE INNER MEDULLARY COLLECTING DUCT (20, 37), SEVERAL INVESTIGATORS REPORTED THAT ANP AFFECTED THE PROXIMAL TUBULE FUNCTION (6, 7, 9, 11, 12). IN THE PRESENT STUDY, WE DEMONSTRATE THAT CYCLIC GMP/PKG-DEPENDENT PROCESSES STIMULATE K⁺ CHANNEL ACTIVITY AND IT IS NO LONGER ACCEPTED THAT THE BASOLATERAL K⁺ CHANNEL ACTIVITY IS REGULATED SIMPLY BY ATP. INSTEAD, IT IS SUGGESTED THAT THE K⁺ CHANNELS ARE REGULATED BY A COMBINATION OF ATP, CYCLIC NUCLEOTIDES, AND PROTEIN KINASES (31, 32, 35).

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that ANP also stimulates the activity via the PKG-mediated process in the human proximal tubule cells.

METHODS

Cell culture. Renal proximal tubule epithelial cells (RPTECs) isolated from the normal human kidney of a 31-yr-old woman (strain 5899, lot 8F0690) were purchased from Clonetics (Walkersville, MD). It is guaranteed that >90% of the cells are positive for γ-GTP, a marker protein specific to the proximal tubule (10). These cells were provided as cryopreserved secondary cultures and maintained in a renal epithelial cell growth medium (Clonetics) in a humidified atmosphere of 5% CO₂-95% air at 37°C. In the experiments, the cells were dispersed from 70–80% confluence at passages 3–6 with trypsin/EDTA, resuspended in the growth medium, and seeded on collagen-coated coverslips (Iwaki Glass, Tokyo, Japan) in 24 multiwells at a density of 2 x 10⁴ cells/well. After a 3- to 7-h incubation, the coverslips were transferred to an open bath-heating chamber (Warner, Hamden, CT).

Scanning electron microscopy. RPTECs incubated for 3 h on the collagen-coated coverslips were prefixed with 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) for 1 h at room temperature. Then, the cells were rinsed with 0.1 M phosphate buffer (pH 7.3) containing 3.3% sucrose and post-fixed with 1% OsO₄ in 0.05 M phosphate buffer (pH 7.3) containing 6.5% sucrose for 1 h at room temperature. The fixed cells were dehydrated in a graded series of ethanol and subjected to the critical point drying. The dried specimens were coated with OsO₄ and viewed in a Hitachi S-4700 scanning electron microscope (Hitachi, Tokyo, Japan).

Solutions. The control bath solution contained (in mM) 140 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 5 glucose, and 10 HEPES. Patch pipettes were filled with a KCl solution, which contained (in mM) 145 KCl, 1 MgCl₂, 1 EGTA, and 1 HEPES. The same KCl solution was employed as the bath solution for inside-out patches. All of these solutions were titrated to pH 7.3 with 5 N NaOH or KOH.

Test substances. MgATP, 8-bromoguanosine 3’,5’-monophosphate (8-BrcGMP), cGMP and human ANP (hANP) were purchased from Sigma (St. Louis, MO). A membrane-permeant PKG-specific inhibitor, KT-5823, and a membrane-permeant PKA-specific inhibitor, KT-5720, were from Calbiochem (La Jolla, CA). PKG was from Promega (Madison, WI). KT-5823 and KT-5720 were dissolved in DMSO as stock solutions, whereas others were dissolved in water. The stock solutions were diluted with appropriate amounts of bath solutions and added to the bath. The final concentration of DMSO in the bath ranged from 0.047 to 0.054%, which had no apparent effect on channel activity. These substances were directly added to the bath by hand-pipetting, except for MgATP, which was added to the bottle of KCl bath solution beforehand.

