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Modulation of Ca^{2+}-Activated Potassium Channels by cGMP-Dependent Signal Transduction Mechanism in Cerebral Arterial Smooth Muscle Cells of the Rabbit

Jin Han, Nari Kim, Kwangbok Lee, and Euiyong Kim

Department of Physiology and Biophysics, College of Medicine, Inje University, Busan 614−735, Korea

The present investigation tested the hypothesis that the activation of protein kinase G (PKG) leads to a phosphorylation of Ca^{2+}-activated potassium channel (K_{Ca} channel) and is involved in the activation of K_{Ca} channel activity in cerebral arterial smooth muscle cells of the rabbit. Single-channel currents were recorded in cell-attached and inside-out patch configurations of patch-clamp techniques. Both molsidomine derivative 3-morpholinosydnonimine-*N*-ethylcarbamide (SIN-1, 50 μM) and 8-(4-Chlorophenylthio)-guanosine-3',5'-cyclic monophosphate (8-pCPT-cGMP, 100 μM), a membrane-permeable analogue of cGMP, increased the K_{Ca} channel activity in the cell-attached patch configuration, and the effect was removed upon washout of the drugs. In inside-out patches, single-channel current amplitude was not changed by SIN-1 and 8-pCPT-cGMP. Application of ATP (100 μM), cGMP (100 μM), ATP+cGMP (100 μM each), PKG (5 U/μl), ATP (100 μM)+PKG (5 U/μl), or cGMP (100 μM)+PKG (5 U/μl) did not increase the channel activity. ATP (100 μM)+cGMP (100 μM)+PKG (5 U/μl) added directly to the intracellular phase of inside-out patches increased the channel activity with no changes in the conductance. The heat-inactivated PKG had no effect on the channel activity, and the effect of PKG was inhibited by 8-(4-Chlorophenylthio)-guanosine-3',5'-cyclic monophosphate, Rp-isomer (Rp-pCPT-cGMP, 100 μM), a potent inhibitor of PKG or protein phosphatase 2A (PP2A, 1 U/ml). In the presence of okadaic acid (OA, 5 nM), PP2A had no effect on the channel activity. The K_{Ca} channel activity spontaneously decayed to the control level upon washout of ATP, cGMP and PKG, and this was prevented by OA (5 nM) in the medium. These results suggest that the PKG-mediated phosphorylations of K_{Ca} channels, or some associated proteins in the membrane patch increase the activity of the K_{Ca} channel, and the activation may be associated with the vasodilating action.

Key Words: Protein kinase G (PKG), Cerebral arterial smooth muscle cells, Phosphorylation, Ca^{2+}-activated potassium channel (K_{Ca} channel), Patch-clamp techniques

INTRODUCTION

Several types of potassium channels have been described in the cerebral circulation. Hyperpolarization of vascular muscle produced by activation of calcium-activated potassium channels (K_{Ca} channels) may be a major mechanism that mediates relaxation of cerebral arteries and arterioles in response to several stimuli (Nelson & Quayle, 1995). Therefore, K_{Ca} channels appear to be negative feedback systems to regulate vascular tone. Activity of K_{Ca} channels is altered in several disease states, including hypertension, diabetes, and atherosclerosis. Alteration of K_{Ca} channels and impairment of vasodilation may possibly contribute to the development or maintenance of cerebral ischemia or vasospasm.

Cyclic GMP (cGMP) has been implicated as an important regulator of smooth muscle tension in response to atrial natriuretic peptide (ANP), nitric oxide (NO), and nitrovasodilators (Gruetter et al, 1979; Winquist et al, 1981). NO and nitrovasodilators

Corresponding to: Euiyong Kim, Department of Physiology and Biophysics, College of Medicine, Inje University, Gaegeum-dong, Busanjin-gu, Busan 614-735, Korea. (Tel) 82-51-890-6714, (Fax) 82-51-894-4500, (E-mail) phykimey@ijnc.inje.ac.kr

have been shown to activate K_{Ca} channels and hyperpolarize the smooth muscle cells (Nelson, 1993). K_{Ca} channels increase their open state probability when the cytosolic Ca^{2+} concentration is raised, but some of them also respond to membrane voltage changes, being activated by depolarization. The linkage between the cytosolic Ca^{2+} and depolarization provokes the suggestion that some channels may function as negative feedback regulators of cytosolic Ca^{2+} by hyperpolarizing the membrane in close proximity to voltage-activated Ca^{2+}channels (Nelson & Quayle, 1995). K_{Ca} channels play an important role in controlling the diameter of small myogenic cerebral arteries (Brayden & Nelson, 1992).

