GABA<sub>Α</sub> Receptors Containing Gamma1 Subunits Contribute to Inhibitory Transmission in the Central Amygdala

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INTRODUCTION

The amygdaloid complex is a temporal lobe structure that is involved in processing emotional information. In particular, it has a key role in the analysis and expression of fear. Disorders within this complex have been associated with a range of anxiety-related disorders such as generalized anxiety, depression, and posttraumatic stress. The treatment of these disorders is complex, with both behavioral and pharmacological treatments playing roles. The benzodiazepines form a class of compounds that have potent anxiolytic actions and are often the drug of choice in the first line of treatment for these disorders. These agents produce their therapeutic actions by enhancing the action of γ-aminobutyric acid (GABA) at GABA<sub>Α</sub> receptors containing γ<sub>1</sub> subunits. Benzodiazepine binding sites are present in the amygdala at high density (Niehoff and Kuhar 1983; Richards and Möhler 1984) and it is likely that the anxiolytic actions of these agents are due to their effects on the amygdala (Pesold and Treit 1994). However, the physiological actions of benzodiazepines are complicated because these agents have effects on GABA<sub>Α</sub> receptors distributed throughout the CNS (Sieghart and Sperr 2002), leading to a range of side effects of systemically administered drugs. Thus the development of more specific compounds for the treatment of these disorders requires an understanding of the molecular composition of GABA<sub>Α</sub> receptors and their distributions in specific circuits within the amygdala.

The GABA<sub>Α</sub> receptor belongs to the Cys-loop superfamily of ligand-gated ion channel and has a heteropolymeric structure that forms a chloride channel (Barnard et al. 1998; Olsen and Tobin 1990). Molecular cloning has so far identified 21 different subunits that can assemble to form ionotropic GABA receptors—6 α, 4 β, 4 γ, 1 δ, 1 ε, 1 π, and 3 ρ—all of which are products of separate genes. Additional heterogeneity is produced by alternative splicing of some of the subunits. These subunits assemble as pentamers and, as for many other ion channels, their biophysical and pharmacological properties are dependent on the subunit stoichiometry (Sieghart and Sperr 2002). Most GABA<sub>Α</sub> receptors in the mammalian CNS are thought to contain α, β, and γ subunits, with the most common receptor having a stoichiometry α1β2γ2 (Möller 2006; Sieghart and Sperr 2002). High-potency benzodiazepine modulation of the GABA<sub>Α</sub> receptor requires the presence of γ2 subunits (Möller 2006). Recent studies have suggested that among the various actions of benzodiazepines, receptors containing α1 subunits are responsible for the sedative/hypnotic actions of benzodiazepines, whereas receptors containing α2 subunits mediate their anxiolytic actions (Rudolph and Möller 2006).

The amygdaloid complex contains 17 different nuclei that have extensive reciprocal connections with cortical and subcortical regions as well as dense internuclear connections (Sah et al. 2003). A variety of GABA<sub>Α</sub> receptor subunits are expressed in all nuclei within this complex and electrophysiological studies, both in vivo and in vitro, have shown synaptic activation of GABA<sub>Α</sub> receptors in several regions (Delaney and Sah 2001; Lang and Paré 1997; Martina et al. 2001; Royer et al. 1999). In this study we have attempted to define the likely subunit composition of GABA<sub>Α</sub> receptors at inhibitory synapses in the central and basolateral amygdaloid. Using real-time polymerase chain reaction (PCR) we first determine the expression levels of different GABA<sub>Α</sub> receptor subunits. We then measure the pharmacological profile of the most likely receptor combinations by expressing the most abundantly expressed subunit combinations in human embryonic kidney (HEK293) cells. We show that inhibitory synapses in the basolateral
amygdala and at synapses formed by medial inputs to neurons in the central amygdala have properties similar to those containing α(1–3)/βγ2 subunits. However, a population of receptors present at lateral inputs to CeA neurons appear to contain γ1 subunits.

METH ODS
Cell culture and transfection
HEK293 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (Gibco). Cells were harvested once each week and seeded at about 50% confluence onto glass coverslips in 35-mm culture dishes. Transfections were performed 24 h after plating.

Foster City, CA). The cells were perfused by a parallel array of microtubular barrels through which solutions were gravity-induced.

