Spinal GABA<sub>A</sub> receptors do not mediate the sympathetic baroreceptor reflex in the rat

ANN K. GOODCHILD, BART T. M. VAN DEURZEN, QI-JIAN SUN, JOHN CHALMERS, AND PAUL M. PILOWSKY

Hypertension and Stroke Research Laboratories, Departments of Physiology and Neurosurgery, University of Sydney, Royal North Shore Hospital, St. Leonards, NSW 2065, Australia

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GABA<sub>A</sub> receptors do not mediate the sympathetic baroreceptor reflex in the rat. Am J Physiol Regulatory Integrative Comp Physiol 279: R320–R331, 2000.—Activation of baroreceptors causes efferent sympathetic nerve activity (SNA) to fall. Two mechanisms could account for this sympathoinhibition: disfacilitation of sympathetic preganglionic neurons (SPN) and/or direct inhibition of SPN. The roles that spinal GABA and glycine receptors play in the baroreceptor reflex (SPN) and/or direct inhibition of SPN. The roles that spinal GABA and glycine receptors play in the baroreceptor reflex were examined in anesthetized, paralyzed, and artificially ventilated rats. Spinal GABA<sub>A</sub> receptors were blocked by an intrathecal injection of bicuculline methiodide, whereas glycine receptors were blocked with strychnine. Baroreceptors were activated by stimulation of the aortic depressor nerve (ADN), and a somatosympathetic reflex was used as control. After an intrathecal injection of vehicle, there was no effect on any measured variable or evoked reflex. In contrast, bicuculline caused a dose-dependent increase in arterial pressure, SNA, phrenic nerve discharge, and it significantly facilitated the somatosympathetic reflex. However, bicuculline did not attenuate either the depressor response or sympathoinhibition evoked after ADN stimulation. Similarly, strychnine did not affect the baroreceptor-induced depressor response. Thus GABA<sub>A</sub> and glycine receptors in the spinal cord have no significant role in baroreceptor-mediated sympathoinhibition.

Sympathetic nerve activity; arterial pressure; phrenic nerve discharge; aortic depressor nerve

Over the last decade, the neural circuitry within the medulla that regulates the sympathetic component of the baroreflex has been extensively investigated (for review, see Refs. 3, 7, 19, 31). This work has led to our current concept of the sympathetic baroreflex. Baroreceptor afferent neurons, that are presumably glutamatergic, terminate in the nucleus of the solitary tract (NTS). From the NTS, there is a direct excitatory projection to inhibitory, presumably GABAergic, neurons in the caudal ventrolateral medulla. These inhibitory interneurons then project rostrally where they inhibit the descending excitatory bulbospinal neurons of the rostral ventrolateral medulla (RVLM), which in turn innervate cardiovascular sympathetic preganglionic neurons (SPN) of the thoracolumbar spinal cord. Thus baroreceptor loading activates this pathway, disfacilitating SPN and causing sympathoinhibition.

However, limited evidence indicates that two alternate pathways may also mediate baroreceptor-evoked sympathoinhibition, with the inhibitory mechanism confined to the spinal cord. First, disfacilitation at the spinal level may occur, because McCall et al. (25) reported that in the cat the activity of interneurons is reduced after carotid artery occlusion. More recent evidence also supports the suggestion that baroreceptor activation may cause direct inhibition of SPN. Activation of baroreceptors by distension of a carotid sinus (10, 27) or by stimulation of the aortic depressor nerve (ADN) (12) in the cat causes hyperpolarization of SPN that is reversible by negative current injection, suggesting direct inhibition. However, the number of neurons sampled is very small. McCall and Humphrey (26) have identified two different latencies of baroreceptor-induced inhibition at 27 and 110 ms and have suggested that they represent inhibition at a spinal and brain stem level, respectively. When averaging the synaptic noise after stimulation of the ADN of the cat, Fedorko et al. (12) also identified a response of similarly short latency. Such studies suggest the short latency response is due to stimulation of a very fast pathway with few synaptic connections. Furthermore, Coote (5) claims that maximal baroreceptor stimulation (using a 200-mmHg rise in systemic arterial pressure) can inhibit glutamate-evoked activity in antidromically identified SPN when the activity is increased from 0 to 31 Hz and suggests that this indicates a baroreceptor spinal inhibitory pathway acting directly on SPN.

