Cyclic GMP-dependent Protein Kinase Activates Cloned BK$_{Ca}$ Channels Expressed in Mammalian Cells by Direct Phosphorylation at Serine 1072*

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Large conductance Ca$_{2+}$-activated K$^+$ (BK$_{Ca}$)$^{1}$ channels are ubiquitously distributed among tissues and are particularly abundant in smooth muscle (1, 2). The activity of BK$_{Ca}$ channels is regulated by membrane potential, intracellular Ca$_{2+}$, and phosphorylation (3, 4). Although BK$_{Ca}$ channels are usually not involved in setting resting potential, they play a key role as a negative feedback mechanism to limit depolarization and contraction (5–7). Activation of BK$_{Ca}$ channels is increased by nitric oxide (NO) and atrial natriuretic peptide, which hyperpolarize the membrane and increase the sensitivity of BK$_{Ca}$ channels to Ca$_{2+}$ (8–11). Membrane hyperpolarization closes voltage-dependent Ca$_{2+}$ channels, reduces Ca$_{2+}$ influx, and leads to a reduction in intracellular Ca$_{2+}$ concentration and relaxation (1). NO has been reported to stimulate BK$_{Ca}$ channels directly as well as through stimulation of guanylate cyclase and the subsequent increase in cGMP (12–15). In addition, activation of BK$_{Ca}$ channels plays an important role in NO-induced relaxation of smooth muscle (16–20). cGMP activates GMP-dependent protein kinase (PKG), which phosphorylates various cytosolic and membrane proteins that regulate smooth muscle tone either directly or indirectly (21, 22). Recent studies in native cells suggest that PKG activates BK$_{Ca}$ channels through phosphorylation of the channel (23). These results are supported by biochemical studies of cloned BK$_{Ca}$ channels, which demonstrate PKG-induced phosphorylation of the channel (24).

The primary sequence of BK$_{Ca}$ has been determined using molecular cloning techniques in Drosophila (25) and mammals (26–28). These studies indicate that BK$_{Ca}$ isoforms belong to the voltage-gated K$^+$ (KV) channel superfamily. The primary sequence of the S1-S6 segment of BK$_{Ca}$ channels is homologous to the corresponding regions in KV channels. The long carboxyl terminus is the region of Ca$_{2+}$-sensing (29, 30), and cslo-contains a single high affinity phosphorylation site for PKG at Ser-1072 (3). However additional putative PKG phosphorylation sites have been identified in other splice variants (31). Expression of the slo channel in Xenopus oocytes or mammalian cells gives rise to voltage-gated, Ca$_{2+}$-sensitive currents with electrophysiological and pharmacological features similar to those of native BK$_{Ca}$ (32–34). However, although many studies of native cells suggest that BK$_{Ca}$ channel activity is also modestly by various protein kinases (35–38), this property has been difficult to reproduce in cloned channels. Two studies in which slo channels have been expressed in either oocytes (27) or Chinese hamster ovary cells (39) have reported that PKG was without effect on slo channel activity. In contrast, Perez et al. (33) reported that an endogenous cAMP-dependent protein kinase-like activity activated dslo-α channels expressed in Xenopus oocytes. A recent study showed that PKG-Iα phosphorylated hsko channels reconstituted into lipid bilayers but had no effect on channel activity in inside-out patches expressed in Xenopus oocytes (24).

The purpose of this study was to examine PKG-induced modulation of cloned BK$_{Ca}$ channels and determine whether direct phosphorylation of the channel was involved. The α-subunit of cslo, a BK$_{Ca}$ channel α-subunit cloned from canine colon, was expressed in HEK293 cells, and currents were measured using both the whole cell mode as well as cell-attached and

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The abbreviations used are: BK$_{Ca}$, large conductance Ca$_{2+}$-activated K$^+$ channel; PKG, cGMP-dependent protein kinase; K$_{a}$ channel, voltage-gated K$^+$ channel; NPo, channel open probability; IBTX, iberiotoxin; SNP, sodium nitroprusside; DETA, diethylenetriamine; PCR, polymerase chain reaction; pS, picosiemens.

