

## $\alpha_2$ Adrenoceptor–mediated Presynaptic Inhibition of Primary Afferent Glutamatergic Transmission in Rat Substantia Gelatinosa Neurons

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**Background:** Although intrathecal administration of norepinephrine is known to produce analgesia, cellular mechanisms for this action have not yet been fully understood.

**Methods:** The actions of norepinephrine (50  $\mu$ M) on glutamatergic transmission were examined by using the whole cell patch clamp technique in substantia gelatinosa neurons of an adult rat spinal cord slice with an attached dorsal root.

**Results:** Norepinephrine inhibited the amplitude of monosynaptically evoked A $\delta$ -fiber and C-fiber excitatory postsynaptic currents in a reversible manner. When compared in magnitude between the A $\delta$ -fiber and C-fiber excitatory postsynaptic currents, the former inhibition (50  $\pm$  4%, n = 20) was significantly larger than the latter one (28  $\pm$  4%, n = 8). Both actions of norepinephrine were mimicked by an  $\alpha_2$  adrenoceptor agonist, clonidine (10  $\mu$ M), and an  $\alpha_{2A}$  agonist, oxymetazoline (10  $\mu$ M), but not by an  $\alpha_1$  agonist, phenylephrine (10  $\mu$ M), and a  $\beta$  agonist, isoproterenol (40  $\mu$ M). The inhibitory actions were antagonized by an  $\alpha_2$  antagonist, yohimbine (1  $\mu$ M), all of the results of which indicate an involvement of  $\alpha_2$  adrenoceptors. Norepinephrine did not affect the amplitude of miniature excitatory postsynaptic current and of a response of substantia gelatinosa neurons to AMPA, indicating that its action on evoked excitatory postsynaptic currents is presynaptic in origin.

**Conclusions:** Norepinephrine inhibits A $\delta$ -fiber– and C-fiber–mediated sensory transmission to substantia gelatinosa neurons through the activation of the  $\alpha_2$  adrenoceptor (possibly  $\alpha_{2A}$  type, based on the current, published behavioral and anatomical data) existing in primary afferent terminals; this action of norepinephrine is more effective in A $\delta$ -fiber than C-fiber transmission. This could contribute to at least a part of inhibitory modulation of pain sensation in the substantia gelatinosa by intrathecally administered norepinephrine.

CATECHOLAMINES, such as epinephrine and phenylephrine, have been used in spinal anesthesia together with local anesthetics with an expectation of a contraction of local vessels by the monoamines resulting in a decrease in the clearance of the anesthetics from the subarachnoid space and thus antinociception,<sup>1,2</sup> while intrathecal administration of norepinephrine itself is

known to have an antinociceptive effect when assessed by the tail-flick and hot-plate tests.<sup>3,4</sup> There is much evidence supporting the latter idea. Nociceptive information is transmitted through thinly myelinated A $\delta$ -afferent and unmyelinated C-afferent fibers from the periphery to the spinal cord, especially substantia gelatinosa (SG) neurons,<sup>5,6</sup> where the information is modulated. Among this modulatory system, there is a descending norepinephrine-containing fiber pathway from cell groups designated A5, A6 (nucleus locus ceruleus), and A7 (subceruleus) in the pons,<sup>7-10</sup> electrical stimulation of which results in behavioral analgesia.<sup>11-14</sup> This norepinephrine pathway is known to be also activated by systemically administered opioids<sup>15,16</sup> or electrical stimulation of the midbrain periaqueductal gray region.<sup>17,18</sup> *In situ* hybridization and immunohistochemical studies have demonstrated the presence of adrenoceptors in dorsal root ganglion and dorsal horn neurons in the rat,<sup>19,20</sup> suggesting a role of norepinephrine at presynaptic and postsynaptic sites in the modulation. Previous studies have reported in SG neurons of the spinal cord and also spinal trigeminal nucleus that norepinephrine hyperpolarizes membrane,<sup>21,22</sup> potentiates inhibitory ( $\gamma$ -aminobutyric acid–mediated and glycinergic) transmission,<sup>23</sup> and inhibits glutamatergic excitatory transmission.<sup>24</sup> The former two actions have been examined in detail, whereas the last one remains to be examined in detail. Although there is known to be a difference between A $\delta$ -fiber and C-fiber excitatory transmission to SG neurons in the action of neuromodulators, such as a  $\gamma$ -aminobutyric acid type B receptor agonist, baclofen,<sup>25</sup> serotonin,<sup>26</sup> nociceptin,<sup>27</sup> and anandamide,<sup>28</sup> and also of capsaicin,<sup>29</sup> this has not yet been revealed for the norepinephrine action because Travagli and Williams<sup>24</sup> examined the action of norepinephrine on excitatory transmission evoked in spinal trigeminal SG neurons by stimulating the trigeminal tract where the stimulation could not be separately given to each of the fibers. It has not yet been unveiled which types of adrenoceptors engage in modulation by norepinephrine of each of the A $\delta$ -fiber and C-fiber transmissions, although the norepinephrine-induced hyperpolarization and presynaptic facilitation of inhibitory transmission are reported to be due to the activation of  $\alpha_2$  and  $\alpha_1$  adrenoceptors, respectively.<sup>21-23</sup> In the current study, we examined the effect of norepinephrine on monosynaptic A $\delta$ -fiber and C-fiber excitatory postsynaptic currents (EPSCs) and its pharmacological property in the adult rat SG using the whole

