Norepinephrine Facilitates Inhibitory Transmission in Substantia Gelatinosa of Adult Rat Spinal Cord (Part 1)

Effects on Axon Terminals of GABAergic and Glycinergic Neurons

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Background: The activation of descending norepinephrine-containing fibers from the brainstem inhibits nociceptive transmission at the spinal level. How these descending noradrenergic pathways exert the analgesic effect is not understood fully. Membrane hyperpolarization of substantia gelatinosa (Rexed lamina II) neurons by the activation of α2 receptors may account for depression of pain transmission. In addition, it is possible that norepinephrine affects transmitter release in the substantia gelatinosa.

Methods: Adult male Sprague-Dawley rats (9–10 weeks of age, 250–300 g) were used in this study. Transverse spinal cord slices were cut from the isolated lumbar cord. The blind whole-cell patch-clamp technique was used to record from neurons. The effects of norepinephrine on the frequency and amplitude of miniature excitatory and inhibitory postsynaptic currents were evaluated.

Results: In the majority of substantia gelatinosa neurons tested, norepinephrine (10–100 μM) dose-dependently increased the frequency of γ-aminobutyric acid (GABA)-ergic and glycine sexually inhibitory postsynaptic currents; miniature excitatory postsynaptic currents were unaffected. This augmentation was mimicked by an α1-receptor agonist, phenylephrine (10–60 μM), and inhibited by α1-receptor antagonists prazosin (0.5 μM) and 2,6-dimethoxyphenylethyl) aminoethyl-1,4-benzodioxane (0.5 μM). Neither postsynaptic responsiveness to exogenously applied GABA and glycine nor the kinetics of GABAergic and glycinergic inhibitory postsynaptic currents were affected by norepinephrine.

Conclusion: These results suggest that norepinephrine enhances inhibitory synaptic transmission in the substantia gelatinosa through activation of presynaptic α2 receptors, thus providing a mechanism underlying the clinical use of α2 agonists with local anesthetics in spinal anesthesia. (Key words: Antinociception; blind patch-clamp recording; descending pain control system; inhibitory transmission; in vitro; transmitter release.)

The sensation of pain is carried to the central nervous system by fine, myelinated (Aβ) and unmyelinated (C) fibers. These fibers terminate in the superficial layers of the spinal cord, particularly the substantia gelatinosa (SG, Rexed lamina II), a region critical for modulating nociceptive information and controlling the activity of projection neurons.1,2 Anatomic studies3,4 show a high concentration of norepinephrine-containing terminals in the superficial laminae of the spinal cord. Activation of these noradrenergic fibers, which originate in the brainstem, can inhibit the transmission of nociceptive signals.

Epinephrine and phenylephrine are administered commonly in combination with local anesthetics in spinal anesthesia and have been shown to prolong the duration of analgesia.3,5 Conventional wisdom has suggested that the beneficial effects of epinephrine and phenylephrine result from local vasoconstriction and a consequent reduction in drug clearance from the subarachnoid space. However, these vasoconstrictors produce analgesia even if administered intrathecally in the absence of local anesthetics,5,6 and they do not appear to significantly alter the clearance of local anesthetics from the subarachnoid space.
In addition, clonidine, an $\alpha_2$ agonist without vasoconstrictive effects, prolongs the duration of analgesia in spinal anesthesia.\(^9\) Thus, it is likely that intrathecal epinephrine and phenylephrine enhance spinal anesthesia via direct actions within the spinal dorsal horn, possibly mimicking the action of descending noradrenergic pathways. However, how descending noradrenergic pathways, and intrathecally administered epinephrine and phenylephrine, inhibit pain transmission at the cellular level is not fully understood. It has been reported that $\alpha_2$ receptors are concentrated in the SG. Membrane hyperpolarization of SG neurons by norepinephrine acting on $\alpha_2$-receptors\(^5\) may account for depression of pain transmission, but it is also possible that norepinephrine affects transmitter release in the SG. The purpose of this study was to determine whether spinally administered norepinephrine acts presynaptically to alter excitatory and inhibitory synaptic transmission and, if so, to identify which receptor subtype is involved. To address this question, we used the blind patch-clamp technique to study the action of norepinephrine on miniature excitatory and inhibitory postsynaptic currents (mEPSCs and mIPSCs) in SG neurons from adult rat spinal cord slices.

