J. Membrane Biol. 141, 43–50 (1994)

The Journal of
Membrane
Biology

Regulation by GTP of a Ca^{2+}-activated K^+ Channel in the Apical Membrane of Rabbit Cortical Collecting Duct Cells

M. Suzuki[1], K. Takahashi[2], O. Sakai[2]

[1]Department of Pharmacology, Jichi Medical University, 3311-1 Yakushiji, Kawachigun, Tochigi, 329-04 Japan
[2]Second Department of Internal Medicine, Jikeikai University, Tokyo, Japan

Received: 14 September 1993/Revised: 4 February 1994

Abstract. Ca^{2+}-activated K^+ channels play an important role in Ca^{2+} signal transduction and may be regulated by mechanisms other than a direct effect of Ca^{2+}. Inside-out patches of the apical membrane of confluent transformed rabbit cortical collecting duct cells cultured on collagen were subjected to patch clamp analysis. Two types of K^+ channel, of medium and high conductance, were observed. The latter channel was characterized by a K^+/Na^+ permeability ratio of 10, an inwardly rectified current, a conductance of 80 pS at 0 mV, and an open probability dependent on both voltage and Ca^{2+}. Guanosine 5′-triphosphate (GTP) but not a guanosine 5′-diphosphate (GDP) analogue, adenosine 5′-triphosphate (ATP), cytidine 5′-triphosphate (CTP), or inosine 5′-triphosphate (ITP), inhibited the activity of this Ca^{2+}-activated K^+ channel. The inhibitory effect of GTP was dose dependent, with a 50% inhibitory concentration of 10^{-5} M in the absence of Mg^{2+}. In the presence of Mg^{2+} (1 mM), which is required for the binding of GTP to G proteins, the 50% inhibitory concentration decreased to 3×10^{-12} M. Pertussis toxin or cholera toxin (each at 10 ng/ml) did not prevent the inhibitory effect of GTP. After removal of GTP from the medium bathing an inhibited channel, subsequent application of Ca^{2+} failed to activate the channel. Ca^{2+}-activated K^+ channels of smooth muscle cells and proximal tubule cells did not respond to GTP. Thus, the Ca^{2+}-activated K^+ channel in the apical membrane of collecting duct cells is inhibited by GTP, which appears to exert its effect via a G protein that is insensitive to both cholera and pertussis toxins.

Correspondence to: M. Suzuki

Key words: Ion channel — Renal tubule — Patch clamp — GTP — G protein

Introduction

K^+ channels activated by intracellular Ca^{2+} are widely distributed in epithelia. Ca^{2+} bound to calmodulin induces phosphorylation of the channel protein, resulting in channel activation [11]. The apical membrane of renal collecting duct cells contains a Ca^{2+}-activated K^+ channel that is believed to play an important role in cell volume regulation [17, 28]. When cells are exposed to hypotonic media, an influx of Ca^{2+} into the cells induces an efflux of K^+ via this channel, which decreases the intracellular K^+ concentration and allows the swollen cells to return to their original volume. Hormones that increase the intracellular free Ca^{2+} concentration may also activate Ca^{2+}-dependent K^+ channels, resulting in depolarization of the resting membrane potential [5, 7, 10]. However, the regulation of Ca^{2+}-activated K^+ channels by mechanisms other than an increase in the cytosolic Ca^{2+} concentration should be considered.

Phosphorylation of channels, a common regulatory mechanism, can be induced by second messengers other than Ca^{2+}. Thus, protein kinase A activated by cyclic AMP and protein kinase C activated by diacylglycerol modulate K^+ channel kinetics in various cell types. Channels can also be regulated by G proteins. Thus, stimulation of K^+ channels in cardiac atrial membranes by muscarinic receptor activation is mediated by a G protein [15]. However, only a few examples of Ca^{2+}-activated K^+ channel regulation by GTP-binding protein have been identified [18, 27], and coupling of

G proteins to K^+ channels in renal cells has not been observed. Thus, we investigated whether the Ca^{2+}-activated K^+ channel in renal epithelia is regulated by a G protein.

Materials and Methods

Cell Isolation, Culture, and Transformation

Primary cultures of renal cortical collecting ducts were prepared as described previously [23]. Briefly, kidneys were removed aseptically from anesthetized male New Zealand White rabbits (body mass, 1–1.5 kg), and cortical collecting ducts were microdissected and transferred to four-well dishes (Nunc) coated with collagen (type IV). Ducts were suspended in a 1:1 (v/v) mixture of HAMF12 and Dulbecco's Minimum Essential Medium supplemented with penicillin (0.2 mg/ml), streptomycin (0.15 mg/ml), and 10% fetal bovine serum (FBS), and incubated at 37°C. After the first few days, during which outgrowth of tubular cells was initiated, cells were cultured in a serum-free hormonally defined medium supplemented with human transferrin (5 μg/ml), 50 nM hydrocortisone, and bovine insulin (5 μg/ml), to enhance growth. The monolayer of cultured cells achieved confluence after 10 to 14 days, after which immortalized cell lines were prepared by transformation of cells with the plasmid pSV-*neo*3.