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PKG Regulation of Cloned BK<sub>Ca</sub> Channel

EXPERIMENTAL PROCEDURES

Expression of cslo-α Channels—The cDNA encoding the α-subunit of the BK<sub>Ca</sub> channel (cslo-α) was previously cloned from canine colonic smooth muscle using reverse transcription and a polymerase chain reaction (GenBank™ accession number U-41001). Northern blot analysis showed that the cslo-α transcripts are expressed in the muscles of the canine gastrointestinal tract and blood vessels (27). The cslo-α construct was subcloned into the mammalian expression vector pZeoSV (Invitrogen, CA).

The S1072A mutation of cslo-α was created by recombinant mutagenesis (40). Briefly, linearized cslo-α plasmid was modified and amplified simultaneously by PCR in two separate reactions. Two primer pairs were used for PCR. In the first amplification reaction, one-half of the plasmid was amplified using a forward primer containing the S1072A mutation, spanning nucleotides 3194 to 3233 (5′-AGTCCTCCAGCAAGAAGAGCGCCTCCGTGCACTCCATCCC-3′) and a reverse primer complementary to the plasmid sequence (5′-CTCCATCCC-3′). The second half of the reaction amplified the remaining half of the plasmid using the reverse-mutating primer (5′-AGTCCTCCAGCAAGAAGAGCGCCTCCGTGCACTCCATCCC-3′) and the forward plasmid-specific primer (5′-ATGGCGGAAAGTTGACCAGTGCCGTTCCGGTGCTCAGCC-3′). Both the mutating and plasmid primers were designed to contain a ≥24-base pair homology at their 5′ ends, which generated an overlap between the ends of the two PCR products. The homologous ends of the PCR products undergo recombination in vivo following transformation of RecA<sup>+</sup> Escherichia coli cells. PCR amplification was performed in 50-μl reactions containing 1× PCR buffer (50 mM KCl, 10 mM Tris-Cl, 1.5 mM MgCl<sub>2</sub> (pH 8.3); Invitrogen), 200 μM each dNTP (Invitrogen), 25 pmol of primer, 2 ng of plasmid template, 10% Me<sub>2</sub>SO, and 2.5 units of Taq polymerase (Promega, Madison, WI). Reactants underwent an initial denaturation (94 °C × 1 min), 30 amplification cycles (94 °C × 30 s, 50 °C × 30 s, and 72 °C × 30 s), and a final extension of 72 °C × 10 min. PCR products were gel-purified, and 2.5 μl of each PCR reaction were mixed and transformed directly into 50 μl of Max Competent™ DH5α E. coli (Life Technologies, Inc) and selected on low salt LB zeocin plates (25 μg/ml). Plasmid DNA was prepared from overnight cultures using the QIAprep Miniprep kit (Qiagen, CA). Plasmid DNA of the correct size was sequenced using the ABI Prism cycle sequencing kit (Perkin-Elmer, CA) and analyzed on a Perkin-Elmer 310 Genetic Analyzer.

HEK293 cells were obtained from ATCC (cell line number CRL-1573, Manassas, VA) and maintained in modified RPMI medium (Life Technologies, Inc) supplemented with 10% heat-inactivated horse serum (Summit Biotechnology, Fort Collins, CO) and 1% glutamine (Life Technologies, Inc) and maintained in modified RPMI medium (Life Technologies, Inc). The cslo-α DNA was transfected into HEK cells by electroporation. Electroporation was performed as follows. After harvesting the HEK cells by trypsin-EDTA, the cells were washed twice with phosphate buffer solution and resuspended in ice-cold phosphate buffer solution at a density of 5 × 10<sup>6</sup> cells/ml in the cuvette for electroporation. Each cuvette was supplemented with appropriate combinations of CD8 (a lymphocyte cell-surface antigen) in the pH3-CD8 plasmid construct as a marker for transfection (4 μg), cslo-α, or S1072A cslo-α in the pZeoSV vector (20 μg). After a 10-min incubation on ice, electroporation was done by applying a 330-V pulse using a pulse generator (Electroporator II, Invitrogen, CA). HEK cells expressing cslo-α were subcultured on glass coverslips for electrophysiological re-
control. mM HEPES, 1 mM IBTX (100 nM). Currents were recorded in cells before and after treatment with IBTX. A, representative traces of rslo-α current expressed in HEK cells before and after treatment with IBTX (100 nM). Currents were recorded in whole cell voltage mode. The membrane potential of the cell was held at -70 mV and depolarized with a series of 200-ms step pulses from -50 mV to +80 mV with 10-mV increments at 15-s intervals. B, current-voltage relationship of rslo-α currents before (●) and after (○) treatment with IBTX. C, average rslo-α currents before and after treatment with IBTX (n = 5). Data shows average peak currents at voltage clamp steps from -70 to +50 mV at 30-s intervals (n = 5). Values are means ± S.E. * p < 0.01 compared with control.

