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Modulation of Recombinant GABA Receptor/Channel Subunits by Domain-specific Antibodies in *Xenopus* Oocytes

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Abstract. To study interaction of specific antibodies with the GABA receptor/channel, antisera were raised against the extracellular domains of the $GABA_A$ receptor/channel β_2 subunit, γ_2 subunit and the $GABA_C$ receptor/channel ρ_1 subunit. The specificity of the antibodies was characterized by immunocytochemistry and by Western blotting of transfected FDC-P1 cells expressing recombinant GABA receptor/channel subunits. The effects of the antibodies on whole-cell currents in *Xenopus laevis* oocytes expressing homomeric recombinant GABA receptor/channel β_2, γ_2, and ρ_1 were studied using two-microelectrode voltage clamp. In the absence of GABA, anti-α_2, anti-γ_2, and anti-ρ_1 antisera elicited whole-cell currents in oocytes expressing β_2, γ_2, and ρ_1 subunits, respectively. The effect of antibody on channel activation was concentration-dependent. The whole-cell currents induced by anti-β_2 and anti-γ_2 were several-fold greater than those induced by application of 100 μM GABA. In *Xenopus* oocytes expressing recombinant ρ_1 subunits, GABA-induced whole-cell currents were inhibited by the anti-ρ_1 antibody. In contrast, the GABA-induced whole-cell currents were potentiated several-fold by anti-β_2 and anti-γ_2 antibodies in *Xenopus* oocytes expressing homomeric β_2 and γ_2 subunits. Our studies indicate that antibodies specific to the N-terminal domain of GABA receptor/channel subunits can modulate the neurotransmitter receptor function.

Key words: Gene expression — Fusion protein — Voltage clamp — Whole-cell current

Introduction

The GABA receptors are the main inhibitory neurotransmitter receptors in the mammalian brain. They have been classified into three categories based on their biophysical and pharmacological properties: $GABA_A$ receptors are sensitive to antagonist bicuculline; $GABA_B$ receptors are sensitive to agonist baclofen; and $GABA_C$ receptors are not sensitive to either bicuculline or baclofen (Study & Barker, 1981; Feigenspan, Wassle & Bormann, 1993; Feigenspan & Bormann, 1994; Matthews, Ayoub & Heidelberger, 1994). $GABA_A$ and $GABA_C$ receptors are ionotropic, forming GABA-gated Cl^- conductive channels. $GABA_B$ receptors are metabotropic, coupling to K^+ and Ca^{2+} channels via G-proteins.

The ionotropic GABA receptor/channel subunits are membrane proteins with a large extracellular N-terminus domain, four transmembrane domains, and an intracellular loop between the third and fourth transmembrane domains. Subunits are held in a putative pentameric arrangement to form a receptor/channel. The extracellular N-terminal domain contains a site for GABA binding as well as binding sites for agonists and modulators. Amongst others, steroids and barbiturates can activate $GABA_A$ receptor/channels in the absence of GABA, and potentiate the GABA response at low concentrations of GABA. The barbiturate and steroid binding sites are distinct from the GABA binding site, and the sites for channel potentiation by steroids and barbiturates are distinct from the sites for channel gating (Macdonald & Olsen, 1994). Other modulators, such as benzodiazepines, have distinct binding sites in the extracellular N-terminus domain (Buhr et al., 1996, 1997; Amin, Brooks-Kayal & Weiss, 1997; Wingrove et al., 1997; Benson et al., 1998; McKernan et al., 1998; Sigel et al., 1998). Unlike the $GABA_A$ receptor/channels, the $GABA_C$ receptor/channel is not modulated by these compounds (DelRaso, Huang & Lu, 1996; Lu, 1997), but zinc can allosterically modulate $GABA_C$ receptor/channel (Wang et al., 1995).

It has been shown that ion channel conductance can be modulated in some cases by antibody binding. Anti-

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body binding inhibits the conductance of chloride channels in *Necturus* gallbladder and in pancreatic zymogen granule membranes (Thevenod, Anderie & Schulz, 1994). The conductance of sodium channels of mammalian nerves is also inhibited by antibody binding (Meiri et al., 1984, 1986, 1987). We have examined the effect of domain-specific antibodies on GABA responses of individual recombinant $GABA_A$ and $GABA_C$ receptor/channel subunits expressed in *Xenopus laevis* oocytes. The antibodies potentiate GABA responses of GABA receptor/channel β_2 and γ_2 subunits, and inhibit GABA responses of the ρ_1 subunit. Furthermore, our results demonstrate that these antibodies were able to directly activate recombinant GABA receptor/channels in oocytes in the absence of GABA.

Materials and Methods

Recombinant DNA Construction

PCR was used to amplify N-terminus cDNA fragments from full-length cDNA. The PCR primers were as follows: β_2 sense, 5′-GGATCCTCTCT-TCAGTGAATCCC-3′; β_2 antisense, 5′-CTCGAGCCAATGTTTCT-TTTCAGCTTA-3′; γ_2 sense, 5′-GGATCCGGCGAGAGGAAAAAAA-AGCG-3′; γ_2 antisense, 5′-CTCGAGCCCCATTCTTCTGCTCAGATC-3′; ρ_1 sense, 5′-GGATCCCCATGTTGGCTGTCCCA-3′; ρ_1 antisense, 5′-GGAGACCCAGGAGAGGATGGTCATGACCATCAGG-GTAGC-3′. The amplified N-terminus sequences (β_2 N′-774 bp, γ_2 N′-837 bp, ρ_1 N′-1.2 Kbp) were subcloned in the multi-cloning site (MCS) of the pGEX vector (Pharmacia, Piscataway, NJ) with the correct reading frame. The constructed cDNA was sequenced to verify the reading frame and the void of mutations.

