Effect of GABA$_B$ receptor agonist on distension-sensitive pelvic nerve afferent fibers innervating rat colon

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Sengupta, Jyoti N., Bidyut K. Medda, and Reza Shaker. Effect of GABA$_B$ receptor agonist on distension-sensitive pelvic nerve afferent fibers innervating rat colon. Am J Physiol Gastrointest Liver Physiol 283: G1343–G1351, 2002; 10.1152/ajpgi.00124.2002.—Spinal afferents innervating the gastrointestinal tract are the major pathways for visceral nociception. Many centrally acting analgesic drugs attenuate responses of visceral primary afferent fibers by acting at the peripheral site. γ-Amino butyric acid (GABA), a major inhibitory neurotransmitter, acts via metabotropic GABA$_A$ and ionotropic GABA$_A$/GABA$_B$ receptors. The aim of this study was to test the peripheral effect of selective GABA$_B$ receptor agonist baclofen on responses of the pelvic nerve afferent fibers innervating the colon of the rat. Distension-sensitive pelvic nerve afferent fibers were recorded from the S$_1$ sacral dorsal root in anesthetized rats. The effect of baclofen (1–300 μmol/kg) was tested on responses of these fibers to colorectal distension (CRD; 60 mmHg, 30 s). A total of 21 pelvic nerve afferent fibers was recorded. Mechanosensitive properties of four fibers were also recorded before and after bilateral transections of T$_{12}$–S$_3$ ventral roots (VR). Effect of baclofen was tested on 15 fibers (7 in intact rats, 4 in rats with transected VR, and 4 in rats pretreated with CGP 54626). In nine fibers (5/7 in intact and 4/4 in VR transected rats), baclofen produced dose-dependent inhibition of response to CRD. Pretreatment with selective GABA$_B$ receptor antagonist CGP 54626 (1 μmol/kg) reversed the inhibitory effect of baclofen. Results suggest a peripheral role of GABA$_B$ receptors in the inhibition of mechanotransduction property of distension-sensitive pelvic nerve afferent fibers.

baclofen; visceral pain; spinal cord; sacral nerve; ventral roots

γ-AMINO BUTYRIC ACID (GABA), a major inhibitory neurotransmitter, plays an important role in antinociception. This effect is largely mediated by GABA$_B$ receptors ubiquitously present in the brain and spinal cord (13, 15, 17, 27, 31, 62, 63). The GABA$_B$ receptor is a unique G protein-coupled receptor that requires dimerization of two subunits (GABA$_{B1}$, GABA$_{B2}$) to form a functional receptor (22, 26, 61). It is thought that GABA$_{B1}$ subunit is essential for binding with the ligand, whereas GABA$_{B2}$ subunit is necessary for the expression of the functional receptor on the cell surface. A number of splice variants of the GABA$_{B1}$ subunit (GABA$_{B1a}$, GABA$_{B1b}$, GABA$_{B1c}$, GABA$_{B1d}$) have been identified in the rat and human, which are differentially expressed in different tissues (20, 24, 26, 42). For example, GABA$_{B1}$ subunit is ubiquitously present in the neurons of CNS and nonneuronal tissues, whereas GABA$_{B2}$ subunit is present only in the neurons of the brain and spinal cord (12). Recent reports indicate that GABA$_B$ receptors play a major role in transient lower esophageal sphincter relaxation (TLESR). Systemic application of GABA$_B$ receptor agonist baclofen decreases the number of TLESR and prevents gastroesophageal acid reflux (7–9, 27, 29). This effect of baclofen occurs partly by attenuating the mechanotransduction property of the vagal afferent fibers innervating the esophagus and the proximal stomach (38, 40, 52).