Patch-clamp technique. Single channel currents were recorded with cell-attached and inside-out patches applied to
the surface membrane of single RPTECs. All patch experiments were performed at 33°C, which was adjusted by a heater platform connected with a controller (TC-324B, Warner). This temperature setting was very suitable for both the gigaseal formation and cell viability. Patch pipettes were fabricated from glass capillaries (Warner), with the resistance ranging from 3 to 5 MΩ when filled with the KCl solution. The pipette holding potential (V_p) was set at 0 mV for cell-attached patches and +50 mV for inside-out patches, unless otherwise stated. Current signals were recorded with a patch-clamp amplifier Axopatch 200B (Axon, Foster City, CA) and stored on a DAT recorder (RD-120TE, TEAC, Tokyo, Japan). The recorded signals were then low-pass filtered (3611 Multifunction Filter, NF Electronic Instruments, Tokyo, Japan) at 500 Hz and digitized at a rate of 10 kHz through an interface (Digidata 1200A, Axon). The acquired data were analyzed with acquisition/analysis software (pCLAMP6, Axon) on an IBM-compatible personal computer. Current traces of downward deflections represented inward currents. Channel activity was determined by NP_o, which was calculated as

$$NP_o = \sum_{n=1}^{N} n \cdot t_n$$

where N is the maximum number of channels observed in the patch, P_o is the open probability, n is the number of channels observed at the same time, and t_n is the probability that n channels are simultaneously open, which was obtained by fitting the amplitude histogram with a Gaussian function of the pSTAT software included in pCLAMP6 (Axon). For convenience, normalized channel activity was calculated by dividing NP_o,e by NP_o,c to compare the channel activity in experimental conditions with controls, where NP_o,e and NP_o,c are the channel activities under control and experimental conditions, respectively. Routinely, we determined NP_o,e from a 20-s sampling period just before the substance was added when the steady state lasted for at least 60 s. Although the time course of the effect of a substance varied in individual patches, which ranged from 15 to 90 s except for hANP (see below), NP_o,e was determined from a 20-s sampling period extracted from the steady state for at least 20–30 s made by the experimental substance. If the 20-s sampling impaired the precise estimation of NP_o because of baseline drift, a few 10-s sampling periods were taken and the averaged NP_o was adopted.

Statistics. Data are expressed as means ± SE from 4–15 patches. Student’s t-test or ANOVA in conjunction with Bonferroni t-test was used for statistical comparisons. A P value <0.05 was considered to be significant.

RESULTS

Figure 1 shows a typical scanning electron microscopic view of a single RPTEC we used in this study. The cell is spherical in form and attaches to the coverslips. Although short microvilli are sparsely distributed over the entire plasma membrane, the apical brush border seen in the proximal tubule cells in situ is not apparent.

As we have demonstrated in the previous paper (24), an inwardly rectifying K⁺ channel is the most frequently observed K⁺ channel in the plasma membrane of RPTECs in cell-attached patches under the control condition. The current-voltage (I-V) relationships of this K⁺ channel are shown in Fig. 2. Representative current traces, which were obtained from an inside-out patch under the symmetrical K⁺ condition, show a typical inward rectification (Fig. 2A). Although data are not shown, there was no significant voltage dependency of channel open probability. Data were pooled from experiments similar to that in Fig. 2A, and an I-V curve was drawn (Fig. 2B, circles). The inward conductance obtained from the slope between −90 and −30 mV of V_p was 42.5 ± 2.9 pS (n = 12), and the outward slope conductance between +15 and +60 mV was 7.8 ± 1.5 pS (n = 12). An asymmetrical K⁺ condition was also employed to estimate the K⁺ selectivity of this channel. When the internal K⁺ concentration was reduced from 15 to 30 mM, the reversal potential shifted to +31 mV (Fig. 2B, squares). According to the Goldman-Hodgkin-Katz voltage equation, the calculated K⁺-to-Na⁺ permeability ratio was ~8.

