Different Subtypes of GABA_B Receptors Are Present at Pre- and Postsynaptic Sites Within the Rat Dorsolateral Septal Nucleus

KEI YAMADA, BAOJIAN YU, AND JOEL P. GALLAGHER
Department of Pharmacology and Toxicology, University of Texas Medical Branch at Galveston, Galveston, Texas 77555-1031

Yamada, Kei, Baojian Yu, and Joel P. Gallagher. Different subtypes of GABA_B receptors are present at pre- and postsynaptic sites within the rat dorsolateral septal nucleus. J. Neurophysiol. 81: 2875–2883, 1999. GABA_B receptor activation modulates neuronal activity mediated by multiple CNS transmitters and can occur at pre- and postsynaptic sites. In low concentrations, baclofen acts presynaptically to diminish transmitter release via both hetero- and autoreceptors, whereas at increasing concentrations, the same compound alters postsynaptic membrane excitability by inducing a membrane hyperpolarization. We have utilized electrophysiological techniques in vitro to focus on the possibility that pharmacologically different subtypes of GABA_B receptors are present on presynaptic sites of glutamatergic terminals when compared with GABA_B receptors on postsynaptic sites within the dorsolateral septal nucleus (DLSN). The glutamatergic terminal within the DLSN originates from a pyramidal cell body located within the hippocampus and most likely terminates on a GABAergic neuron from which recordings were made. Whole cell patch voltage-clamp methods were employed to record pharmacologically isolated excitatory postsynaptic currents (EPSCs) from DLSN neurons as an index of glutamatergic transmission. Using a modified internal pipette solution containing QX-314 and in which CsGluconate and GDPbS replaced Kgluconate and GTP, respectively, we recorded isolated monosynaptic EPSCs. The GABA_A receptor antagonists bicuculline and picrotoxin were included in the external standard superfusion solution. Application of the GABA_B receptor agonists, (±)-baclofen, CGP44533, and CGP35024 (10 nM to 10 μM) depressed glutamate-mediated EPSCs in a concentration-dependent manner. With the use of this combination of solutions, CGP44533 did not produce postsynaptic membrane property changes. Under these conditions, both (±)-baclofen and CGP35024 still induced increases of postsynaptic membrane conductance associated with an outward current. The GABA_B receptor antagonist CGP55845A (1 μM) blocked the presynaptic CGP44533-mediated depressant effects of EPSCs, whereas CGP35348 (100 μM) or barium (2 mM) was ineffective. Furthermore, both CGP35348 (100 μM) and CGP55845A (1 μM) were effective in blocking the postsynaptic conductance changes associated with baclofen and CGP35024, whereas barium was ineffective. Our results demonstrate a distinct pharmacology for GABA_B agonists acting at putative subtypes of GABA_B receptors located on presynaptic sites of a glutamatergic terminal versus GABA_B receptors on postsynaptic sites of a DLSN neuron. Furthermore, our results also suggest a different pharmacology and/or coupling of a GABA_B receptor to different effectors at postsynaptic sites within the DLSN. Thus there may be three or more pharmacologically distinct GABA_B receptors or receptor complexes associated with DLSN neurons: at least one pre- and two postsynaptic. If this distinct pharmacology and GABA_B receptor distribution also extends to other CNS structures, such differences could provide development of selective drugs to act at these multiple sites.

INTRODUCTION

GABA receptors in the mammalian CNS are currently classified into three pharmacological types: GABA_A, GABA_B, and GABA_C. Multiple neurochemical studies at a variety of CNS preparations in which transmitter release has been monitored suggest that subtypes of GABA_B receptors are present on various nerve terminals. Furthermore, molecular biological cloning of GABA_B receptors has provided additional support to the concept of multiple GABA_B receptors. The initial cloning of a GABA_B receptor that demonstrated the presence of two splice variants (GABA_B1a and GABA_B1b) (Kaupman et al. 1997), has recently been expanded by the demonstration of a second and different GABA_B clone: GABA_B2. This latter discovery was especially exciting in that four different laboratories, almost simultaneously, reported their similar results (Jones et al. 1998; Kaupmann et al. 1998; Kuner et al. 1999; White et al. 1998). Furthermore, the discovery of GABA_B2 adds a new concept to the field of G protein–coupled receptors in that the combination, as a heterodimer, of these two clones (GABA_B1 + GABA_B2) is required when expressed in a cell line with an inward rectifier potassium channel (GIRK) to observe full biological activity comparable with that recorded from native tissue. It may be possible that the requirement for this combination of two different clones may lead to the further discovery of additional GABA_B receptors, which may involve the formation of novel heterodimers.

