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Baclofen Reduces $GABA_A$ Receptor Responses in Acutely Dissociated Neurons of Bullfrog Dorsal Root Ganglia

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ABSTRACT The effect of baclofen on the function of the γ-aminobutyric $acid_A$ ($GABA_A$) receptor was examined in acutely dissociated neurons of bullfrog dorsal root ganglia (DRG) by using the whole-cell voltage-clamp method. Baclofen (0.1–100 μM) depressed the inward currents produced by GABA (100 μM) and muscimol (100 μM). Baclofen shifted the concentration-response curve for GABA (1 μM–1 mM) downward. Baclofen decreased the maximum response (V_{max}) to GABA without changing the apparent dissociation constant (K_d), suggesting a noncompetitive antagonism. The effect of baclofen on the GABA current was blocked by antagonists for the $GABA_B$ receptor; the rank order of potency was P-[3-Aminopropyl]-P-diethoxymethylphosphinic acid (CGP 55845A) ≫ 3-N[1-(S)-(3,4-dichlorophenyl)ethyl]amino-2-(S)-hydroxypropyl-P-benzyl-phosphinic acid (CGP 35348) > saclofen ≫ phaclofen. Baclofen produced an irreversible depression of the GABA current in neurons dialyzed with an internal solution containing guanosine 5′-O-(3-thiotriphosphate) (GTPγS, 100 μM). Intracellular guanosine 5′-O-(2-thiodiphosphate) (GDPβS, 100 μM) blocked the inhibitory effect of baclofen on the GABA current. Forskolin (10 μM) and dibutyryl N^6, 2′-O-dibutyryladenosine 3':5′-cyclic monophosphate (db-cyclic AMP) (200 μM) depressed the GABA current. N-(2-aminoethyl)-5-isoquinolinesulfonamide (H-9, 40 μM) and N-(2-guanidinoethyl)-5-isoquinolinesulfonamide (HA-1004, 50 μM), protein kinase A (PKA) inhibitors, reduced the depressant effect of baclofen on the GABA current. The baclofen-induced depression of the GABA current was blocked by PKI(5–24), a specific PKA inhibitor, but not by PKC(19–36), a specific protein kinase C (PKC) inhibitor. We suggest that $GABA_B$ receptors regulate the $GABA_A$ receptor function through a G-protein linked to the adenylyl cyclase-PKA pathway in bullfrog DRG neurons. **Synapse 26:165–174, 1997.**

INTRODUCTION

γ-aminobutyric acid (GABA) is a major inhibitory transmitter in neurons of the vertebrate central nervous system (CNS). Morphological and pharmacological studies have identified two subtypes of GABA ($GABA_A$ and $GABA_B$) receptors in various neuronal tissues (Bormann, 1988; Mody et al., 1994). The $GABA_A$ receptor belongs to the superfamily of ligand-gated ion channels which comprise five subunits (Bormann, 1988; Schofield et al., 1987; Smith and Olsen, 1995), whereas the $GABA_B$ receptor is coupled to potassium channels and calcium channels as well as to second messenger systems by guanine nucleotide-binding (G) proteins (Bowery, 1989). The $GABA_A$ receptor possesses binding sites for GABA and its allosteric modulators, such as hypnotic, anesthetic, and anticonvulsant drugs (reviewed in Bormann, 1988; DeLorey and Olsen, 1992; Macdonald and Olsen, 1994; Sieghart, 1992). Recent functional studies have suggested that neurotransmitters also modulate the function of $GABA_A$ receptors in central neurons (Akhondzadeh and Stone, 1994; Chen and Wong, 1995) and of $GABA_C$ receptors in retinal bipolar cells (Feigenspan and Bormann, 1994; Wellis and Werblin, 1995).

$GABA_A$ and $GABA_B$ receptors are colocalized on cells in the dorsal root ganglion (DRG) and on terminals of primary afferents (Désarmenien et al., 1984a,b; Price et al., 1987). Such a colocalization raises the possibility of

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interactions between these two receptors. Baclofen, a prototypical agonist for $GABA_B$ receptors, has been shown to inhibit muscimol-stimulated $^{36}Cl^-$ uptake in cultured cerebellar granule cells (Kardos, 1989) and mouse cerebellum (Hahner et al., 1991). In this study, we investigated the effect of baclofen on $GABA_A$ receptor-activated currents in freshly dissociated neurons of bullfrog DRG by whole-cell patch-clamp techniques. The results showed that baclofen depressed $GABA_A$ receptor responses through a G-protein-cyclic AMP-dependent pathway.

