Differential Regulation of GABA<sub>B</sub> Receptor Subunit Expression and Function

S. A. SANDS, K. E. MCCARSON, and S. J. ENNA

Department of Pharmacology, Toxicology and Therapeutics, Kansas University School of Medicine, Kansas City, Kansas

Received October 31, 2002; accepted December 30, 2002

ABSTRACT

The GABA<sub>B</sub> receptor is a G protein-coupled heterodimer composed of GABA<sub>B1</sub> and GABA<sub>B2</sub> subunits. In the present study, experiments were undertaken to examine the relationship between GABA<sub>B</sub> receptor function and subunit expression in the rat lumbar spinal cord following pharmacological and physiological manipulation of this receptor system. Although formalin-induced hind paw inflammation increases the production of GABA<sub>B1</sub> and GABA<sub>B2</sub> protein in the spinal cord within 24 h, there is no change in receptor function, as measured by the baclofen-stimulated guanosine 5'-O-(3-[<sup>35</sup>S]thiotriphosphate) ([<sup>35</sup>S]GTP<sub>S</sub>) binding assay. Conversely, although chronic (7 days) administration of baclofen, a GABA<sub>B</sub> receptor agonist, abolishes baclofen-stimulated [<sup>35</sup>S]GTP<sub>S</sub> binding in the spinal cord tissue, causes tolerance to the sedative and antinociceptive effects of the drug, increases the number of formalin-induced hind paw flinches, and induces mechanical hyperalgesia, this treatment had no effect on the levels of GABA<sub>B1</sub> or GABA<sub>B2</sub> mRNAs in the lumbar spinal cord. The results indicate a lack of concordance between expression of GABA<sub>B1</sub> and GABA<sub>B2</sub> subunits and GABA<sub>B</sub> receptor function, suggesting these subunit proteins may serve multiple functions in the cells. Moreover, these findings indicate that nongenomic mechanisms are primarily responsible for the GABA<sub>B</sub> receptor desensitization that occurs during prolonged exposure to receptor agonist.

The metabotropic GABA<sub>B</sub> receptor was the first heterodimeric, G protein-coupled neurotransmitter site to be identified in mammalian tissue (Bowery and Enna, 2000). Studies on wild-type GABA<sub>B</sub> receptors, and in recombinant systems, reveal that GABA<sub>B</sub> receptor function requires the presence and dimerization of two subunits, GABA<sub>B1</sub> and GABA<sub>B2</sub>, which are distinct gene products (Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998; Chronwall et al., 2001). Whereas subunit splice variants have been identified, with GABA<sub>B1v</sub> and GABA<sub>B2v</sub>, being the most characterized, GABA<sub>B</sub> receptor responses are consistently detected only in systems expressing both GABA<sub>B1</sub> and GABA<sub>B2</sub> proteins. Thus, neither GABA<sub>B1v</sub> nor GABA<sub>B2v</sub> homodimers, nor the individual subunits themselves, yield a functional GABA<sub>B</sub> receptor. Although other proteins have been identified that structurally resemble GABA<sub>B</sub> subunits, none yield functional GABA<sub>B</sub> receptors when expressed alone, or with either the GABA<sub>B1</sub> or GABA<sub>B2</sub> subunit (Mezler et al., 2001). Accordingly, current data suggest that dimerization of a GABA<sub>B1</sub> and a GABA<sub>B2</sub> subunit is required for receptor function.

Although the heterodimeric structure of the GABA<sub>B</sub> receptor is well established, little is known about the pharmacological properties and regulation of this site. Although there are reports that pharmacological selectivity varies with the GABA<sub>B1</sub> splice variant, this finding remains controversial (Lanneau et al., 2001; Ng et al., 2001). Indeed, the requirement for a union of the GABA<sub>B1</sub> and GABA<sub>B2</sub> subunits limits significantly the possibility of pharmacologically distinct receptor subtypes (Enna, 2001). Nonetheless, efforts continue to discover proteins that may combine to form pharmacologically distinct subclasses of GABA<sub>B</sub> receptors and to identify agents that may influence GABA<sub>B</sub> receptors in a selective manner (Urwyler et al., 2001).