A major function of GABA_B receptors is to modulate transmitter release (Alford and Grillner 1991; Bowery et al. 1980; Davies et al. 1991; Kombian et al. 1996; Mouginot and Gähwiler 1996; Thompson et al. 1993). Activation of nerve terminal GABA_B receptors inhibits the release of various transmitters including biogenic amines, GABA, and glutamate. Reports have implicated CNS GABA_B receptors with several CNS disorders, for example, epilepsy, specifically absence seizures (Crunelli and Leresche 1991; Malcangio and Bowery 1995), drug abuse (Roberts and Andrews 1997; Roberts et al. 1996; Shoji et al. 1997), and other CNS disorders (Bowery et al. 1997). In animal models, GABA_B receptor antagonists block absence-epilepsy seizures, whereas spike-and-wave discharges were suppressed by injection of CGP35348 intraperitoneally (Smith and Fisher 1996). Experiments by Roberts et al. (1996) and Roberts and Andrews (1997) demonstrated that acute treatment with baclofen suppressed cocaine self-administration in rats, whereas Gudeman et al. (1996) and Ling et al. (1998) reported that baclofen may act as a cocaine anti-craving medication in cocaine-dependent individuals. These and other results suggest that GABA_B receptor agonists or antagonists
could prove to be potentially useful therapies for a variety of CNS disorders. Because GABA<sub>B</sub> receptors exist in various parts of the nervous system, it is difficult to predict where GABA<sub>B</sub> receptor agonists or antagonists act and whether they do so selectively.

The possibility that multiple GABA<sub>B</sub> receptor subtypes are found in various synapses in brain has been suggested (Beck et al. 1995; Bonanno and Raiteri 1992, 1993a,b; Calabresi et al. 1991; Cunningham and Enna 1996; Dutar and Nicoll 1988; Gemignani et al. 1994; Howel et al. 1987; Pham and Lacaille 1996; Pham et al. 1998). In particular, using both baclofen and GABA, older GABA<sub>B</sub> receptor antagonists (phaclofen, 5-OH-saclofen, CGP35348) were shown to antagonize less effectively postsynaptic responses to GABA versus similar responses due to exogenous application of baclofen (Jarolimek et al. 1994). More recently, Pham et al. (1998) suggested that there were two distinct GABA<sub>B</sub> receptors located postsynaptically on hippocampal pyramidal cells, and that these receptors were linked to two different K<sup>+</sup> conductances.

In addition, application of expression cloning had demonstrated two splice variants of the first GABA<sub>B</sub> receptor clone (Bowery and Brown 1997; Kaupmann et al. 1997). Data suggested that one splice variant (GABA<sub>B</sub>1a) was located presynaptically, whereas the other (GABA<sub>B</sub>1b) was located postsynaptically. Furthermore, Shoji et al. (1997) suggested two different subtypes of GABA<sub>B</sub> receptors within the same synapse at pre- and postsynaptic sites. The results of Shoji's experiments are consistent with several prior studies (Deisz et al. 1993, 1997; Dutar and Nicoll 1988; Pitzler and Alger 1994; Thompson and Gähwiler 1992). These results focused on the different subtypes of GABA<sub>B</sub> receptor within GABAergic synapses. On the other hand, there has been a limited number of reports (Huston et al. 1995; Yoon and Rothman 1991; for review see Thompson et al. 1993) that investigated the pharmacology of GABA<sub>B</sub> heteroreceptors at glutamatergic synapses. A major limitation has been the unavailability of novel GABA<sub>B</sub> agonists other than baclofen, and potent antagonists.

In this study we demonstrate with electrophysiological techniques a pharmacological distinction between subtypes of GABA<sub>B</sub> receptors at pre- versus postsynaptic sites in the dorsolateral septal nucleus (DLSN). Our data also support the recent findings of Pham et al. 1998, namely, that two postsynaptic GABA<sub>B</sub> receptors are coupled differentially to potassium channels. Thus this glutamatergic terminal onto presumptive GABAergic neurons within the DLSN provides us with potentially three different GABA<sub>B</sub> receptor subtypes. These are distinguished by their pharmacological sensitivity to GABA<sub>B</sub> agonists and antagonists; at least one presynaptic type with two others located postsynaptically.

