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Norepinephrine Facilitates Inhibitory Transmission in Substantia Gelatinosa of Adult Rat Spinal Cord (Part 2)

Effects on Somatodendritic Sites of GABAergic Neurons

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Background: **It has been reported previously that norepinephrine, when applied to the spinal cord dorsal horn, excites a subpopulation of dorsal horn neurons, presumably inhibitory interneurons. In the current study, the authors tested whether norepinephrine could activate inhibitory interneurons, specifically those that are "GABAergic."**

Methods: **A transverse slice was obtained from a segment of the lumbar spinal cord isolated from adult male Sprague-Dawley rats. Whole-cell patch-clamp recordings were made from substantia gelatinosa neurons using the blind patch-clamp technique. The effects of norepinephrine on spontaneous GABAergic inhibitory postsynaptic currents were studied.**

Results: **In the majority of substantia gelatinosa neurons tested, norepinephrine (10–60 μM) significantly increased both the frequency and the amplitude of GABAergic inhibitory postsynaptic currents. These increases were blocked by tetrodotoxin (1 μM). The effects of norepinephrine were mimicked by the α_1-receptor agonist phenylephrine (10–80 μM) and inhibited by the α_1-receptor antagonist WB-4101 (0.5 μM). Primary-afferent–evoked polysynaptic excitatory postsynaptic potentials or excitatory postsynaptic currents in wide-dynamic-range neurons of the deep dorsal horn were also attenuated by phenylephrine (40 μM).**

Conclusion: **The observations suggest that GABAergic interneurons possess somatodendritic α_1 receptors, and activation of these receptors excites inhibitory interneurons. The α_1 actions reported herein may contribute to the analgesic action of intrathecally administered phenylephrine. (Key words: Antinociception; blind patch; clamp recording; intracellular recording; IPSC.)**

IN the companion article by Baba *et al.*,[1] we demonstrated that norepinephrine increases the release of both γ-aminobutyric acid (GABA) and glycine through presynaptic mechanisms, without influencing postsynaptic GABA or glycine receptor sensitivity. To extend these observations, we performed an additional series of experiments to evaluate whether norepinephrine acts at somatic or dendritic sites to directly depolarize "GABAergic" interneurons. Here we report that norepinephrine activates α_1 receptors on either the soma or the dendrites of GABAergic neurons to elicit tetrodotoxin-sensitive, large-amplitude inhibitory postsynaptic currents (IPSCs) in substantia gelatinosa (SG) neurons. Norepinephrine acts through α_1 receptors located on presynaptic nerve terminals and on the soma or dendrites of GABAergic interneurons to suppress the activity of wide-dynamic-range (WDR) neurons in the deep dorsal horn.

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Materials and Methods

Spinal Cord Slice Preparation and Patch-clamp Recording from Substantia Gelatinosa Neurons

This study was approved by the Animal Care and Use Committee at the Niigata University School of Medicine. Methods for preparing adult rat spinal cord slices and patch-clamp recording from SG neurons are described in detail in the companion article.[1] In some experiments, a dorsal root (L4) was preserved to permit stimulation of primary-afferent fibers. Unless otherwise indicated, GABAergic IPSCs were recorded in the presence of DL-2-amino-5-phosphonovarelic acid (APV, 25 μM), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM), and strychnine (1 or 2 μM), to block *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and glycine receptors, respectively.

Intracellular and Patch-clamp Recording from Deep Dorsal Horn Neurons

Conventional intracellular "sharp" electrode recordings were made from neurons located in lamina IV–V using an Axoclamp 2A (Axon Instruments, Foster City, CA). The resistance of a typical sharp electrode was 150–200 MΩ when filled with 4 M potassium acetate. In some cases, blind patch-clamp recordings were also made from lamina IV–V neurons using an Axopatch 200A amplifier (Axon Instruments). The internal solution contained 135 mM potassium gluconate, 5 mM KCl, 0.5 mM $CaCl_2$, 2 mM $MgCl_2$, 5 mM EGTA, 5 mM HEPES, 5 mM adenosine triphosphate magnesium salt, and 0.5 mM guanosine triphosphate sodium salt. The resistance of a typical patch pipette was 5–10 MΩ. Neurons were voltage-clamped to −70 mV for recording excitatory postsynaptic currents (EPSCs). Dorsal roots were stimulated using a suction electrode.[2] Minimum stimulus intensities necessary to activate Aα or β (10 μA, 0.05 ms), Aδ (30 μA, 0.05 ms) and C (200 μA, 0.5 ms) fibers were determined previously by extracellular recording of compound action potentials from the dorsal root near the dorsal root entry zone.[1] Signals were filtered at 2 kHz and digitized at 5 kHz.