The receptors for cGMP documented in mammalian cells include cGMP-dependent protein kinase (PKG), cation channel protein, and a family of cGMP-dependent phosphodiesterases (PDEs) (Lincoln et al, 1976; Beavo, 1988; Kaupp et al, 1989; Wolf et al, 1989). PKG is known to be a potential receptor for the cGMP-dependent relaxation of the smooth muscle (Francis et al, 1988). Indeed, significant amounts of PKG are found in smooth muscles (Keilbach et al, 1992). Immunocytochemical studies have shown that vascular smooth muscle cells are particularly rich in PKG activity (Ives et al, 1980). Activation of PKG may lead to a relaxation of vascular smooth muscle by lowering the cytosolic Ca^{2+} concentration and membrane hyperpolarization through activation of potassium channels (Ruth et al, 1993; Ruth, 1999). It is, therefore, important to investigate the interaction between the NO/cGMP signaling cascade and the K_{Ca} channel activity in cerebral arterial smooth muscle cells. The aim of the present study was to investigate if there exist any interaction between the NO/cGMP signaling cascade and the K_{Ca} channel in cerebral arterial smooth muscle cells. We tested the hypothesis that the activation of PKG promotes phosphorylation of K_{Ca} channel and is involved in the activation of K_{Ca} channel activity in cerebral arterial smooth muscle cells of the rabbit.

METHODS

Single cell isolation

Single vascular myocytes were isolated from middle cerebral arteries of the rabbits by enzymatic dissociation, as described previously (Hamill et al, 1981). Rabbits (New Zealand white rabbit, 0.8~1.2 Kg) were anaesthetized with sodium pentobarbital (10 mg/kg i.v.) and exsanguinated. Middle cerebral arteries were removed and cleaned of extraneous connective tissue in a dissecting solution containing (in mM): 137 NaCl, 5.6 KCl, 0.42 Na_2HPO_4, 0.44 NaH_2PO_4, 4.17 $NaHCO_3$, 1 $MgCl_2$, 2.6 $CaCl_2$, and 10 N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES). The pH was adjusted to 7.3 with NaOH. The arteries were then transferred to the isolation solution (in mM): 55 NaCl, 6 KCl, 88 L-glutamic acid, 10 HEPES, and 10 glucose. The pH was adjusted to 7.3 with NaOH, for 20 min. Cerebral arteries were digested for 10 min in an isolation solution containing (in mg/ml): 1 albumin, 1 papain, and 1 dithioerythritol, and then for 10 min in the isolation solution containing (in mg/ml): 1 albumin, 1 collagenase Type F, and 1 hyaluronidase Type I-S. Single smooth muscle cells were obtained by gentle trituration with a wide-bore pipette in the fresh isolation solution with albumin (1 mg/ml), stored at 4°C and used within 12 hours.

Electrophysiological methods

Single-channel currents were measured in cell-attached and inside-out patch configurations of the patch-clamp technique (Han et al, 1993) by using a patch-clamp amplifier (Axopatch-1D, Axon Instruments, Foster City, CA, USA). Giga-ohm seals were obtained using pipettes of 5~10 MΩ resistance pulled from borosilicate glass capillaries (Clark Electrochemical, Pangbourne, England) with a vertical puller (Narishige PP-83, Tokyo, Japan). Their tips were coated with Sylgard and fire polished. After a patch was obtained, an equilibration period of ~5 min was allowed. The patches that showed large fluctuations in channel activity over this period were discarded. The channel activity was recorded continuously until the end of the experiment. Drug actions were observed for 5~10 min after the addition of the drug to the bath solution either by pipette (at 1% of the bath volume) or by perfusion (in a volume of at least 10 times the bath volume). Single-channel currents were digitized at a sampling rate of 48 kHz and stored in digitized format on digital audio tapes using a Biologic DTR-1200 recorder (Grenoble, France). For the analysis, the data were transferred to a computer (IBM-PC, Pentium III)

with pCLAMP version 6.03 software (Axon Instruments Inc., Burlingame, CA, USA) through an analogue-to-digital converter interface (Digidata-1200, Axon Instruments Inc., Burlingame, CA, USA). In most experiments the DAD-12 superfusion system was used for the rapid change of the bath solution and drugs.

Data analysis and quantification of single channel activity

The threshold for judging the open state was set at half of the single-channel amplitude (Colquhoun & Sigworth, 1983). The open time histogram was formed from continuous recordings of more than 60 sec. The open probability (Po) was calculated using the formula:

$$P_O = (\sum_{j=1}^{N} t_j j)/(T_d N)$$

where t_j is the time spent at current levels corresponding t_j = 0, 1, 2, ... N channels in the open state, T_d is the duration of the recording and N is the number of channels active in the patch. The number of channels in a patch was estimated by dividing the maximum current that observed by the mean unitary current amplitude. Po was calculated over 30 sec records.

