Various brain K+ channels, which may normally exist as complexes of α (pore-forming) and β (auxiliary) subunits, were subjected to regulation by metabotropic glutamate receptors. Kv1.1/Kvβ1.1 is a voltage-dependent K+ channel composed of α and β proteins that are widely expressed in the brain. Expression of this channel in Xenopus oocytes resulted in a current that had fast inactivating and noninactivating components. Previously we showed that basal and protein kinase A-induced phosphorylation of the α subunit at Ser-446 decreases the fraction of the noninactivating component. In this study we investigated the effect of protein kinase C (PKC) on the channel. We showed that a PKC-activating phorbol ester (phorbol 12-myristate 13-acetate (PMA)) increased the noninactivating fraction via activation of a PKC subtype that was inhibited by staurosporine and bisindolylmaleimide but not by calphostin C. However, it was not a PKC-induced phosphorylation but rather a dephosphorylation that mediated the effect. PMA reduced the basal phosphorylation of Ser-446 significantly in plasma membrane channels and failed to affect the inactivation of channels having an α subunit that was mutated at Ser-446. Also, the activation of coexpressed mGluR1a known to activate phospholipase C mimicked the effect of PMA on the inactivation via induction of dephosphorylation at Ser-446. Thus, this study identified a potential neuronal pathway initiated by activation of metabotropic glutamate receptor 1a coupled to a signaling cascade that possibly utilized PKC to induce dephosphorylation and thereby to decrease the extent of inactivation of a K+ channel.

It is now well accepted that modulation of activity of voltage-gated K+ channel by protein kinases and phosphatases can regulate neuronal excitability (for recent reviews see Refs. 1 and 2). Previously, we showed that basal activity of an unidentified protein kinase endogenous to Xenopus oocytes as well as activation of protein kinase A (PKA) and PKC modulate the delayed rectifier-type of current through a Kv1.1 (α subunit) homomultimeric channel (3–5). We could correlate the modulation of the channel activity by the unidentified kinase and by PKA with phosphorylation of Ser-446 on the cytoplasmic C terminus of the α subunit. In the case of PKC, however, mutations of the numerous putative phosphorylation sites on the α subunit did not eliminate the modulation by PKC (5).

Mammalian Shaker family homologues such as Kv1.1 may normally exist as heteromultimers of α with β subunits that supply the pore-occluding domain that confers fast inactivation upon coexpression of the two subunits in heterologous systems (6, 7). Recently we showed (8) that the extent of fast inactivation of the heteromultimeric Kv1.1/Kvβ1.1 (αβ) channel expressed in Xenopus oocytes is regulated by the basal and PKA-induced phosphorylations of the α subunits that affect the interaction of the channel with microfilaments. Part of the interaction is probably mediated by a native post-synaptic density-95-like protein of the oocyte that recognizes the C-terminal end of the α subunit (9).

In this work we studied the effect of PKC on the extent of fast inactivation of the Kv1.1/Kvβ1.1 channel. We showed that the PKC effect is opposite that of PKA, and it is mediated by dephosphorylation of Ser-446 that is phosphorylated by PKA.

Glutamate is a major excitatory neurotransmitter in the brain. mGluRs participate in synaptic plasticity, both in long term potentiation and long term depression, as well as in neurotoxicity and neuroprotection (for reviews see Refs. 11–16). mGluRs were shown to inhibit several types of K+ currents like the M-type current, the Ca2+-activated current (I_{Ca,ATP}), a voltage-dependent K+ current (I_{K,slow}), and resting K+ currents. mGluRs were shown to activate K+ currents in cerebellar granule cells (17). They belong to the superfAMILY of G protein-coupled seven transmembrane receptors (14, 18) and comprise eight members encoded by distinct genes, mGluR1–mGluR8. Group I of mGluRs comprises mGluR1 and mGluR5 (the longer splice variant is mGluR1a) and mGluR5 that activate phospholipase C (probably via coupling to Gs class of G proteins) and therefore activate a large endogenous Ca2+-activated chloride current (19, 20) and a PKC subtype, possibly PKC-μ (21), when expressed in oocytes. In this study we identify a potential physiological pathway initiated by activation of mGluR1a coupled to a signaling cascade that possibly utilizes PKC to modulate the extent of inactivation of Kv1.1/Kvβ1.1 channel.