Given the apparently fixed stoichiometry of the system, and the seemingly absolute requirement for combining GABA<sub>B1</sub> and GABA<sub>B2</sub> subunits, production of these proteins should be modified as the need for receptors ebbs and flows and should track with changes in receptor function that occur due to long-term perturbations of the system. In particular, such a finding would be expected if GABA<sub>B1</sub> and GABA<sub>B2</sub> function only as components of GABA<sub>B</sub> receptors. One way to test this is to modify receptor function while monitoring the production of the subunits. Previous work established that...
GABA<sub>B</sub> receptor subunit expression in the rat spinal cord changes during subchronic formalin-induced inflammatory pain (McCarson and Enna, 1999), whereas chronic administration of a GABA<sub>B</sub> receptor agonist, such as baclofen, results in tolerance to the sedative and antinociceptive effects of this agent (Enna et al., 1998). Using these physiological and pharmacological approaches, the present study was undertaken to characterize the relationship between the expression of GABA<sub>B</sub> receptor subunits and GABA<sub>B</sub> receptor activity. The results reveal no correlation between subunit expression and receptor function, suggesting that not all GABA<sub>B</sub> receptor subunits are incorporated into functional GABA<sub>B</sub> receptors and that nongenomic mechanisms play a role in regulating receptor availability and function, even after prolonged agonist stimulation.

Materials and Methods

**Animals.** Pathogen-free male Sprague-Dawley rats (250–300 g; Harlan, Indianapolis, IN) were used for all experiments. The animals were maintained on a 12-h light/dark cycle in the Kansas University Medical Center animal care facility with free access to standard rat chow and water. The experiments were approved by the Kansas University Medical Center Animal Care and Use Committee and were performed in accord with institutional guidelines regarding the ethical treatment of animals and the use and disposal of hazardous waste.

**Drug Administration and Tissue Preparation.** For some experiments, 5 mg/kg baclofen (β-chlorophenyl GABA), a selective GABA<sub>B</sub> receptor agonist, was administered (i.p.) twice daily for 7 consecutive days. Previous work demonstrated that this treatment regimen results in tolerance to the antinociceptive and sedative effects of baclofen (Enna et al., 1998). Twenty-four hours after the last dose of baclofen and immediately after any behavioral tests, the rats were decapitated and spinal cord tissues rapidly removed by a forceful injection of ice-cold isotonic saline into the caudal end of the vertebral canal using a 60-ml syringe attached to a 16-gauge needle. For measurement of mRNA, the lumbar portion of the vertebral column was rapidly dissected and frozen at −70°C until assayed. For immunohistochemistry studies the lumbar region was immediately immersed in 4% paraformaldehyde and refrigerated at 4°C until assayed. Lumbar spinal tissue for measuring [35S]GTP binding was immediately placed on dry ice and then stored at −70°C until assayed.

To study nociceptive responses, dilute (5%) formalin (100 μl) was injected subcutaneously into the planar aspect of one or both hind paws. The animals were decapitated and their spinal cords removed 24 h after formalin treatment, during which time their nociceptive threshold was measured. The lumbar portion of the spinal cords was dissected and stored as described above.

**Nociceptive Tests.** Nociception was assessed by measuring the time to withdrawal of the paw following a mechanical pinch to the dorsal surface of the hind paw of unrestrained rats using a small vascular clamp calibrated to 250 g/mm² (McCarson and Goldstein, 1991).

For some experiments late-phase nociceptive behaviors were monitored for 10 min beginning 30 to 40 min after injection of formalin. The rats were monitored in a 256-square inch observation chamber, and hind paw flinches were counted for 1-min periods by an observer blind to treatments (Wheeler-Aceto et al., 1990).

**Analysis of GABA<sub>B</sub> Receptor mRNAs.** Total RNAs were obtained from rat lumbar spinal cord tissue using a rapid guanidinium isothiocyanate-phenol/chloroform extraction and subsequent precipitation with sodium acetate and ethanol (Chomczynski and Sacchi, 1987). One dorsal quarter of the spinal cord lumbar enlargement routinely provided approximately 50 to 100 μg of total RNA. The level of specific mRNAs for each GABA<sub>B</sub> receptor subunit was quantified using solution hybridization-nucleic protection assays.