Solutions and drugs

For cell-attached and inside-out patches, the bath solution was (in mM): 135.0 KCl, 2.0 $MgCl_2$, 1.2 KH_2PO_4, 1.8 or 2.2 $CaCl_2$, 3.0 EGTA and 10.0 HEPES, and pH 7.2. Free Ca^{2+} in this solution was either 250 nM ($CaCl_2$=1.8 mM) or 500 nM ($CaCl_2$= 2.2 mM). In inside-out patches, the Ca^{2+}, EGTA ratio was adjusted to give a pCa of 6×10^{-7} at which simultaneous opening of 1 to 3 channels could be typically observed. The pipette solution contained (in mM): 136.0 NaCl, 5.0 KCl, 10.0 HEPES, 1.0 $MgCl_2$, 1.8 $CaCl_2$, with pH adjusted to 7.4 using NaOH solution.

All chemicals and drugs were obtained from Sigma Chemical (St. Louis, MO, USA). Experiments were done at a room temperature of 25±2°C.

RESULTS

Effect of molsidomine derivative 3-morpholinosydnonimine-N-ethyl carbamide (SIN-1) on K_{Ca} channel activity in cell-attached patch configuration

Initially we investigated the effects of SIN-1 on K_{Ca} channels in cell-attached patch configuration in order to test the hypothesis that K_{Ca} channels were involved in the actions of NO. Extracellular SIN-1 has been known to increase NO in the bath. After giga-seal

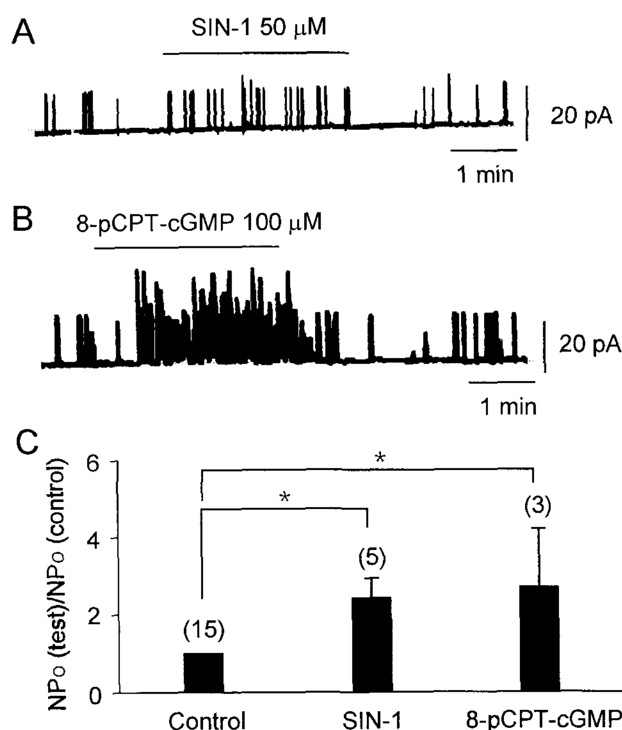

Fig. 1. (A) Effect of the molsidomine derivative 3-morpholinosydnonimine-N-ethyl carbamide (SIN-1) on Ca^{2+}-activated K^+ channel (K_{Ca}) activity. Single-channel current trace obtained from a cell-attached patch configuration held at +50 mV. Application of SIN-1 caused an increase in K_{Ca} channel activity. (B) Representative record of the effect of 8-(4-chlorophenylthio)-guanosine 3',5'-cyclic monophosphate (8-pCPT-cGMP, 100 μM) on K_{Ca} channel activity. Single-channel current trace obtained from a cell-attached patch configuration held at +50 mV. 8-pCPT-cGMP caused a marked increase of K_{Ca} channel activity. Data were sampled at 400 Hz and filtered at 1 kHz. Dashed line indicates the zero current level. (C) Histogram showing effect of SIN-1 and 8-pCPT-cGMP on K_{Ca} channel activity. The effect compared with control condition (normalized to 1). Numbers above each bar indicate number of experiments.