**Materials and Methods**

**Spinal Cord Slice Preparation**

This study was approved by the Animal Care and Use Committee at Niigata University School of Medicine. A portion of the lumbosacral spinal cord (2.0–2.5 cm) was removed from an adult rat (9–10 weeks of age, 250–300 g) during urethane anesthesia (1.5–2.0 g/kg, intraperitoneal). The isolated spinal cord was then placed in preoxygenated ice-cold Krebs solution (2–4°C). After removal of the dura mater, all ventral and dorsal roots were cut and the pia-arachnoid membrane was removed. The spinal cord was placed in a shallow groove formed in an agar block and glued to the bottom of the microslicer stage with cyanoacrylate adhesive. Under a dissecting microscope with transmitted illumination, the SG was clearly discernible as a relatively translucent band across the dorsal horn. However, the contour of individual SG neurons cannot be visualized under these conditions; therefore, gigaohm sealing (attaching electrode to the cell with a resistance of at least 1 GΩ) was performed blindly.\(^4\) Patch pipettes were fabricated from thin-walled, borosilicate, glass-capillary tubing (1.5 mm OD; World Precision Instruments, Sarasota, FL). After establishing the whole-cell configuration, voltage-clamped neurons were held at either −70 or 0 mV for recording mEPSCs and mIPSCs, respectively (fig. 1B). The reversal potentials of EPSCs and IPSCs in SG neurons are 0 and −70 mV, respec...
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Fig. 2. Miniature postsynaptic currents recorded from substantia gelatinosa neurons. (A) At a holding potential of \(-70\) mV, only mEPSCs were recorded as downward deflections in the membrane current trace. (B) At a holding potential of \(-30\) mV, both mEPSCs and mIPSCs were recorded as downward and upward deflections, respectively. mIPSCs are indicated by arrowheads. (C) At a holding potential of 0 mV, only mIPSCs were recorded as upward deflections. Two distinct types of mIPSCs (GABAergic and glycinergic) could be distinguished according to their decay time courses. Note that GABAergic mIPSCs (indicated by arrowheads) have longer duration than glycine centered mIPSCs. (D) Norepinephrine (NE, 10\(\mu\)M) was applied just after establishing the whole-cell configuration. Norepinephrine did not increase the frequency of mEPSCs recorded at \(-70\) mV (top), but significantly increased the frequency of mIPSCs (bottom). Baseline currents of both traces were elevated by the activation of \(\alpha_1\) receptors. Top and bottom traces were obtained from two different neurons. (E) The effect of norepinephrine (20 and 100 \(\mu\)M) on the frequency of mEPSCs. Norepinephrine did not change or slightly decreased the frequency of mEPSCs \((P = 0.08\) for norepinephrine 20 \(\mu\)M, \(n = 17\); \(P = 0.09\) for norepinephrine 100 \(\mu\)M, \(n = 8\), paired \(t\) test). (F) The effect of norepinephrine (20 \(\mu\)M) on the frequency of mIPSCs. Norepinephrine markedly increased the frequency of mIPSCs \((P < 0.0001\), \(n = 18\), paired \(t\) test).

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without altering the perfusion rate and the temperature. The time necessary for the drug-containing solution to flow from the three-way stopcock to the recording chamber was approximately 3 s. Drugs used were norepinephrine (WAKO, Osaka, Japan), 6-cyano-7-nitroquinoxaline-2,3-dione (Tocris Cookson, Ballwin, MO), strychnine (Sigma, St. Louis, MO), bicuculline (Sigma), tetrodotoxin (WAKO), GDP-β-S (Sigma), chloroethylclonidine (RBI, Natick, MA), prazosin (Sigma), clonidine (Sigma), phenylephrine (Sigma), isoproterenol (Sigma), yohimbine (WAKO), 2-(2,6-dimethoxyphenoxoxyethyl)aminomethyl-1,4benzodioxane (WB-4101; Sigma), γ-aminobutyric acid (GABA; WAKO), glycine (WAKO), and propranolol (Sigma).