Plasmid DNA was isolated from *Escherichia coli* containing pSV-*neo*3, which possesses the Simian virus 40 large T antigen (early region promoter) gene and confers resistance to the aminoglycoside antibiotic G418 (neomycin), by the alkaline lysis method [19], digested with Bam HI, and analyzed by agarose gel electrophoresis. Plasmid DNA (1 mg/ml, in distilled water) was then mixed with an equal volume of lipofectin. Cell monolayers were incubated for 24 hr in serum- and hormone-free medium containing the DNA-lipofectin mixture (10 μg DNA/ml). After a further 24 hr in medium containing 10% FBS, the transformed cells were selected by incubation in hormone-free medium containing 10% FBS and neomycin (40 μg/ml). After the first passage, surviving cells were subcultured in a neomycin-containing medium, which was used in all subsequent passages. The subcultured cells achieved confluence after two to three weeks. Cells were used in experiments after three to five passages.

Measurement of Cyclic AMP

Cell monolayers were washed with ice-cold Krebs-Henselite buffer [KHB (mM): 125 NaCl, 1 $CaCl_2$, 1.2 $MgCl_2$, 1 Na_2HPO_4 and 5 HEPES-NaOH, pH 7.4], and incubated for 10 min at room temperature with 500 μl of KHB containing 0.1 mM 3-isobutyl-1-methyl xanthine and either parathyroid hormone, arginine vasopressin or isoproterenol. The reaction was terminated by adding 0.1 M HCl. Cyclic AMP accumulation in the medium and cells combined was determined with a radioimmunoassay kit (Yamasa, Chiba, Japan).

Peanut Agglutinin Binding

Cells cultured on collagen-coated coverslips were washed three times with ice-cold phosphate-buffered saline. Cells were treated with absolute ethanol for 10 min at 4°C, and then incubated 1 hr at room temperature with acridine orange-conjugated peanut agglutinin (20 μg/ml in phosphate-buffered saline). Finally, cells were then washed with phosphate-buffered saline and examined by fluorescence microscopy (Axoplan; Zeiss, Germany).

Patch Clamp Experiments

Patch clamp recordings were obtained as described previously [20, 24]. Currents were recorded at room temperature (20–24°C) with an EPC-7 patch clamp amplifier (List Electronic, Germany), and data were stored on a DAT-200 recorder (Sony, Tokyo, Japan). Records were sampled at 2,000 points/sec and analyzed on a Sanyo MBC AX computer with Axon ver. 5.5.1 software. The data were then transferred to a Macintosh SE/30 computer and analyzed with Excel. 2.2 software. Mean open probability (NP_o) was calculated from $NP_o = \Sigma\, nt_n$, where N is the number of functional channels in the patch, P_o is the single-channel open probability, n represents the state of the channels (0 closed; 1, one open channel; and so on), and t_n is the length of time in state n.

Cells were bathed in oxygenated Krebs-Henselite buffer, and the pipette contained a filtered solution of 150 mM KCl and 5 mM HEPES (pH 7.4). For inside-out patches, 150 mM KCl and 5 mM HEPES (pH 7.4), with or without 1 mM $MgCl_2$, was used as the bath solution. Reagents were dissolved in bath solution and delivered by a separate glass pipette located near the patch-clamped membrane.

Materials

All chemicals were from Sigma (St. Louis, MO), except Bam HI and lipofectin (both from BRL, Bethesda, MD). Smooth muscle (A10) and lung epithelial (W126 VA4) cells, and plasmid pSV-*neo*3, were from American Type Culture Collection (Rockville, MD); the cell lines were maintained in appropriate media. The proximal tubule cell line was generated by transformation with pSV-*neo*3; the characteristics of the cells have been described previously [25].

Statistics

Data are expressed as means ± SE and were analyzed by Student's t-test, chi-square test, or F test (ANOVA) as appropriate. A P value of < 0.05 was considered statistically significant.

Results

Characterization of Collecting Duct Cells

Transformed renal cortical collecting duct (CCD) cells were routinely cultured in medium containing FBS. Growth curves over two days after exchange of medium were well fitted to an exponential function, with doubling times of 2.04 days in FBS-containing medium and 4.83 days in hormone-supplemented FBS-free medium. The cells survived as long as 10 days in FBS-free medium. During the fourth or fifth passage, cells were dispersed and cloned by limiting dilution to an average plating density of 0.5 cells per well in 384 wells. Ten colonies were successfully cloned; two were negative for peanut agglutinin binding, and the other eight colonies included peanut agglutinin-binding cells after incubation with 1 nM retinol for three days. Because Ca^{2+}-activated K^+ channels are present in the luminal membrane of principal cells, we studied the peanut agglutinin-negative colonies.

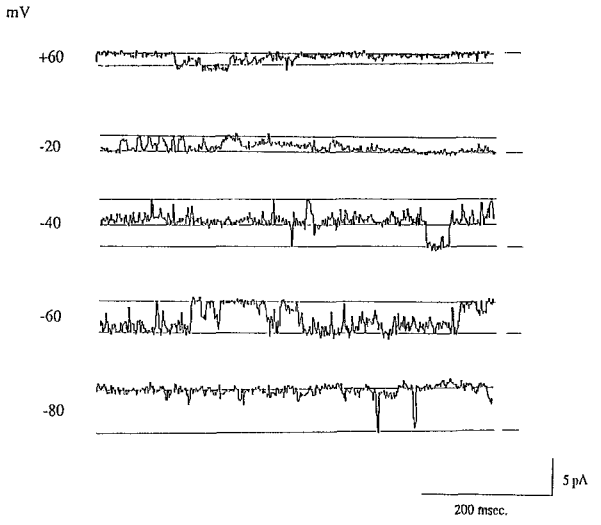

Fig. 1. Effect of membrane potential on Ca^{2+}-activated K^+ channel activity in the apical membrane of CCD cells. The cells were bathed in Krebs-Henselite buffer and the pipette contained 150 mM KCl and 5 mM HEPES. A typical recording of current to negative value of applied voltage (membrane potential) from the apical membrane of a CCD cell in the cell-attached mode is shown.

