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Role of protein kinase C in opioid modulation of glycine-gated Cl^- current in rat periaqueductal gray neuron

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Abstract

The Role of protein kinase C in the modulatory effect of a μ-opioid receptor agonist, [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]enkephalin (DAMGO), on the glycine-gated Cl^- current was examined in acutely dissociated rat periaqueductal gray neurons. Using the nystatin-perforated patch-clamp technique, the neurons were voltage-clamped at −60 mV. The glycine-gated Cl^- current (I_{Gly}) was sensitive to strychnine. On pretreatment with 1 μM DAMGO, the 30-μM glycine response increased with time and showed a maximum amplitude of 209 ± 37% of control. After a protein kinase C activator, phorbol-12-myristate-13-acetate (PMA, 0.1 μM) as pretreatment, I_{Gly} increased to 138 ± 6% of control. The DAMGO potentiation of I_{Gly} was not altered by coapplication with PMA. Although protein kinase C inhibitors, chelerythrine (3 μM) and 2-[1-(3-dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl) maleimide (GF109203X, 1 μM), did not alter I_{Gly}, the DAMGO-induced potentiation of I_{Gly} was reduced to 161 ± 21% or 164 ± 31% of the control after coapplication with chelerythrine or GF109203X, respectively. These results indicate that the potentiation of I_{Gly} by a μ-opioid receptor agonist is partly mediated by activation of protein kinase C.

Keywords: Periaqueductal gray; Cl^- current, glycine-gated; Protein kinase C; Opioid

1. Introduction

The endogenous pain control system (the descending pain control system) consists of three major components including the midbrain periaqueductal gray, the rostral ventral medulla including the nucleus raphe magnus and the spinal dorsal horn (Basbaum and Fields, 1984). This pain inhibitory neuronal system is activated by several factors such as stress, electroacupuncture and local electrical stimulation of the periaqueductal gray (Millan et al., 1980, 1987; Xie et al., 1983).

It has been postulated that opioid peptides and opiates play an important role in analgesia by activating the descending pain modulatory pathways, especially at the level of the periaqueductal gray (Duggan, 1983; Fields et al., 1991; Renno et al., 1992). Analgesia can be generated from all regions of the periaqueductal gray, but it is suggested that the ventrolateral region is most effective (Gebhart and Toleikis, 1978). Among opioid receptors, μ-type receptors are involved in analgesic effects predominantly at the periaqueductal gray region (Mansour et al., 1995).

Glycine is one of the major inhibitory neurotransmitters in the central nervous system (Yoshimura and Nishi, 1995). It has become evident that in mammalian neurons, phosphorylation of the glycine receptor by intracellular second messenger-triggered protein kinases causes an alteration in the functional characteristics of glycine receptor-mediated events. The glycine-gated Cl^- current (I_{Gly}) in neurons can be modulated by protein kinase A (Song and Huang, 1990; Agopyan et al., 1993; Vaello et al., 1994; Gu and Huang, 1998). Several studies have shown contradictory results about the effects of protein kinase C on I_{Gly} in various tissue preparations. The activation of protein kinase C suppresses I_{Gly} in *Xenopus* oocytes (Vaello et al.,

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1994; Nishizaki and Ikeuchi, 1995), and potentiates I_{Gly} in various neurons (Schönrock and Bormann, 1995; Nabekura et al., 1996; Xu et al., 1996; Gu and Huang, 1998; Han and Slaughter, 1998; Nabekura et al., 1999) and in *Xenopus* oocytes (Nishizaki and Ikeuchi, 1995). When the activity of protein kinase C is inhibited, I_{Gly} is usually not altered (Nabekura et al., 1996; Xu et al., 1996) or slightly attenuated (Nabekura et al., 1999).

In the periaqueductal gray neurons, Min et al. (1996) studied the modulatory effect of a μ-type opioid receptor agonist on I_{Gly} and found that a μ-type opioid receptor agonist potentiates glycine responses by the inhibition of cyclic AMP-dependent protein kinase A. However, it has not yet been investigated whether the change in protein kinase C activity in the periaqueductal gray neurons alters both the neuronal I_{Gly} itself and the potentiation effect of a μ-type opioid receptor agonist. On this basis, we designed the present study to elucidate the roles of protein kinase C in the modulatory effect of a μ-type opioid receptor agonist on the glycine-gated Cl^- current in acutely dissociated rat periaqueductal gray neurons using the nystatin-perforated whole cell patch-clamp technique under voltage-clamp conditions.