Drug Application

Drugs were applied by exchanging the perfusion solution with a solution that contained a known drug concentration, without altering the perfusion rate or the temperature. All drugs were from Sigma (St. Louis, MO) unless otherwise specified. The following drugs were used: norepinephrine (WAKO, Osaka, Japan), CNQX (Tocris Cookson, Ballwin, MO), APV, strychnine, bicuculline, tetrodotoxin (WAKO), 2-(2,6-Dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride (WB-4101), propranolol, yohimbine (WAKO), phenylephrine, clonidine, and isoproterenol.

Statistical Analysis

Numerical data are presented as the mean ± SD (unless otherwise stated). Modulation of the frequency of postsynaptic currents was analyzed using the Student paired *t* test. The effects of tetrodotoxin and selective antagonists were analyzed using analysis of variance, with the Scheffé test for *post hoc* comparison. The Kolmogorov-Smirnov test was used to compare the effect of norepinephrine on IPSC amplitude distributions. $P < 0.05$ was considered significant and is indicated by an asterisk in the figures.

Results

Norepinephrine Increases the Frequency and Amplitude of Spontaneous Inhibitory Postsynaptic Currents

As shown in figure 1, in the absence of tetrodotoxin, norepinephrine markedly increased the frequency of spontaneous GABAergic IPSCs. This effect of norepinephrine is distinct from the effect on miniature IPSCs that is observed in the presence of tetrodotoxin.[1] In the absence of tetrodotoxin, norepinephrine elicited repetitive large-amplitude IPSCs that were not present in the control state. The baseline frequency of IPSCs was 6.5 ± 3.1 Hz (n = 32; range 2.2–15.6 Hz). Norepinephrine (10–60 μM) increased the frequency of IPSCs in 46 of 51 SG neurons tested (587 ± 212% of control for norepinephrine 20 μM; n = 12; $P < 0.0001$; paired *t* test). For the neuron in figure 1, there was a persistent elevation in the baseline current after norepinephrine application that most likely reflects IPSC summation because it was tetrodotoxin-sensitive (data not shown). Norepinephrine-induced IPSCs were $GABA_A$ receptor-mediated because they were eliminated by bicuculline (20 μM; n = 5; fig. 2A). Norepinephrine-induced large-amplitude IPSCs also were eliminated by tetrodotoxin (fig. 2B); however, the increase in small-amplitude IPSC frequency persisted in the presence of tetrodotoxin (fig. 2B, bottom; see Baba *et al.*[1]).

The effects of norepinephrine and tetrodotoxin on

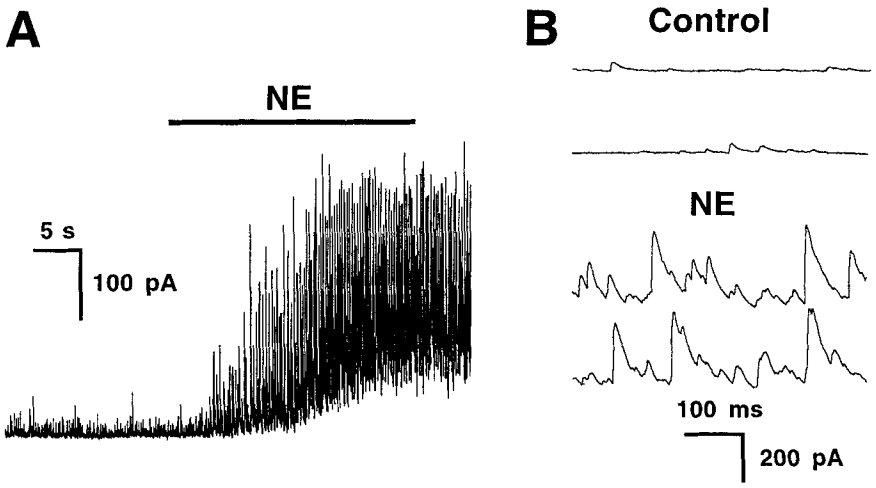

Fig. 1. Norepinephrine increases the frequency of spontaneous "GABAergic" inhibitory postsynaptic currents (IPSCs). Records shown were obtained from one neuron. (*A*) At a holding potential of 0 mV, IPSCs were recorded as upward deflections in the membrane current trace. Bath application of norepinephrine (20 μM) evoked repetitive large-amplitude IPSCs. In this cell, a prominent summation of IPSCs produced a persistent elevation of the baseline current. The bar above each trace indicates the application of norepinephrine. (*B*) Two sets of traces show IPSCs recorded in control and during application of norepinephrine on an expanded time base.