Fusion Proteins

Ten ng plasmid DNA was transformed into 200 μl SRP-84 competent *E. coli.* Single colonies 2–3 mm in diameter were chosen to grow in 5 ml LB/Amp medium overnight at 37°C with vigorous shaking. The 5-ml overnight culture was transferred into 250 ml LB/Amp and incubated with shaking for 4–6 hr at 37°C. Isopropyl-β-D-thiogalactoside (IPTG) was supplemented to a final concentration of 0.1 mM to induce fusion protein synthesis, and the cells were cultured overnight at 30°C with vigorous shaking. The cells were collected by centrifugation at 8000 rpm for 10 min. 50 μl 1XPBS was added per ml of culture, and the cells were lysed by sonicating on ice. The lysate was centrifuged at 12,000 rpm for 10 min and the supernatant was collected. The supernatant was transferred into a glutathione sepharose 4B (Pharmacia) column (1 ml glutathione sepharose 4B per 6 ml sonicate) and incubated overnight with mild shaking at 4°C. The column was washed 5 times with 1XPBS, and the fusion protein was eluted with a glutathione elution buffer (10 mM reduced glutathione in 50 mM Tris-HCL [pH 8.0]). The fusion protein was concentrated by dialysis and quantified on a PAGE gel.

Antiserum Production

The purified fusion protein was concentrated to 1 mg/ml in 1XPBS and shipped to Research Genetics, Huntsville, AL, for inoculation in animals. Inoculation and bleeding of the animals was according to standard procedure. Anti-β_2 and anti-γ_2 antisera were raised in rabbits, while anti-ρ_1 antiserum was raised in guinea pigs. For rabbits, test bleeds were performed at 4 and 8 weeks followed by a final bleed at 10 weeks. For guinea pigs, a single exsanguination was performed after 10 weeks.

Immunocytochemistry on Transfected FDCP-1 Cells

FDCP-1 cells were cultured and aliquoted into six electroporation vials (1×10^7 cells per vial). Full-length β_2, γ_2, and ρ_1 cDNA subcloned in PCR3 vector were added to the electroporation vials to a final concentration of 0.125 ng/μl. The FDCP-1 cells were transfected with cDNA by electroporation at 200 V. The transfected cells were cultured for 18 hr, washed in PBS and fixed with 4% paraformaldehyde solution. Primary antibody (anti-β_2, anti-γ_2, and anti-ρ_1) was diluted to 1:15,000, and incubated overnight at 4°C. Cells were rinsed 5 times with PBS to wash out the primary antibody. Secondary antibody (fluorescene-conjugated anti-rabbit IgG or rhodamine-conjugated anti-guinea pig IgG) was added to a 1:50 dilution, and incubated at room temperature for 1 hr. The secondary antibody was washed by PBS rinses for 4 times followed by one rinse in TBS-T. The cells were mounted on glass slides, allowed to air-dry, covered with coverslip, and observed using the fluorescence microscopy.

Western Blot

FDCP-1 cells were transfected with full-length α_1, β_2, γ_2, and ρ_1 cDNA in PCR3 plasmid. The cells were cultured for 18 hr post-transfection and collected by centrifugation. The cells were then treated with 100 μl urea-Tris (0.1% Triton X100, Tris, 9 M urea) for 30 min. An equal volume of SDS-PAGE sample buffer was added and the mixture was boiled for 5 min, followed by centrifugation at 12,000 rpm for 10 min to collect the supernatant. 25 μl of supernatant was loaded onto 12% SDS-PAGE gels and electrophoresed for 1 hr at 200 V. Gels were blotted onto nitrocellulose membranes at 150 V for 1 hr. The membranes were blocked in 10% milk and 3% albumin for 1 hr at room temperature. Primary antibody (1:15,000–1:20,000) was added in blocking buffer and incubated overnight at 4°C with shaking. The membranes were then washed 4 times in TBS-T, secondary antibody (1:1000 of AP-linked anti-rabbit IgG or anti-guinea pig IgG) added, and incubated at room temperature for 1 hr on a rocking table.

In Vitro Transcription

The cDNA fragments of β_2, γ_2, and ρ_1 subunits were subcloned into the multiple cloning site of the expression vector PCR3 in the T7 to SP6 orientation. Plasmid DNAs with insert were linearized with PstI (New England Biolabs, Beverly, MA). The T7 in vitro transcription kit (Invitrogen, Carlsbad, CA) was used in all transcription reactions. Transcription reactions were carried out in the presence of the cap analog diguanosine triphosphate, and were catalyzed by T7 RNA polymerase. The cRNA was quantified by agarose gel electrophoresis and photospectroscopy (Pharmacia, Piscataway, NJ) and dissolved in DEPC-treated RNase-free water to a final concentration of ~2 μg/μl.