2. Materials and methods

2.1. Isolation of periaqueductal gray neurons

The periaqueductal gray neurons were dissociated with techniques described previously (Han et al., 1999). In brief, 10- to 14-day-old Sprague–Dawley rats of both sexes were decapitated under Zoletil 50® anesthesia (50 mg/kg). The brain was removed and transverse slices (400-μm thickness) were made with a microslicer (DTK-1000, DSK, Japan). Slices were preincubated in the incubation solution that had been well saturated with 95% O_2 and 5% CO_2 at room temperature for 30 min. Then, the slices were treated with pronase (protease XIV, 1 mg/5 ml of the oxygenated incubation solution) for 40–80 min at 32 °C and subsequently with thermolysin (protease X, 1 mg/5 ml) for 10–20 min at 32 °C. After enzyme treatments, the slices were kept in the enzyme-free incubation solution for 1 h. The ventrolateral region of the periaqueductal gray was identified in a 60-mm culture dish coated with silicon under a binocular microscope (SZ-ST, Olympus, Tokyo, Japan), and was micropunched out from the slices with an electrolytically polished injection needle. The micropunched-out pieces were mechanically dissociated in a different dish with fire-polished fine glass Pasteur pipettes in 35-mm plastic culture dishes (3801, Falcon, Becton Dickinson, USA) filled with the standard external solution. The dissociation procedure was done under an inverted phase-contrast microscope (CK-2, Olympus). The dissociated neurons usually adhered to the bottom of the dish within 20 min. These cells remained viable for electrophysiological studies for up to 6 h after dissociation.

2.2. Electrical measurements

Electrical recordings were performed in the nystatin-perforated patch-clamp recording mode (Akaike and Harata, 1994) under voltage-clamp conditions. Patch pipettes were prepared from glass capillaries (1.5 mm o.d., 1 mm i.d.; G-1.5, Narishige, Tokyo, Japan) in two stages on a vertical pipette puller (PP-83, Narishige). The patch pipette was positioned on the neuron, using a water-driven micromanipulator (WR-60, Narishige). The resistance between the recording electrode filled with the internal pipette solution and the reference electrode was 5–7 MΩ. The neurons were visualized with phase-contrast equipment on an inverted microscope (IX-70, Olympus). Electrical stimulation, current recordings and filtration of currents (at 1 kHz) were done with an EPC-9 patch-clamp amplifier (HEKA Electronik, Lambrecht, Germany) linked to a Macintosh Quadra 650 computer controlled by HEKA software. The current and voltage were also monitored on a pen recorder (Recti-Horiz-8K, NEC San-ei, Tokyo, Japan) and stored on videotapes with a data recorder (RD-120TE, TEAC, Japan). All experiments were performed at room temperature (22–25 °C).

2.3. Solutions

The ionic composition of the incubation solution was (in mM): NaCl 124, KCl 5, KH_2PO_4 1.2, $MgSO_4$ 1.3, $CaCl_2$ 2.4, glucose 10 and $NaHCO_3$ 24. The pH was adjusted to 7.4 by continuous bubbling with 95% O_2 and 5% CO_2. The standard external solution was (in mM): NaCl 150, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, glucose 10 and *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid (HEPES) 10. The pH was adjusted to 7.4 with Tris–hydroxymethylaminomethane (Tris–base). The composition of the internal pipette solution for nystatin-perforated recording was (in mM): KCl 150, HEPES 10. The pH was adjusted to 7.2 with Tris–base. The nystatin–stock solution containing 10 mg/ml of nystatin in methanol was prepared and then diluted with the internal pipette solution to a final concentration of 200 μg/ml.

2.4. Drugs

Zoletil 50® (tiletamine HCl 125 mg/5 ml + zolazepam HCl 125 mg/5 ml) was purchased from Virbac laboratory (06516 Carros, France). Pronase, thermolysin, nystatin, dimethyl sulfoxide (DMSO), [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]enkephalin acetate (DAMGO), chelerythrine chloride, 4α-phorbol and phorbol-12-myristate-13-acetate (PMA) were purchased from Sigma (St. Louis, MO, USA). 2-[1-(3-dimethylaminopropyl)indol-3-yl]-3-(indol-3-yl) malei-

mide (GF109203X) was purchased from Tocris Cookson (Bristol, United Kingdom). Drugs were added to external solutions at the final concentrations shown in the text and vehicle concentrations never exceeded 0.01%. Drugs were applied using a rapid application system termed the "Y-tube method" as described elsewhere (Min et al., 1996).

2.5. Statistical analysis

Data are presented as means ± S.E.M. and Student's *t*-test was used for statistical analyses. P values less than 0.05 were considered significant.

