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Physiology

Dual modulation of renal ATP-sensitive K^+ channel by protein kinases A and C

(ion channels/cortical collecting duct/rat kidney)

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ABSTRACT **A small-conductance K^+ channel in the apical membrane of rat cortical collecting duct (CCD) cells controls K^+ secretion in the kidney. Previously, we observed that the activity of the channel is stimulated by cAMP-dependent protein kinase A (PKA)-induced channel phosphorylation. We now have applied the patch-clamp technique to study the effects of protein kinase C (PKC) on the secretory K^+ channel of rat CCD. In cell-attached patches, application of phorbol 12-myristate 13-acetate progressively reduced the open probability and current amplitude of the K^+ channel. In inside-out patches, administration of PKC reversibly decreased the channel open probability (P_o) without changing the channel conductance. The PKC-induced inhibition of channel activity was Ca^{2+} dependent: P_o decreased 42%, 23%, and 11% in the presence of 1000 nM, 100 nM, and 10 nM free Ca^{2+}, respectively. We also demonstrate that PKC antagonizes the stimulatory effect of PKA on the apical K^+ secretory channel of rat CCD. These results suggest regulation of K^+-channel activity by two separate sites of phosphorylation with distinct and opposite effects on channel activity.**

K^+ secretion in renal cortical collecting tubules (1) is controlled in part by the activity of 35 pS K^+ channels in the apical membrane of principal cells (2–4). These K^+ channels have a high open probability (P_o), discriminate poorly between K^+ and Rb^+ (5), and are inhibited by acidic cell pH as well as by barium and 1 mM ATP (6). The channels are insensitive to cytoplasmic changes of Ca^{2+} in inside-out patches and are not affected by changes of the membrane potential (6). Channel activity declines frequently in excised patches but can be promptly restored by addition of cAMP, noninhibitory concentration of ATP, and protein kinase A (PKA) (7). It appears safe to conclude that PKA-induced phosphorylation is essential for maintaining this K^+ channel in open states.

The observation that tumor-promoting phorbol esters reduced K^+ secretion in cortical collecting ducts (CCDs) of the rabbit kidney (8) suggests that protein kinase C (PKC) may have an inhibitory effect on the activity of the secretory K^+ channel in principal cells of CCD. Bradykinin, a hormone produced in the kidney (9), activates PKC in cultured CCD cells (10). We have now investigated the effects of PKC on the activity of the secretory K^+ channel to obtain information on the molecular mechanism that regulates K^+ secretion in renal tubules.

METHODS

CCDs were dissected and prepared from rat kidney as described (6, 7). Two types of cells can be identified in CCDs—i.e., principal and intercalated cells (3). In this study, only the principal cells were patched. All experiments were performed at 35°C–37°C. The patch-clamp technique was used to record channel currents that were low-pass-filtered at 1000 Hz. The data were analyzed by using the PCLAMP software system and SCAP software (written by M. Hunter, Leeds University, U.K.). The pipette solution contained 140 mM KCl, 1.5 mM $MgCl_2$, 1 mM EGTA, and 10 mM Hepes·KOH (pH 7.4). The bath solution for the cell-attached patch was composed of 140 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 1.8 mM $MgCl_2$, and 10 mM Hepes·NaOH (pH 7.4). The bath solution for inside-out patches was the same as described above, except for Ca^{2+}, which was chelated to the desired concentration with 1 mM EGTA. Phorbol 12-myristate 13-acetate (PMA) was purchased from Sigma and was dissolved in dimethyl sulfoxide (DMSO). A concentrated stock solution of PMA in DMSO was prepared fresh each day and was stored at 4°C. Portions of the solutions were added to the bath solution and the final concentration of DMSO was 0.1%, which did not affect channel activity. PKC isolated from rat brain (11) was purchased from Lipidex (Westfield, NJ). Data are presented as mean ± SEM. Where appropriate, Student's *t* tests for paired data were used to assess significance of difference.