MATERIALS AND METHODS

Dorsal root ganglia were rapidly isolated from bullfrogs (*Rana catesbeiana*) after decapitation and were incubated in a Ringer solution containing trypsin (Sigma type XI (St. Louis, MO) 1.4 mg/ml) and collagenase (Sigma type I; 0.6 mg/ml) for 2 h at 30°C. The Ringer solution had the following composition (in mM): NaCl, 112; KCl, 2; $CaCl_2$, 1.8; and $NaHCO_3$, 2.4. The pH of the solution was adjusted to 7.2. Neurons were dissociated by gentle pipetting and were collected by centrifugation (80*g* for 4–5 min). Dissociated cells were cultured for 1–3 days in Leibovitz's L-15 medium (Gibco 320-1415AG, Gaithersburg, MD) with 20% fetal bovine serum (Gibco 200-6140AG). The cultured ganglion cells were continuously superfused (2–4 ml/min) with the Ringer solution in the recording chamber (0.5 ml total volume). Pipettes for whole-cell voltage-clamp had a tip resistance of 3–6 MΩ, when filled with a internal solution having the following compositions (in mM): KCl, 100; $MgCl_2$, 4; adenosine 5′-triphosphate sodium (Na_2-ATP), 5; 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid, tetrapotassium salt (BAPTA), 1; and N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] (HEPES-sodium salt), 2.5 (pH adjusted with KOH to 7.0). The membrane patch was ruptured by increasing negative pressure in the pipette after gigaohm-seal contact (≥1 gigaohm) was established between the pipette and the cell membrane. A sample-and-hold/voltage-clamp amplifier (Axoclamp 2A, Axon Instruments, Foster City, CA) was used at the switching frequency of 7–10 kHz. Head-stage current gain of the amplifier was 0.1, indicating that clamping current as large as ±20 nA could be applied to the cell. Axodata and Axograph software programs (Axon Instruments) operating a Macintosh Centris 650 computer (Apple Computer, Inc., Cupertino, CA) through an ITC-16 computer interface (Instrutech Corp., Great Neck, NY) were used to command the membrane potential and to analyze membrane current. Drugs used in the present study included: muscimol, 4-amino-3-[4-chlorophenyl]-butanoic acid (baclofen), bicuculline, 2-hydroxy saclofen, phaclofen, adenosine-5′-O-(3-thiotriphosphate) (ATPγS), guanosine 5′-O-(3-thiotriphosphate) (GTPγS), guanosine 5′-O-(2-thiodiphosphate) (GDPβS), forskolin, N^6,2′-O-dibutyryladenosine 3′:5′-cyclic monophosphate (db-cyclic AMP) and N-(6-aminohexyl)-5-chloro-1-naphthalene-sulfonamide (W-7). All were purchased from Sigma Chemical Company (St. Louis, MO). 1-(5-isoquinoline-sulfonyl)-2-methylpiperazine (H-7), N-(2-aminoethyl)-5-isoquinolinesulfonamide (H-9), and N-(2-guanidino-ethyl)-5-isoquinolinesulfonamide (HA-1004) were obtained from RBI (Natick, MA). cAMP-dependent protein kinase (PKA) peptide inhibitor, PKI(5–24), was purchased from Promega Co. (Madison, WI). PKC(19–36) was from Peninsula Laboratories, Inc. (Belmont, CA). γ-aminobutyric acid (GABA) and picrotoxin were from Wako Pure Chemical Industries (Osaka, Japan). P-[3-aminopropyl]-P-diethoxymethylphosphinic acid (CGP 55845A) and 3-N[1-(S)-(3,4-dichlorophenyl)ethyl-]amino-2-(S)-hydroxypropyl-P-benzyl-phosphinic acid (CGP 35348) were gifts from CIBA-Geigy (Basle, Switzerland). All recordings were carried out at 22–24°C. The data are presented as mean ± SE.

RESULTS

Effects of baclofen on $GABA_A$ receptor responses

Under whole-cell patch-clamp, bath-application of GABA (100 μM) for 5–10 sec caused an inward current with amplitude of 2.1 ± 0.3 nA in acutely dissociated DRG neurons (n = 18) (Fig. 1). Bicuculline (50 μM) and picrotoxin (20 μM, $GABA_A$ receptor antagonists, completely blocked the inward current produced by GABA (GABA current) (Fig. 1A). Muscimol (100 μM), a $GABA_A$ receptor agonist, also produced an inward current (Fig. 1B). The amplitude of the muscimol (100 μM) current was similar to that of the GABA (100 μM) current. The muscimol-induced current was strongly depressed by bicuculline (50 μM) or picrotoxin (20 μM). Baclofen (up to 500 μM) produced no obvious inward current in dissociated bullfrog DRG neurons. These results suggest that the $GABA_A$ receptor is predominantly responsible for the inward current produced by GABA in DRG neurons of bullfrogs. Figure 2 shows the effect of baclofen (10 μM) on the GABA (100 μM) current. Application of baclofen to the Ringer solution for 2–5 min depressed the GABA current by about 30% of the control current in 85% of the DRG neurons tested (Fig. 2A). The depression of the GABA current persisted as long as baclofen was present in the superfusing solution. The GABA current recovered within 20 min after washout of baclofen. At a concentration of 10 μM, baclofen caused 28 ± 3% (n = 28) depression of the GABA current. The effect of baclofen (0.1–100 μM) on the GABA current was concentration-dependent (Fig. 2B). The minimum effective concentration of baclofen was 0.1 μM, producing 5 ± 1% (n = 8) depression of the GABA current. The maximum depression (42 ± 4%, n = 12) of the GABA current was produced by 100 μM baclofen. Figure 3 shows the effect of baclofen on the concentration-response relation for GABA. Baclofen (10 μM) shifted downward the sigmoidal concentration-re-