**Analogue of Frequency and Amplitude of Miniature Postsynaptic Currents**

The elementary unit of neurotransmitter release is the content of a single synaptic vesicle. The amplitude of an evoked EPSC or IPSC is some multiple of the postsynaptic current in response to the transmitter content of a single vesicle. The notion of quantal transmission originally was derived from synaptic release at the neuromuscular junction, and considerable evidence has accumulated that also supports the quantal hypothesis at central synapses. At many synapses, exocytosis of synaptic vesicles occurs spontaneously at a low rate, even in the absence of presynaptic stimulation. In the presence of tetrodotoxin, postsynaptic responses to spontaneously released transmitter can be detected as relatively small amplitude miniature postsynaptic currents (mPSCs) (fig. 1B).

The strength (efficacy) of synaptic transmission can be altered through modulation of both "transmitter release probability" and "postsynaptic responsiveness." Analysis of frequency and amplitude distributions of mPSCs has been used to distinguish between pre- and postsynaptic loci of experimental manipulations, including volatile anesthetics. From the quantal hypothesis, only presynaptic actions can affect the probability of release. Thus, changes in the frequency of mPSCs indicate a postsynaptic effect, if recruitment of latent (silent) receptors can be ruled out. In this study, we used GDP-β-S in the intrapipette solution to eliminate possible postsynaptic effects (changes in postsynaptic responsiveness or recruitment of silent synapses) by norepinephrine; therefore, changes in the frequency of mPSCs can be attributed only to a presynaptic effect of norepinephrine. Alterations in mPSC peak amplitude can be explained only by changes in postsynaptic responsiveness. The mean (or median) amplitude can also be altered by changes in postsynaptic responsiveness. However, the shape of the amplitude distribution can be skewed by the existence of synapses that are unaffected by the treatment, resulting only in an apparent change in the mean (median) amplitude.

**Statistical Analysis**

Numeric data are presented as the mean ± SD (unless otherwise stated). Analyses of the modulation of the frequency of mPSCs were performed using a paired t test. The effects of selective agonists and antagonists were analyzed using the one-way analysis of variance, and statistical significance was further evaluated using the Scheffé test for post hoc comparison. The Kolmogorov-Smirnov test was used to compare the effect of norepinephrine on the amplitude distribution of postsynaptic currents. Differences for which P < 0.05 were considered significant and are indicated by asterisks in the figures. Dose-response data were fitted using logistic equations. Curve fitting was accomplished using Origin 4.1 software (Microcal Software, Northampton, MA).

