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Regulation of Renal Potassium Channels by Protein Kinases and Phosphatases

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1. Introduction

Ion channels are well-known functional proteins, and their activity can be directly estimated by monitoring ion currents through channel proteins using the patch-clamp technique (Hamill et al., 1981), which is a fine tool for the investigation of not only ion channels, but also the membrane current and membrane potential of the whole cell. Many investigators have applied the patch-clamp technique to various cells, and found that many kinds of ion channel are present in the cell membrane.

Classical electrophysiological studies have demonstrated that the membrane potential of epithelial cells is indispensable to drive electrolytes and solute transport across cell membranes (Bello-Reuss & Weber, 1986; Beck et al., 1991; Fujimoto et al., 1991). It has also been demonstrated that the major part of the membrane potential consists of ion conductance (Kubota et al., 1983; Kubokawa et al., 1990), and that several factors regulate the membrane potential by changing the ion conductance of the cell membrane (Kubota et al., 1983; Hagiwara et al., 1990). The development of the patch-clamp technique has revealed that ion conductance of the cell membrane is mainly facilitated by current passing through the ion channels present in the membrane (Edelman et al., 1986; Fujimoto et al., 1991; Kubokawa et al., 1998). Irrespective of the recent advances in molecular technology, investigation of the functional significance of channel proteins is largely dependent on experiments using the patch-clamp technique.

Among the many kinds of ion channel, potassium selective channels (K^+ channels) are the most abundant channels in both excitable and non-excitable cells, and they play important roles in the formation of the membrane potential. It is generally accepted that the membrane potential of epithelial cells is indispensable as the driving force for electrogenic transport, such as Na^+ -coupled solute co-transport. Since the driving force is largely dependent on the membrane potential, electrogenic transport is mediated, at least in part, by its potential. Namely, it is conceivable that membrane hyperpolarization stimulates and depolarization suppresses such transport. Moreover, since the membrane potential of the epithelial cells is mainly formed by the activity of K^+ channels and Na^+ - K^+ -ATPase, investigation of the mechanisms leading to changes in K^+ channel activity using the patch-clamp technique would provide important knowledge for the regulation of epithelial transport systems.

2. Distribution of renal K⁺ channels along the nephron

In the kidney tubules, epithelial transport plays important roles in body fluid homeostasis, such as the electrolyte and acid-base balance (Giebisch, 1998; Gennari & Maddox, 2005). It has been demonstrated that several types of K⁺ channel are present in the apical and basolateral membranes of tubular epithelia, and the functional importance of these K⁺ channels in epithelial transport has also been reported in individual nephron segments (Guggino et al., 1987; Gögelein, 1990; Palmer, 1992; Giebisch, 1995; Quast, 1996). The most frequently observed types of K⁺ channel with the patch-clamp technique are Ca²⁺-activated channels and inwardly rectifying channels regulated by cytosolic ATP (Ohno-Shosaku, et al., 1989; Giebisch, 1995; Quast, 1996; W. Wang et al., 1997). The former are mainly present in the apical membranes (Merot et al., 1989; Hirano et al., 2001) and the latter are in the basolateral membranes (Ohno-Shosaku et al., 1990; Robson & Hunter, 1997; Kubokawa et al., 1998; Nakamura et al., 2001) of the proximal tubule cells, the principal cells of the cortical collecting duct (CCD) (Hirsch et al., 1993), the apical membranes of the thick ascending limb of Henle (TAL) (W. Wang, 1994), and the principal cells of CCD (Giebisch, 1995; W. Wang et al., 1997; Kawahara & Anzai, 1997). Studies using molecular techniques have also demonstrated the structures (Ho et al., 1993) and tissue distribution of cloned K⁺ channels in several nephron segments of the kidney (Boim et al., 1995; McNicholas et al., 1996; Derst et al., 2001). It has also been reported that K⁺ channels requiring ATP to maintain their activity possess protein kinase-mediated phosphorylation sites in cloned K⁺ channels (Ho et al., 1993; Hebert et al., 2005). Although ATP-sensitive K⁺ (K_{ATP}) channels in the kidney are usually inhibited by an increase in cytosolic ATP (W. Wang & Giebisch, 1991a; Tsuchiya et al., 1992; Welling, 1995; Kawahara & Anzai, 1997; Mauere et al., 1998a), several lines of evidence strongly suggest that ATP-regulated channels with ATP-dependent activation are mediated by protein kinases (W. Wang & Giebisch, 1991b; McNicholas et al., 1994; Levitan, 1994; Kubokawa et al., 1997; Nakamura et al., 2001) and phosphatases (Kubokawa et al., 1995a; 2000). Namely, the ATP-regulated K⁺ channels are mainly mediated by protein kinases and phosphatases. To date, ATP-regulated K⁺ channels have been found in the proximal tubule cells, TAL, and principal cells of CCD. Thus, we have focused on ATP-regulated K⁺ channels in these tubule cells, and reviewed the involvement of protein kinases and phosphatases in the regulation of these channels and their importance in kidney functions.

2.1 Roles of K⁺ channels in the proximal tubule cells

A model of ion transport in the proximal tubule is shown in Fig. 1A. An explanation of the individual transporters, pump, and ion channels in the apical and basolateral cell membranes is presented in the lower part of the figure. The filtered Na⁺ and many solutes such as glucose and amino acids are reabsorbed into the proximal tubule cells by co-transporters according to the electrochemical gradient for Na⁺ across the apical membrane (Weinstein, 2000). The basolateral Na⁺-K⁺-ATPase (Na⁺-K⁺ pump) plays a crucial role in the formation of the Na⁺ and K⁺ gradient across the apical and basolateral membranes. The basolateral ATP-regulated K⁺ channels are indispensable for the negative cell potential, which is largely dependent on the K⁺ gradient across the cell membrane and K⁺ channel activity, and serves as the driving force for the apical Na⁺-coupled transporters. The Na⁺ that enters the cell is excluded to the interstitial space by basolateral Na⁺-K⁺ pump (Na⁺-K⁺

pump). Thus, the major part of the ATP produced in the cell is used for transport by $\text{Na}^+\text{-K}^+$ pump and ATP-regulated K^+ channels in renal tubule cells (Beck et al., 1991). Several protein kinases are involved in the regulation of the basolateral ATP-regulated K^+ channels, as described below (Kubokawa et al., 1997; Mauerer et al., 1998b; Mori, et al, 2001, Nakamura et al., 2002). The transport of other ions, such as H^+ or HCO_3^- , is mediated mainly by Na^+ -coupled processes. It has also been reported that some K^+ channels are present in the apical membrane of proximal tubule cells (Merot et al., 1989). Although the precise role of the apical K^+ channel is still unknown, the K^+ channel requires a high intracellular Ca^{2+} concentration $[\text{Ca}^{2+}]_i$ to open (Hirano et al., 2001). Thus, the open probability (P_o) of the apical K^+ channel would usually be low under the normal conditions.

