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ERK Integrates PKA and PKC Signaling in Superficial Dorsal Horn Neurons. I. Modulation of A-Type K^+ Currents

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Hu, Hui-Juan, Kathi S. Glauner, and Robert W. Gereau IV. ERK integrates PKA and PKC signaling in superficial dorsal horn neurons. I. Modulation of A-type K^+ currents. *J Neurophysiol* 90: 1671–1679, 2003. First published May 15, 2003; 10.1152/jn.00340.2003. The transient outward potassium currents (also known as A-type currents or I_A) are important determinants of neuronal excitability. In the brain, I_A is modulated by protein kinase C (PKC), protein kinase A (PKA), and extracellular signal-related kinase (ERK), three kinases that have been shown to be critical modulators of nociception. We wanted to determine the effects of these kinases on I_A in superficial dorsal horn neurons. Using whole cell recordings from cultured mouse spinal cord superficial dorsal horn neurons, we found that PKC and PKA both inhibit I_A in these cells, and that PKC has a tonic inhibitory action on I_A. Further, we provide evidence supporting the hypothesis that PKC and PKA do not modulate I_A directly, but rather act as upstream activators of ERKs, which modulate I_A. These results suggest that ERKs serve as signal integrators in modulation of I_A in dorsal horn neurons and that modulation of A-type potassium currents may underlie aspects of central sensitization mediated by PKC, PKA, and ERKs.

INTRODUCTION

Many chronic pain conditions are an expression of neuronal plasticity. Strong input from the periphery to the spinal cord leads to central sensitization, which is associated with hyperexcitability of dorsal horn neurons that likely contributes to increased pain sensitivity (Eide 1998). A large number of studies show that several protein kinases are critical in the modulation of nociception. In particular, strong evidence implicates protein kinase C (PKC), protein kinase A (PKA), and the extracellular signal-regulated kinases (ERKs) in the establishment and/or maintenance of central sensitization (Ji and Woolf 2001; Karim et al. 2001). Although several studies have addressed the modulation of pain behaviors by these kinases, little is known about the phosphorylation targets that underlie nociceptive central sensitization (Ji and Woolf 2001).

Increases in neuronal excitability, such as those associated with central sensitization, frequently result from phosphorylation-dependent modulation of ion channels. Voltage-gated potassium (K^+) channels are critical determinants of neuronal excitability in the central nervous system (CNS). Thus changes in K^+ channel function are likely to contribute to the increased excitability in central sensitization, yet to date the role of these channels in dorsal horn neurons has not been studied in detail.

Transient potassium currents (A-type currents, I_A) have been described in neurons from many regions of the CNS (Hoffman and Johnston 1998; Song et al. 1998; Wolff et al. 1998). These currents are often defined by their sensitivity to millimolar concentrations of 4-aminopyridine (4-AP), and their rapidly activating and fast inactivating characteristics. A-type channels are prominently expressed in dorsal horn neurons (Grudt and Perl 2002; Wolff et al. 1998), where their activation may reduce the slope of the rising phase of synaptic potentials (Yoshimura and Jessell 1989b). Finally, A-type potassium channels in other regions of the CNS are modulated by PKC, PKA, and ERKs (Adams et al. 2000; Anderson et al. 2000; Hoffman and Johnston 1998; Watanabe et al. 2002; Yang et al. 2001; Yuan et al. 2002). However, little is known about the modulation of potassium channel function in dorsal horn neurons by these kinases. In the present study, we tested the effects of PKC, PKA, and ERKs on A-type K^+ currents in mouse superficial dorsal horn neurons to determine whether modulation of A currents by these kinases could be involved in central sensitization.

METHODS

Cell culture

Primary cultures of spinal cord dorsal horn neurons were prepared from 5- to 10-day-old ICR mice using a procedure modified from a previously described method (Hugel and Schlichter 2000). Briefly, after decapitation a laminectomy was performed and the spinal cord was carefully removed. Spinal cord superficial dorsal horn was dissected with a surgical blade cut in approximately lamina III. This results in cultures composed primarily of neurons from laminae I–II, although the presence of lamina III neurons cannot be entirely ruled out. The superficial dorsal horn strips were then cut into 2- to 3-mm fragments, which were incubated for 45 min at 37°C in Hanks' balanced salt solution (HBSS; Invitrogen Life Technologies, Carlsbad, CA) (in mM: 137 NaCl, 5.4 KCl, 0.4 KH_2PO_4, 1 $CaCl_2$, 0.5 $MgCl_2$, 0.4 $MgSO_4$, 4.2 $NaHCO_3$, 0.3 Na_2HPO_4, 5.6 glucose) containing papain (15 U/ml; Worthington Biochemical, Lakewood, NJ), rinsed 3 times with HBSS, and placed in culture medium containing Neurobasal (Invitrogen Life Technologies), fetal calf serum (5%, Invitrogen Life Technologies), heat-inactivated horse serum (5%, Invitrogen Life Technologies), penicillin (100 U/ml, Invitrogen Life Technologies) and streptomycin (100 μg/ml), L-glutamax-1 (2 mM, Invitrogen Life Technologies), B-27 (1%, Invitrogen Life Technologies), and glucose (20 mM, Sigma-Aldrich, St. Louis, MO). The

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fragments were mechanically dissociated by gently triturating with a fire-polished Pasteur pipette. The resulting cell suspension was plated onto 12-mm poly-D-lysine– and collagen-coated coverslips, and cultured for 1 to 2 days in humidified air with 5% CO_2 at 37°C.

Electrophysiological recording

Whole cell recordings were made by standard procedures at room temperature with an AXOPATCH 200B amplifier and CLAMPEX 8.0 software (Axon Instruments, Union City, CA). Electrodes were pulled from filamented borosilicate glass and fire-polished. Pipette resistances were 3–6 MΩ. Most neurons had series resistances around 6–10 MΩ (range, 5–18 MΩ), which were compensated ≥65%. Input resistance was 1.00 ± 0.05 GΩ (n = 45). Most neurons had leak currents <100 pA (at −80 mV), which were not subtracted on-line. The bath solution (HBSS) contained 500 nM TTX and 2.5 mM $CoCl_2$ to block voltage-gated Na^+ currents, Ca^{2+} currents, and Ca^{2+}-activated K^+ currents. The electrode solution contained (in mM): 140 KCl, 1 $MgCl_2$, 0.5 $CaCl_2$, 5 EGTA, 10 HEPES, 3 Na_2ATP, 0.3 Na_2GTP, pH adjusted to 7.4 with KOH. The membrane voltage was held at −80 mV and transient potassium currents (I_A) were isolated by a two-step voltage protocol. To determine the voltage dependency of activation, voltage steps of 500 ms were applied at 5-s intervals in +10-mV increments to a maximum of +70 mV. To determine the voltage dependency of inactivation, conditioning prepulses ranging from −100 to +30 mV were applied at 5-s intervals in +10-mV increments for 150 ms followed by a voltage step to +40 mV for 500 ms.