**METHODS**

**Preparation of brain slices**

Male Sprague-Dawley rats (100–250 g; Harlan) were decapitated, and their brains were rapidly dissected out and placed in cold artificial cerebrospinal fluid (ACSF) of the following composition (in mM): 117 NaCl, 4.7 KCl, 1.2 MgCl<sub>2</sub>, 2.5 CaCl<sub>2</sub>, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, 11.5 glucose, and 25 NaHCO<sub>3</sub>, prebubbled with 95% O<sub>2</sub>-5% CO<sub>2</sub>, pH 7.4. Transverse blocks of tissue containing the septum were serially cut on a Vibroslice (model 752 M; Campden Instruments, London), into 500-μm slices. A single slice was submerged and held between two nylon nets in a recording chamber that was superfused with ACSF bubbled with 95% O<sub>2</sub>-5% CO<sub>2</sub> at 30–32°C. Only neurons with stable membrane potentials of ~50 mV maintained over a minimum period of 10 min, and that yielded postsynaptic potentials (or currents) evoked by electrical focal fimbrial stimulation, were included in this study.

**Recording from brain slices**

DLSN neurons were recorded using the blind whole cell patch-clamp recording technique (Blanton et al. 1989; Zheng et al. 1995). Whole cell patch recordings were obtained with 5–7 MΩ electrodes filled with (in mM) 122 KGluconate, 5 NaCl, 0.3 CaCl<sub>2</sub>, 2 MgCl<sub>2</sub>, 1 ethylene glycol-bis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 5 Na<sub>2</sub>ATP, 2 guanosine 5'-O-(2-thiodiphosphate). Instead of using our standard pipette solution, we used CsGluconate, GDPβS, and 3 mM QX-314 in place of potassium gluconate and guanosine triphosphate (GTP). This combination has been suggested to block postsynaptic potassium channel conductance changes induced by GABA<sub>B</sub> receptor activation (McLean et al. 1996; Nathan et al. 1990; Whittington et al. 1992). We assayed for the effectiveness of GDPβS by testing for the loss of a serotonin (5HT-1A) (Twery et al. 1991) mediated hyperpolarization; once lost other GTP-dependent responses (baclofen) would also be absent.

Current signals and applied voltages were generated and recorded with an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA). The output amplifier was DC-coupled to a storage oscilloscope (model 220; Gould, Cleveland, OH). A model 4208 Panametrics video cassette recorder (A. R. Vetter, Redersburg, PA) was used to capture all tracings for off-line analysis and archiving. The stored signal could be played back and analyzed using pClamp (version 6.3.1) software, with a DigiData 1200 interface to a Gateway 2000 4DX2–66V computer. Orthodromic stimuli were delivered with square-wave pulses (4–40 V; 0.1 ms) via a concentric bipolar electrode placed focally within the DLSN.

**Drug application**

(±)-Baclofen, CGP44533, CGP35024, bicuculline methiodide, picROTOXIN, 6,7-dimethoxinoline-2,3-dione (DNQX), D(-)-2-amino-5-phosphono pentanoic acid (d-APV), CGP35348, and CGP55845A were all applied by bath superfusion to achieve steady-state concentrations within the recording chamber. (±)-Baclofen, bicuculline methiodide, and picROTOXIN were obtained from Sigma. DNQX and d-APV were obtained from RBI. QX-314 bromide was supplied by ASTRA. Novartis (Basel) kindly supplied CGP44533, CGP35024, CGP35348, and CGP55845A. (Note that CGP35024 is identical to SKF97541. Figures, legends, and text will only refer to CGP35024, although identical results have also been collected by us and reported by others with SKF97541. All results reflect the effect of drugs applied to only one neuron from each brain slice.)

**Data analysis**

A concentric electrode positioned focally at the dorsolateral horn of the septum (where hippocampal input entered via the fimbria) evoked synaptic currents. The intensity of a test stimulus was chosen to yield a half-maximal amplitude excitatory postsynaptic current (EPSC). Changes in postsynaptic conductance resulting from drug application were monitored by application of a hyperpolarizing voltage step (~5 mV). EPSCs were isolated by using the modified internal solution with addition of bicuculline (10 μM) and picROTOXIN (10 μM) to the standard external solution. Individual EPSCs were recorded at 0.3 Hz, with 10 EPSCs collected and averaged. Data from different slices were pooled and presented as means ± SE.
GABA<sub>R</sub> agonists were added in the superfusion solution and applied for 5–10 min. GABA<sub>R</sub> receptor antagonists were applied in advance of GABA<sub>B</sub> receptor agonists for 10 min and then co-applied with agonists.

Statistical analyses employed a one-way repeated measures ANOVA using SigmaStat for Windows, V. 1.0. Multiple comparisons were made to control values using Dunnett’s test for statistical significance determined at the level of \( P < 0.05 \), when \( \alpha = 0.05 \). Graphs were generated using SigmaPlot (Windows version 4.0) software (SPC Software, Santa Clara, CA), with a Hewlett Packard LaserJet 4 printer.