IPSC amplitude were further analyzed by constructing amplitude histograms. As shown in figure 3A (top), most IPSCs were relatively small, clustering primarily between 0 and 20 pA. Overall, the median IPSC amplitude was 10.1 ± 1.5 pA (n = 6; range 8.5–12.5 pA) during control conditions. In marked contrast, the presence of norepinephrine shifted IPSC amplitude distribution to the right. The majority of events were greater than 20 pA (fig. 3A, middle). This shift is not caused by temporal summation of increased IPSCs because the amplitude of each IPSC was measured from the initial deflection point (not from the baseline) to the peak of the event (fig. 3B). Norepinephrine increased the median amplitude of IPSCs in all cells tested (n = 6; 337 ± 98% of control; range, 201–458%). This change in amplitude distribution is clearly shown in the cumulative histogram (fig. 3C). In the presence of norepinephrine, the relative frequency curve was significantly shifted to the right ($P < 0.0001$ in all six cells tested; Kolmogorov-Smirnov test), and in each instance this shift was inhibited by tetrodotoxin. It is unlikely that large-amplitude IPSCs were miniature IPSCs of quantal size (*q*) following a Gaussian distribu-

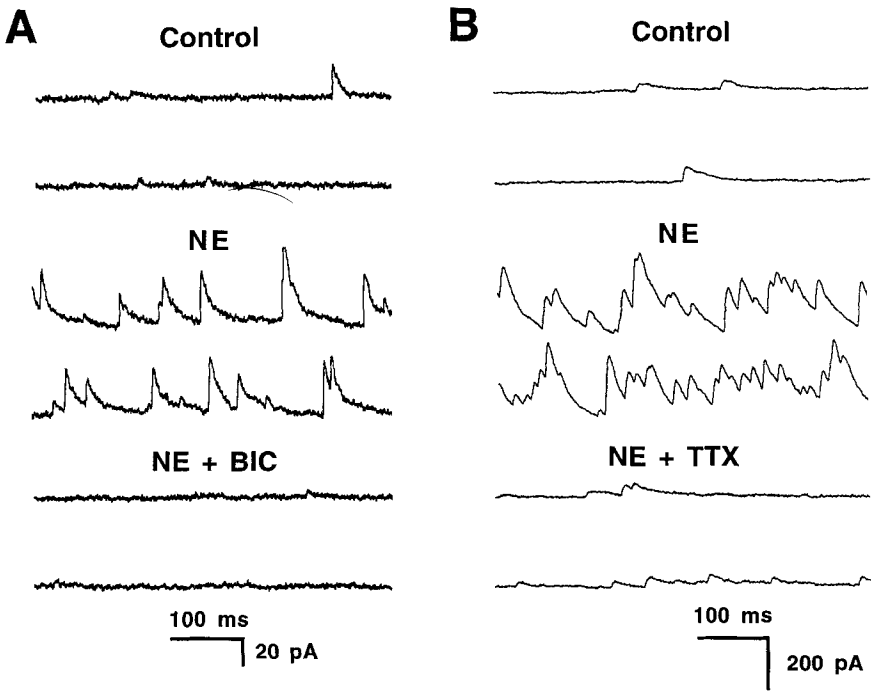

Fig. 2. The effects of bicuculline and tetrodotoxin on norepinephrine-induced inhibitory postsynaptic currents (IPSCs). Records shown were obtained from two different neurons (*A* and *B*). (*A*) Three sets of traces show IPSCs recorded with control, norepinephrine (20 μM), and norepinephrine with bicuculline (BIC, 20 μM) bath solutions. Norepinephrine increased the frequency of IPSCs, and all IPSCs were eliminated by the simultaneous application of bicuculline. (*B*) Norepinephrine (30 μM) increased IPSC frequency. Increased IPSC frequency was reduced, but not completely blocked, by tetrodotoxin (1 μM). Norepinephrine increased the frequency of spontaneous IPSCs from 6.5 to 47.3 Hz, and the norepinephrine-induced increase in IPSC frequency was reduced by tetrodotoxin to 10.6 Hz. Note that norepinephrine-induced IPSCs with large amplitude were not seen in control and that these large IPSCs were eliminated by tetrodotoxin.