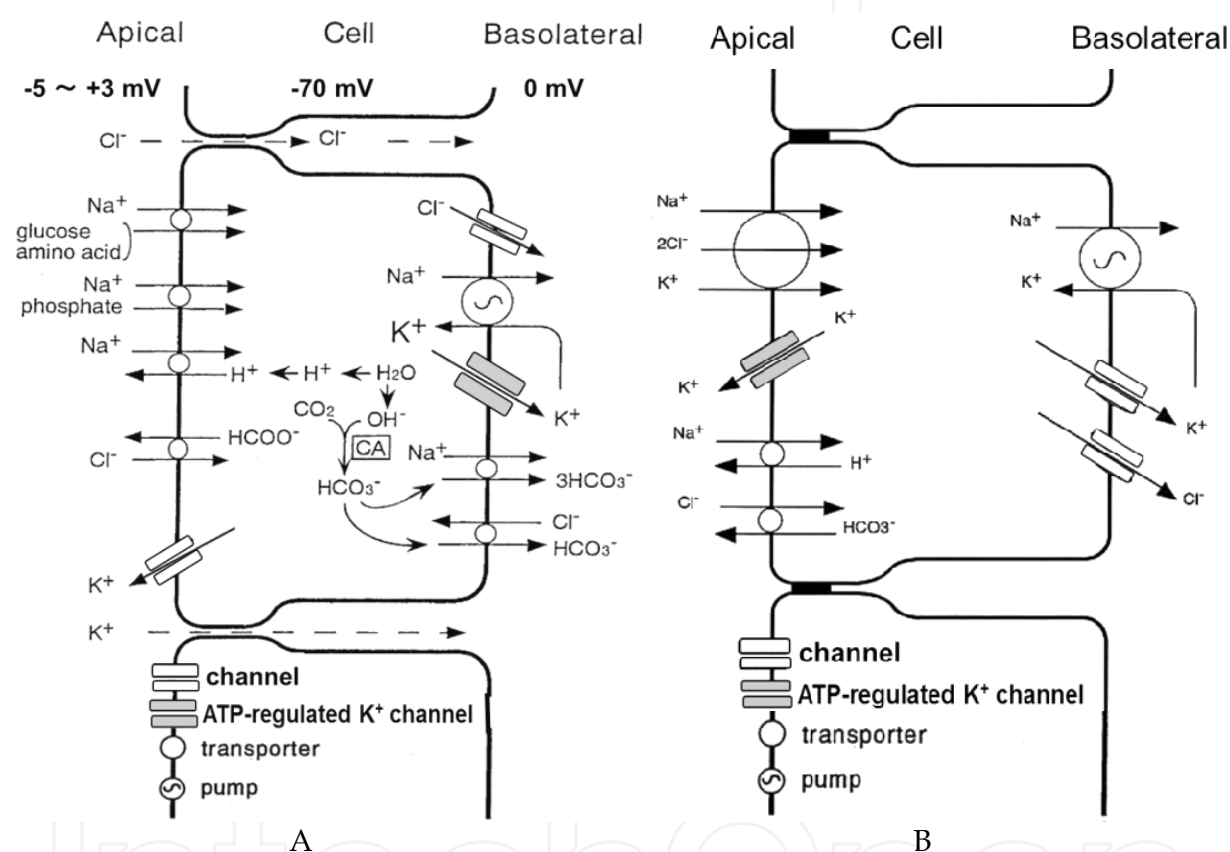


Fig. 1. Simplified models of the ion transport processes in the proximal tubule (A) and TAL (B). The double rectangles indicate ion channels (the gray double rectangles are ATP-regulated K^+ channels). The circles are transporters and the pump, as depicted in the lower part of the figures.

2.2 Roles of K^+ channels in TAL

The functional significance of TAL is to dilute the luminal fluid by the re-absorption of Na^+ and Cl^- without H_2O , which results in elevation of the interstitial osmolarity. As shown in Fig. 1B, the $\text{Na}^+/\text{K}^+/\text{Cl}^-$ co-transporter plays a major role in the entry of these ions into cells. For the sufficient entry of these ions via the $\text{Na}^+/\text{K}^+/\text{Cl}^-$ co-transporter, a K^+ supply to the lumen is required. Thus, the role of the K^+ channel in the apical membrane of TAL is to move K^+ into the lumen from the cytosol. Two types of apical K^+ channel were reported in

TAL (W. Wang, 1994). Both of these channels were inhibited by a high concentration of ATP, but one is an ATP-regulated channel with a high P_o , which is activated by protein kinase A (W. Wang, 1994). These results suggest that the apical ATP-regulated K^+ channel with a high open probability is a major candidate for the K^+ channel which supplies the lumen with K^+ .

In the basolateral membrane of TAL, an Na^+ - K^+ pump and K^+ conductance were observed, as has been shown in normal transporting epithelia. However, little information is available regarding the basolateral K^+ channel in TAL, although the properties of basolateral Cl^- channels have been reported (Winters et al., 1999).

2.3 Roles of K^+ channels in the principal cells of CCD

The renal collecting duct including CCD, and inner and outer medullary collecting duct (IMCD and OMCD, respectively) are the most important nephron segments for determining the final urine conditions to maintain body fluid homeostasis. It has been demonstrated that the Na^+ and K^+ concentrations of blood are largely dependent on Na^+ re-absorption and K^+ secretion in CCD (Malnic et al., 2000). Na^+ is reabsorbed from the lumen to cell mainly through the apical epithelial Na^+ channel (ENaC) (Palmer & Carty, 2000). It is also generally accepted that K^+ is secreted from the cell to lumen mainly through the apical ATP-regulated K^+ channel (Giebisch, 1995) (Fig.2), although a few reports have demonstrated that the Ca^{2+} -activated maxi- K^+ channel is a major candidate for K^+ secretion in CCD (Taniguchi & Imai, 1998; Woda et al., 2001). The apical K^+ channels are very important to maintain the normal K^+ level of blood (Giebisch, 1998). If the apical K^+ channel is suppressed, the blood K^+ level is elevated, which often suppresses the cardiac function. In contrast, massive K^+ secretion through K^+ channels in CCD results in hypokalemia.

A K^+ channel similar to the apical ATP-regulated K^+ channel in CCD was cloned from the outer medulla of the rat kidney, which is named ROMK (Ho et al., 1993). Analyses of the cloned K^+ channel yielded several important findings regarding the apical K^+ channel. One of the most significant findings was that this ATP-regulated channel possessed separate PKA- and PKC-mediated phosphorylation sites. As reported previously, the apical K^+ channel in CCD was stimulated by PKA and suppressed by PKC (W. Wang & Giebisch, 1991b), suggesting that the roles of PKA and PKC in the modulation of the channel were different. Molecular analyses of the cloned K^+ channel supported the view obtained from observing the native K^+ channel that the PKA-mediated site is clearly distinct from PKC-mediated sites (Ho et al., 1993). Moreover, several subtypes of the ATP-regulated K^+ channel were cloned from the distal nephron segment including TAL (Hebert et al., 2005). These channels possess similar properties, such as conductance of about 30 pS, being ATP-regulated, and PKA-activated channels (Hebert et al., 2005). ATP-regulated K^+ channels in mammalian proximal tubule cells are also PKA-activated channels, although the conductance of these channels was greater (40 -90 pS) than that of the cloned channels (Kubokawa et al., 1997; Nakamura et al., 2001).