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cell patch clamp technique under the condition of a blockade of the hyperpolarizing action.

## Materials and Methods

### *Preparation of Spinal Cord Slices*

This study was approved by the institutional Animal Use and Care Committee at Saga Medical School (Saga, Japan). The technique used for obtaining slice preparations from the rat spinal cord was the same as that described elsewhere.<sup>27,30</sup> Briefly, adult male Sprague-Dawley rats (7–8 weeks old) were deeply anesthetized with urethane (1.2 g/kg). The lumbosacral spinal cord (L1–S3) was then removed and placed in preoxygenated (95% O<sub>2</sub> and 5% CO<sub>2</sub>) Krebs solution (117 mM NaCl, 3.6 mM KCl, 2.5 mM CaCl<sub>2</sub>, 1.2 mM MgCl<sub>2</sub>, 1.2 mM NaH<sub>2</sub>PO<sub>4</sub>, 25 mM NaHCO<sub>3</sub>, and 11 mM glucose) at 1–3°C; the rats were then immediately sacrificed by exsanguination. After cutting all of ventral and dorsal roots near the root entry zone, except for the L4 or L5 dorsal root on one side, the pia-arachnoid membrane was removed. The spinal cord was mounted on a Vibratome Series 1000® (Technical Products International, O'Fallon, MO), and then a 600- to 650- $\mu$ m-thick transverse slice was cut with an attached dorsal root having a length of 6–14 mm. The slice was placed on a nylon mesh in the recording chamber and then perfused at a rate of 15–20 ml/min with Krebs solution maintained at 36  $\pm$  1°C. Before the start of the experiment, the slice was preincubated for at least 1 h with Krebs solution.