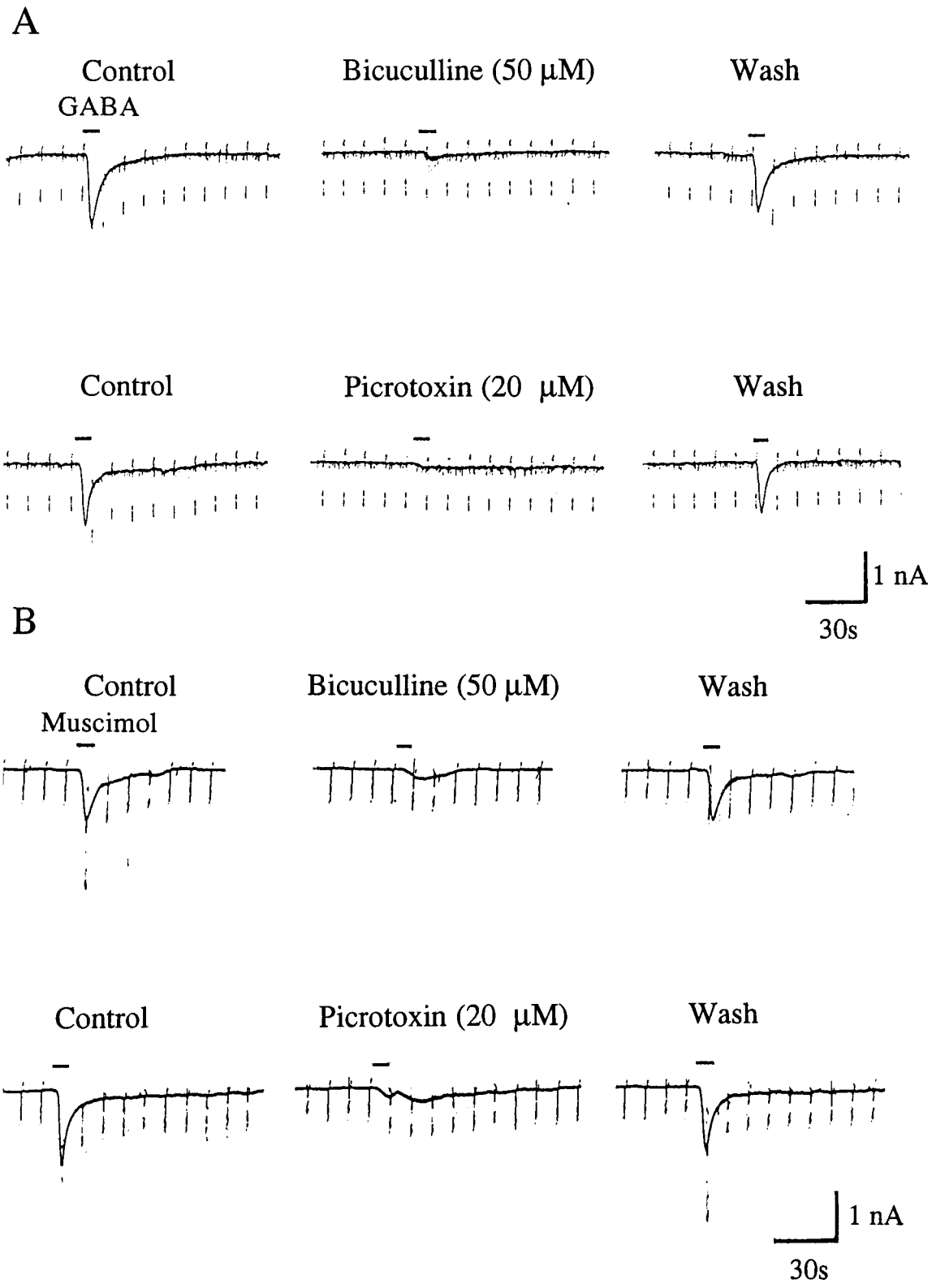


Fig. 1. Effects of bicuculline (50 μM) and picrotoxin (20 μM) on inward currents produced by GABA (**A**) and muscimol (**B**) in DRG neurons. Horizontal bars indicate application of GABA (100 μM) and muscimol (100 μM) to the Ringer solution. Bicuculline (50 μM) and picrotoxin (20 μM) were applied for 10 min to the superfusing (Ringer) solution. Downward deflections indicate inward currents produced by hyperpolarizing voltage-commands (40 mV for 200 msec).

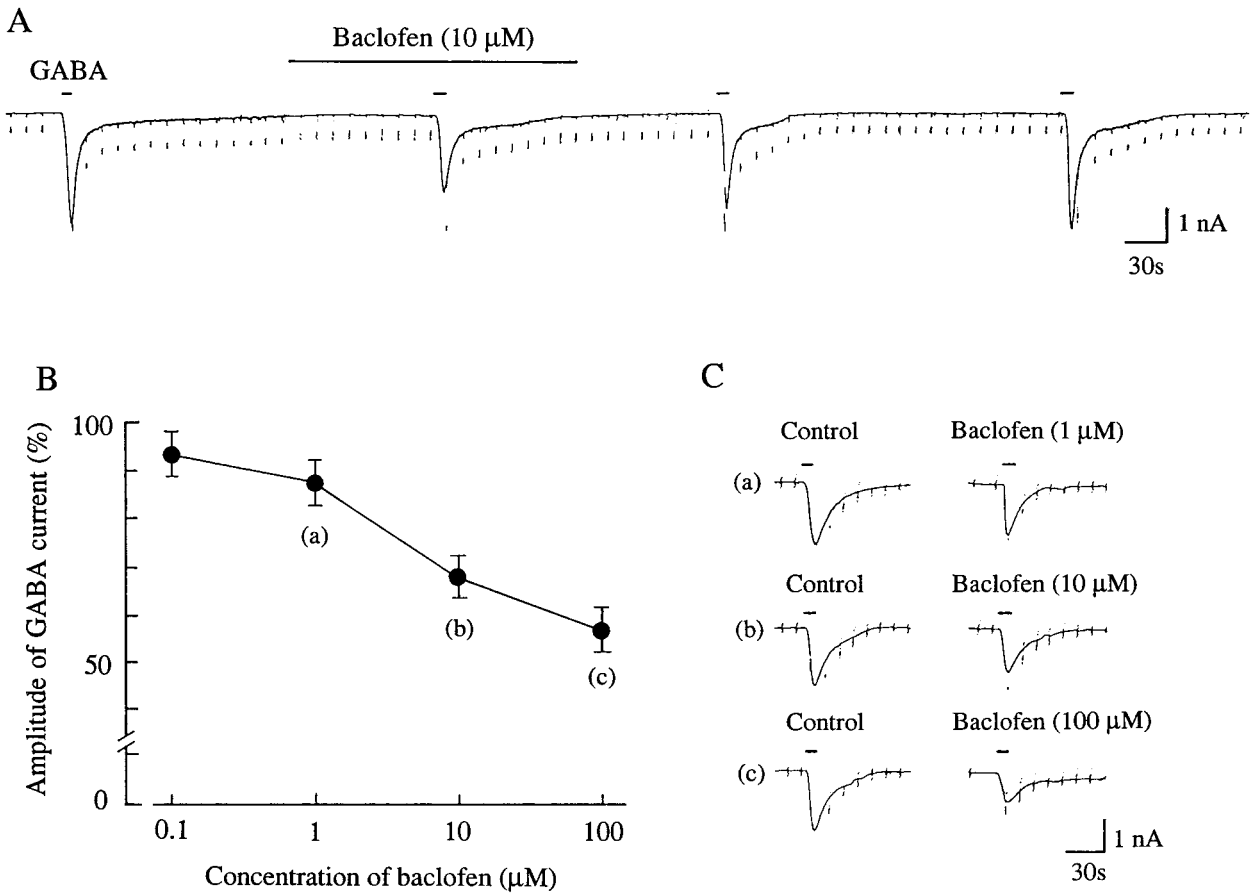


Fig. 2. Effect of baclofen on GABA current. **A:** Consecutive recording of inward currents produced by repeated applications of GABA (100 μM). Short and long horizontal bars indicate periods of applications of GABA and baclofen (10 μM), respectively. Downward deflections on current recordings were inward currents produced by hyperpolarizing voltage-command pulses (40 mV for 200 msec). **B:** Graph shows concentration-dependent inhibition of GABA current produced by baclofen (0.1–100 μM). Solid circles and vertical lines indicate mean amplitude of the GABA current and the SE of the mean. Ordinate indicates relative amplitudes of GABA current, where the amplitude of GABA currents recorded before application of baclofen represents 100%. **C:** Sample records of depressions of GABA current produced by the concentrations of baclofen (1–100 μM) shown in B.

sponse curve (Fig. 3B). The maximum response to GABA (V_{max}) and the apparent dissociation constant (K_d) were estimated by Lineweaver-Burk plot, a double-reciprocal plot of the concentration-response curve (Fig. 3C). Baclofen (10 μM) reduced the V_{max} of GABA to 76 ± 3% (n = 4). The K_d were 48 ± 6 μM (n = 4) and 52 ± 7 μM (n = 4) in the respective absence and presence of baclofen (10 μM). There was no statistical significance between these data. These results suggest that baclofen inhibits the GABA current in a noncompetitive manner.