RESULTS

Isolation of EPSCs using a modified pipette solution and GABA<sub>A</sub> receptor antagonists in the external solution

Results were obtained from 52 DLSN neurons using blind whole cell patch-clamp recording. These cells had resting potentials ranging from \(-58 \) to \(-76 \) mV (\(-60.6 \pm 0.6 \) mV, mean \( \pm \) SE). Input resistance of the cells ranged between 75.0 and 625 M\( \Omega \) (233.4 \( \pm \) 24.9 M\( \Omega \), \( n = 44 \)). Fimbrial stimulation produced triphasic postsynaptic currents, consisting of a fast-EPSC, fast-inhibitory postsynaptic current (f-IPSC), and slow-inhibitory postsynaptic current (s-IPSC) (Fig. 1A). The f-IPSC was completely blocked by the combination of bicuculline (10 \( \mu M \)) and picrotoxin (10 \( \mu M \)). The s-IPSC was blocked by CGP35348 (100 \( \mu M \)) or CGP55845A (1 \( \mu M \)) (Bon and Galvan 1996; Pham et al. 1998) or by barium (1 mM; Fig. 1B) (Pham et al. 1998). Using sharp recording electrodes, we previously reported that the s-IPSC in the DLSN was also blocked by barium (2 \( mM \)) (Yamada and Gallagher 1997).

f-IPSCs and s-IPSCs need to be blocked to investigate monosynaptic glutamatergic transmission (Fig. 1D). Elimination of these two IPSCs was achieved by modification of the standard pipette solution and external ACSF, while not including a GABA<sub>B</sub> antagonist in the superfusion solution. Addition of GABA<sub>B</sub>, antagonists would mask a potential, selective presynaptic action of GABA<sub>B</sub> agonists. K-glucuronate was replaced with CsGluconate to block potassium-sensitive channels coupled to the GABA<sub>B</sub> postsynaptic receptor, and GTP was replaced with GDP\( \beta \)S to uncouple the postsynaptic G protein coupled to its effector, a potassium channel. QX-314 was added to block action potentials evoked by supramaximal EPSCs and to block potassium channels coupled to the postsynaptic GABA<sub>B</sub> receptor (Nathan et al. 1990). In our hands, the use of either QX-314 or GDP\( \beta \)S alone was inadequate to block the s-IPSC. Furthermore, this combination was unable to block a baclofen (or CGP55204)-induced postsynaptic membrane hyperpolarization (see Effects of GABA<sub>B</sub> agonists on postsynaptic membrane conductance and Fig. 4). Bicuculline (10 \( \mu M \)) and picrotoxin (10 \( \mu M \)) were applied in the external solution to eliminate f-IPSCs. This combination of antagonists exhibited no change in holding current or conductance. Immediately after impalement with the electrode containing the modified pipette solution, the postsynaptic response consisted of a series of triphasic postsynaptic currents (Fig. 1A). After 30 min of impalement with the modified internal solution in the recording electrode and application of bicuculline and picrotoxin, an EPSC was isolated (Fig. 1D). The amplitude of isolated EPSCs ranged between 25.8 and 173.8 pA (92.4 \( \pm \) 5.3 pA; \( n = 68 \)) and were blocked completely by DNQX (50 \( \mu M \)) and d-APV (25 \( \mu M \)).

Activation of presynaptic GABA<sub>R</sub> receptors depressed EPSCs

To study the function of presynaptic GABA<sub>B</sub> receptors in glutamatergic synapses, we used the modified internal pipette solution (see above and METHODS), while bicuculline and picrotoxin were applied in the external solution. Upon elimination of all IPSCs evoked by fimbrial stimulation, a series of three consecutive monosynaptic EPSCs were recorded and served as controls. After these control EPSCs were recorded, (+)-baclofen (10 nM to 10 \( \mu M \)) was applied to the external ACSF solution for 6–10 min. During this period, postsynaptic membrane properties (membrane conductance and holding current) were monitored continuously while applying a negative cathodal voltage step command (–5 mV).