**Results**

Whole-cell patch-clamp recordings were made from 109 SG neurons. In the presence of tetrodotoxin (1 μM), all SG neurons tested exhibited mEPSCs and mIPSCs. At holding potentials of −70 mV, only mEPSCs were observed (figs. 2A and D; top). At holding potentials more positive than −60 mV, mEPSCs and mIPSCs both were observed (fig. 2B). However, at 0 mV, only mIPSCs could be observed (fig. 2C). mEPSCs observed at −70 mV were completely blocked by 6-cyano-7-nitroquinoxaline-2,3-dione, suggesting that they were mediated by non-NMDA (AMPN/kainate) receptors. Two distinct types of mIPSCs could be distinguished based on the decay time course (fig. 2C). One type of mIPSC had a short duration (10–20 ms) and was antagonized by a glycine receptor antagonist: strychnine (1–2 μM). The other had a relatively long duration (50–100 ms) and was antagonized by a GABAA receptor antagonist, bicuculline (10 μM), suggesting that the two types of mIPSCs were mediated by glycine and GABAA receptors, respectively. Shortly after establishing the whole-cell patch-clamp configuration, norepinephrine elicited outward currents at −70 mV in the majority of SG neurons (18 of 24 cells; fig. 2D), in agreement with previous reports. This effect was mimicked by clonidine (10 μM) and...
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Fig. 3. The effect of norepinephrine on mIPSC frequency was mediated by $\alpha_1$ receptors. (A) Clonidine (10 $\mu$m) and isoproterenol (40 $\mu$m) failed to increase the frequency of mIPSCs; norepinephrine (NE, 10 $\mu$m) increased mIPSC frequency. The data were obtained from the same neuron as in figure 2D (bottom), but 15 min after establishing whole-cell configuration. Note that $\alpha_2$-receptor-mediated baseline elevation (outward current) could no longer be elicited by clonidine or norepinephrine when diffusion of GDP-$\beta$S was complete. (B) The effects of $\alpha_1$, $\alpha_2$, and $\beta$ agonists on the frequency of mIPSCs. The frequency of mIPSCs was significantly increased by phenylephrine (10 $\mu$m, n = 5) but not by clonidine (10 $\mu$m, n = 6) or isoproterenol (40 $\mu$m, n = 6). *P < 0.01 compared with clonidine and isoproterenol groups, one-way analysis of variance. (C) The effects of $\alpha_1$, $\alpha_2$, and $\beta$ antagonists on the facilitatory effect of norepinephrine. The effect of norepinephrine (20 $\mu$m) was significantly decreased by the $\alpha_1$-receptor antagonist prazosin (0.5 $\mu$m, n = 5) and WB-4101 (0.5 $\mu$m, n = 6). An $\alpha_2$ antagonist, yohimbine (1 $\mu$m, n = 6), and a $\beta$ antagonist, propranolol (1 $\mu$m, n = 5), failed to block the norepinephrine effect. The $\alpha_{1\alpha}$- and $\alpha_{1\beta}$-receptor antagonist chloroethylclonidine (10 $\mu$m, n = 6) was also without effect. *P < 0.005 compared with control group (norepinephrine 20 $\mu$m, n = 18); one-way analysis of variance.

Norepinephrine Increases the Frequency of mIPSCs through Activation of $\alpha_1$ Receptors

In 26 SG neurons, membrane potentials were clamped to $-70$ mV, and the effect of norepinephrine on the frequency of mIPSCs was evaluated. The baseline frequency of mEPSCs was 20.9 $\pm$ 15.2 Hz (n = 26; range, 3.7-64.5 Hz). The frequency of mEPSCs was not significantly affected by norepinephrine (10-100 $\mu$m, 92 $\pm$ 19% of control for norepinephrine 20 $\mu$m; P = 0.08, n = 17, paired t test; figs. 2D and E). In sharp contrast, norepinephrine markedly increased the frequency of mIPSCs (figs. 2D and F). The baseline frequency of mIPSCs was 2.9 $\pm$ 1.5 Hz (n = 59; range, 0.5-6.2 Hz). Norepinephrine (10-100 $\mu$m) increased the frequency of mIPSCs in 55 of 59 SG neurons tested (459 $\pm$ 167% of control for norepinephrine 20 $\mu$m; P < 0.0001, n = 18; paired t test; fig. 2F). In 8 of 18 cells in which norepinephrine elicited outward currents at $-70$ mV, the membrane potentials were also clamped at 0 mV, and the effect of norepinephrine on mIPSCs was evaluated. In all eight cells, norepinephrine elicited an increase in the frequency of mIPSCs. The effect of norepinephrine on mIPSC frequency reached a steady state within 2 min and showed no evidence of desensitization over at least 30 min. After norepinephrine washout, IPSC frequency slowly returned to baseline (recovery time was usually > 5 min).