$GABA_B$ receptors mediate depression of $GABA_A$ current

Effects of $GABA_B$ receptor antagonists, such as CGP 55845A, CGP 35348, saclofen, and phaclofen (Bittiger et al., 1993; Bonanno and Raiteri, 1993; Bowery, 1989; Brugger et al., 1993; Davies et al., 1993; Jarolimek et al., 1993; Malcangio et al., 1991; Soltesz et al., 1988), were examined in the baclofen-induced depression of the GABA current. When CGP 55845A (20 μM), a selective and potent $GABA_B$ antagonist, was applied to the Ringer solution, the amplitude of the GABA current was increased by 19 ± 3% (n = 7) (Fig. 4B). The baclofen (100 μM)-induced depression of the GABA current was almost completely prevented by CGP 55845A (1–20 μM). CGP 35384, at a relatively high concentration (100 μM), increased the amplitude of the GABA current and suppressed the inhibitory effect of baclofen (10 μM) on the GABA current (Fig. 4C). 2-hydroxy saclofen (100 μM), a commonly used $GABA_B$ antagonist, also inhibited the depressant effect of baclofen (100 μM) on the GABA current (Table I). Phaclofen (5 mM) produced only a partial inhibition of the effect of baclofen (100 μM) (Table I). The rank order of potency was CGP 55845A ≫ CGP 35348 > 2-hydroxy saclofen ≫ phaclofen. The effect of baclofen (0.1–100 μM) on the inward current produced by muscimol (muscimol current) was examined in DRG neurons. Baclofen (10 μM) depressed the muscimol (100 μM) current by 31 ± 3% (n = 4). Interestingly, application of

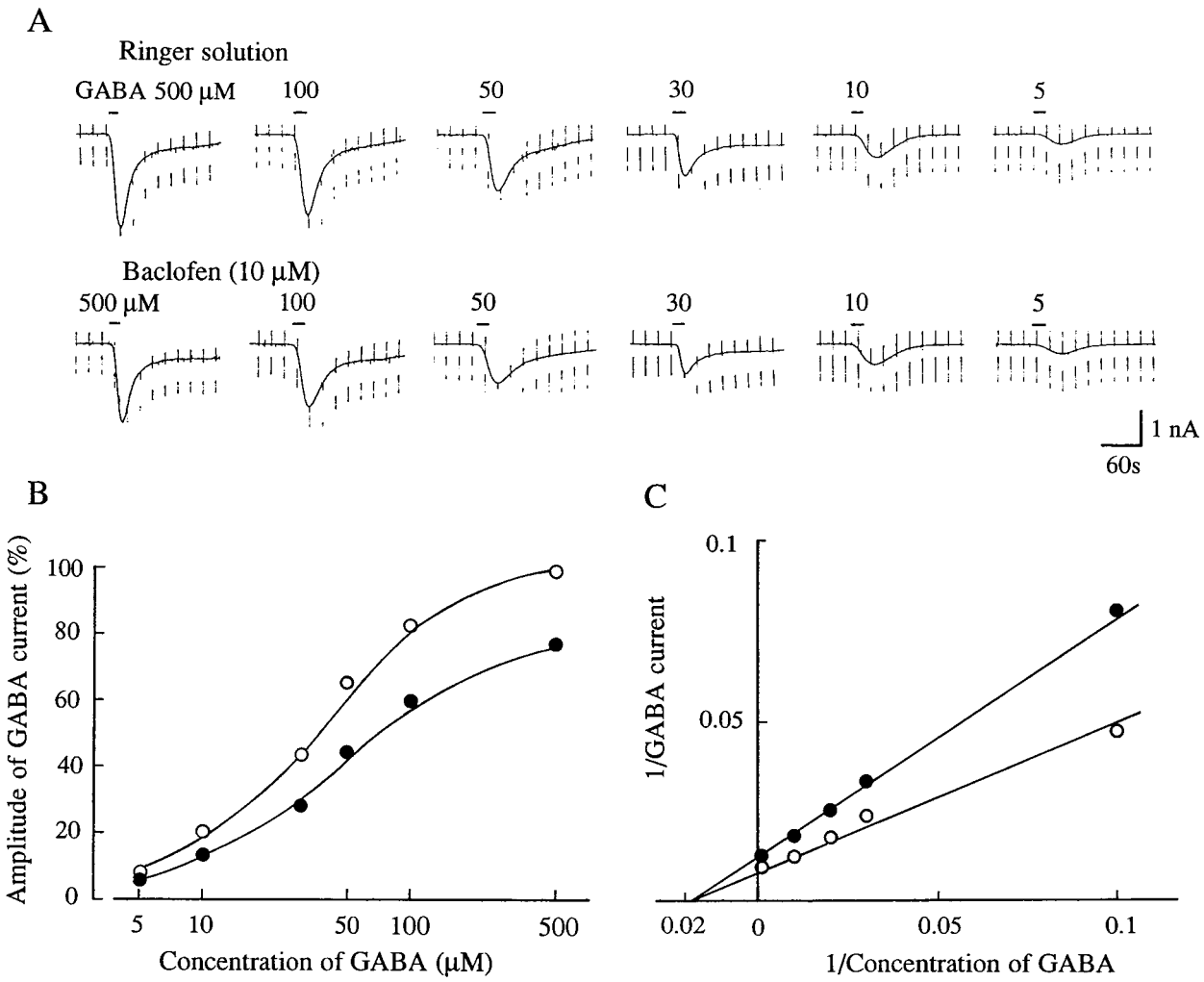


Fig. 3. Effect of baclofen (100 μM) on concentration-response relationship for GABA. **A:** Sample records of GABA (5–500 μM) current recorded in Ringer solution in absence (upper traces) and presence (lower traces) of baclofen (10 μM). **B:** Baclofen (10 μM) shifted the concentration-response curve downward. Ordinate and abscissa indicate amplitudes of GABA currents and concentrations of GABA, respectively. The inward current produced by 500 μM GABA represents 100%. **C:** Double-reciprocal plot of concentration-response data. Intersections of the double-reciprocal plot with the abscissa and the ordinate indicate apparent dissociation constant (K_d) and maximum response (V_{max}), respectively. In B and C, open and solid circles indicate responses obtained in the absence and presence of baclofen (100 μM), respectively. Data were taken from A.