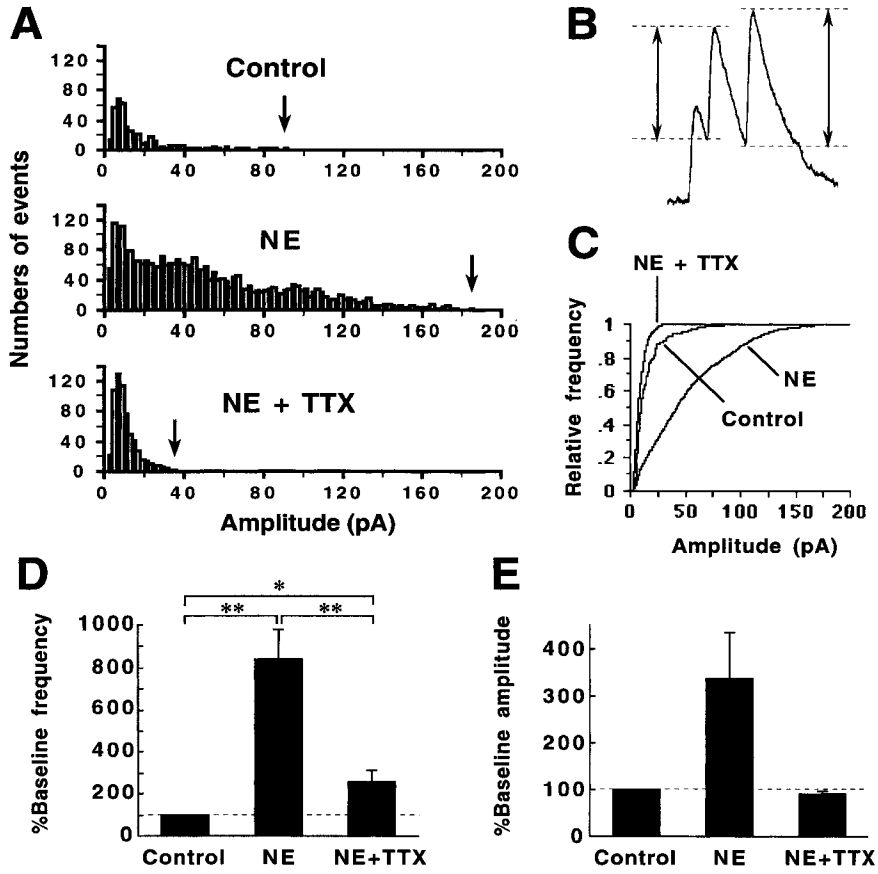

Fig. 3. The effect of norepinephrine and tetrodotoxin on amplitude distribution of inhibitory postsynaptic currents (IPSCs). (*A*) An example of amplitude analysis. The *top, middle,* and *bottom* show, respectively, the amplitude histograms for IPSCs recorded with control, norepinephrine (40 μM), and norepinephrine with tetrodotoxin (1 μM) bath solutions. Each histogram was constructed from 60 s of continuous recording. (*Top*) In the control bath, the median IPSC amplitude was 9.3 pA and the maximum value was 90.0 pA. (*Middle*) In the presence of norepinephrine, the median and maximum IPSC amplitudes were increased to 47.3 pA and 185.8 pA, respectively. (*Bottom*) tetrodotoxin reduced the median amplitude and maximum values to 9.0 pA and 36.0 pA, respectively. The norepinephrine-induced large-amplitude IPSCs were eliminated by tetrodotoxin, but small-amplitude IPSCs persisted. The IPSC amplitude maxima are indicated by arrows. (*B*) An illustration showing how the amplitude of each IPSC was measured, especially in cases in which the baseline current was elevated by IPSC summation. (*C*) Cumulative histograms (for the data in *A*) of IPSC amplitude before and during norepinephrine application, and coapplication of norepinephrine and tetrodotoxin. Norepinephrine significantly shifted the curve to the right (Kolmogorov-Smirnov test; $P < 0.0001$; 389 and 2,835 events analyzed for control and norepinephrine, respectively), which was reversed by tetrodotoxin (635 events analyzed). (*D*) The effect of norepinephrine (40 μM) and tetrodotoxin (1 μM) on IPSC frequency. Norepinephrine significantly increased the frequency of IPSCs; this increase in frequency was reduced, but not completely blocked, by tetrodotoxin (n = 6; **$P < 0.0001$; *$P < 0.05$; analysis of variance). Note that the frequency of IPSCs in the presence of norepinephrine and tetrodotoxin was significantly higher than in the control. (*E*) The effect of norepinephrine (30 μM) and tetrodotoxin (1 μM) on the median amplitude of IPSC in six cells. Norepinephrine significantly increased the median amplitude of IPSCs, although, in the presence of norepinephrine and tetrodotoxin, IPSC amplitude was reduced to less than the control value in all cells tested. The graph shows the mean ± SD of median amplitudes for six cells.

tion. More likely, large-amplitude IPSCs represent coordinated synaptic release caused by norepinephrine-induced firing of GABAergic interneurons. Large-amplitude IPSCs (> 40 pA) were abolished by the addition of tetrodotoxin (1 μM) to the bathing medium (fig. 3A, bottom), indicating that norepinephrine-enhanced GABA release is *via* action-potential propagation. The norepinephrine-induced increase in total IPSC frequency, however, could not be completely blocked by tetrodotoxin (256 ± 57% of control frequency, fig. 3D) because the frequency of the relatively small amplitude IPSCs (< 40 pA) remained elevated even in the presence of tetrodotoxin. This is most likely caused by the facilitatory effect of norepinephrine on quantal release from presynaptic axon terminals, as illustrated in the article Baba *et al.*[1]

Norepinephrine Increases Large-amplitude Spontaneous Inhibitory Postsynaptic Currents via α_1 *Receptors*