In the basolateral membrane of the principal cells, two types of K^+ channel have been reported. One is the Ca^{2+} -activated channel (Hirsch et al., 1993) and the other is the PKG-mediated channel (Hirsch & Schlatter, 1995). The former requires a high $[Ca^{2+}]_i$ to open, but the latter is able to open under normal conditions. Furthermore, the latter channel is considered to be an ATP-regulated channel, since it was modulated by a protein kinase,

PKG (Hirsch & Schlatter, 1995). Thus, it is suggested that the ATP-regulated (PKG-mediated) K^+ channel is one of the major candidates for the formation of the membrane potential of the principal cells.

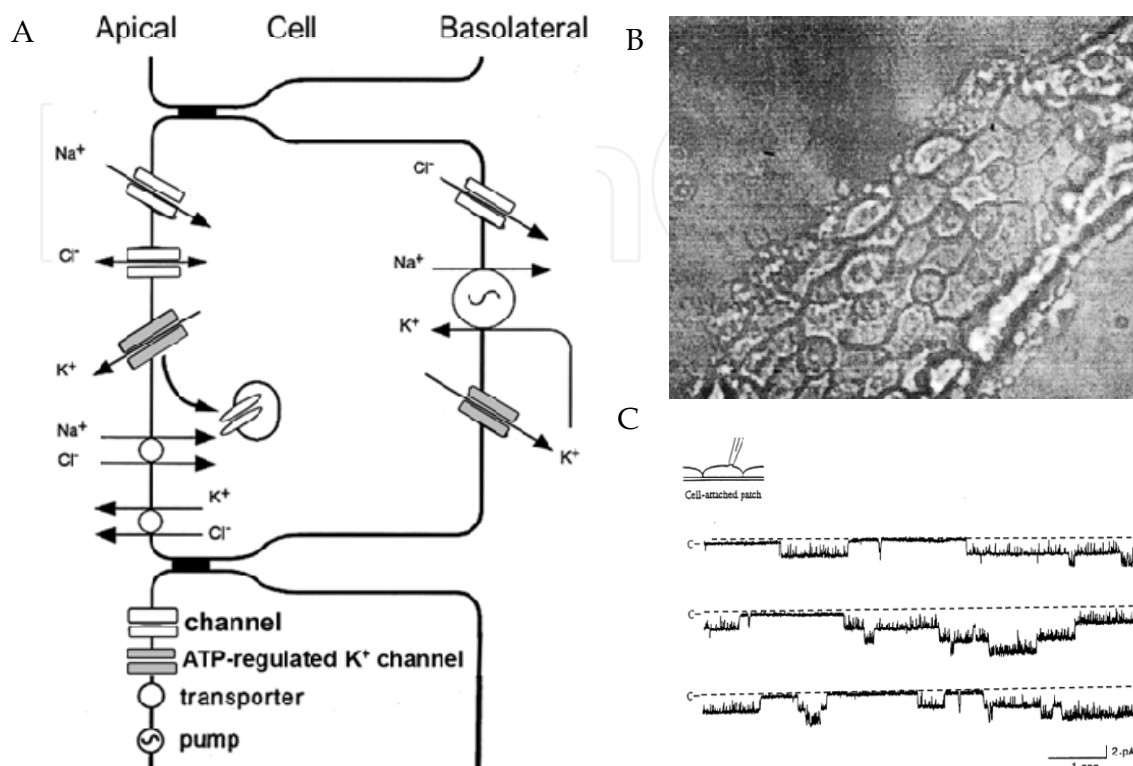


Fig. 2. A model of ion transport in the principal cell of CCD (A), the apical surface of CCD (B), and channel currents obtained from the apical membrane of the principal cell. ATP-regulated K^+ channels are present in both apical and basolateral membranes (A). A microscopic view of the apical surface of CCD revealed that two types of cell, the principal cell (large cells) and intercalated cell (small, round cells), are present (B). The represented current trace was the apical ATP-regulated K^+ channel observed in a cell-attached patch (C).

3. Mechanisms of activation and suppression of renal ATP-regulated K^+ channels by protein kinases and phosphatases

3.1 ATP-dependency of the renal ATP-regulated K^+ channels

In general, ATP-regulated K^+ channels in renal tubule cells were previously believed to be ATP-sensitive (ATP-inhibitable) channels (W. Wang & Giebisch, 1991a; Tsuchiya et al., 1992; Welling, 1995; Robson & Hunter, 1997; Maurer et al., 1998a), since a high concentration of internal ATP inhibited the activity of these channels. However, most of these channels require relatively low concentrations of ATP to maintain their activity (Kubokawa et al., 1995a; Maurer et al., 1998b; Nakamura et al., 2001). The ATP-regulated K^+ channels cloned from the renal outer medulla also require ATP to maintain their activity (Ho et al., 1993). Although high concentrations of ATP may suppress the activity of the ATP-regulated channels including a cloned renal K^+ channel (McNicholas et al., 1996), a relatively low concentration of ATP is indispensable for maintenance of the channel activity. Thus, the renal K^+ channels regulated by ATP are usually ATP-dependent channels.