**EXPERIMENTAL PROCEDURES**

**Materials**—Chemicals were from Sigma unless stated otherwise. Vanadate (sodium orthovanadate) and okadaic acid were from Alomone Labs (Jerusalem); bisindolylmaleimide (BIS) and calyculin A were from Calbiochem; cyclosporin A was a gift from Sandoz Pharmaceutical, Basel, Switzerland. [35S]Methionine/cysteine mix and [γ-32P]ATP were from Amersham Corp. Kv1.1 antisera was generated against a 23-amino acid peptide that corresponds to the N terminus of Kv1.1 (SGENADEASAPGHQPQDSYPRQ), as described (3). Kv1.1, S46A and
PMA Decreases the Extent of Inactivation of the Kv1.1/Kv1.1 Channel—Previous (5) we showed that oocytes injected with Kv1.1 (α) RNA and assayed by the two-electrode voltage clamp technique express a delayed rectifier type of K+ current (α current) that is modulated by PMA. Incubation with 10 μM PMA causes gradual reduction of the α current amplitude (Ref. 5; c.g., Fig. 1D) that is mediated by activation of the oocyte’s PKC. When Kv1.1 is coexpressed with Kvβ1.1 (β) a heteromultimeric αβ channel is formed that expresses a rapidly inactivating current that has a fast inactivating component (Ii) and a non-activating sustained current component (Is). In the absence of PMA, 10 μM PMA reduces the peak αβ current amplitude; the time course and the extent of the reduction (reaching about 50% of control after 20 min) were similar to those of α current expressed in oocytes taken from the same frogs. PMA did not affect any endogenous currents in oocytes un.injected with RNA (Fig. 1A, inset). In addition, PMA caused a gradual decrease in the extent of inactivation (Fig. 1B), which is depicted in Fig. 1E as an increase in the Is fraction determined in currents where Ii was larger than 400 nA (after leak subtraction, see “Experimental Procedures”) to preclude any interference from endogenous currents activated by PMA. The initial Is fraction was 0.41 ± 0.02 before the addition of PMA and increased to more than 150% of its original value after 20 min. The time constant of inactivation changed slightly from 6.8 ± 0.5 ms before PMA to 8.9 ± 0.4 ms 20 min after the start of PMA (seven oocytes assayed; p > 0.05). No change in Ii, or the extent of inactivation could be detected in the absence of PMA (Fig. 1, C and E, respectively) or in the presence of 10 μM α-FMA, an isomer that does not activate PKC (not shown). As expected, separate analysis of the effect of PMA on each of the two apparent current components (Fig. 1D) shows that the increase in Is fraction is due to the fact that within 20 min the reduction of Ii (-40% of its initial value) was larger than the reduction of Is (-65% of its initial value). Actually, the reduction of Ii was preceded by an early increase (-130% of its initial value) that peaked at -10 min when Ii was still constant and Is had already started to decrease. Taken together, one may deduce that two overlapping effects are initiated by PMA as follows: 1) as already described for the α current (5) which is a PKC-mediated reduction of the total current amplitude consist-
FIG. 1. Modulation of αβ current by PMA. A, two-electrode voltage clamp recordings in one oocyte injected with α and β RNA that was exposed to continuous bath application of 10 nm PMA. αβ currents were elicited by a 160-ms voltage step from −80 mV to +50 mV, before (control) and 13 and 25 min after the start of PMA application. Inset shows currents elicited by the same voltage protocol as in A in oocytes uninjected with RNA in the absence and presence of 10 nm PMA (two overlapping current traces). I\_1, I\_p, and I\_s illustrate definitions in the text for the inactivating, noninactivating, and total currents, respectively. B, the same currents superimposed (each normalized to its peak). C, time course of the effect of PMA on the peak amplitude (I\_p) of αβ (filled squares) and αβ (filled circles) currents; shown are average normalized values from 9 and 14 oocytes, respectively, taken from the same 6 frogs. Open squares are of αβ currents in the absence of PMA (4 oocytes taken from the same frogs). Inset shows activation curves of I\_p of αβ current before (control; circles) and 20 min after (+PMA; squares) the start of PMA application (at t = 0). The currents were elicited by 160-ms voltage pulses to the indicated voltages, in 10-mV increments, every 20 s (to allow for recovery from inactivation); the elicited currents were normalized to maximal current at +60 mV. D, time course of the effect of PMA on the sustained (I\_s; circles) and inactivating (I\_i; squares) current components in the same oocytes of C, E, time course of the changes in I\_i fraction (I\_i/I\_p) of αβ currents in the presence (circles) and in the absence (squares) of PMA (same oocytes as in C). F, the effect of PMA on I\_i/I\_p of αβ currents in the presence of protein kinase inhibitors (see "Experimental Procedures"). The effect of each inhibitor was evaluated by comparing the increase in I\_i/I\_p after 20 min application of PMA in the presence (shaded bars) and in the absence (white bars) of the inhibitor (see "Experimental Procedures") in oocytes taken from the same frogs. ss, staurosporine; bis, bisindolylmaleimide; cc, calphostin C. Numbers above bars denote number of assayed oocytes taken from 2 frogs. *, p < 0.05; **, p < 0.003: significantly different from values before the application of PMA (C, D and E), or from control (F).