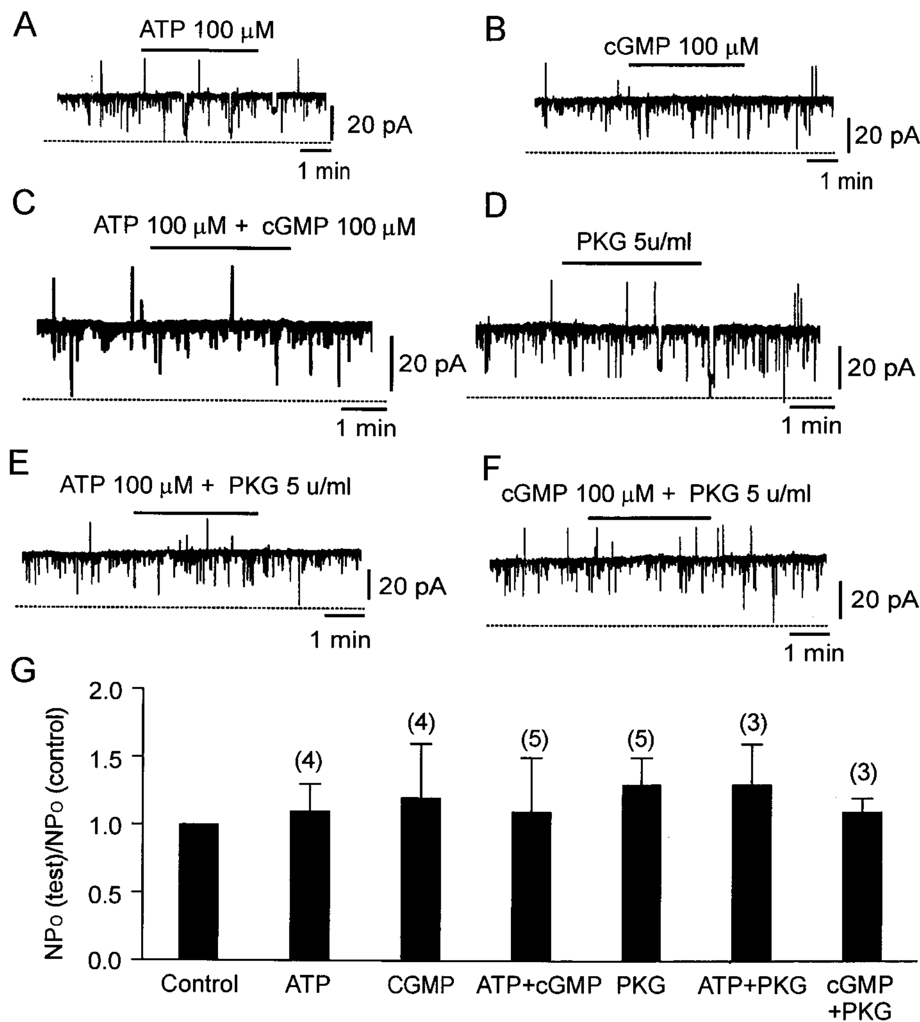

Fig. 2. Effect of ATP, cGMP and PKG on the K_{Ca} channel activity in rabbit cerebral arterial smooth muscle cells. Current recording from an inside-out patch configuration held at +50 mV. Data were sampled at 400 Hz and filtered at 1 kHz. Dashed line indicates the zero current level. (A) Effect of ATP on the K_{Ca} channel activity. Intracellular ATP (100 μM) had no effect on the activity of K_{Ca} channels compared with control. (B) Effect of cGMP on the K_{Ca} channel activity. Intracellular cGMP (100 μM) had no effect on the activity of K_{Ca} channels compared with control. (C) Effect of ATP plus cGMP on the K_{Ca} channel activity in rabbit cerebral arterial smooth muscle cells. Intracellular ATP (100 μM) and cGMP (100 μM) together had no effect on the activity of K_{Ca} channels compared with control. (D) Effect of PKG (5 U/μl) on the K_{Ca} channel activity in rabbit cerebral arterial smooth muscle cells. PKG (5 U/μl) had no effect on the activity of K_{Ca} channels compared with control. (E) Effect of ATP and PKG on the K_{Ca} channel activity in rabbit cerebral arterial smooth muscle cells. Intracellular ATP (100 μM) and PKG (5 U/μl) together had no effect on the activity of K_{Ca} channels compared with control. (F) Effect of cGMP and PKG on the K_{Ca} channel activity in rabbit cerebral arterial smooth muscle cells. Intracellular cGMP (100 μM) and PKG (5U/μl) together had no effect on the activity of K_{Ca} channels compared with control. (G) Histogram showing effect of ATP, cGMP, or PKG on K_{Ca} channel activity. The effect compared with control condition (normalized to 1). Numbers above each bar indicate number of experiments.

the pipette potential was set to −50 mV and the bath soluiton was switched from the normal Tyrode solution to the high K^+ solution. In order to minimize changes in calcium flux across the membrane, we bathed the cells in low extracellular calcium and examined K_{Ca} channels at membrane potential near the calcium equilibrium potential (+50 mV). Under these conditions, SIN-1 (50 μM) increased activity of K_{Ca} channels (Fig. 1A). The same result was observed in four other patches (Fig. 1C). This increase was reversed by washout of SIN-1. Single-channel current amplitude was not changed by SIN-1.

