Modulation of Inwardly Rectifying ATP-Regulated K⁺ Channel by Phosphorylation Process in Opossum Kidney Cells

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Abstract: The role of protein phosphorylation in modulating an inwardly rectifying ATP-regulated K⁺ channel with inward conductance of about 90 pS was examined using the patch-clamp technique on opossum kidney (OK) cells. The activity of the inwardly rectifying K⁺ channel observed in cell-attached patches rapidly declined (channel "run-down") upon excision of the membrane into inside-out patches in a control bath solution (3 mM Mg²⁺, ATP-free). The declined channel activity was partially restored by applying ATP to the bath, and the ATP-induced channel restoration reached the near maximal level at an ATP concentration of 3 mM. The channel activity maintained by 3 mM ATP in inside-out patches was inhibited by K-252a (10 μM), a non-specific protein kinase inhibitor, or KT5720 (200 nM), a specific inhibitor of cyclic AMP (cAMP)-dependent protein kinase (PKA), and was further stimulated by the addition of a catalytic subunit of PKA (20 nM). In cell-attached patches, the channel activity was also inhibited by K-252a (10 μM) or KT5720 (200 nM). The application of dibutyl-cAMP (100 μM) alone failed to enhance channel activity, but significantly stimulated channel activity after the pretreatment of cells with Ro-20-1724 (100 μM), an inhibitor of cAMP-specific phosphodiesterase. These results suggest that maintenance of the activity of ATP-regulated K⁺ channels in OK cells requires protein kinase-mediated phosphorylation with ATP-hydrolysis, and that phosphorylation is mainly induced by PKA. [Japanese Journal of Physiology, 47, 111–119, 1997]

Key words: proximal tubule cell, inwardly rectifying K⁺ channel, patch-clamp, cAMP-dependent protein kinase, phosphorylation.

The functional significance and distinct characteristics of inwardly rectifying K⁺ channels regulated by cytosolic ATP have been investigated for a variety of cell membranes using the patch-clamp technique. In the tubular epithelium of mammalian kidneys, several types of inwardly rectifying ATP-regulated K⁺ channels are found in the basolateral membrane of the proximal tubule [1, 2], the apical membrane of the thick ascending limb [3], and the apical and basolateral membranes of the collecting duct [4–6]. These K⁺ channels play important roles in the reabsorption or secretion processes of K⁺ or other ions along the nephron [7–9]. Previous reports have demonstrated that an inwardly rectifying ATP-regulated K⁺ channel having inward conductance of 90 pS was present in the membrane of cultured opossum kidney (OK) cells [10, 11] which had been used as a model system for proximal tubular epithelia [12].

It has been shown that protein kinase-mediated phosphorylation is one of the important processes in the modulation of a number of ion channels including the ATP-regulated K⁺ channels in renal tubule cells [13, 14]. The ATP-regulated K⁺ channel present in the apical membrane of the principal cell of rat cortical collecting duct (CCD) is activated by protein kinase A (PKA) and inactivated by Ca²⁺-dependent protein kinase C (PKC) [15] and Ca²⁺/calmodulin-dependent protein kinase II (CaMK II) [16]. A cloned ATP-regulated K⁺ channel from rat renal outer medulla (ROMK1 K⁺ channel) [17], which has been proven to be distributed in the collecting duct [18], is also modulated by PKA-mediated protein phosphorylation when expressed in Xenopus oocytes [19]. However, in the ATP-regulated K⁺ channel of the proximal tubule...
cells, little is known about the phosphorylation process in modulating channel activity. In this study, we explore the role of protein phosphorylation in modulating inwardly rectifying ATP-regulated K⁺ channel in OK cells using the patch-clamp technique, demonstrate that protein kinase-mediated phosphorylation is essential for maintaining channel activity, and provide evidence that cAMP-dependent protein kinase (PKA) is involved in this phosphorylation process.

MATERIALS AND METHODS

Cell culture. OK cells were maintained in Dulbecco’s modified Eagle’s medium (Dainippon Pharma- ceutical, Tokyo, Japan) supplemented with 10% fetal bovine serum at 37°C in 5% CO₂, as reported previously [11]. In the experiments, cells were isolated from confluent monolayers at passages 18–48 with trypsin and incubated in a culture medium for 4–8 h before use. All experiments were performed at room temperature (approx. 24°C).