In addition, BIS was also injected into the oocytes before PMA application. PMA effects with and without the blockers were compared in oocytes from the same frogs because of variable effects by PMA among oocytes from different frogs (Fig. 1F). Staurosporine and BIS significantly suppressed the decrease in inactivation; however, neither a 4-h incubation with 5 μM calphostin C nor injection of this blocker into the oocytes suppressed the PMA effect on the extent of inactivation.

PMA Brings about Dephosphorylation of the α Protein—Previously (3, 4), we showed that the α protein is extensively phosphorylated in its basal state in the oocyte and that this phosphorylation is manifested as a shift in its migration in SDS-PAGE; the nonphosphorylated form migrates as a 54-kDa protein, whereas the phosphorylated form migrates as a 57-kDa protein. This phosphorylation was totally abolished when Ser-446 was replaced by alanine (S446A mutant). In this study we looked for PKC-induced phosphorylation of the channel proteins first in homogenates of oocytes and then under in vivo conditions. Fig. 2 shows an experiment in which the oocyte's
PKC was stimulated in homogenates of oocytes expressing α or αβ channels by stimulating with added 100 nm PMA and 1.5 mM CaCl₂, in the presence of added γ-ATP, as described by Beguin et al. (23) for the Na,K-ATPase. To our surprise, PKC activation brought about dephosphorylation, rather than phosphorylation, of the α protein both in α and αβ channels. Dephosphorylation of the basally phosphorylated α protein at Ser-446 was evident from SDS-PAGE analyses of 32P-labeled or [35S]Met/Cys-labeled channel proteins that were coimmunoprecipitated by an antibody from homogenates of oocytes injected (3 days before) with either α alone or α and β mRNAs together, and from homogenates of uninjected oocytes (c). Homogenates (prepared in the presence of protease inhibitors) of either [35S]Met/Cys-labeled (left panel) or unlabeled (right panel) oocytes were incubated for 40 min with either 3 mM ATP or 50 μM [γ-32P]ATP, respectively, in the presence (+) or absence (−) of CaCl₂ and PMA (as indicated above the lanes), followed by addition of phosphatase inhibitors. Thick arrows point to α and β proteins; thin arrows point to the migration of molecular mass markers in kDa. Bottom row of left panel denotes for each of the lanes the ratio of labeling intensities of the 57-kDa over the 54-kDa bands that represents the ratio of the phosphorylated over the nonphosphorylated forms of the α protein (the extent of phosphorylation; see text).

We then asked whether PMA induces dephosphorylation in vivo by testing the effect of a 25-min incubation of intact oocytes with PMA. Fig. 3 shows an SDS-PAGE analysis of α and β proteins immunopurified separately from plasma membranes and from internal fractions of oocytes labeled with [35S]Met/Cys (see “Experimental Procedures”). In oocytes treated with PMA (10 or 100 nm; no significant difference between the effects of the two concentrations was detected) the relative intensity of the 57-kDa band decreased, as compared with untreated oocytes, indicating that PMA induced dephosphorylation of the α protein. Dephosphorylation occurred both in the plasma membranes and in the internal fractions. In three similar experiments (including that shown in Fig. 3) the PMA-induced dephosphorylation was quantified as the reduction in the extent of phosphorylation. Thus, in the plasma membranes of oocytes treated with PMA the extent of phosphorylation (ratio of labeling intensities of 57- over 54-kDa bands) was 0.74 ± 0.05 of those of untreated oocytes, and in the internal fractions it was 0.41 ± 0.005. The larger phosphorylation in the plasma membranes versus the internal fractions probably relates to the phenomenon described by us previously that α protein phosphorylated at Ser-446 tends to accumulate into plasma membrane (4). However, in five of nine additional experiments, in which channel proteins were immunopurified from whole oocytes, PMA decreased the extent of phosphorylation to 0.68 ± 0.07 of control. In one experiment we checked if the dephosphorylation could occur in less than 5 min incubation with PMA and found that already within 5 min significant dephosphorylation occurred (not shown). It is noteworthy that
PMA did not alter the β-binding capacity of α, as verified in all the experiments by a quite constant ratio of intensities between the α and the β proteins coprecipitated with the α antibody (Fig. 3).