Most recently, Lewis and Coote (21) and Coote and Lewis (6) reported that an excitatory response evoked in the renal nerve, after stimulation of the dorsolateral funiculus of the spinal cord, was inhibited by baroreceptor activation. They found that ~40% of the inhib-
tion was mediated by spinal glycinergic mechanisms, and the remaining inhibition was due to spinal GABAergic mechanisms. Furthermore, they suggested that the latter spinal component was dependent on descending excitatory pathways originating in the brain stem, but the spinal glycinergic mechanism acted independently of the RVLM. Lewis and Coote (20) further suggested that because electrical stimulation of the NTS could evoke inhibition in SPN that was dependent on either glycine or GABA, this was evidence in support of spinally mediated inhibition.

Lewis and Coote (21) and Coote and Lewis (20) thus suggest that essentially all baroreceptor-induced inhibition of a spinally evoked reflex is due to spinal inhibitory mechanisms. In direct contrast, the vasomotor component of the baroreceptor reflex is abolished or at least severely reduced after bilateral injections of GABA antagonists into the RVLM (33), suggesting that disfacilitation of a descending excitatory pathway is the principal mode of action of the arterial baroreceptors.

Thus we sought to determine the contribution that spinal GABA_A and glycine pathways make to the sympathoinhibition evoked by baroreceptor activation in the rat. We activated baroreceptors directly by electrical stimulation of the ADN, which in the rat is purely barosensory (19). The somatosympathetic reflex was used as a control to evaluate the effectiveness of the spinal GABA_A receptor blockade evoked by the intrathecal injection of bicuculline methiodide (BMI). Multiple doses of bicuculline were used to determine the maximal effects on baseline variables of cardiorespiratory function.

**METHODS**

**General preparation.** Wistar (n = 17) and Sprague-Dawley (n = 3) rats weighing 350–790 g were anesthetized with either pentobarbital sodium (60 mg/kg ip, n = 3) or ethyl carbamate (urethan, 1.3 g/kg ip, n = 17). Additional doses of anesthetic were administered intravenously to maintain stable levels of arterial pressure: pentobarbital sodium 3–6 mg/kg or ethyl carbamate 0.13 g/kg. The right femoral artery and vein were cannulated to enable the recording of arterial pressure and the administration of drugs, respectively. A tracheostomy tube was inserted for artificial ventilation. Rectal temperature was maintained and maintained at 37°C with the use of a thermostatically controlled electric blanket and/or infrared heating source.

The left aortic depressor and phrenic nerves were exposed in the neck with the use of a dorsolateral approach (28). The nerves were isolated, cut distally, and ligated to permit stimulation or recording. In five animals, the rostral branch of the left splanchnic nerve was exposed with the use of a retroperitoneal approach, cut distally, and prepared for recording. Recordings were made from the preganglionic nerves of paraffin oil. The vagus nerves were kept intact.

In all 20 animals, an intrathecal catheter was implanted. The catheter (OD 0.5 mm, ID 0.2 mm) was inserted via the atlantooccipital membrane in the first five animals. In the remaining 15 animals, the intrathecal catheter was implanted after a laminectomy at L3. Using this lumbar approach further minimized the risk of drug flowing rostrally via capillary action along the catheter tubing. The tips of the catheters were all located between T8 and T11. The patency of the catheter was tested immediately after insertion by withdrawal of cerebrospinal fluid (CSF). A drop of cyanoacrylate glue placed at the site of the catheter insertion held it in position and prevented leakage of CSF.

The animals were placed prone in a stereotaxic frame with the head above the level of the spinal cord. This position reduced the possibility of drug access to the upper cervical spinal cord and brain stem. Animals were paralyzed with pancuronium bromide (1.6 mg/kg iv), and supplemental doses were administered when necessary (0.8 mg/kg iv). The animals were artificially ventilated with the use of room air with additional oxygen (60–70 cycle/s, 2.7–4 ml/cycle). Peak expired CO2 was monitored and maintained between 3.8 and 4.5% by varying the frequency and tidal volume.

Fluid losses were replaced intravenously with a 5% glucose solution at a rate of ~1 ml/h. At the end of the experiment, animals were killed with an overdose of anesthetic.

**Intrathecal injection.** The volume of each catheter was measured before insertion (range 10–14 µl) and this volume was then used to flush the catheter. Drugs were administered in 5-µl volumes with the use of a 25-µl Hamilton syringe. In all experiments, an initial injection of 10 mM PBS (pH 7.4) (the vehicle of the injectant) preceded the drug injection. At the conclusion of each experiment, 5 µl of pentamine sky blue solution (~2% in PBS) were injected. The extent of the spread of the dye in the intrathecal space and the position of the catheter tip were examined postmortem.