### *Whole Cell Recordings from Substantia Gelatinosa Neurons and Stimulation of the Dorsal Root*

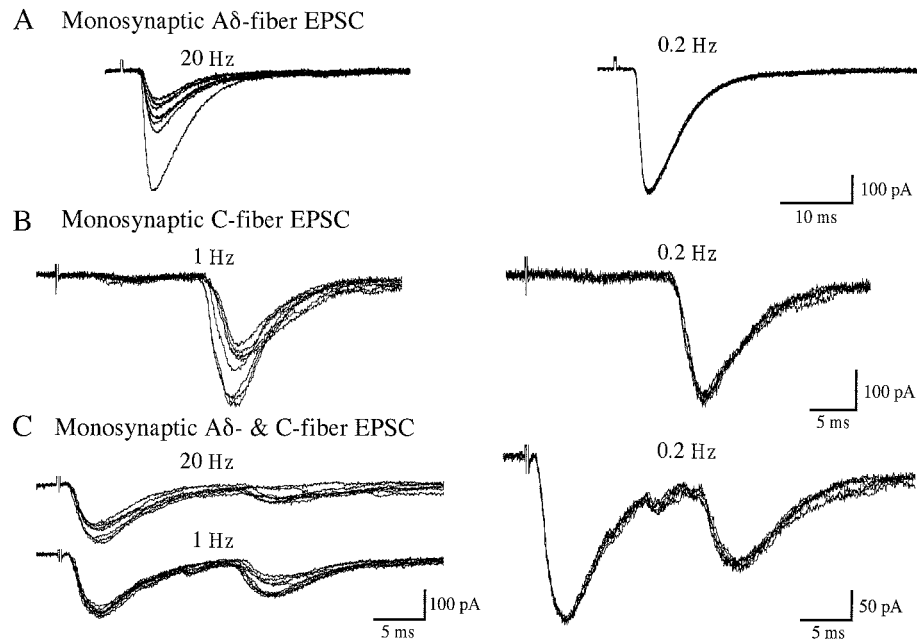
Substantia gelatinosa neurons were identified by their location under a binocular microscope with light transmitted from below as reported previously<sup>25,27,31</sup>; the SG was discernible as a relatively translucent band. Blind whole cell voltage clamp recordings were made from neurons that are located at the center of SG, with a patch pipette that was made up of a thin-walled fiberglass (1.5-mm OD) using a single-stage horizontal puller (P-97; Sutter Instrument, Novato, CA). The patch pipettes had a tip resistance of 5–10 M $\Omega$  when filled with a solution having the following composition: 110 mM Cs<sub>2</sub>SO<sub>4</sub>, 0.5 mM CaCl<sub>2</sub>, 2 mM MgCl<sub>2</sub>, 5 mM EGTA, 5 mM HEPES, 5 mM Mg-ATP, 5 mM tetraethylammonium, and 1 mM guanosine 5'-O-(2-thiodiphosphate), where guanosine 5'-O-(2-thiodiphosphate) and K<sup>+</sup>-channel blockers (Cs<sup>+</sup> and tetraethylammonium) were added to inhibit a hyperpolarizing effect of norepinephrine through the action of G proteins and to block an activation of K<sup>+</sup> channels, respectively. After making a rigid seal (resistances: 5–20 G $\Omega$ ) in the cell-attached mode by a gentle suction given into the patch pipette, the membrane patch was ruptured by a brief period of more powerful suction, resulting in the

whole cell configuration. Signals were amplified with a patch clamp amplifier (Axopatch 200B; Axon Instruments, Foster City, CA) in the voltage clamp mode. Data were low-pass filtered at 5 kHz, digitized at 333 kHz with an A/D converter, and stored and analyzed with a personal computer using the pCLAMP data acquisition program (version 6.0; Axon Instruments). The program used for analyzing miniature EPSCs (mEPSCs) detects spontaneous events if the difference between the baseline and a following current value exceeds a given threshold of 6 pA and separating valleys are less than 50% of adjacent peaks; the validity of the method was confirmed by measuring visually individual mEPSCs on a fast time scale in several cases.<sup>30</sup>

Orthodromic stimulation of the dorsal root to elicit EPSCs was performed with a suction electrode at 0.2 Hz unless otherwise mentioned. The strength of stimuli used was 1.2 times the threshold to elicit EPSCs, fearing a conduction block of action potentials in the dorsal root. The holding potential (V<sub>H</sub>) used was –70 mV, at which glycine and  $\gamma$ -aminobutyric acid type A receptor-mediated synaptic currents were invisible. The duration of stimuli used was 0.1 ms throughout the experiments, and conduction velocities were calculated from the latency of monosynaptic EPSC and the length of dorsal root. A $\delta$ -fiber and C-fiber evoked EPSCs were distinguished from each other on the basis of the conduction velocity of afferent fibers and stimulus threshold; they were considered as monosynaptic in origin when the latency remained constant and there was no failure during stimulation at 20 Hz for 1 s or when failures did not occur during repetitive stimulation at 1 Hz for 20 s, respectively, as reported previously.<sup>25,26,32,33</sup> During these stimulations, a conduction block of action potentials did not occur when examined by using rat dorsal root ganglion neurons.<sup>25,33</sup> Although in some neurons monosynaptic A $\delta$ -fiber or C-fiber EPSCs were accompanied by polysynaptic A $\delta$ -fiber or C-fiber EPSCs, such neurons were not used for analysis if the peak of the monosynaptic EPSCs was contaminated by the polysynaptic EPSCs.

### *Application of Drugs*

Drugs were dissolved in Krebs solution and then applied to the SG by an exchange of superfusing solution *via* a three-way stopcock with one containing them at known concentrations without a change in superfusion rate and thus in temperature. Solutions in the recording chamber completely altered within 20 s. The drugs used in this work were ( $\pm$ )-norepinephrine (Aldrich Chemical Co., Milwaukee, WI), yohimbine hydrochloride, (–)-phenylephrine (Wako, Osaka, Japan),  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), (–)-isoproterenol hydrochloride, clonidine hydrochloride, oxymetazoline hydrochloride, guanosine 5'-O-(2-thiodiphosphate) (Sigma, St Louis, MO), and 6-cyano-7-nitroquinoxaline-2,3-dione (Tocris Cookson, St. Louis, MO).