CGP 55845A did not increase but slightly depressed the muscimol current (Fig. 5B).

G-protein-coupling in baclofen-induced modulation of GABA current

The contribution of a G-protein to the inhibitory effect of baclofen on GABA$_A$ receptor function was examined in DRG neurons. Guanosine 5′-O-(3-thiotriphosphate) (GTPγS), a GTP analogue which irreversibly activates G-proteins, was applied to the intracellular space of DRG neurons through the recording patch pipette. Application of GABA (100 μM) caused an inward current with amplitudes of 2.1 ± 0.8 nA (n = 4) immediately after the beginning of whole-cell recording. The first application of baclofen (100 μM) produced a typical depression (26 ± 3%, n = 4) of the GABA current. However, the depression did not completely recover but progressively increased in GTPγS-treated neurons, even when baclofen was removed from the superfusing solution. In neurons that had been treated with GTPγS (100 μM) for 30 min, the GABA current was depressed to 0.9 ± 0.2 nA (n = 6) (Fig. 6). A third application of baclofen produced no depression of the GABA current. GDPβS (100 μM), a pseudosubstrate for G-protein, was applied to the intracellular space of DRG neurons to block the action of the G-protein. Baclofen produced no depression of the GABA current (Fig. 6). The amplitude of the GABA current was 1.7 ± 0.2 nA (n = 4) when baclofen (100 μM) was applied to DRG neurons that had been dialyzed with GDPβS (100

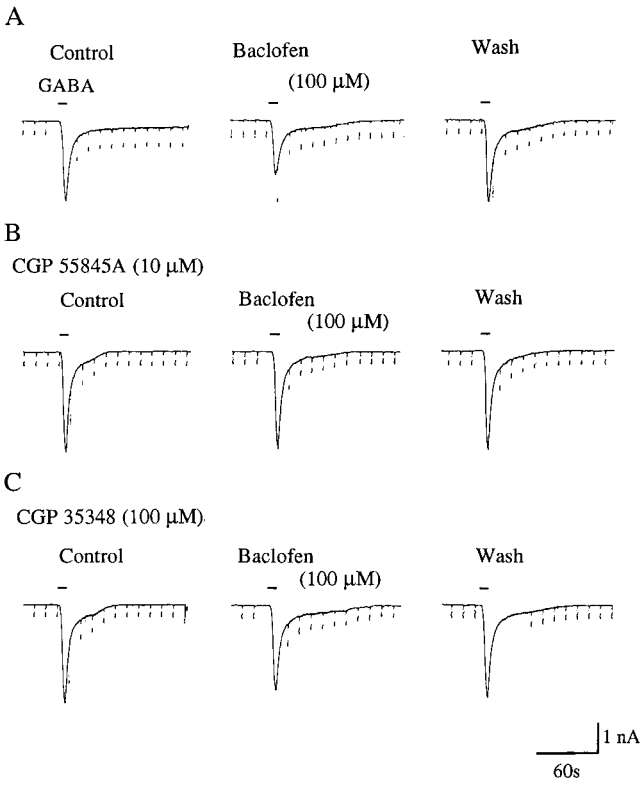


Fig. 4. Effects of $GABA_B$ receptor antagonists on baclofen-induced depression of GABA current. GABA (100 μM) was applied to the neuron for 10 sec. **A:** Baclofen-induced depression of GABA current obtained in Ringer solution. **B:** Effect of baclofen on GABA current in the presence of CGP 55845A (10 μM). **C:** Effect of baclofen on GABA current in the presence of CGP 35348 (100 μM). All records were taken from the same cell.

TABLE I. Block by $GABA_B$ receptor antagonists of baclofen-induced depression of GABA current[1]

	Depression of GABA current by baclofen (100 μM) (%)
Control	39 ± 3 (n = 8)
CGP 55845A (10 μM)	4 ± 1 (n = 6)**
CGP 35348 (100 μM)	12 ± 4 (n = 5)**
Saclofen (100 μM)	17 ± 3 (n = 5)**
Phaclofen (5 mM)	21 ± 5 (n = 4)*

[1]Drugs were applied to the superfusing solution. Number (n) of experiments is shown in parentheses. Asterisks indicate significant differences between drugs vs. control calculated with Student's t-test.
*$P < 0.05$.
**$P < 0.01$.