Oocyte Preparation and Microinjection of cRNA

Xenopus laevis (*Xenopus* I, Dexter, MI) were anesthetized by immersion in 0.15% ethyl 3-aminobenzoate methanesulfonic acid salt (Tri-

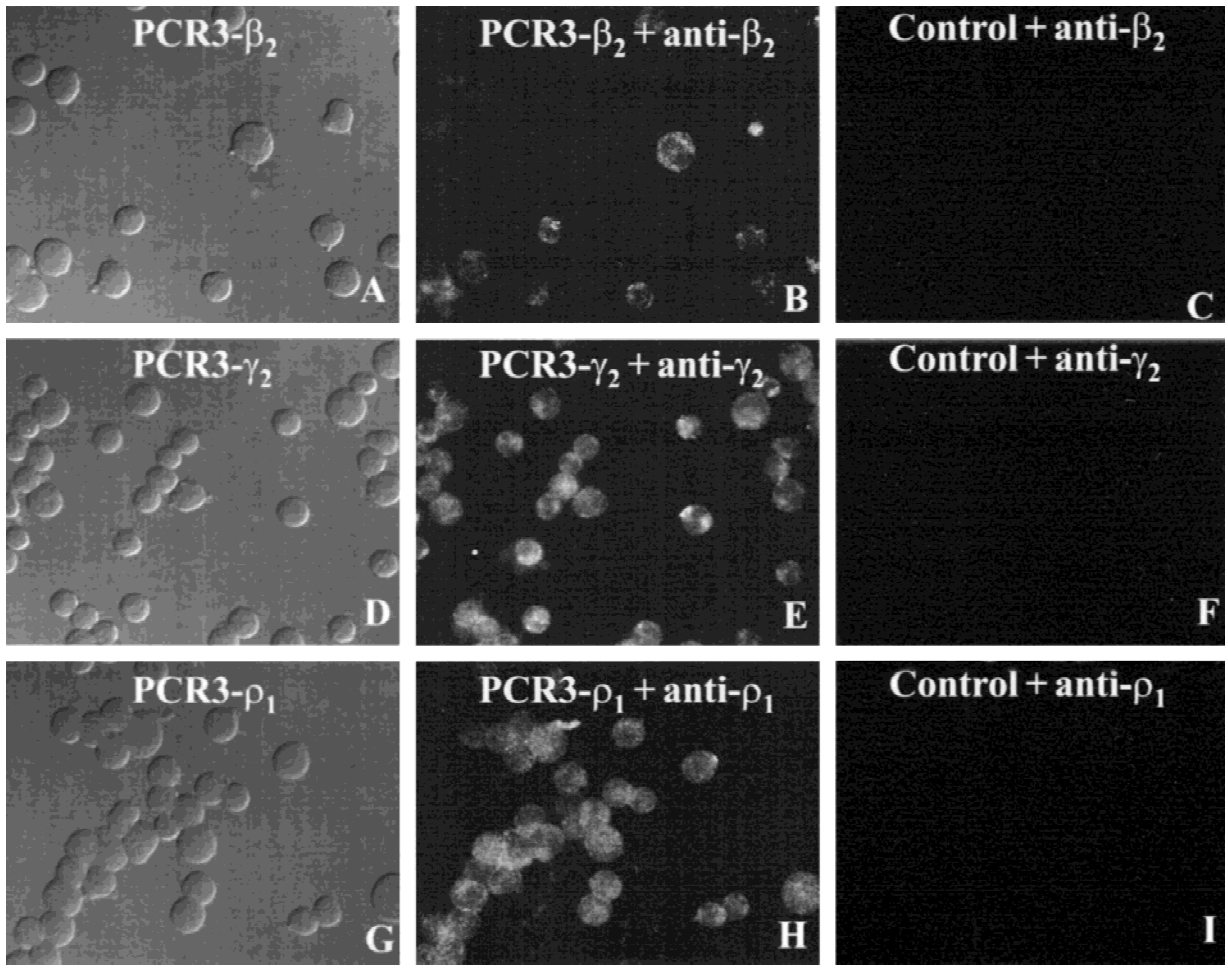

Fig. 1. Determination of antibody affinity by immunostaining of FDC-P1 cells. The FDC-P1 cells were transfected with cDNA encoding full-length GABA β_2, γ_2, or ρ_1 subunits in PCR3 by electroporation and grown in culture for 18 hr (panels *A, D, G*). Punctuate immunofluorescence staining occurs on the plasma membrane of some FDC-P1 cells, corresponding to specific staining of recombinant GABA receptor/channels (panels *B, E, H*). Untransfected FDC-P1 cells (control cells) showed a background staining without immunofluorescence activity (panels *C, F, I*). The transfected cells were cultured for 18 hours, washed in PBS and fixed with 4% paraformaldehyde solution. Primary antibody (anti-β_2, anti-γ_2, and anti-ρ_1) was added to a 1:10000 dilution. Secondary antibody (fluorescene-conjugated anti-rabbit IgG or rhodamine-conjugated anti-guinea pig IgG) was added to a 1:50 dilution. The cells were mounted on glass slides, and observed using fluorescence microscopy.