PCR reactions were performed in a 20-ml volume containing 1 U Taq (Fisher Biotech), 1.5 mM MgCl2, 0.2 mM dNTP, and 10 pmol of specific GABA receptorsubunit forward and reverse primers with 50 ng of template. Samples were made ≤20 ml with RNase, DNase-free water. Reactions were carried out using a Quant Tect SYBR Green kit (Qiagen) in a Roter-Gene (Corbell Research) PCR machine as follows: an initial 95°C denaturation step for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s repeated 40 times. The amplification products were analyzed on 2% agarose gels stained with ethidium bromide. Primers used for real-time PCR are listed in the Supplemental Materials.1

Relative expression data were quantified using 2^(-ΔΔCt) where Ct is the cycle threshold. Relative standard curves were generated by plotting the threshold value (Ct) versus the log of the amount of total cDNA added to the reaction and used to check the efficiency of primers. Calculations of Ct, standard curve preparation, and quantification of mRNA in the samples were performed by software provided with the Rotor-Gene 3000 (version 6). All target genes were normalized to the β-actin housekeeping gene.

Slice recordings
Wistar rats, aged postnatal day 21 (P21) to P28 were anesthetized with halothane and decapitated. The brain was immediately immersed in ice-cold carbogenated (95% O2-5% CO2) Ringer, containing (in mM): 118 NaCl, 2.5 KCl, 25 NaHCO3, 1.2 NaH2PO4, 1.3 MgCl2, 2.5 CaCl2, and 10 d-glucose. Coronal slices (350 μM) containing the amygdala nuclei were cut with the vibratome in the preceding solution. Slices were then placed onto nylon net and submerged in bubbled Ringer at 34°C for 30 min. Slices were allowed to recover for ≥30 min at room temperature (24°C) before recording.

For recordings, slices were superfused in the preceding solution at 200 ml/h. Kynurenic acid (3 mM) was included in the external solution to block excitatory glutamatergic transmission. Inhibitory postsynaptic currents (IPSCs) were recorded from principal neurons in the basolateral amygdala (BLA) (Faber et al. 2001) and lateral division of the central amygdala (CeA) (Delaney and Sah 1999) using whole-cell patch-recording techniques using infrared-differential interference contrast optics. Borosilicate glass electrodes (3–5 MΩ) were filled with high chloride internal solution containing (in mM) 130 CsCl, 1 MgCl2·6H2O, 10 EGTA, 10 HEPES, and 2 Mg2ATP, and 0.2 Na2GTP (pH 7.3 with CsOH, 290 mosM). IPSCs were recorded at a holding potential of ~60 mV and were evoked by electrical stimulation applied locally within the BLA nucleus or laterally along the edge of lateral division of CeA and medially in central nucleus medial sector, as in Delaney and Sah (2001), and isolated by the addition of glutamate receptor blockers (kynurenic acid or/and NBQX). Signals were recorded using a Multiclamp 700A (Molecular Devices), filtered at 5 kHz, and digitized at 10 kHz (ITC 16; Instruchtech). Data were acquired with Axograph (Molecular Devices) on a Macintosh OS X computer. Series resistance (10–30 MΩ) was monitored on-line throughout the experiment and experiments were rejected if resistance changed by >10%. No series resistance compensation was used. IPSC peak amplitude and decay time constants were analyzed using Axograph 4.9 and compiled and statistically analyzed using Microsoft Excel or Prism. All values are expressed as means ± SE and all statistical comparisons were done using Student’s t-test. All experiments were done at room temperature (21–24°C).

Drugs and reagents
Bicuculline methiodide, diazepam, flunitrazepam, zolpidem, DMCM (β-carboline methyl-4-ethyl-6, 7-dimethoxy-β-carboline-3-carboxylate), GABA, and kynurenic acid were all obtained from Sigma. Tetrodotoxin (TTX) and 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo-

1 The online version of this article contains supplemental data.
quinoxaline-2,3-dione (NBQX) were obtained from Alomone and Tocris Laboratories, respectively. Pentobarbitone was a gift from Dr. Mark Bellingham.

Data analysis

The empirical Hill equation, fitted by a nonlinear least-squares algorithm (SigmaPlot 9.0; Jandel Scientific, San Rafael, CA), was used to calculate the half-maximal response concentration (EC50), the half-maximal inhibitory concentration (IC50), and the Hill coefficient (nH) of excitatory and inhibitory dose–response curves. Statistical significance was determined by a one-way ANOVA, with P < 0.05 representing significance.