Drug application

Phorbol 12-myristate 13-acetate (PMA) and PD98059 were purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in DMSO as stock solutions. 4α-PMA, forskolin (FSK), IBMX, PKI(6–22) amide, KT5720, U-0126, GF 109203X, and Ro31-8220 were obtained from Biomol (Plymouth Meeting, PA); 1,9-dideoxyforskolin from CN Biosciences (San Diego, CA); U0124 from Calbiochem (La Jolla, CA), all of which were prepared as concentrated stock solutions in DMSO. All of these were diluted to final concentrations in HBSS. Coverslips were placed in a small laminar flow perfusion chamber and continuously perfused at approximately 2–3 ml/min.

Data analysis

Off-line evaluation was done using clampfit 8.0 software (Axon Instruments) and Origin (Microcal Software, Northampton, MA). Data are expressed as original traces or as means ± SEM. The voltage dependency of activation and inactivation of I_A was fitted with the Boltzmann function. For activation, peak currents were converted to conductances (G) by the formula $G = I/(V_m - V_{rev})$, where V_m is the membrane voltage of depolarization pulses and V_{rev} is the calculated potassium reversal potential (−84 mV). The function $G/G_{max} = 1/(1 + \exp[(V_{1/2} - V)/k])$ was used, where G_{max} is the maximal conductance obtained with a depolarizing pulse to +70 mV, $V_{1/2}$ is the half-maximal voltage, and k is the slope factor. For inactivation, $I/I_{max} = 1/(1 + \exp[(V_{1/2} - V)/k])$ was used, where I_{max} is the maximal current obtained with a −100 mV prepulse. Treatment effects were statistically analyzed by one-way ANOVA followed by Bonferroni's multiple comparison test. Paired or two-sample t-test was used when comparisons were restricted to two means. Error probabilities of $P < 0.05$ were considered statistically significant.

RESULTS

To begin dissecting the roles of protein kinases in modulation of A currents in superficial dorsal horn neurons, we had to first isolate the A current from the myriad of currents activated by a voltage step in these cells. With Na^+ and Ca^{2+} currents eliminated pharmacologically (see METHODS), a large outward current was evoked by a command potential of +40 mV from a holding potential of −80 mV in cultured mouse spinal cord dorsal horn neurons (laminae I–II). The typical current profile observed in these neurons exhibits a rapidly inactivating component along with a sustained component (Fig. 1). This large transient current was almost completely blocked by application of 5 mM 4-AP (inhibition 87.0 ± 2.0%, $P < 0.01$, n = 10). The A-type current was dissected away from the sustained

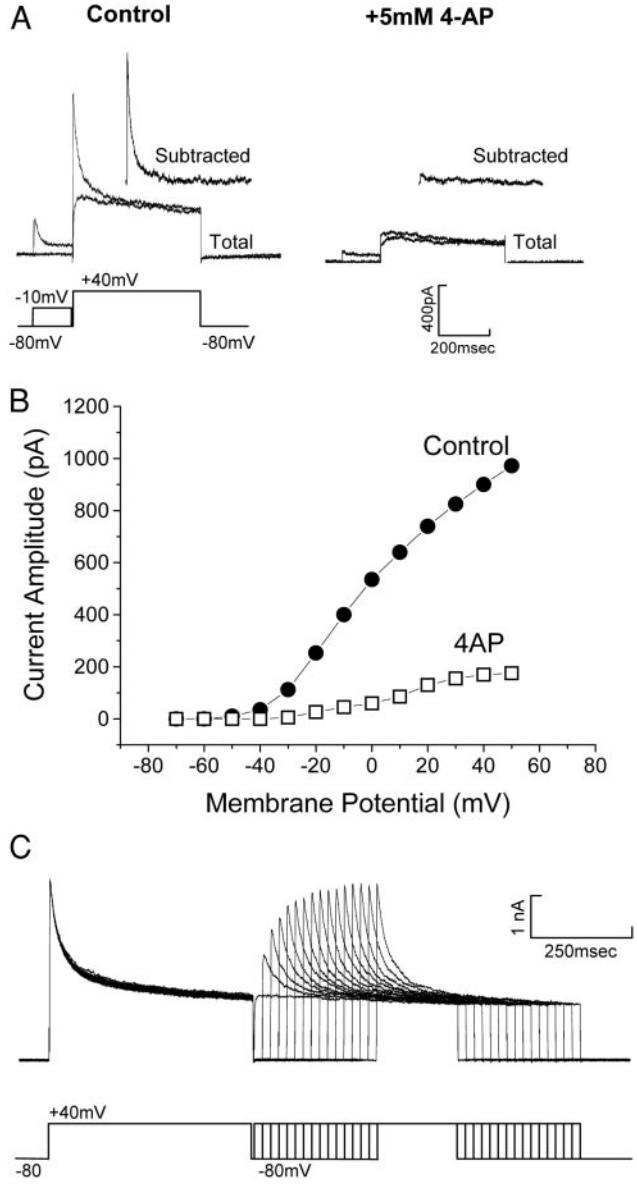

FIG. 1. Depolarization-activated K^+ currents in spinal cord dorsal horn neurons. Na^+ currents, Ca^{2+} currents, and Ca^{2+}-activated K^+ currents have been eliminated (see METHODS). *A*: representative recording from dorsal horn neuron before (*left*) and after 5 mM 4-aminopyridine (4-AP) application (*right*). *Inset*: remaining current after off-line subtraction of noninactivating portion of current remaining after brief prepulse to −10 mV. This protocol is used for isolation of A-type currents in all figures. *B*: *I–V* curve of peak amplitude of current vs. test voltage for control and 5 mM 4-AP. *C*: representative recording from a neuron showing rapid recovery from inactivation typical of A-type currents in these cells. *Inset*: protocol for recovery from inactivation, where first duration of voltage step from 40 to −80 mV is 3 ms; incremental duration, 20 ms.