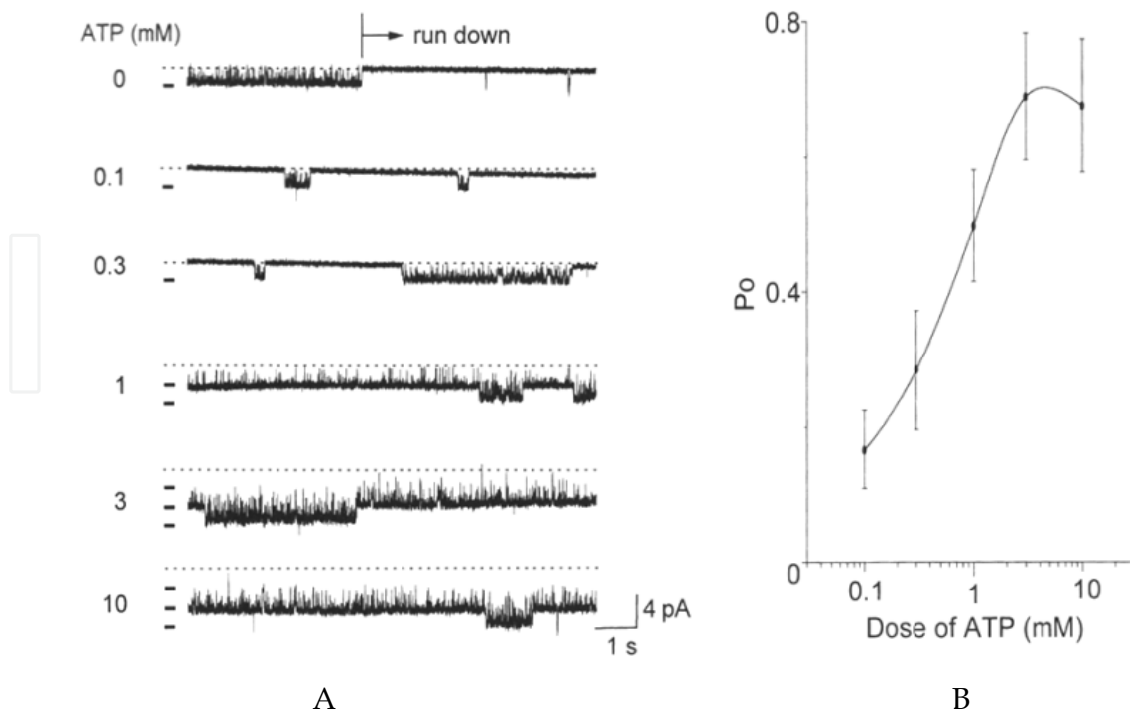


Fig. 3. ATP-dependency of the ATP-regulated K^+ channel in cultured human proximal tubule cells. ATP is required to maintain channel activity, and an increase in ATP on the cytosolic surface of the patch membrane enhanced channel activity (A). The dose-response curve indicates that the maximal P_o was observed around 3 mM ATP (B). [Reprinted from Nakamura, K., Hirano, J., & Kubokawa, M. (2001). An ATP-regulated and pH-sensitive inwardly rectifying K^+ channel in cultured human proximal tubule cells. *Jpn. J. Physiol.*, Vol. 51, No. 4, pp. 523-530, ISSN: 0021-521X with permission, Copyright 2001]

In the inside-out mode of the patch-clamp technique, ATP-dependent channel activity was suppressed by protein kinase inhibitors (Kubokawa et al., 1997; Nakamura et al., 2001). Since protein kinase induces protein phosphorylation, inhibitors of protein kinase suppress the phosphorylation processes. Moreover, it is well-known that the cell membranes bind to many protein kinases. Thus, ATP is required to induce the protein kinase-mediated phosphorylation of the channel or associated protein in inside-out patches. Cytosolic ATP would act as a donor of phosphate for protein phosphorylation. After the removal of ATP, channel activity is rapidly reduced as shown in the top trace of Fig. 3A, which is usually called "run-down". Such channel run-down would result from phosphatase-mediated protein dephosphorylation, since the run-down observed in the inside-out patch can be blocked by phosphatase inhibitors (Kubokawa et al., 1995a). Moreover, closed channels after run-down can be re-activated by the addition of ATP. Thus, it is suggested that not only protein kinases but also phosphatases are bound to the inside of the cell membrane (Kubokawa et al., 1995a, 2000).

3.2 Protein kinase-mediated activation and phosphatases-mediated suppression of the renal ATP-regulated K^+ channels

Among several kinds of protein kinase, cAMP-dependent PKA is known to stimulate the ATP-regulated K^+ channels in proximal tubule cells (Kubokawa et al., 1997; Nakamura et al.,

2001), TAL (W. Wang et al., 1994), and principal cells of CCD (W. Wang & Giebisch, 1991a; Kubokawa et al., 1995a), including the cloned renal K⁺ channel (McNicholas et al., 1994). Thus, PKA-mediated channel activation seems to be a common characteristic of the renal ATP-regulated K⁺ channels.

As shown in Fig. 4A, the application of membrane-permeant 8Br-cAMP to the bath elevated the K⁺ channel activity of the proximal tubule cells in a cell-attached patch (Kubokawa et al., 1997; Nakamura et al., 2001). This result suggested that an increase in cytosolic cAMP stimulated PKA, and then PKA-mediated phosphorylation resulted in channel activation. Indeed, as shown in Fig. 4B, the direct application of PKA to the cytosolic surface of the patch-membrane in an inside-out patch elevated the activity of the ATP-regulated K⁺ channel in the proximal tubule cells (Nakamura et al., 2001). As described above, PKA-