The GABA<sub>B</sub> receptor subunit-encoding mRNAs were provided by Dr. Klemens Kaumpmann (Novartis, Basel, Switzerland). Analysis of GABA<sub>B1a</sub> and GABA<sub>B1b</sub> subunit-encoding mRNAs used a coding region plasmid pBS GABA<sub>B1a</sub>Rpan [+2462 → +3234], whereas the GABA<sub>B2</sub> subunit-encoding mRNAs were analyzed using the coding region plasmid pBS GABA<sub>B2</sub>R2[+555 → +1246]. Analysis of β-actin mRNA was performed using a coding region plasmid pBS-βactin(lj). All constructs were used to transcribe antisense probes as well as message-sense cRNAs.

Antisense 32P-labeled cRNA probes were synthesized using [α-32P]UTP. The plasmids were linearized with restriction enzymes and probes generated using T3 or T7 RNA polymerases. Unlabeled message-sense cRNAs were used as quantification standards in the nuclelease protection assays. All transcription reactions were conducted according to procedures recommended by Promega (Madison, WI). Template DNA was subsequently digested using RQ1 RNase. Samples of total RNA were assayed for GABA<sub>B</sub> receptor subunit and β-actin mRNAs using a solution hybridization-nucleic protection assay, as described previously (McCarson and Krause, 1994, 1995). Briefly, 2 × 10<sup>6</sup> dpm of the specific 32P-labeled antisense cRNA probe was coprecipitated with 25 μg of total cellular RNA, 5 to 200 pg of cRNA quantification standards, or Escherichia coli tRNA for negative controls (each sample less than 25 μg of RNA was made up to 25 μg with E. coli tRNA). The RNA – [32P]cRNA coprecipitates were each resuspended in 10 μl of hybridization buffer [40 mM PIPES, pH 6.4, 400 mM NaCl, 1 mM EDTA, and 80% (v/v) deionized formamide], and the mRNAs were allowed to anneal with cRNA probes for 16 to 20 h at 45°C. Annealed portions of the mRNAs were protected from nuclease digestion by incubating for 20 min at 37°C with nucleases A (4.0 μg/ml) and T<sub>1</sub> (0.2 μg/ml), followed by a 15-min digestion at 37°C with proteinase K (100 μg/sample). To control for loading and spectrophotometer error, β-actin mRNA was assayed in a similar manner using only 2 μg of total RNA, 2 × 10<sup>6</sup> dpm of 32P-labeled antisense probe and digestion with nucleases A and T<sub>1</sub>. In all cases, the digestion reaction products were precipitated with an equal volume of 2-propanol before resuspension and electrophoresis at 25 V/cm on 6% acrylamide gels. The gels were fixed, dried, and exposed to phosphor plates (Amersham Biosciences, Inc., Sunnyvale, CA) for 16 to 48 h. Densitometric images were generated and analyzed using a PhosphoImager SP (Amersham Biosciences, Inc.). Densitometric signals for the total RNA samples were compared by linear regression analyses to those for the message-sense cRNA quantification standards to calculate the relative amount of specific mRNA in each total RNA sample. Data are reported as picograms of specific RNA per nanogram of β-actin mRNA.