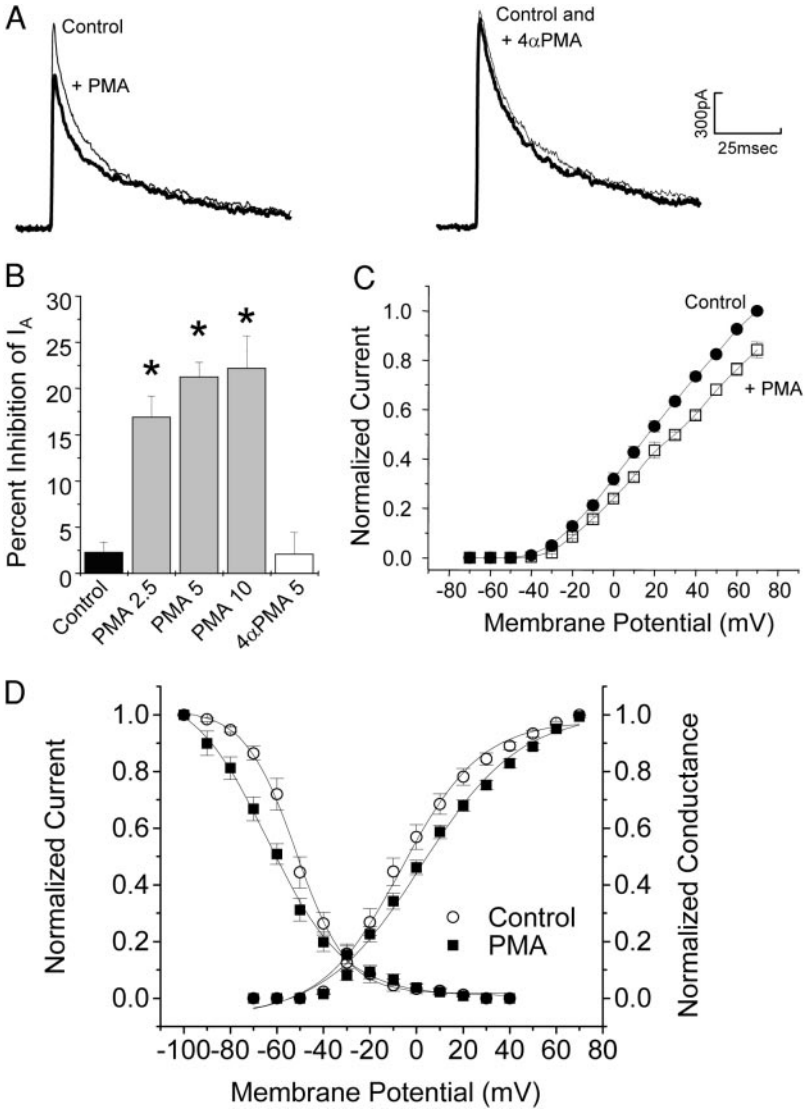

FIG. 2. Protein kinase C (PKC) activation suppresses A-type potassium current (I_A). *A*: representative examples of I_A (isolated from sustained current by voltage protocol shown in *inset* of Fig. 1) recorded before (control) and after 5-min bath-application of 5 μM PMA (PKC activator) or 4α-PMA (negative control analog). *B*: inhibition of peak amplitude of A-type current by 2.5–10 μM PMA and 4α-PMA (5 μM). Values are means ± SE; n = 5–10 neurons each. *$P < 0.05$, ANOVA compared with the control. *C*: mean current–voltage plot of peak amplitude of current vs. test voltage for control and 5 μM PMA. *D*: steady-state inactivation and activation curves before (open circles) and after (filled squares) 5-min bath-application of 5 μM PMA. Curves (here and in Figs. 3, 5, 6) are fits of data to Boltzmann functions (see METHODS).

current by the voltage protocol shown in Fig. 1. A 150-ms prepulse to −10 mV allowed the transient channels to inactivate, leaving only the sustained current. Subtraction of the sustained current from the total current isolated a transient, A-type current. The A-type currents recorded from these cells consistently demonstrated fast recovery from inactivation, as shown in Fig. 1*C*.

PKC decreases A-type potassium currents in dorsal horn neurons

As mentioned above, PKC has been implicated in central sensitization (Coderre 1992) and also shown to be a modulator of the A current in hippocampal dendrites (Hoffman and Johnston 1998; Yuan et al. 2002). To ascertain the effects of PKC activation on A currents in dorsal horn neurons, we

TABLE 1. *Effects of PMA, FSK/IBMX, GF109203x, and PD098059 on the voltage dependency of I_A activation and inactivation*

	Activation			Inactivation		
Sample	$V_{1/2}$	k	n	$V_{1/2}$	k	n
Pre-PMA	−5.8 ± 2.3	17.1 ± 0.4	12	−51.2 ± 2.4	10.5 ± 1.7	8
PMA	1.6 ± 2.3*	21.2 ± 0.8*	12	−63.3 ± 4.3*	17.3 ± 2.2*	8
Pre-GF	−4.2 ± 2.3	19.1 ± 1.3	7	−50.7 ± 1.2	10.6 ± 1.4	7
GF	−13.0 ± 2.0*	22.2 ± 2.3	7	−43.2 ± 2.9*	22.3 ± 2.3*	7
Pre-FSK	−3.8 ± 1.0	17.1 ± 0.8	6	−49.0 ± 1.8	9.3 ± 1.1	6
FSK	0.3 ± 1.3*	19.4 ± 0.3	6	−58.4 ± 2.2*	10.7 ± 1.3	6
Pre-PD	−1.9 ± 1.6	18.5 ± 1.4	12	−53.1 ± 2.3	9.3 ± 0.9	12
PD	−8.2 ± 1.3*	19.2 ± 1.2	12	−45.9 ± 1.9*	10.7 ± 1.4	12

Values are means ± SE; n = number of neurons. $V_{1/2}$, voltage of half-maximal activation or inactivation; k, slope factor.

bath-applied the PKC activator PMA (2.5–10 μM) and measured the changes in the peak amplitude of A currents. We found that 5 μM PMA was the lowest concentration necessary to elicit the maximal effect on I_A, decreasing the peak current (measured at a step of depolarization to +40 mV from −80 mV holding potential) by 22.1 ± 2.0% (Fig. 2, *A–C*). The decay rate of A-type current was best fitted with double exponential (at +40 mV). Neither the fast nor the slow time constant was changed after PMA treatment (Table 2). The control analog for PMA, 4α-PMA (5 μM), had no effect on A-type currents (Fig. 2, *A* and *B*). Steady-state inactivation and activation curves generated from these experiments show a shift of the activation curve to the right 7.3 mV and a shift of the inactivation curve to the left 12.4 mV in response to 5 μM PMA (Fig. 2*D*, Table 1). The slope factors of both the activation and the inactivation curves were significantly changed (Table 1). These data indicate that PMA modulates A-type potassium channels, at least in part, by reducing both the total current produced by the channels and the percentage of channels available for activation.

To confirm that the effect of PMA on I_A was a direct result of PKC activation, we tested the effects of the PKC inhibitor GF 109203X (GF) (Toullec et al. 1991) on A currents. Bath-application of a range of concentrations (0.25–2 μM) of GF increased I_A in a dose-dependent manner (Fig. 3, *A* and *B*). The decay kinetics were changed by higher concentrations of GF. The slow time constant was decreased from 120 ± 9 to 76 ± 4 ms after 1 μM GF treatment without changing the fast time constant (Table 2). A significant shift of the activation curve to the left 8.8 mV and of the inactivation curve to the right 7.5 mV (Fig. 3*C*, Table 1) resulted from application of 1 μM GF. The slope factor of the inactivation curve was also significantly changed (Table 1). Application of 5 μM GF produced a smaller enhancement of the A current (15.5 ± 2.3, $n = 4$, $P <$ 0.05 compared with 2 μM GF 49.3 ± 5.0), likely as a result of nonspecific effects. In contrast to the effect of PMA on A currents, GF enhanced the activation of A-type channels, increasing A currents.