RESULTS

Fig. 1 shows a representative recording of the effects of PMA, a tumor-promoting phorbol ester, on the activity of the K^+ channel in CCD principal cells. In this experiment on cell-attached patches, P_o of the channel in control conditions was 0.95 ± 0.03 (n = 10). Exposure of CCD cells to 10 μM PMA-containing solutions progressively and significantly ($P < 0.001$) reduced the P_o to 0.56 ± 0.04 (n = 4) within 6–10 min. The current amplitude of the K^+ channel in the cell-attached patches also decreased because the driving force of K^+ was reduced with depolarization of the membrane potential. Fig. 2 illustrates recordings from a representative experiment in which we examined the effect of exogenous PKC on channel activity in inside-out patches. To activate PKC, ATP, phosphatidylserine, or diacylglycerol as well as tumor-promoting phorbol esters are required. We used ATP (0.1 mM), 1 μM 1-oleoyl-2-acetyl-*sn*-glycerol, or 1 μM PMA and PKC (1 unit/ml) (4 nM), with the cell membrane serving as the source of phospholipid. We noted that in the presence of 1 μM Ca^{2+}, activation of PKC reduced the P_o of the K^+ channel from a control value of 0.95 ± 0.02 to 0.42 ± 0.05 (n = 6; $P < 0.001$) within 30 sec.

In additional experiments, we observed that the PKC-induced inhibition of channel activity was significantly affected by the cytoplasmic Ca^{2+} concentration. Fig. 3 summarizes data from experiments in which the Ca^{2+} concentration was changed in the presence of a constant PKC

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Abbreviations: P_o, channel open probability; PKA, protein kinase A; PKC, protein kinase C; CCD, cortical collecting duct; PMA, phorbol 12-myristate 13-acetate.

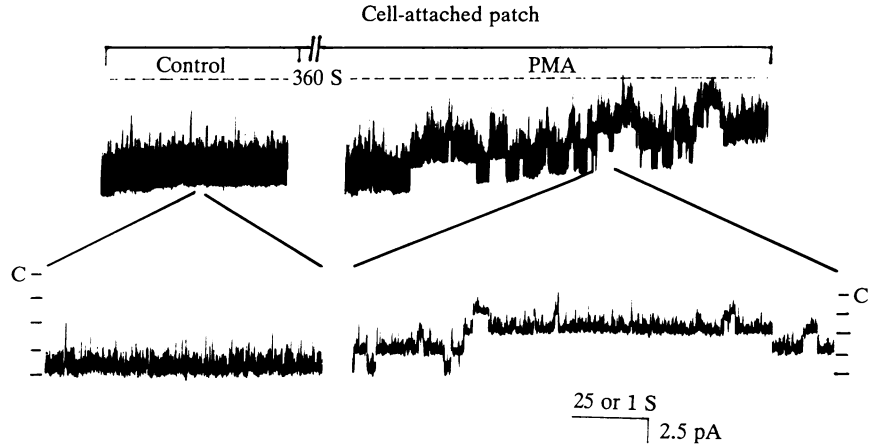

FIG. 1. Current records from a cell-attached patch of the apical membrane of a rat CCD principal cell demonstrating inactivation of the K^+ secretory channel by PKC activator. (*Upper*) Continuous recording with a slow time course. (*Lower*) Parts of channel tracing from *Upper* are displayed with fast time resolution. In the control condition, four channels with P_o of 0.95 were present. Application of 10 μM PMA progressively reduced P_o and the current amplitude of the channel within 360 sec. The holding potential of the patch was 0 mV.

concentration (1 unit/ml). We observed that at a low concentration of Ca^{2+} (10 nM), PKC induced only a slight inhibition of channel activity (11% ± 1%; $P < 0.01$; $n = 3$). When we increased the bath concentration of Ca^{2+} to 100 nM or 1 μM, PKC reduced the P_o of the channel to 23% ± 2% ($P < 0.001$; $n = 5$) and 42% ± 5% ($P < 0.001$; $n = 6$), respectively. The PKC-induced inhibition was reversible regardless of the bath Ca^{2+} concentration. Fig. 2 shows the reversibility of the PKC effect at 1 μM Ca^{2+} concentration within 30 sec.