**Immunohistochemistry.** The paraformaldehyde fixed lumbar spinal cord tissues were sectioned (50 μm) on a vibratome and placed into phosphate-buffered saline (PBS). Floating sections were treated with 3% H<sub>2</sub>O<sub>2</sub> in absolute methanol (1:4) for 5 min to block endogenous peroxidases and 10% normal goat serum for 15 min to reduce nonspecific binding of the antibodies. Tissue slices were then incubated for 48 h at 4°C with either guinea pig anti-GABA<sub>B1</sub> or guinea pig anti-GABA<sub>B2</sub> diluted 1:1000 in PBS containing 0.2% Triton X-100 (PBS-TX). Both primary antibodies were donated by Gordon Ng (Merck Frosst, Kirkland, ON, Canada). After this exposure, the sections were washed three times in PBS-TX for 5 min each and then incubated in PBS-TX at room temperature for 1 h in goat anti-guinea pig secondary antibody conjugated to horseradish peroxidase (1:50; Jackson Immunoresearch Laboratories, Inc., West Grove, PA). The sections were then washed for 5 min in PBS-TX, followed by two 5-min washes in 0.1 M Tris-saline. Antigen-antibody complexes were made visible by incubation in 3,3-diaminobenzidine in 0.1 M Tris-saline containing 0.001% H<sub>2</sub>O<sub>2</sub> for 10 to 15 min, after which the sections were rinsed in PBS. The sections were mounted on glass slides coated with gelatin, air-dried, cover-slipped in Permount (Fisher Scientific Co., Pittsburgh, PA), and then analyzed microscop-
ically with the results quantified using a single illumination setting. Selected regions with immunoreactive product were outlined and measured using a circle drawing command, and the density of the immunoreactivity within the region quantified. The background level was subtracted before calculating a final mean value for the pooled data from the selected regions. For data analysis, the procedure of Beatty et al. (1998) was used, with some modifications. The optical density of the dianisobenzodiazepine precipitate was used to compare the level of GABA$_{B}$ subunits between groups. The image analysis system consisted of a Dage/MII 72 CCD camera mounted on the trinocular port of an Axiosplan microscope (Carl Zeiss, Inc., Thornwood, NJ). The camera was connected to a Matrix MVP-AT array processor installed in a 486-based PC with IM3000B image processing and analysis software (Belvoir Consulting, Long Beach, CA).

**Western Blot Analysis.** Dorsal lumbar spinal cords were minced using a sterile blade and placed in ice-cold protein extraction buffer I (20 mM Hepes, 6 mM MgCl$_2$, 1 mM EDTA and 250 mM sucrose, pH 7.4). Tissues were homogenized with a Polytron at maximum speed for 30 s, followed by centrifugation at 1,700g for 10 min. The resultant supernatant was centrifuged at 100,000 g for 30 min, and the pellet was resuspended in 50 to 100 μl of protein extraction buffer II (20 mM Hepes, 6 mM MgCl$_2$, 1 mM EDTA, and 1 mM EGTA, pH 7.4). Portions of this suspension were taken for protein quantification using a bicinechonic acid assay kit (Sigma-Aldrich, St. Louis, MO). Protein extracts were stored at −20°C until analysis. For assay, portions of the protein extracts (50–100 μg) were subjected to electrophoresis in a 7.5% polyacrylamide gel and transferred to nitrocellulose membranes using an electrophoretic gel-transfer apparatus (Bio-Rad, Hercules, CA). The blots were incubated for 1 h at room temperature in blocking buffer (5% dry milk-Tris-buffered saline-0.1% Tween 20), followed by a 2-h incubation in the guinea pig anti-GABA$_{B}$R1 diluted 1:10,000. After a 1-h exposure to the goat anti-guinea pig secondary antibody conjugated to horseradish peroxidase, blots were developed using chemiluminescence markers following the manufacturer’s protocol (PerkinElmer Life Sciences, Boston, MA). The blots were apposed to X-ray film, and densitometric images were generated and analyzed using a scanning densitometer (Amersham Biosciences Inc.). Densitometric signals for the protein samples were quantified using IP LabGen (National Institutes of Health).