To test whether PKC inhibitors block the inhibition of I_A by PMA, we first applied GF and subsequently tested PMA's ability to reverse the GF-mediated enhancement of I_A. Application of 1 μM GF induced a time-dependent increase in A currents that reached a plateau at 37.1 ± 7.0% above the basal current after 5 min. Consistent with our hypothesis that the inhibition of I_A by PMA was attributed to PKC activation, PMA did not significantly decrease I_A in the presence of GF (Fig. 4, *B–D*). Another selective PKC inhibitor, Ro31-8220 (1 μM) (McKenna and Hanson 1993; Shen and Glazer 1998), also enhanced A currents (by 18.5 ± 4.6%), decreased the slow time constant (Table 2), and prevented PMA's effect on the A current (Fig. 4*D*).

TABLE 2. *Effects of PMA, GF109203x, Ro31-8220, FSK, FSK with PKI(6–22)amide, and PD098059 on inactivation time constants*

Sample	Time Constant (ms) τ_1	τ_2	n
Pre-PMA	9.9 ± 1.2	100 ± 13	10
PMA	9.8 ± 1.7	88 ± 11	10
Pre-GF	13.6 ± 2.0	120 ± 9	7
GF	12.5 ± 2.4	76 ± 4*	7
Pre-Ro	10.1 ± 1.1	106 ± 13	7
Ro	8.9 ± 0.8	88 ± 12*	7
Pre-FSK	10.9 ± 0.8	121 ± 15	8
FSK	5.6 ± 0.5*	73 ± 8*	8
Pre-8Br-cAMP	11.1 ± 1.0	99 ± 4	5
8Br-cAMP	10.3 ± 0.9	90 ± 6	5
Pre-PKI + FSK	13.0 ± 2.1	99 ± 10	6
PKI + FSK	6.9 ± 2.1*	69 ± 5*	6
Pre-PD	13.8 ± 0.8	99 ± 9	9
PD	12.8 ± 0.7	90 ± 8	9

Values are means ± SE; n = number of neurons.

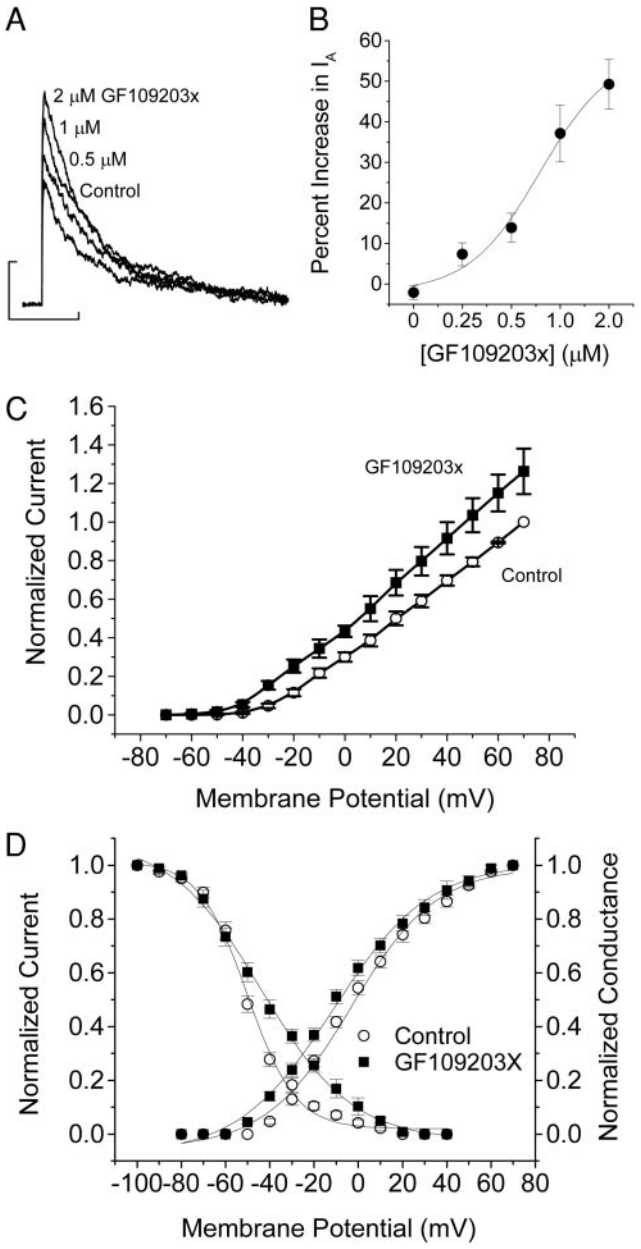

FIG. 3. Inhibition of PKC increases A-type potassium current. *A*: representative example of I_A recorded before and after exposure to increasing concentrations of GF109203X (GF) (A-type currents were isolated from sustained current by voltage protocol shown in *inset* of Fig. 1). *B*: dose–response curve showing mean ± SE percentage change in I_A; n = 6–10 neurons for each point. *C*: mean current–voltage plot of peak amplitude of current vs. test voltage for control and 1 μM GF. *D*: steady-state inactivation and activation curves before (open circles) and after (filled squares) 5 min bath-application of 1 μM GF. Scale bar in *A* is 500 pA and 50 ms.

