Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 11751–11755, December 1995
Neurobiology

A single amino acid in γ-aminobutyric acid ρ1 receptors affects competitive and noncompetitive components of picrotoxin inhibition

(*Xenopus* oocytes/mutagenesis/chloride channel/ligand-gated neuroreceptor)

TIAN-LI WANG*†, ABIGAIL S. HACKAM†, WILLIAM B. GUGGINO*, AND GARRY R. CUTTING†‡§

Departments of *Physiology, ‡Pediatrics, and the †Center for Medical Genetics, Johns Hopkins University, School of Medicine, Baltimore, MD 21205

Communicated by George Wald, Harvard University, Cambridge, MA, August 11, 1995

ABSTRACT **A class of bicuculline-insensitive γ-aminobutyric acid (GABA) receptors, $GABA_C$, has been identified in retina. Several lines of evidence indicate that $GABA_C$ receptors are formed partially or wholly of GABA ρ subunits. These receptors generate a Cl^- current in response to GABA but differ from $GABA_A$ receptors in a number of ways. Picrotoxin, widely accepted as a noncompetitive antagonist of $GABA_A$ receptors, displays competitive and noncompetitive antagonism of $GABA_C$ receptors in perch and bovine retina and GABA ρ1 receptors expressed in *Xenopus* oocytes. The aim of this study was to identify the molecular basis of the two components of picrotoxin inhibition of GABA ρ1 receptors. By using a domain-swapping and mutagenesis strategy, a difference in picrotoxin sensitivity between ρ1 and ρ2 receptors was localized to a single amino acid in the putative second transmembrane domain. Substitution of this amino acid with residues found in the analogous position in highly picrotoxin-sensitive glycine α and $GABA_A$ subunits increased the sensitivity of ρ1 mutants 10- to 500-fold. Importantly, the competitive component of picrotoxin inhibition of the ρ1 mutant receptors was almost eliminated. These findings demonstrate that an amino acid in the putative channel domain of GABA ρ1 receptors influences picrotoxin sensitivity and mediates agonist binding by an allosteric mechanism.**

Picrotoxin, a plant-derived compound, is a noncompetitive antagonist of γ-aminobutyric acid type A ($GABA_A$) receptors and certain glycine receptors (1). Picrotoxin may exert its effect on these receptors by blockade of the ion conduction pore (1–4). However, in crustacean muscle, picrotoxin has been observed to inhibit $GABA_A$ receptors in a competitive manner, suggesting an allosteric mechanism of action (5, 6). Binding studies of picrotoxin analogues and single-channel studies of picrotoxin action on rat sympathetic neurons are consistent with this concept (1, 7). Recently, a class of picrotoxin-inhibitable GABA receptors has been characterized in the retina of several species (5, 8–11). These receptors, variously termed bicuculline-insensitive, ρ-like, and $GABA_C$, generate a Cl^- conduction in response to GABA that is insensitive to bicuculline, benzodiazepines, and barbiturates (5, 12, 13). These receptors are referred to as $GABA_C$, although their precise definition remains a matter of debate. GABA ρ subunits cloned from retina share many of these pharmacologic properties, suggesting that $GABA_C$ receptors are composed partially or entirely of ρ subunits (9, 13–18).

The picrotoxin sensitivity of $GABA_C$ receptors is of interest because it differs from $GABA_A$ receptors in several ways. First, $GABA_C$ receptors in mammalian retina are less sensitive to picrotoxin than $GABA_A$ receptors located in retina or in cortex (9, 12). Second, the inhibitory effects of picrotoxin have a weak use-dependent component compared to $GABA_A$ receptors (12). Third, picrotoxin inhibition of $GABA_C$ receptors in perch and bovine retina depends on agonist concentration, suggesting a complex type of antagonism (12, 19). Together, these data suggest that the site and mechanism of picrotoxin action on $GABA_C$ receptors may differ from $GABA_A$ receptors.

The goal of the present study was to identify amino acids underlying the picrotoxin inhibition of GABA receptors formed of ρ1 subunits. Like $GABA_C$ receptors, GABA ρ1 and ρ2 receptors expressed in *Xenopus* oocytes are considerably less sensitive to picrotoxin than homooligomeric or heterooligomeric receptors formed of $GABA_A$ subunits, and GABA ρ1 receptors display both noncompetitive and competitive components of picrotoxin inhibition (18–20). Differences in picrotoxin sensitivity of ρ1 and ρ2 homooligomeric GABA receptors facilitated identification of a single position in the ρ1 subunit critical for picrotoxin sensitivity. Mutating this residue to amino acids found in the analogous position of $GABA_A$ or glycine subunits significantly increased picrotoxin sensitivity over wild-type ρ1 and virtually abolished the agonist-dependent elements of picrotoxin inhibition. Therefore, both components of picrotoxin of inhibition of GABA ρ1 receptors are determined by the same amino acid.