Dephosphorylation of Ser-446 of the α Protein Underlies the Modulation of Extent of Inactivation by PMA—Next we tested the possibility that dephosphorylation of Ser-446 underlies the decrease in the extent of inactivation by PMA. We performed two experiments in which the modulation by PMA of wild-type (αWTβ) channels was compared with that of the mutant (αS446Aβ) channels where Ser-446 in α was replaced by alanine and thereby rendered the channel totally non-phosphorylated in its basal state (4). Although the peak amplitude of αS446Aβ was decreased by PMA to the same extent as that of αWTβ (Fig. 4A), the extent of inactivation of the mutant was unaffected compared with WT where the extent of inactivation decreased (as shown in the increased Ic fraction; Fig. 4C). The inactivation of two other phosphorylation-irrelevant serine mutants S489I and S322A (first in the C terminus and second in the loop between S4 and S5 transmembrane segments, respectively; Ref. 5) was not reduced by PMA (Fig. 4D). It is noteworthy that the Ic current component of αS446Aβ did not exhibit biphase modulation by PMA; rather, PMA caused an apparent decrease of Ic that was larger than that of αWTβ (Fig. 4B). This substantiates the notion that a PMA-induced increase of Ic that overlaps a PMA-induced reduction of total current (including Ii and Ic) underlies the decrease in the extent of inactivation of the WT channel caused by PMA.

These experiments indicated that a dephosphorylating activity underlies the modulation by PMA, so we tested the possible involvement of phosphatases. Previously, we showed in in vitro studies that 10 nm okadaic acid (a selective inhibitor of protein phosphatase-1 (PP-1)) inhibits spontaneous dephosphorylation of the α protein in the oocyte homogenate and that protein phosphatase-2B (PP-2B) can dephosphorylate its immunopurified form (4). Here we tested the effect of PMA in the presence of several phosphatase inhibitors according to experimental protocols used successfully by others in different cells (10, 38). However, a 2–4-h preincubation with either 2 μM okadaic acid and protein phosphatase-2A (PP-2A; Refs. 25 and 26), or 0.3 μM calyculin A (a selective inhibitor of PP-1 and PP-2A; Refs. 27 and 28), or 250 μM cyclosporin A (a PP-2B inhibitor; Ref. 29) did not inhibit the effect of PMA on the currents; each inhibitor was tested in oocytes of at least three different frogs.

mGluR1α Mimics the Effect by PMA—Since mGluR1α activates phospholipase C and lately was shown to activate PKC in oocytes (21), we tested whether activation of mGluR1α, coexpressed with the channel in oocytes, will mimic the modulation by PMA. Coinjection of α, β, and mGluR1α RNAs into oocytes gave rise to an αβ current with an average amplitude of 53 ± 0.04% of the current elicited in oocytes that were injected with the same RNA amounts of α and β without mGluR1α (51 and 61 oocytes tested, respectively; 4 frogs; p < 0.001). The reduced amplitude could be due to lower expression of the αβ proteins in oocytes injected with mGluR1α; however, no evidence for this was obtained in concomitant biochemical analyses of the level of expression of the proteins in six experiments (see below). The other alternative would be that the reduced amplitudes were intrinsic to the receptor modulation of the current (see below) due to enough receptor molecules being spontaneously active without agonist, thereby continually activating at a significant level a signaling cascade that utilizes PKC (cf. Ref. 30). This notion is supported by the observation that in two experiments the currents elicited in oocytes injected with mGluR1α had significantly larger (∼140%) Ic fractions as compared with those in oocytes not injected with the receptor.