**[^35S]GTPγS Binding Assay.** Snap-frozen lumbar spinal cords were cryostat-sectioned (20μm), thaw-mounted onto gelatin-coated glass microscope slides, and then stored at −70°C until use. For assay, the slides were brought to room temperature and then placed into assay buffer (4 mM MgCl$_2$, 160 mM NaCl, 0.267 mM EGTA, and 67 mM Tris, pH 7.4) for 10 min, after which they were exposed for 15 min to 2 mM GDP. Incubation buffers (1.25 ml of assay buffer, 1.25 ml of 8 mM GDP, 1.25 ml of[^35S]GTPγS (27,500–30,000 dpm), and 1.25 ml of 4 mM baclofen) were prepared in 15-ml plastic slide containers (mailer vials), as were nonspecific binding (40 μM nonradioactive GTPγS) and basal (no baclofen) controls. The slides were incubated for 2 h at room temperature, and the reaction terminated by two ice-cold rinses in 50 mM Tris-HCl (pH 7.4), and one rinse in room temperature distilled water. The slides were air-dried overnight and then apposed to autoradiographic film for 24 to 48 h. The films were developed in a Kodak X-ray developer, with images quantified by densitometric analysis (Scion Image). The digitized images of the[^35S]GTPγS autoradiograms were captured with a Dage/MTI model 72 CCD camera using the NIH Image software package, with the density of the signal overlying the spinal cord slice analyzed using Scion Image. Basal and baclofen-stimulated[^35S]GTPγS binding levels were measured in lamina I and II of the spinal cord in six to eight sections per animal, the mean of which was used as the value for each subject. Basal[^35S]GTPγS binding was defined as the binding density across the superficial dorsal horns of spinal cord sections incubated in assay buffer with no baclofen. Nonspecific binding (optical density of sections exposed to buffers only) was subtracted from basal[^35S]GTPγS levels to calculate the specific binding. The results were compared with the results quantified using a single illumination setting. Statistical Analyses. The results are reported as the mean ± S.E.M. of values obtained from multiple observations. Differences between means were considered statistically significant when p ≤ 0.05. When appropriate, comparisons between groups were made by an analysis of variance or analysis of covariance, with a Dunnett’s test or Fisher’s protected least significant difference used for post hoc comparisons.

**Results**

**Effect of Formalin Administration on GABA$_{B}$ Receptor Subunit Immunoreactivity.** To assess the effect of persistent nociceptive activation of the GABAergic system on GABA$_{B}$ receptor subunit proteins, 5% formalin (100 μl) was injected subcutaneously into the rat hind paw, with lumbar spinal cord GABA$_{B1}$ and GABA$_{B2}$ proteins examined histochemically 2 h later (Fig. 1). The results revealed a significant increase in both GABA$_{B1}$ and GABA$_{B2}$ protein in this region of the spinal cord (Fig. 1, panels B and D) compared with untreated control animals (Fig. 1, panels A and C). Although formalin treatment increased GABA$_{B1}$ and GABA$_{B2}$ protein levels, there was no significant difference in the regional distribution of these proteins between the two groups, with the highest density found in the dorsal horn (Fig. 1). Densitometric analyses revealed a 33 ± 7 and 51 ± 10% increase in GABA$_{B1}$ and GABA$_{B2}$ protein levels, respectively, in the formalin-treated subjects compared with controls.

Western blot analysis confirmed the change in GABA$_{B2}$ subunit protein in response to formalin (Fig. 2).
there was a 66% increase in the level of GABA B2 protein detected 24 h after the subcutaneous injection of 5% formalin into both hind paws. Because the GABA B1 antibody was inadequate for Western blot analysis, it was not possible to use this analytical approach for this subunit.

Effect of Baclofen Administration on GABA B Receptor Subunit mRNA Levels. Rats were injected (i.p.) with baclofen (5 mg/kg) twice daily for 7 consecutive days, and GABA B subunit mRNAs were quantified in the lumbar dorsal horn of the spinal cord 24 h after the last injection (Fig. 3). Although it has been established that this dosing schedule results in tolerance to the sedative and antinociceptive effects of baclofen (Enna et al., 1998), no effect on mRNA levels for either the GABA B1 or GABA B2 subunit, compared with saline-treated control subjects, was noted under these conditions (Fig. 3).

Acute Administration of Formalin or Chronic Administration of Baclofen on Agonist-Stimulated [35S]GTPγS Binding. To assess the effects of prolonged stimulation on GABA B receptor function, baclofen-stimulated [35S]GTPγS binding was assayed in rat lumbar spinal cord slices 24 h after a single injection of 5% formalin (100 μl) into the hind paws and 24 h after the last injection of baclofen in rats treated for 7 consecutive days with 5 mg/kg (b.i.d.) of the GABA B1 agonist (Fig. 4). In the presence of a saturating concentration of baclofen (1 mM), [35S]GTPγS binding nearly doubled in the vehicle-treated control slices and in slices taken from animals treated with formalin. Thus, formalin treatment had no effect on GABA B receptor function. In contrast, baclofen-stimulated [35S]GTPγS binding was abolished in lumbar spinal cord tissues obtained from rats treated chronically with baclofen (Fig. 4).