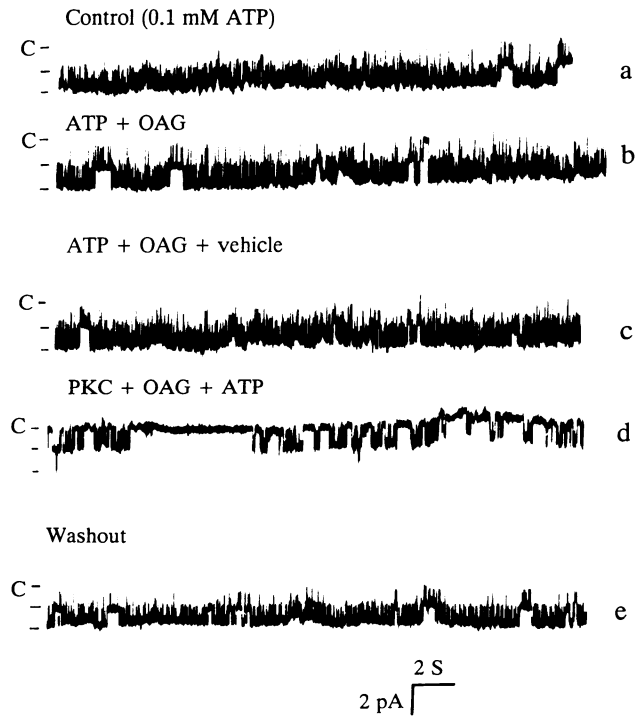

FIG. 2. Channel recording made in an inside-out patch displaying the effect of PKC on the apical K^+ secretory channel of CCD principal cells. (*a*) Control (0.1 mM ATP). Two channels were present in the patch and the P_o was 0.95. In the bath, 1 μM 1-oleoyl-2-acetyl-*sn*-glycerol (OAG) + 0.1 mM ATP (*b*) and the vehicle (*c*) were present. P_o was not significantly changed. (*d*) Thirty seconds after application of PKC (1 unit/ml) to the bath, P_o was reduced to 0.38. (*e*) Thirty seconds after washout. The bath Ca^{2+} concentration was 1 μM. The closed states of the channel are indicated by C. The holding potential of the pipette was 30 mV.

In previous experiments on excised membrane patches, we observed that potassium channel activity decreased progressively and eventually ceased. Such "channel rundown" was effectively reversed by application of cAMP-dependent protein kinase in the presence of a low concentration (<0.1 mM) of Mg-ATP (7). In additional experiments, we explored the interrelationship between PKA and PKC on the activity of the K^+ secretory channel (Fig. 4). It is apparent that after excision of the membrane patch, channel activity had immediately disappeared, even in the presence of 0.1 mM Mg-ATP; yet channel activity was fully restored by administration of PKA (20 units/ml) ($P_o = 0.94 \pm 0.02$) within 90 sec. The subsequent application of PKC (1 unit/ml) in the presence of 0.1 mM ATP, 1 μM PMA, and 500 nM Ca^{2+} reduced P_o from 0.94 to 0.68 ± 0.05 ($P < 0.001$; $n = 4$).

DISCUSSION

Complex modulation of ion-channel activity by PKC has been reported in several tissues (12). PKC activates Cl^- channels in airway epithelia (13, 14) at low Ca^{2+} concentrations (<10 nM) but inactivates chloride channels at high Ca^{2+} concentrations (>1 μM) (13). Two effects of PKC activators on ATP-sensitive K^+ channels in insulin-secreting cells were also reported (15–17). Short-term exposure (100–300 sec) of cells to tumor-promoting phorbol esters or to diacylglycerol derivatives inhibited ATP-sensitive K^+ channels (15). In contrast, long-term exposure (5–10 min) resulted in activa-

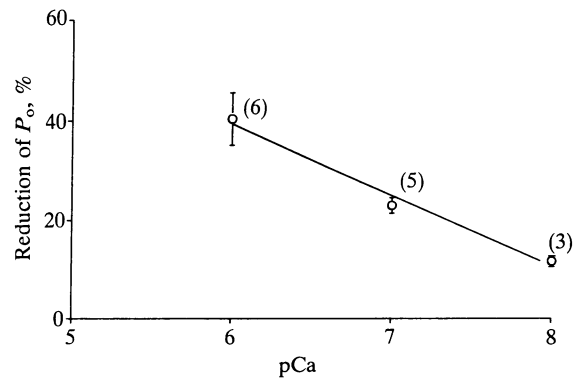

FIG. 3. Relationship between free Ca^{2+} concentration and percentage of PKC-induced decrease of P_o. Number of observations is indicated in parentheses. The scale of abscissa is the negative value of the logarithms of free Ca^{2+} concentrations (molar) in the bath (pCa). Data are expressed as mean ± SEM.