The accumulation of cyclic AMP by confluent peanut agglutinin-negative cells cultured on collagen in FBS-free medium for three days with three reagents was examined. Arginine vasopressin (F test, $P < 0.05$), but neither parathyroid hormone nor isoproterenol, stimulated production of cyclic AMP: control, 0.27 ± 0.04; arginine vasopressin (10 nM), 0.7 ± 0.07 parathyroid hormone (0.1 μM), 0.4 ± 0.06; isoproterenol (0.1 μM), 0.21 ± 0.03 pmol/well ($n = 6$). The potential difference of cell monolayers grown on a filter membrane was −36 ± 5.2 mV, apical side negative, which decreases to −16 ± 5.6 mV after addition of 10 nM arginine vasopressin on the basolateral side ($n = 4$). Thus, transformed cell clones maintained, at least in part, characteristics of the principal cells of the collecting duct [1, 3]. The apical side of cells was patch-clamped in the following experiments.

Characterization of the Ca^{2+}-activated K^+ Channel in CCD Cells

We first examined the characteristics of K^+ channels in the apical membrane of CCD cells in the cell-attached patch clamp mode. The pipette solution contained 150 mM KCl and the bath contained Krebs-Henselite buffer. At least two types of K^+ channel were detected. The larger conductance was activated by Ca^{2+}. This channel was rarely detected in growing cells or in cells not cultured on a collagen base (2/30 patches); it was observed more frequently in cells that had achieved confluence on a collagen base (20/58 patches; χ^2, $P <$

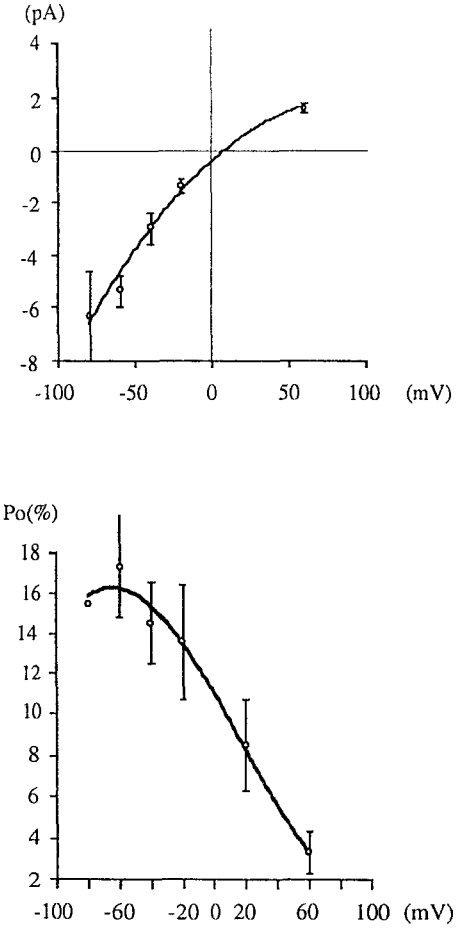

Fig. 2. Current-voltage relation (upper) and open probability *vs.* membrane potential (lower) of single Ca^{2+}-activated K^+ channels in the cell-attached mode. The cells were bathed in Krebs-Henselite buffer and the pipette contained 150 mM KCl and 5 mM HEPES (pH 7.4). Recordings were obtained from the apical membrane of CCD cells in the cell-attached mode. Current was measured by Gaussian analysis, and open probability was calculated as described in Materials and Methods. Each current amplitude and the corresponding open probability were analyzed and then plotted against membrane potential ($n = 4$).

0.05). The K^+ channel with smaller conductance was not voltage activated and was blocked by 1 mM ATP in the bath. The large-conductance K^+ channel was examined in inside-out patches with 150 mM KCl in both sides. The current-voltage (*I-V*) relation was inwardly rectified and the open probability was dependent on membrane voltage (Figs. 1 and 2). The conductance at 0 mV was 80 pS. The channel was activated by bath Ca^{2+} in a dose-dependent manner; maximal channel opening was apparent at 1 mM Ca^{2+}. Substitution of 150 mM KCl in the bath with 75 mM NaCl plus 75 mM KCl, or 100 mM NaCl plus 50 mM KCl, revealed a K^+/Na^+ permeability ratio (P_K/P_{Na}) of 10 ($n = 3$). In outside-out patches, charybdotoxin at 10^{-8} M inhibited channel activity by ~50%. Therefore, the large-con-