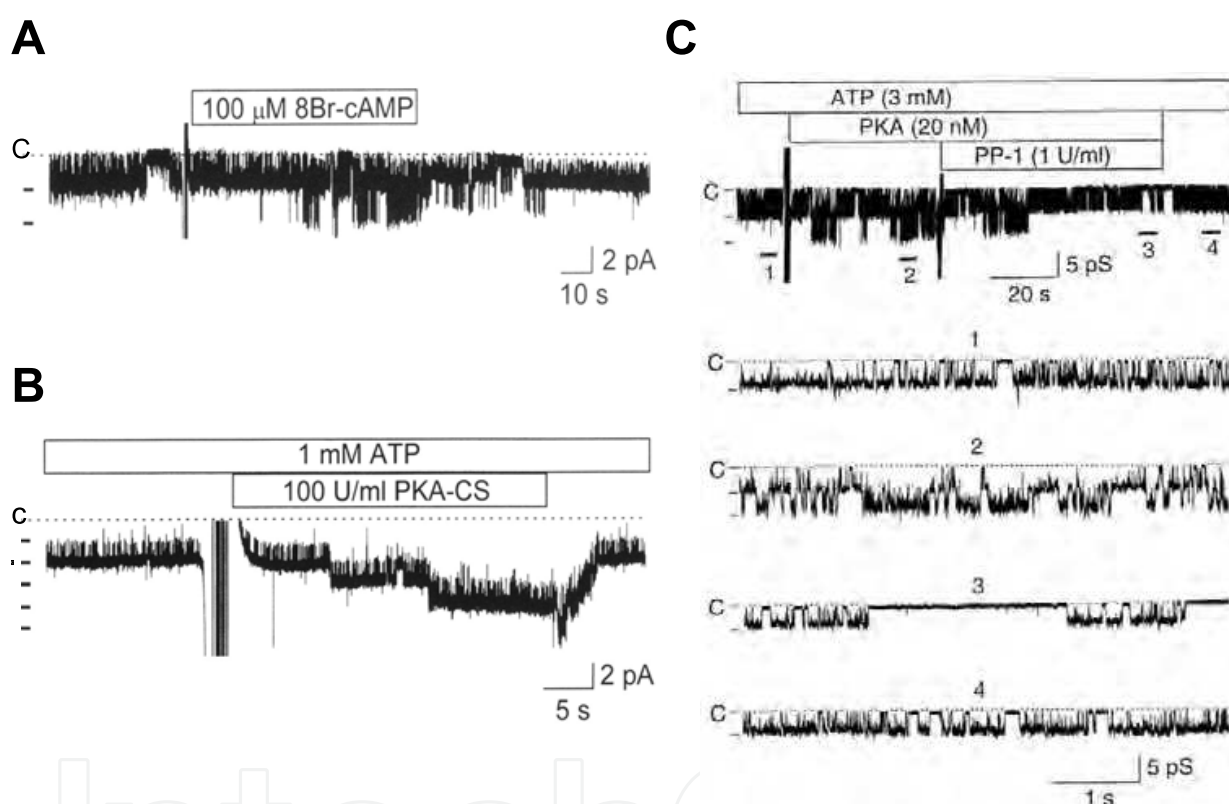


Fig. 4. Examples of the effects of cAMP-dependent PKA and protein phosphatase type 1 (PP-1) on the activity of the renal ATP-regulated K⁺ channels in mammalian proximal tubule cells. The addition of membrane-permeant 8Br-cAMP to the bath enhanced channel activity in the cell-attached patch (A), and a catalytic subunit of PKA (PKA-CS) in the presence of ATP stimulated activity in the inside-out patch (B). In contrast, enhanced channel activity induced by PKA was inhibited by the addition of PP-1 in an inside-out patch (C). [A and B are reprinted from Nakamura, K., Hirano, J. & Kubokawa, M. (2001). An ATP-regulated and pH-sensitive inwardly rectifying K⁺ channel in cultured human proximal tubule cells. *Jpn. J. Physiol.*, Vol. 51, No. 4, pp. 523-530, ISSN: 0021-521X with permission, Copyright 2001, and C is reprinted from Kubokawa, M., Nakamura, K., Hirano, J., Yoshioka, Y., Nakaya, S., Mori, Y., & Kubota, T. (2000). Regulation of inwardly rectifying K⁺ channel in opossum proximal tubule cells by protein phosphatases 1 and 2A. *Jpn. J. Physiol.*, Vol. 50, No. 2, pp. 249-256, ISSN: 0021-521X, Copyright 2000]

induced channel activation is a common property of the renal ATP-regulated K^+ channel in both proximal and distal nephron segments. Moreover, a similar property was observed not only in human (Nakamura et al., 2001) but also opossum kidney proximal tubule cells (Kubokawa et al., 1997). These results suggest that the regulatory mechanisms of renal ATP-regulated K^+ channels along the nephron are similar.

In addition to PKA, cGMP-dependent protein kinase (PKG) was reported to enhance the activity of the ATP-regulated K^+ channel in the basolateral membrane of the proximal tubule (Kubokawa et al., 1998; Nakamura et al., 2002) and principal cells of CCD (Hirsch & Schlatter, 1995). Although the effect of cGMP or PKG on the activity of other renal K^+ channels has not been examined, PKG would also be an important protein kinase in the regulation of channel activity.

As mentioned above, phosphorylated protein would be dephosphorylated by some phosphatases. The importance of some protein phosphatases in regulating channel activity has been demonstrated in neuronal NMDA receptor channels (L. Wang et al., 1994; Lieberman & Mody, 1994). Also, in renal tubules, the effects of a protein phosphatase on the activity of ATP-regulated K^+ channels have been demonstrated, as shown in Fig. 4C. In this experiment, PP-1 inhibited the enhanced channel activity brought about by PKA in the proximal tubule cells of the opossum kidney (OK) in an inside-out patch (Kubokawa et al., 2000). Moreover, PP-2A also has an inhibitory effect on channel activity in OK proximal tubule cells (Kubokawa et al., 2000). However, the ATP-regulated K^+ channel in the apical membrane of the principal cells of CCD was not inhibited by PP-1, but inhibited by PP-2A (Kubokawa et al., 1995a). Thus, the types of protein kinase and phosphatase affecting the activity of renal ATP-regulated K^+ channel are not always the same.

Roles of protein kinases and phosphatases in modulating the renal ATP-regulated K^+ channel are schematically shown in Fig. 5. Namely, as mentioned above, the ATP-regulated K^+ channels in the proximal tubule cells are activated by PKA- and PKG-mediated phosphorylation of the channel or associated protein. The apical ATP-regulated K^+ channel in TAL and the principal cell of CCD is activated by PKA, and the basolateral channel in the

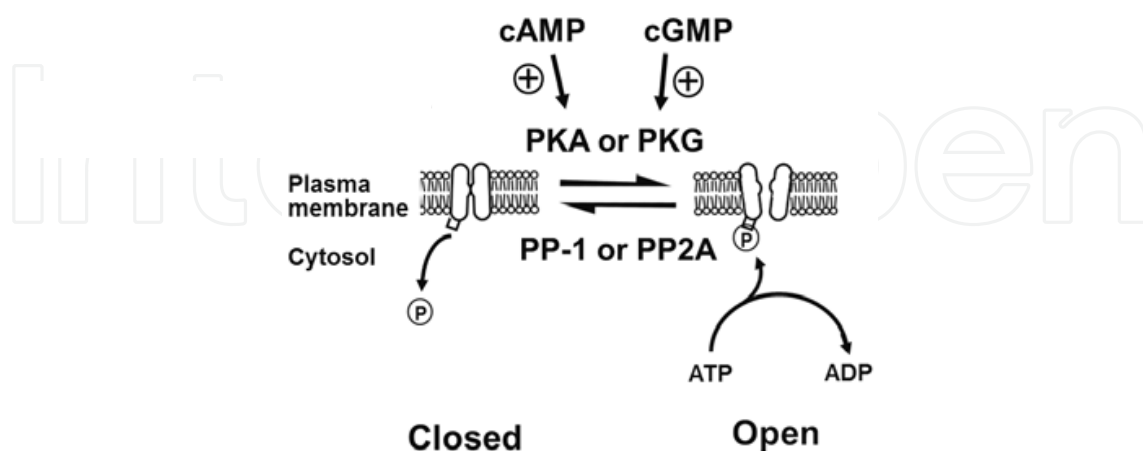


Fig. 5. A schematic representation of the roles of protein kinase-mediated phosphorylation and phosphatase-mediated dephosphorylation in modulation of the renal ATP-regulated K^+ channel. PKA- and/or PKG-mediated phosphorylation lead the channel to open, whereas PP-1 and/or PP-2A-mediated dephosphorylation promote the closed state of the channel.

principal cell is activated by PKG. Taken together, cAMP-dependent PKA or cGMP-dependent PKG induced phosphorylation which elevates the channel activity, and PP-1 or PP-2A induced dephosphorylation which lowers the channel activity. Thus, the phosphorylation and dephosphorylation induce the reversible alteration of channel activity.