MATERIALS AND METHODS

Construction of Chimeric cDNAs. Chimeric DNA fragments coding for the indicated regions of ρ1 and ρ2 as shown in Fig. 1 were generated by two-step PCR (21) on ρ1 and ρ2 cDNA templates by using three oligonucleotide primers. The primers used for constructing the ρ1ρ2-A chimeric cDNA were as follows (boldface indicates ρ1 cDNA sequence):

5′-ρ1 primer: 5′-ATAGGATC**ATGTTGGCTGTCCCAAAT**-3′
*Bam*HI

ρ1ρ2-A hybrid primer:
5′-**CAGACGGTTGTACCAGCCA**GTGCTGCTGTAGAAAGCCAGT-3′

3′-ρ2 primer: 5′-TATCTGCAGCCCCCCTCGATGTCTA-3′
*Pst*I

The first step of PCR amplified an 850-bp fragment using 0.1 μM 5′-ρ1 primer and 0.01 μM ρ1ρ2-A hybrid primer and ρ1 cDNA template. Since the hybrid primer contained 20 bp of ρ2 cDNA sequences (boldface), the resulting 850-bp PCR product contained this sequence at the 3′ end and served as the 5′ primer for a second PCR using 0.1 μM 3′-ρ2 primer and 1 ng of ρ2 cDNA as template. The presence of the 5′-ρ1 primer in the reaction mixture enabled the amplification of a 1568-bp chimeric cDNA (Fig. 1). The ρ1ρ2-A chimeric cDNA was

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GABA, γ-aminobutyric acid.

§To whom reprint requests should be addressed at: Center for Medical Genetics, Johns Hopkins University, School of Medicine, CMSC 1004, 600 North Wolfe Street, Baltimore, MD 21287.

produced by the same procedure using the following PCR primers (boldface indicates ρ1 cDNA sequence):

5′-p2 primer: 5′-CGGCGGATCCACAGGCTACTGGAAAGCAGC-3′
*Bam*HI

ρ1ρ2-A hybrid primer:
5′-CTGGCCTTCTACAGCAGCAC**AGGCTGGTACAACCGTCTC**-3′

3′-ρ1 primer: 5′-**CTGCAGCTTTCCAAAGCT**-3′

After PCR amplification, both ρ1ρ2-A and ρ2ρ1-A chimeras were subcloned into the PCRII vector (Invitrogen) and completely sequenced. PCR errors were corrected by site-directed mutagenesis techniques described below.

***In Vitro* Mutagenesis.** Oligonucleotide-mediated site-directed mutagenesis was performed on uracil-containing single-stranded DNA by the method of Kunkel (22). Mutated clones were identified by hybridization with the radiolabeled oligonucleotides. All positive mutants were verified by dideoxynucleotide sequencing of the mutated region. Five mutagenic oligonucleotides were used, with nucleotides producing the mutation underlined:

P309A 5′-GATACCTAAGGCGACTCTGGC-3′
P309G 5′-GTGATACCTAAGCCGACTCTGGCAGG-3′
P309S 5′-GAGATACCTAAGGAGACTCTG-3′
P309V 5′-TGTGATACCTAAGACGACTCTGGCAGG-3′
S319T 5′-GATGGTGGTCATGGTTAGCAC-3′