RESULTS

Functional GABA\(_{\text{A}}\) receptors present at synapses are thought to be heteromultimers of \(\alpha\), \(\beta\), and \(\gamma\) subunits (Sieghart

![Figure 1](image1.png)

FIG. 1. Expression levels of \(\gamma\)-aminobutyric acid type A (GABA\(_{\text{A}}\)) receptor subunits in the rat amygdala. A and B: real-time polymerase chain reaction (PCR) results for GABA\(_{\text{A}}\) receptor subunits in the basolateral amygdala (BLA) and central amygdala (CeA). As can be seen, the expression level of \(\gamma_{1}\) is higher than that of \(\gamma_{2}\) in the CeA. The expression of \(\gamma_{3}\) is at a low level in both nuclei. In the CeA real-time PCR results reveal that \(\alpha_{2}\), \(\alpha_{4}\), \(\beta_{1}\), \(\beta_{3}\), \(\gamma_{1}\), and \(\gamma_{2}\) subunits are expressed at levels higher than that of other subunits. The \(\alpha_{1}\), \(\alpha_{5}\), \(\beta_{2}\), and \(\gamma_{3}\) are the next most abundant subunits expressed in CeA. Panels on the right show expression levels of \(\delta\), \(\theta\), \(\epsilon\), \(\rho_{2}\), and \(\rho_{3}\).

![Figure 2](image2.png)

FIG. 2. GABA and bicuculline sensitivity of recombinantly expressed GABA\(_{\text{A}}\) receptors in human embryonic kidney (HEK293) cells. Whole cell voltage-clamp recordings from HEK293 cells transiently transfected with the indicated subunits. A: GABA was applied at the indicated concentrations (\(\mu\)M) using a flow pipe. B: GABA was applied at EC20 and bicuculline applied at the indicated concentrations. C: the normalized dose–response activation curve for the action of GABA. D: normalized dose–response inhibition curve for the action of bicuculline. Data have been fit with a Hill equation (see METHODS). Half-maximal response concentration (EC50) values for each subunit combination are given in Table 1. Scale bars represent 100 pA and 30 s.
and Sperr 2002). To begin to determine the subunit stoichiometry of GABA_A receptors present in the amygdala we first mapped expression of different subunits using real-time PCR (Fig. 1). These results indicate that in the basolateral amygdala the relative expression levels of GABA_A receptor subunits are α2 > α1, β1, β3 ≥ β2, γ1 < γ2, whereas and in the central amygdala the expression pattern is α2 > α1, β1, β3 ≥ β2, γ1 > γ2. As can be seen in Fig. 1, some expression of δ, θ, ε, ρ2, and ρ3 subunits was also detected, although their expression levels were very low in both the BLA and CeA. No ρ1 expression was detectable in either nucleus. It is clear that GABA_A receptors consisting of different subunits show distinct pharmacologies (Sieghart and Sperr 2002). To determine the subunit stoichiometry of receptors at a particular synapse, specific pharmacological tools are required to discriminate between different GABA_A receptor stoichiometries. To date, the pharmacological profiles of different subunit combinations have been tested by heterologous expression in Xenopus oocytes as well as mammalian expression systems (Sieghart and Sperr 2002). However, there are clear differences in the pharmacology of expressed GABA_A receptors between oocytes and mammalian cells (Yamashita et al. 2006); thus we first evaluated the properties of receptors of different subunit combinations expressed in mammalian HEK293 cells.

Pharmacology of recombinantly expressed GABA_A receptors

GABA SENSITIVITY. From the expression profile of GABA_A receptor subunits in the amygdala the different subunit combinations were transiently expressed in HEK293 in a 1:1:3 ratio. Combinations tested were α1β1, α2β1, α1β1γ1, α1β1γ2, α2β1γ1, and α2β1γ2 GABA_A receptors. We first evaluated the GABA sensitivity of each expressed receptor type. Sample current responses from cells expressing α1β1, α2β1, and α2β1γ2 receptors are shown in Fig. 2A and averaged dose responses for all six receptors are shown in Fig. 2B. Mean parameters of best fit to the individual dose–response curves are presented in Table 1. In agreement with previous studies on GABA_A receptors expressed in Xenopus oocytes (Sigel and Baur 2000; Wafford et al. 1993), α1β1 and α2β1 GABA_A receptors were significantly (P < 0.01) more sensitive to GABA relative to α1β1γ1, α2β1γ1, α1β1γ2, and α2β1γ2 GABA_A receptors. The averaged GABA EC50 values for the α1β1γ1 and α1β1γ2 GABA_A receptors were significantly (P < 0.01) reduced relative to those of the α2β1γ1 and α2β1γ2 GABA_A receptors. In all pharmacological experiments described in the following text, GABA was applied at the EC20 concentration at each receptor type. This corresponded to 1 μM GABA for α1β1 and α2β1 receptors, 3 μM GABA for α1β1γ1 and α1β1γ2 receptors, and 5 μM GABA for α2β1γ1 and α2β1γ2 receptors.