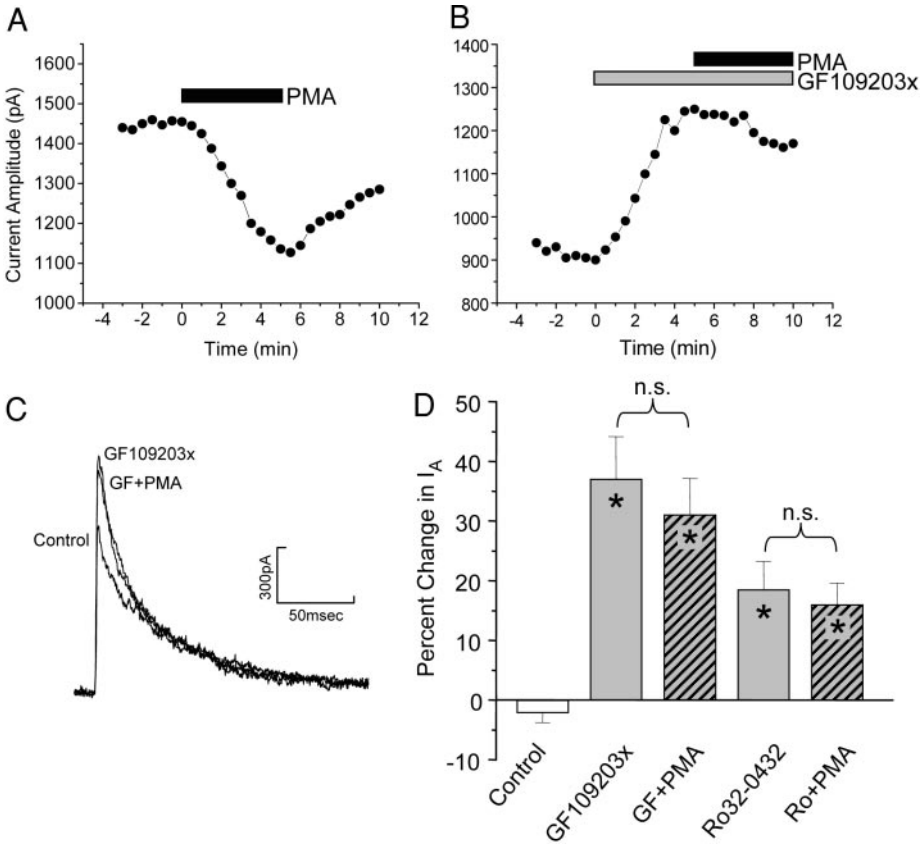

FIG. 4. Inhibition of PKC blocks effect of PMA on I_A. *A*: representative time course of inhibition by 5 μM PMA on I_A. *B*: representative time course of increase in I_A induced by GF. PMA (5 μM) did not decrease I_A in presence of 1 μM GF. *C*: representative recording of I_A under control conditions, in presence of 1 μM GF, and in presence of 5 μM PMA + GF (A-type currents were isolated from sustained current by voltage protocol shown in *inset* of Fig. 1). *D*: summary of effects of GF and Ro31-8220 in absence and presence of PMA on peak amplitude of A-type currents (using protocol shown in *B*). Values are means ± SE; n = 7–10. *$P < 0.05$, ANOVA. Asterisks within bars indicate significant change compared with control. n.s., not significant.

PKA activation suppresses A-type currents

Activation of PKA is also implicated in central sensitization (Malmberg et al. 1997; Sluka 2002). To determine whether PKA modulates A-type currents in dorsal horn neurons, we examined the effects of forskolin (FSK), an adenylyl cyclase activator, on these channels. The A-current peak amplitude was reduced by 18.3 ± 2.9% after application of 50 μM FSK with 50 μM IBMX (Fig. 5, *A* and *D*). The decay time constants (both of fast and slow) were reduced after FSK treatment (Table 2). FSK caused a slight but significant rightward shift in the activation curve ($V_{1/2}$, −3.8 ± 1.0 and 0.3 ± 1.3 mV for control and FSK, respectively, $n = 6$, $P < 0.05$; Fig. 5*C*, Table 1). We also found the inactivation curve to be shifted toward the left −9.4 mV (Fig. 5*C*, Table 1). Similarly, 1 mM 8Br-cAMP, a membrane-permeable analog of cAMP, significantly reduced the peak amplitude of the A current by 14.6 ± 1.6% ($n = 5$, $P < 0.05$; Fig. 5, *A* and *D*), but did not change its kinetics (Table 2). The negative control for FSK, 1,9-dideoxyforskolin (50 μM) did not decrease the A current (Fig. 5, *A* and *D*). KT5720 (1 μM), a specific inhibitor of PKA (Hidaka et al. 1984), had no significant effect on the A current when applied alone, but blocked the inhibition of I_A by FSK (Fig. 5*D*). When 50 μM PKI(6–22) amide, another specific PKA inhibitor (Kemp et al. 1988), was added to the patch pipette, bath-application of FSK had no effect on A currents (Fig. 5, *A* and *D*), but still significantly decreased both the fast and slow time constants. These results indicate that inhibition of peak amplitude of A current by FSK is attributable to PKA activation, and the change in kinetics is likely a nonspecific effect on the channel.

Inhibition of ERK signaling enhances A-type currents

We previously demonstrated that ERK activation is necessary for dorsal horn plasticity in inflammatory pain (Karim et al. 2001). Because there are no direct activators or inhibitors of ERK, to test whether ERK activation modulates the A-type currents in dorsal horn, we applied PD98059 and U0126 (inhibitors of mitogen-activated/ERK kinase (MEK), the upstream activator of ERKs) to the neurons. PD98059 (20 μM) significantly increased A currents by 21.7 ± 3.8% without changing their kinetics (Table 2), and shifted the activation curve toward the left −6.3 mV (Fig. 6*C*, Table 1) while shifting the inactivation curve toward the right 7.2 mV (Fig. 6*C*, Table 1). Similarly, 0.5 μM U0126 increased the A current by 20.7 ± 4.7% (Fig. 6, *A* and *B*). Bath-application of U0124 (0.5 μM), a negative control analog of U0126, had no significant effect on I_A (Fig. 6, *A* and *B*). These results suggested that there is basal ERK activity in dorsal horn neurons that tonically inhibits I_A in these neurons.

ERK inhibitors block PKC and PKA inhibition of A-type currents

The results discussed above show that the effect of ERK is similar to that of PKA and PKC, acting to inhibit the A current. This led us to question the pathway by which the kinases were acting. Both PKA and PKC activation can stimulate ERK (Roberson et al. 1999; Yuan et al. 2002). Do PKA and PKC act through ERK, or do these kinases directly modulate A-type potassium channels? To determine whether the PKC- and PKA-induced suppression of the A current depends on activa-