Oocyte Expression and Electrophysiology. Oocyte injection and a two-electrode voltage-clamping technique were performed as described (18). RNA injection was used for ρ1, ρ1 mutants, and the ρ1ρ2-A chimera. The amount of RNA injected in a 50-nl vol was as follows: ρ1, 5 ng; ρ1ρ2-A, 5 ng; P309G, 5 ng; P309S, 10 ng; P309A, 25 ng. Nuclear injection of the ρ2 cDNA and ρ2ρ1-A chimeric cDNA was performed to achieve efficient expression of ρ2 and ρ2ρ1-A. The 3-kb ρ2 cDNA fragment was subcloned into the *Sca* I site of the nuclear injection vector pMT3 and the ρ2ρ1-A chimera was subcloned into the *Eco*RI site of the pMT3 vector (23). For studies of picrotoxin sensitivity, the amount of complementary RNA or pMT3 cDNA injected was varied to obtain similar whole-cell currents (150–300 nA). For nuclear injection, 1.5 ng of pMT3-ρ2 or 1.5 ng of pMT3-ρ2ρ1-A was injected. Two to 4 days after RNA or cDNA injection, oocytes were recorded in ND96 solution under voltage-clamp measurements with an Axoclamp-2A amplifier (Axon Instrument, Burlingame, CA). ND96 contains 96 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM Hepes·NaOH (pH 7.4). GABA and picrotoxin were purchased from Sigma. GABA was dissolved in bathing solution (ND96). Picrotoxin was dissolved in dimethyl sulfoxide (DMSO) as 5 M stock solution and was diluted in ND96 into test concentrations. The final concentration of DMSO was kept below 0.1%.

Curve Fit and Statistics. The dose-dependent inhibition data were best-fitted by inverse Hill equation using the *N*-fit program (University of Texas, Galveston). The equation was $I(x) = I_{max}/1+(x/K_d)^n$, where x represents the drug concentration, K_d is the drug concentration for eliciting half-maximal response, and n is the Hill coefficient. Student's t test was used to determine the statistical significant difference between two sample groups. In this study, independent sampling and comparison were used and $P < 0.01$ was set as a significant level.

RESULTS

The Difference in Picrotoxin Sensitivity Between ρ1 and ρ2 Receptors Is Conferred by a Single Residue in the Second Membrane-Spanning Domain (M2). GABA ρ2 receptors were consistently more sensitive to picrotoxin than GABA ρ1 receptors in oocytes expressing either ρ1 or ρ2 receptors with similar whole-cell GABA-gated currents (range, 150–200 nA) (Fig. 1*A*). Picrotoxin did not alter the linearity of the whole-cell current, indicating that the blockage is voltage independent (data not shown). The IC_{50} (concentration of picrotoxin that inhibits 50% of maximal currents) derived from these curves differed significantly between ρ1 and ρ2 receptors ($P < 0.01$) (Table 1). The minimal picrotoxin concentration that inhibited ρ1 and ρ2 currents was 1 μM. Total inhibition of ρ2 currents was observed when picrotoxin concentration reached 30 μM, whereas 250 μM was required for complete inhibition of ρ1 receptors. The Hill coefficients of picrotoxin inhibition for ρ2 and ρ1 receptors were also significantly different ($P < 0.01$) (Table 1).

Two chimeras were generated to determine regions conferring the different picrotoxin sensitivity to ρ subunits. The ρ1-ρ2-A chimera included the cDNA sequence of the N-terminal extracellular domain of the ρ1 subunit fused with the C-terminal half of ρ2 containing putative membrane-spanning domains M1–M4 (Fig. 1*B*). A reciprocal chimera, ρ2ρ1-A, was also created that contains the N-terminal domain of the ρ2 subunit fused with the C-terminal half of the ρ1 subunit. The ρ1ρ2-A chimera produced robust currents (200 nA to 1 μA; $n = 9$) when expressed in oocytes similar to those observed with the ρ1 subunit. However, receptors formed from this chimera were 10-fold more sensitive to picrotoxin than wild-type ρ1 receptors with an IC_{50} value and Hill coefficient close to that obtained from ρ2 receptors (Fig. 1*B*). Nuclear expression of the ρ2ρ1-A chimeric cDNA produced current levels (150–200 nA; $n = 6$) similar to those observed in ρ2 cDNA-injected oocytes. However, unlike ρ2 receptors, the ρ2ρ1-A chimeric receptors were more resistant to picrotoxin blockage with an IC_{50} value and Hill coefficient similar to that of ρ1 receptors (Fig. 1*B*). These data indicate that the C-terminal half of ρ subunits containing M1–M4 determine picrotoxin sensitivity in ρ subunits.