**Drugs used.** The convulsant alkaloid bicuculline competitively interacts with GABA at the GABA_A receptor and is a specific GABA_A antagonist (Merck). Bicuculline methiodide (Sigma) was used as it probably does not pass the blood brain barrier (16). Strychnine is also a convulsant alkaloid, and its antagonism of glycine defines a specific glycine receptor subtype (the strychnine-sensitive glycine receptor) (Merck). Strychnine (n = 1) or strychnine hydrochloride (n = 2) (Sigma) were used.

All drugs were dissolved in PBS except strychnine. This was partially dissolved in dimethyl sulfoxide and diluted in PBS to form a suspension, which was subsequently injected. All drugs were administered intrathecally in volumes of 5 µl containing 5, 10, 20, 50, or 100 nmol of the active component. No more than two concentrations of the drugs were administered to any one animal, and the lower concentration always preceded the highest. In each animal, injections were separated by 60–90 min. In one of three animals receiving strychnine, bicuculline injection preceded the use of strychnine hydrochloride.

In two animals, the effects of intravenous administration of 100 nmol bicuculline were assessed.

**Nerve recording.** Phrenic nerve discharge and splanchnic nerve activity were recorded with the use of bipolar silver wire electrodes. The signals were amplified and acquired online with the use of a CED 1401 plus and SPIKE2 software (CED, Cambridge, UK). Neurograms were acquired at a sampling rate of 1–1.5 kHz.

**ADN stimulation.** The ADN was stimulated with the use of a bipolar silver electrode to evoke the baroreceptor reflex. The voltage threshold for evoking a depressor response was determined with the use of a 5-s train of pulses at 50 Hz and 0.1-ms duration. In 16 animals, this threshold was <1.5 V and usually in the range 0.3–0.5 V. The stimulus used to evoke a depressor response throughout each experiment was three times threshold, except in two animals when this was 5 and 10 times the threshold. These latter two animals were not used in studies with sympathetic nerve recording, and...
the blood pressure results obtained from them were not pooled with other animals.

Two stimulation protocols were used to determine the effects of ADN stimulation on splanchnic nerve activity. First, with the use of the 5-s train ADN stimulus paradigm, the effects of bicuculline on the frequency response of the sympathetic nerve activity (SNA) were assessed in three Sprague-Dawley rats. In these three animals, the stimulus intensity used ranged between 0.75 and 3.5 V. The frequency response was determined at 10, 25, 50, 75, and 100 Hz. It is important to note the short duration of the stimulus pulses (0.1 ms).

The second stimulus paradigm was used to determine the sympathoinhibition evoked before and after intrathecal drug injection in five animals. This second stimulus was used to determine any latency differences in the sympathoinhibitory response evoked after bicuculline or strychnine. Furthermore, because the large bursts of activity evoked by bicuculline may have influenced the inhibition evoked by baroreceptor stimulation, a response averaged over some time was investigated. For this paradigm, the stimulus intensity used in all five animals was three times the threshold. The ADN was stimulated with three pulses separated by 2.5 ms (400 Hz) and a 0.1-ms pulse duration. The train repetition rate was 1.25 Hz (800 ms between each burst of 3 pulses). We shall refer to this as the 1.25-Hz stimulus paradigm. Due to the low-train repetition rate, little or no change in arterial pressure was elicited.

Analysis of nerve activity. To assess the effect of the drugs on splanchnic nerve activity, mean levels were calculated before and after drug administration. An average of rectified activity was taken over a 300-ms period every 800 ms for a total period of 30–60 s.

For the splanchnic nerve activity acquired with the use of the 5-s ADN stimulus paradigm at various frequencies, the nerve activity was rectified and a 5-s moving average was calculated. A total of 10 s of nerve activity was analyzed 5 s before and 5 s during the stimulus.

For splanchnic nerve activity acquired before and during 1.25-Hz stimulation of the ADN, stimulus-triggered moving averages of 300 ms every 800 ms were calculated over 100 s before and at regular intervals after drug administration.

The change in nerve activity was then calculated as the difference in the level of SNA before and after the stimulus, divided by the level of SNA before the stimulus, and expressed as a percentage.

Evoking a somatosympathetic reflex. A somatosympathetic reflex was evoked either by tail pinch, with the use of a pair of hemostats, or by electrical stimulation of the foot. Two needle electrodes were implanted between the toes of the foot, and a stimulus (0.1-ms pulses at 50 Hz for 5 s) was applied. Voltage was increased until a small effect on arterial pressure was detected or the maximal stimulus of 99 V was attained.