In our previous study, we showed that the channel is activated by PKA-mediated phosphorylation (24). To

![Fig. 3. Representative current traces showing effects of a membrane-permeant analog of cGMP, 8-bromoguanosine 3',5'-monophosphate (8-BrcGMP, A), and a PKG-specific inhibitor (KT-5823; B), on channel activity. Each current recording was obtained from separate cell-attached patches at V_p of 0 mV. 8-BrcGMP was added to the bath at 100 μM, and KT-5823 was added at 1 μM. C: summary of the effects of 8-BrcGMP and KT-5823. Data were obtained from 15 cell-attached patches for 8-BrcGMP and another 15 for KT-5823. NP_o,e and NP_o,c, channel activities under experimental and control conditions, respectively; N, maximum no. of channels observed in the patch; P_o, open probability **Significantly different (P < 0.01) compared with respective initial control levels.](http://ajprenal.physiology.org/download/FIG3.jpg)
extend our knowledge about the regulation of this K⁺ channel, the involvement of the cGMP/PKG pathway was explored. A representative current recording of the K⁺ channels in response to a membrane-permeant cGMP analog, 8-BrcGMP, in a cell-attached patch is shown in Fig. 3A. Application of 8-BrcGMP (100 μM) to the bath caused an increase in the number of active channels compared with control. This result suggests that elevation of intracellular cGMP activates the K⁺ channels. Because cGMP activates PKG, we tested the effect of a membrane-permeant PKG-specific inhibitor, KT-5823, on channel activity to examine whether PKG is involved in the regulation of the channel. As the results show in Fig. 3B, bath application of KT-5823 (1 μM) inhibited channel activity in a cell-attached patch. Summarized data on effects of 8-BrcGMP and KT-5823 on channel activity are shown in Fig. 3C. 8-BrcGMP significantly stimulated, and KT-5823 significantly suppressed, channel activity. These data suggest the involvement of PKG-mediated phosphorylation processes in activation of the K⁺ channel.

Because the PKA-mediated phosphorylation process can also activate the K⁺ channel in RPTECs (24), it is possible that the stimulatory effect of 8-BrcGMP would have some relationship to the PKA-mediated process. In fact, it has been demonstrated that cGMP causes inhibition of phosphodiesterase type 3 (PDE3), which subsequently increases intracellular cAMP, resulting in stimulation of PKA activity (4). Therefore, the following experiment was designed to examine whether the stimulatory effect of cGMP on channel activity was observed even in the presence of a PKA-specific inhibitor. A channel recording obtained with a cell-attached patch is shown in Fig. 4A. A PKA-specific inhibitor, KT-5720 (500 nM), suppressed channel activity observed under the control condition, and the following addition of 8-BrcGMP (100 μM) restored the activity. The restored channel activity was again suppressed by KT-5823. These results suggest that channel activation induced by 8-BrcGMP is not dependent on the PKA-mediated process but for the most part on PKG-mediated phosphorylation. Similar results were obtained from another six patches, and the data are summarized in Fig. 4B.

Next, we examined effects of the cytoplasmic cGMP and PKG on channel activity in inside-out patches under the symmetrical KCl condition at a Vₚ of +50 mV. The bath solution contained 1 mM MgATP, because this K⁺ channel requires cytoplasmic ATP to maintain its activity in inside-out patches (24). Figure 5A shows a representative current trace in response to cytoplasmic cGMP and PKG in the presence of MgATP. Addition of cGMP (100 μM) alone to the bath had little effect on channel activity, whereas the subsequent addition of PKG (500 U/ml) enhanced it. After the washing out of both cGMP and PKG, channel activity was reduced to the control level. Data from eight patches were pooled and summarized in Fig. 5B. There was no statistically significant difference between the control and cGMP-treated groups, whereas addition of PKG significantly stimulated channel activity in the presence of cGMP and MgATP.

Finally, we tested a guanylate cyclase-activating peptide, hANP, in cell-attached patches. Addition of hANP (20 nM) to the bath caused a reversible channel activation, as shown in Fig. 6A. This peptide required a relatively longer time to express its effect compared with other test substances. A time course experiment, where five cell-attached patches were exposed to hANP for up to 5 min, revealed that the hANP-induced channel activation began at ~2.5 min after addition of hANP and reached a maximal plateau in 4 min (Fig. 6B). On the basis of this result, we examined the effect of hANP for ~4-min exposure in additional six patches. Summarized data from 11 patches are shown in Fig. 6C. The effect of a PKG-inhibitor, KT-5823, on hANP-AJP-Renal Physiol • VOL 283 • OCTOBER 2002 • www.ajprenal.org
induced channel activation was also explored. This inhibitor suppressed the channel activated by hANP (Fig. 7A). Furthermore, hANP failed to reactivate the channel suppressed by KT-5823 (Fig. 7B). Summarized data are shown in Fig. 7C. It is apparent that KT-5823 significantly suppresses the effect of hANP, whether this inhibitor was added before or after hANP. These results strongly suggest that hANP-induced channel activation is PKG-dependent.