Electrophysiological Recording—The patch-clamp technique was used to measure membrane currents in whole cell and single cell configuration. Patch pipettes were made from borosilicate grass capillaries pulled with a three-stage micropipette puller (P-80/PC, Sutter, CA) and heat-polished with a microforge (MF-83, Narishige, Japan). The pipettes had tip resistances of 2 to 5 megaohm for whole cell recordings and 8 to 10 megaohm for single-channel recordings. Coverslips containing HEK cells were placed in a recording chamber (volume 1.0 ml) mounted on the stage of an Olympus inverted microscope and superfused with bath solution at a rate of 1.0 ml/min. Standard gigaohm seal patch-clamp recording techniques were used to measure the currents of whole cell, cell-attached, and excised inside-out configurations. An Axopatch 200A patch-clamp amplifier (Axon Instruments, CA) was used to measure whole cell and single-channel recordings. Capacitance and series resistance compensation were performed. The output signals were filtered at 1 kHz with an 8-pole Bessel filter, digitized at a sampling rate of 3 kHz, and stored on the hard disk of a computer for off-line analysis. Data acquisition and analysis were performed with pClamp software (version 6.0.4., Axon Instruments). Channel open probability (NPo) in patches was determined from recordings of more than 3 min by fitting the sum of Gaussian functions to an all-points probability histogram plot at each potential. Single channel conductance was determined using Student's t test for paired observations.

RESULTS

Characterization of rslo-α Currents Expressed in HEK Cells—Membrane currents of nontransfected native HEK cells were measured in whole cell voltage clamp configuration. Depolarizing steps triggered an outward current in native HEK cells. The current showed little or no inactivation during the pulse. The steady-state current at +50 mV was 0.29 ± 0.04 nA (n = 10). This current was suppressed by the K⁺ channel blocker 4-aminopyridine (0.09 ± 0.03 nA at 1 mM; n = 4) but was unaffected by the specific BKCa channel inhibitor iberiotoxin (IBTX 100 nM; n = 3). Expression of CD8 DNA (marker plasmid) in HEK cells had no effect on the current (n = 6).

The amplitude of outward current in HEK cells expressing rslo-α was considerably larger than in native HEK cells. Mean current amplitude obtained under steady-state conditions at +50 mV in cells expressing rslo-α was 4.56 ± 0.42 nA (n = 10).
Representative whole cell currents obtained in transfected and native cells are shown in Fig. 1A. The current-voltage relations of transfected and native cells are shown in Fig. 1B. Membrane conductance plotted as a function of voltage in HEK cells expressing cslo-a is shown in Fig. 1C. Discernible conductance was apparent at potentials positive to 240 mV, and maximum conductance was reached at approximately 160 mV. The $V_{0.5}$ was 120.3 mV, and the slope was 15.1.

Effect of IBTX on Whole Cell cslo-a Currents—BKCa channels are specifically blocked by IBTX purified from venom of the scorpion (43). Experiments were therefore undertaken to determine whether outward currents recorded in cslo-a cells were blocked by IBTX. The addition of IBTX (100 nM) to the bathing solution produced a marked reduction in outward current at all voltages tested as seen in Fig. 2, A and B. In 5 cells expressing cslo-a, IBTX significantly ($p < 0.01$) reduced current amplitude by greater than 90% (Fig. 2C). The current remaining in the presence of IBTX was not different from that of native currents recorded in HEK cells ($p > 0.05$).

Single Channel Recordings of cslo-a Current—To further examine the properties of outward currents in cells transfected with cslo-a, single channel activity was recorded in inside-out patches in a symmetrical KCl solution (140 mM KCl). Channel openings could be detected at membrane potentials ranging from −60 to +60 mV. The membrane potential was clamped at +40 mV. D, open-state probability versus cytosolic Ca$^{2+}$ concentration at membrane potential +40 mV. The line is the Boltzmann fit to the data.