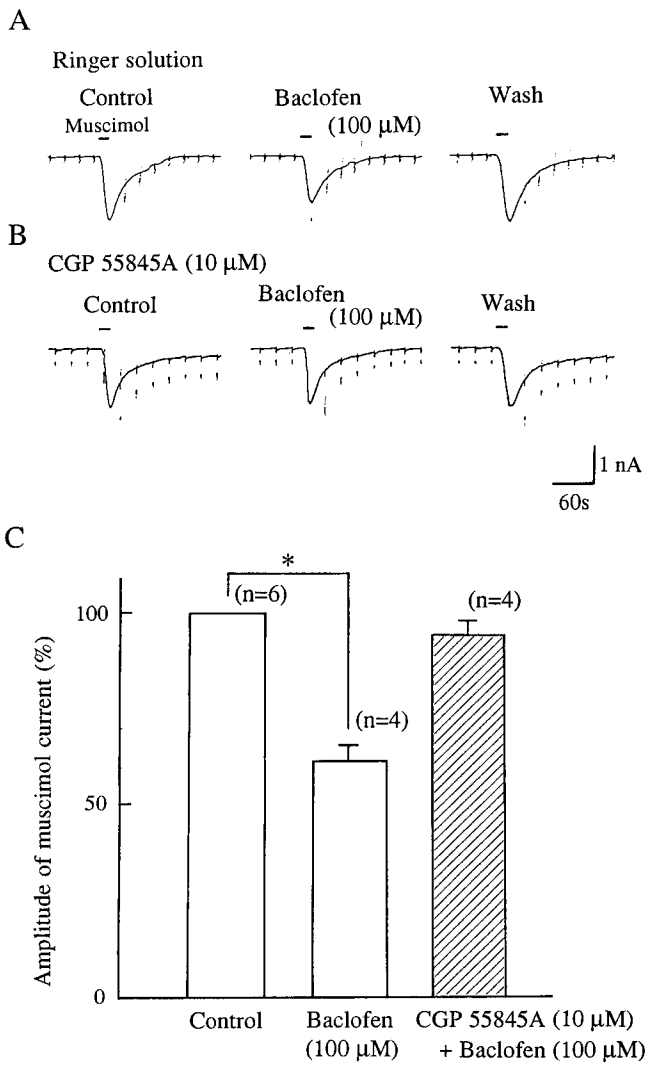


Fig. 5. Effect of baclofen on muscimol-induced current. **A:** Sample records of inhibitory effect of baclofen (100 μM) on muscimol current. Horizontal bar indicates period of bath-application of muscimol (100 μM). **B:** Effects of CGP 55845A (10 μM) on baclofen-induced depression of muscimol current. Muscimol was applied to Ringer solution for 10 sec. Note that CGP 55845A did not increase the amplitude of the muscimol current. **C:** Numerical data for baclofen-induced depression of muscimol current obtained from six cells. Control amplitude of muscimol current obtained before application of baclofen (100 μM) represents 100%. Vertical line on top of the bars indicates SE of the mean. Number of experiments is shown in parentheses.

μM) for 30 min (Fig. 6). GDPβS may block the baclofen-induced modulation of the GABA current.

Role of protein kinases in depression of GABA current

The contribution of cyclic AMP to the $GABA_B$-receptor-mediated depression of $GABA_A$ receptor function was examined in DRG neurons. Bath-application of forskolin (10 μM), an activator of adenylyl cyclase, decreased the amplitude of GABA currents by 37 ± 3% (n = 5) (Fig. 7). The effect of forskolin on the GABA current was restored, when DRG neurons were superfused with recovery solution (Ringer solution) for 20 min. Dibutyryl cyclic AMP (db-cyclic AMP, 200 μM), a membrane-permeable cyclic AMP analogue, also reversibly reduced the amplitude of GABA currents by 33 ± 4% (n = 10) (Fig. 7). We next examined the contribution of protein kinases to the modulation of $GABA_A$ receptor function. H-9 (40 μM), an inhibitor of protein kinase A (PKA), was applied to the intracellular space of DRG neurons through a recording patch pipette. The GABA current showed no obvious "run-down" during the whole-

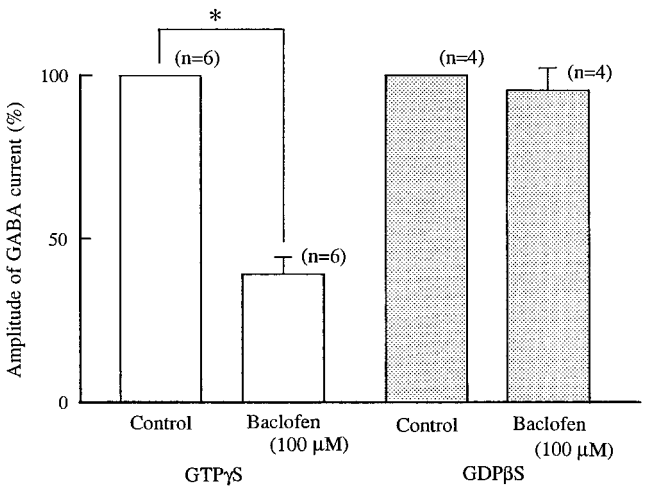


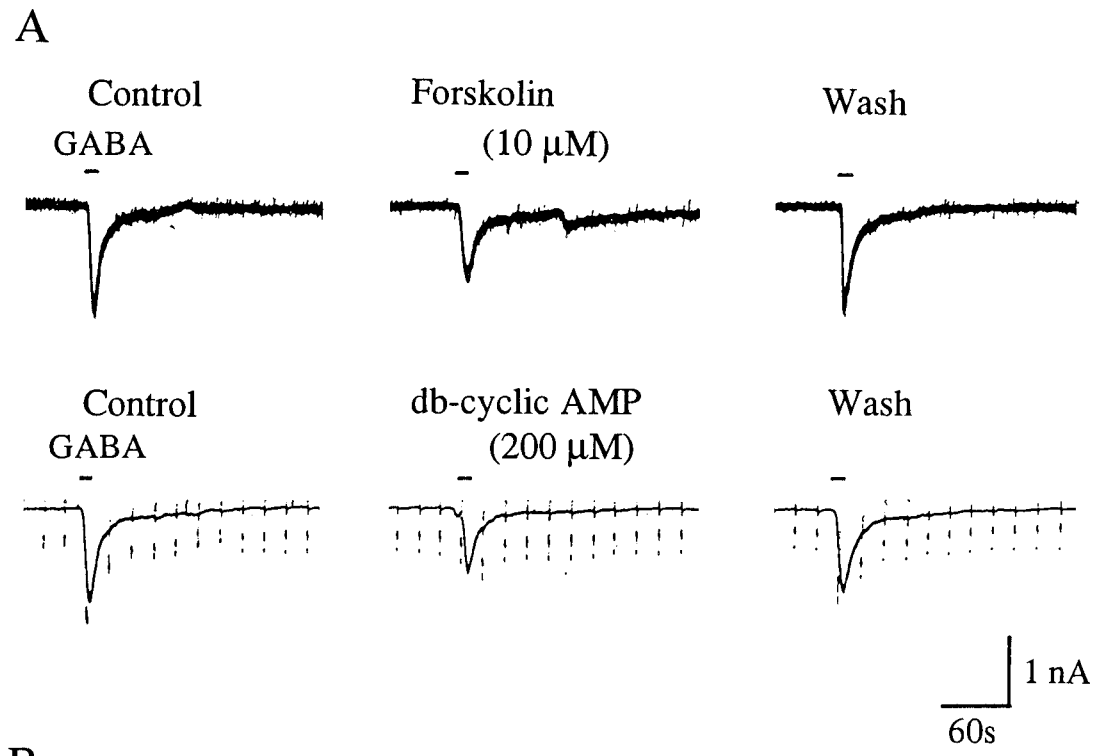
Fig. 6. Effect of baclofen (100 μM) on GABA current in DRG neurons treated with GTPγS (open columns) and GDPβS (stippled columns). Amplitude of GABA currents obtained before application of baclofen (control) was normalized as 100%. Number of experiments is shown in parentheses. Vertical line on top of the bars indicates SE of the mean.