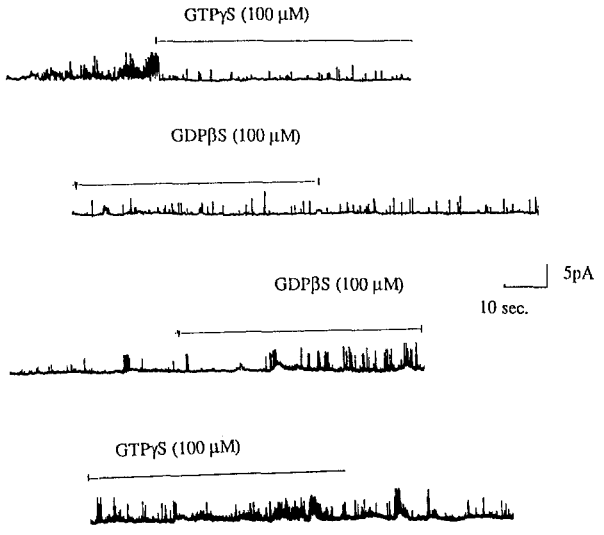

Fig. 3. Effects of GTPγS and GDPβS on Ca^{2+}-activated K^+ channel activity. Both bath and pipette contained 150 mM KCl and 5 mM HEPES (pH 7.4). Inside-out membranes patches were formed and voltage was held at +50 mV. (Upper trace) GTPγS (100 μM) and subsequently GDPβS (100 μM) were added to the inside face of the membrane as indicated. Mean open probability per minute was 42 ± 3.6% for control, 2.5 ± 1.5% ($P < 0.01$) after addition of GTPγS, and 5.6 ± 3.6% after subsequent addition of GDPβS ($n = 4$). (Lower trace) GDPβS (100 μM) and subsequently GTPγS (100 μM) were added to the inside face of the membrane as indicated. Mean open probability per minute was 40 ± 5.6% for control, 62 ± 3.8% ($P < 0.05$) after addition of GDPβS, and 56 ± 4.5% after subsequent addition of GTPγS ($n = 4$).

ductance K^+ channel present in the apical membrane of CCD cells was shown to be a Ca^{2+}-activated K^+ channel, as suggested previously [4, 7] from analysis of the luminal membrane of cortical collecting ducts. Subsequent patch clamp studies were thus performed on the apical membrane of CCD cells at confluence.

Inactivation of the Ca^{2+}-activated K^+ channel by GTP

We investigated the effects of guanosine 5′-*O*-(3-thiotriphosphate) (GTPγS), a nonhydrolyzable analogue of GTP, on the Ca^{2+}-activated K^+ channel. The channel was observed at a holding potential of +50 mV in inside-out patches with 150 mM KCl of both sides of the membrane. Although the channel spontaneously fluctuated between open and closed states, the mean open probability over 1 min was stable. Therefore, we used open probability per minute as a parameter of channel activity. GTPγS (100 μM) added to the bath almost completely inhibited the opening of the Ca^{2+}-activated K^+ channel, and addition of the GDP analogue guanosine 5′-*O*-(2-thiodiphosphate) (GDPβS) did not restore activity (Fig. 3). GDPβS alone potentiated channel opening, and subsequent addition of GTPγS did not inhibit channel activity. The mean open probability was also determined for 1 min before and 1 min after the addition of GTP, ATP, ITP, or CTP to the bath. Only GTP showed an inhibitory effect on channel activity (Table 1) and the removal of GTP by washout did not restore the channel activity. Therefore, GTP/GDP is a specific compound among the nucleotides that regulates the Ca^{2+}-activated K^+ channel.

Table 1. Effects of nucleotide triphosphate on Ca^{2+}-activated K^+ channel activity.

Nucleotide	NP_o	
	Control	Nucleotide (300 μM)
GTP	45.6 ± 9.8	4.52 ± 5.5*
ATP	42.2 ± 10.2	38.9 ± 6.8
ITP	44.2 ± 12.3	45.6 ± 10.1
CTP	35.2 ± 10.0	38.6 ± 6.8

$n = 4$. *$P < 0.01$.

Effect of Mg^{2+} on Channel Inhibition by GTP

Because GTP binds to G proteins more effectively in the presence of Mg^{2+}, the dose-response relation for the inhibitory effect of GTP on the Ca^{2+}-activated K^+ channel was examined in the presence and absence of Mg^{2+}. Again, channel activity was observed in inside-out patches and the mean open probability was determined for 1 min before and 1 min after the addition of GTP. The 50% inhibitory concentration (IC_{50}) for GTP was 10^{-5} M in the absence of Mg^{2+}, and 3×10^{-12} M in the presence of 1 mM Mg^{2+} (Fig. 4). The IC_{50} in the presence of Mg^{2+} was similar to the dose of GTP required for G-protein activation. We also examined the effect of addition of $Al^-F_4^-$, which possesses γ-glutamyl moiety and is known to activate G proteins directly, to the cytoplasmic face of the membrane patched. $Al^-F_4^-$ (10 mM) significantly inhibited Ca^{2+}-activated K^+ channel activity (NP_o decreased from 42 ± 12.2% to 5.3 ± 4.5%; $n = 3$, $P < 0.01$) and the removal of $Al^-F_4^-$ did not restore the channel activity. Thus, the inhibitory effect of GTP on Ca^{2+}-activated K^+ activity appeared to be mediated by G proteins.