Spinal cord transaction. In four animals, the spinal cord was transected at the C8 level. A laminectomy at the C8 segment was performed, and the membranes were removed. Fine diathermy occluded the major blood vessel, and fine forceps were used to slowly transect the spinal cord over a period of 5 min. After complete transection, verified visually, a small wad of gelfoam was placed between the cut ends of the cord.

RESULTS

The effects of intrathecal bicuculline or strychnine on arterial pressure and expired CO₂ were evaluated in 18 rats. The effects of baroreceptor stimulation (in all animals) and the effects of noxious stimuli (in 14 animals) were assessed after drug injection. In addition to arterial pressure and expired CO₂ (monitored in all animals), phrenic nerve activity was measured in nine urethan-anesthetized rats and splanchnic SNA was monitored in eight urethan-anesthetized animals.

Pentobarbital sodium was used in initial experiments, but the dramatic effects of high doses of bicuculline on arterial pressure made it difficult to assess the adequacy of anesthesia over the duration of experimentation. This led to the change to urethan. Although no statistical tests were conducted between the pentobarbital sodium (n = 3) and urethan (n = 15) groups, the effects were qualitatively the same and quantitatively similar.

Effects of Intrathecal Administration of Bicuculline

Figures 1A and 4 show the effects of 10 and 100 nmol bicuculline, respectively, injected intrathecally at the T8-T11 spinal levels. Large increases in arterial pressure, SNA, phrenic nerve activity, and expired CO₂ (not shown) were elicited. The rate of increase of the variables measured and the duration of the response varied slightly between experiments and also with the dose of bicuculline injected. Arterial pressure consistently began to rise within 3 min of injection.

Arterial pressure. The mean level of arterial pressure before 100 nmol bicuculline in 15 animals was 107 ± 6 mmHg (means ± SE). The peak level of mean arterial pressure reached after bicuculline injection was 158 ± 4 mmHg (means ± SE). Systolic, diastolic, and pulse pressure all increased after bicuculline injection (Fig. 1A).

Figure 1, B and D, shows that an intrathecal injection of PBS, which was used as the vehicle, does not affect arterial pressure or SNA measured at any time after injection. In contrast, dose-related pressor responses were evoked by 5, 10, and 100 nmol bicuculline. The pressor response evoked by 5 nmol BMI was 15 ± 4 mmHg (n = 3). The levels of arterial pressure reported are the largest detected after injection. The peak response in arterial pressure usually occurred within 15 min of injection. In most experiments, more than one dose of bicuculline was administered, so the dose-response graph in Fig. 1B may represent a cumulative effect. However, in four animals, a dose of 100 nmol of bicuculline administered first caused a rise of 65 ± 16 mmHg (means ± SE), indicating that a noncumulative dose-response effect exists.

The lability of arterial pressure also increased after injection (Figs. 1, A and C, 2, and 4). The fluctuations occurred irregularly, varying in frequency from 2 to 20 per minute. Oscillations began within 2–5 min of injection, peaked, and gradually decreased in frequency, persisting for ~1 h. Fluctuations in efferent SNA matched the undulations in arterial pressure (Fig. 1C).

SNA. The effects of intrathecal bicuculline on SNA were assessed by monitoring preganglionic greater splanchnic SNA. Bicuculline applied intrathecally increased the mean level of SNA (Figs. 1, A and D, 4, and...
6A) as well as its lability (Figs. 1C and 4). The increases in SNA were dose dependent, with the largest average increase of 40 ± 15% (means ± SE) elicited by 100 nmol bicuculline (Fig. 1D). Even 1 nmol bicuculline evoked a rise in SNA in two animals tested.

Phrenic nerve activity. The effects of intrathecal bicuculline, with the catheter tip placed at T8-T11 spinal levels, on phrenic nerve activity were determined (Figs. 1A and 2). Bicuculline increased the amplitude of phrenic nerve activity (Figs. 1A and 2) on average by 44 ± 7% (means ± SE, 100 nmol bicuculline). Figure 2 shows the effects of bicuculline on phrenic nerve discharge and also the effect of transection of the spinal cord at C8 on the bicuculline-induced phrenic activity. Irregularities in rhythmicity are also evident after bicuculline injection because the duration and interval of the bursts become variable (Fig. 2A). Bicuculline-induced effects on phrenic nerve activity persist at least as long as the effects on SNA. Transection of the spinal cord at the C8 level was conducted in four animals, and in all cases the bicuculline-induced bursts of activity were eliminated after transection.
with a return of regular, rhythmic discharge in phrenic nerve activity (Fig. 2B). This restoration of a rhythmic discharge after transection indicates that the drug was not directly affecting phrenic motoneurons. Although SNA was attenuated after spinal cord transection, bicuculline-induced bursts of activity were still evident.