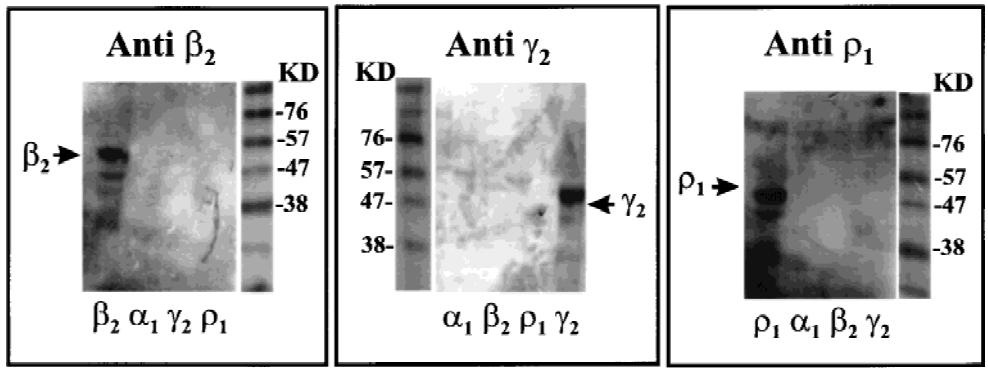

Fig. 2. Determination of antibody specificity by Western blot. FDC-P1 cells were transfected with full-length α_1, β_2, γ_2, or ρ_1 cDNA in PCR3 plasmid. Cells expressing each subunit were lysed and loaded onto individual lanes of PAGE gel for Western analysis. Primary antibody dilution was at 1:15,000–1:20,000, and secondary antibody dilution was at 1:1000. The different antibodies detected major bands corresponding to the subunits against which they were raised, respectively, and showed no cross-reactivity at these dilutions. Protein markers are presented to indicate molecular weight in KDa.

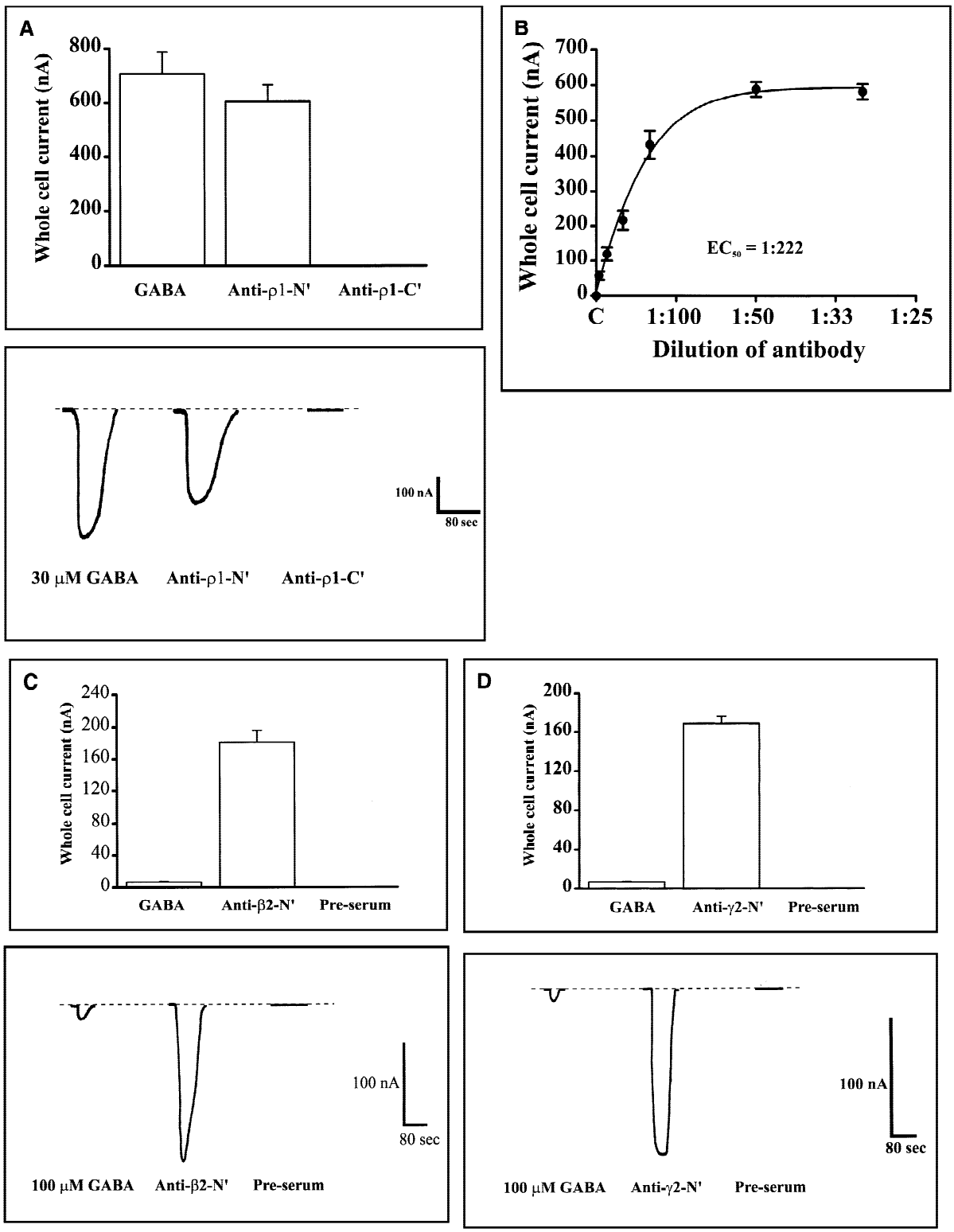

Fig. 3.