Baclofen produces antinociception in the rat model of visceral pain (1, 19). Abelli et al. (1) demonstrated that in conscious rats subcutaneous injection of baclofen prevents the behavioral responses of pain produced by instilling xylene into the urinary bladder. Similarly, intrathecal injection of baclofen increases the threshold for visceromotor response (VMR) to colorectal distension (CRD) in a dose-dependent fashion (19). Expression of c-fos in the lumbo-sacral spinal cord to mustard oil-induced inflammation of the colon can be markedly reduced by intraperitoneal injection of baclofen (30). This effect of baclofen occurs primarily via the activation of presynaptic GABA$_B$ receptors that regulate the release of substance P (SP) from the primary afferent terminals in the spinal cord (4, 31, 33, 44). In addition, baclofen reduces the c-fos expression and internalization of neurokinin (NK)$_1$ receptor in lamina I neurons even when SP is exogenously administered, suggesting a direct postsynaptic site of action on the dorsal horn neurons.

Recent studies have documented that baclofen and other GABA$_B$ receptor agonists have a peripheral site of action. Baclofen dose dependently attenuates responses of vagal mucosal and muscle tension-sensitive afferent fibers innervating the esophagus and proximal stomach (38, 40, 52). Results strongly suggest the pres-
ence of functional GABA_B receptors at the receptive terminals of the vagal afferent fibers. Retrograde labeling of the vagal afferents from the proximal stomach and subsequent immunocytochemical reaction for GABA_B receptors have confirmed the presence of GABA_B receptors in the cell bodies of the nodose ganglion (3, 52). Like in the vagus nerve, GABA_B receptor subunits and their mRNAs are also present in the dorsal root ganglion (18, 34, 43, 59). Therefore, it is conceivable that functional GABA_B receptors may be present at the receptive terminals of the spinal primary afferent fibers, and selective agonist for the GABA_B receptor may attenuate responses of these afferent fibers. Therefore, the aim of the present study was to evaluate the effect of GABA_B receptor agonist baclofen on responses of mechanosensitive pelvic nerve afferent fibers innervating the colon of the rat. Preliminary data of this work were presented in abstract form at the annual meeting of American Gastroenterology Association (49).

MATERIALS AND METHODS

Animals

Male Long-Evans rats (Taconic, New York) weighing 400–450 g were used in this study. Food, but not water, was withhold 12 h before the experiment. All procedures were approved by The Institutional Animal Care and Use Committee of the Medical College of Wisconsin.

General Procedures

Rats were anesthetized initially with pentobarbital sodium (40–45 mg/kg ip) and maintained with supplemental doses of 5–10 mg·kg⁻¹·h⁻¹. The trachea was cannulated for mechanical ventilation with room air. The left common carotid artery was cannulated for recording blood pressure. A femoral artery and vein were catheterized for injection of drug and anesthetic, respectively. The rat was paralyzed with pancuronium bromide (0.3 mg/kg iv) and ventilated with room air (55–60 strokes/min and 3- to 4-ml stroke volume). Additional doses of pancuronium bromide (0.2–0.3 mg·kg⁻¹·h⁻¹) were given to maintain paralysis during the course of the experiment. Mean arterial blood pressure was monitored continuously and maintained at >80 mmHg with supplemental intravenous injection of 5% dextrose in saline given in a bolus of 1–1.5 ml as required. At the end of an experiment, the rat was euthanized by injecting 1 ml of Beuthanasia-D (50 mg pentobarbital) over a black microbase plate immersed in warm (37°C) mineral oil. The dorsal rootlet was split into thin bundles, and a fine filament was isolated from the bundle to obtain a single unit. Electrical activity of single units was recorded monopolarly by placing a teased fiber over one arm of a bipolar platinum electrode; a fine strand of connective tissue was placed across the other pole of the electrode. Action potentials were monitored continuously by analog delay and displayed on a storage oscilloscope after initial amplification through a low-noise AC differential amplifier. Action potentials were processed through a window discriminator, and the frequency of firing was analyzed (1-s binwidth) on line using the spike2 (version-3.21/CED 1401 data acquisition program. Peristimulus time histograms (PSTHs), colonic distending pressures, and blood pressure were displayed on line.