3. Results

3.1. Effect of a μ-opioid receptor agonist on the glycine-gated Cl^- currents

The periaqueductal gray neurons were kept at the holding potential (V_H) of −60 mV, and glycine at the concentration of 30 μM was applied briefly every 3 min. Glycine induced a rapidly activating inward current with gradual desensitization. The glycine-gated Cl^- current (I_{Gly}) was blocked by pretreatment with 1 μM strychnine for 1 min (1 ± 1% of the initial current; $n = 4$; Fig. 1A and C). After wash out of strychnine, I_{Gly} gradually restored the initial amplitude for 9 min. As the control, after more than three consecutive currents induced by glycine showed identical amplitudes, we examined the change in amplitude of the glycine-gated Cl^- current with time. The amplitudes of the 30 μM glycine-gated Cl^- currents did not change significantly for at least 30 min ($n = 14$; Fig. 1B and C).

A previous report had shown that at the holding potential of −40 mV, a μ-type opioid receptor agonist, DAMGO (1 μM), potentiated the periaqueductal gray neuronal glycine-gated Cl^- currents at the low concentration of 1–10 μM but slightly reduced I_{Gly} at the high concentration of 100 μM (Min et al., 1996). In the present experiment, the holding potential and the concentration of glycine were changed to −60 mV and 30 μM, respectively. Thus, it was necessary to evaluate the characteristics of the modulatory effect of DAMGO on I_{Gly} under these conditions. After the periaqueductal gray neurons were pretreated with 1 μM DAMGO for 5 min, the peak current of I_{Gly} increased with time. The DAMGO-induced facilitation of I_{Gly} was maximal at 21 min after the application of DAMGO (209 ± 37% of control; $n = 8$; Fig. 2).

3.2. Effect of protein kinase C activation on the glycine-gated Cl^- current

To elucidate whether the periaqueductal gray neuronal I_{Gly} was affected by protein kinase C activation, we examined the effect of a membrane-permeable activator of protein kinase C, phorbol-12-myristate-13-acetate (PMA), on I_{Gly}. With pretreatment of 0.1 μM PMA for 3 min, the 30 μM glycine-gated Cl^- current increased to 121 ± 7% of the control just after the pretreatment ($n = 5$; Fig. 3). Thereafter, I_{Gly} showed a gradual and slow increase for 30 min. At 27 min after PMA-pretreatment, the amplitude of

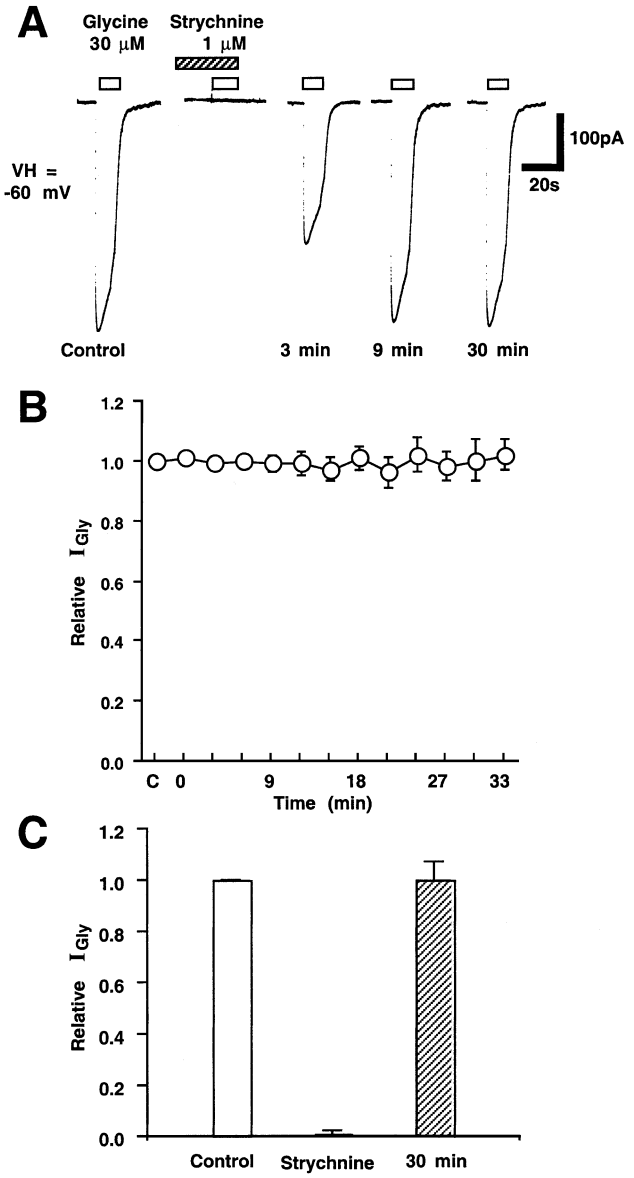

Fig. 1. The control response of the glycine-gated Cl^- current (I_{Gly}) in the periaqueductal gray neuron. (A) Chart records showing the currents gated by 30 μM glycine. The I_{Gly} was blocked by the pretreatment of 1 μM strychnine. Open bars above each current trace indicate the application of glycine. Holding potential was −60 mV. (B) The time course of I_{Gly}. The peak amplitude of each current was normalized to that of the initial current. The glycine responses did not change significantly for at least 30 min. *C* means the control current. Each value is the mean ± S.E.M. for 14 cells. (C) I_{Gly} was completely blocked by strychnine and the amplitude of I_{Gly} did not change significantly at 30 min. Each value is the mean ± S.E.M. for three (strychnine) and 14 cells (at 30 min).