Fig. 3. Single channel recordings of cslo-a current in inside-out patches of HEK cells. A, representative recording of cslo-a channel recorded from an inside-out excised membrane patch at membrane potentials ranging from −60 to +60 mV. Cytosolic Ca$^{2+}$ concentration was maintained at 10$^{-5}$ M. C indicates closed. B, current-voltage relationship of cslo-a channel current from inside-out patches of HEK cells using symmetrical 140 mM KCl solution. C, a representative recording of cslo-a channel recorded from an inside-out excised membrane patch at cytosolic Ca$^{2+}$ concentration ranging from 10$^{-8}$ to 10$^{-5}$ M. The membrane potential was clamped at +40 mV. D, open-state probability versus cytosolic Ca$^{2+}$ concentration at membrane potential +40 mV. The line is the Boltzmann fit to the data.
SNP significantly enhances whole cell cslo-activity, which returned to near control levels 10–15 min after wash-out (Fig. 5). The relationship between Ca\(^{2+}\) concentration and Po of the cslo- channel at +40 mV is shown in Fig. 3B.

**Effect of SNP on Whole Cell cslo-Currents**—The NO donor sodium nitroprusside (SNP) increased the activity of native BK\(_{Ca}\) channels in smooth muscle (13–18). Experiments were therefore undertaken to determine the action of SNP on cells expressing cslo- using the whole cell patch-clamp mode. The addition of SNP (10\(^{-4}\) M) to the bathing solution led to a marked increase in cslo- channel activity, ranging from 10 to 10\(^{-4}\) M, channel activity increased dramatically (holding potential = +40 mV; see Fig. 4C). The relationship between Ca\(^{2+}\) concentration and Po of the cslo- channel at +40 mV is shown in Fig. 3D.

**Effect of NO Donors on cslo-Channel Activity**—Because SNP significantly enhances whole cell cslo- activity, additional experiments were performed to determine whether changes also occur in single channel activity recorded in cell-attached patches. The addition of SNP (10\(^{-4}\) M) to the bathing solution led to a marked increase in cslo- channel activity, which returned to near control levels 10–15 min after wash-out (Fig. 5, A and C). In Fig. 5B, the time course of changes in cslo- activity after the addition of SNP is shown. SNP increased NPo from 0.092 to 0.656 in this cell. In 8 cells, SNP significantly (p < 0.01) increased NPo 3.3-fold (holding potential = +40 mV; see Fig. 5C) but had no effect on unitary current amplitude (control 253 ± 11.3, SNP 262 ± 13.1, wash-out 258 ± 8.8 pS, n = 8, p > 0.05). SNP had no significant effect on cslo- channels in inside-out patches (NPo; control 0.117, SNP 0.249, 0.05). Additional experiments were performed with the NO donor, DETA/NO (300 μM), because there is evidence that different donors may have differing effects upon potassium channels (44). In contrast to SNP, DETA/NO increased cslo- channel activity in the absence (NPo; control 0.036 ± 0.023; DETA/NO, 0.072 ± 0.029, n = 6, p < 0.05) and in the presence (NPo; control 0.152 ± 0.121, DETA/NO, 0.249 ± 0.117, n = 8, p < 0.05) of KT5823 in cell-attached patches.

**Effect of PKG-I\(\alpha\) on cslo-Channel Activity**—Our experiments with SNP suggest that cslo- channels are activated by the cGMP/PKG pathway. To provide more direct evidence for PKG-induced modulation of cslo-, we examined the effects of PKG-I\(\alpha\) on cslo- channel activity in inside-out patches. Application of PKG-I\(\alpha\) to the cytosolic side of the membrane did not modify cslo- currents (Fig. 6, B and C). The effect of ATP (1 mM) on cslo- channel was variable (11 of 21 increased, 7 of 21