Since prior studies had suggested that picrotoxin may block the ion conduction pore, we compared the amino acid sequence of the putative channel-lining M2 domains of ρ1 and ρ2 subunits. Two differences were noted: a proline at codon 309 of ρ1, whereas ρ2 has a serine in the analogous position, and a serine at codon 319 of ρ1, while ρ2 has a threonine. To determine whether either residue altered picrotoxin sensitivity, two mutant ρ1 subunits were created. In one mutant, the proline residue at position 309 of ρ1 was changed to serine (P309S) and, in the other, the serine residue at position 319 was substituted with threonine (S319T). Both mutants were expressed readily in oocytes with whole cell currents ranging from 200 nA to 1 μA. Picrotoxin inhibition of currents generated by the S319T mutant was indistinguishable from ρ1 (Table 1). However, the IC_{50} of P309S to picrotoxin is significantly different from ρ1 ($P < 0.01$) but very similar to ρ2 receptors (Table 1). Furthermore, the P309S and ρ2 receptors have Hill coefficients for picrotoxin around 2, which is 2-fold larger than that of ρ1 receptors (Table 1). Therefore, mutation of this single amino acid to that found in the ρ2 subunit altered the picrotoxin interaction of ρ1 to match that of ρ2.

Picrotoxin Sensitivity of ρ1 Receptors Is Increased by Substituting the Residue at Position 309 with Those Found in Picrotoxin-Sensitive Glycine α and $GABA_A$ β and *Rdl* Receptor Subunits. To assess the relationship between the nature of the amino acid at position 309 of ρ1 and picrotoxin sensitivity, mutants P309G, P309V, and P309A were constructed to correspond with residues found in the analogous position in glycine and $GABA_A$ α, β, and wild-type *Rdl* subunits, respectively. (P309S corresponds with the serine residue found in $GABA_A$ γ and δ subunits.) Two mutants, P309A and P309G, expressed GABA-gated currents that differed markedly in picrotoxin sensitivity (Table 1 and Fig. 2). For example, 5 μM picrotoxin did not inhibit ρ1 currents, whereas the same concentration of picrotoxin decreased P309G currents by ≈30% and almost totally abolished currents generated by