Effects of intrathecal administration of bicuculline. In two animals, 100 nmol bicuculline were injected intravenously, before any intrathecal injection. No effect on any measured variable was detected.

Effects of intrathecal administration of bicuculline on the cardiovascular component of the somatosympathetic reflex. Two stimuli were used to evoke the somatosympathetic reflex in 14 rats; either the tail was pinched or the foot was stimulated electrically between two toes. Tail pinch on average elicited a small increase in arterial pressure (~10 mmHg) before bicuculline injection (Fig. 3, A and B). In contrast, stimulation of the footpad evoked either small depressor or pressor responses (range ~7 to 23 mmHg, Fig. 3, C and D).

Five to ten minutes after intrathecal bicuculline, a large pressor response was consistently evoked by pinching of the tail or stimulation of the foot pad [Fig. 3, A-D; in Fig. 3D, P = 0.0046 when the 10-min sample is compared with the prebicuculline sample (paired t-test)]. It can be seen that the facilitation of the cardiovascular response to somatosympathetic reflex stimulation is dose dependent (compare Fig. 3, A and C, with Fig. 3, B and D, respectively). The duration of the facilitatory response lasted for up to 75 min and was also dose related.

In two experiments, the effects of innocuous stimuli were assessed by stroking the hair on the back or side of the animal. Before intrathecal administration of bicuculline, no cardiovascular response could be evoked by such stimuli, but after drug injection pressor responses of the order of ~20 mmHg were elicited.

Effects of intrathecal administration of bicuculline on the baroreceptor reflex. The effects of intrathecal bicuculline on the arterial pressure changes and/or SNA evoked by baroreceptor stimulation were assessed in 18 rats. Figure 4 shows the effect of 10 nmol bicuculline on the responses evoked by stimulation of the ADN in one animal.

Arterial pressure. Stimulation of the ADN (with the use of a 5-s train at 50 Hz and 0.1-ms duration) consistently evoked a depressor response of between ~25 and ~50 mmHg before administration of bicuculline (Fig. 5). Figures 4, B, D, and F, and 5A show the effect of stimulation before drug administration, at the peak level of arterial pressure evoked after bicuculline injection, and during the recovery period in two experiments. A large depressor response was evoked at each time. The grouped data in Figs. 5, B-D, show the depressor responses evoked by stimulation of the ADN in one animal.
6D, P = 0.37 when the 10-min sample is compared with the prebicuculline sample (paired t-test)].

SNA. Figure 4 shows the effect in one animal on SNA before and after bicuculline with the use of two different paradigms of ADN stimulation. Figure 4, B, D, and F, shows that a 5-s stimulation (at 3 times the threshold and 50 Hz) of the ADN evoked a dramatic inhibition of splanchnic nerve activity, even though there is some
contamination by stimulus artifact. Figure 4, C, E, and G, shows the filtered, rectified, and averaged sympathetic nerve responses to 1.25-Hz stimulation of the ADN with the use of a train of three pulses separated by 2.5 ms. A distinct inhibition of SNA was evoked at a latency of ~85 ms. The inhibition was not attenuated over the 25 min shown. The sympathoinhibition evoked by both stimulus paradigms was never attenuated or altered in this animal.

The frequency response effect of ADN stimulation with the use of the 5-s ADN stimulus paradigm was evaluated in three animals (Fig. 6). Figure 6A shows that before drug administration, a distinct frequency-dependent sympathoinhibitory response was evoked in splanchnic nerve activity after ADN stimulation. Then after an intrathecal injection of 5 nmol bicuculline, the sympathoinhibition evoked in all animals was never attenuated. In one animal, a dose of 50 nmol was also tested. The sympathoinhibition evoked was larger after bicuculline administration than before injection. The 75-Hz response illustrated in Fig. 6B shows an inhibition of splanchnic nerve activity of 73%, 15 min after bicuculline was injected. Before bicuculline, a 47% fall in activity was obtained.