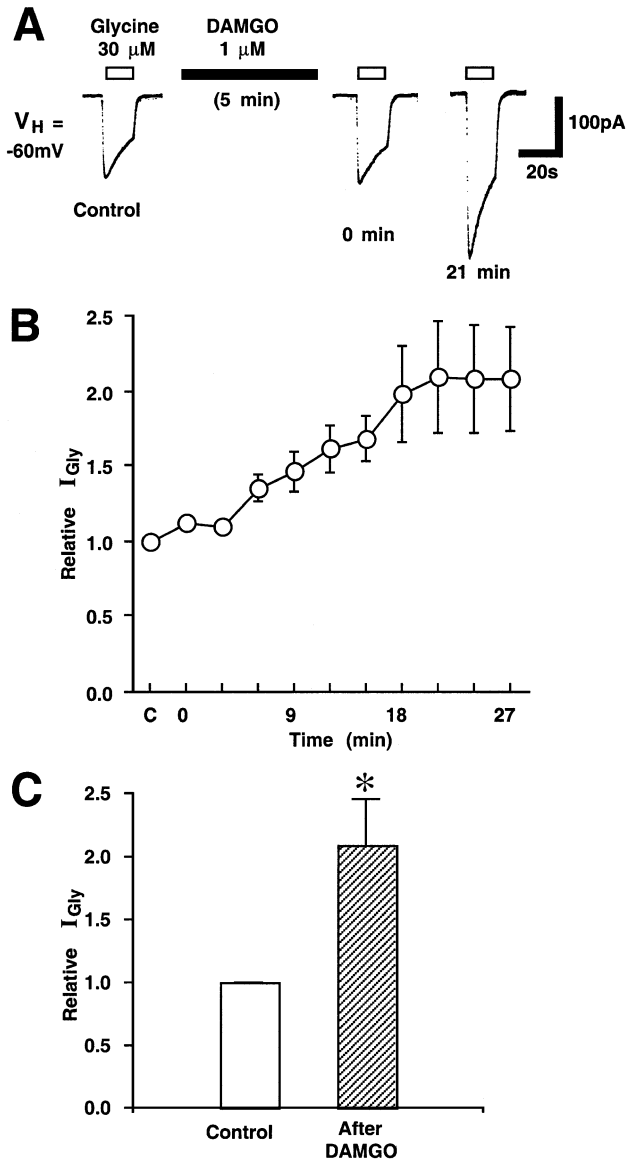

Fig. 2. Effect of a μ-opioid receptor agonist, DAMGO, on the glycine-gated Cl^- currents. (A) After pretreatment with DAMGO (1 μM) for 5 min, the 30-μM glycine response increased with time. The holding potential was −60 mV. Open and closed bars indicate the application of glycine and DAMGO, respectively. (B) The time course of the potentiation effect of DAMGO on I_{Gly}. The peak amplitude of each current was normalized to that of the initial current. *C* means the control current. Each value is the mean ± S.E.M. for eight cells. (C) I_{Gly} increased significantly at 21 min after DAMGO pretreatment. Asterisk (*) indicates $P < 0.05$.

I_{Gly} increased to 138 ± 6% of the control. However, phorbol esters may have a direct action on glycine receptor channels independent of protein kinase C activation (Nishizaki and Ikeuchi, 1995). To rule out this possibility, we examined the effect of an inactive phorbol ester, 4α-phorbol. On pretreatment with 4α-phorbol at the same concentration (0.1 μM) for 3 min, I_{Gly} did not change significantly for at least 21 min (data not shown).

3.3. Effect of protein kinase C inhibition on the glycine-gated Cl^- currents

The effects of protein kinase C inhibitors on I_{Gly} were examined to elucidate whether the periaqueductal gray neuronal I_{Gly} was affected by protein kinase C inhibition. After the periaqueductal gray neurons were pretreated with a membrane-permeable, specific protein kinase C inhibitor (chelerythrine, 3 μM) for 3 min, I_{Gly} did not change significantly for at least 21 min (95 ± 2% of control; $n = 5$; Fig. 4). Even though the duration of chelerythrine pretreatment was increased to 5 min, there was no signifi-