caine; Aldrich, Milwaukee, WI) for 20 min. Ovarian lobes were surgically excised and immersed in a Ca^{2+}-free OR-2 solution (in mM: 100 NaCl, 2 KCl, 1 $MgCl_2$, and 5 Hepes-Tris; pH 7.5). Oocytes were defolliculated by incubation with Ca^{2+}-free OR-2 solution containing 2 mg/ml collagenase (Type 1A; Sigma, St. Louis, MO) for 3 hr at room temperature. Following defolliculation, oocytes were washed 5 times with Ca^{2+}-free OR-2 and 5 times with a modified Barth's solution (MBS; in mM: 88 NaCl, 1 KCl, 2.4 $NaHCO_3$, 0.3 $Ca(NO_3)_2$, 0.4 $CaCl_2$,

Fig. 3. Induction of whole-cell currents by domain-specific antibodies in *Xenopus* oocytes expressing homomeric recombinant GABA receptor/channel subunits. (*A*) Induction of whole-cell currents by anti-ρ_1 antibody. A 1:250 dilution of anti-ρ_1 antiserum induced robust whole-cell currents in *Xenopus* oocytes expressing the homomeric ρ_1 subunit. The mean value of these currents was only slightly lower than that of currents evoked by application of 30 μM GABA. Application of antiserum raised against an intracellular loop between the M3- and M4-transmembrane domains of the ρ_1 subunit did not evoke currents in these oocytes. (*B*) Induction of currents in *Xenopus* oocytes by antisera is concentration-dependent. Anti-ρ_1 antiserum was added at decreasing dilutions to evoke whole-cell currents in oocytes expressing the homomeric ρ_1 subunit. The whole-cell currents in the presence and absence (*C*) of the antiserum were recorded, and the mean values showed a concentration-dependent response of anti ρ_1, with an EC_{50} of 1:222 dilution of the antiserum. (*C*) Induction of whole-cell currents by anti-β_2 antibody. A 1:250 dilution of anti-β_2 antiserum induced robust whole-cell currents in *Xenopus* oocytes expressing the homomeric β_2 subunit. The mean value of these currents was more than 18 times greater than that evoked by application of 100 μM GABA. Application of pre-immune serum from the same animal did not evoke currents in these oocytes. (*D*) Induction of whole-cell currents by anti-γ_2 antibody. A 1:250 dilution of anti-γ_2 antiserum induced robust whole-cell currents in *Xenopus* oocytes expressing the homomeric γ_2 subunit. The mean value of these currents was about 17 times greater than that evoked by application of 100 μM GABA. Application of pre-immune serum from the same animal did not evoke currents in these oocytes.

0.8 $MgSO_4$, 15 Tris-HCl; pH 7.6). Stage 5–6 oocytes were selected and incubated at 18°C in MBS for 24 hr, followed by injection of cRNA. Microinjection of cRNA into oocytes was done by positive displacement using a 10-μl micropipette (Drummond Scientific Inc., Broomal, PA). Fifty oocytes were injected with 50 ng of ρ_1 cRNA, and two additional groups of 50 oocytes each were injected with 50 ng of β_2 cRNA or γ_2 cRNA. Oocytes were further incubated for 72 hr before voltage-clamp recordings.

Voltage Clamp

Two-microelectrode voltage-clamp recordings were carried out at room temperature (22°C) with continuous perfusion (10 ml/min). Glass microelectrodes were made using a horizontal puller (PD-5; Narishige), and were filled with 3 M KCl (resistance ~2.0 MΩ). The perfusion bath was connected to the voltage-recording amplifier (Axoclamp 2A; Axon Instruments, Foster City, CA) by an Ag-AgCl-Agar-3 M KCl bridge. Data were filtered with a 4-pole Bessel filter at 500 Hz. Data acquisition was performed with the pCLAMP software (Axon Instruments).

Two separate methods were used in examining the effect of antibodies on GABA response. In the first method, whole-cell currents were recorded from oocytes under perfusion with 30 μM GABA by using voltage clamp. After an initial whole-cell recording, the oocytes were incubated for 30 min to 1 hr with 1:1000 dilution of anti-ρ_1, and for 1 hr with anti-β_2, or anti-γ_2 antibody in the perfusion chamber. After each incubation, the antibody was washed out by perfusion with 10 ml of low-Ca^{2+} oocyte Ringers solution. Whole-cell current was then recorded under perfusion with 30 μM GABA. Due to a low viability rate of oocytes kept 1 hr or more in the perfusion chamber, a new method was developed. In this method, antibody was added to the oocytes in Petri dishes for 1 hr, and then GABA-evoked whole-cell currents were recorded. GABA-evoked whole-cell currents were also recorded from a group of control oocytes that were not incubated in antibody. The mean whole-cell current values from both groups of oocytes were determined and statistically analyzed for significant variability.