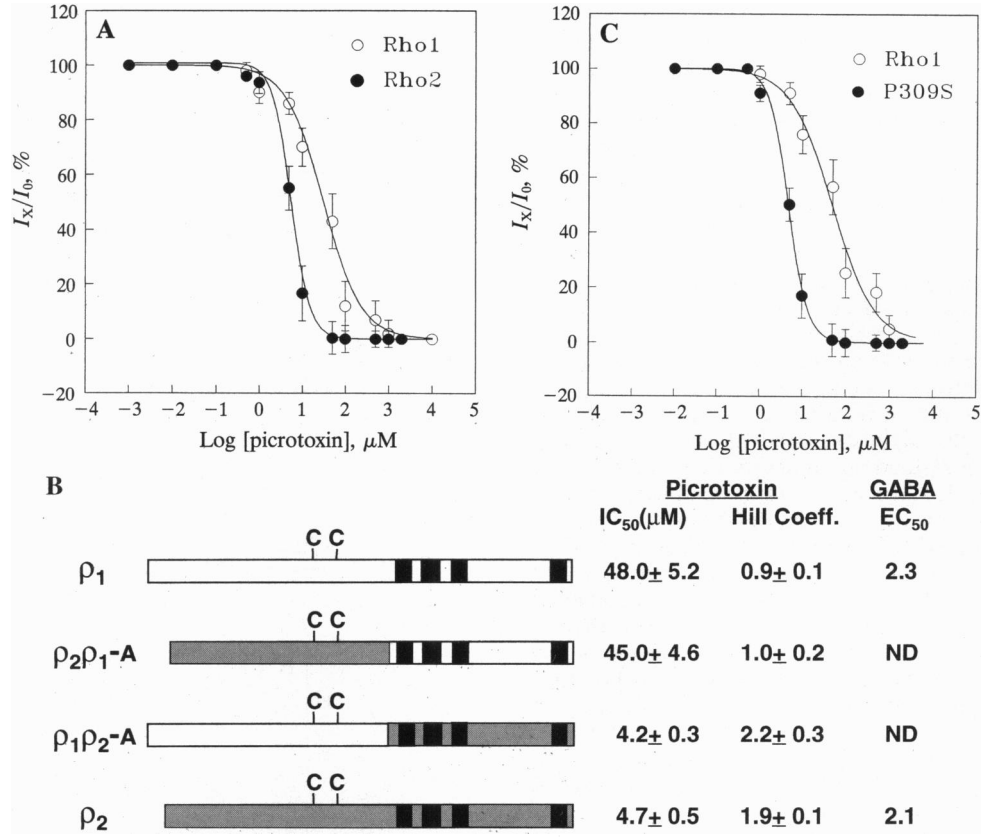

FIG. 1. Inhibitory effects of picrotoxin on ρ1 and ρ2 receptors. (*A*) Picrotoxin dose-dependent inhibition curve of ρ1 and ρ2 receptors. ○, ρ1 (n = 3); ●, ρ2 (n = 3). Data are expressed as I_X/I_0 vs. concentration of picrotoxin. I_0, current elicited by 20 μM GABA; I_X, current evoked by different concentrations of picrotoxin coapplied with 20 μM GABA. Data are means ± SE. (*B*) Comparison of picrotoxin sensitivity between ρ1ρ2-A chimera and ρ2ρ1-A chimera. Structures of ρ1 (open rectangle) and ρ2 (shaded rectangle) and chimeric channels composed of ρ1 and ρ2 sequences are shown on the left, with the four putative transmembrane segments aligned. The responses of each construct to picrotoxin (IC_{50} values and Hill coefficients) are shown as means ± SE (n = 3). (*C*) Dose-dependent picrotoxin inhibition curves of receptors formed from ρ1 or the P309S mutant. I_0, current activated by 20 μM GABA; I_X, current elicited by coapplication of picrotoxin and GABA. Data are expressed as normalized picrotoxin response (I_X/I_0) ± SE vs. log scale of picrotoxin concentration. ○, ρ1 (n = 3); ●, P309S mutant (n = 3). I_0 and I_X are the same as in A.

P309A, the most sensitive mutant. Unlike the P309S mutant, however, the Hill coefficients for picrotoxin inhibition of the P309G and P309A mutants were near unity, similar to wild-type ρ1 (Table 1). Oocytes injected with the P309V mutant failed to express GABA-gated channels (n = 5). To determine whether a stable P309V mutant subunit was translated in oocytes, complementary RNAs synthesized from P309V and from ρ1 were coinjected at ratios of 1:1 and 4:1. At the higher ratio, the expressed currents were 4-fold more sensitive to picrotoxin than ρ1 alone (n = 5), suggesting that the P309V mutant, although nonfunctional as a homooligomer, could interact with ρ1 subunits and form heterooligomeric receptors with increased picrotoxin sensitivity (Table 1).

Table 1. Comparisons of picrotoxin and GABA responses in ρ1, ρ2, and ρ1 mutants

	Picrotoxin		GABA
	IC_{50}, μM	Hill coefficient	C_{50}, μM
GABA ρ1	48.0 ± 5.0	0.9 ± 0.1	2.3 ± 0.5
GABA ρ2	4.7 ± 0.5	1.9 ± 0.1	2.1 ± 0.3
P309S	4.8 ± 0.8	1.8 ± 0.2	6.7 ± 1.1
P309G	5.8 ± 1.2	0.9 ± 0.2	6.2 ± 1.5
P309A	0.1 ± 0.4	0.9 ± 0.3	7.2 ± 1.2
P309V	—	—	—
S319T	47.2 ± 6.1	1.0 ± 0.1	
GABA ρ1 + P309V*	11.3 ± 4.0	0.8 ± 0.1	34.5 ± 5.4

Data are expressed as means ± SE (n = 3). —, No current.
*Recordings after coinjection of 5 ng of ρ1 and 20 ng of P309V per oocyte.

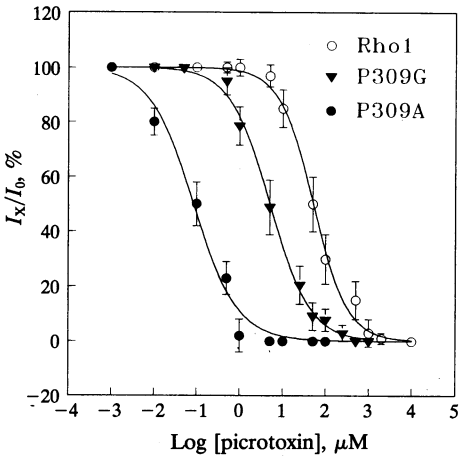

FIG. 2. Dose-dependent inhibition curves of picrotoxin generated by ρ1 receptors and mutant ρ1 receptors P309G and P309A. Responses to a wide concentration range of picrotoxin were collected from oocytes expressing homooligomeric channels generated from mutants P309G (▼) (n = 5), P309A (●) (n = 5), and ρ1 (○) (n = 5). The current elicited by 20 μM GABA without picrotoxin is the maximal current response (I_0). The current response after picrotoxin application is represented as I_X. Normalized current (I_X/I_0) vs. log scale of picrotoxin concentration is plotted ± SE.