Effects of Toxins on Ca^{2+}-activated K^+ Channel Activity

We next attempted to characterize the G protein that mediates the inhibitory effect of GTP on the Ca^{2+}-activated K^+ channel. Pertussis toxin (PTX) catalyzes the ADP-ribosylation of G_i and G_o proteins, and cholera toxin (CTX) catalyzes the ADP-ribosylation of G_s. In the presence of these toxins, GTP has no effect on the mod-

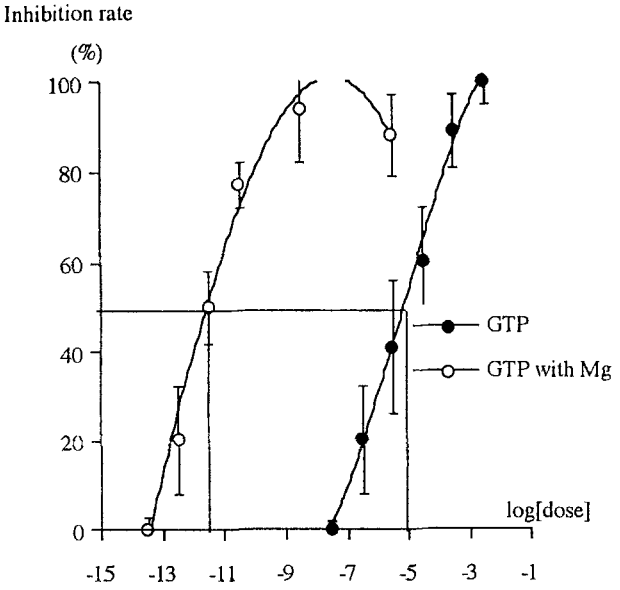

Fig. 4. Effect of Mg^{2+} on the inhibitory effect of GTP on Ca^{2+}-activated K^+ channel activity. The pipette contained 150 mM KCl plus 5 mM HEPES (pH 7.4) and the bath contained 150 mM KCl plus 5 mM HEPES (pH 7.4) with (open circles) or without (filled circles) 1 mM $MgCl_2$. Inside-out membrane patches were formed, and voltage was held at +50 mV. The mean open probability per minute was determined before and after addition of various concentrations of GTP. Percent inhibition was calculated and plotted against the concentration of GTP (n = 5).

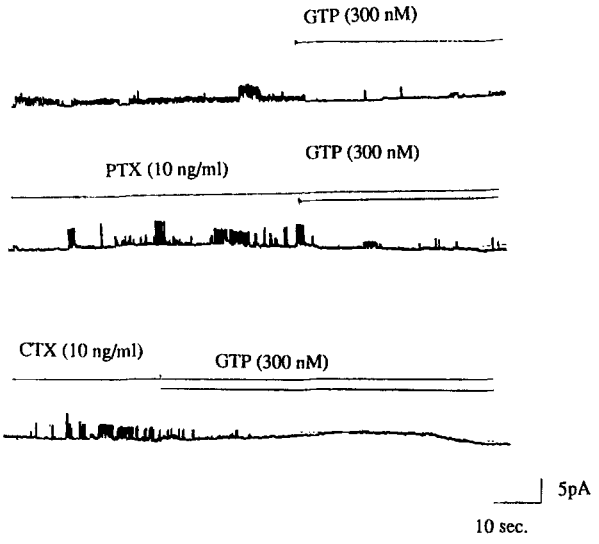

Fig. 5. Effect of PTX and CTX on the inhibitory action of GTP on Ca^{2+}-activated K^+ channel activity. Bath and pipette solutions contained 150 mM KCl, 5 mM HEPES (pH 7.4) and 1 mM $MgCl_2$. Inside-out membrane patches were formed, and voltage was held at +50 mV. (Upper trace) GTP (300 nM) was added to the cytoplasmic face of the membrane in the presence of NAD (0.1 mM) and dithiothreitol (0.1 mM). (Middle trace) GTP (300 nM) was added to the cytoplasmic face of the membrane in the presence of PTX (10 ng/ml), NAD (0.1 mM) and dithiothreitol (0.1 mM). (Lower trace) GTP (300 nM) was added to the cytoplasmic face of the membrane in the presence of CTX (10 ng/ml), NAD (0.1 mM) and dithiothreitol (0.1 mM).

ified G proteins. We thus added the toxins (10 ng/ml) together with the cofactor of NAD^+ (0.1 mM) and dithiothreitol (0.1 mM) to the bath solution containing 150 mM KCl and 1 mM $MgCl_2$. Addition of either toxin to the cytoplasmic face of an inside-out patch did not affect channel activity and did not present the inhibitory action of GTP (300 nM) (Fig. 5 and Table 2). Because G_s requires an ADP-ribosylation factor for activation by GTP, the effect of CTX may not be apparent in excised membranes. The effect of CTX on the Ca^{2+}-activated K^+ channel was thus also examined in the cell-attached patch mode. However, no effect of CTX on channel activity was apparent over a 10-min period [NP_o: control, 26.3 ± 1.6%; CTX, 25.3 ± 8.9% (n = 3)]. The membrane patch was then detached from the cells and GTP (300 nM) was added to the bath. Inhibition of channel activity by GTP was still apparent [NP_o: control, 36.5 ± 12.5%; GTP, 2.5 ± 1.2% (n = 3, P < 0.01)]. Thus, the G protein that mediates the inhibitory effect of GTP on the Ca^{2+}-activated K^+ channel did not appear to be sensitive to either PTX or CTX.