The effects on SNA were also determined with the use of the 1.25-Hz ADN stimulus paradigm in five animals. Individual examples of the sympathoinhibition are shown in Figs. 4 and 7A. The grouped data are shown in Fig. 7, B and C. In the experiment depicted in Fig. 7A, the effects of bicuculline on arterial pressure, SNA, and phrenic nerve activity persisted for ~90 min. In all animals, despite the sympathetic activation that occurred after bicuculline, the sympathoinhibition evoked by ADN stimulation was neither attenuated nor was its latency affected. In several cases, the sympathoinhibition appeared facilitated. However, this apparent facilitatory effect did not reach statistical significance [Fig. 7C, P = 0.06, when the 10- to 15-min sample is compared with the prebicuculline sample (paired t-test)]. In all experiments, the latency of the ADN-evoked response was consistently between 80

Fig. 6. The frequency response of splanchnic nerve activity to a 5-s train of 0.1-ms pulses applied to the ADN before and after intrathecal bicuculline (5 and 50 nmol). A: frequency response of sympathoinhibition evoked before and after 5 nmol bicuculline (means ± SE; n = 3). Attenuation of the sympathoinhibition was never seen. B: response to a 5-s burst of 0.1-ms pulses at 75 Hz, 15 min after 50 nmol intrathecal bicuculline. Horizontal bar, period of stimulation. This shows an inhibition of splanchnic nerve activity of 73%. Before bicuculline, an inhibition of 47% was evoked at 75 Hz. Attenuation of the frequency response to ADN stimulation was never seen.
and 90 ms. Importantly, no evidence of any other inhibition is seen between 10 and 300 ms after the stimulus. No reduction in the sympathoinhibitory response or alteration of latency was ever observed, even when doses as high as 100 nmol bicuculline were administered.

Effects of Intrathecally Administered Strychnine

In three animals, the effects of intrathecally administered strychnine ($n = 1$) or its more soluble salt strychnine hydrochloride ($n = 2$) were assessed. The data are shown in Fig. 8. Doses of 100 nmol evoked a negligible effect on either arterial pressure, SNA (Fig. 8, A and B), and phrenic nerve discharge (data not shown).

Similarly, intrathecal strychnine had no effect on the depressor response evoked by stimulation of the ADN, certainly no attenuation of the response was seen (Fig. 8C). The cardiovascular response to nociceptive stimuli showed no significant change after strychnine (Fig. 8D). In two animals, in which strychnine hydrochloride was administered, the effects of innocuous stimuli were determined by stroking the fur. No cardiovascular responses could be evoked before administration of the drug, but 10–20 min after strychnine administration pressor responses of 20–25 mmHg were evoked.

DISCUSSION

Three main results arise from the present study. First, blockade of GABA<sub>A</sub> receptors in the spinal cord increases arterial pressure and SNA as well as phrenic nerve discharge, whereas glycinergic blockade has little effect. Second, the cardiovascular effects evoked by nociceptive and possibly nonnociceptive stimuli are facilitated by the application of intrathecal bicuculline methiodide. Third, there is no effect on either the depressor response or the sympathoinhibition evoked by stimulation of the ADN after the application of bicuculline or strychnine at the T8-T11 spinal levels.

Effects of Intrathecal BMI

Arterial pressure and SNA. The finding that arterial pressure increases after the intrathecal administration of bicuculline confirms previous reports (14–16). Gordon (14) further confirmed the role of the GABA<sub>A</sub> receptors in this response by showing that the GABA agonist, muscimol, applied intrathecally reduced arterial pressure and SNA, and these effects were reversed by the subsequent administration of BMI. The findings of the present study extend these observations by showing that spinal administration of bicuculline increases SNA. Curiously, Coote and Lewis (6) and Lewis and Coote (21) failed to evoke an increase in SNA with a similar dose to one used here, although they do describe a bursting pattern of activity in two animals. These investigators made no mention of arterial pressure levels after bicuculline administration.

Blockade of GABA<sub>A</sub> receptors also increased the lability of arterial pressure first described by Hara et al. (15). In the present study, bursts of SNA precede the increases in arterial pressure. The origin of these fluctuations may be related to activation of a spinal network, dependent on the sodium pump, similar to that described in neonatal rat spinal motoneurons after GABA receptor blockade (2).

As reported previously and seen in the present study, there is good evidence that the responses evoked
arise only from blockade of GABA_A receptors in the spinal cord. First, no peripheral effects appear to contribute because intravenous bicuculline has no effect (6, 14, 16, 21). Most convincingly perhaps, the effects of C8 transection indicate that the drug is not reaching the phrenic motoneurons located between C4 and C6 let alone any more rostrally located structures, suggesting that the drug is having its action in the thoracic spinal cord. Furthermore, if bicuculline had reached the RVLM for example, the baroreceptor reflex would be blocked and stimulation of the ADN would have little or no effect on arterial pressure or SNA (8, 19, 35). Also dye injected (5 µl) at the conclusion of each experiment was always restricted to the lowest cervical and thoracic segments. Thus the effects demonstrated do not appear to depend on supraspinal structures.