Next, the identity of the adrenergic receptor subtype responsible for the norepinephrine-induced increase in large-amplitude spontaneous IPSCs was evaluated. In all SG neurons tested (n = 8), the α_1-receptor agonist phenylephrine (10–80 μM) increased the frequency of spontaneous IPSCs and elicited large-amplitude IPSCs, which were not present in the control state (fig. 4A). However, neither the α_2-receptor agonist clonidine (10–40 μM n = 12) nor the nonselective β-receptor agonist isoproterenol (10–100 μM; n = 9) had any effect (data not shown). Together, this suggests that the norepinephrine-induced increase in IPSCs was mediated by α_1 receptors. To confirm these observations, we tested the effects of

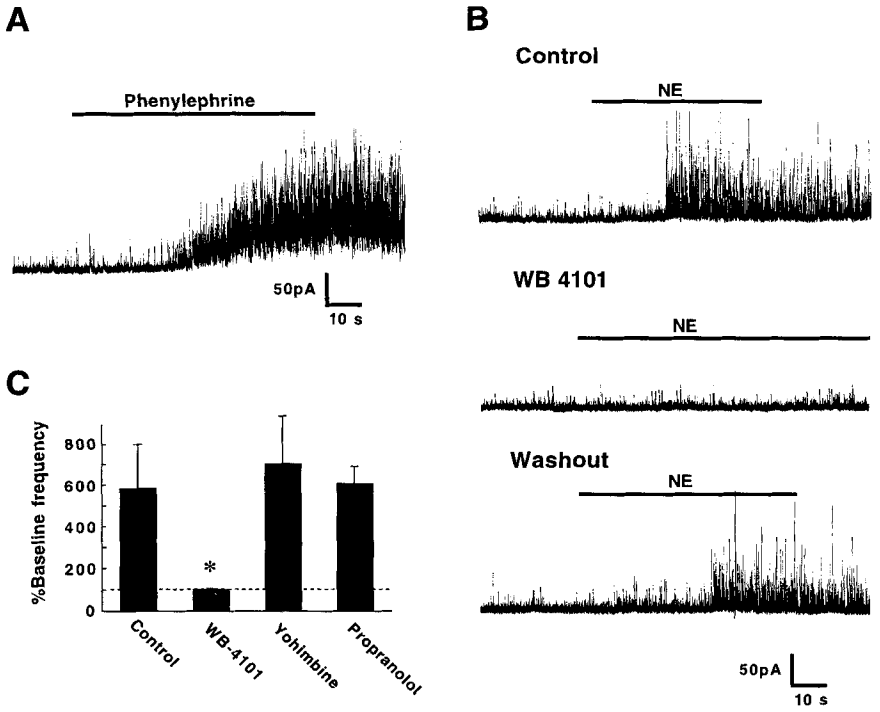

Fig. 4. The effect of norepinephrine on inhibitory postsynaptic current (IPSC) frequency is mediated by α_1 receptors. Records *A* and *B* were from two different neurons. (*A*) The frequency of IPSCs was increased by α_1-receptor agonist phenylephrine (40 μM). (*B*) The frequency of IPSCs were significantly increased by norepinephrine (20 μM; *top*), which was reversibly blocked by α_1-receptor antagonist WB-4101 (0.5 μM; *middle*, *bottom*). (*C*) The effects of α_1, α_2, and β antagonists on the facilitatory effect of norepinephrine. The effect of norepinephrine (20 μM; control group; n = 12) was significantly antagonized by the α_1-receptor antagonist WB-4101 (0.5 μM; n = 7). The α_2 antagonist yohimbine (1 μM; n = 5) and the β antagonist propranolol (1 μM; n = 5) failed to block the effect of norepinephrine. *$P < 0.01$, compared with the control group; analysis of variance test.

selective adrenergic receptor antagonists on norepinephrine-induced increases in IPSC frequency. WB-4101, an α_1- receptor antagonist (0.5 μM; n = 7), reversibly blocked the norepinephrine-induced increase in IPSC frequency and the induction of large-amplitude IPSCs (figs. 4B and C). Neither the α_2-receptor antagonist yohimbine (1 μM) nor the nonselective β-receptor antagonist propranolol (1 μM) blocked the effects of norepinephrine (fig. 4C), confirming the role of α_1 receptors.

α_1-Receptor Agonist Attenuates Polysynaptic EPSPs and EPSCs in Deep Dorsal Horn Neurons