We next tested the effect of bicuculline at all six GABA_A receptor subunits. Examples of bicuculline dose–response relationships for α1β1, α2β1γ1, and α2β1γ2 GABA_A receptors are shown in Fig. 2B with averaged dose–response relationships for all six receptors plotted in Fig. 2D. The mean parameters of best fit to individual dose–response curves are summarized in Table 1. Although there was generally a significant increase in bicuculline sensitivity in α2–relative to α1-containing receptors, this difference was too small for use to pharmacologically discriminate among the GABA_A receptors investigated here.

### Table 1. Mean Hill equation parameters of best fit for pharmacological compounds investigated in this study

<table>
<thead>
<tr>
<th>Receptor and Compound</th>
<th>EC50/IC50†</th>
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<td><strong>GABA, μM</strong></td>
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<tr>
<td>α1β1</td>
<td>3.00 ± 0.60</td>
<td>1.03 ± 0.21</td>
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</tr>
<tr>
<td>α2β1</td>
<td>3.90 ± 0.30</td>
<td>0.97 ± 0.07</td>
<td>8</td>
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<tr>
<td>α1β1γ1</td>
<td>22.40 ± 2.00**</td>
<td>1.11 ± 0.10</td>
<td>10</td>
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<tr>
<td>α2β1γ1</td>
<td>110.30 ± 1.70**</td>
<td>0.98 ± 0.12</td>
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</tr>
<tr>
<td>α1β1γ2</td>
<td>19.40 ± 1.40**</td>
<td>1.09 ± 0.07</td>
<td>6</td>
</tr>
<tr>
<td>α2β1γ2</td>
<td>12.30 ± 0.90**</td>
<td>0.86 ± 0.05</td>
<td>6</td>
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<td><strong>Bicuculline, μM</strong></td>
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<tr>
<td>α1β1</td>
<td>1.90 ± 0.16</td>
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<td>7</td>
</tr>
<tr>
<td>α2β1</td>
<td>0.83 ± 0.09**</td>
<td>−0.70 ± 0.05</td>
<td>7</td>
</tr>
<tr>
<td>α1β1γ1</td>
<td>1.59 ± 0.17</td>
<td>−0.73 ± 0.05</td>
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<tr>
<td>α2β1γ1</td>
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<td>2.49 ± 0.40</td>
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<tr>
<td>α2β1γ2</td>
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<td>−1.09 ± 0.10</td>
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<td><strong>Diazepam, μM</strong></td>
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<td>0.71 ± 0.09</td>
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<td><strong>Zolpidem, nM</strong></td>
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<tr>
<td>α1β1</td>
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<tr>
<td>α2β1</td>
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<tr>
<td>α1β1γ1</td>
<td>182.00 ± 36.00</td>
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<td>α1β1γ2</td>
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<td>α2β1γ2</td>
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<td>1.45 ± 0.57</td>
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<td><strong>DMMC, nM</strong></td>
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</tr>
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<td>5</td>
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<tr>
<td>α2β1</td>
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<tr>
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<tr>
<td>α2β1γ2</td>
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<td>−0.35 ± 0.02</td>
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<td>α1β1</td>
<td>919.00 ± 267.00</td>
<td>−0.50 ± 0.08</td>
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†Values for bicuculline and DMMC represent IC50; all others represent EC50. *P < 0.05, **P < 0.01, ***P < 0.001 relative to α1β1 GABA_A receptors using one-way ANOVA.