Effects of membrane-permeable cGMP analogue on K_{Ca} channel in cell-attached patch configuration

The above results suggest that NO may activate K_{Ca} channels in cerebral arterial smooth muscle cells. Since NO activates guanylate cyclase, one would expect that an elevation of intracellular cGMP could activate K_{Ca} channels. Therefore, we addressed the question of whether this channel activity is involved in the action of cGMP-dependent protein kinase (PKG). In experiments evaluating the involvement of PKG in the stimulation of K_{Ca} channel activity, we used 8-(4-chlorophenylthio) guanosine 3',5'-cyclic monophosphate (8-pCPT-cGMP). As showing Fig. 1B, 8-pCPT-cGMP (100 μM) applied extracellularly activated the K_{Ca} channel activity, and the effect was reversed by washout of the drug. The same result was observed in two other patches (Fig. 1C). 8-pCPT-cGMP had no effect on the unitary currents.

Effect of PKG activation on K_{Ca} channel in an inside-out patch configuration

The activation of K_{Ca} channels by 8-pCPT-cGMP in the cell-attached patches suggests the possibility that PKG activates K_{Ca} channel. It is, therefore, particularly important to know whether there is any interaction between cGMP/PKG signaling pathway and K_{Ca} channels in excised inside-out patches. Initially, application of 100 μM ATP (Fig. 2A) or 100 μM cGMP (Fig. 2B) had no effect on the K_{Ca} channel activity in an inside-out patch configuration. Also, the application of 100 μM ATP and 100 μM cGMP together (Fig. 2C) or PKG (5 U/μl) alone did not change the channel activity (Fig. 2D). Likewise, application of 100 μM ATP and PKG (5 U/μl) together (Fig. 2E) or 100 μM cGMP and PKG (5 U/μl) together had no apparent effect of the channel activity (Fig. 2F). The same result was observed in all patches (Fig. 2G). However, PKG (5 U/μl) increased the channel activity in the presence of cGMP (100 μM) and ATP (100 μM) together (Fig. 3B). The same result was observed in ten other patches (Fig. 3D). Thus, the activation of K_{Ca} channel by PKG required both cGMP and ATP.

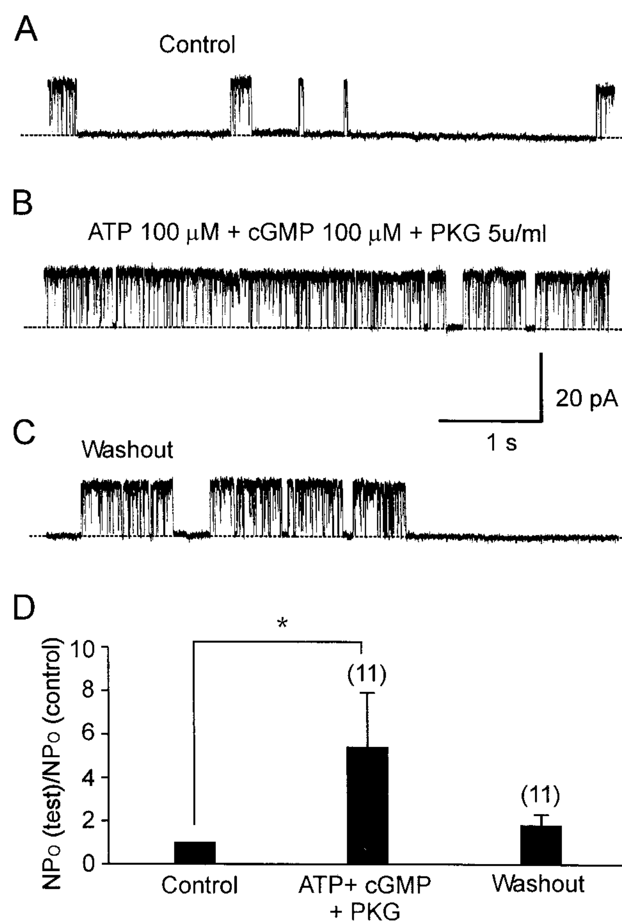

Fig. 3. Effect of PKG activation on the K_{Ca} channel activity in rabbit cerebral arterial smooth muscle cells. Current recording from an inside-out patch configuration held at +50 mV. PKG (5 U/μl) caused an increase in the channel activity in the presence of 100 μM ATP and 100 μM cGMP. Data were sampled at 400 Hz and filtered at 1 kHz. Dashed line indicates the zero current level. (D) Histogram showing effect of PKG activation on K_{Ca} channel activity in the presence of ATP and cGMP. The effect compared with control condition (normalized to 1). Numbers above each bar indicate number of experiments.

Specificity of the PKG in stimulating K_{Ca} channel activity

In order to confirm the stimulating effect of PKG on the channel activity, we conducted experiments with heat-inactivated PKG. Fig. 4A shows a representative result obtained in an inside-out patch exposed to ATP (100 μM) and cGMP (100 μM) at the intracellular surface. The application of heated PKG (5 U/μl) to the intracellular surface failed to enhance the channel activity. Similar results were observed in three other patches (Fig. 4C).