The effect of norepinephrine was mimicked by the $\alpha_1$ agonist phenylephrine (10-60 $\mu$m; n = 11), but not by the $\alpha_2$ agonist clonidine (10-40 $\mu$m; n = 10) or the $\beta$ agonist isoproterenol (40 $\mu$m; n = 6) (fig. 3B). The $\alpha_1$-receptor antagonists prazosin (0.5 $\mu$m; n = 5) and WB-4101 (0.5 $\mu$m; n = 6) reversibly antagonized the norepinephrine effect; the $\alpha_{1\beta}$ and $\alpha_{1\beta}$ antagonist chloroethylclonidine (10 $\mu$m; n = 6) had no significant effect (fig. 3C). In addition, the $\alpha_2$- and $\beta$-receptor antagonists yohimbine (1 $\mu$m; n = 6) and propranolol (1 $\mu$m; n = 5), respectively, were without effect (fig. 3C).

Norepinephrine Facilitates Glycinergic and GABAergic mIPSCs

Because mIPSCs consist of glycinergic and GABAergic components, we evaluated which type of mIPSCs were

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facilitated by norepinephrine. In the presence of strychnine (2 \( \mu \)M), norepinephrine increased the remaining mIPSCs (\( n = 12 \)). These mIPSCs were completely abolished by the simultaneous application of strychnine (2 \( \mu \)M) and bicuculline (10 \( \mu \)M), confirming that facilitated mIPSCs were GABAergic (fig. 4A). Conversely, mIPSCs recorded in the presence of bicuculline were facilitated by norepinephrine (\( n = 10 \); fig. 4B). Thus, norepinephrine increased the frequency of both GABAergic and glycineuric mIPSCs.

Facilitation of GABAergic and glycineuric mIPSC frequency by norepinephrine was concentration dependent (fig. 5). Concentration-response curves for GABAergic (\( n = 7 \)) and glycineuric (\( n = 8 \)) mIPSCs were well-fitted by logistic equations. The estimated EC\(_{50}\) values for the effect of norepinephrine on GABAergic (29.5 \( \pm \) 12.2 \( \mu \)M) and glycineuric (38.8 \( \pm \) 37.3 \( \mu \)M) mIPSCs were similar, as were the Hill coefficients (GABAergic: 2.8 \( \pm \) 1.4; glycineuric: 2.8 \( \pm \) 1.5). Norepinephrine facilitates GABAergic mIPSCs with an approximately twofold greater efficacy than for glycineuric mIPSCs (GABAergic: 1.094 \( \pm \) 342\%; glycineuric: 541 \( \pm \) 403\%).

Amplitude histograms were constructed for each set of GABAergic and glycineuric mIPSCs. In all cells tested, amplitude distribution analyses showed a slight but statistically significant norepinephrine-induced increase in the median amplitude of GABAergic (\( n = 7 \)) and glycineuric (\( n = 5 \)) mIPSCs (fig. 6). However, the peak amplitude of both types of mIPSCs was not increased by norepinephrine. The effect of norepinephrine was particularly prominent between 10 and 15 \( \mu \)A for GABAergic and glycineuric mIPSCs. This change in the amplitude distribution is clearly shown in the cumulative histogram (figs. 6B and D). In the presence of norepinephrine, the relative frequency curves were significantly shifted to the right in all cells tested.

**Norepinephrine Does Not Affect Postsynaptic Responsiveness to GABA and Glycine**

The norepinephrine-induced changes in amplitude distributions might be attributable to an increase in postsynaptic sensitivity to GABA and glycine under conditions in which GDP-\( \beta \)-S diffusion was incomplete. Alternatively, an unknown mechanism not mediated by G protein might mediate a postsynaptic effect of norepinephrine. To rule out these possibilities, we evaluated whether norepinephrine affected postsynaptic sensitivity to exogenously applied GABA and glycine under our recording conditions (i.e., with an internal solution con-
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Control

Concentration of NE (μM)