A direct action of bicuculline on SPN is supported by both anatomic and electrophysiological data. Iontophoresis of GABA or bicuculline on identified SPN reduces or increases their activity, respectively (1). The inhibitory action of GABA is probably via the generation of inhibitory postsynaptic potentials, which are blocked by bicuculline (18). GABA_A binding sites are evenly distributed throughout the spinal cord (24). GABA immunoreactive terminals innervate many SPN (22), and GABA receptors are found on SPN (4). Nevertheless, involvement of spinal interneurons cannot be discounted.

Most significantly, these results indicate that there is a high degree of GABA_A receptor-mediated inhibition of SPN and furthermore that this inhibition is tonically active. The origin of this activity is not known.

Phrenic nerve activity. An unanticipated finding in this study is that phrenic nerve activity increased after a blockade of spinal GABA_A receptors. A direct effect of the drug on phrenic motoneurons is not possible because C8 transection immediately after bicuculline application abolishes the bicuculline-induced phrenic bursting. Furthermore, the phrenic and cardiovascular responses evoked had similar latencies and time courses, suggesting a similar distance of drug diffusion. It has been shown that intrathecal administration of drugs does not spread more than a few segments away from the site of administration (34), and furthermore the penetration is ~2 mm (35). The increase in phrenic nerve discharge is not due to the increase in arterial pressure because phrenic nerve discharge would normally decrease when arterial pressure increases.

A direct effect of bicuculline on intercostal and abdominal motoneurons thereby limiting lung inflation leading to an increase in phrenic nerve output is not possible, because the animals were paralyzed and artificially ventilated. In the present study, bicuculline caused both an increase in the amplitude of phrenic discharge and an alteration in the pattern of discharge. The mechanisms underlying these effects are unknown. Conceivably, blockade of spinal GABA receptors affects sensory and intraspinal pathways possibly modulating the spinal central pattern generator, but further studies are necessary.

**Effects of Intrathecal Strychnine**

**Arterial pressure, SNA, and phrenic nerve activity.** In the present study, intrathecal strychnine had little effect on arterial pressure, SNA, or phrenic nerve discharge. In previous studies, intrathecal administration of strychnine at the T2 level elicited a small increase in
heart rate, but when given at T9, arterial pressure (17) and renal nerve activity (6) were unaffected. Thus there is little, if any, tonic glycine-mediated inhibition of sympathetic outflow. However, there is evidence suggesting that inhibition can occur. Glycine hyperpolarizes SPN, and application of strychnine to identified SPN abolishes inhibitory postsynaptic potentials evoked by stimulation of the lateral funiculus of the cat (18). Furthermore, glycine receptors are found on SPN (4).

Effects of Intrathecal Bicuculline on the Somatosympathetic Reflex

In the present study, two stimuli were used to evoke the somatosympathetic reflex; both responses were facilitated in the presence of intrathecal bicuculline. Electrical stimulation of the footpad provided a more controlled stimulus than tail pinch, but both stimuli were used because different afferent pathways may have been involved. A larger pressor response was obtained by tail pinch before drug administration. However, inconsistent cardiovascular responses evoked by painful stimuli have been reported (23). With the use of either stimulus, the degree and time course of the facilitation of the cardiovascular response after bicuculline injection were very similar.

There is considerable evidence to implicate spinal GABA_A receptors in both antinociception and allostynia (13, 23, 24). The antinociceptive effects of GABA are exerted in the dorsal horn, which contains large numbers of GABA immunoreactive interneurons and binding sites for GABA agonists. Bicuculline increases the evoked activity of dorsal horn cells to noxious stimuli and light touch (24, 30). Blockade of spinal GABA_A receptors thus permits an increase in nociceptive traffic in the spinothalamic tract. Nociceptive stimuli excite sympathoexcitatory neurons in the RVLM and ventromedial medulla, and this excitation precedes increases in SNA and arterial pressure (23, 28, 32).

For the purpose of the present study, the facilitatory response of the cardiovascular changes associated with nociceptive (and innocuous) stimuli evoked by intrathecal bicuculline demonstrates that bicuculline penetrated the spinal cord and blocked GABA_A receptors.

Effects of Intrathecal Strychnine on the Somatosympathetic Reflex and Baroreceptor Reflex

After intrathecal strychnine, an increase in arterial pressure was evoked after innocuous, but not painful, stimuli. There is little evidence to support a role for glycine in antinociception, whereas strychnine-sensitive modulation in the spinal cord appears selective for nonnoxious sensory input, at least in the rat (29). The alldynic effects of intrathecal strychnine confirms previous findings (29). This demonstrates that strychnine penetrated the spinal cord and blocked strychnine-sensitive glycine receptors there.