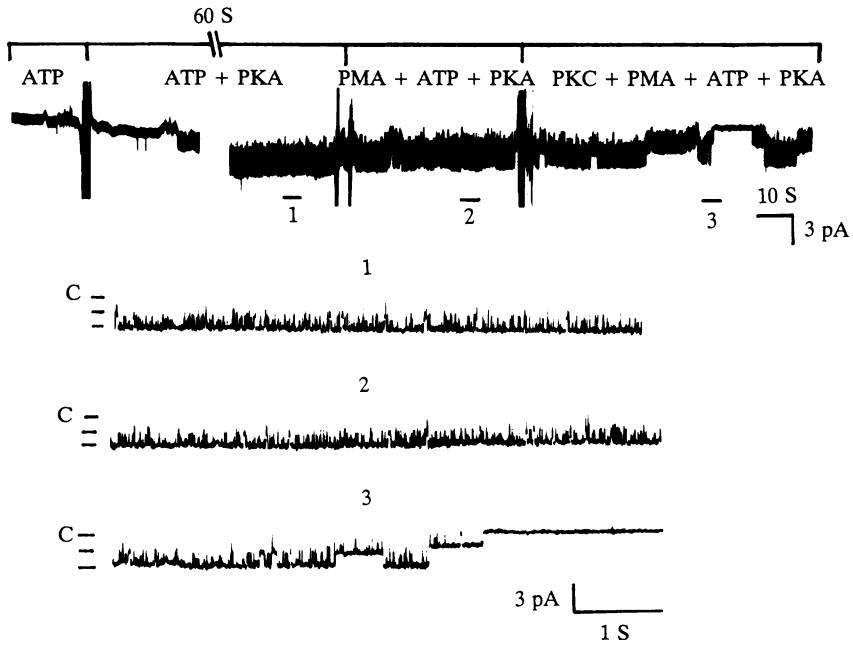

FIG. 4. Channel recording made in an inside-out patch illustrating the inhibitory effect of PKC on the PKA-activated K^+ channel of rat CCD principal cells. (*Upper*) Continuous channel recording. (*Lower*) Three parts indicated by a short bar are expanded. The holding potential of the pipette was 30 mV. ATP (0.1 mM), PKA (20 catalytic units), PMA (1 μM), and PKC (1 unit) were used during the experiments. Bath Ca^{2+} concentration was 500 nM.

tion of ATP-sensitive K^+ channels (16, 17). It was suggested that the dual effects of PKC activators on ATP-sensitive K^+ channels of β cells could result from the transition from up- to down-regulation of PKC by its activator because the prolonged association of PKC–PKC activator with membrane initiated the progressive degradation of PKC (18, 19). We did not observe such a biphasic effect of PKC activators in our experimental setting, even when cells of CCD were exposed to PKC activator for 20 min. However, our experiments demonstrate a significant synergism between Ca^{2+} mobilization and PKC activation. This behavior of PKC was also reported in several tissues other than renal cells (20, 21). The PKC molecules used in this study were a mixture of different subspecies. Since distribution of different PKC isoforms is qualitatively and quantitatively variable among different tissues and cells, it is unknown which type of PKC dominates in principal cells of CCDs. However, the observation that application of tumor-promoting phorbol ester or diacylglycerol derivative is able to inhibit channel activity indicates strongly that PKC plays an important role in regulation of the K^+ secretory channel of CCD.

It is of interest that in inside-out patches K^+ channel activity is not Ca^{2+} dependent. This conclusion is based on our previous observation that in excised patches without exogenous PKC, Ca^{2+} had no effect on channel activity (6). This finding suggests that the PKC molecule is not present in membranes of excised patches and that translocation of PKC from cytoplasm to the cell membrane is necessary for activation of PKC (22). Recently, we observed that an increase of intracellular Ca^{2+} concentration with ionomycin significantly reduced the P_o of the K^+ secretory channel of CCDs in cell-attached patches (unpublished observation). This finding further implies that the modulation of K^+ channels by intracellular Ca^{2+} is indirect and most likely occurs through the PKC signal transduction pathway. Further experiments are needed to confirm this hypothesis.

Dual modulation of ion channels by PKA and PKC has been demonstrated in Cl^- channels of airway epithelial cells in which PKA activates and PKC, at a high concentration of Ca^{2+}, inactivates the Cl^- channel (14). Our results suggest that the 35 pS K^+ secretory channel of rat CCD principal cells also has two phosphorylation sites, which could be located either directly in the channel protein or, alternatively, in a closely associated protein. On the one hand, the cAMP-dependent, PKA-induced phosphorylation opens the channel. This mechanism is physiologically significant because it could mediate the well-documented stimulatory effect of vasopressin on renal potassium secretion (23). Recent observations that vasopressin activates potassium channels in principal cells (A. Cassola, G.G., and W.W., unpublished observations) supports this view. On the other hand, we now demonstrate that stimulation of PKC produces channel inhibition by activation of a separate phosphorylation mechanism. It is likely that the PKC-dependent phosphorylation mechanism is involved in the reduction of K^+ secretion that has been observed after PKC activation in the rabbit CCD (8). We suggest that the two separate activation pathways of PKA and PKC are an integral part of the regulation of renal tubule K^+ secretion in the mammalian kidney.

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