EFFECTS OF BENZODIAZEPINES. The effects of the classical non-selective benzodiazepine receptor agonist diazepam were investigated at concentrations between 0.01 to 1.00 μM on currents activated by EC20 GABA at each of the six receptor subtypes. Examples of the diazepam dose responses at α1β1γ1 and α1β1γ2 GABA_A receptors are shown in Fig. 3A, left. Diazepam dose–response curves were measured for all receptors with averaged diazepam EC50 and nH values of best fit summarized in Table 1. These results agree with previous studies (Pritchett et al. 1989; Walters et al. 2000) showing that γ-subunit incorporation conferred a significant increase in diazepam sensitivity. Furthermore, as previously observed in Xenopus oocyte-expressed GABA_A receptors (Walters et al. 2000), 0.1 μM diazepam potentiated γ2-containing receptors to a significantly greater extent than γ1-containing receptors (Fig. 3A, right). As expected, α1β1 and α2β1 receptors were not significantly potentiated by 0.1 μM diazepam. This response profile was largely conserved at 1 μM diazepam, although at 10 μM diazepam the α1β1 and α2β1 GABA_A receptors were also strongly potentiated (Fig. 3A, right). These results indicate that diazepam at concentrations of 0.1–1 μM can be used as a selective pharmacological marker for γ-subunit–containing GABA_A receptors.
Effects of the benzodiazepine type I-selective imidazopyridine, zolpidem (MacDonald and Olsen 1994; Pritchett et al. 1989), were tested at concentrations of 10 nM to 100 µM on currents induced by EC20 GABA at each of the six receptors. Examples of zolpidem effects on α2β1γ1 and α1β1γ2 GABA_A receptors are shown in Fig. 3B, left. Zolpidem enhanced GABA-induced currents in γ-containing receptors but it had no significant effect on α1β1 or α2β1 receptors at any concentration tested. Thus dose–response curves for zolpidem were quantitated for γ-containing receptors only, with averaged EC50 and nH values of best fit summarized in Table 1. It is apparent in Fig. 3B, right, that 0.1 and 10 µM zolpidem potentiated α1β1γ2 receptors to a significantly greater extent than α1β1γ1 receptors. Thus zolpidem can be used to pharmacologically identify the presence of γ subunits and can discriminate between γ1- and γ2-containing GABA_A receptors.

DMCM. The β-carboline DMCM is an inverse benzodiazepine receptor agonist (Stevenson et al. 1995). The effects of this compound were tested at concentrations from 1 nM to 10 µM on currents activated by EC20 GABA at each of the six receptors used in this study. Examples of the effects of DMCM on α1β1γ1 and α1β1γ2 GABA_A receptors are shown in Fig. 3C. DMCM had no consistent effect on α1β1, α2β1, α1β1γ1, or α2β1γ1 GABA_A receptors at any concentration (Fig. 3C). However, it potently inhibited both the α1β1γ2 and α2β1γ2 GABA_A receptors with IC50 values in the high nanomolar range (Fig. 3C, Table 1). The reduction in GABA-evoked currents at 10 nM was significantly greater for α1β1γ2 receptors than for α2β1γ2 receptors. However, at 1,000 nM DMCM, its differential inhibitory effects were largely reduced (Fig. 3C, right). These inhibitory effects of DMCM agree with previous studies (Stevenson et al. 1995). Studies on oocyte-expressed GABA_A receptors have shown a concentration-dependent switch from inverse agonist activity to agonist activity (Sigel et al. 1990; Stevenson et al. 1995); however, we did not see this effect on receptors expressed in HEK293 cells and DMCM uniformly acted as an antagonist at the receptors we tested (e.g., Fig. 3C).
Pharmacology of synaptic GABA$_A$ receptors

In agreement with previous studies (Sieghart and Sperk 2002), Fig. 1 shows that in the basolateral and central amygdala, the dominant subunits expressed are α2 and β3 with α1 expression particularly low in the CeA. Both γ1 and γ2 subunits are also expressed in both nuclei, although there were clear differences in the expression levels of the two receptors (Fig. 1). In the CeA, relative expression levels of γ1 and γ2 subunits were 803 ± 43 and 560 ± 62 units, respectively (P < 0.05, n = 3), whereas for the BLA these levels were 342 ± 9 and 1,308 ± 25 (P < 0.05, n = 3). Thus expression of γ1 subunits is significantly higher than that of γ2 subunits in the CeA, whereas the relative levels are reversed in the BLA. Interestingly, synaptic GABA$_A$ receptors containing γ1 subunits have not been previously described; we thus tested whether γ1 subunits may be present at synapses in the CeA. We compared the pharmacology of inhibitory synaptic currents at three distinct synapses: 1) those made by local interneurons onto principal neurons in the basolateral amygdala (BLA), 2) those made by inputs arising from the stria terminalis onto neurons in the CeA (the medial input) (Delaney and Sah 1999), and 3) inhibitory inputs made by intercalated cell masses onto neurons in the CeA (the lateral input) (Delaney and Sah 1999). The medial and lateral inputs to the CeA are defined as CeAM and CeAL, respectively.