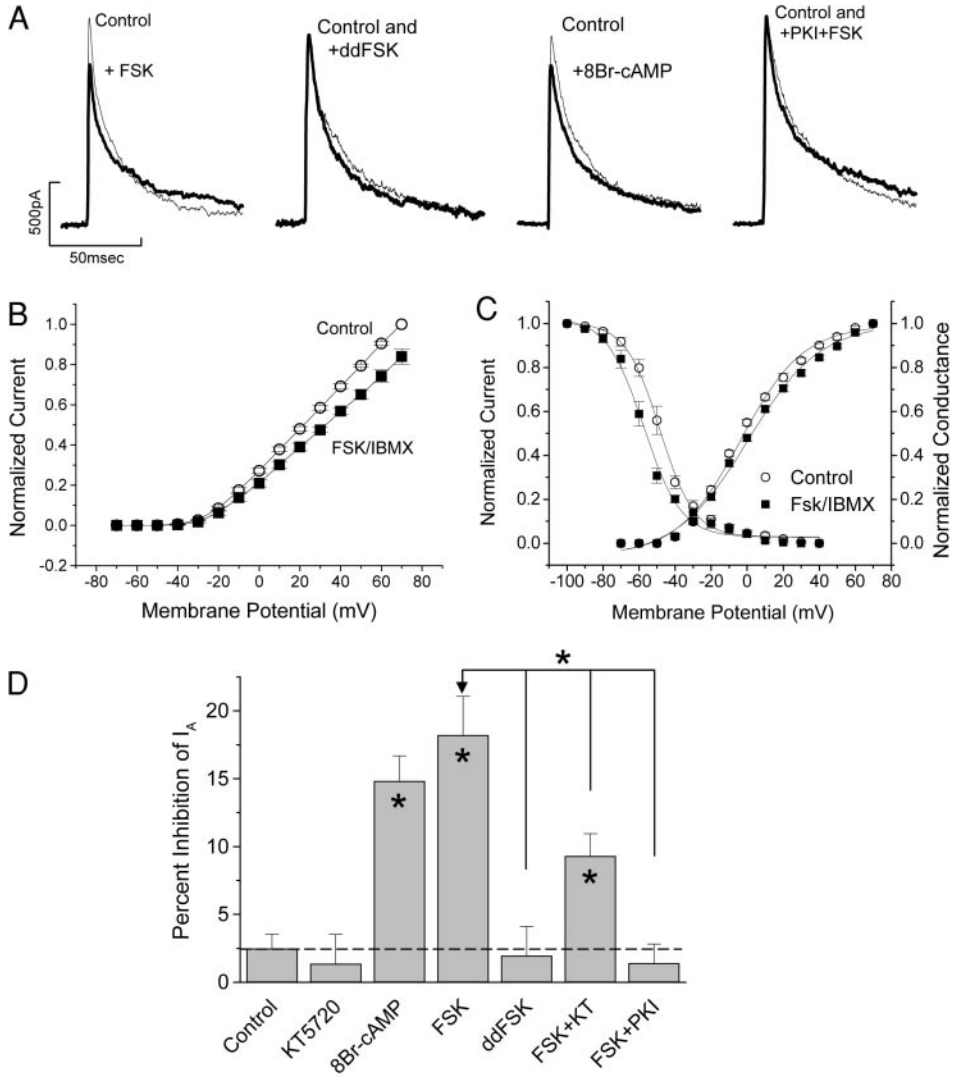

FIG. 5. Protein kinase A (PKA) activation suppresses A-type potassium current. *A*: representative examples of I_A recorded before and after 5-min bath-application of 50 μM FSK + 50 μM IBMX, 50 μM dideoxyfoskolin [negative control analog for forskolin (FSK)], 1 mM 8Br-cAMP, or FSK + IBMX in presence PKI(6–22) amide (in recording pipet) (A-type currents were isolated from sustained current by voltage protocol shown in *inset* of Fig. 1). *B*: mean current–voltage plot of peak amplitude of current vs. test voltage for control and 50 μM FSK. *C*: steady-state inactivation and activation curves before (open circles) and after 5-min bath-application of 50 μM FSK with 50 μM IBMX (filled squares). *D*: summary of inhibition of peak amplitude of A-type currents by 50 μM FSK, 1 μM KT 5720, 1 mM 8Br-cAMP, 50 μM dideoxyforskolin, and 50 μM FSK in presence of KT 5720 or PKI(6–22) amide (in recording pipet). Values are means ± SE; n = 6–10 neurons each. *$P <$ 0.05, ANOVA. Asterisks within bars indicate significant change compared with control. Asterisks above bars indicate significant difference between groups. Dashed line represents mean amount of channel rundown recorded under control conditions.

tion of the ERK pathway, we examined the effects of the MEK inhibitor PD98059 on the PMA- and FSK-mediated decrease in A currents. As shown in Fig. 7, neurons were exposed to 20 μM PD98059 for 5 min to inhibit activation of ERK. This led to a gradual increase in I_A that reached a plateau (Fig. 7, *A* and *B*). Neither PMA nor FSK reduced A currents in the presence of PD98059, indicating that blocking ERK activation also blocks the effects of PKC and PKA on A currents (Fig. 7*C*).

Finally, we tested the effect of the PKC inhibitor GF on the PD98059-mediated increase in A currents. As shown in Fig. 8, 2 μM GF elicited an increase in the A current of 43.2 ± 3.9%. PD98059 (20 μM) did not produce a further increase in I_A in the presence of GF. These results are consistent with the hypothesis that PKC is acting upstream of ERK to modulate I_A.

DISCUSSION

PKA, PKC, and ERK have been shown to be involved in the regulation of A-type potassium channels in hippocampal CA1 pyramidal neurons (Hoffman and Johnston 1998; Johnston et al. 1999). Furthermore, PKA, PKC, and ERK are localized in dendrites along with the A-type potassium channels, and inhibition and activation of these kinases modulates A currents (Yuan et al. 2002). ERK is known to phosphorylate Kv4.2, an A-type potassium channel subunit (Adams et al. 2000), and was shown to modulate the effects of GDNF on transient potassium channel currents (Yang et al. 2001). Because PKC, PKA, and ERK play important roles both in nociception and modulation of A-type potassium channels, we sought to test whether these kinases modulate A-type potassium channels in spinal cord dorsal horn neurons.

Our patch-clamp data demonstrate that outward currents recorded in our conditions are consistent with those of A-type channels in rat spinal dorsal horn neurons in slice (Wolff et al. 1998; Yoshimura and Jessell 1989a). The A-type K^+ channel subunit, Kv4.2, has been shown to localize to some central neurons (Alonso and Widmer 1997; Sheng et al. 1992), and Kv4.2 is a substrate for PKA- and ERK-mediated phosphorylation (Adams et al. 2000; Anderson et al. 2000). Our data show that A currents in dorsal horn neurons have many pharmacological and biophysical properties, such as activation, inactivation, and recovery from inactivation, that are similar to those of Kv4 subunits (Bahring et al. 2001; Tseng et al. 1996), indicating that Kv4 channels may be expressed in dorsal horn