20 μM

60 μM

Fig. 5. Norepinephrine increased the frequency of mIPSCs in a concentration-dependent manner. (A) An example of GABAergic mIPSCs and the effects of increasing concentrations of norepinephrine. (B) The concentration–response curves of GABAergic (n = 7) and glycineric (n = 8) mIPSCs. Concentration–response data were fit by logistic equations. GABAergic and glycineric curves had similar EC50 values (GABAergic: 29.5 ± 12.2 μM; glycineric: 38.8 ± 37.3 μM) and Hill coefficients (GABAergic: 2.8 ± 1.4; glycineric: 2.8 ± 1.5). The efficacy of the norepinephrine effect was greater for GABAergic (1,094 ± 342%) than glycineric (541 ± 403%) mIPSCs. P < 0.05, unpaired t test.

Discussion

Increase in the frequency of mIPSCs

We showed that norepinephrine increases the frequency of mIPSCs in the majority (> 90%) of SG neurons tested, without any significant effect on postsynaptic responsiveness. In contrast, norepinephrine did not change or slightly decreased the frequency of mEPSCs. These data indicate that norepinephrine selectively facilitates the quantal release of inhibitory transmitters from presynaptic terminals of inhibitory interneurons. This study shows that norepinephrine increases the frequency of both GABAergic and glycineric mIPSCs. This may not be surprising in light of the observation that GABA and glycine can be colocalized, at least in a subpopulation of dorsal horn neurons, and cotransmission of GABA and glycine can occur.28 However, norepinephrine facilitated GABAergic mIPSCs with greater efficacy than glycineric mIPSCs. The reason for this difference has yet to be determined, but it may result from a differential distribution of α1 receptors on GABAergic and glycineric terminals.

The facilitatory effect of norepinephrine was mediated via adrenergic α1 receptors, because phenylephrine (an α1-receptor agonist) mimicked norepinephrine, and prazosin and WB-4101 (α1-receptor antagonists) inhibited the norepinephrine effect. Thus, GABA- and glycine-containing neurons are endowed with α1 receptors on the axon terminals, and activation of these receptors enhances GABAergic and glycineric inhibitory transmission in the SG. Currently, at least three native α1-adrenergic receptor subtypes (α1A, α1B, and α1D) have been identified pharmacologically (α1C is lacking).29 Both α1B and α1D receptors are characterized by a high sensitivity to chloroethylclonidine. The action of norepinephrine on mIPSC frequency was highly sensitive to WB-4101 (an α1A-receptor antagonist), but resistant to a relatively high concentration (10 μM) of chloroethylclonidine (an α1B and α1D-receptor antagonist). Therefore, the most likely α1-adrenergic receptor responsible for norepinephrine action is the α1A subtype.
The norepinephrine-induced increase in mIPSC frequency could be secondary to changes in the postsynaptic neuron. If norepinephrine produces an increase in the sensitivity of postsynaptic GABA_ and glycine receptors, it could potentiate a population of subliminal mIPSCs, such that they would become visible, yielding an apparent increase in mIPSC frequency. Furthermore, if norepinephrine results in the recruitment of "silent" inhibitory synapses in a manner analogous to that described for excitatory synapses^{23,30,51} the apparent mIPSC frequency would increase. It is unlikely that these types of postsynaptic changes mediate the facilitatory effects of norepinephrine because all known adrenergic receptors are coupled to ion channels via G proteins, and G proteins were inhibited in postsynaptic (recorded) neurons by GDP-β-S. In addition, the amplitude of outward currents elicited by exogenously applied GABA and glycine was not altered by norepinephrine, nor were kinetics of mIPSCs. Together, these observations indicate that norepinephrine does not alter postsynaptic responsiveness to GABA and glycine. Therefore, it is surprising that the amplitude-distribution analysis showed a significant norepinephrine-induced increase in the median amplitude of GABAergic and glycinergic mIPSCs and a shift in the cumulative histogram curves to the right. This most likely results, however, from a relatively selective increase in a subpopulation of mIPSCs with amplitudes between 10 and 15 pA (figs. 6A and C). SG neurons receive numerous types of inhibitory inputs from interneurons in the SG and the surrounding laminae (laminae I, III, IV, and so forth).^{32} The effect of norepinephrine may be selective for a subpopulation of these inputs. If norepinephrine facilitates quantal release, especially in a population of inhibitory synapses...
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Fig. 7. Norepinephrine does not affect postsynaptic responsiveness to GABA and glycine. (A, B) Exogenously applied GABA (500 $\mu$M) and glycine (200 $\mu$M) elicited outward currents. The peak amplitude of the currents evoked by GABA or glycine were virtually identical before and during application of norepinephrine (NE, 20 $\mu$M). (C) Norepinephrine did not significantly affect the peak outward currents elicited by GABA (500 $\mu$M, $n = 6$) or glycine (200 $\mu$M, $n = 6$). Open bars and solid bars represent control (C) and norepinephrine (20 $\mu$M), respectively. (D) The kinetics of GABAergic (top) and glycine (bottom) mIPSCs before and during norepinephrine application were nearly identical, as shown by superimposed records. mIPSCs from each group were averaged and scaled to equal amplitudes. mIPSCs were averaged from the same cells as shown in figure 6.