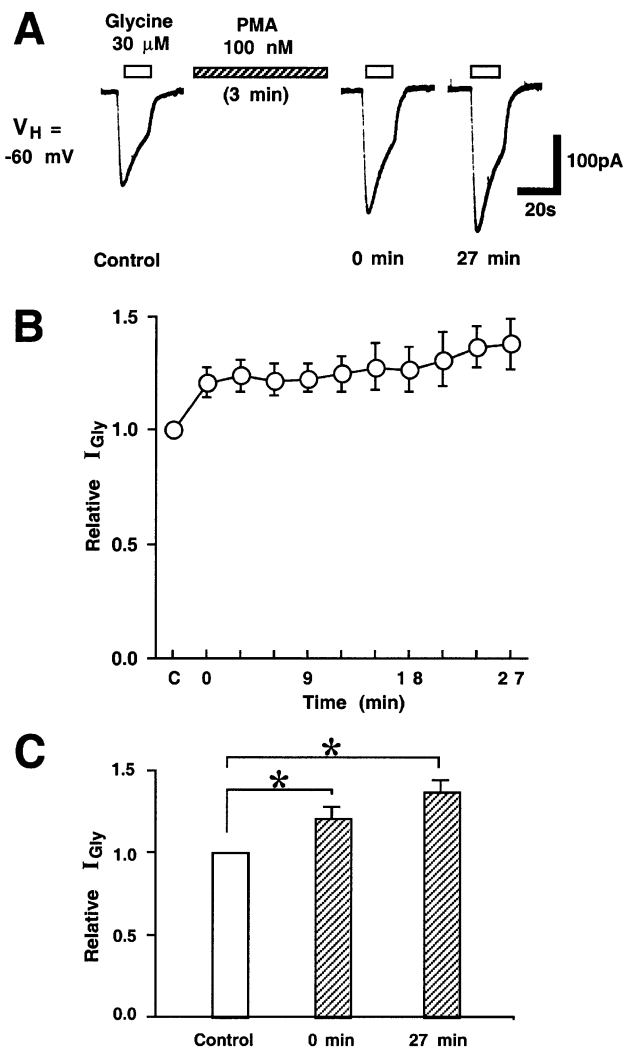

Fig. 3. Effect of a protein kinase C activator, PMA, on the glycine-gated Cl^- currents. (A) After pretreatment with PMA (0.1 μM) for 3 min, the 30-μM glycine response increased with time. The periaqueductal gray neurons were kept at the holding potential of −60 mV. Open and shaded bars indicate the application of glycine and PMA, respectively. (B) The time course of the facilitatory effect of PMA on I_{Gly}. The peak amplitude of each current was normalized to that of the initial current. *C* means the control current. Each value is the mean ± S.E.M. for five cells. (C) I_{Gly} increased significantly at 0 and 27 min after PMA pretreatment. Asterisks (*) indicate $P < 0.05$.