DISCUSSION

The single RPTEC used in this study seemed not to be well polarized. This cell attached to the coverslip, but the sparsely distributed short microvilli, which are far from the apical brush border seen in proximal tubule cells in situ, would suggest the lack of an apparent distinction between the apical and basolateral membrane. In support of this notion, it has been reported that cell-cell interactions were required for the polarization of a basolateral protein in Madin-Darby canine kidney (MDCK) cells (30). The same situation might hold true for the single RPTEC. Thus it is not known whether the inwardly rectifying K\$/H channel in RPTECs originated from the basolateral or the apical membrane. However, we have previously reported that this K\$ channel is pH sensitive and requires PKA-mediated phosphorylation processes to maintain its activity (24). These properties are very similar to those
Traces were recorded with separate cell-attached patches at 
K/H11001 inhibited by high doses (experiments similar to those in 
K/H11001 KT-5823. Data for hANP and hANP 
respectively. C: summary of the interaction between hANP and 
K/H11001 KT-5823. Data for hANP and hANP+KT-5823 were obtained from 4 
experiments similar to those in A, and those for KT-5823 and KT-
5823+hANP were from 5 experiments similar to those in B. **Signi-
ficantly different (P < 0.01) compared with respective initial con-
trol levels. †Significantly different (P < 0.01) compared with hANP.

reported previously in the basolateral K+ channel of 
proximal tubule cells (21). Thus we assume that the K+
channel in single RPTECs would correspond to the 
basolateral K+ channel in human proximal tubule 
cells. Nonetheless, we must notice that the properties 
of this K+ channel are not always identical to those of 
K+ channels in the native proximal tubule. For exam-
ple, the activity of the K+ channel in RPTECs is not 
inhibited by high doses (~10 mM) of ATP (24), which is 
different from the ATP sensitivity of basolateral K+
channels in proximal tubule cells reported by several 
investigators (1, 8, 29, 33, 34). Such a difference might 
result from alterations in channel composition induced 
by cell culture, although the ATP-sensitivity of human 
K+ channels in situ is unknown.

Our present data demonstrate that regulation of the 
K+ channel in RPTECs involve PKG-mediated pro-
cesses. Channel activity in cell-attached patches was 
activated by 8-BrcGMP, whereas it was suppressed by 
a PKG-specific inhibitor. Activation of the channel by 
8-BrcGMP was induced even in the presence of a PKA-
specific inhibitor. Moreover, although internal cGMP 
alone in the presence of MgATP had no significant 
effect on channel activity in inside-out patches, con-
comitant application of PKG significantly enhanced it. 
Thus it is suggested that PKG-dependent phosphory-
lation processes besides PKA-dependent ones enhance 
K+ channel activity. A similar dual modulation of 
channel activity has been demonstrated in the in-
wardly rectifying K+ channel of opossum kidney prox-
imal tubule cells (16, 17).

It has been reported that intracellular cGMP in-
duced not only activation of PKG but also inhibition of 
activity of PDE3 (4). The latter would lead an increase in 
cAMP and hence may stimulate the other cyclic nucleotide-dependent protein kinase, PKA. However 
as mentioned above, 8-BrcGMP stimulated channel 
activity even in the presence of a PKA-specific inhibi-
tor, suggesting that cGMP-induced channel activation 
was not due to stimulation of PKA. Furthermore, a 
direct effect of cGMP on channel activity was also 
reported, and such a cGMP-gated K+ channel was 
expressed in the kidney (5, 36). This type of K+ channel 
contains a cGMP-binding site related to channel activ-
ation (5, 36). In immortalized human proximal tubule 
cells, Hirsch et al. (14) demonstrated the existence of a 
cGMP-regulated K+ channel, which was inhibited by 
cGMP without PKG-mediated phosphorylation. In our 
study, however, direct application of cGMP alone had 
no significant effect on channel activity in inside-out 
patches. Taken together, it can be concluded that the 
stimulatory effect of cGMP on the K+ channel in RPT-
ECs is mainly produced through activation of PKG, 
which is independent of PKA-mediated processes. 
However, the site of PKG-mediated phosphorylation in 
our study is presently unknown. PKG and PKA might 
share the same phosphorylation site because of a sim-
ilarity between the substrate-recognition sequences for 
these two protein kinases (27). It is also possible that 
PKG would indirectly activate the K+ channel through 
the phosphorylation of channel-associated proteins 
rather than the channel protein itself as reported in 
the maxi-K+ channel in a rat pituitary cell line (35).