Solutions. The control bath solution contained (in mM) NaCl 140, KCl 5, CaCl₂ 2, MgCl₂ 1, glucose 5, and N-2-hydroxyethylpiperazine-N’-2-ethanesulfonylic acid (HEPES) 10. The KCl solution used in the bath for inside-out patches contained (in mM) KCl 115, MgCl₂ 3, KOH 30, ethylene glycol-bis (β-aminoethoxy ether)-N, N, N’, N’-tetraacetic acid (EGTA) 10, and HEPES 5 with 10⁻⁷-M free-Ca²⁺. In some experiments, a NaCl solution with low free-Ca²⁺ was used, which contained (in mM) NaCl 110, KCl 5, MgCl₂ 1, NaOH 30, EGTA 10, and HEPES 5 with 10⁻⁷-M free-Ca²⁺. The free-Ca²⁺ concentration was obtained according to the computer program of Oki and Okada [20], and was adjusted using the absolute values of the stability constant of EGTA for binding of Ca²⁺, Mg²⁺, and H⁺ [21]. The actual concentration of free-Ca²⁺ was checked using Ca²⁺-selective micro-electrodes [22] made with a Ca²⁺-ion exchanger (ETH-1001, Science Trading, Frankfurt, Germany). The solutions with ATP were made by adding Na₂ATP (Nacalai Tesque, Kyoto, Japan). All of these solutions were adjusted to pH 7.3 with a minute amount of HCl, NaOH, or KOH at 24°C.

Chemicals. Dibutyryl-cAMP (N⁶,2’-O-dibutyryladenosine 3’,5’-cyclic monophosphate) was obtained from Sigma (St Louis, USA). The catalytic subunit of protein kinase A (PKA), K-252a, KT5720, and Ro-20-1724 (4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone) were purchased from Calbiochem (La Jolla, USA).

Patch-clamp technique. Patch-clamp experiments were performed by methods described previously [9]. Patch-clamp electrodes were pulled from hemocytocapillaries (Nichiden Rika, Hyogo, Japan) and filled with a filtered solution containing (in mM) KCl 145, MgCl₂ 1, and HEPES 10 (pH 7.3). The electrode resistance ranged between 4 and 8 MΩ. Electric currents measured with a patch-clamp amplifier (AXOPATCH 200A, Axon, Foster City, CA, USA) were stored on a DAT recorder (Model 5870, NF, Tokyo, Japan). The current records were then low-pass filtered (902LPF, Frequency Devices, Haverhill, USA) at 0.3–1 kHz and digitized using a computer (Macintosh IIfx, Apple, Cupertino, CA, USA) equipped with an interface (ITC-16, Instrutech, Elmont, NY, USA) and data acquisition software (AXODATA, Axon). The data obtained were analyzed using the AXO-GRAPH program system (Axon). Values for the potential represent the voltages of the pipette (Vp). Currents flowing from the cytoplasmic to the extracellular side were defined as outward and shown as upward deflections in the figures.

Channel activity was determined by the mean channel-open probability (P₀), which was calculated as,

\[ P₀ = \frac{1}{N} \sum_{n=1}^{N} t_n \]

where N is the number of active channels in the patch and \( t_n \) (t₁, t₂, ..., tₙ) is the fractional open time at each current level. Normalized channel activity was calculated by the ratio \( P₀_{\text{exp}} \). \( P₀_{\text{cont}} \) and \( P₀_{\text{exp}} \) are the mean channel activities of the control and experimental conditions, respectively. \( P₀_{\text{cont}} \) was determined from a 20 s data sampling period just before adding chemicals, and \( P₀_{\text{exp}} \) was determined from a 10 to 20 s data sampling period 10 to 50 s after adding each chemical.

Statistics. Data are expressed as mean±SE. Where appropriate, Student’s t-test for paired or unpaired data was used to assess significance of difference.

RESULTS

In a previous paper, Oino-Shosaku et al. [10] demonstrated that an inwardly rectifying ATP-regulated K⁺ channel with inward conductance of about 90 pS was present in the membrane of OK cells. Thus, at first, we investigated the basic properties of the ATP-regulated K⁺ channel in OK cells to examine whether the K⁺ channel observed in our experiment was the same channel demonstrated previously.