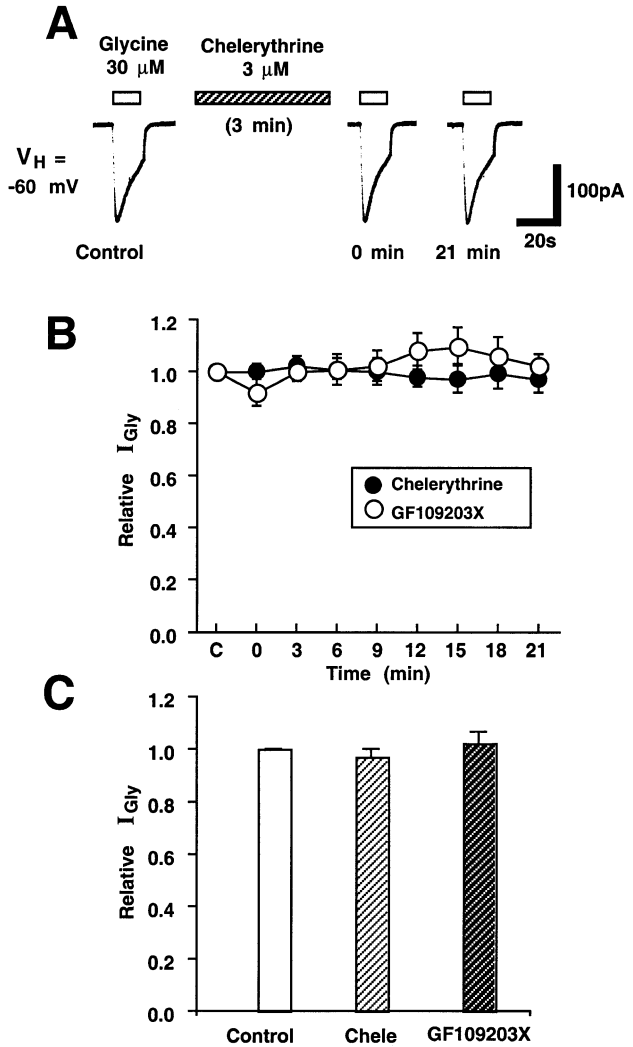

Fig. 4. Effects of protein kinase C inhibitors, chelerythrine and GF109203X, on the glycine-gated Cl^- currents. (A) Representative current traces showing the effect of chelerythrine on I_{Gly}. After pretreatment with chelerythrine (3 μM) for 3 min, the 30-μM glycine response did not change. Holding potential was −60 mV. Open and shaded bars indicate the application of glycine and chelerythrine, respectively. (B) The time courses denote the changes of I_{Gly} after the application of chelerythrine (filled circle) or GF109203X (open circle) for 3 min. The peak amplitude of each current was normalized to that of the initial current. *C* means the control current. Each value is the mean ± S.E.M. for five (chelerythrine) and six (GF109203X) cells. (C) A histogram shows that I_{Gly} did not change significantly at 21 min after the application of chelerythrine or GF109203X.

cant change in the amplitude of the glycine-gated Cl^- current (data not shown).

However, recent studies have indicated that chelerythrine does not inhibit protein kinase C (Lee et al., 1998), and under some circumstances may activate it (Yu et al., 2000). To confirm the effect of chelerythrine as a protein kinase C inhibitor, we examined the effect of a structurally unrelated protein kinase C inhibitor, GF109203X, on the glycine-gated Cl^- current. As shown for the effect of chelerythrine, application of 1 μM GF109203X for 3 min did not alter the glycine response for at least 21 min (102 ± 5% of control; $n = 6$; Fig. 4B and C). Even though GF109203X was applied for 5 min, the glycine response did not change (data not shown).

3.4. Effect of protein kinase C activation on the DAMGO-induced potentiation of I_{Gly}

To elucidate the possible role of protein kinase C in the DAMGO-induced potentiation of I_{Gly}, we needed to examine whether the maximum amplitude of I_{Gly} potentiated by DAMGO would be altered by activation of protein kinase C with PMA. After the neurons were pretreated with the external solution containing both 1 μM DAMGO and 0.1 μM PMA for 5 min, the amplitudes of I_{Gly} were measured every 3 min. The maximum potentiation of the periaqueductal gray neuronal I_{Gly} was 191 ± 19% of the control ($n = 5$; Fig. 5). The maximum amplitude of I_{Gly} induced

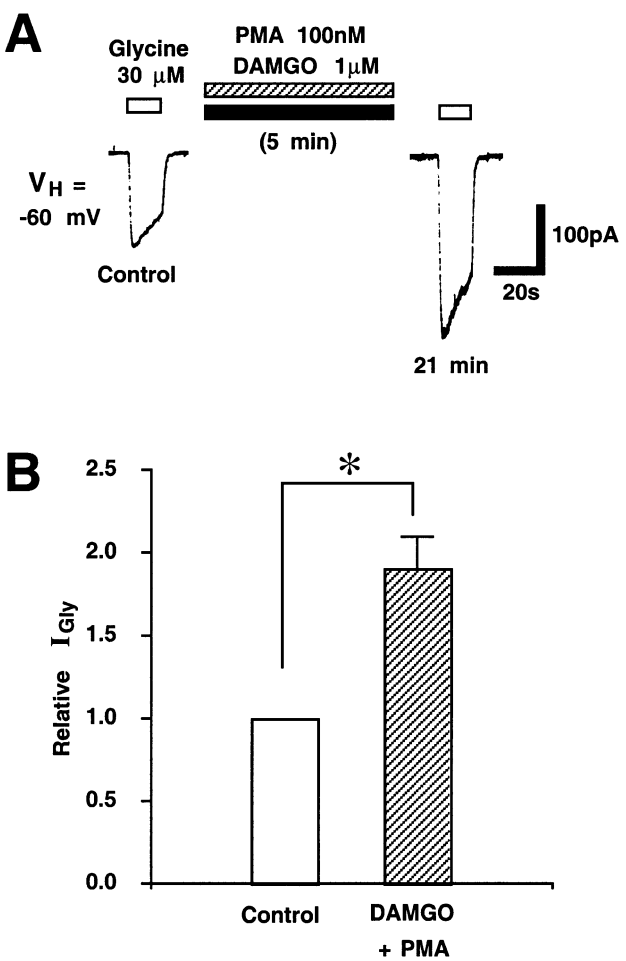

Fig. 5. Effect of PMA on the μ-opioid receptor agonist-induced potentiation of I_{Gly}. (A) Representative current traces showing the potentiation of 30 μM glycine response by coapplication of 1 μM DAMGO and 0.1 μM PMA for 5 min. The holding potential was −60 mV. Open, closed and shaded bars indicate the application of glycine, DAMGO and PMA, respectively. (B) Relative I_{Gly} facilitated by coapplication of DAMGO and PMA. Each value is the mean ± S.E.M. for five cells. Asterisks (*) indicate $P < 0.05$.

by coapplication of DAMGO and PMA was similar to that induced by DAMGO alone (Fig. 2).