Bath application of (+)-baclofen (10 nM to 10 \( \mu M \)) depressed the amplitude of EPSCs in a concentration-dependent manner (Fig. 2, top, \( n = 10 \)). (+)-baclofen at 10 nM depressed EPSCs by 11.4 \( \pm \) 7.2\% (\( q' = 1.27; P = 3 \); N.S.) whereas at 10 \( \mu M \), EPSCs were depressed by 81.4 \( \pm \) 5.3\% (\( q' = 8.02; P = 5 \); \( P \leq 0.05 \)). The onset of the depressant effect was observed after 3–5 min, whereas recovery occurred only after 10–30 min of wash out. Another GABA<sub>R</sub> receptor agonist, CGP44533 (Froestl et al. 1995a), which is less potent when compared with...
(--)-baclofen in binding studies (IC$_{50}$ = 152 and 32 nM, respectively), was applied in the same manner. At 10 nM, CGP44533 depressed EPSCs by 0.4 ± 3.7% (q' = 0.03; P = 3; N.S.), whereas at 10 µM, EPSCs were depressed by 78.2 ± 5.5% (q' = 6.64; P = 5; P ≤ 0.05; Fig. 2, middle). The presynaptic inhibitory potency of (--)-baclofen and that of CGP44533 were very similar (Fig. 3).

CGP35024 is reported to be one of the most potent GABA$B$ receptor agonists, being 10 times more potent than (--)-baclofen (Froestl et al. 1995a). CGP35024 (10 nM) depressed EPSCs by 28.2 ± 11.4% (q' = 2.03; P = 3; N.S.), and at 10 µM depressed EPSCs by 86.2 ± 4.5% (q' = 7.32; P = 5; P ≤ 0.05; Fig. 2, bottom). The depressant effect induced by CGP35024 was observed after 3 min exposure, and recovery occurred only after >30–60 min of wash out. The IC$_{50}$ of CGP35024 was 10 times less than that of (--)-baclofen or CGP44533 (Fig. 3).

Presynaptic depressant effect on EPSCs induced by CGP44533 persists in the presence of different GABA$B$ receptor or channel antagonists, but not in CGP55845A

There are three reports describing the sensitivity of postsynaptic GABA$B$ receptors to GABA$B$ receptor agonists or antagonists within the DLSN (Bon and Galvan 1996; Hasuo and Gallagher 1988; Stevens et al. 1985). But the sensitivity of presynaptic GABA$B$ receptors on glutamatergic neurons (heteroreceptors) to GABA$B$ receptor agonists or antagonists has not been reported. On the basis of our results showing a lack of postsynaptic conductance change by CGP44533 (Fig. 4), we utilized the CGP44533-induced presynaptic depression of orthodromically induced EPSCs as an index of GABA$B$ antagonist efficacy at presynaptic GABA$B$ receptors. After 10 min pretreatment with GABA$B$ receptor or channel antagonists, CGP44533 was co-applied with three different antagonists for 6–10 min. During application of barium (2 mM), at a concen-
tration sufficient to eliminate the s-IPSCs in the DLSN, CGP44533 (10 nM to 10 μM) still depressed EPSCs in a concentration-dependent manner (Figs. 5 and 6, n = 6). We assayed for the effectiveness of barium by monitoring the prolongation of a direct cathodally induced action potential. In the absence of barium, CGP44533 (10 nM) depressed EPSCs by 0.43 ± 3.7%, whereas at 10 μM it depressed EPSCs by 78.2 ± 5.5%. Similarly, but in the presence of barium, CGP44533 (10 nM) continued to depress EPSCs by 0.9 ± 5.5%, whereas at 10 μM it also depressed EPSCs by 75.3 ± 7.4%. CGP35348 (100 μM), at a concentration sufficient to block baclofen-induced hyperpolarizations and s-IPSCs (Bon and Galvan 1996; Yamada and Gallagher 1997), did not suppress the ability of CGP44533 to induce its presynaptic depressant effect (Figs. 5 and 6, n = 8). There was no statistical difference between results obtained in the presence or absence of CGP35348 (P > 0.05). Furthermore, all cells showed slightly potentiated EPSCs, +5.5 ± 2.2% at 1 μM (q = 3.10, P = 4), and +4.9 ± 1.7%, at 10 μM (q = 10.69, P = 4) in the presence of CGP55845A after 6–10 min co-application of CGP44533 and CGP55845A (Fig. 6, bottom). This potentiation of the EPSC by CGP55845A may have been due to its disinhibition of presynaptic GABAB receptor function by CGP55845A. CGP44533 by itself or co-application of CGP44533 with CGP35348, CGP55845A, or barium did not affect postsynaptic membrane properties under these recording conditions (data not shown). However, using sharp electrodes filled with KAc, we have recorded a direct

![FIG. 4. Concentration-response curve for postsynaptic effects of GABA_B agonists. Plots of change of postsynaptic membrane conductance compared with control membrane conductance vs. the concentration of each GABA_B agonist: CGP44533, at all 4 concentrations, P > 0.05, N.S.; CGP35348, at 0.01 and 0.1 μM, P > 0.05, N.S.; at 1 μM, P ≤ 0.05 (q = 3.45, P = 4); and at 10 μM, P ≤ 0.05 (q = 3.56, P = 5). (±)-Baclofen at 0.01 and 0.1 μM, P > 0.05, N.S.; at 1 μM, P ≤ 0.05 (q = 2.64, P = 4), and at 10 μM, P ≤ 0.05 (q = 4.18, P = 5). Concentration-dependent persistent outward current associated with an increased postsynaptic conductance is still induced by baclofen (A and B) and CGP35024 (C), even while recording with the modified pipette solution.