Chronic Administration of Baclofen and the Nociceptive Response to Formalin. The effect of chronically administered baclofen on nociceptive threshold was examined in rats treated for seven consecutive days with 5 mg/kg baclofen (i.p., b.i.d.) by measuring their responses to painful stimuli 24 h after the last injection of the GABA B agonist (Fig. 5). The results indicate that baclofen treatment causes a slight, but significant, increase in the number of late-phase formalin-induced hind limb flinches measured 30 to 40 min after formalin injection (Fig. 5).
after injection of the chemogenic inflammatory stimulus. Likewise, there was a significant reduction in the latency to hind paw withdrawal in the mechanical pinch test after formalin administration to animals previously treated with baclofen, compared with those receiving formalin alone (Fig. 5). In this case, there was a nearly 50% reduction in latency observed with animals treated chronically with the GABAB receptor agonist.

Discussion

The heterodimeric nature of the GABAB receptor distinguishes it from most other G protein-coupled sites. Indeed, GABAB receptor function seems to be absolutely dependent upon the expression of two separate gene products and their ability to dimerize and remain united after insertion into the cell membrane (Kaupmann et al., 1998; Chronwall et al., 2001). This multistep process may render the GABAB receptors more vulnerable to disruption, and perhaps to pharmacological manipulation, than other G protein-coupled sites.

The present study was conducted to examine whether GABAB1 and GABAB2 subunits are used solely for the formation of GABAB receptors by assessing the relationship between subunit expression and GABAB receptor function. The dorsal horn of the rat lumbar spinal cord was selected for study because it is anatomically well defined and activation of GABAB receptors in this region alters nociception, an easily measured behavioral endpoint. The experiments were designed to determine whether changes in the production of these subunits are accompanied by a change in GABAB receptor function, and vice versa, which should occur if the subunits are used only for this purpose, and only these two particular subunits dimerize to form a functional GABAB site. The results indicate a lack of concordance between subunit expression and GABAB receptor function, suggesting that GABAB1 and GABAB2 may serve other functions besides forming GABAB receptors and indicating that nongenomic mechanisms play an important role in the long-term downregulation of GABAB sites.

Earlier work revealed that formalin-induced hind paw inflammation increases both GABAB1 and GABAB2 subunit mRNA levels in the rat spinal cord (McCarson and Enna, 1999). This observation was extended in the present study with the discovery that formalin treatment increases GABAB1 and GABAB2 protein levels as measured by immunohistochemistry and, for GABAB2 at least, by Western blot analysis. Thus, the pain-induced increase in GABAB receptor subunit gene expression evoked by persistent inflammatory nociception is accompanied by an increase in protein synthesis. It was suggested previously that this change in subunit expression may be due to a prolonged and massive release of GABA at these synapses in response to the peripheral pain stimulus (McCarson and Enna, 1999). However, GABAB receptor occupancy, per se, apparently cannot fully explain the enhanced expression of the subunits because the results of the present study reveal that chronic (7 days) administration of baclofen, a selective GABAB receptor agonist, has no effect on either GABAB1 or GABAB2 subunit expression in the spinal cord.

Previous work indicated that chronic administration of baclofen decreases GABAB receptor number and causes tolerance to the pharmacological effects of this agent (McCarson et al., 1995; Enna et al., 1998). In the present study, experiments were performed to define more precisely changes in GABAB receptor activity under this condition using in vitro and in vivo techniques. The results revealed that baclofen-stimulated [35S]GTPγS binding was abolished in lumbar spinal cord slices taken from baclofen-tolerant rats, indicating receptor desensitization. Moreover, formalin-evoked, spontaneous pain-related behaviors and hyperalgesia were augmented significantly in the baclofen-tolerant animals. These findings, along with the [35S]GTPγS binding data, indicate a significant reduction in GABAB receptor function after chronic administration of baclofen in the absence of a change in spinal cord mRNA levels for the GABAB receptor subunits.