Inhibitory synaptic currents were evoked in voltage clamp after blockade of excitatory transmission with kynurenic acid. At principal neurons in the BLA, application of bicuculline (10 μM) almost fully blocked the IPSC (90 ± 2% n = 5, P < 0.05). However, as previously described (Delaney and Sah 2001) for CeL neurons, whereas IPSCs originating from the medial inputs were almost completely blocked by bicuculline, those activated by lateral inputs were not. When bicuculline was applied at 10 μM, the medially evoked IPSC was blocked to 93 ± 1% of control (n = 6, P < 0.05), whereas the laterally evoked IPSC was only partially blocked (to 64 ± 4% of control, n = 6, P < 0.05). Consistent with the incomplete block of the IPSC at this lateral input, increasing the stimulus intensity in the presence of 10 μM bicuculline increased the amplitude of the laterally evoked IPSC but had no effect on the IPSC evoked from the medial input or the IPSC evoked in the BLA (data not shown).

EFFECTS OF BENZODIAZEPINES. Diazepam was tested at two concentrations: 1 and 10 μM. In the CeA, diazepam had distinct actions at the medial and lateral inputs (Fig. 4, A–E). For the medial input, diazepam at both concentrations had little effect on IPSC amplitude (amplitude 109 ± 14% of control and 112 ± 19% of control for 1 and 10 μM, respectively; P > 0.1, n = 11). The decay time constant of the IPSC was slightly enhanced by 1 μM diazepam (from 41 ± 5 to 51 ± 7 ms, P < 0.05), whereas it was significantly enhanced at 10 μM (to 54 ± 4 ms, P < 0.04, Fig. 4E). In comparison, the lateral input to CeAL neurons was largely insensitive to diazepam with no effect on either amplitude or decay time constant (Fig. 4, B, D, and E). At 1 μM, peak amplitude was 95 ± 10% of control (P > 0.1, n = 11) and the decay time constant changed from 38 ± 4 to 40 ± 4 ms (P > 0.1), whereas 10 μM diazepam caused a modest reduction in peak amplitude to 87 ± 10% of control (P > 0.05, n = 11) and the decay time constant increased to 45 ± 4 ms (P > 0.05, n = 11). In contrast to the CeAL, in the BLA, diazepam enhanced the amplitude of the IPSC at 1 μM (to 139 ± 14% of control, P < 0.01) and slightly slowed the decay time constant from 38 ± 4 to 47 ± 6 ms (P < 0.05, n = 11; Fig. 4, C–E). At 10 μM the amplitude of the IPSC increased to 139 ± 14% (P < 0.05), but had little further effect on the decay time constant to 50 ± 6 ms (P > 0.05, n = 11; Fig. 4, C–E).

Zolpidem is a benzodiazepine that potentiates responses to α1-containing receptors to a greater extent than those containing α2 subunits (Korpi et al. 2002; Möhler et al. 2002). Overall, we found it had similar effects to diazepam (Fig. 5, A–E). In the CeA, for the medial input the amplitude of the IPSC was scarcely affected by zolpidem at 0.1 μM (to 114 ± 9% of control, P > 0.05) but enhanced to 120 ± 9% of control at 1 μM zolpidem (P < 0.05; n = 7); however, there was a minimal effect on the decay time constant, changing from 39 ± 7 to 46 ± 9 and 43 ± 7 ms (27%, P > 0.1) for the low and high concentration, respectively (n = 9). Similar to the actions of diazepam, at lateral inputs, zolpidem has no effect on the peak amplitude of the IPSC at either low or high concentrations (92 ± 7% of control at 0.1 μM and 82 ± 2% of control at 1 μM, P > 0.1, n = 7). The decay time constant was not affected by either concentration of zolpidem (from 33 ± 2 to 33 ± 3 ms for 0.1 μM and to 37 ± 2 ms for 1 μM, P > 0.05 for each case, n = 7). In contrast, in the BLA, IPSCs were significantly
enhanced. Zolpidem at 0.1 µM increased the peak amplitude of IPSCs to 132 ± 3% of control (P > 0.05) and the decay time constant was little affected (from 34 ± 4 to 35 ± 3 ms, P > 0.05), whereas at 1 µM, zolpidem increased the peak amplitude of IPSCs to 117 ± 5% of control (P < 0.05) and decay time constant to 40 ± 3 ms (P < 0.05; n = 9).