3.5. Effect of protein kinase C inhibition on the DAMGO-induced potentiation of I_{Gly}

If it is true that the facilitating effect of DAMGO on the periaqueductal gray neuronal I_{Gly} is partly mediated by DAMGO-induced activation of protein kinase C, the DAMGO potentiation should be partly inhibited when protein kinase C is inhibited. This suggestion led us to examine the effects of protein kinase C inhibitors, chelerythrine and GF109203X, on the DAMGO-induced potentiation. After the periaqueductal gray neurons were superfused with the external solution containing both DAMGO and each protein kinase inhibitor for 5 min, the amplitude of I_{Gly} was measured every 3 min. After the coapplication of 1 μM DAMGO and 3 μM chelerythrine, the maximum amplitude of I_{Gly} was 161 ± 21% of the control ($n = 5$; Fig. 6A and B). To confirm the effect of protein kinase C inhibition by chelerythrine, we examined the effect of GF109203X. Even though 1 μM GF109203X was substituted for chelerythrine, the amplitude of I_{Gly} was similar to the result with chelerythrine (164 ± 31% of control; $n = 6$; Fig. 6B).

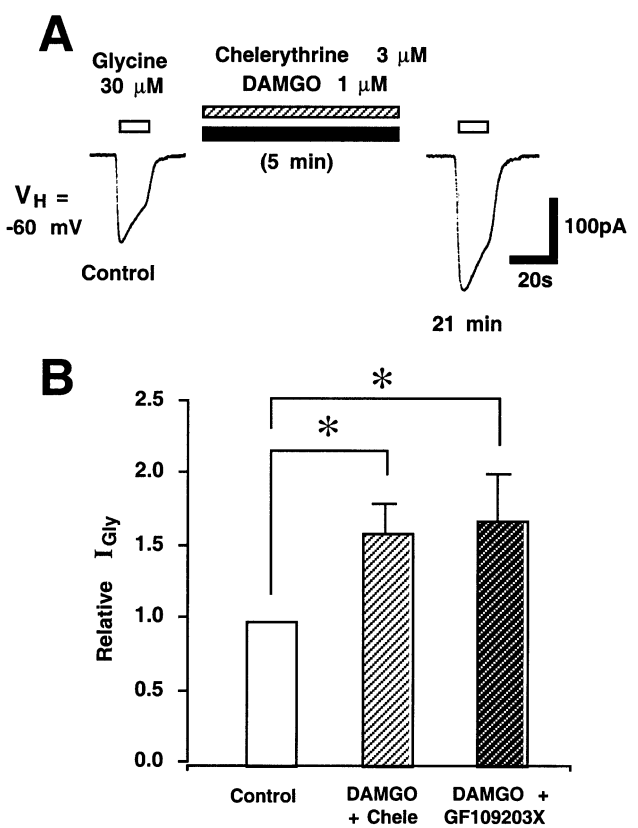

Fig. 6. Effects of chelerythrine and GF109203X on the μ-opioid receptor agonist-induced potentiation of I_{Gly}. (A) Representative current traces showing the potentiation of the 30-μM glycine response by coapplication of 1 μM DAMGO and 3 μM chelerythrine for 5 min. The holding potential was −60 mV. Open, closed and shaded bars indicate the application of glycine, DAMGO and chelerythrine, respectively. (B) Relative I_{Gly} potentiated by coapplication of DAMGO and a protein kinase inhibitor (3 μM chelerythrine or 1 μM GF109203X). Each value is the mean ± S.E.M. for five (chelerythrine) and six (GF109203X) cells. Asterisks (*) indicate $P < 0.05$.

4. Discussion

We had designed the present study to elucidate the role of protein kinase C in the modulatory effect of a μ-type opioid receptor agonist on the glycine-gated Cl^- current in the rat periaqueductal gray neurons. The neurons were acutely dissociated after enzyme treatment. Using the nystatin-perforated whole cell patch-clamp technique, the neurons were voltage-clamped at the holding potential of −60 mV. In the previous report, also concerning acutely dissociated periaqueductal gray neurons, when the concentration of glycine and the holding potential were 10 μM and −40 mV, respectively, the maximal facilitatory effect (213 ± 22.7% of control) appeared 15 min after the application of 1 μM DAMGO (Min et al., 1996). Because the amplitude of the glycine-gated Cl^- current at the concentration of 10 μM is relatively small and the kinetics of activation and desensitization of the currents are not clear, we used 30 μM glycine in the present experiment. At a holding potential of −60 mV, 1 μM DAMGO gradually facilitated the glycine-gated Cl^- current and the maximal potentiation (209 ± 37% of control) was at 21 min (Fig. 2). Although the experimental conditions were different, the maximally potentiated current of I_{Gly} in the present study was very similar to that in the report of Min et al. (1996).