Experimental Protocol

Pelvic nerve input to the S1 dorsal root was identified first by electrical stimulation of the pelvic nerve (a single 0.5-ms square-wave pulse at 5–8 mA). Fibers were classified on the basis of their conduction velocities; those with conduction velocities <2.5 m/s were considered unmyelinated C-fibers and those with conduction velocities >2.5 m/s were considered thinly myelinated Aδ-fibers. If a fiber responded to CRD, a stimulus-response function (SRF) to graded phasic distending pressures of 5, 10, 20, 30, 40, 60, 80, and 100 mmHg was constructed. The duration of distension was 30 s, and the interstimulus time interval was 180 s. In some experiments, SRF was repeated after bilateral transsection of ventral roots (VR: T12–S3).

The effect of baclofen (GABA_B agonist) was tested on the responses of distension-sensitive pelvic nerve afferent fibers to nociceptive CRD (60 mmHg, 30 s). The interstimulus time interval was 3 min. Baclofen was injected intra-arterially 90 s before distension. A cumulative dose-response function was constructed by giving 1, 3, 10, 30, and 300 μmol/kg baclofen. To test whether the inhibitory effect of baclofen was mediated via spinal motor neurons, the effect of baclofen was tested in rats with bilateral VR transsection (T12–S3).
To examine the GABA<sub>B</sub> receptor-mediated effect of baclofen, cumulative doses of baclofen were injected into rats pretreated with the GABA<sub>B</sub> receptor-selective antagonist CGP 54626 (1 μmol/kg iv). CGP 54626 was injected after recording two reproducible responses to CRD (60 mmHg). Response of the fiber to another distension was recorded after CGP 54626 injection to test the effect of CGP 54626. This was followed by a cumulative dose-response function of baclofen. Because the inhibitory effect of baclofen lasted >120 min, the dose-response function of baclofen could not be tested before transection VR or before CGP 54626 application.

**Data Analysis**

Resting activity of a fiber was counted for 60 s and the firing frequency was represented as impulses/s. To determine the actual response of the fiber to CRD the resting firing frequency was subtracted from the firing frequency during the CRD. SRF-to-graded CRD was plotted for individual fiber, and a least squares regression line was obtained from the linear part of the SRF. The regression line was then extrapolated to the ordinate (representing distension pressure), and the intercept point was considered as estimated response threshold. The effect of baclofen was determined by measuring the firing frequencies of the fiber to CRD (30 s) subtracted from the baseline activity.

All data were expressed as means ± SE. Results were compared statistically using Student’s t-test or repeated-measures ANOVA. Doses to produce 50% inhibition of response (ID<sub>50</sub>) were calculated from the 20–80% regression by fitting the data of the cumulative dose-response curve to logarithmic best fit line (58). The following equation was derived from this line: \( y = y_0 + a \cdot \ln(x) \), where \( a \) is slope. Setting the \( y \) value to 50 and using the \( y_0 \) and \( a \) coefficients from the logarithmic best fit line accomplished the \( x \) variable in the equation. A value of \( P < 0.05 \) was considered statistically significant.

**Drugs**

Baclofen (213.7 mol wt; Sigma) was dissolved in saline, and the pH was set to 7.00 by adding a few drops of acetic acid. CGP 54626 (444.7 mol wt; Tocris Cookson) was dissolved in polyethylene glycol.

**RESULTS**

**Sample**

A total of 21 pelvic nerve afferent fibers was identified in the S<sub>1</sub> sacral dorsal root that responded to CRD. Seven of these were unmyelinated C-fibers (mean: 1.85 ± 0.12 m/s, range: 1.55–2.50 m/s) and 14 were thinly myelinated A<sub>δ</sub>-fibers (mean: 5.11 ± 1.55 m/s, range: 2.60–17.50 m/s). Eighteen fibers had low, ongoing spontaneous activity (mean: 1.66 ± 0.64 impulses/s) with the balloon in the descending colon, and three fibers had no spontaneous activity. Responses of four fibers to CRD were tested before and after bilateral transection of VR (T<sub>12</sub>-S<sub>3</sub>). The mean resting activity of these fibers did not change significantly after VR transection (2.20 ± 0.33 impulses/s vs. 1.56 ± 0.56 impulses/s, \( P > 0.05 \)).