**Fig. 1.** Monosynaptic excitatory postsynaptic currents (EPSCs) evoked in substantia gelatinosa neurons by stimulating the dorsal root. (*A and B*) Two types of monosynaptic EPSCs evoked at higher frequencies (*left*: 20 Hz in *A* and 1 Hz in *B*) and 0.2 Hz (*right*), which are different in latency from an artifact of the stimulus. A $\delta$ -fiber and C-fiber EPSCs were distinguished from each other on the basis of the conduction velocity of afferent fibers and stimulus threshold (9.6 m/s and 62  $\mu$ A and 0.5 m/s and 560  $\mu$ A in *A* and *B*, respectively). The A $\delta$  or C responses, respectively, were considered as monosynaptic in origin when there were no failures and the latency remained constant during stimulation at 20 Hz or when failures did not occur during stimulation at 1 Hz. Both of the monosynaptic A $\delta$ -fiber and C-fiber EPSCs were observed in 31% of neurons examined (*C*; stimulus strength, 600  $\mu$ A). Each of the records is a superimposition of seven or eight traces of EPSCs;  $V_H = -70$  mV.

#### Statistical Analysis

Data are presented as mean  $\pm$  SEM. Statistical significance was determined as  $P < 0.05$  using either the paired or unpaired Student  $t$  test unless otherwise mentioned. The Kolmogorov-Smirnov test was also used to compare mEPSCs in the absence and presence of norepinephrine in the distributions of their amplitudes and interevent intervals. In all cases,  $n$  refers to the number of neurons studied.

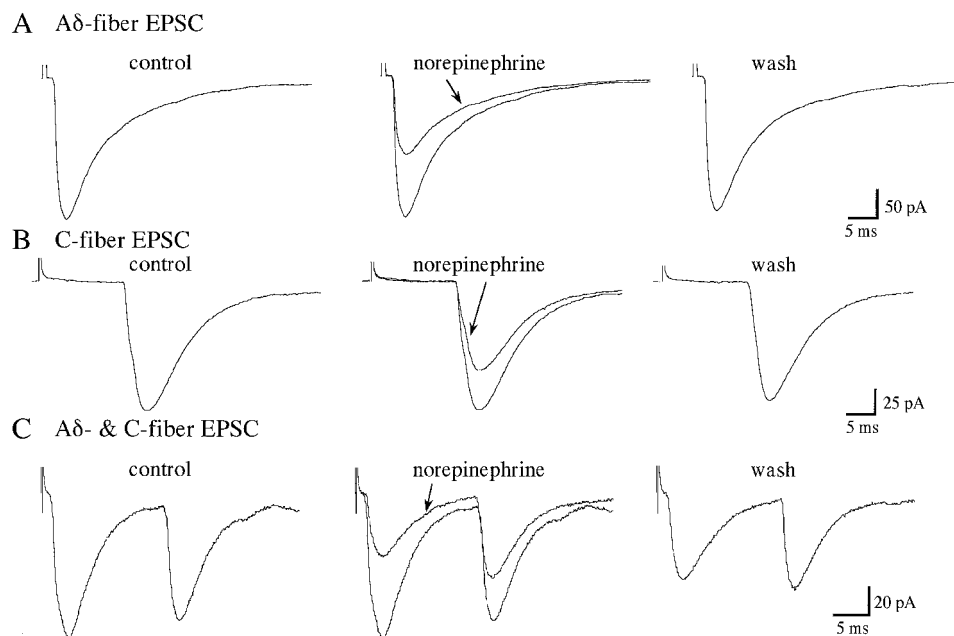
## Results

Whole cell patch clamp recordings were made from a total of 158 SG neurons. Stable recordings could be obtained from neurons in spinal cord slices maintained *in vitro* for more than 12 h, and recordings were made from single neurons for up to 4 h. All experiments were performed at least 10 min later, enough time for guanosine 5'-O-(2-thiodiphosphate) and  $K^+$ -channel blockers in patch pipette solutions to diffuse into SG neurons, after the establishment of whole cell configuration; norepinephrine (50  $\mu$ M) did not change holding currents at  $-70$  mV. In all SG neurons tested, mEPSCs were downward at  $-70$  mV and were blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (10  $\mu$ M;  $n = 2$ ), as reported previously,<sup>27,30</sup> indicating the activation of AMPA receptors.