In order to reaffirm the stimulating effect of PKG on the channel activity, we tested the effect of Rp-pCPT-cGMP, a selective inhibitor of PKG. Fig. 4B illustrates that in excised inside-out patch configuration, PKG (5 U/μl) caused an increase in K_{Ca} channel activity in the presence of ATP and cGMP. Addition of exogenous Rp-pCPT-cGMP (100 μM) resulted in a reversal of the PKG activation, returning the channel activity to near the control level. The same result was observed in three other patches (Fig. 4C).

PKG-induced activation of K_{Ca} channel reversed by protein phosphatase

The activation of K_{Ca} channel by PKG occurred in the presence of cGMP and ATP, suggesting that cGMP stimulated PKG, which in turn activating the channel by phosphorylation. We sought to investigate whether exogenous PP2A, applied intracellularly during PKG activation, could impair the K_{Ca} channel activation. Fig. 5A shows that in excised inside-out patch configuration, PKG (5 U/μl) caused an increase in K_{Ca} channel activity in the presence of ATP and cGMP. Addition of PP2A (1 U/ml) led to a reversal of the PKG-induced K_{Ca} channel activation. The same result was observed in five other patches (Fig. 5C).

In order to investigate whether the PKG-mediated phosphorylation of the K_{Ca} channel underlies the PKG-induced activation of K_{Ca} channels, further experiments were performed with okadaic acid (OA), a potent inhibitor of type 1 and 2A protein phosphatases (Fig. 5B). OA was used at a low concentration (5 nM) to specifically block the activity of PP2A in excised inside-out patches. In the three patches tested, application of PKG in the presence of ATP caused an increase in the channel activity. If ATP, cGMP and PKG were washed out and OA was

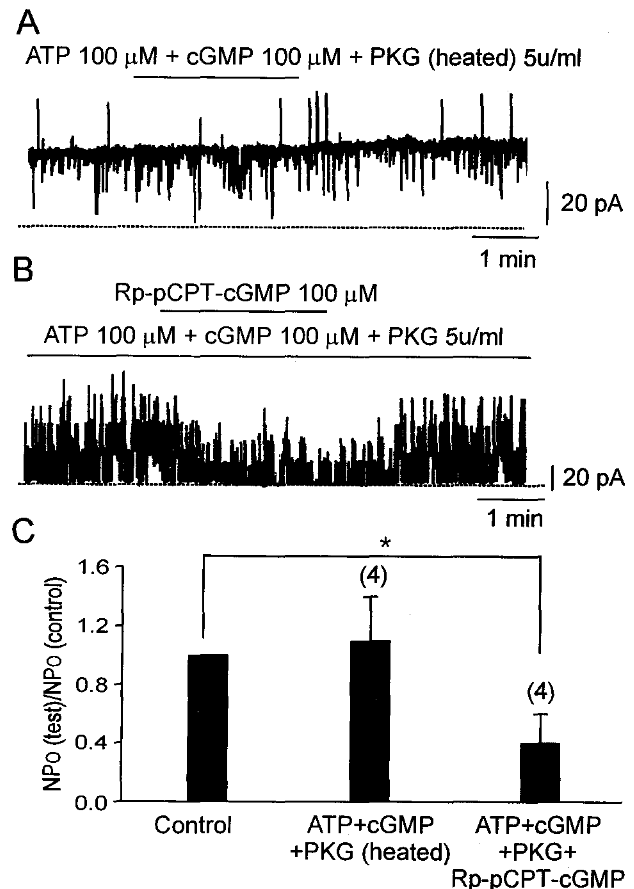

Fig. 4. (A) Effect of heated PKG on the K_{Ca} channel activity in rabbit cerebral arterial smooth muscle cells. Current recording from an inside-out patch configuration held at +50 mV. Heated PKG had no effect on the channel activity in the presence of 100 μM ATP and 100 μM cGMP. (B) Effect of PKG inhibitor on PKG activation of the K_{Ca} channel activity in the presence of ATP and cGMP. Current recording from an inside-out patch configuration held at +50 mV. Representative record of the effect of Rp-pCPT-cGMP on PKG activation of the K_{Ca} channel activity. Application of PKG (5 U/μl) caused an increase in the channel activity in the presence of ATP and cGMP together (100 μM each). In the same patch, Rp-pCPT-cGMP (100 μM) was added to bath solution in the presence of ATP+cGMP+PKG. Note that Rp-pCPT-cGMP (100 μM) reversed the stimulatory effects of PKG on K_{Ca} channel activity. Data were sampled at 400 Hz and filtered at 1 kHz. Dashed line indicates the zero current level. Note that stimulatory effect of PKG on K_{Ca} channel activity results from its enzymatic activity. (C) Histogram showing effect of heated PKG and PKG inhibitor on K_{Ca} channel activity. The effect compared with control condition (normalized to 1). Numbers above each bar indicate number of experiments.