For recordings to examine antibody-modulation of recombinant channels, *Xenopus laevis* oocytes were microinjected with 50 ng β_2, γ_2, or ρ_1 cRNA and incubated at 18°C for 72 hr in modified Barth's solution. Oocytes were perfused with a 1:2000 dilution of antibody in low-Ca^{2+} oocyte Ringer's solution, and whole-cell currents were recorded.

Results

In immunocytochemistry studies, cDNA encoding GABA β_2, γ_2, and ρ_1 subunits were subcloned into PRR3 expression vector containing CMV promoter and transfected into cultured mouse myeloblastic FDC-P1 cells by electroporation. Anti-β_2, anti-γ_2, and anti-ρ_1 antisera showed punctuate staining of recombinant receptors expressed in FDC-P1 cells (Fig. 1). Control experiments were done by using untransfected FDC-P1 cells that were stained with respective antibodies. The untransfected cells did not show the specific staining patterns. In additional control experiments, GABA β_2, γ_2, and ρ_1 subunit-transfected FDC-P1 cells incubated with pre-immune serum had no punctuate staining (*data not shown*). Western blot analysis was performed to detect recombinant GABA receptor subunits expressed in FDC-P1 cells. For this, transfected FDC-P1 cell lysates were run on PAGE gels, transferred to PVDF membranes and exposed to anti-β_2, anti-γ_2, and anti-ρ_1. Using low concentrations of primary antibody (1:15,000–1:20,000 dilution), each antibody detected the specific subunit that it was raised against without cross reacting with other subunits (Fig. 2).

All N-terminus-derived antibodies that were raised against the entire N-terminal half (the extracellular domains) of subunits induced currents in *Xenopus* oocytes expressing homomeric recombinant GABA receptor/channel subunits. The whole-cell currents in oocytes expressing the ρ_1 subunit were immediately evoked upon application of antibody against the entire N-terminal half of the ρ_1 subunit, and decayed when the antibody was washed off with Ringers solution (Fig. 3*A*). Activation of the whole-cell current in oocytes expressing the ρ_1 subunit was dependent on the antibody concentration. An increased activation of the current was observed in oocytes perfused with the anti-ρ_1 antibody when the concentration of the anti-ρ_1 antibody was increased. Amplitude of the current reached a saturation level at the full strength of the antibody (Fig. 3*B*). Another anti-ρ_1 antibody was raised against the intracellular-loop domain between the third (*M3*) and fourth (*M4*) transmembrane segments of the ρ_1 subunit. However, this antibody did not induce channel opening in *Xenopus* oocytes expressing recombinant ρ_1 subunits. In a similar approach, oocytes expressing β_2 subunit were perfused with anti-β_2 antibody (Fig. 3*C*) and oocytes expressing γ_2 subunit

were perfused with anti-γ_2 antibody (Fig. 3*D*). Both antibodies induced robust whole-cell currents. In addition, pre-immune sera were collected from rabbits in which anti-β_2 and anti-γ_2 antibodies were raised against the entire N-terminal half of β_2 or γ_2 subunits. These pre-immune sera did not induce channel opening in *Xenopus* oocytes expressing recombinant β_2 or γ_2 subunits, respectively.

Moreover, the effect of anti-ρ_1 antibody on GABA-induced whole-cell currents was examined in *Xenopus* oocytes expressing homomeric recombinant ρ_1 subunits. The anti-ρ_1 antibody inhibited GABA-induced ρ_1 currents in oocytes expressing the ρ_1 subunit (Fig. 4*A*). The inhibitory effect of the antibody was time-dependent. After 1 hr incubation of oocytes in the presence of anti-ρ_1 antibody, the amplitude of GABA-induced whole-cell current was reduced to 36 ± 4% of the average currents induced by application of 30 μM GABA in the absence of anti-ρ_1 antibody.

However, both anti-β_2 and anti-γ_2 antibodies potentiated GABA-induced whole-cell currents in *Xenopus* oocytes expressing recombinant β_2 or γ_2 subunits. Oocytes expressing the β_2 subunit were incubated in the presence of anti-β_2 antibody for 1 hr and whole-cell currents induced by perfusion with 100 μM GABA were recorded (Fig. 4*B*). The mean value of GABA-induced current was increased more than 5 times with the antibody incubation compared with the mean value of the whole-cell current elicited by application of 100 μM GABA alone. Similarly, following 1 hr incubation in the presence of anti-γ_2 antibody, oocytes expressing γ_2 subunit were perfused with 100 μM GABA. The mean value of GABA-induced currents was increased more than 3-fold compared with the value obtained from application of 100 μM GABA in absence of the antibody (Fig. 4*C*).