![FIG. 5. CGP44533 continued to depress EPSCs in the presence of GABA_B antagonists, but not in the presence of CGP55845A. Top: CGP44533 (100 nM to 10 μM) in Ba⁺ (2 mM). Middle: CGP44533 (100 nM to 10 μM) in CGP35348 (100 μM). Bottom: CGP44533 (100 nM to 10 μM) on EPSC in CGP55845A (1 μM). The depressant effect on EPSC by CGP44533 was blocked completely by CGP55845A at all concentrations of CGP44533.](http://jn.physiology.org/)
postsynaptic membrane hyperpolarization associated with a conductance increase after superfusion with CGP44533 (10 μM) (Yamada and Gallagher 1997); this postsynaptic action of CGP44533 was blocked by CGP33548 (100 μM). We have repeated these experiments using the whole cell recording technique and demonstrated (Fig. 1C) the ability of CGP44533 to induce an outward current using a control pipette recording solution; this response and its associated increase in conductance is lost with the modified pipette recording solution (Fig. 4).

Effects of GABA_B agonists on postsynaptic membrane conductance

When focusing on the postsynaptic membrane conductance changes induced by GABA_B receptor agonists, we observed that (±)-baclofen and CGP35024 continued to produce a concentration-dependent increase of postsynaptic membrane conductance, even under conditions when using the modified internal pipette solution with externally applied bicuculline and picrotoxin. (±)-Baclofen (10 nM) produced a 11.8 ± 4.5% increase, whereas at 10 μM it produced a 35.8 ± 14.5% increase of postsynaptic membrane conductance (Fig. 4). Similarly, CGP35024 (10 nM) produced a 1.6 ± 1.5% change, and at 10 μM it produced a 20.4 ± 6.0% increase of postsynaptic membrane conductance (Fig. 4). Under these recording conditions, both baclofen and CGP35024 still induce a concentration-dependent and persistent outward current (Fig. 4, A–C) associated with an increased postsynaptic conductance. CGP35348 of CGP55845A blocked this conductance change induced by each of these GABA_B agonists, whereas barium was ineffective (Yamada and Gallagher, unpublished data). These results suggest that (±)-baclofen and CGP35024 activate GABA_B receptors on the postsynaptic membrane that may be coupled to potassium channels that are Cs and Ba insensitive, while also QX-314 resistant. Results with these GABA_B agonists applied exogenously differ from data collected with blockade of the s-IPSC. A distinction between blockade of synaptic responses versus inability to block exogenous application of GABA responses has been reported in CA1 of the hippocampus (Pham and Lacaille 1996). Subsequently (Pham et al. 1998), these authors have suggested a differential distribution of GABA_B receptors coupled to different K+ channels that are present at subsynaptic versus extrasynaptic sites. Synaptically released GABA could activate only the subsynaptic sites, whereas exogenous superfusion of GABA and/or GABA released by high-intensity or high-frequency stimuli could activate both receptors (however, see Pham et al. 1998). Another possibility is that the receptors are similar but exhibit a different pharmacology due to a difference in their effectors or coupling machinery.

In terms of postsynaptic conductance changes, only CGP35024 and baclofen, not CGP44533, continued to produce significant conductance changes even under the conditions of using the modified internal pipette solution with externally applied bicuculline and picrotoxin (Fig. 4). CGP44533 at 10 nM produced a 7.6 ± 3.6% increase in conductance, whereas at 10 μM only a 7.2 ± 12.6% change was recorded. These data suggest that CGP44533, under our recording conditions, is a selective presynaptic GABA_B heteroreceptor agonist at glutamatergic nerve terminals.