Mutation of Position 309 in ρ1 Influences the Agonist-Dependent (Competitive) Component of Picrotoxin Inhibition. The channel properties and responses of the residue 309 mutants to GABA were determined to assess whether the mutations had any effect on other receptor properties. Mutants P309S, P309G, and P309A have linear *I–V* relationships with a reversal potential close to the Cl^- equilibrium potential (data not shown). The three mutants had the same anion selectivity series as ρ1 receptors; $Cl^- > Br^- > I^-$ when measuring the slopes of the cord conductance, and $Cl^- > Br^- > I^-$ when measuring shifts in reversal potential ($n = 3$ for each). The discrepancies in selectivity series measured by these two different methods are similar to previous reports of GABA-gated Cl^- currents from isolated neurons (24). These data suggest that intrinsic biophysical properties of the Cl^- channel formed by the ρ1 mutants were not significantly altered. However, the agonist-dependent activation differs between ρ1 and the residue 309 mutants. Regardless of the substituted amino acid, all mutants have significantly higher EC_{50} values for GABA activation than ρ1 ($P < 0.01$) (Table 1). These results prompted an analysis of the sensitivity of ρ1 mutants to picrotoxin at submaximal concentrations of GABA. Picrotoxin inhibition curves for P309S and P309G at three different GABA concentrations are compared with ρ1 in Fig. 3. ρ1 receptors showed a 30-fold increase in the picrotoxin IC_{50} between 1 and 5 μM GABA applications (18). In contrast, P309S and P309A had minor changes in picrotoxin IC_{50} values between these two concentrations of GABA. For P309S, the IC_{50} value for picrotoxin was 1.0 ± 0.6 μM at 1 μM GABA ($n = 5$) and 3.1 ± 0.8 μM ($n = 5$) at 5 μM GABA, and it reached 4.8 ± 0.7 μM ($n = 5$) at a saturating level of GABA (20 μM). Similar minor changes in the IC_{50} of picrotoxin between 1 μM [1.0 ± 0.8 μM ($n = 3$)], 5 μM [2.5 ± 0.7 μM ($n = 3$)], and 20 μM [5.4 ± 0.5 μM ($n = 3$)] GABA concentrations were observed for the P309A mutant.

DISCUSSION

This report identifies a molecular basis for the picrotoxin sensitivity of GABA ρ1 receptors. Substitution of proline-309 of ρ1 with a serine (P309S), the corresponding residue in ρ2, decreased the IC_{50} and increased the Hill coefficient of this mutant to precisely match ρ2 receptors. This suggested that the difference between ρ1 and ρ2 subunits in picrotoxin sensitivity is primarily determined by a single amino acid in the M2 domain. Two lines of evidence indicate that this single mutation is specific for picrotoxin interaction. First, several important properties of the mutant receptor (current–voltage relationship, Cl^--reversal potential, anion selectivity) were unaltered. Second, this mutation produced a gain in function (i.e., increased sensitivity to picrotoxin) rather than a loss of function. The distinction is critical because increased function provides evidence that the structural integrity of the domain that interacts with the compound has not only been preserved but has been altered specifically to enhance its interaction with the compound.

The nature of the amino acid at position 309 accounts for the differences in picrotoxin inhibition not only between ρ1 and ρ2 receptors but also among GABA ρ1 receptors and $GABA_A$ receptors. Alignment of the amino acid sequences of the M2 domain of mammalian $GABA_A$ receptor subunits and ρ subunits reveals that the amino acid residue at this position is highly diverse. Our data demonstrate that substituting the proline residue of ρ1 with valine, alanine, serine, or glycine, the amino acids found in $GABA_A$ subunits and the glycine α subunit, increased the sensitivity toward picrotoxin. They also suggest a starting point to assess the differences in the picrotoxin blocking effect between mammalian retinal $GABA_C$ receptors and those characterized in perch, salamander, and catfish retina (9, 10, 12, 19, 20, 25). Identification of the residues occupying position 309 in ρ subunits from these species helps determine whether variation at this location accounts for the increased sensitivity of nonmammalian $GABA_C$ receptors.