Discussion

In many cases, recombinant transmembrane receptor and ion channel proteins expressed in *E. coli* retain their structure, and they can be functional when purified and reconstituted in lipid vesicles (Furukawa & Haga, 2000; Grisshammer & Tate, 1995; Marullo et al., 1988). We expressed the extracellular N-terminus domain of β_2, γ_2, and ρ_1 subunits of GABA receptor/channel in SRP-84 *E. coli* cells, and used these recombinant domains as immunogens to raise polyclonal antibodies. This method is advantageous in that the epitopes on the recombinant subunits expressed in *E. coli* were reserved in their native configuration. Polyclonal antibodies thus raised will likely bind at multiple native binding sites, including sites for agonist binding and sites for allosteric modulation. The specific binding of antibodies at the agonist site caused gating of the receptor/channel subunits, so that the antibodies mimic the agonist effect. Likewise, binding of antibodies at the sites for modulation may cause receptor/channel potentiation in the absence of allosteric modulators.

It has been known that either sole expression of one $GABA_A$ receptor subunit or coexpression of the α_1 and β_2 subunits in oocytes result in a very small GABA-induced current (Machu, Olsen & Browning, 1993; Thompson & Stephenson, 1994). By using exogenous expression of GABA receptor/channel subunits in *Xenopus* oocytes, the effect of antibodies on GABA receptor/channel subunits can be studied. It suggests that the antibodies against specific regions of GABA receptor/channels may provide a useful tool to further study mechanisms of GABA receptor/channel gating and modulation. Although it does still not know how the antibodies affect function of both recombinant $GABA_A$ and $GABA_C$ receptor/channels, it is possible that an antibody-receptor/channel interaction induces modulations of the receptor/channel protein causing functional changes. Modulation of GABA receptor/channel by protein kinase C (PKC) activator, 4β-phorbol 12-myristate 13-acetate (PMA), decreases GABA response and causes internalization of the receptor/channels. This is due to PKC-mediated phosphorylation of an unidentified protein(s) (Chapell et al., 1998; Filippova, Dudley & Weiss, 1999; Filippova et al., 2000; Leidenheimer & Chapell, 1997). Interestingly, the internalization of membrane receptors in some cases may also be mediated by antibody clustering. It is known that the internalization of receptors by antibody binding is not due to simple antibody pinocytosis (Meiri et al., 1986). In the present case, we do not have evidence to show the receptor/channel internalization, however, modulation occurring in GABA receptor/channels in *Xenopus* oocytes by application of antibodies seems to mimic the decline in GABA response mediated by PKC phosphorylation and is similar to the effect of antibodies on other membrane receptors.

The decline in whole-cell currents by antibody binding in *Xenopus* oocytes expressing recombinant ρ_1 subunits is consistent with internalization of these receptor/channels. Interestingly, antibody binding resulted in potentiation of whole-cell currents in *Xenopus* oocytes expressing homomeric recombinant β_2 and γ_2 subunits. We attempt here a logical explanation of this disparity by considering a fundamental difference between the ρ_1 subunit and the β_2 and γ_2 subunits. $GABA_A$ receptor/channels can be modulated by a wide variety of compounds, while the $GABA_C$ receptor/channels don't show such wide modulation. While both β_2 and γ_2 subunits of the $GABA_A$ receptor/channel have sites for different modulators, the $GABA_C$ receptor/channel ρ_1 subunit does often not contain these sites. On the other hand, the effect of potentiation by antibody binding to modulation sites is greater than the inhibitory effect, indicating that the net effect is a potentiation of the whole-cell currents.