Effect of GTP on Ca^{2+}-activated K^+ Channel Activity in Other Cell Types

G proteins influence the activities of ion channels in various tissues [6]. We therefore examined the effect of GTP on Ca^{2+}-activated K^+ channels of various cell lines. Inside-out patches were prepared from the apical membrane of rabbit CCD cells (control), rat smooth muscle cells (A10) [11], and rabbit proximal tubule cells [9], as well as from the basolateral side of human lung epithelial (WI26 VA4) cells [16]. All cells were grown on a collagen base and allowed to achieve confluence in appropriate FBS-containing media. Addition of Ca^{2+} to the cytoplasmic face of the membrane significantly activated K^+ channels in all four cell types [NP_o: CCD cells, from 15 to 62%; A10 cells, from 30 to 62%; W126VA4 cells, from 10 to 42%; proximal tubule cells, from 20 to 82% (n = 3; t-test, P < 0.01)]. GTP (300 nM), however, suppressed channel activity only in CCD cells [NP_o: CCD cells; to 3.5% (P < 0.01); A10 cells, to 52%; W126VA4 cells, to 32%; proximal tubule cells, to 82% (n = 3, t-test, P > 0.05) (Fig. 6). After removal of the bath solution containing the added Ca^{2+} and GTP, Ca^{2+} (1 μM) was added back to the cytoplasmic face of the CCD cell membrane; Ca^{2+} was no longer able to activate the K^+ channel (NP_o, 3.3 ± 2.5 to 3.3 ± 3.0%, n = 4). The NP_o after a second addition of Ca^{2+} was also determined for a membrane that had not been exposed to GTP (NP_o, 35 ± 5.6%; n = 4). Thus, GTP prevents the direct activation of the channel by Ca^{2+}.

Table 2. Effect of pertussis (PTX) and cholera toxin (CTX) on Ca^{2+}-activated K^+ channel activity

	NP_o			
	Control	Toxin	Toxin+GTP (300 nM)	*n*
PTX+NAD+DTT	54.6 ± 10.2	64.4 ± 8.2	38.2 ± 8.5*	6
PTX+NAD	45.6 ± 12.2	48.6 ± 6.3	21.5 ± 6.5*	3
CTX+NAD	52.3 ± 12.4	50.3 ± 10.5	6.2 ± 3.5**	3

*$P < 0.05$, **$P < 0.01$ *vs.* control.

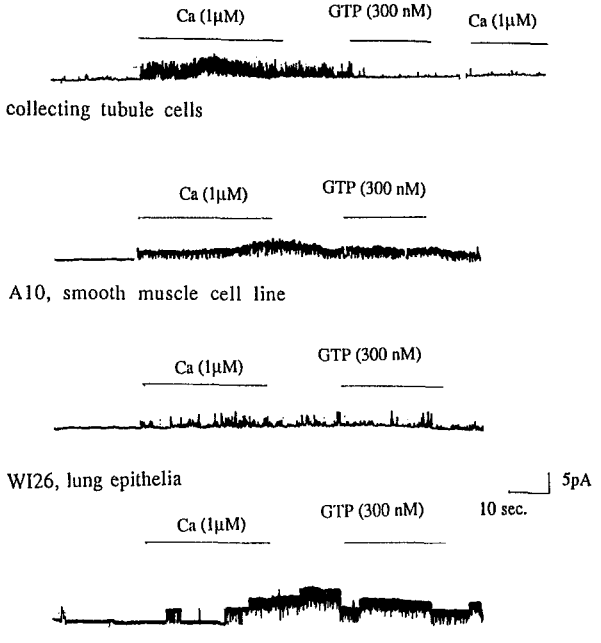

Fig. 6. Effects of Ca^{2+} and GTP on Ca^{2+}-activated K^+ channels in various cell types. Bath and pipette solutions contained (mM) 150 KCl, 5 HEPES (pH 7.4) and 1 $MgCl_2$. Inside-out patches from the apical membrane of CCD cells (upper trace), smooth muscle A10 cells (upper middle trace), and proximal tubule cells (lower trace), and from the basolateral membrane of W126VA4 lung epithelial cells (lower middle trace), were formed, and voltage was held at +50 mV. $CaCl_2$ (final, 1 μM) was added to the bath. After observation of the effect of Ca^{2+}, GTP (300 nM) was added. In the case of CCD cells, Ca^{2+} and GTP were washed out and a second addition of Ca^{2+} was performed.

Discussion

Previous studies have indicated the presence of K^+ channels in the apical membrane of the renal CCD cells. Cortical collecting ducts are composed of two cell types: principal cells and intercalated cells. The principal cells express an apical K^+ conductance and are characterized by the fact that they do not bind peanut agglutinin. Recently, the two cell types have been sorted on the basis of peanut agglutinin binding and cultured as individual cells [3]. Intercalated cells form a dome as they grow to confluence and can be converted to principal cells. In contrast, principal cells appear to maintain their characteristics during growth even when subcultured. Furthermore, the cloned M-1 cell line shows characteristics of principal cells [21]. Therefore, the cloned CCD cells in our study which do not bind peanut agglutinin may maintain characteristics of principal cells and possess K^+ channels qualitatively comparable to those in the luminal membrane of principal cells in cortical collecting ducts.

At least three types of K^+ channel have been described in the apical membrane of CCD cells; a small-conductance (4 pS) K^+ channel, an ATP-inhibitable K^+ channel, and a Ca^{2+}-activated K^+ channel. We detected two types of K^+ channel in our cells with characteristics similar to those of the ATP-inhibitable and Ca^{2+}-activated channels. Hunter et al. [7] have characterized a Ca^{2+}-activated K^+ channel in the apical membrane of the rabbit cortical collecting duct in detail: the current was inwardly rectified, the single channel conductance was 46 pS, and the open probability was dependent on voltage and cytoplasmic Ca^{2+}. Although the properties of the channels described by Hunter et al. [7] and in our study differed quantitatively, the basic characteristics of the two channels, including voltage dependence, Ca^{2+} dependence, and rectification, are similar.