**Responses to CRD**

SRFs of 21 fibers were tested to graded isobaric CRD (5–100 mmHg). All fibers exhibited a linear increasing response to graded distension. Figure 1 illustrates the example of intensity-dependent response of a pelvic nerve afferent fiber to graded CRD. These fibers typically exhibited a slowly adapting response to a 30-s period of CRD. At greater intensities (>40 mmHg),
these fibers exhibited a dynamic response at the beginning of the distension, which then adapted to a tonic response.

Figure 2A shows the individual SRFs of 17 pelvic nerve afferent fibers with intact VR. Figure 2B shows the mean SRF of these fibers. The threshold for response of each fiber was estimated by extrapolating the linear regression line to the x-axis. The estimated mean threshold for response of these fibers was 4.7 ± 1.14 mmHg (range: 0–18 mmHg).

To examine the influence of tonic effect of the spinal motorneurons innervating the colon, SRF was repeated after bilateral transection of VR (n = 4). Figure 3 shows examples of responses of a fiber before and after transection of the VRs. Figure 2C shows individual SRFs of four fibers before and after VR transection.
Mean SRF plots of these fibers before and after VR transection were very similar (Fig. 2D). Slopes of the mean SRFs were quite identical (0.95 and 0.97), suggesting that VR transection did not affect the mechanosensitive properties of these fibers to isobaric distension. The mean estimated thresholds for response before and after ventral root transection were 2.02 ± 0.70 (range: 0–3.06 mmHg) and 3.41 ± 0.33 mmHg (range: 0–3.77 mmHg), respectively.

Effect of GABA<sub>B</sub> Receptor Agonist

The effect of selective GABA<sub>B</sub> agonist baclofen on responses to distension were tested on afferent fiber recorded in intact (n = 7) and bilateral VR transected rats (n = 4). Of seven fibers tested with baclofen in intact rats, responses of five (71%) to CRD (60 mmHg, 30s) were dose dependently inhibited. Baclofen also progressively inhibited the ongoing resting activity of these fibers. Figure 4 illustrates an example of the effect of incrementing doses (1–300 μmol/kg) of baclofen on responses of a distension-sensitive pelvic nerve afferent fiber from an intact rat. The mean maximum response to a 300 μmol/kg cumulative dose of baclofen was 8.24 ± 8.24% (n = 5) of the control response. The mean calculated ID<sub>50</sub> of baclofen that produced responses to 60-mmHg CRD was 9.84 ± 3.18 μmol/kg. In the majority of cases, the inhibitory effect of baclofen lasted >120 min.

Like in intact rats, baclofen produced dose-dependent inhibition of response of the fibers to CRD in rats with VR transected (Fig. 5). The mean maximum response to a cumulative dose of 300 μmol/kg was 20.38 ± 2.35% (n = 4) of the control response. The mean calculated ID<sub>50</sub> of baclofen that produced responses to 60-mmHg CRD was 11.62 ± 2.35 μmol/kg, which was not significantly (P < 0.05) different from the VR intact rats (see Fig. 7).