**EFFECT OF DMCM.** Application of DMCM to IPSCs in the BLA led to a clear reduction in their peak amplitude (Fig. 6). At a concentration of 10 µM DMCM reduced the peak amplitude to 55 ± 4% of control (P < 0.01, n = 13) with no effect on the decay time constant (from 55 ± 4 to 59 ± 4 ms, n = 13, P > 0.05). In the CeA, application of DMCM reduced the peak amplitude of the medially evoked IPSC by 63 ± 5% but having no effect on the amplitude of the IPSC evoked by lateral stimulation (IPSC in DMCM: 94 ± 16%, n = 6, P > 0.05; Fig. 6). As in the BLA, DMCM had no effect on the decay time constant of the IPSC at either the medial (control: 40 ± 5 ms; DMCM: 47 ± 8 ms, n = 11) or the lateral input (control: 31 ± 2 ms; DMCM: 28 ± 2 ms, P > 0.05, n = 9).

**DISCUSSION**

The amygdala is a temporal lobe structure that plays a key role in emotional behavior and dysfunction within the amygdala is thought to contribute to a range of anxiety and mood disorders (Davis and Whalen 2001; Drevets 2003). Benzodiazepines, commonly used as therapeutic agents in the pharmacological management of anxiety-related disorders, are well known to act at ionotrophic GABA<sub>A</sub> receptors (Rudolph and Möhler 2006). However, the therapeutic actions of these agents are complicated by their diverse side effects due to the widespread distribution of GABA<sub>A</sub> receptors throughout the CNS. Because ionotropic GABA<sub>A</sub> receptors are heterooligomeric pentamers that are expressed in different stoichiometries...
throughout the CNS (Sieghart and Sperk 2002), the design of more specific therapeutic agents requires a clear understanding of the subunit composition of receptors at specific synapses in central circuits. In this study we have attempted to determine the subunit composition of GABA<sub>A</sub> receptors at inhibitory inputs in the basolateral and central amygdala. Our results show that at GABAergic synapses onto principal neurons in the BLA, the postsynaptic ionotropic receptors that are present are consistent with a composition α2β3γ2. In the lateral sector of the central amygdala, medial inputs, presumably arising from the bed nucleus of stria terminalis also contain receptors that are composed of α2β2γ2. However, lateral inputs, thought to arise from the intercalated cell masses (Delaney and Sah 2001), express receptors that have a distinctly different pharmacology. Although the exact composition of these receptors is currently unclear, our pharmacological analysis suggests that they contain γ1 subunits.

GABA<sub>A</sub> receptors present at inhibitory synapses in the BLA are potently blocked by biccuculline and are clearly potentiated by both type I and type II benzodiazepines, diazepam and zolpidem, indicating that γ2 subunits are present at these synapses. This is similar to the pharmacology of GABA<sub>A</sub> receptors that has been described at many synapses in the mammalian CNS that are thought to be pentamer-containing α1/2β1/3γ2 subunits (Möller 2006; Sieghart and Sperk 2002). Consistent with this we find that application of the β-carbolines DMCM also reduced the amplitude of the IPSC at these synapses. Both mRNA (Fujimura et al. 2005; Pirker et al. 2000) and protein (Marowsky et al. 2004) levels of α2 subunits are relatively high in the BLA. Together with the relatively higher levels of β1 and γ2 subunits, our data suggest that inhibitory synapses on principal neurons in the BLA most likely have a stoichiometry of α2β1/2γ2.

In the lateral sector of the CeA we have described two different GABAergic inputs to these neurons. One, the “medial” input, defined by the site of stimulation in acute coronal slices, is thought to originate in the bed nucleus, whereas the “lateral” input arises from the intercalated neuron masses and are thought to mediate inhibition of amygdala output during extinction of conditioned fear (Likhitik et al. 2008). Thus specific modulators of these receptors open the possibility of a selective treatment for anxiety-related disorders.

**References**