Ca^{2+}-activated K^+ channels have been observed in other nephron segments. For example, the proximal tubules of *Necturus* [8] and rabbit [9, 26] possess a Ca^{2+}-activated K^+ channel in the apical membrane. However, those channels show a linear current-voltage relation and a conductance >150 pS in detached membrane patches. The Ca^{2+}-activated K^+ channel of rabbit proximal tubule cells was frequently observed in the cell-attached mode at a holding potential of −80 mV and the current was outwardly rectified [26], whereas the K^+ channel in CCD cells was apparent in the cell-attached mode at a holding potential of +50 mV, and the current was inwardly rectified. Furthermore, in contrast to CCD cells, GTP did not inhibit the activity of the Ca^{2+}-activated K^+ channel in rabbit proximal

tubule cells in our present study. Therefore, different types of Ca^{2+}-activated K^+ channels, with different responses to GTP, are present in these two nephron segments.

Regulation of K^+ channels by G proteins has been demonstrated in excitable membranes, such as in heart or in neuronal cells [29]. Few examples of such regulation have been observed in nonexcitable epithelial cells; however, Ca^{2+}-activated K^+ channels in fibroblasts and endothelial cells are activated by GTP-binding proteins. Transformation of fibroblasts with the *ras* oncogene resulted in the appearance of a Ca^{2+}-activated K^+ channel that was not present in nontransformed cells [18]; together with the observation that this Ca^{2+}-activated K^+ channel is important in growing of fibroblasts. In contrast to the observation with fibroblasts, GTP, presumably via a G protein, inhibited, rather than stimulated, the activity of the Ca^{2+}-activated K^+ channel in CCD cells. Furthermore, the Ca^{2+}-activated K^+ channel characterized in our study was frequently observed in confluent, quiescent cells.

We failed to identify the G protein that inhibits the Ca^{2+}-activated K^+ channel in CCD cells. Neither PTX nor CTX prevented the inhibitory effect of GTP on the Ca^{2+}-activated K^+ channel. Although the inhibitory effect of GTP appeared reduced after treatment of membrane with PTX, the dose of PTX used may have resulted in a nonspecific effect on G proteins other than the normal targets of the toxin. GTP-binding proteins not susceptible to PTX and CTX and which therefore may mediate the effect of GTP on the CCD cells' Ca^{2+}-activated K^+ channel include *ras* or Gp.

G proteins couple various hormone receptors to enzymes or ion channels that generate intracellular second messengers. Thus, in renal tubules, G proteins are located in the hormone-binding basolateral membrane. However, G_i proteins have been detected in the apical membrane of canine renal tubule cells cultured on membrane-filter support [2]. G proteins have also been located in the luminal membrane, as well as in the basolateral membrane, of noncultured renal tubules along the nephron [22]. What is the role of luminal G proteins? Light et al. [12–14] have shown that a luminal G protein regulates a Na^+ channel [12–14]: cyclic GMP regulates the activity of the channel via a G_i protein. Thus, luminal G proteins may not be coupled with hormone receptors but rather may directly couple with effectors, ion channels. The Ca^{2+}-activated K^+ channel in cortical collecting ducts may be regulated in a similar manner. Thus, an unknown second messenger may activate a G protein, which in turn inhibits the Ca^{2+}-activated K^+ channel.

In summary, we have demonstrated the presence of K^+ channel that is activated by Ca^{2+} and inhibited by GTP in the apical membrane of CCD cells. The regulation of this channel by a G protein may contribute to the physiological regulation of K^+ transport in this nephron segment.