cell recording (for 30–40 min). In these neurons, baclofen (100 μM) produced 20 ± 3% (n = 4) depression of the GABA current (Table II). Neurons were also treated with HA-1004 (50 μM), a relatively selective inhibitor of PKA (Hidaka et al., 1984). Baclofen (100 μM) produced depression of the GABA current by 17 ± 3% (n = 4) in neurons treated with HA-1004 (Table II). The depression of GABA currents was statistically significant ($P < 0.05$) (Table II). H-7 (200 μM), a broad-spectrum protein kinase inhibitor, and W-7 (100 μM), a Ca^{2+}-calmodulin kinase inhibitor, showed no significant effect on the baclofen-induced depression of GABA current (Table II). We next examined the effects of specific inhibitors for protein kinases on the baclofen-induced depression of GABA current. Cyclic-AMP-dependent protein kinase (PKA) peptide inhibitor, a peptide known as PKI(5–24) (10 μM), was applied to the intracellular space of DRG neurons. Baclofen produced only 8 ± 3% (n = 6) depression of the GABA current in DRG neurons treated with PKI(5–24). In contrast, PKC(19–36) (20 μM), a specific PKC inhibitor, applied intracellularly to DRG neurons, did not prevent the baclofen-induced depression of GABA current. Data for these experiments are shown in Table II. The forskolin-induced depression of GABA current was reduced to 7 ± 4% (n = 3) in neurons intracellularly dialyzed with an internal solution containing PKI(5–24) (10 μM). It has been demonstrated that dephosphorylation of GABA$_A$ receptors by a Ca^{2+}-dependent phosphatase, calcineurin, results in the "run-down" of GABA$_A$ receptor responses (Chen and Wong, 1995; Chen et al., 1990; Stelzer et al., 1988). To test this possibility of the inhibitory effect of baclofen on GABA current, DRG neurons were treated with ATPγS (5 mM) or okadaic acid (3 μM), which block phosphatase activity. Baclofen (100 μM) produced typical depressions of the GABA current (Table II) in neurons internally dialyzed with a pipette solution containing either ATPγS or okadaic acid.

DISCUSSION

The present study shows that treatment of DRG neurons with baclofen depresses the inward currents produced by GABA and muscimol in acutely dissociated DRG neurons of bullfrogs. Baclofen shifted the concentration-response curve for GABA downward. A Lineweaver-Burk plot showed that baclofen (10 μM) reduced the V_{max} without changing the apparent dissociation constant (K_m). These results suggest that baclofen acts at an allosteric site rather than at the specific binding site for GABA/muscimol on the GABA$_A$ receptor-ionic channel complex. CGP 55845A, a selective and potent GABA$_B$ receptor antagonist (Bittiger et al., 1993; Brugger et al., 1993; Davies et al., 1993; Jarolimek et al., 1993), prevented the baclofen-induced depression of the GABA current. At a relatively higher concentration, GABA$_B$ antagonists, such as CGP 35348 (Bittiger et al., 1993; Bonanno and Raiteri, 1993; Malcangio et al., 1991) and 2-hydroxy-saclofen (Bittiger et al., 1993), also blocked the inhibitory action of baclofen on the GABA current. Phaclofen (5 mM) (Soltesz et al., 1988), another GABA$_B$ receptor antagonist, was less effective in producing depression of the GABA current. These results indicate that the activation of GABA$_B$ receptors downregulates the function of GABA$_A$ receptors in bullfrog DRG neurons. Interestingly, the application of CGP 55845A (20 μM) and CGP 35348 (100 μM) increased the amplitude of GABA currents, while they did not affect the muscimol-induced current. Since GABA is a mixed GABA$_A$ and GABA$_B$ receptor agonist, application of GABA would simultaneously depress the GABA$_A$ receptor response, even when baclofen was absent.

The results of the present study suggest that a G-protein-coupled second messenger system transduces the baclofen-induced depression of the GABA current. Baclofen irreversibly depressed the GABA current in DRG neurons that had been dialyzed with a GTPγS-containing solution. Inactivation of G-protein by intracellular application of GDPβS resulted in the block of the baclofen-induced depression of the GABA current. Application of forskolin and db-cyclic AMP depressed the GABA current in DRG neurons. These results suggest that a G-protein, probably Gs, mediates the baclofen-induced modulation of the GABA$_A$ receptor in DRG neurons. It has been demonstrated that the GABA$_A$ receptor function is modulated through second messengers, such as PKC and PKA (Browning et al., 1990; DeLorey and Olsen, 1992; Harrison and Lambert, 1989; Moss et al., 1992; Nestler and Greengard, 1984; Porter et al., 1990; Tehrani et al., 1989). Intracellular application of PKA inhibitors, such as H-9, and HA-