DISCUSSION

We investigated the effects of GABA_B receptor agonists and antagonists on monosynaptic glutamatergic transmission in the DLSN. Focal fimbrial stimulation of the DLSN slice preparation produced a composite of triphasic synaptic currents, namely, an EPSC, f-IPSC, and s-IPSC. To isolate monosynaptic glutamatergic transmission, inhibitory postsynaptic responses were eliminated by the use of a modified pipette solution including cesium, GDPβS and QX-314, with external application of bicuculline and picrotoxin. After 30 min of impalement, the f-IPSC and s-IPSC were absent, and an isolated EPSC became apparent. The EPSC was blocked completely by DNQX and t-APV. These results suggested that this isolated EPSC was mediated by non-NMDA and NMDA re-
GABA<sub>B</sub> antagonists differentiate between presynaptic GABA<sub>B</sub> receptors and postsynaptic GABA<sub>B</sub> receptors

Two GABA<sub>B</sub> receptor antagonists, CGP35348 (100 μM), CGP55845A (1 μM) (Bon and Galvan 1996), or barium (2 mM) (Yamada and Gallagher 1997) depressed baclofen-induced membrane hyperpolarizations and blocked the s-IPSP in the DLSN. The most potent GABA<sub>B</sub> receptor antagonist tested, CGP55845A, not only blocked the CGP44533 depressant action on EPSCs, but it also resulted in a potentiation in the amplitude of EPSCs. These results suggest that CGP55845A blocked the CGP44533-induced depression of EPSCs by blocking the presynaptic GABA<sub>B</sub> receptor and increasing glutamate release. A similar result has been reported in the supraoptic nucleus (Kombian et al. 1996). There, another GABA<sub>B</sub> receptor antagonist, CGP36742, also enhanced EPSCs significantly without exhibiting any postsynaptic membrane changes or altering postsynaptic responses induced by exogenous application of glutamate agonists (Froestl et al. 1995b; Kombian et al. 1996). This result adds further support for a differential GABA<sub>B</sub> pharmacology at glutamatergic terminals.

Neither CGP35348 nor barium altered the ability of CGP44533 to depress EPSCs in the DLSN. Barium has been shown to be ineffective in blocking the presynaptic actions of baclofen at other sites (Thompson et al. 1993). On the other hand, CGP35348 has been reported to block the release of glutamate and somatostatin in cerebral cortex synaptosomes (Bonanno and Raiteri 1993b). Together these data indicate that, within glutamatergic synapses, the sensitivity of CGP35348 varies, depending on the respective sites examined. Our data support the earlier suggestions (Bonanno and Raiteri 1993a) that there are at least two kinds of GABA<sub>B</sub> heteroreceptor subtypes on glutamate nerve terminals in the CNS, each differing in their sensitivity to CGP35348. Bonanno and Raiteri went on to propose a classification to differentiate GABA<sub>B</sub> receptors. GABA<sub>B1</sub> are baclofen sensitive, whereas GABA<sub>B2</sub> are baclofen insensitive, but GABA-sensitive. Pham et al. 1998 have extended this differentiation to suggest that GABA<sub>B1</sub> are coupled to barium-sensitive K<sup>+</sup> conductances and are found at subsynaptic sites, whereas GABA<sub>B2</sub> are coupled to barium-insensitive K<sup>+</sup> conductances found at extrasynaptic sites.

The fact that inhibition of glutamate release induced by all three GABA<sub>B</sub> agonists tested in the DLSN also persists in the presence of extracellular barium supports the concept that calcium, rather than potassium channels (Takahashi et al. 1998), mediate this inhibitory action in the DLSN. Baclofen has been shown to depress specific types of calcium currents differentially in thalamocortical neurons. N-, L-, and R-type calcium currents were recorded, with the following depression of each of these currents observed: 0, 30, and 100% depression of L, N, and R, respectively (Guyon and Leresche 1995). CGP35348 (100 μM) or CGP55845A (100 nM) reversed depression by baclofen of the N-type current. On the other hand, only CGP55845A (100 nM) blocked the baclofen effect on the R-type current; CGP35348, up to 100 μM, was ineffective (Guyon and Leresche 1995). It is possible that presynaptic GABA<sub>B</sub> receptors within the DLSN may also be coupled with R-type calcium channels because the presynaptic depression by baclofen was not blocked by CGP35348, but was sensitive to CGP55845A. Another possibility is that a nerve terminal GABA<sub>B</sub> receptor may be coupled to a barium-resistant K<sup>+</sup>-channel, and thus is similar to a barium-resistant GABA<sub>B</sub> receptor complex postulated for the postsynaptic membrane.