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**Fig. 4. Effect of SNP on cslo-Currents expressed in HEK cells.** A, representative traces of cslo-Currents expressed in HEK cells before and after application of SNP (10\(^{-3}\) M). Currents were recorded in whole cell voltage mode. The membrane potential of the cell was held at −70 mV and depolarized with a series of 200-ms step pulses from −50 mV to +80 mV with 10-mV increments at 15-s intervals. B, current-voltage relationship of cslo-Currents before (○) and after (●) application of SNP. C, average cslo-Currents of control, SNP and after washout (WO) of SNP. Data shows average peak currents at voltage clamp steps from −70 to +50 mV at 30-s intervals. Values are means ± S.E. (n = 8). *p < 0.01 compared with control.
PKG phosphorylation at Ser-1072. Thus a point mutation of cslo phosphorylates at Ser-1072. Thus a point mutation of cslo at Ser-1072 was created in which Ser-1072 was replaced by Ala. The point mutation on the other kinases or related proteins. To clarify this point, we made a point mutation of PKG-I. PKG-I may indirectly activate BK Ca channels through cGMP, 252, 252 M) plus cGMP (0.1 mM) also had no effect on mutated cslo channels (control 253 pS, n = 10, p > 0.05). Effect of PKG-I on Mutated cslo-a Channel Activity—Activation of PKG may indirectly activate BKCa channels through other kinases or related proteins. To clarify this point, we made a point mutation on the cslo-a channel. The amino acid sequence of cslo-a has only one optimal consensus sequence for PKG phosphorylation at Ser-1072. Thus a point mutation of cslo-a was created in which Ser-1072 was replaced by Ala. The general characteristics of mutated cslo-a channels is shown in Fig. 7, A–C. The mutated cslo-a channel was activated by membrane depolarization, and its conductance (247.2 ± 13 pS, n = 10) was not different (p > 0.05) from that of wild-type cslo-a channels. Increases in Ca^2+ concentration at the cytosolic surface activated the channel in a concentration-dependent manner. These characteristics of the mutated cslo-a channel were comparable with wild-type cslo-a channels. However, application of PKG-I in the presence of ATP (1 mM) plus cGMP (0.1 mM) was without effect on mutated cslo-a channel activity (n = 10; Fig. 7, D and E). The single channel conductance of the cslo-a channel was also unchanged by PKG (n = 10; Fig. 7F). SNP also had no effect on mutated cslo-a channel activity in cell-attached patches (NPo: control, 0.049 ± 0.042; SNP, 0.054 ± 0.051, n = 6, p > 0.05).

**DISCUSSION**

This study provides direct evidence that cloned BKCa channels expressed in HEK cells can be activated by cGMP-dependent protein kinase. Activation required ATP and cGMP, suggesting that PKG stimulates BKCa channels through phosphorylation. Mutation of cslo-a at Ser-1072, the only optimal consensus phosphorylation site for PKG on cslo-a, abolished the stimulatory effect of PKG on cslo-a channels. These results indicate that PKG activates cslo-a channels through direct phosphorylation at Ser-1072.

Native outward currents in HEK cells were small and inhibited by 4-aminopyridine but not by IbTx, suggesting that these currents were largely because of delayed rectifier-type K^+ channels with little contribution from BKCa channels. This result agrees with another recent study of these cells (45). Several kinds of Cl^- channels also contribute to native HEK cell currents (46). However, under the conditions of our experiments, both delayed rectifier and chloride currents were minimal compared with currents recorded in cells transfected with...
cslo-a. Whole cell outward currents recorded in transfected cells were blocked by IBTX and exhibited a voltage dependence comparable with native BKCa channel currents. cslo-a channel activity recorded in single channel mode was enhanced by membrane depolarization and by increases in Ca$^{2+}$ concentration at the cytosolic surface. Half-maximal activation of cslo-a for Ca$^{2+}$ at +40 mV was $10^{-2}$ M. This Ca$^{2+}$ sensitivity is comparable with the sensitivity observed by others when only slo-a is expressed (3, 27, 39) but is 10 to 20 times less than the Ca$^{2+}$ sensitivity observed when slo-a is co-expressed with the $\beta$-subunit (27) or when native BKCa channel currents are recorded (47). In addition, the single channel conductance of cslo-a (253 pS) was similar to that of native BKCa channels. These results indicate that cslo-a current expressed in HEK cells exhibits functional features of native BKCa channels in smooth muscle cells.