3.3 Effects of Ca^{2+} -dependent protein kinase and phosphatase on the activity of renal ATP-regulated K^+ channels

It has been demonstrated that an increase in $[\text{Ca}^{2+}]_i$ suppresses the renal ATP-regulated K^+ channels, which is mainly induced by the activation of Ca^{2+} -dependent protein kinase C (PKC) in the proximal tubule cells (Mauerer et al., 1998; Mori et al., 2001) and the apical membrane of principal cells of CCD (W. Wang & Giebisch, 1991). Moreover, it was reported that Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) also suppressed the activity of the ATP-regulated K^+ channels in the basolateral membrane of the proximal tubule cells (Kubokawa et al., 2009) and the apical membrane of the principal cells in CCD (Kubokawa et al., 1995b). Indeed, the Ca^{2+} -induced K^+ channel inhibition was not blocked by PKC inhibitor alone, but was almost completely blocked by KN-62 (Tokumitsu et al., 1990), a CaMKII inhibitor, in addition to PKC inhibitor (Kubokawa et al., 1995b, 2009). Thus, it is possible that both PKC and CaMKII are involved in K^+ channel regulation.

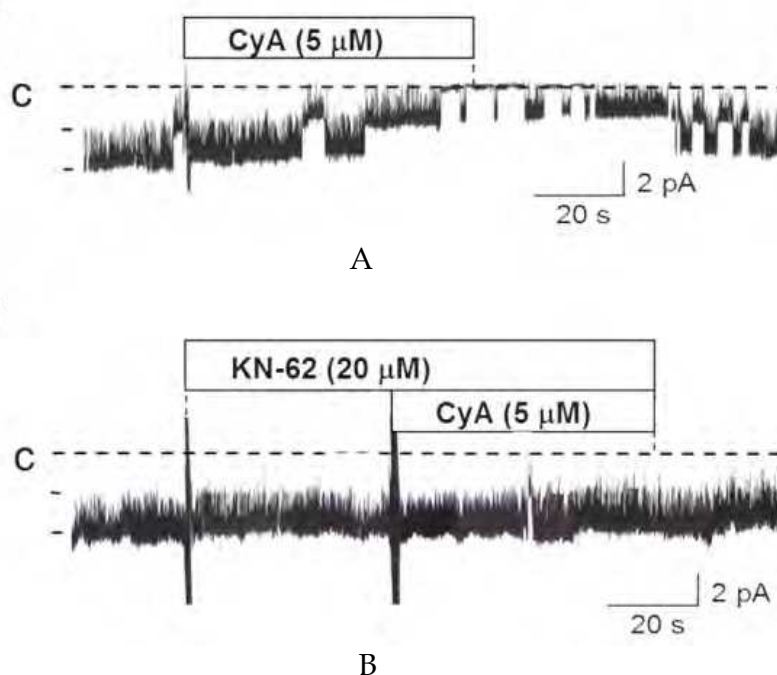


Fig. 6. Representative current recordings of the changes in activity of the K^+ channel in response to cyclosporin A (CyA) in the presence and absence of an inhibitor of CaMKII, KN-62, in cell-attached patches. Channel activity was suppressed by CyA alone (A), but the suppression was blocked in the presence of KN62 (B). [Modified from Kubokawa, M., Kojo, T., Komagiri Y. & Nakamura, K. (2009). Role of calcineurin-mediated dephosphorylation in modulation of an inwardly rectifying K^+ channel in human proximal tubule cells. *J. Membr. Biol.*, Vol. 231, No 2-3, pp. 79-92, ISSN: 0022-2631, with kind permission from Springer Science, Copyright 2009]

It has also been demonstrated that cyclosporin A (CyA), an inhibitor of Ca^{2+} /calmodulin-dependent phosphatase, calcineurin (CaN) (Hemenway & Heitman, 1999), inhibited the activity of the K^+ channel in both proximal tubule (Ye et al., 2006; Kubokawa et al., 2009) and principal cells of CCD (Ling & Eaton, 1993). Cyclosporin A is a well-known immunosuppressive agent (Morris, 1981; White & Calne, 1982; Cohen, 1984), and affects various functions of the kidney other than K^+ channels (Epting et al., 2006; Tumlin, 1993; Grinyó & Cruzado, 2004; J. Wang et al., 2009; Damiano et al., 2010). Since the inhibition of CaN may induce a phosphorylation-dominant state, a Ca^{2+} /calmodulin-dependent kinase, such as CaMKII may be stimulated in this condition. This was supported by experiments using cultured human renal proximal tubule cells. That is, CyA-induced channel suppression was blocked by a CaMKII inhibitor, KN-62, as shown in Fig. 6 (Kubokawa et al., 2009). Moreover, Western blot analysis also revealed that CyA increased phospho-CaMKII, an active form of CaMKII (Gerges et al., 2005), as shown in Fig. 7 (Kubokawa et al., 2009). Taken together, these results strongly suggest that CaMKII-mediated phosphorylation suppresses channel activity, and CaN-mediated dephosphorylation reversibly elevates activity of the renal ATP-regulated K^+ channel. It was also demonstrated that CyA increased $[\text{Ca}^{2+}]_i$ in various cells, including renal tubule cells (Gordjani et al., 2000; Frapier et al., 2001; Bultynck et al., 2003; Kubokawa et al., 2009). Thus, the CyA-induced increase in $[\text{Ca}^{2+}]_i$ may enhance the activity of CaMKII.

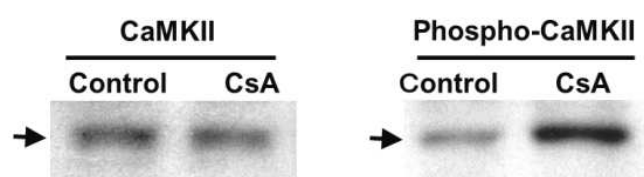


Fig. 7. Effects of CyA on CaMKII and phospho-CaMKII (Thr 286) in human kidney tubule cells. Western blot showing the effect of CyA (5 μM) on CaMKII detected with CaMKII antibody (A). CyA induced no appreciable change in CaMKII protein. Western blot showing the effect of CyA (5 μM) on phospho-CaMKII detected with a specific phospho-CaMKII antibody (Thr 286) (B). CyA clearly increased 50-kDa phospho-CaMKII (Thr 286). [Reprinted from Kubokawa, M., Kojo, T., Komagiri, Y. & Nakamura, K. (2009). Role of calcineurin-mediated dephosphorylation in modulation of an inwardly rectifying K^+ channel in human proximal tubule cells. *J. Membr. Biol.*, Vol. 231, pp. 79-92, ISSN: 0022-2631, with kind permission from Springer Science, Copyright 2009]

3.4 Possible relationship between CaMKII and CaN in modulation of the renal ATP-regulated K^+ channels

Protein kinase-mediated phosphorylation and phosphatase-mediated dephosphorylation are coupled mediators for the regulation of functional protein. It has been reported that CaN is an important phosphatase in regulating several cell functions (Rusnak & Merts, 2000). The above data suggest that CaMKII and CaN are coupled mediators in modulating the ATP-regulated K^+ channels. However, it has been reported that the target phosphorylation site which was dephosphorylated by CaN is mainly mediated by PKA (Santana et al., 2002). This report demonstrated that the functional coupling of CaN and PKA modulated Ca^{2+} release in ventricular myocytes (Santana et al., 2002). It has also been demonstrated that Na^+ - K^+