The existence of multiple subtypes of GABA<sub>B</sub> receptors has been suggested in the CNS (Bonanno and Raiteri 1993a; Dutar and Nicoll 1988; Pham and Lacaille 1996). Many reports have focused on differences between pre- and postsynaptic GABA<sub>B</sub> receptors (Deisz et al. 1993, 1997; Dutar and Nicoll 1988; Pittler and Alger 1994; Thompson and Gähwiler 1992). We report different subtypes of GABA<sub>B</sub> receptor at an excitatory glutamatergic terminal within the DLSN. In the DLSN, although the postsynaptic GABA<sub>B</sub> receptor responsible for the synaptically mediated s-IPSC is sensitive to barium, CGP35348, and CGP55845A, the presynaptic GABA<sub>B</sub> receptor is only sensitive to CGP55845A and not to barium or CGP35348.

However, over the same concentration range, both CGP35024 and (±)-baclofen also produced postsynaptic membrane conductance increases. These latter results are consistent with a previous report (Bon and Galvan 1996) that demonstrated the following IC<sub>50</sub>s for these agonists to induce a membrane hyperpolarization in the DLSN: CGP35024 (0.05 μM) and (±)-baclofen (0.55 μM). The reversal potential of the membrane hyperpolarization induced by (±)-baclofen or CGP35024 in DLSN neurons was the same as the equilibrium potential of potassium (Bon and Galvan 1996; Stevens et al. 1985). This result indicates that postsynaptic GABA<sub>B</sub> receptors in the DLSN are coupled solely with potassium channels. However, even though CsGluconate, QX-314, and GDPβS were employed to eliminate postsynaptic GABA<sub>B</sub> responses, both baclofen and CGP35024 still caused an increase in membrane conductance and an associated outward current at a time when the synaptic s-IPSC was blocked. We have conducted extrapolated reversal potentials of these outward currents, which yield values between ~90 to ~100 mV; these data suggested a K<sup>+</sup> reversal. Experiments with altered extracellular [K<sup>+</sup>] concentrations will be conducted to verify this. These latter, planned experiments are especially critical due to the high cesium and gluconate in our recording electrode; the high cesium may pass through K<sup>+</sup> channels, whereas the high gluconate may produce a large D.C. offset that is difficult to compensate. We will conduct a thorough analysis of the reversal potential of these responses as part of another study.

These data suggest that there are two types of potassium channels coupled to a postsynaptic GABA<sub>B</sub> receptor in the rat DLSN. One is sensitive to intracellular cesium, QX-314, and/or GTPβS. This GABA<sub>B</sub> receptor is intrasynaptic and responsible for mediating the synaptic response. Another is insensitive to intracellular cesium and QX-314, extracellular barium, and possibly coupled to its receptor via a membrane...
delimited G protein; this latter receptor is situated extrasynaptically. Baclofen-activated cesium-insensitive potassium channels have been reported (Wagner and Dekin 1993, 1997). These cesium-insensitive potassium channels are classified as outward rectifying or “KORs”. We propose that the coupling mechanism for KORs may be membrane delimited (Hille 1992; Isaacs and Hille 1997), a property that could explain why the response we recorded persisted even when the cell is filled with GDPβS. KORs are distinguished from the more common inwardly rectifying potassium channels or “GIRKs”, which are cesium sensitive. Taken together, these data could also explain the results of Pham and Lacaille (1996), who reported a barium-resistant postsynaptic response at a time when the synaptic s-IPSC was blocked; they (Pham et al. 1998) suggested that this barium-resistant response is situated extrasynaptically.

A teleological question may be asked of why have two postsynaptic GABA_B receptors coupled to different potassium channels. One suggestion could be that they are situated separately to function under different circumstances; one, when little GABA is released within the synapse, and the other, when excessive GABA is released. Under this latter situation when the synaptic receptors are saturated, and if additional inhibition is required, the extrasynaptic receptors are activated to enhance/prolong inhibition.

These results support the concept that there are at least three pharmacologically different subtypes of GABA_B receptors present within a glutamatergic DLSN synapse; two are located at postsynaptic sites, whereas a third is found on the nerve terminal. It may be possible that a similar distribution of GABA_B receptors could be found at other CNS synapses.

We are grateful to Dr. W. Froestl for reviewing this manuscript and supplying critical Novartis compounds.

The National Institutes of Health and the John Sealy Foundation supported this work.

Address reprint requests to J. P. Gallagher.

Received 15 May 1998; accepted in final form 22 February 1999.

REFERENCES


DIFERENT SUBTYPES OF GABA<sub>B</sub> RECEPTORS IN DLSN


Wagner, P. G. and Dekin, M. S. cAMP modulates S-type-K<sup>+</sup> channel coupled to GABA<sub>B</sub> receptors in mammalian respiratory neurons. *Neuroreport* 8: 1667–1670, 1997.