Residue 309 of ρ1 corresponds to one of the amino acids in glycine receptors shown to affect picrotoxin sensitivity. Mutation of 13 residues in the putative channel-lining M2 domain

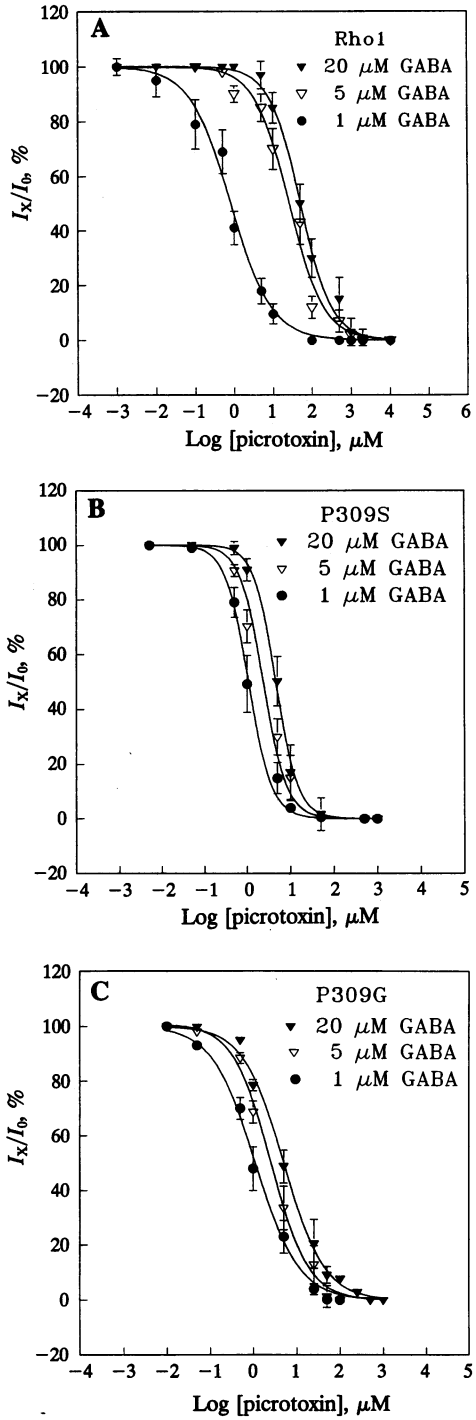

FIG. 3. Agonist-dependent picrotoxin inhibition curves of ρ1 and ρ1 mutants P309S and P309G. (*A*) Picrotoxin inhibition responses of the ρ1 receptor observed at three GABA concentrations: 1, 5, and 20 μM ($n = 3$ for each). Data are expressed as I_X/I_0 vs. concentration of picrotoxin. I_0, current response to 1, 5, or 20 μM GABA; I_X, current response to 1, 5, or 20 μM GABA coapplied with a certain concentration of picrotoxin. (*B*) Picrotoxin inhibition curves for the P309S mutant at the same three concentrations of GABA as in *A* ($n = 5$ for each). (*C*) Picrotoxin inhibition curves for the P309G mutant at the same three concentrations of GABA as in *A* ($n = 3$ for each). Data are means ± SE.

of the β glycine receptor subunits to those found in the α_3 subunit increased picrotoxin sensitivity of α_3/β receptors to levels observed in α_3 homooligomeric receptors (3). It also is the same residue that is altered in a $GABA_A$ receptor subunit of *Drosophila Rdl*. This naturally occurring point mutation confers insecticide resistance to the *Rdl* (resistance to dieldrin) strain (4, 26). Since picrotoxin and the cyclodiene insecticides compete for the same binding site (27), this amino acid substitution identified a biologically important location for picrotoxin interaction with invertebrate $GABA_A$ receptors. These observations are consistent with the concept that picrotoxin noncompetitively inhibits $GABA_A$, glycine, and GABA ρ1 receptors by interacting with residues forming the ion pore.

One of the aims of this study was to identify residues facilitating competitive picrotoxin antagonism of ρ1 receptors. Our results suggest that the nature of the amino acid at position 309 plays a critical role in this process. The presence of a proline residue confers a competitive component to the GABA ρ1 receptor. Substitution with residues found in the analogous location in $GABA_A$ receptors eliminates this component. The residue 309 location is distinct from the putative agonist binding region of ρ1 receptors (28). Thus, picrotoxin may inhibit GABA activation of ρ1 receptors by an allosteric mechanism in addition to blockade of the open channel. This could explain why picrotoxin acts noncompetitively on $GABA_A$ receptors but displays mixed antagonism of GABA ρ and $GABA_C$ receptors. Characterization of the molecular composition of retinal $GABA_C$ receptors will help confirm this hypothesis.