It has been reported that norepinephrine also inhibits WDR neurons located in the deep dorsal horn.[3] To determine whether α_1 receptors also mediate this action of norepinephrine, conventional intracellular sharp electrode recordings and blind patch-clamp recordings were made in the absence of APV and CNQX (n = 15; fig. 5A). According to synaptic responses to graded intensity stimulation of the dorsal root, 11 of 15 deep dorsal horn neurons tested were classified as WDR; the remainder were low-threshold neurons. C-fiber electrical stimulation of the dorsal root usually evoked early, fast monosynaptic excitatory postsynaptic potentials and EPSCs [EPSP(C)s] and late, slow polysynaptic EPSP(C)s in WDR neurons (figs. 5B and C). In the majority of WDR neurons, phenylephrine (40 μM) reversibly attenuated polysynaptic EPSP(C)s, whereas monosynaptic EPSP(C)s appeared to be unchanged (fig. 5B, n = 9). In the remaining two WDR neurons, EPSP(C)s evoked by dorsal root stimulation (which had both fast and slow components) were unaffected by phenylephrine (data not shown). Synaptic responses in low-threshold neurons evoked by C-fiber intensity stimulation (n = 4) were essentially the same as those evoked by low-threshold afferent fiber (A-fiber) stimulation, consisting usually of fast, short-lasting (< 200 ms) monosynaptic EPSP(C)s (fig. 5D1). These monosynaptic EPSP(C)s were unaffected by phenylephrine (n = 4; fig. 5D2).

Discussion

Mechanisms of adrenergic modulation of GABAergic synaptic transmission in the SG of the rat spinal dorsal horn were evaluated. In the presence of glutamate and glycine receptor antagonists, norepinephrine increased the amplitude and frequency of $GABA_A$ receptor-mediated IPSCs in the majority of SG neurons tested. The appearance of large-amplitude IPSCs was tetrodotoxin sensitive, whereas the increase in IPSC frequency was only partially tetrodotoxin sensitive. The facilitatory effect of norepinephrine on GABAergic transmission was