Exposure of these oocytes to 100 μM glutamate for 1 min caused a Ca2+-dependent Cl− current as described previously for oocytes injected with mGluR1α alone (20, 39). To observe the effect of glutamate on the αβ channel without the interfer-

**FIG. 4.** Mutation of Ser-446 abolishes the effect of PMA on the extent of inactivation. A–C, time course of the effect of continuous bath application of 10 nM PMA on Ip (A), Is, and Ii (B), and Ip/Ip (C) of αWTβ (open squares) and αS446Aβ (filled circles) currents elicited in 8 and 10 oocytes, respectively, taken from two frogs (as described in legend to Fig. 1, D–F). Shown are values normalized to that before the start of PMA application (at t = 0 min). D, the maximal effect of PMA on currents through channels mutated at the α subunit, normalized to the effect of PMA on WT currents elicited in oocytes (number denoted above bars) taken from the same frogs (two for S446A, two for S489I, and three for S322A). *, p < 0.05; **, p < 0.007, significantly different from values before the application of PMA (A–C) or from WT (D); *, p < 0.03, a significant difference between S446A and WT.
Fig. 5. Activation of mGluR1α mimics the effect of PMA on inactivation; mutation of Ser-446 abolishes the effect. A–C, time course of the effect of application of glutamate for 1 min (at t = 0 min) on I_p (A), I_s (B), and I_s/I_p (C) of αWTβ (filled circles) and αS446Aβ (open squares) currents elicited (as described in legend to Fig. 1) in 14 and 8 oocytes taken from 5 and 2 frogs, respectively, that were injected with mGluR1α and a and β mRNAs. *, p < 0.002, significantly different from values before the application of glutamate (A–C); p < 0.03, a significant difference between WT and S446A (C).

Discussion

This study shows that in Xenopus oocytes activation of PKC by PMA decreases the extent of inactivation (increases the I_s fraction) of rat brain Kv1.1/Kvβ1.1 (αβ) current. It further suggests that the metabotropic glutamate receptor mGluR1α that is coupled to phospholipase C is a plausible candidate to initiate such a cellular process and to modulate the inactivation of the αβ channel in a physiologically relevant environment. Several interesting aspects to this modulation are as follows: (i) it does not involve PKC-mediated phosphorylation but rather a PKC-mediated dephosphorylation of the channel; (ii) it is opposite to the modulation by PKA that increases the extent of inactivation of the channel (8); (iii) PKA and PKC signaling pathways converge onto the same site bringing about phosphorylation and dephosphorylation of Ser-446, respectively.

PKC-induced Dephosphorylation of Ser-446 Decreases the Extent of Inactivation of the αβ Channel—An intriguing mode of modulation of an ion channel is described here that involves induction of a dephosphorylating activity by activation of PKC. Involvement of PKC is evident from the inhibition of the PMA effect on inactivation by two potent blockers, staurosporine (a wide specificity kinase blocker) and BIS (a specific PKC blocker). The fact that calphostin C (a specific PKC blocker) did not inhibit the effect may be interpreted as an indication of the PKC subtype involved, as detailed in the following. PKC isoenzymes have been classified into three groups with different
structure and cofactor regulation (31, 32). Activation by PMA makes the involvement of “atypical” PKC isoenzymes unlikely. Ca\(^{2+}\) chelation that did not impair the effect by mGluR1a leaves out the possibility of “conventional” PKCs. Of the “new” PKCs that are left as candidates, PKC-\(\mu\) is the most plausible isoenzyme to mediate the decrease in inactivation as it is insensitive to calphostin C (33).

Correlation between the decrease in the extent of inactivation and dephosphorylation of Ser-446 of the \(\alpha\beta\) subunit was suggested by SDS-PAGE analysis of the \(\alpha\beta\) proteins after PMA treatment. First, it eliminated the possibility that decreased \(\beta\)-binding capacity of \(\alpha\) was the cause of the decreased inactivation by PMA. Second, it demonstrated that PMA did not phosphorylate but rather dephosphorylated the channel by decreasing the basal phosphorylation of Ser-446. This analysis was verified specifically for channel proteins residing in the plasma membrane. Final verification of a causal relationship between dephosphorylation and decreased inactivation was provided by biophysical analysis that showed that PMA reverts the phosphatases PP-1, PP-2A, or PP-2B, as the corresponding antibody from homogenate of the mouse brain. It is evident from this study that activation of mGluR1a by glutamate mimics the effect of PMA on inactivation, the onset being faster (4 min to reach 40% of response as compared with 10 min for PMA) and the response saturating. The fast onset of the response could be due to colocalization of the receptor, the channel, and the oocyte's signaling molecules involved in the response in submembranous sites targeted by protein(s) which serve as a scaffold (for review see Ref. 44). Notably, a post-synaptic density-95-like endogenous protein was shown by us to interact with the channel and to affect its extent of inactivation (9). The effect of glutamate on the current amplitudes was small compared with that of PMA, possibly because it was somewhat occluded by the effect exerted by spontaneous coupling of the channel without the signaling machinery, since coexpression of the receptor with the channel reduced the current amplitudes significantly even in the absence of agonist.