#### More Inhibition by Norepinephrine of Monosynaptic A $\delta$ -fiber Than C-fiber EPSCs Elicited in Substantia Gelatinosa Neurons

When stimulating the dorsal root with a strength of less than 200  $\mu$ A, 82% ( $n = 75$ ; this percentage was almost comparable to a value [79%] obtained previously<sup>27</sup>) of 92 neurons examined could elicit monosynaptic EPSCs that had no failure and no change in latency when elicited at 20 Hz (fig. 1A, left). Conduction velocity values (3.8–15 m/s), estimated from the latency of EPSC and the length of dorsal root, were almost within a range of those of A $\delta$ -fibers obtained from the experiment in dorsal root ganglion neurons, as reported previously.<sup>25,32</sup> Monosynaptic A $\delta$ -afferent EPSCs evoked at 0.2 Hz had a mean amplitude of  $279 \pm 23$  pA (range, 59–884 pA;  $V_H = -70$  mV; fig. 1A, right). On the other hand, when stimulated with a strength of more than 200  $\mu$ A, 83% ( $n = 53$ ; this percentage was also similar to a value [80%] obtained previously<sup>27</sup>) of 64 neurons tested exhibited monosynaptic EPSCs that had no failures in response to stimuli at 1 Hz (fig. 1B, left), albeit a variability in EPSC latency was observed in some neurons.<sup>25</sup> The EPSCs originated from afferent fibers whose conduction velocities were 0.3–0.8 m/s, a range of those of C fibers.<sup>25,32</sup> The amplitude of monosynaptic C-afferent EPSCs evoked at 0.2 Hz averaged to be  $253 \pm 26$  pA (range, 96–714 pA;  $V_H = -70$  mV; fig. 1B, right). Thirty-one percent ( $n = 20$ ) of the neurons tested exhibited both of the monosynaptic





**Fig. 2.** Actions of norepinephrine (50  $\mu\text{M}$ ) on monosynaptic excitatory postsynaptic currents (EPSCs) evoked in substantia gelatinosa neurons. (A and B) A $\delta$ -fiber and C-fiber EPSCs, respectively, in the absence (left and right) and presence (middle; where control EPSC is superimposed for comparison) of norepinephrine. Note that the A $\delta$ -fiber EPSC was more sensitive to norepinephrine than the C-fiber EPSC. This was so in a neuron in which both of the A $\delta$ -fiber and C-fiber EPSCs were observed (C). Each record in A, B, and C (stimulus strength: 52, 250, and 790  $\mu\text{A}$ , respectively) shows an average of 12 traces of EPSCs;  $V_H = -70$  mV.

A $\delta$ -fiber and C-fiber EPSCs, as seen in figure 1C. These EPSCs were completely inhibited by 6-cyano-7-nitroquinoxaline-2,3-dione (10  $\mu\text{M}$ ;  $n = 2$ ), indicative of an involvement of AMPA receptors, as reported previously.<sup>27,32</sup>

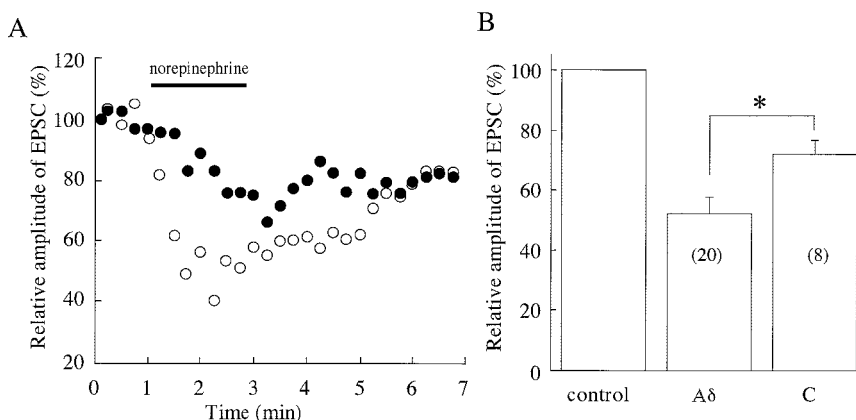
Effects of norepinephrine (50  $\mu\text{M}$ ) on the monosynaptic A $\delta$ -fiber and/or C-fiber EPSCs were examined in a total of 24 SG neurons. As seen from figures 2A-C, each of the EPSCs was inhibited in amplitude by norepinephrine in a reversible manner. These effects on A $\delta$ -fiber and C-fiber EPSCs were, respectively, maximal at approximately 1 and 2 min following the application of norepinephrine (fig. 3A). When examined in many neurons, the inhibitions (measured at approximately 2 min following norepinephrine superfusion) of the peak amplitudes of A $\delta$ -fiber and C-fiber EPSCs were, respectively,  $50 \pm 4\%$  ( $n = 20$ ) and  $28 \pm 4\%$  ( $n = 8$ ), with the values being significantly different from each other ( $P < 0.01$ ), as