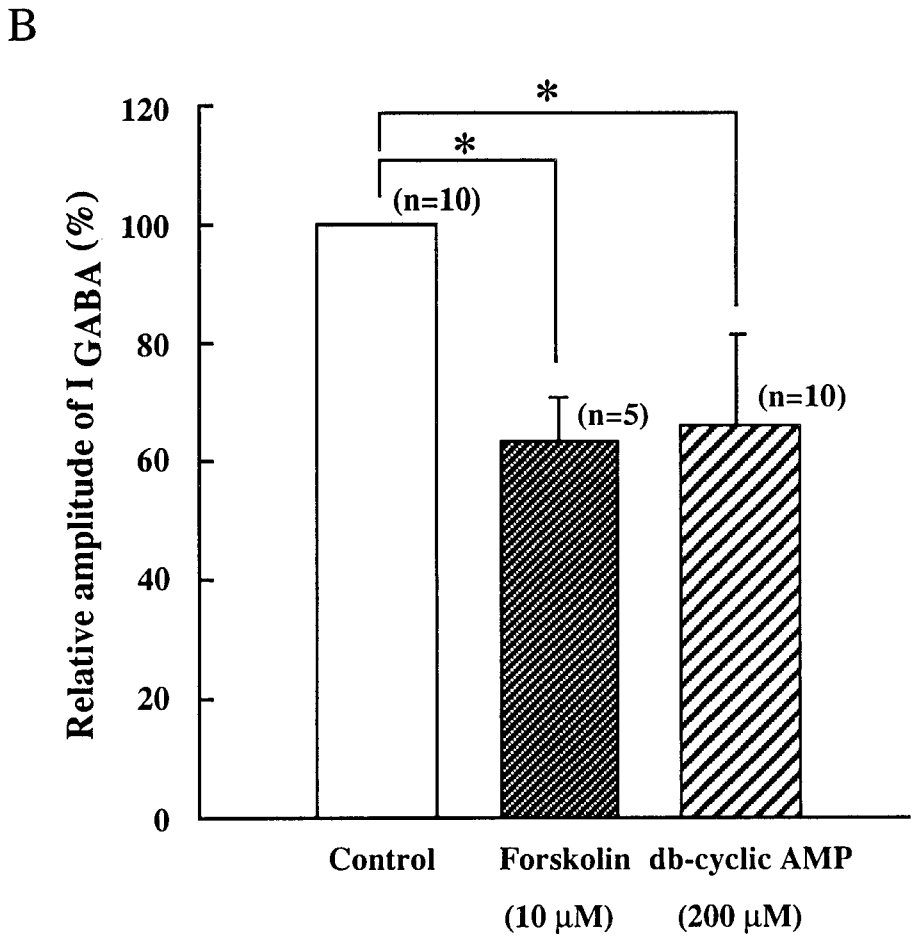


Fig. 7. Effects of forskolin (upper traces) and db-cyclic AMP (lower traces) on GABA current. **A:** Sample records of these experiments. Forskolin (10 μM) and db-cyclic AMP (200 μM) were applied to Ringer solution for 10 min. Horizontal bar indicates period of application of GABA (100 μM). **B:** Numerical data for inhibitory effects of forskolin and db-cyclic AMP on GABA current. Amplitude of GABA currents taken before application of forskolin or db-cyclic AMP was normalized as 100%. Vertical line on top of the bars indicates SE of the mean. Asterisks indicate statistical significance ($P < 0.01$).

TABLE II. Effects of protein kinase inhibitors, phosphatase inhibitors, and ATPγS on baclofen-induced inhibition of GABA current[1]

	Depression of GABA current by baclofen (10 μM) (%)
Control	28 ± 2 (n = 8)
H-9 (40 μM)	20 ± 3 (n = 4)*
HA-1004 (50 μM)	17 ± 3 (n = 4)*
PKI (5–24) (10 μM)	8 ± 3 (n = 6)**
H-7 (200 μM)	23 ± 3 (n = 4) n.s.
PKC (19–36) (20 μM)	25 ± 4 (n = 6) n.s.
Okadaic acid (3 μM)	29 ± 4 (n = 6) n.s.
W-7 (50 μM)	25 ± 2 (n = 3) n.s.
ATPγS (5 mM)	26 ± 2 (n = 3) n.s.

[1]All drugs except GABA were included in the pipette solution. n.s., statistically not significant. Asterisks indicate significant differences between drugs vs. control calculated with Student's t-test.
*$P < 0.05$.
**$P < 0.01$.

1004 (Hidaka et al., 1984), significantly inhibited the effect of baclofen in depressing the GABA current. H-7, a broad-spectrum protein kinase inhibitor effective on PKC (Hidaka et al., 1984), did not block the baclofen-induced depression of GABA current. PKA peptide inhibitor PKI(5–24), that inhibits phospholylation of target proteins by binding to the protein-substrate site of the catalytic subunit of PKA, significantly reduced the effect of baclofen in depressing the GABA current. PKC(19–36), a specific PKC inhibitor, did not prevent the inhibitory effect of baclofen on the GABA current. These results suggest that PKA mediates the transduction pathway of the baclofen-induced depression of the GABA current in DRG neurons.

Recently, Chen and Wong (1995) reported that NMDA suppresses GABA responses via dephosphorylation of $GABA_A$ receptors mediated by the Ca^{2+}-dependent phosphatase calcineurin in guinea-pig hippocampal neurons. ATPγS has been known to thiophosphorylate the substrate protein, which is resistant to hydrolysis by phosphatase (Eckstein, 1985). Okadaic acid is also known to be an inhibitor of phosphatase (Bialojan and Takai, 1988). If dephosphorylation is involved in the baclofen-induced depression of the GABA current, ATPγS and okadaic acid are expected to reduce the depressant effect of baclofen. In the present study, replacement of intracellular ATP with ATPγS did not prevent the baclofen-induced depression of the GABA current. Baclofen produced depression of the GABA current in neurons treated with okadaic acid. Therefore it seems unlikely that dephosphorylation of $GABA_A$ receptors mediates the baclofen-induced depression of GABA current.

It has been shown that baclofen does not depress the $GABA_A$-receptor response produced by rapid (0.1–0.6 sec) application of GABA in rat cortical neurons (Howe et al., 1987) and hippocampal neurons (Harrison et al., 1988). Baclofen had no effect on the GABA receptor function at short flux time of $^{36}Cl^-$ uptake in central neurons (Allan et al., 1980; Mehta and Ticku, 1990). In contrast, baclofen strongly reduced muscimol-stimulated $^{36}Cl^-$ uptake by cerebellar granule cells at the relatively prolonged baclofen exposure times of 7 or 14 sec (Hahner et al., 1991; Kardos, 1989). The present study showed that bath-application of baclofen for 5–10 min suppressed the inward current produced by a 5-sec exposure of neurons to GABA. Cyclic AMP has been shown to increase the rate of $GABA_A$ receptor desensitization in chick cortical neurons through a G-protein-coupled pathway (Tehrani et al., 1989). These results imply that cyclic AMP-dependent desensitization of GABA receptors (Dingledine and Korn, 1985; Hahner et al., 1991) is involved in the baclofen-induced depression of the GABA current in bullfrog DRG neurons.

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