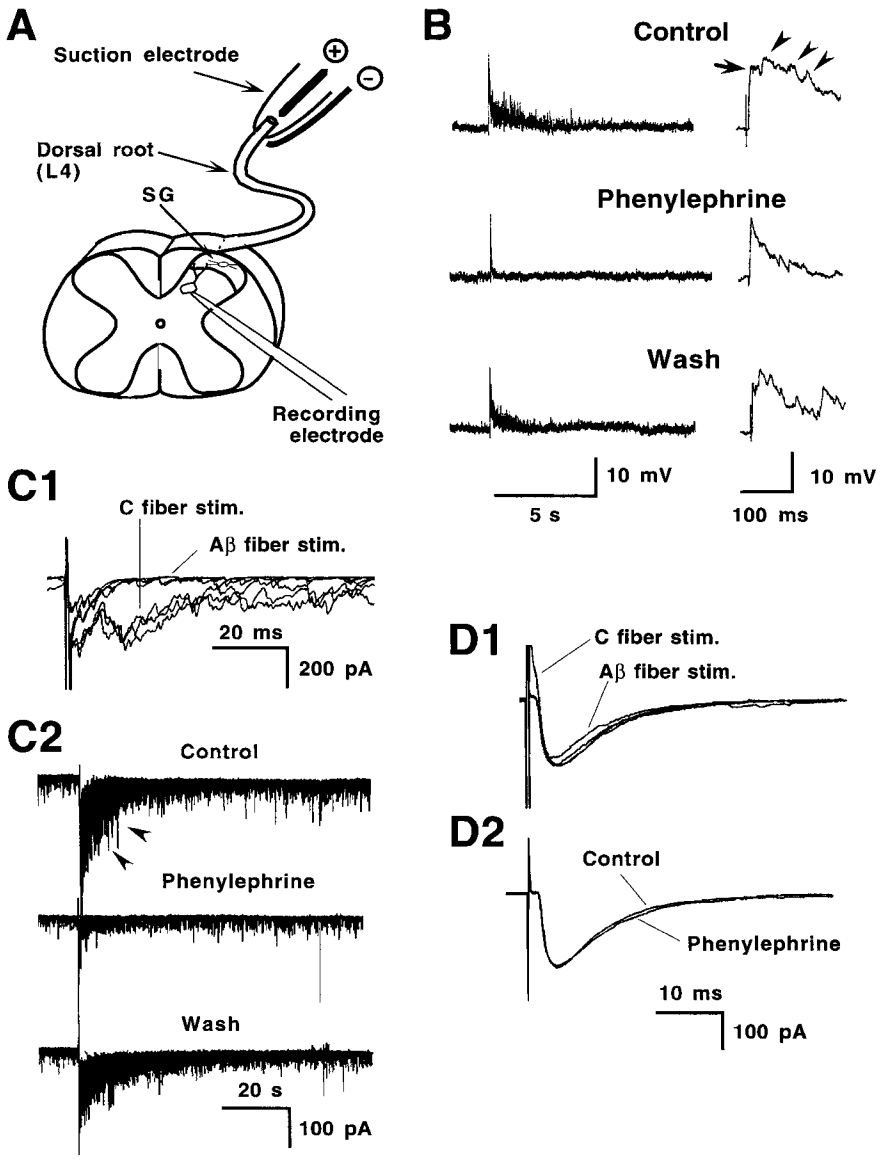

Fig. 5. α_1 Agonist attenuates dorsal root stimulation–evoked polysynaptic excitatory postsynaptic potential (EPSP) and excitatory postsynaptic current (EPSC) in lamina IV–V neurons. (*A*) Schematic diagram of intracellular or whole-cell patch-clamp recording from a lamina IV–V neuron. The dorsal roots (L4, 15–20 mm) were stimulated using a suction electrode. (*B*) An example of an intracellular recording from a wide-dynamic-range neuron in current-clamp configuration. (*Right*) Shows the records on an expanded time base. (*Top*) Single dorsal root stimulation at intensities sufficient to activate C fibers (1 mA, 0.5 ms) evoked monosynaptic (arrow) and polysynaptic EPSPs (arrow head). (*Middle*) Phenylephrine (40 μM) attenuated the polysynaptic EPSP, whereas the monosynaptic EPSP was unaffected. (*Bottom*) The effect of phenylephrine was reversible. (*C*) An example of whole-cell voltage-clamp recording from a wide-dynamic-range neuron. (*C1*) The amplitude and duration of EPSCs were augmented by an increase in stimulus intensity (Aβ ~ C-fiber intensity). (*C2*) Single dorsal root stimulation at C-fiber intensity (1 mA, 0.5 ms) elicited fast EPSC and long-lasting polysynaptic responses (arrow head, *top*), which were reversibly attenuated by phenylephrine (40 μM; *middle*, *bottom*). (*D*) An example of whole-cell voltage-clamp recording from a low-threshold neuron. (*D1*) Fast monosynaptic EPSCs were evoked by Aβ and C-fiber intensity stimulation. The synaptic responses evoked by C-fiber stimulation were essentially the same as that evoked by Aβ stimulation. (*D2*) The fast monosynaptic EPSCs evoked by Aβ-fiber stimulation (25 μA, 0.05 ms) were unaffected by phenylephrine (40 μM).

mimicked by phenylephrine and antagonized by WB-4101, whereas clonidine, isoproterenol, yohimbine, and propranolol each had no effect. Those observations indicate that adrenergic modulation of GABA-mediated fast synaptic transmission results from the activation of α_1 receptors located primarily at somatodendritic sites of GABAergic interneurons and, to a lesser degree, on the presynaptic terminals of GABAergic interneurons.

Adrenergic Excitation of GABAergic Neurons

The amplitude histogram for IPSCs recorded in the presence of norepinephrine showed an increase in large-amplitude IPSCs. The majority of these large-amplitude events appeared to result from propagation of action potentials from the soma of GABAergic interneurons to presynaptic terminals because they were blocked by tetrodotoxin. These large-amplitude IPSCs suggest the possibility that GABAergic interneurons are depolarized by norepinephrine acting *via* α_1 receptors (fig. 6), a mechanism that has been reported in other central nervous system regions.[4-6] The norepinephrine-induced increase in overall IPSC frequency, however, could not be completely blocked by tetrodotoxin: the frequency of the relatively small-amplitude IPSCs remained elevated