In this study, SNP activated whole cell BKCa channel currents and increased NPo of cslo-a channels in cell-attached patches without a change in single channel conductance. There are three possible mechanisms by which SNP could activate the cslo-a channel. First, NO derived from SNP may directly activate the cslo-a channel (12, 39). Second, NO may activate PKG, which then leads to direct phosphorylation of the cslo-a channel. Third, activation of PKG by NO may lead to stimulation of a phosphatase (possibly phosphoprotein phosphatase 2A), which dephosphorylates the channel (39, 48). Our results suggest that the mechanism involved in activation of cslo is dependent upon the NO donor used. In the case of SNP, activation of cslo appears to be because of a PKG-dependent mechanism without an appreciable contribution from the direct activation of channels by NO. This conclusion was reached because 1) SNP was without effect when applied to the cytosolic surface of the membrane in inside-out patches, 2) the stimulatory effect of SNP was blocked by the PKG inhibitor KT5823 in cell-attached patch recordings, and 3) SNP was without effect on mutated cslo-a channel activity. Further support for this conclusion comes from studies by other laboratories suggesting that the cGMP-PKG pathway is functional in HEK cells (49–52). In contrast to SNP, the effect of DETA/NO was not blocked by the PKG inhibitor KT 5823, suggesting that this NO-donor may have direct effects upon the cslo-a channel. This conclusion is in good agreement with studies by Zhou et al. (39) who recently reported that hsl-o-a channels expressed in Chinese hamster ovary cells are directly activated by NO derived from diethylamine/NO via S-nitrosylation. The differences between SNP and DETA/NO may be because of the differences in the redox state of NO generated by these NO-donors as suggested by others (44).
Additional experiments were undertaken to distinguish between direct PKG-mediated phosphorylation of the channel versus more indirect effects of PKG. Exposure of the cytosolic surface of inside-out patches to PKG-Iα in the presence of ATP plus cGMP increased NPo of cslo-a channels as previously reported for native BK channels (10, 23). This suggests that the action of PKG involves a phosphorylation event. In a previous study by our laboratory using cslo-a (27) and a study using hsla-a channels (24) expressed in oocytes, PKG did not activate slo channels, although, interestingly, PKG-induced regulation was observed when the hsla-channels were reconstituted in a lipid bilayer (24).

In our studies, neither ATP alone nor ATP plus cGMP increased the activity of channels, suggesting that significant quantities of PKG are not bound to the cytoplasmic surface of the isolated patch. This result differs from a study by Fujino et al. (14), who reported that cGMP plus ATP increased BKca channel activity in isolated patches of porcine coronary artery myocytes. However, it is in agreement with studies of vascular (7, 10) and tracheal (36) smooth muscles in which cGMP plus ATP were without affect in isolated patches. These disparate results suggest that mammalian expression systems and different smooth muscle preparations may contain differing amounts of bound PKG.

PKG has been reported to phosphorylate many proteins that regulate smooth muscle tone (21, 22), and it is possible that PKG could regulate BKCa channel activity indirectly by phosphorylating a protein that then regulates channel activity. A particularly intriguing target in this regard are phosphatases that could regulate channel activity through dephosphorylation (39, 48, 53). To investigate whether PKG directly acts on the channel or, alternatively, requires some intermediary protein, we mutated the single optimal consensus sequence for PKG phosphorylation in the carboxyl-terminal region of the cslo-a channel (i.e. KKSS at 1069–1072). Mutation of Ser-1072 abolished PKG-induced modulation of channel activity but did not change the electrophysiological characteristics of the channel. The mutated cslo-a channels exhibited all of the features.
described for wild-type channels, i.e. they were activated by membrane depolarization and by elevation of Ca\textsuperscript{2+} on the cytosolic side of the membrane and had the same single channel conductance as the wild-type cslo-a channel. Thus, the lack of effect of PKG on mutated channels could not be attributed to general channel dysfunction. These mutation experiments suggest that PKG enhances channel activity through direct phosphorylation of the channel rather than requiring the actions of a phosphatase. In studies of reconstituted hso-a channels, it was also concluded that activation of channels by PKG involved phosphorylation rather than dephosphorylation (24). Furthermore, the cloned human sloc channel hso-a has the same optimal consensus phosphorylation site as cslo-a, and this channel has been reported to be directly phosphorylated by PKG-I\textalpha (24).

In summary, we have found that the cslo-a channel activity recorded in whole cell and single channel configuration is increased by the NO donor SNP, presumably through activation of PKG. Direct application of PKG-I\textalpha also activated cslo-a channels but only in the presence of ATP and cGMP. A point mutation at the only optimal consensus phosphorylation site for PKG on cslo-a abolished the stimulatory effects of PKG. From these results we conclude that PKG activates cslo-a channel by direct phosphorylation at serine 1072.

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