Figure 1A shows a recording of the ATP-regulated
Modulation of K⁺ Channel in OK Cells

![Graphs and Image]

Fig. 1. A: Single-channel recordings of an inwardly rectifying ATP-regulated K⁺ channel in an inside-out patch with symmetrical KCl solution in pipette and bath. The bath solution contained 3 mM MgCl₂ and 1 mM ATP. Membrane potential (−Vₒ) in mV is indicated on top of each trace, and closed channel states are indicated by dotted lines and C. B: Current-voltage (I-V) curves of the K⁺ channel in inside-out patches with symmetrical KCl solution (open circles) and NaCl solution with low-free Ca²⁺ containing 5 mM KCl, 1 mM MgCl₂, and 1 mM ATP in the bath (closed circles). The curves through the mean values were drawn by hand.

K⁺ channel of an OK cell at 4 different holding potentials in an inside-out patch with symmetrical 145 mM KCl solutions in the bath and pipette. The bath solution contained 3 mM Mg-ATP to maintain channel activity. It was apparent that the inward channel current was larger than the outward current. Figure 1B shows the current-voltage (I-V) relations of the ATP-regulated K⁺ channel in inside-out patches with symmetrical 145 mM K⁺ solutions and 140 mM Na⁺ solutions with 5 mM K⁺ in the bath. In symmetrical 145 mM KCl solutions, the inward slope conductance was 90.5 ± 2.4 pS (n = 6) and outward slope conductance was 34.9 ± 1.4 pS (n = 6); similar to data reported in a previous paper [10]. Thus, we confirmed that the ATP-regulated K⁺ channel observed in our experiment was the same K⁺ channel demonstrated by Ohno-Shosaku et al. [10, 11]. Switching the bath from the 145 mM KCl solution to the 140 mM Na⁺ solution with 5 mM K⁺, the I-V relation was shifted by about 50 mV (Fig. 1B). We calculated the channel selectivity ratio (P_K/P_Na) using the equation,

\[ E_{\text{rev}} = 59 \log \frac{145(P_K/P_Na)}{140 + 5(P_K/P_Na)} \]

where \( E_{\text{rev}} \) is the shift of the reversal potential. A K⁺/Na⁺ selectivity of about 9 was obtained.

In an ATP-free bath solution with Mg²⁺, the inwardly rectifying K⁺ channel observed in a cell-attached patch of OK cell rapidly declined (channel "rundown") upon excision of the membrane into an inside-out patch, and the addition of ATP (3 mM) to the bath partially restored channel activity as shown in Fig. 2. In this experiment, KCl solution was used for the bath in which 3 mM Mg²⁺ was present.

The changes in channel activity in response to various concentrations of ATP in an inside-out patch are shown in Fig. 3A. A KCl solution with 3 mM Mg²⁺ was used for the bath. Channel activity was increased by adding ATP in a dose-dependent manner, and \( P_0 \) reached to almost the maximal level in the presence of 3 mM ATP (0.56 ± 0.06, n = 4) as shown in Fig. 3B. These results indicate that ATP simply has a stimulatory effect and no inhibitory effect on channel activity. Since channel activity was almost maximally activated by 3 mM ATP, the following experiments in inside-out patches were carried out with 3 mM Mg-ATP in the bath.

To examine the involvement of protein kinase-mediated phosphorylation in maintaining channel activity in inside-out patches, we applied protein kinase inhibitors to a bath solution in which Mg-ATP was present. As shown in Fig. 4, the channel activity maintained by 3 mM Mg-ATP was significantly inhibited by
Fig. 3. The dose-dependent effect of ATP on channel activity in the cytoplasmic surface of membrane. A: Single-channel recordings with various concentrations of ATP in inside-out patches with a pipette holding potential of 50 mV. The current level of the K⁺ channel is indicated by short bars (left), and closed channel states are indicated by the dotted lines and C. B: Concentration response of single-channel open probabilities (Pₒ) by ATP. The curve through the mean values was drawn by hand.

Fig. 4. Changes in channel activity in response to K-252a (A) and KT5720 (B) in inside-out patches with a pipette holding potential of 50 mV. The individual current levels of the K⁺ channels are indicated by short bars (left), and closed channel states are indicated by the dotted lines and C.