Furthermore, channel activation by PKG-mediated 
phosphorylation was reversible in most cases, as 
shown in Figs. 3 and 5, suggesting that the phos-
phatase-mediated dephosphorylation would be in-
volved in this reversibility. Thus channel activity at a 
given time would be determined by the dynamic bal-
ance between protein kinase activity and protein phos-
phatase activity. The time course of effects of test 
substances may well also be affected by this balance. If 
phosphatase activity was potentially high, the effect of 
a protein kinase inhibitor on the open channels would 
be rapidly manifested, as seen in Fig. 4A. In contrast, 
if PKG activity was fairly predominant, a relatively 
longer time would be required for the expression of the 
effect of a protein kinase inhibitor, as shown in Fig. 7B.
Taken together, it is highly likely that protein phosphatases, as well as protein kinases, are the key factors in regulating channel activity in RPTECs. Further studies will be necessary to clarify what types of phosphatases would be involved in the suppression of the PKG-dependent channel activity.

The present study showed that hANP also activated the K\(^+\) channel in RPTECs through PKG-mediated phosphorylation. Although the major sites of ANP action in the kidney are thought to be the glomeruli and the inner medullary collecting duct (20, 37), several investigators have demonstrated the existence of guanylate cyclase-coupled ANP receptors in the proximal tubule (12, 28). In addition, it was reported that ANP caused accumulation of intracellular cGMP in this nephron segment (3, 25). Therefore, it is likely that hANP-induced changes in channel activity would be elicited by binding of this peptide to its specific receptor(s). The slow time course of hANP action observed in our study suggests that the production of an effective concentration of cGMP would take a relatively long time.

As for the effect of ANP on the K\(^+\) channel in proximal tubule cells, two reports are now available. One is that ANP inhibited the activity of an apical cGMP-regulated K\(^+\) channel in immortalized human proximal tubule cells (14). In contrast, the other report showed that ANP stimulated the activity of an ATP-regulated K\(^+\) channel in the surface membrane of opossum kidney proximal tubule cells (17). The present data are consistent with the results shown in the latter report. Considering the role of basolateral K\(^+\) channels in the proximal tubule, ANP-induced channel activation seems to be contradictory to the natriuretic property of this peptide. However, some investigators reported that ANP alone did not affect the reabsorption of fluid in the rat proximal tubule, whereas this peptide inhibited the angiotensin II-induced increase in fluid reabsorption (6, 7, 11). Another group demonstrated that ANP suppressed Na\(^+\)-coupled P\(_i\) reabsorption and Na\(^+\)-H\(^+\) exchange but had no effect on Na\(^+\)-coupled glucose and proline uptake (9). Although the physiological role of ANP in the human proximal tubule is still obscure, one plausible explanation for our finding is that ANP-induced channel activation might prevent the excessive loss of Na\(^+\) and water by increasing the driving force for Na\(^+\) transport in this nephron even when certain kinds of Na\(^-\)coupled transporters would be suppressed by ANP.

In summary, the ATP-regulated inwardly rectifying K\(^+\) channel in human proximal tubule cells is under the stimulatory control of PKG-mediated phosphorylation processes, besides PKA-mediated ones. Moreover, PKG-mediated phosphorylation is important for ANP to activate this K\(^+\) channel.

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**REFERENCES**


**EFFECT OF PKG ON HUMAN RENAL K⁺ CHANNEL**