References

1. Dietl, P., Kizer, N., Stanton, B.A. 1992. Conductive properties of a rabbit cortical collecting duct cell line: regulation by isoproterenol. *Am. J. Physiol.* **262:**F578–F582
2. Ercolani, L., Stow, J.L., Boyle, J.F., Holtzman, E.J., Lin, H., Grove, J.R., Ausiello, D.A. 1990. Membrane localization of the pertussis toxin-sensitive G-protein subunits αi-2 and αi-3 and expression of a metallothionein-αi-2 fusion gene in LLC-PK1 cells. *Proc. Natl. Acad. Sci. USA* **87:**4637–4639
3. Fejes-Toth, G., Naray-Fejes-Toth, A. 1992. Differentiation of renal β-intercalated cells to α-intercalated and principal cells in culture. *Proc. Natl. Acad. Sci USA* **89:**5487–5491
4. Geibel, J., Zweifach, A., White, S., Wang, W.H., Giebisch, G. 1990. K^+ channels of the mammalian collecting duct. *Renal Physiol. Biochem.* **13:**59–69
5. Guggino, S.E., Suarez-Isla, B.A., Guggino, W.B., Sacktor, B. 1985. Forskolin and antidiuretic hormone stimulate a calcium activated potassium channel in cultured kidney cells. *Am. J. Physiol.* **249:**F448–F455
6. Hescheler, J., Rosenthal, W., Trautwein, W., Schultz, G. 1990. Receptor-ion channel coupling through G proteins. *In:* G Proteins. R. Iyenger, L. Birnbaumer, editors. pp. 384–403. Academic, San Diego
7. Hunter, M., Lopes, A.G., Boulpaep, E., Giebisch, G. 1986. Regulation of single potassium ion channels from apical membrane of rabbit collecting tubule. *Am. J. Physiol.* **251:**F725–F733
8. Kawahara, K., Hunter, M., Giebisch, G. 1987. Potassium channels in *Necturus* proximal tubule. *Am. J. Physiol.* **251:**F488–F494
9. Kawahara, K., Ogawa, A., Suzuki, M. 1991. Hypoosmotic activation of Ca-activated K channels in cultured rabbit kidney tubule cells. *Am. J. Physiol.* **260:**F27–F33
10. Klaerke, D.A., Jørgensen, P.L. 1988. Role of Ca^{2+}-activated K^+ channels in regulation of NaCl reabsorption in thick ascending limb of Henle's loop. *Comp. Biochem. Physiol. A* **90:**757–765
11. Kume, H., Takai, A., Tokuno, H., Tomita, T. 1989. Regulation of Ca^{2+}-dependent K^+-channel activity in tracheal myocytes by phosphorylation. *Nature* **341:**152–154
12. Light, D.B., Ausiello, D.A., Stanton, B.A. 1989. Guanine nucleotide-binding protein, alpha i-3, directly activates a cation channel in rat renal inner medullary collecting duct cells. *J. Clin. Invest.* **84:**352–356
13. Light, D.B., Schwiebert, E.M., Karlson, K.H., Stanton, B.A. 1989. Atrial natriuretic peptide inhibits a cation channel in renal inner medullary collecting duct cells. *Science* **243:**383–385
14. Light, D.B., Corbin, J.D., Stanton, B.A. 1990. Dual ion-channel regulation by cyclic GMP and cyclic GMP-dependent protein kinase. *Nature* **344:**336–339
15. Mattera, R., Yatani, A., Kirsch, G.E., Graf, R., Okabe, K., Olate, J., Codina, J., Brown, A.M., Birnbaumer, L. 1989. Recombinant alpha i-3 subunit of G protein activates Gk-gated K^+ channels. *J. Biol. Chem.* **264:**465–471
16. McCann, J.D., Matsuda, J., Garcia, M., Kaczorowski, G., Welsh, M.J. 1990. Basolateral K^+ channels in airway epithelia. I. 1990. Regulation by Ca^{2+} and block by charybdotoxin. *Am. J. Physiol.* **258:**L334–L342
17. Montrose, R.C., Guggino, W.B. 1990. Cell volume regulation in the nephron. *Annu. Rev. Physiol.* **52:**761–772
18. Rane, S.G. 1991. A Ca^{2+}-activated K^+ current in ras-transformed

fibroblast is absent from nontransformed cells. *Am. J. Physiol.* **260:**C104–C112

19. Sambrook, J., Fritsch, E.F., Maniatis, T. 1989. Molecular Cloning. A Laboratory Manual. pp. 1.25–1.33. Cold Spring Harbor Laboratory, New York
20. Sakmann, B., Neher, E. 1988. Single-Channel Recording. pp. 237–250. Plenum, New York.
21. Schuster, V.L., Fejes-Toth, G., Naray-Fejes-Toth, A., Gluck, S. 1991. Colocalization of H^+-ATPase and band 3 anion exchanger in rabbit collecting duct intercalated cells. *Am. J. Physiol.* **260:**F506–F517
22. Stow, J.L., Sabolic, I., Brown, D. 1991. Heterogeneous localization of G protein α-subunits in rat kidney. *Am. J. Physiol.* **261:** F831–F840
23. Suzuki, M., Caparelli, A., Jo, O.D., Kawaguchi, Y., Ogura, Y., Miyahara, T., Yanagawa, N. 1988. Phosphate transport in the in vitro cultured rabbit proximal convoluted and straight tubules. *Kidney. Int.* **34:**268–272
24. Suzuki, M., Morita, T., Hanaoka, K., Kawaguchi, Y., Sakai, O. 1991. A Cl channel activated by parathyroid hormone in rabbit renal proximal tubule cells. *J. Clin. Invest.* **88:**735–742
25. Suzuki, M., Morita, T., Hasegawa, H., Miyazaki, K., Ikeda, M., Hanaoka, K., Watabe, K., Kawaguchi, Y., Sakai, O. 1992. Transformed cloned cells from isolated tubules (Proximal tubules) *Jikeikai. Med. J.* **39:**119–126
26. Suzuki, M., Morita, T., Kawaguchi, Y., Kurihara, S., Sakai, O. 1991. Vitamin D metabolites increase cytoplasmic free calcium concentration in cultured rabbit proximal straight tubule cells. *Am. J. Physiol.* **260:**F757–F763
27. Vaca, L., Schilling, W.P., Kunze, D.L. 1992. G-protein-mediated regulation of a Ca(2+)-dependent K^+ channel in cultured vascular endothelial cells. *Pfluegers Arch.* **422:**66–74
28. Volkl, H., Paulmichl, M., Lang, F. 1988. Cell volume regulation in renal cortical cells. *Renal Physiol. Biochem.* **11:**158–173
29. Yatani, A., Cadina, J., Brown, A.M. 1990. G-protein mediated effects on ionic channels. *In:* G. Proteins, R. Iyenger, L. Birnbaumer, editors. pp. 243–261. Academic, San Diego