Fig. 5. The stimulatory effect of a catalytic subunit of PKA (20 nm) on channel activity in an inside-out patch in the presence of 3 mM Mg-ATP. The pipette holding potential was 50 mV. The current level of the K⁺ channel is indicated by short bars (left), and closed channel states are indicated by the dotted lines and C.

either K-252a (10 μM), a non-specific protein kinase inhibitor [23] (Fig. 4A), or KT5720 (200 nM), a potent specific inhibitor of cAMP-dependent protein kinase (PKA) [24] (Fig. 4B). These recordings were obtained before and 10 s after the application of individual inhibitors and 10 s after washing out the inhibitors.

In the next step, we examined the effect of exogenous PKA on channel activity in inside-out patches in the presence of 3 mM Mg-ATP. The channel activity maintained by Mg-ATP was further stimulated by the application of a catalytic subunit of PKA (20 nm) to the cytoplasmic surface of the membrane (Fig. 5). A marked stimulation in channel activity, as shown in
Fig. 6. The effects of K-252a (10 μM), KT5720 (200 nM), and PKA (20 nM) on channel activity in inside-out patches. Data are represented as normalized channel activities from control values. **, *** Significantly different from control values on paired samples at levels of p<0.01 and p<0.001, respectively.

Fig. 5, was observed about 10 s after the application of PKA.

Figure 6 represents the summarized data obtained by experiments similar to those shown in Figs. 4 and 5. K-252a (10 μM) and KT5720 (200 nM) significantly (p<0.001) inhibited channel activity to 11.9±2.6% (n=4) and 25.1±3.8% (n=5) of the control value, respectively. These results suggest that channel activity in inside-out patches is maintained mainly by membrane-bound PKA. The application of a catalytic subunit of PKA (20 nM) significantly (p<0.01) stimulated channel activity to 187.7±13.7% (n=4) of the control value, supporting the view that PKA-mediated phosphorylation is important in stimulating channel activity.

The effects of protein kinase inhibitors on channel activity were examined also in cell-attached patches. Figure 7 shows representative recordings of the inwardly rectifying K⁺ channel in the membrane of OK cell in cell-attached patches during control conditions, 20 s after the application of individual protein kinase inhibitors, and 30 s after washing out each inhibitor. The application of 10 μM K-252a (Fig. 7A) or 200 nM KT5720 (Fig. 7B) reduced channel activity. The bath solutions used were control NaCl Ringer solutions. The moderate reductions in magnitude of the single-channel currents during the application of K-252a and KT5720 were probably due to a decrease in the driving force for K⁺ across the OK cell membrane, which was induced by depolarization.

Finally, the effect of dibutyryl-cAMP (db-cAMP), a membrane-permeable cAMP analogue, on channel activity was examined for cell-attached patches. As shown in Fig. 8A, channel activity under control conditions was slightly increased by the application of db-cAMP (100 μM) to the bath. The recordings were obtained before and 30 s after the application of db-cAMP. The maximal effect of db-cAMP on channel activity was observed within 1 min after the addition of db-cAMP, and long-time exposure (for more than 3 min) of the cells to db-cAMP did not produce a more significant effect on channel activity (data are not shown). Furthermore, as shown in Fig. 8B, we examined the effect of Ro-20-1724 (100 μM), a selective inhibitor of cAMP-specific phosphodiesterase [25], with or without db-cAMP on channel activity. The application of Ro-20-1724 for 30 s moderately stimulated channel activity, and the subsequent application of db-cAMP remarkably stimulated channel activity within 30 s.
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Fig. 8. Changes in K⁺ channel currents in response to db-cAMP (A) and db-cAMP with Ro-20-1724, a selective inhibitor of cAMP-specific phosphodiesterase (B). The channel recordings were made in cell-attached patches with 50 mV pipette holding potential. The individual current levels of the K⁺ channels are indicated by short bars (left), and closed channel states are indicated by the dotted lines and C.

The data obtained by experiments similar to those demonstrated in Figs. 7 and 8 are summarized in Fig. 9. K-252a (10 μM) and KT5720 (200 nM) significantly (p<0.001 and 0.01) inhibited channel activity to 14.8±2.8% (n=4) and 23.6±6.0% (n=4) of the control value, respectively. In addition, although db-cAMP (100 μM) or Ro-20-1724 (200 nM) alone produced no significant increase in channel activity (110.4±7.1%, n=7 and 115.1±7.9%, n=4 of the control value, respectively), the application of db-cAMP after pretreatment of Ro-20-1724 for 20 to 30 s significantly (p<0.05) stimulated channel activity to 133.5±8.6% (n=4) of the control value. These results suggest that channel activity is stimulated by cAMP-dependent protein kinase (PKA), and that both exogenous cAMP and the inhibition of cAMP-specific phosphodiesterase are required to efficiently stimulate the activity of PKA in OK cells.