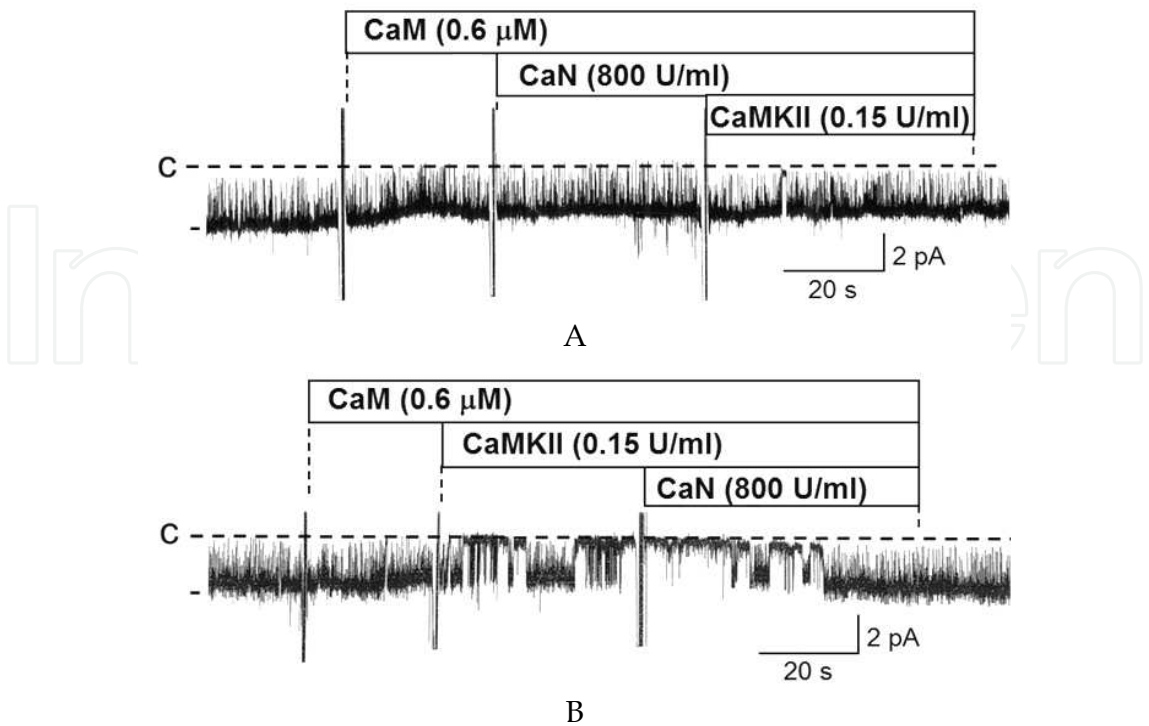


Fig. 8. Effects of CaMKII and CaN on activity of the ATP-regulated K⁺ channel in the human proximal tubule cells in inside-out patches. [Reprinted from Kubokawa, M., Kojo, T., Komagiri, Y. & Nakamura, K. (2009). Role of calcineurin-mediated dephosphorylation in modulation of an inwardly rectifying K⁺ channel in human proximal tubule cells. *J. Membr. Biol.*, Vol. 231, pp. 79-92, ISSN: 0022-2631, with kind permission from Springer Science, Copyright 2009]

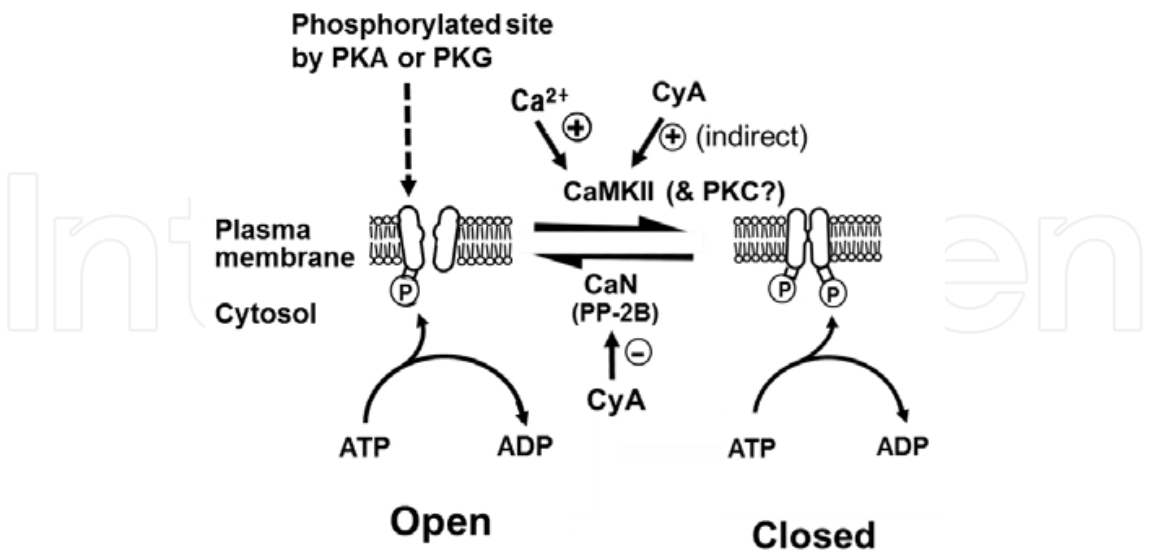


Fig. 9. A schematic representation of Ca²⁺ (/calmodulin)-dependent phosphorylation and dephosphorylation in modulation of the renal ATP-regulated K⁺ channel. CaMKII- (PKC-) mediated phosphorylation leads the channel close, whereas CaN-mediated dephosphorylation causes the channel to open.

pump at the basolateral membrane of kidney tubular epithelia was inhibited by CyA (Tumlin & Sands, 1993) and stimulated by PKA (Carranza et al., 1998). On the other hand, a cardiac $\text{Na}^+/\text{Ca}^{2+}$ exchanger was reported to be regulated by CaN and PKC (Shigekawa et al., 2007). A cellular process which depended on mitogen-activated protein kinase was reported to be negatively regulated by CaN (Tian & Karin, 1991). Thus, the protein kinases opposing CaN-mediated processes would not act in unity. Only a few reports have suggested that CaMKII-mediated processes are abolished by CaN (Wu et al., 2002; Gerges et al., 2005). Despite the variety of coupled mediators for CaMKII or CaN, experiments showing the effects of CaMKII on channel activity were abolished in the presence of CaN and that CaMKII-induced channel inhibition was restored by CaN in inside-out patches (Fig. 8) indicate the coupling of CaMKII and CaN in modulating the activity of the ATP-regulated K^+ channel. A model of the modulation of the ATP-regulated K^+ channel is schematically represented in Fig.9.