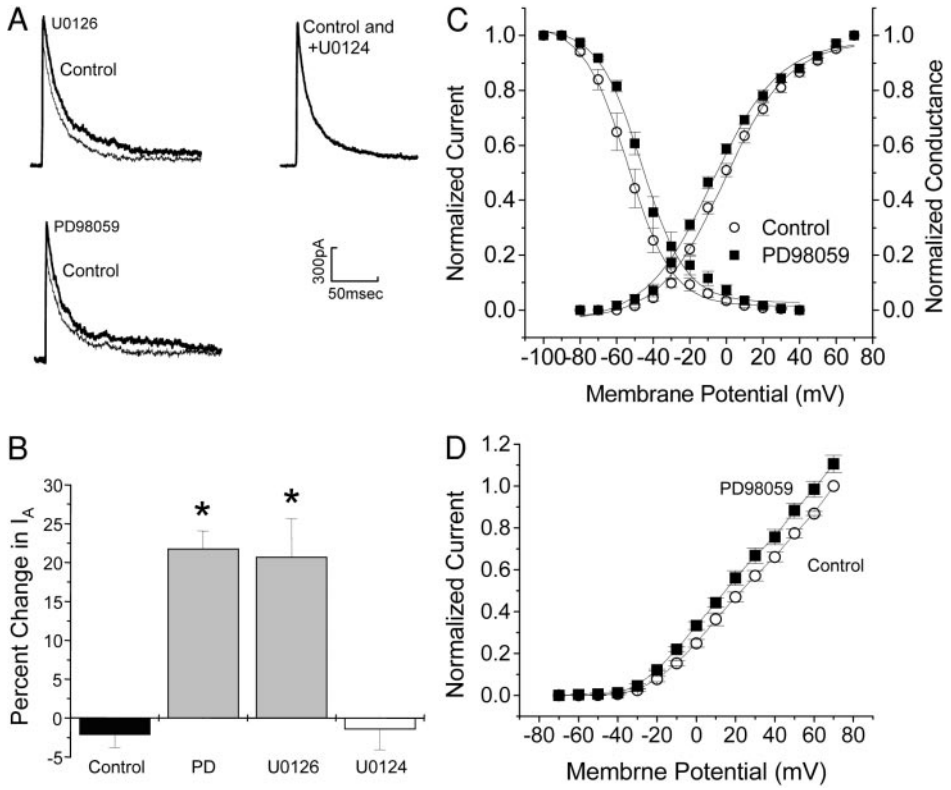

FIG. 6. Extracellular signal-related kinase (ERK) inhibition increases A-type potassium current. *A*: representative examples of I_A recorded before and after 5-min bath-application of 20 μM PD98059, 500 nM U0126, or 500 nM U0124 (negative control analog) (A-type currents were isolated from sustained current by voltage protocol shown in *inset* of Fig. 1). *B*: summary of percentage changes of peak amplitude of I_A by PD98059, U0126, and U0124. Values are means ± SE; $n = 8–12$. *C*: steady-state inactivation and activation curves before (open circles) and after 5-min bath-application of 20 μM PD98059 (filled squares). *D*: mean current–voltage plot of peak amplitude of current vs. test voltage for control and 20 μM PD98059.

neurons. Further studies using anatomical and genetic approaches are needed to identify the exact Kv subunits that underlie I_A in dorsal horn neurons, and further which subunits mediate the kinase-dependent regulation of I_A demonstrated here.

This study provides strong support for the hypothesis that PKC, PKA, and ERK are involved in modulation of A-type currents in spinal cord superficial dorsal horn neurons. Activation of PKC had significant effects on I_A, reducing its amplitude without changing current kinetics, and shifting the activation and the inactivation curves. In contrast, inhibition of PKC dramatically and dose-dependently increased A-type currents and shifted the activation and inactivation curves in the opposite direction. Interestingly, PKC inhibitors increased the rate of current decay for the slow component but not the fast component. This change in current decay kinetics may be a nonspecific effect of the PKC inhibitors because, as mentioned above, PKC activators had no effect on current decay kinetics.

Activation of PKA by forskolin also decreased A currents and shifted the activation and inactivation curves. This effect was mimicked by the PKA activator 8-Br-cAMP and blocked by the specific peptide inhibitor of PKA, PKI(6–22) amide. Forskolin has been shown to have nonspecific effects on some types of potassium channels (Herness et al. 1997). We observed this nonspecific effect as an increasing current decay rate of both the fast and slow components, which was not blocked by PKI(6–22) amide. MEK inhibitors also resulted in an increase in A-type currents and shifted both the activation and inactivation curves without changing channel kinetics, whereas a negative control analog had no effect on A-type currents.

Our data suggest that PKC, PKA, and ERK modulate A-current activation and inactivation. It is likely that these gating changes are at least in part responsible for the inhibition of peak currents we observed. It is interesting to note that activators of PKC and PKA appear to induce nonparallel shifts in the activation and inactivation curves. This suggests that there may be heterogeneity in the channels that are mediating the A currents in these neurons, and that these activators may have differential or voltage-dependent effects on these channels. Future studies will endeavor to identify the specific Kv subunits that mediate the A currents in dorsal horn neurons.

Experiments in rat primary sensory neurons separated the PKC, PKA, and ERK pathways to hyperalgesia by showing that inhibition of the ERK pathway did not affect hyperalgesia produced by PKA or PKC activation and also that PKA and PKC inhibitors were unable to block hyperalgesia mediated through the ERK pathway (Aley et al. 2001). This was not necessarily the case in dorsal horn neurons. We addressed the issue by combining inhibitors of ERK with activators of PKC or PKA, and our data demonstrate that ERK inhibition blocks the effects of both PKC and PKA on A-type currents. This finding is consistent with the idea that ERK activation and modulation of I_A are downstream of both PKA and PKC, as shown in the model depicted in Fig. 8*C*. Alternatively, it is also possible that PKA and PKC modulation of K_A requires prior phosphorylation by ERK to achieve this modulation. Our data cannot rule out either model. The potential mechanism of activation of ERK by PKA and PKC in dorsal horn neurons is unknown. Recent studies suggest that PKC might act on Raf, and PKA on B-Raf through Rap1 to activate ERK (Gutkind 2000). Thus the PKA- and PKC-mediated signaling pathways converge onto ERKs, which we suggest phosphorylate A-type K^+ channel subunits in dorsal horn neurons.