Several types of \(K^+\) channels have been shown to be regulated variably by activation of group I of mGluRs in neurons of different brain areas (reviewed in Ref. 15). However, the exact mechanisms involved in the regulations have not been clearly established. A PKC-mediated regulation was inferred only in the inhibition by mGluR1 of \(IK_{\text{ATP}}\) in dentate granule neurons (45) but was shown not to be involved in the same effect in CA3 pyramidal neurons (46). In every expression system examined group I receptors stimulate phosphorylase C as revealed by an increase in phosphoinositide turnover and Ca\(^{2+}\) release from internal stores (15). In Xenopus oocytes PKC (probably PKC-\(\mu\)) was explicitly shown to mediate inhibition by mGluR1a of an inwardly rectifying \(K^+\) channel (21). In this study the signaling pathway underlying the effect of mGluR1a on the extent of inactivation seems to be mediated by PKC (possibly PKC-\(\mu\)) and involves dephosphorylation of Ser-446. It differs from the signaling pathway underlying the effect on amplitudes which is not eliminated in the \(\alpha_{\text{S446A}\beta}\) channel and thus does not involve dephosphorylation.

**Modulation of the Extent of Inactivation by Dephosphorylation, Possible Mechanisms**—It is evident from this study that stimulation of PKC causes a decrease in the extent of inactivation of the \(\alpha\beta\) current by dephosphorylating Ser-446. This effect is opposite that shown by us for a constitutively active kinase (yet unidentified) or for a stimulated PKA that causes an increase in the extent of inactivation by phosphorylating Ser-446 that impairs interaction between the channel and the microfilaments (8). Part of the interaction with the microfilaments was shown to be mediated via a post-synaptic density-95-like protein that interacts with the C-terminal end of Kv1.1 (9). A similar phenomenon was described in mammalian cells.
for the K+ channel Kir 2.3 that dissociates from post-synaptic density-95 upon PKA phosphorylation of a serine residue at its C terminus (47). We proposed and have now confirmed a kinetic model that assumes two modes of gating of the αβ channel, inactivating and a noninactivating. In the noninactivating mode the channel's interaction with microfilaments results in impaired inactivation and gives rise to the sustained current component (Is), whereas in the inactivating mode the channel does not interact with the microfilaments and gives rise to the inactivating current component (Ii); the equilibrium between the modes is influenced by the extent of phosphorylation of Ser-446. In this context it is expected that the PKC-induced dephosphorylation shifts the equilibrium toward the noninactivating mode which is manifested in the increase in the sustained fraction of the current.

Another mechanism that can possibly underlie the increase in the sustained fraction by PKC-induced dephosphorylation is suggested by the biphasic response to PMA that exhibited an initial increase followed by a decrease in the sustained current amplitude. It was converted in the mutant channel that is not phosphorylated (α9446β) to a monophasic exhibiting only reduction of amplitude (Ia)

In conclusion, activation of PKC induces dephosphorylation of Ser-446 upon PKA phosphorylation of a serine residue at its C terminus (47). We proposed and have now confirmed a kinetic model that assumes two modes of gating of the αβ channel, inactivating and a noninactivating. In the noninactivating mode the channel's interaction with microfilaments results in impaired inactivation and gives rise to the sustained current component (Is), whereas in the inactivating mode the channel does not interact with the microfilaments and gives rise to the inactivating current component (Ii); the equilibrium between the modes is influenced by the extent of phosphorylation of Ser-446. In this context it is expected that the PKC-induced dephosphorylation shifts the equilibrium toward the noninactivating mode which is manifested in the increase in the sustained fraction of the current.

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