Intrathecal strychnine failed to affect the depressor response to stimulation of the ADN despite the effective blockade of spinal glycine receptors. This suggests that spinal glycineergic synapses do not play a significant role in mediating the sympathoinhibition associated with baroreceptor activation. This is in contrast to the findings of Coote and Lewis (6) and Lewis and Coote (21). Furthermore, because the dose of strychnine used in the present study was 10 times the dose used by Coote and Lewis (6) and Lewis and Coote (21), a concentration-dependent effect cannot be considered to be the reason for the difference between the two findings.

Effects of Intrathecal Bicuculline on the Baroreceptor Reflex

In the present study, a large depressor response and sympathoinhibition evoked by stimulation of the ADN persisted after intrathecal administration of bicuculline. No attenuation of either variable was seen. Frequency response characteristics and latency of the splanchnic nerve response to ADN stimulation were determined with the use of two different stimulus paradigms. These results show that neither the frequency response nor the latency of response of SNA to baroreceptor stimulation were altered after the blockade of spinal GABA_A receptors. These data suggest that spinal GABA_A receptors are not involved in the baroreceptor pathway evoked by this stimulus. This is consistent with many earlier studies that show a blockade of GABA_A receptors in the RVLM abolishes, or greatly reduces, the depressor response, sympathoinhibition, and pulse modulation of sympathetic nerve discharge evoked by activation of baroreceptors (3, 7, 8, 31, 35). Thus disfacilitation of bulbospinal sympathoexcitatory neurons provides the major route by which baroreceptor-evoked sympathoinhibition is mediated. Results from the present study do not contradict these earlier findings.

In the present study, the latency of the sympathoinhibition evoked by baroreceptor activation is ~85 ms. This compares well with latencies of ~150 ms in the renal nerve in rabbits (see Ref. 19) and 100 ms in the cat measured at the T2 level or the inferior cardiac nerve (26), taking into account animal size and different nerve recording sites. Such response latencies indicate a polysynaptic pathway for which there is much anatomic and electrophysiological evidence. Inhibition at shorter latencies was never detected in the present study. Latencies in the order of ~25 ms have been previously reported in the cat (12, 26). The cat ADN contains not only baroreceptive but also nociceptive and chemoreceptive fibers (19), which may account for the short latency inhibition detected. Furthermore, short latency responses indicate a monosynaptic pathway to the SPN for which there is no anatomic evidence. Direct inhibition of SPN is difficult to demonstrate and can rarely be recorded either during spontaneous or evoked activity (9, 27). However, two cases of baroreceptor-evoked inhibition of SPN have been reported (10, 27), but only a single example (from a very small sample of neurons) has been published (27). Even in this example, baroreceptor activation led
to a gradual hyperpolarization of the SPN rather than distinct inhibitory postsynaptic potentials. Thus taken together, the evidence for direct spinal inhibition playing a role in baroreceptor-mediated inhibition is weak.

On the other hand, Coote and Lewis (6) and Lewis and Coote (21) suggest that direct inhibition at the level of the spinal cord plays a very significant role in mediating the baroreceptor-evoked inhibition of a spina

elically elicited refex. They suggest that both GABA_A and glycine receptors mediate this inhibition. These data are completely at odds with the findings presented here. We used several doses of bicuculline, including the dose used by Lewis and Coote (21), so that the results cannot be explained merely by concentration differences. Several differences between our study and the work of Coote and Lewis (6) and Lewis and Coote (21) should be noted. They determined the effect of indirect baroreceptor stimulation on a spinally evoked response. Stimulation of the dorsolateral funiculus of the cervical spinal cord activates a whole gamut of inhibitory and excitatory, ascending and descending pathways whose summed effects may be excitatory to the renal nerve. No examples of nerve responses after bicuculline injection are actually presented for analysis. A further difference is that they evoked a baroreceptor response with the use of very large doses of phenylephrine that are likely to be much less specific than ADN stimulation.

In conclusion, the results of the present study suggest that spinal GABA_A receptors play little role in baroreceptor-mediated sympathoinhibition in the anesthetized rat. However, this statement is made cautiously. Pathways activated by carotid sinus baroreceptors may differ from those activated by aortic arch baroreceptors. Similarly, the contribution of spinal GABA_A receptors to baroreceptor-mediated sympathoinhibition may be unmasked by removal of the normal disfacilitatory mechanisms of SPN that originate in the brain stem. Finally, results of the present study show that there is a large tonically active GABAAergic input to the SPN. Further investigation into the origin and role of these inhibitory pathways is essential.

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