Prolonged activation of nociceptive primary afferents by

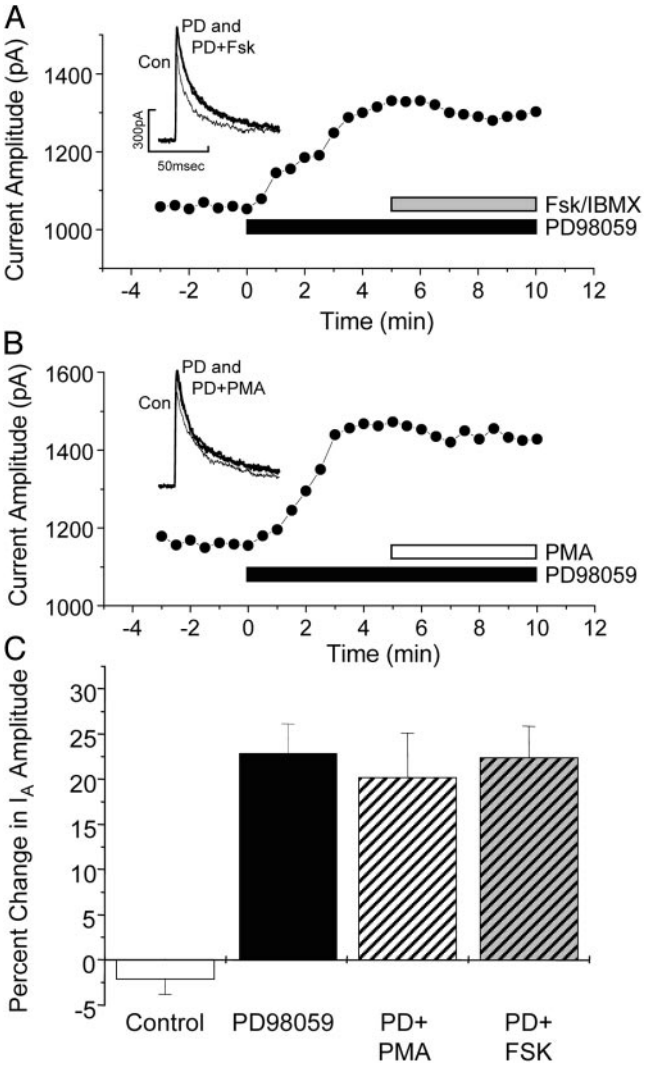

FIG. 7. ERK inhibition blocks effects of PKC and adenylyl cyclase activators on A-type current. *A*: representative time course of enhancement of I_A by 20 μM PD98059. FSK (50 μM) with IBMX did not decrease I_A in presence of PD98059. *Inset*: representative *traces* (isolated from sustained current by voltage protocol shown in *inset* of Fig. 1) recorded in control solution, 20 μM PD98059, and 5 μM PMA + PD98059. *B*: representative time course of effect of 20 μM PD98059 on I_A. PMA (5 μM) did not decrease I_A in presence of PD98059. *Inset*: representative *traces* recorded in control solution, 20 μM PD98059, and 5 μM PMA + PD98059. *C*: summary of data for *A* and *B*. Values are means ± SE; n = 6–12 neurons. All three groups showed significant change compared with control ($P < 0.05$), and there were no significant differences between PD98059 group and either PD98059 + PMA or PD98059 + FSK groups.

noxious stimuli can increase the excitability of nociceptive neurons in the dorsal horn (Ji and Woolf 2001). This central sensitization is the result of the activation of several kinase cascades, some of which have been linked in this study to modulation of the A-type potassium current by ERK. Studies presented in the accompanying report show that the changes in K_A reported here are paralleled by the predicted changes in action firing properties, leading to a dramatic increase in excitability of dorsal horn neurons (Hu and Gereau 2003). We propose that ERK modulation of A-type potassium channels may play an important role in establishing central sensitization associated with injury and disease.

The results obtained here from cultures of superficial dorsal horn neurons leave some open questions. For example, are similar changes seen in identified laminae I–II neurons in acute slices? Although we isolated only the most superficial portion of the dorsal horn, our cultures may contain some cells from deeper laminae. Will similar neuromodulatory effects be

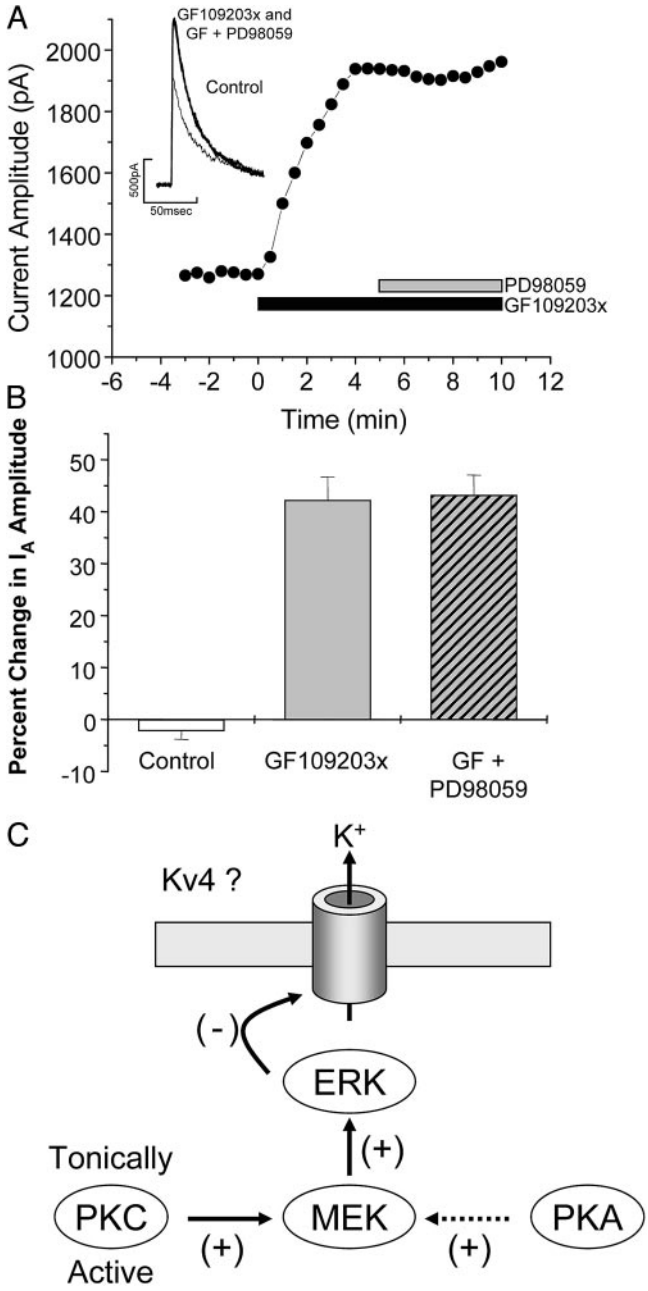

FIG. 8. Potentiation of A currents by inhibition of PKC and ERK are nonadditive. *A*: representative time course showing enhancement of I_A by 2 μM GF109203X. PD98059 (20 μM) did not increase I_A in presence of GF. *Inset*: representative *traces* recorded in control solution, 2 μM GF, and 20 μM PD98059 + GF. *B*: summary of data for *A*. Values are means ± SE; n = 6–10. Both groups showed significant change compared with control ($P < 0.05$), whereas there was no significant difference between GF and GF + PD98059 groups. *C*: proposed model for mechanism of modulation of K_A channels in superficial dorsal horn neurons by PKA, PKC, and ERKs. In this model, PKA and PKC inhibit K_A by activating MEK (through a series of as yet unidentified signaling molecules). Our data suggest some level of tonic PKC and MEK/ERK activity in these cells (solid arrows), but no tonic PKA activity (indicated by broken arrow). An alternative model is mentioned in DISCUSSION.

seen in cells from more mature animals? The roles of PKC, PKA, and ERK in modulating pain perception were established in adult animals, whereas the cultures used here were prepared from very young animals. Are A-type currents seen mostly in glutamatergic cells or do they and their modulation also have an impact on the physiological properties of inhibitory interneurons? All of these questions are difficult to answer using cell culture systems. These studies must be extended to recordings from visually identified and characterized neurons in acute slices from more mature animals. Such efforts will help to answer these questions and provide a more direct link between the types of neuromodulation we have identified here and the modulation of nociception identified at the behavioral level.

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DISCLOSURES

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