DISCUSSION

Protein phosphorylation is an important mechanism for the regulation of several kinds of ion channels. This study has shown that modulation of the inwardly rectifying ATP-regulated K⁺ channel with inward conductance of about 90 pS in the membrane of OK cells involves a protein kinase-mediated phosphorylation process. The channel activity in inside-out patches essentially required ATP to maintain activity (Figs. 2 and 3), and ATP-dependent channel opening was blocked by an inhibitor of PKA and enhanced by a catalytic subunit of PKA (Figs. 5 and 6). The channel activity in cell-attached patches was also reduced by the PKA inhibitor (Figs. 7 and 9) and stimulated by application of db-cAMP with an inhibitor of cAMP-phosphodiesterase (Figs. 8 and 9), indicating that PKA-mediated phosphorylation is important in modulating channel activity.

OK cells have been used as a model system for the proximal tubule cell [10, 12], and the surface membrane of this single cell corresponds largely to the basolateral membrane [11]. The basolateral K⁺ channels in the proximal tubule cell are important in K⁺ recycling across the basolateral membrane coupled to the basolateral Na⁺/K⁺ ATPase [1, 2, 26] and in the pro-
duction of the membrane potential difference, which serves as the driving force for several ions across the apical and basolateral membranes [27].

In rabbit proximal tubule, it has been demonstrated that the ATP-regulated K⁺ channel present in the basolateral membrane is inhibited by cytosolic ATP [1, 2]. The ATP-regulated K⁺ channel with an ATP-inhibitable nature, named "ATP-sensitive K⁺ channel," was first reported by Noma in cardiac myocyte [28], and some of the ATP-regulated K⁺ channels in epithelial cells are ATP-sensitive channels [13]. In insulin-secreting cell lines, it has been demonstrated that ATP mediates both activation and inhibition of the ATP-sensitive K⁺ channel: that is, a low concentration of ATP (1–10 μM) stimulates, and a high concentration of ATP (more than 25 μM) reduces channel activity [29]. Similar results were reported for the ATP-regulated K⁺ channel in the apical membrane of CCD principal cell [4]. These K⁺ channels are also categorized as ATP-sensitive K⁺ channel [13], and the mechanism of ATP-induced channel inhibition has been investigated and discussed [4, 29, 30].

Recently, McNicholas et al. [31] provided evidence from the molecular structure of a cloned renal K⁺ channel, ROMK2 (an isofrom of ROMK1), that the ATP-sensitive renal K⁺ channel possesses a specific ATP-binding site in addition to the phosphorylation site in the channel-forming polypeptide. It has been also suggested that ATP-induced channel activation or inhibition is produced by the balance between protein phosphorylation and the direct binding of ATP to the specific site, the former stimulating and the latter inhibiting channel activity [31]. In the ATP-regulated K⁺ channel in OK cells, the channel activity observed in cell-attached patches rapidly declined (channel "rundown") upon excision of the membrane into inside-out patches in the control bath solution with Mg²⁺, and activity was partially restored by adding ATP to the bath as shown in Fig. 2. Furthermore, channel activity was simply stimulated but not inhibited by ATP, inasmuch as the ATP concentration up to 9 mM did not reduce channel activity (Fig. 3). Although the presence of a specific ATP-binding site of the ATP-regulated K⁺ channels in OK cells has not been examined, these results suggest that the ATP effect on channel activity is mainly produced by a phosphorylation process, and that the ATP-regulated K⁺ channel in OK cells is not identified as an ATP-sensitive K⁺ channel but as an ATP-dependent K⁺ channel at the present time. An ATP-regulated K⁺ channel which is not inhibited by a high concentration (5 mM) of ATP has also been demonstrated in the basolateral membrane of rat CCD [6].