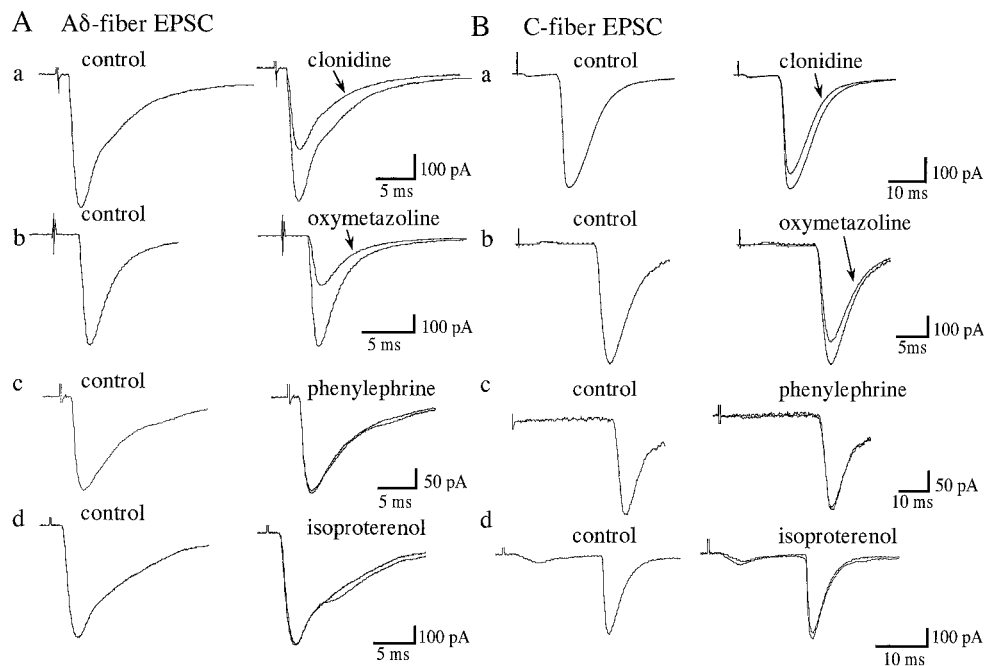
seen in figure 3B. This greater sensitivity of A $\delta$ -fiber EPSC than C-fiber EPSC to norepinephrine was also observed in a neuron in which both of the EPSCs were elicited ( $n = 5$ ; for example, see fig. 2C).

*Involvement of  $\alpha_2$  Adrenoceptors in Norepinephrine-induced Inhibition of Monosynaptic A $\delta$ -fiber and C-fiber EPSCs Evoked in Substantia Gelatinosa Neurons*

We next examined which subtypes of adrenoceptors are involved in the norepinephrine-induced inhibition of A $\delta$ -fiber and C-fiber EPSCs by use of their agonists and antagonists. When superfused for 2 min, an  $\alpha_2$  adrenoceptor agonist, clonidine (10  $\mu\text{M}$ ), as well as norepinephrine, inhibited the peak amplitude of monosynaptically evoked A $\delta$ -fiber and C-fiber EPSCs (by  $24 \pm 2\%$  [ $n = 13$ ] and  $19 \pm 3\%$  [ $n = 11$ ], respectively), as seen in figures