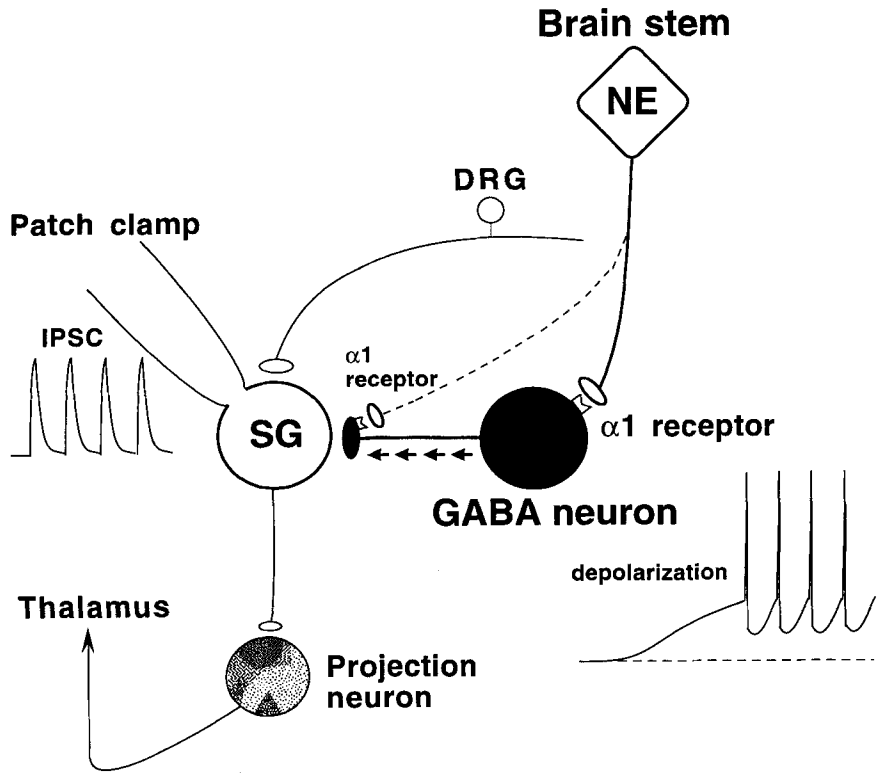

Fig. 6. Schematic diagram of proposed synaptic circuitry. In the dorsal horn, norepinephrine is released by terminals of noradrenergic neurons located in pontine nuclei onto "GABAergic" neurons at both the soma and the dendrites and axon terminals. Activation of postsynaptic α_1 receptors at somatodendritic sites depolarizes the GABAergic neuron sufficient to generate an orthodromic action potential. This action potential invades presynaptic axon terminals and causes coordinated release of γ-aminobutyric acid (GABA), which in turn produces the observed large-amplitude inhibitory postsynaptic currents (IPSCs) in substantia gelatinosa (SG) neurons. Activation of presynaptic α_1 receptors at axon terminals also facilitates the release of GABA from presynaptic terminals of GABAergic neurons. GABA-evoked currents hyperpolarize SG interneurons, thereby making generation of an action potential in response to nociceptive primary-afferent inputs less likely. This decrease in SG interneuron firing is consistent with the loss of polysynaptic, but not monosynaptic EPSPs, in lamina IV–V neurons after dorsal root stimulation.

even in the presence of tetrodotoxin. This result is consistent with the observation in Baba *et al.*[1] that norepinephrine facilitates quantal release of GABA from presynaptic terminals. Thus, norepinephrine activates GABAergic neurons at somatodendritic sites and at axon terminals.

The excitatory effects of norepinephrine appear to be selective for inhibitory neurons because the frequency of EPSCs recorded at −70 mV in the absence of glutamate receptor antagonists was unaffected (H. Baba, unpublished observations). The effects of norepinephrine reported herein seem to result from the activation of α_1 receptors on GABAergic interneurons because our data were obtained in the presence of glutamate receptor antagonists (therefore precluding a polysynaptic, or indirect, effect). Although the exact location of the GABAergic interneurons affected by norepinephrine is unknown, it is possible that they reside within the SG because some cell bodies and terminals of SG neurons are thought to contain GABA.[7] Indeed, norepinephrine has been shown to depolarize a subset of SG neurons through α_1-receptor activation,[8] and norepinephrine produced inward currents in some spinal SG neurons when guanosine-5′-0-(2-thiodiphosphate) was omitted from the pipette solution (H. Baba, unpublished observation).

As discussed in the the article by Baba *et al.*,[1] the majority of SG neurons are local interneurons and do not project to the thalamus.[9] The main projections of SG neurons are to lamina I and to the deep dorsal horn (lamina IV-V), where the projection neurons to the thalamus are located. Therefore, in the current study, we tested whether α_1-receptor activation inhibits nociceptive input to deep dorsal horn neurons. Our data show that α_1-receptor activation inhibited polysynaptic, but not monosynaptic, input to deep dorsal horn neurons.

Based on these considerations, we propose the model circuit shown in figure 6. Nociceptive information, conveyed *via* Aδ and C fibers, is transmitted to neurons within the SG, the first relay for such information in the central nervous system. SG neurons in turn transmit this information to projection neurons located in lamina I or lamina IV-V and, in so doing, create a simple polysynaptic pathway. Release of norepinephrine by descending adrenergic fibers, however, suppresses the feed-forward aspects of the circuit by activating α_1 receptors located on both the somatodendritic sites and the axon terminals of GABAergic interneurons. Activation of α_1 receptors on soma or dendrites of GABAergic interneurons leads to depolarization, which in turn can promote to release of GABA onto SG relay neurons. The SG relay neuron is

hyperpolarized after the opening of $GABA_A$-receptor channels, and the resting membrane potential is shifted away from the action potential threshold, thereby decreasing the likelihood that the original nociceptive signal will be transmitted to the thalamic projection neuron. Thus, norepinephrine might, by directly stimulating inhibitory interneurons, inhibit nociceptive polysynaptic input to lamina IV–V, while leaving monosynaptic responses unchanged.

Functional Consideration

As discussed in the article by Baba *et al.*,[1] GABA may play an important role in spinal antinociception. GABAergic interneurons may be responsible for suppressing evoked excitatory responses. Increased activity of these inhibitory interneurons may decrease the excitability of SG neurons, thereby increasing the threshold for noxious inputs. Our current data suggest that the GABAergic system is, in turn, regulated by adrenergic inputs acting through α_1 adrenoreceptors. Given that nearly all norepinephrine-containing synaptic terminals in the dorsal horn of the spinal cord are supraspinal in origin,[10,11] these descending adrenergic fibers may represent an intrinsic system for the regulation of GABAergic dorsal horn neurons. The α_1 action on somatodendritic sites of GABAergic interneurons, together with the facilitatory action on axon terminals, may contribute to the analgesic action of intrathecally administered phenylephrine.

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