As for the mechanism of channel rundown, it is suggested that Mg²⁺-dependent dephosphorylation is involved, since the removal of Mg²⁺ prevented the rundown of the ATP-regulated K⁺ channel in OK cells [10, 11] and the decreased channel activity after rundown in the presence of Mg²⁺ was restored by ATP-induced rephosphorylation [10]. The involvement of Mg²⁺-dependent dephosphorylation in the rundown process has also been suggested for other renal ATP-regulated K⁺ channels, such as the ATP-regulated K⁺ channel in rat CCD [32] and cloned ROMK1 K⁺ channel [19].

This study demonstrated that both K-252a, a non-specific protein kinase inhibitor, and KT5720, a specific inhibitor of cAMP-dependent protein kinase (PKA), significantly inhibited ATP-dependent channel opening in inside-out patches (Figs. 4 and 6). These results suggest that protein kinase-mediated phosphorylation plays a key role in maintaining channel activity in inside-out patches, and that ATP-dependent channel opening mainly results from protein phosphorylation by membrane-bound PKA with ATP-hydrolysis. Furthermore, as shown in Figs. 5 and 6, the addition of a catalytic subunit of PKA to the cytoplasmic face of the patch membrane significantly stimulated channel activity; results which are consistent with the data that an inhibitor of PKA reduced channel activity. The importance of PKA in the stimulation of renal K⁺ channels has already been demonstrated in the apical membrane of the thick ascending limb of Henle's loop [3] and the principal cell of CCD [4]. Thus, PKA might be a common modulator for some of the ATP-regulated K⁺ channels in these nephron segments.

The channel activity in cell-attached patches was also significantly inhibited by a non-specific protein kinase inhibitor or a specific inhibitor of PKA (Fig. 7), suggesting that channel activity in intact OK cells as well as in inside-out patches is maintained at least in part by PKA-mediated phosphorylation. Since the activity of intracellular PKA is dependent on cAMP, which leads the enzyme to dissociation into a regulatory subunit and a catalytically active subunit, it was predicted that an increase in intracellular cAMP would enhance PKA activity and result in the stimulation of channel activity. Thus, in the next step, we examined the effects of db-cAMP, a membrane-permeable cAMP analogue, and Ro-20-1724, an inhibitor of cAMP-specific phosphodiesterase (both of which can increase intracellular cAMP) on channel activity. As the result, however, the application of db-cAMP or Ro-20-1724 alone failed to significantly stimulate the channel activity in cell-attached patches (Figs. 8 and 9). These results are inconsistent with the data that
PKA inhibitor reduces channel activity and the data that the direct application of a catalytic subunit of PKA to inside-out patches significantly stimulates channel activity.

Although the precise mechanism for the above discrepancy is still unknown, it has been demonstrated in unstimulated cells that PKA activity in response to an elevated cAMP concentration is usually lower than that expected as a result of interaction between the catalytic subunit of PKA and a specific protein kinase inhibitor (PKI), which compete for the substrate binding site [33, 34]. Thus, it is conceivable that the minor action of db-cAMP on channel activity in cell-attached patches resulted from the interaction of PKI with a newly dissociated catalytic subunit of PKA. The significant stimulation of channel activity by the direct application of a catalytic subunit of PKA in inside-out patches supports the presence of such interaction in the cell since intracellular PKI would be absent in inside-out patches. Further investigation of this hypothesis is required.

In our final experiment, the channel activity in cell-attached patches was significantly stimulated only when db-cAMP was applied in the presence of Ro-20-1724 (Figs. 8 and 9). An elevated intracellular cAMP level by the combined use of db-cAMP and Ro-20-1724 was much higher than the application of db-cAMP or Ro-20-1724 alone. Therefore, it is suggested that an efficient increase in intracellular cAMP level to produce significant action of PKA on channel activity requires both db-cAMP and Ro-20-1724. An efficient increase in cAMP may elicit enhanced PKA activity over the interaction of PKI.

In summary, we have demonstrated in this study that PKA-mediated phosphorylation plays an important role in the modulation of inwardly rectifying ATP-regulated K+ channel in the membrane of OK cells. Again, our data suggest that ATP-dependent channel opening in inside-out patches results mainly from protein phosphorylation by membrane-bound PKA. However, the involvement of pH and Ca2++, both of which have been previously reported to modulate the ATP-regulated K+ channel in OK cells [10, 11], in the phosphorylation or dephosphorylation process remains to be examined.

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