Effect of Selective Antagonist CGP 54626

To determine whether the inhibitory effect of baclofen was produced by activation of GABA<sub>B</sub> receptors, baclofen was tested on responses of four fibers in four rats pretreated with the selective GABA<sub>B</sub> receptor antagonist CGP 54626 (1 μmol/kg iv). Because the inhibitory effect of baclofen was long lasting (>120 min), a control dose response of baclofen before the effect of CGP 54626 was not performed. The reversal by CGP 54626 was compared with that of the fibers tested only to baclofen. CGP 54626 was injected 4–5 min before cumulative injection of baclofen (1–100 μmol/kg). Figure 6 shows an example of reversal of the inhibitory effect of baclofen by the antagonist. CGP 54626 produced a brief increase in firing but no attenuation of response to CRD. The inhibitory effect of baclofen was markedly reversed by CGP 54626. The mean maximum of response at a cumulative dose of 300 μmol/kg baclofen was 71.36 ± 17.85% (n = 4), suggesting a significantly less inhibition of response by baclofen in the presence of CGP 54626 (Fig. 7).
DISCUSSION

Results show that distension-sensitive pelvic nerve afferent fibers exhibit a linear increasing response to graded CRD. All fibers exhibit a slowly adapting response to phasic distension. The present study also shows that the threshold for response and the slope of the SRF of these afferent fibers do not change after bilateral VR transection. Previous studies (46, 47, 50) have documented that in Sprague-Dawley rats, 25–30% of pelvic nerve afferent fibers supplying the descending colon and the urinary bladder have high thresholds (>30 mmHg) for response. In the present study in Long-Evans rats, however, all fibers exhibited low thresholds (<5 mmHg) for response. This difference in results could be a strain-related phenomenon.

A comparative study (36) has indicated that the mechanical stimulation of the plantar skin by von Frey filaments has significantly lowered baseline responses in Long-Evans rats compared with Sprague-Dawley and Wistar rats. This strain has the highest percentage of rats that develops mechanical allodynia to spinal cord injury. Results suggest that sensory thresholds of Long-Evans rats are relatively lower than other strains of rats.

The present study documents that selective GABA_b receptor agonist baclofen dose dependently (1–300 μmol/kg) attenuates responses of distension-sensitive afferent fibers in the pelvic nerve. The effect appears to be a direct peripheral site of action of the drug at the sensory nerve terminals. It is quite unlikely that inhibitory effects of baclofen on responses of distension-sensitive pelvic nerve afferent fibers could be mediated by spinal mechanisms.
VR transected animals were 9.84 ± 2.35/H9262 tested on distension, and of baclofen was tested to isobaric (a constant pressure) saline of inhibitory response by selective GABAB receptor the spinal motorneurons for two reasons: smooth muscle and/or by inhibiting the tonic control of changes in compliance of the lumen by relaxing the distension-sensitive afferent fibers in bilateral VR transected rats. Reversal of inhibitory response by selective GABAB receptor antagonist CGP 54626 indicates that the action of baclofen is mediated via GABAB receptors. Results also indicate there is no correlation between the effect of baclofen and conduction velocity of the nerve fiber.

**Effect of Baclofen in Visceral Pain**

There are reports that baclofen has an effect on visceral pain. In the conscious, freely moving rat model of visceral pain induced by instilling xylene into the urinary bladder, subcutaneous injection of baclofen reduces the behavioral (licking of lower abdomen or perineal region, hind paws in hyperextension) responses (1). Similarly, visceral pain produced by noxious CRD is dose dependently reduced by intrathecal administration of baclofen into the lumbarosacral segment of the spinal cord (19). Systemic application of Baclofen markedly reduces the c-fos expression in the lumbarosacral spinal cord in capsaicin-induced irritation of the urinary bladder or mustard oil-induced inflammation of the colon (5, 30). The antinociception and reduction of c-fos expression by baclofen are thought to be principally due to the reduction of SP release from the presynaptic terminals in the spinal cord (44). The present study, however, documents that the antinociceptive effect of baclofen may also be, in part, due to direct effects on the mechanotransduction properties of visceral afferent fibers. This result is inconsistent with those of Blackshaw and colleagues (38, 40, 52), who reported a peripheral inhibitory effect of baclofen on responses of vagal afferent fibers innervating the esophagus and proximal stomach.