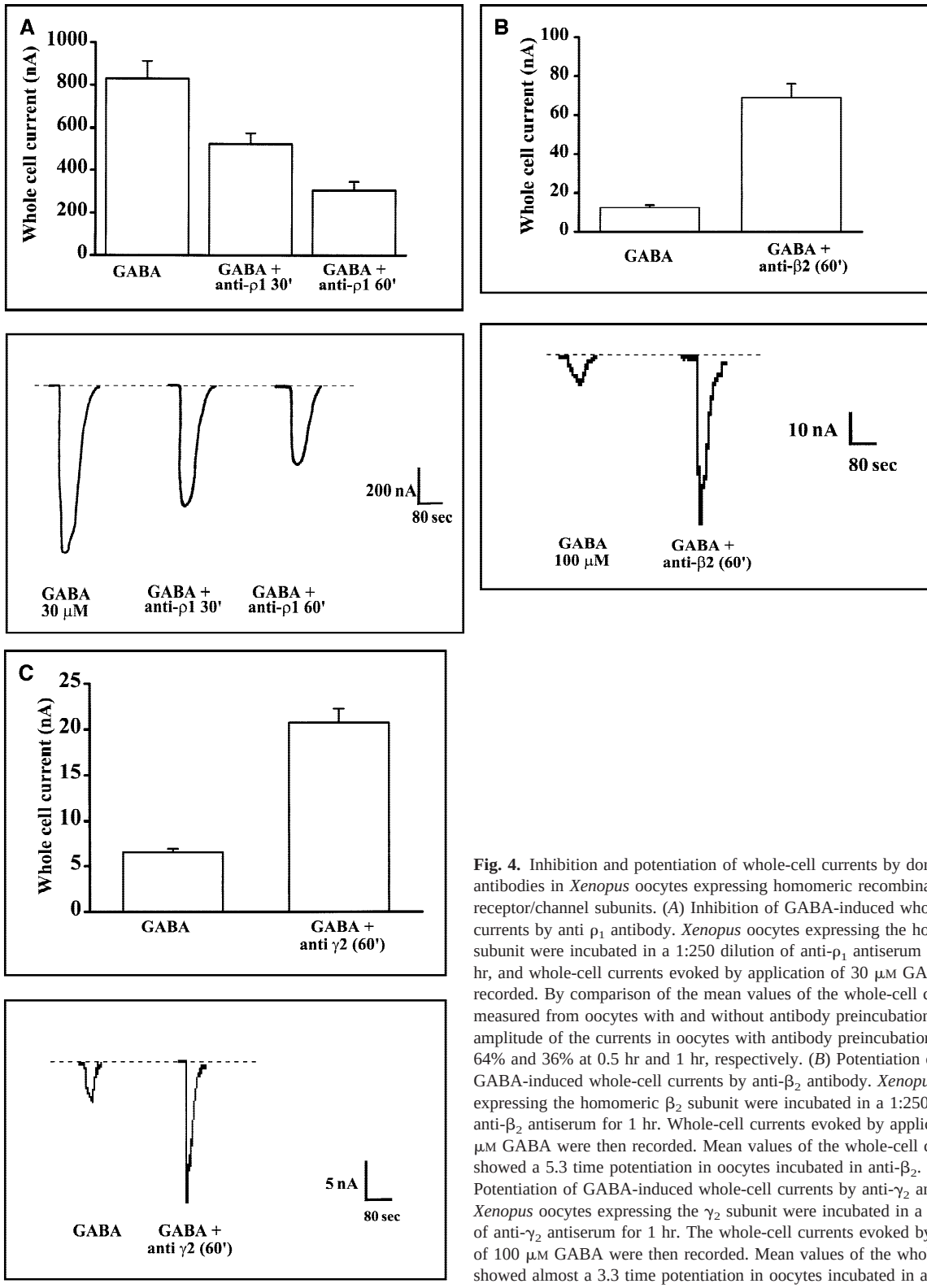

Fig. 4. Inhibition and potentiation of whole-cell currents by domain-specific antibodies in *Xenopus* oocytes expressing homomeric recombinant GABA receptor/channel subunits. (*A*) Inhibition of GABA-induced whole-cell currents by anti ρ_1 antibody. *Xenopus* oocytes expressing the homomeric ρ_1 subunit were incubated in a 1:250 dilution of anti-ρ_1 antiserum for 0.5 and 1 hr, and whole-cell currents evoked by application of 30 μM GABA were recorded. By comparison of the mean values of the whole-cell current measured from oocytes with and without antibody preincubation, the amplitude of the currents in oocytes with antibody preincubation decreased to 64% and 36% at 0.5 hr and 1 hr, respectively. (*B*) Potentiation of GABA-induced whole-cell currents by anti-β_2 antibody. *Xenopus* oocytes expressing the homomeric β_2 subunit were incubated in a 1:250 dilution of anti-β_2 antiserum for 1 hr. Whole-cell currents evoked by application of 100 μM GABA were then recorded. Mean values of the whole-cell current showed a 5.3 time potentiation in oocytes incubated in anti-β_2. (*C*) Potentiation of GABA-induced whole-cell currents by anti-γ_2 antibody. *Xenopus* oocytes expressing the γ_2 subunit were incubated in a 1:250 dilution of anti-γ_2 antiserum for 1 hr. The whole-cell currents evoked by application of 100 μM GABA were then recorded. Mean values of the whole-cell current showed almost a 3.3 time potentiation in oocytes incubated in anti-γ_2.

The induction of whole-cell currents by our antibodies in *Xenopus* oocytes expressing homomeric recombinant β_2, γ_2, and ρ_1 subunits is likely due to antibody binding to specific sites in the N-terminal domain of the receptor/channel. The whole-cell currents in oocytes expressing β_2 and γ_2 subunits induced by application of anti-β_2 and anti-γ_2 antibodies in the absence of GABA are several-fold greater than those induced by application of GABA. However, whole-cell currents induced by the anti-ρ_1 antibody in oocytes expressing ρ_1 subunit in the absence of GABA were slightly less than those induced by application of GABA. This disparity in antibody gating between the $GABA_C$ ρ_1 subunit and the $GABA_A$ β_2 and γ_2 subunits is consistent with the theory that anti-β_2 and anti-γ_2 antibodies act as modulators by binding to specific sites causing modulation on the β_2 and γ_2 subunits.

It is also possible that the modulation of whole-cell currents in the presence or absence of GABA in *Xenopus* oocytes by these antibodies is due to different mechanisms from what we have hypothesized. However, these subunit N-terminus-specific antibodies activate the receptor/channels in the absence of GABA, and potentiate or inhibit the GABA response in a subunit-specific manner, providing us with an important and interesting tool for further exploring the modulation of the GABAergic system. It has been known that neurological disorders are often seen in patients suffering from autoimmune diseases. The observation that antibodies against the N-terminal domain of GABA receptor/channel subunits modulate function of the receptor/channel suggests a possible cause of neurological disorders in these patients. Thus, results from the present study provide important information for understanding related neurological diseases and for developing drugs to ameliorate these diseases.

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