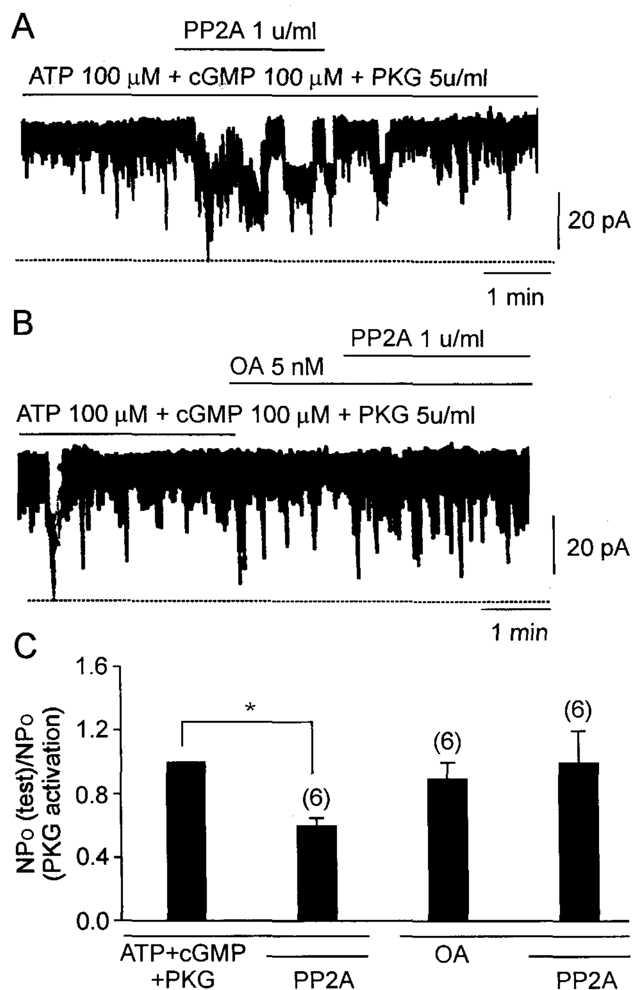

Fig. 5. (A) Effect of exogenous protein phosphatase 2A (PP2A) on the K_{Ca} channel activity stimulated by PKG in rabbit cerebral arterial smooth muscle cells. Current recording from an inside-out patch configuration held at +50 mV. PKG (5 U/μl) in the presence of ATP (100 μM) and cGMP (100 μM) caused an increase in the channel activity. In the same patch, PP2A (1 U/ml) was added to bath solution. PP2A caused an inhibition of the channel activity. (B) Effect of exogenous PP2A on the K_{Ca} channel activity stimulated by PKG in the presence of okadaic acid (OA), a potent inhibitor of PP2A. Current recording from an inside-out patch configuration held at +50 mV. In the presence of ATP+cGMP, PKG caused an increase in the channel activity. Upon removal of ATP +cGMP+PKG and exposure to OA (5 nM), activation of K_{Ca} channel activity persisted. PP2A applied intracellularly in the presence of OA was ineffective. Data were sampled at 400 Hz and filtered at 1 kHz. Dashed line indicates the zero current level. (C) Histogram showing effect of PP2A and OA on K_{Ca} channel activity. The effect compared with PKG activation (normalized to 1). Numbers above each bar indicate number of experiments.

applied to the patches, K_{Ca} channel activity was maintained high. The application of PP2A did not alter the channel activity. The same result was observed in five other patches (Fig. 5C).

DISCUSSION

We demonstrated in the present study that SIN-1 and the membrane permeable cGMP analogue (8-pCPT-cGMP) could open K_{Ca} channels. Furthermore, we provided (a) direct evidence that K_{Ca} channels in cerebral arterial smooth muscle cells could be opened through the activation of cGMP-dependent protein kinase (PKG). The PKG activation of K_{Ca} channels required both cGMP and ATP, suggesting that cGMP stimulated the PKG, which in turn activated the channel by phosphorylation. The data in the present study strongly favor this possibility. (1) OA, at a concentration of 5 nM, prevents the spontaneous reversal of PKG-induced activation of K_{Ca} channels; (2) Application of exogenous PP2A in the presence of PKG activation reversed the PKG-induced activation of K_{Ca} channels. These data suggest that an endogenous membrane-associated PP2A is responsible for the reversal of PKG-induced activation of K_{Ca} channels in accordance with the hypothesis that K_{Ca} channels are under the control of both PKG and PP2A in cerebral arterial smooth muscle cells of the rabbit. It may be that the processes of phosphorylation and dephosphorylation could dynamically regulate the activity of K_{Ca} channels in cerebral arterial smooth muscle cells, and provide a mechanism by which K_{Ca} channel activity and hence cellular excitability can be controlled reversibly.