**Fig. 3.** Comparison of the action of norepinephrine (50  $\mu\text{M}$ ) between A $\delta$ -fiber and C-fiber excitatory postsynaptic currents (EPSCs) in substantia gelatinosa neurons. (A) Time courses of changes in the peak amplitude of A $\delta$ -fiber EPSC ( $\circ$ ) and C-fiber EPSC ( $\bullet$ ) under the action of norepinephrine, relative to that in the control. There was a time delay of 1–2 min before the norepinephrine action shows a maximal effect. Each of them was obtained from a different neuron. Each point is an average of the amplitudes of three consecutive EPSCs. (B) Relative peak amplitude of A $\delta$ -fiber and C-fiber EPSC under the action of norepinephrine to that in the control. The number of neurons examined is shown in parentheses;  $*P < 0.01$ ;  $V_H = -70$  mV.





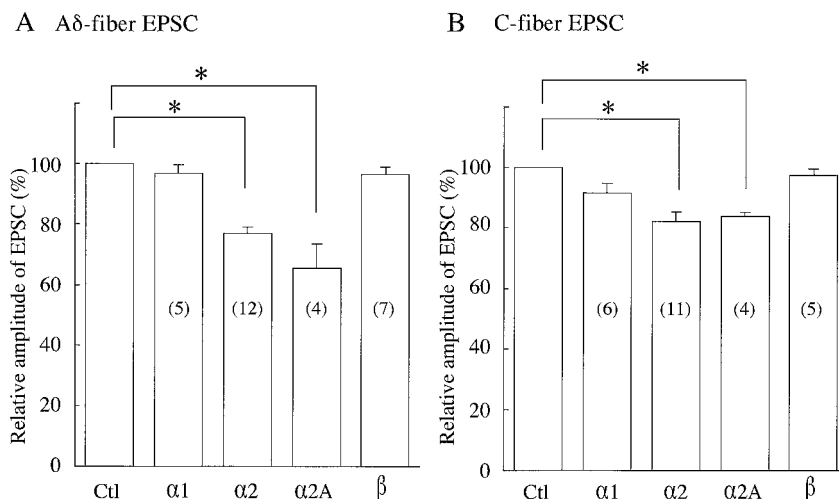
**Fig. 4.** A $\delta$ -fiber excitatory postsynaptic currents (EPSCs) (**A**) and C-fiber EPSCs (**B**) evoked in substantia gelatinosa neurons in the absence (*left*) and presence (*right*; where control EPSC is superimposed for comparison) of an  $\alpha_2$  agonist, clonidine (10  $\mu\text{M}$ ; **a**), an  $\alpha_{2A}$  agonist, oxymetazoline (10  $\mu\text{M}$ ; **b**), an  $\alpha_1$  agonist, phenylephrine (10  $\mu\text{M}$ ; **c**), and a  $\beta$  agonist, isoproterenol (40  $\mu\text{M}$ ; **d**). Note that both of the EPSCs were depressed by clonidine and oxymetazoline but not phenylephrine and isoproterenol. Each of the records is an average of 12 traces of EPSCs;  $V_H = -70$  mV.

4Aa and Ba. An agonist of  $\alpha_{2A}$  adrenoceptor, oxymetazoline (10  $\mu\text{M}$ ),<sup>34</sup> exhibited a similar action on A $\delta$ -fiber and C-fiber EPSCs (inhibition:  $34 \pm 9\%$  [ $n = 4$ ] and  $16 \pm 2\%$  [ $n = 4$ ], respectively; these were not different from each other;  $P > 0.05$ ; figs. 4Ab and Bb). On the other hand, an  $\alpha_1$  adrenoceptor agonist, phenylephrine (10  $\mu\text{M}$ ), and a  $\beta$  adrenoceptor agonist, isoproterenol (40  $\mu\text{M}$ ), were without the inhibition on A $\delta$ -fiber and C-fiber EPSCs, as seen in figures 4Ac, Bc, Ad, and Bd. Figure 5 summarizes the effects of the adrenoceptor agonists on A $\delta$ -fiber and C-fiber EPSCs. With respect to antagonists, an  $\alpha_2$  adrenoceptor antagonist, yohimbine (1  $\mu\text{M}$ ), superfused for 5 min prior to the application of norepinephrine (50