The authors wish to thank Dan Raben, William Agnew, and Peter Maloney for their thoughtful critiques and Janice Staton for secretarial assistance. This work was supported by National Institutes of Health Grants HL 47122 (W.B.G.) and EY09531 (G.R.C.).

1. Ticku, M. J. (1986) in *Convulsant Binding Sites on the Benzodiazepine/GABA Receptor*, eds. Olsen, R. W. & Venter, J. C. (Liss, New York), pp. 195–207.
2. Inoue, M. & Akaike, N. (1988) *Neurosci. Res.* **5,** 380–394.
3. Pribilla, I., Takagi, T., Langosch, D., Bormann, J. & Betz, H. (1992) *EMBO J.* **11,** 4305–4311.
4. Ffrench-Constant, R., Mortlock, D. P., Shaffer, C. D., MacIntyre, R. J. & Roush, R. T. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 7209–7213.
5. Qian, H. & Dowling, J. E. (1993) *Nature (London)* **361,** 162–164.
6. Smart, T. G. & Constanti, A. (1986) *Proc. R. Soc. London B* **227,** 191–216.
7. Newland, C. F. & Cull-Candy, S. G. (1992) *J. Physiol.* **447,** 191–213.
8. Polenzani, L., Woodward, R. M. & Miledi, R. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 4318–4322.
9. Feigenspan, A., Wassle, H. & Bormann, J. (1993) *Nature (London)* **361,** 159–161.
10. Dong, C., Picaud, S. A. & Werblin, F. S. (1994) *J. Neurosci.* **14,** 2648–2658.
11. Lukasiewicz, P. D. & Werblin, F. S. (1994) *J. Neurosci.* **14,** 1213–1223.
12. Woodward, R. W., Polenzani, L. & Miledi, R. (1992) *Mol. Pharmacol.* **42,** 165–173.
13. Woodward, R. W., Polenzani, L. & Miledi, R. (1993) *Mol. Pharmacol.* **43,** 609–625.
14. Cutting, G. R., Lu, L., O'Hara, B. F., Kasch, L. M., Montrose-Rafizadeh, C., Donovan, D. M., Shimada, S., Antonarakis, S. E., Guggino, W. B., Uhl, G. R. & Kazazian, H. H., Jr. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 2673–2677.
15. Cutting, G. R., Curristin, S., Zoghbi, H., O'Hara, B., Seldin, M. F. & Uhl, G. R. (1992) *Genomics* **12,** 801–806.
16. Shimada, S., Cutting, G. R. & Uhl, G. R. (1992) *Mol. Pharmacol.* **41,** 683–687.
17. Kusama, T., Spivak, C. E., Whiting, P., Dawson, V. L., Schaeffer, J. C. & Uhl, G. R. (1993) *Br. J. Pharmacol.* **109,** 200–206.
18. Wang, T.-L., Guggino, W. B. & Cutting, G. R. (1994) *J. Neurosci.* **14,** 6524–6531.
19. Qian, H. & Dowling, J. E. (1994) *J. Neurosci.* **14,** 4299–4307.
20. Sigel, E., Baur, R., Trube, G., Mohler, H. & Malherbe, P. (1990) *Neuron* **5,** 703–711.
21. Yon, J. & Fried, M. (1989) *Nucleic Acids Res.* **17,** 4895.
22. Kunkel, T. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 488–492.
23. Swick, A. G., Janicot, M., Cheneval-Kastelic, T., McLenithan, J. C. & Lane, M. D. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 1812–1816.
24. Bormann, J., Hamill, O. P. & Sakmann, B. (1987) *J. Physiol.* **385,** 243–286.
25. Lukasiewicz, P. D., Maple, B. R. & Werblin, F. S. (1994) *J. Neurosci.* **14,** 1202–1212.
26. Ffrench-Constant, R. H., Rocheleau, T. A., Steichen, J. C. & Chalmers, A. E. (1993) *Nature (London)* **363,** 449–451.
27. Matsumura, F. & Ghiasuddin, S. M. (1983) *J. Environ. Sci. Health* **B18,** 1–14.
28. Amin, J. & Weiss, D. S. (1994) *Recept. Channels* **2,** 227–236.