that generate mIPSCs with an amplitude of 10–15 pA, the amplitude distribution could be skewed. Although norepinephrine facilitates inhibitory transmitter release via presynaptic $\alpha_1$ receptors, it may not influence all presynaptic terminals of inhibitory interneurons in the SG in a homogeneous fashion. Alternatively, temporal summation of mIPSCs might result in an increase in the median amplitude of mIPSCs. We measured the amplitude of mIPSCs from the initial inflection point (not from the baseline) to avoid effects of temporal summation on the amplitude distribution. However, in cases in which two (or more) quantal events occur at exactly the same moment in time, they cannot be distinguished; therefore, they may have been measured as a single mIPSC. This type of error is more likely to occur during conditions in which the frequency of mIPSCs is increased.
Nonetheless, we believe this to be rare because carbachol, a muscarinic agonist, also increased the frequency of mIPSCs but did not affect the amplitude distribution. 

**Functional Consideration**

Almost all norepinephrine-containing terminals in the dorsal horn of the spinal cord are supraspinal in origin. Currently, our knowledge of how these descending pain-control pathways inhibit nociceptive transmission at the spinal level is not defined clearly, but there are several possible mechanisms. First, activation of noradrenergic descending systems releases norepinephrine, which can directly hyperpolarize a proportion of SG neurons that may be excitatory interneurons in the pain pathway (postsynaptic inhibition). Second, norepinephrine can inhibit excitatory transmitter (glutamate, substance P, calcitonin gene-related peptide [CGRP], and so forth) release from primary afferent terminals or presynaptic terminals of excitatory interneurons. However, our data do not support this type of mechanism in the SG. Third, norepinephrine could depolarize inhibitory interneurons that contain GABA, glycine, or other inhibitory peptides. Iontophoretic application of norepinephrine near nociceptive dorsal horn neurons generally inhibits background activity of these cells and the responsiveness to excitatory amino acids. This inhibition most likely results from α2-receptor activation, which increases K+ conductance, thereby evoking a membrane hyperpolarization. However, norepinephrine (and brainstem stimulation) also has been reported to produce excitatory effects. The neurons excited by iontophoretically applied norepinephrine and electrical stimulation of the periaqueductal gray were low-threshold cells, possibly inhibitory interneurons that synapse onto high-threshold and wide-dynamic-range neurons. In the accompanying article, we directly address whether norepinephrine depolarizes inhibitory interneurons that synapse onto SG neurons.