A lack of correlation between subunit gene expression and GABAB receptor function was also noted when the system was activated physiologically. Thus, there was a significant increase in GABAB receptor subunit mRNA levels (McCarson and Enna, 1999) and protein in the lumbar-sciatic cord 24 h after the injection of formalin into the hind paw, with no change in baclofen-stimulated [35S]GTPγS binding. In this case, the increased production of subunits does not seem to translate into an increase in GABAB receptor function, at least as it relates to G protein activation.

The finding that GABAB receptor activity, as measured by [35S]GTPγS binding, is unmodified even though receptor subunit expression increased suggests that the GABAB1 and GABAB2 subunits may serve other functions in addition to forming GABAB receptors. Indeed, reports indicate that GABAB1 and GABAB2 dimerize with a number of different cellular proteins, including transcription factors (White et al., 2000). It is also conceivable that changes in the post-translational or post-transcriptional processing of the GABAB receptor subunits may change their pharmacological selectivity, rendering new sites insensitive to baclofen, and therefore undetectable by the assays performed in this study. Studies with GABAB1 knockout mice suggest, however, that this subunit is required for GABAB receptor activity (Schuler et al., 2001), suggesting that any changes in the molecular structure of the receptor would be due primarily to a change in the GABAB2 component.

As for GABAB receptors in baclofen-tolerant animals, the present findings reveal that receptor number and function can be reduced significantly in the absence of any change in GABAB receptor subunit expression. Although a number of nongenomic mechanisms, such as internalization and degradation, are important for short-term (minutes to hours) desensitization of G protein-coupled receptors in vitro (Perkins et al., 1991), it seems longer term (hours to days) modifications are due, at least in part, to genomic changes in receptor expression (Nishikawa et al., 1993; Karoor et al., 1996). This does not seem to be the case for GABAB receptors. Thus, the lack of a change in GABAB receptor subunit expression after chronic (7 days) administration of baclofen suggests that genomic mechanisms responsible for the production of GABAB1 and GABAB2 proteins are not involved in the regulation of GABAB receptor sensitivity that occurs in response to prolonged activation by this exogenously administered agonist, although it remains possible that the recovery of GABAB receptor subunit gene expression occurs within 24 h after the last dose of agonist. Some possible nongenomic mechanisms for desensitization include an increase in recep-
tor degradation or phosphorylation. In the latter case, it has been reported that phosphorylation is required for maintaining GABA_B receptor function, with dephosphorylation leading to desensitization (Couve et al., 2002). It is also possible that the reported change in the number of GABA_B binding sites (Malcangio et al., 1995), and the decline in receptor function noted in the present study, could be due to a post-transcriptional regulation of GABA_B1 or GABA_B2 subunit proteins. The lack of a decline in subunit gene expression, at a time when receptor activity is diminished, further supports the notion that these proteins serve functions other than formation of GABA_B receptors. A better understanding of the mechanisms responsible for inducing and maintaining GABA_B receptor desensitization should be of value in developing strategies for developing GABA_B agonists that may be less likely to desensitize the receptor system (Enna et al., 1998).

In summary, the results of the present study reveal that changes in GABA_B receptor subunit expression is not necessarily accompanied by a change in receptor function, nor is a change in function indicative of a modification in the expression of these receptor subunits. Thus, the results indicate that agonist-induced desensitization, even when prolonged, does not seem to be accompanied by genomic changes in receptor availability. Rather, the decline in receptor function seems likely due to an enhanced sequestration, degradation, or dephosphorylation of the receptor dimer.

Acknowledgments

We thank Jason Moran, Michelle Winter, and Dr. Maya Gadhvi Pursiainen for technical assistance.

References


Address correspondence to: Dr. S. J. Enna, Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, G034 Breidenthal, 3901 Rainbow Blvd., Kansas City, KS 66160-7424. E-mail: senna@kumc.edu