It has been shown that NO does not hyperpolarize the vascular muscle in large cerebral arteries (Plane & Garland, 1992). Dilatation of cerebral arterioles in rabbits in response to nitroprusside and acetylcholine is not inhibited by iberiotoxin and charybdotoxin (Taguch et al, 1995), suggesting that cerebral vasodilation in response to the stimulation of guanylate cyclase is not dependent on the activity of K_{Ca} channels. In contrast, we provide here the evidence that K_{Ca} channels in cerebral arterial smooth muscle of the rabbit can be opened through the activation of PKG. Whether there are any species or segmental differences in the effect of PKG on the activity of K_{Ca} channels in cerebral vessels is not known. Nevertheless, there are many evidences supporting that the

K_{Ca} channel is regulated by PKG in vascular smooth muscle cells. It was demonstrated that K_{Ca} channels could be activated by ANF, nitroprusside, nitroglycerin and 8-Br-cGMP, which increases cGMP, in vascular smooth muscle cells (Williams et al, 1988; Fujino et al, 1991). Here, in cell-attached patches, we used a membrane-permeant analogue of cGMP (8-pCPT-cGMP) that, unlike 8-Br-cGMP or other cGMP analogues, is very specific for PKG and is resistant to degradation by cGMP phosphodiesterases. Our findings suggest that the activation of K_{Ca} channels by 8-pCPT-cGMP or SIN-1 is probably mediated via the activation of PKG. In addition, we directly tested the effects of PKG in inside-out patches. Although Robertson et al (1993) directly tested the effects of PKG on K_{Ca} channels, in our knowledge, our present results provide further direct evidence that K_{Ca} channels in cerebral arterial smooth muscle cells can be opened through PKG-dependent phosphorylation. The observed effects of PKG, OA, or PP2A in inside-out patches suggest that an endogenous membrane-associated PP2A is responsible for the reversal of PKG-mediated activation of K_{Ca} channels and that K_{Ca} channels are under the control of both PKG and PP2A. Furthermore, the experiments with the expressed K_{Ca} channels from human myometrium favor a direct regulation of the channel by PKG Type I (Alioua et al, 1998). In most cases, PKG activation resulted in the activation of K_{Ca} channel (Zhou et al, 1996; Zhou et al, 1998), which is activated already at lower cytosolic calcium concentration. The resulting hyperpolarization impairs Ca^{2+} influx via voltage-dependent L-type Ca^{2+} channels and relaxes the smooth muscle.

Atrial natriuretic peptide, NO and nitrovasodilators elevate intracellular cGMP and cause relaxation of tension in vascular smooth muscles (Gruetter et al, 1979; Winquist et al, 1981). First evidence for the involvement of PKG in the effect of cGMP came from in vitro studies. Cyclic nucleotide analogs that potently activate purified PKG Type I induce a relaxation of vascular smooth muscle tone with a similar pattern of potency, suggesting that the activation of PKG mediates the relaxation in response to an increase in intracellular cGMP (Francis et al, 1988). Recent studies in mice carrying null mutations of the genes coding the endothelial NO synthase (eNOS) (Huang et al, 1995) and the receptor GC-A (Lopez et al, 1995) clearly showed that the NO/cGMP signalling cascade mediates basal vasodilation in vivo, since these mice are hypertensive. Deletion of the PKG Type I gene in mice resulted in a similar elevation of blood pressure (Pfeifer et al, 1998), suggesting that cGMP modulates vascular resistance via PKG and not via other cGMP receptor proteins such as the cGMP-dependent PDEs. Although smooth muscle contains a number of proteins that appear to be preferentially phosphorylated by PKG Type I, a causal link between the phosphorylation of a specific protein and the relaxation of smooth muscle could not be established. Activation of PKG Type I lowers cytosolic calcium concentrations in smooth muscles and various other cell types (Cornwell & Lincoln, 1989; Ruth et al, 1993), an effect that is compatible with the smooth muscle relaxing activity of the enzyme. Several mechanisms have been proposed to attribute to the reduction of cytosolic calcium concentration by PKG. In conclusion, activation of PKG contributes to phoshorylation of K_{Ca} channel and is involved in the activation of K_{Ca} channel activity in cerebral arterial smooth muscle cells of the rabbit. Phosphorylation of the channel or a channel-associated protein may be a general mechanism for PKG to activate K_{Ca} channel in cerebral arterial smooth muscle cells of the rabbit. These results suggest that activation of K_{Ca} channels may be involved in the vasodilating action of NO in cerebral arterial smooth muscle cells of the rabbit.

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