Here, we describe a distinct type of mechanism: norepinephrine acts at pre- and postsynaptic axon terminals of GABAergic and glycinergic interneurons to facilitate inhibitory transmitter release. We also demonstrated that some SG neurons display both an α2-receptor-mediated outward current and an α1-receptor-mediated increase in mIPSC frequency (fig. 2D). Because 80% of SG neurons are hyperpolarized (or display an outward current) via α2-receptor activation, and more than 90% show an increase in mIPSC frequency (current study), it is likely that the excitability of most SG neurons is inhibited by exogeneously applied norepinephrine (and probably by epinephrine) through α2- and α1-receptor-mediated mechanisms. Finally, it should be noted that norepinephrine may change postsynaptic sensitivity to GABA and glycine. In the current study, we blocked postsynaptic effects of norepinephrine using GDP-β-S to evaluate the presynaptic action. However, in intact SG neurons, norepinephrine may increase postsynaptic responsiveness to inhibitory neurotransmitters by G protein-coupled mechanisms.

γ-Aminobutyric acid and glycine have been shown to be present in cell bodies and terminals in the superficial dorsal horn and are thought to be involved in spinal antinociception. Electrical stimulation of primary afferent fibers evokes mono- and polysynaptic excitatory postsynaptic potentials (EPSPs) that are then augmented in both amplitude and duration by perfusion with GABA and glycine receptor agonists. In addition, intrathecal administration of GABA and glycine receptor agonists or glycine is antinociceptive, and antagonists of GABA and glycine receptors produce allodynia and hyperalgesia in animals. These observations suggest that GABA- and glycine-containing interneurons are responsible for discontinuing evoked excitatory responses. The facilitatory effect of norepinephrine on inhibitory interneurons may decrease excitability of dorsal horn neurons, which in turn would increase the threshold for transmission of noxious information.

As reviewed by Willis and Coggshall, SG neurons receive substantial primary afferent input from nociceptive Aδ and C fibers (these fibers can, however, also form synapses directly onto projection neurons). In addition to nociceptive inputs, the SG receives descending adrenergic input from a number of pontine nuclei. With respect to output from this region, the majority of SG neurons are local interneurons and do not project to the thalamus, although there are a few exceptions. The main projections of SG neurons are to lamina I and to deep dorsal horn neurons, with cell bodies in laminae IV and V, where the projection neurons to the thalamus are located. Unfortunately, in the current study, the phenotype of the SG neurons from which we recorded is unknown; some SG neurons might produce excitatory, and others inhibitory, effects on projection neurons in laminae IV and V. In the companion article, we address whether α1-receptor activation inhibits transmission of nociceptive inputs to deep dorsal horn neurons.

Although the vasoconstrictive effects of epinephrine and phenylephrine in the spinal cord have not been...
established, these drugs are sometimes added to local anesthetics in spinal anesthesia to prolong the duration of analgesia.\textsuperscript{5,6} It has been reported that subarachnoid epinephrine and phenylephrine do not affect spinal cord blood flow.\textsuperscript{19,50} Another study suggested that at a relatively high dose, phenylephrine decreased blood flow.\textsuperscript{31} Furthermore, it has been reported that epinephrine and phenylephrine do not significantly affect the clearance of local anesthetics from the subarachnoid space.\textsuperscript{9,10}

Thus, it remains controversial whether the vasoconstrictive actions of epinephrine or phenylephrine contribute to the prolongation of analgesia in spinal anesthesia. The mechanism of epinephrine action in spinal anesthesia can be accounted for, at least in part, by activation of $\alpha_2$ receptors.\textsuperscript{7,8,11} As discussed previously, exogenously applied epinephrine (a mixed $\alpha_1$ and $\alpha_2$ agonist) can decrease the excitability of SG neurons via $\alpha_1$ and $\alpha_2$ receptors. Interestingly, phenylephrine (a pure $\alpha_1$ agonist) can also enhance spinal anesthesia. Considering the evidence that phenylephrine does not affect the clearance of local anesthetics, the action of phenylephrine in spinal anesthesia must be caused by a direct action on spinal neurons via $\alpha_1$ receptors. The presynaptic $\alpha_1$ action reported here may account for the prolongation of analgesia by $\alpha_1$ agonists in spinal anesthesia.

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