4. Conclusion

Among the many kinds of protein kinase, PKA- and/or PKG-mediated phosphorylation induces opening (an active state) of the renal ATP-regulated K^+ channel in proximal tubule cells (Kubokawa et al., 1997; Maurer et al., 1998; Kubokawa et al., 1998), TAL (W. Wang,

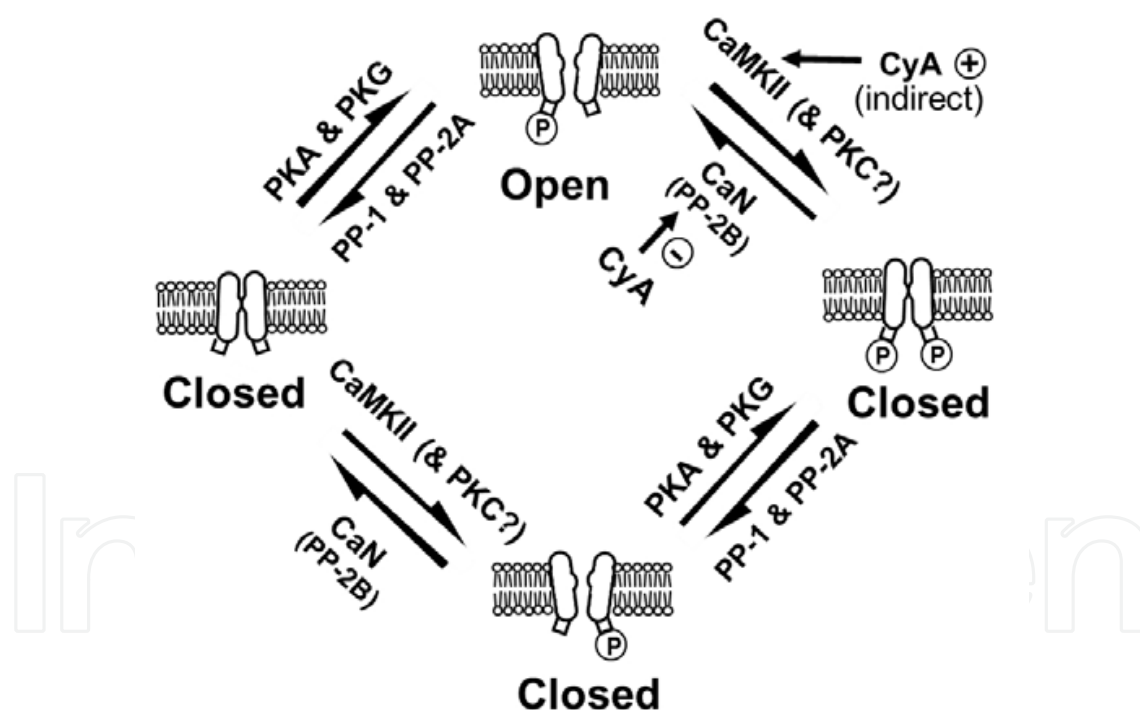


Fig. 10. A schematic representation of the 4-state phosphorylation model of the K^+ channel observed in the proximal tubule cells. CaN is often called protein phosphatase-2B (PP-2B). The circled "P" indicates phosphate. The circled "+" and "-" indicate stimulation and inhibition, respectively. [Reprinted from Kubokawa, M., Nakamura, K. & Komagiri, Y. (2011) Functional relationships between Ca^{2+} /calmodulin-dependent kinase and phosphatase in the regulation of K^+ channel activity and intracellular Ca^{2+} in kidney tubule cells. *J. Iwate med. Assoc*, Vol. 63, No4, pp. 209-218, with kind permission from Iwate Medical Association, Copyright 2011]

1994), and CCD principal cells (W. Wang & Giebisch, 1991; Kubokawa et al., 1995). In contrast, the open channels are closed by PP-1 and/or PP-2A (Kubokawa et al., 1995; Kubokawa et al., 2000), suggesting that PKA- or PKG-mediated phosphorylation was dephosphorylated by PP-1 or PP-2A. In addition, it is strongly suggested that CaMKII or PKC phosphorylates the other site, resulting in a closed state, while CaN-mediated dephosphorylation results in channel opening (Kubokawa et al., 2009). CyA directly inhibits CaN, and indirectly increases the active type of CaMKII, phospho-CaMKII, through the inhibition of CaN-mediated dephosphorylation processes (Kubokawa et al., 2009). A certain CaM-kinase kinase may be involved in the phosphorylation of CaMKII. Taken together, at least 2 phosphorylation sites are present at the cytosolic site of the channel or its associated protein, and individual phosphorylation sites are independently phosphorylated or dephosphorylated. Thus, the 4 different states of phosphorylation and dephosphorylation conditions would occur in the channel or the associated proteins in the regulatory processes of channel activity (Kubokawa et al., 2011). A putative 4-state model for the mechanism of K⁺ channel regulation by phosphorylation and dephosphorylation is shown in Fig. 10. Although the channel state at the CaMKII-mediated phosphorylation alone is still unclear, these states would determine the channel activity at least in phosphorylation and dephosphorylation levels.

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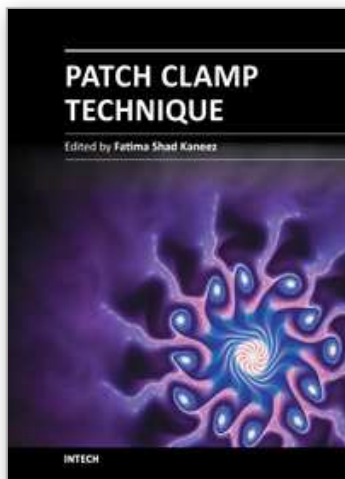
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Patch Clamp Technique

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This book is a stimulating and interesting addition to the collected works on Patch clamp technique. Patch Clamping is an electrophysiological technique, which measures the electric current generated by a living cell, due to the movement of ions through the protein channels present in the cell membrane. The technique was developed by two German scientists, Erwin Neher and Bert Sakmann, who received the Nobel Prize in 1991 in Physiology for this innovative work. Patch clamp technique is used for measuring drug effect against a series of diseases and to find out the mechanism of diseases in animals and plants. It is also most useful in finding out the structure function activities of compounds and drugs, and most leading pharmaceutical companies used this technique for their drugs before bringing them for clinical trial. This book deals with the understanding of endogenous mechanisms of cells and their receptors as well as advantages of using this technique. It covers the basic principles and preparation types and also deals with the latest developments in the traditional patch clamp technique. Some chapters in this book take the technique to a next level of modulation and novel approach. This book will be of good value for students of physiology, neuroscience, cell biology and biophysics.

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