4.1. The periaqueductal gray neuronal I_{Gly} is facilitated by activation of protein kinase C but not by inhibition of protein kinase C

There is much evidence that the characteristics of the glycine-gated Cl^- current are altered by protein kinase C. Activation of protein kinase C potentiates I_{Gly} in cultured rat hippocampal neurons (Schönrock and Bormann, 1995), in substantia nigra neurons (Nabekura et al., 1996), in salamander retinal ganglion cells (Han and Slaughter, 1998), and in the rat sacral dorsal commissural neurons (Xu et al., 1996; Nabekura et al., 1999). Activation of endogenous protein kinase C by serum increases the glycine-gated Cl^- current in *Xenopus* oocytes (Nishizaki and Ikeuchi, 1995) and intracellular application of either protein kinase A or protein kinase C potentiates I_{Gly} in trigeminal neurons (Gu and Huang, 1998). In contrast, inhibition of protein kinase C does not significantly alter I_{Gly} in substantia nigra neurons (Nabekura et al., 1996) or in rat sacral dorsal commissural neurons (Xu et al., 1996; Nabekura et al., 1999). In the present study, the periaqueductal gray neuronal I_{Gly} was increased by a protein kinase C activator, PMA, but not altered by the protein

kinase C inhibitors, chelerythrine and GF109203X. These results are well consistent with the reports mentioned above and indicate that I_{Gly} in the periaqueductal gray neurons is potentiated by activation of protein kinase C, but not altered by inhibition of protein kinase C. However, other reports have shown contradictory results that activation of protein kinase C with phorbol ester reduces I_{Gly} in *Xenopus* oocytes expressing glycine receptor subunits (Vaello et al., 1994; Nishizaki and Ikeuchi, 1995). These conflicting results could be due to regional differences in the expression of subunits making up the glycine receptor complex or to heterogeneity of the subunit combinations of glycine receptors in different brain areas (Betz, 1991).

4.2. The DAMGO-induced facilitation of I_{Gly} is partly mediated by activation of protein kinase C

A number of neurotransmitters, neuromodulators and neuropeptides may modulate the inhibitory responses elicited by glycine acting as an important inhibitory neurotransmitter in central nervous system neurons. The roles of protein kinase C in the agonist-induced modulation of glycine response are diverse. Norepinephrine facilitates the glycine receptor-mediated taurine responses in substantia nigra neurons. This facilitation is potentiated by protein kinase C activation with PMA and blocked by protein kinase C inhibition with staurosporin (Nabekura et al., 1996). In rat sacral dorsal commissural neurons, 5-HT-induced potentiation of I_{Gly} is mimicked by PMA and blocked by chelerythrine (Xu et al., 1996). However, experiments of other reports also performed in sacral dorsal commissural neurons have shown that, even in the same neurons, if the agonist types are different, the roles of protein kinase C in the agonist-induced modulation of I_{Gly} might be diverse. In these reports, the potentiation of the sacral dorsal commissural neuronal I_{Gly} by noradrenaline (Nabekura et al., 1999) and kainate (Xu et al., 1999) is not affected in the presence of PMA or chelerythrine.

In the present study, the potentiating effect of a μ-opioid receptor agonist on the periaqueductal gray neuronal I_{Gly} was not affected by PMA, but was partly blocked by chelerythrine or GF109203X. PMA itself exerted a facilitating effect on the periaqueductal gray neuronal I_{Gly}, and the current amplitude blocked by chelerythrine or GF109203X was similar to the current potentiated by the activation of protein kinase C with PMA. Thus, it might be suggested that the reason for PMA not exerting any influence on the potentiation effect of DAMGO is that DAMGO exerts its potentiation effect on the periaqueductal gray neuronal I_{Gly} through the same pathway as PMA.

The cDNAs coding for nine different protein kinase C isoenzymes have so far been cloned from different species and tissues or cell lines. They can be divided into two main groups: (1) Ca^{2+}-dependent or conventional protein kinase Cs and (2) Ca^{2+}-independent or novel protein kinase C (Hug and Sarre, 1993). Members of the novel protein kinase C group do not require Ca^{2+} for activation, but require either diacylglycerol or PMA. In the present experiments, the periaqueductal gray neuronal I_{Gly} was directly increased by PMA. Thus, a hypothesis can be formulated from the present results that the μ-opioid-induced potentiation of the periaqueductal gray neuronal I_{Gly} is partly mediated by activation of protein kinase C, mainly the novel protein kinase C. To confirm this hypothesis, however, more studies should be done to elucidate the effects of diacylglycerol and intracellular Ca^{2+}-dependent protein kinase C on the periaqueductal gray neuronal I_{Gly}.

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