*Peripheral Site of Action*

It is known that systemic applications of many of the centrally acting drugs exert inhibitory effects by acting at the peripheral sites. For example, κ-opioid receptor agonist (KORA), tricyclic antidepressants (TCAs), and N-methyl-D-aspartate open channel blockers produce inhibition of responses of mechanosensitive afferent fibers in the vagus and pelvic nerves (21, 35, 37, 51, 53–56). However, the mechanism of action of many of these centrally acting drugs at peripheral sites is an enigma and may not be always specific-receptor mediated. For example, KORA-induced inhibition of responses of the mechanosensitive vagus and pelvic nerve afferent fibers are not completely reversed by nonselective opioid antagonist naloxone or by selective KOR antagonist nor-BNI (37, 51, 53, 54, 56). A recent report indicates that the inhibition of response by KORA occurs by blocking the Na⁺-channels in the peripheral site not via KOR (57). It should be noted that in the behavioral study, KORA asimadoline, which is thought to be a peripherally restricted drug, produces visceral antinociception at a significantly lower dose than the dose that attenuates responses of pelvic nerve afferent fibers (48). Our recent study (48) documents that intravenous asimadoline-induced visceral antinociception and diuretic effects in rats can be blocked by intracerebroventricular application of selective KOR antagonist nor-BNI, suggesting that asimadoline crosses the blood-brain barrier to produce antinociception and diuresis. Similarly, the exact mechanism of action of the TCAs at the peripheral site is not known. It has been proposed that in the formalin-induced paw flinch model TCA amitryptilline produces antinociception by activating adenosine A1 receptors located on the somatic primary afferent fibers (45). These investigators have also observed a local anesthetic effect of TCAs, which can be explained by the fact that TCAs (amitryptilline, doxepin, and desipramine) block Na⁺ inward current of the voltage-gated Na⁺ channels (13, 39).

Our results show that selective GABA_B receptor antagonist CGP 54626 can reverse the inhibitory effect of baclofen at the peripheral site of the pelvic nerve afferent fibers, confirming a GABA_B receptor-mediated effect of baclofen. This is consistent with the previous reports in the vagal afferent fibers (10, 38, 40, 52). The exact mechanisms by which GABA_B receptors attenuate responses of the mechanosensitive pelvic nerve...
afferent fibers are yet to be studied. Three possible mechanisms can be discerned for GABAB-mediated action: 1) a reduction of neurotransmitter (e.g., SP, CGRP) release from the primary afferent nerve terminals that in turn may excite the fibers, 2) inhibition of Ca2+-channel coupled to the GABAB receptor, and 3) activation of G protein couple inwardly rectifying K+ channels (GIRK). Although there is no direct evidence of release of SP and/or CGRP from the receptive terminals to mechanical distension, this possibility cannot be excluded. Electrophysiological studies (23) have shown that NK2 receptor antagonist SR 142,801 can attenuate mechanotransduction properties of the pelvic nerve afferent fibers innervating the colon but not the urinary bladder. Therefore, SP and/or NK2 may be involved in excitation of spinal afferents to mechanical distension. However, evidence is strongest that regulations of Ca2+ and K+ channels coupled to GABAB receptors are the principal mechanisms by which baclofen attenuates responses of these afferent fibers. There is ample evidence that baclofen inhibits N- and P/Q-type Ca2+ channels in the sensory neurons and activates the GIRK (11, 15, 60).

In conclusion, the present study provides direct evidence that GABAB receptor agonist baclofen can modulate visceral nociception by attenuating responses of pelvic nerve afferent fibers at the peripheral site. Like KOR, antinociceptive effect of baclofen occurs at multiple sites and, more importantly, at the level of the spinal cord. Use of baclofen for visceral pain may be associated with a number of undesirable effects (e.g., sedation, tolerance, respiratory depression, constipation, etc.). However, recent identifications of GABAB receptor subunits (GABAB1, GABAB2), their splice variants, and specific location in the tissues are encouraging. This may lead to the design of subtype-targeted drugs for more effective therapy.

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