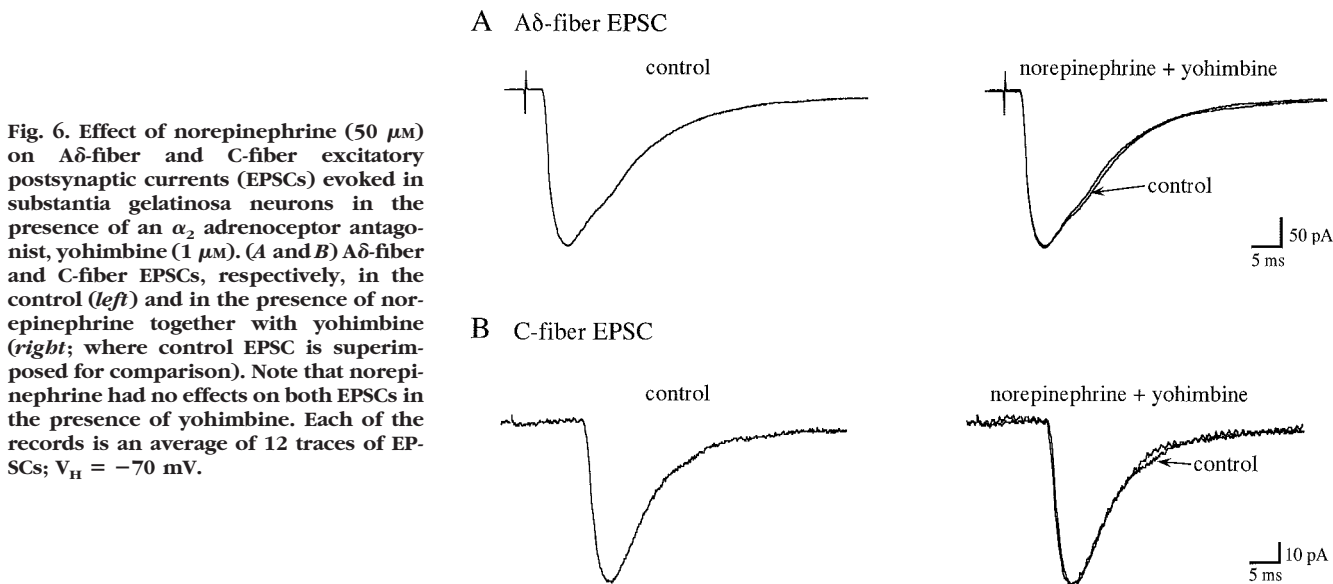
$\mu\text{M}$ ), greatly depressed its inhibitory effect on A $\delta$ -fiber or C-fiber EPSCs (peak amplitude:  $95 \pm 3\%$  [ $n = 12$ ] and  $97 \pm 6\%$  [ $n = 4$ ] of control, respectively, in the presence of the antagonist), as seen in figures 6A and B. Altogether, these results indicate that the suppressive actions of norepinephrine on A $\delta$ -fiber and C-fiber EPSCs are mediated by  $\alpha_2$  adrenoceptors.

#### Lack of the Effect of Norepinephrine on AMPA Responses in Substantia Gelatinosa Neurons

Figures 7A and B demonstrate the effect of norepinephrine (50  $\mu\text{M}$ ) superfused for 2 min on mEPSCs recorded from SG neurons. Both mEPSC amplitude and



**Fig. 5.** Effects of various  $\alpha$  adrenoceptor agonists and  $\beta$  adrenoceptor agonist on A $\delta$ -fiber and C-fiber excitatory postsynaptic currents (EPSCs) in substantia gelatinosa neurons. (**A** and **B**) Relative peak amplitudes of A $\delta$ -fiber EPSC (**A**) and C-fiber EPSC (**B**) in the presence of an  $\alpha_1$  agonist, phenylephrine (10  $\mu\text{M}$ ;  $\alpha_1$ ), an  $\alpha_2$  agonist, clonidine (10  $\mu\text{M}$ ;  $\alpha_2$ ), an  $\alpha_{2A}$  agonist, oxymetazoline (10  $\mu\text{M}$ ;  $\alpha_{2A}$ ), or a  $\beta$  agonist, isoproterenol (40  $\mu\text{M}$ ;  $\beta$ ) to that in the control (Ctl). Note that the  $\alpha_2$  and  $\alpha_{2A}$  but not  $\alpha_1$  and  $\beta$  agonists significantly inhibited both EPSCs. The number of neurons examined is shown in parentheses; \* $P < 0.01$ .



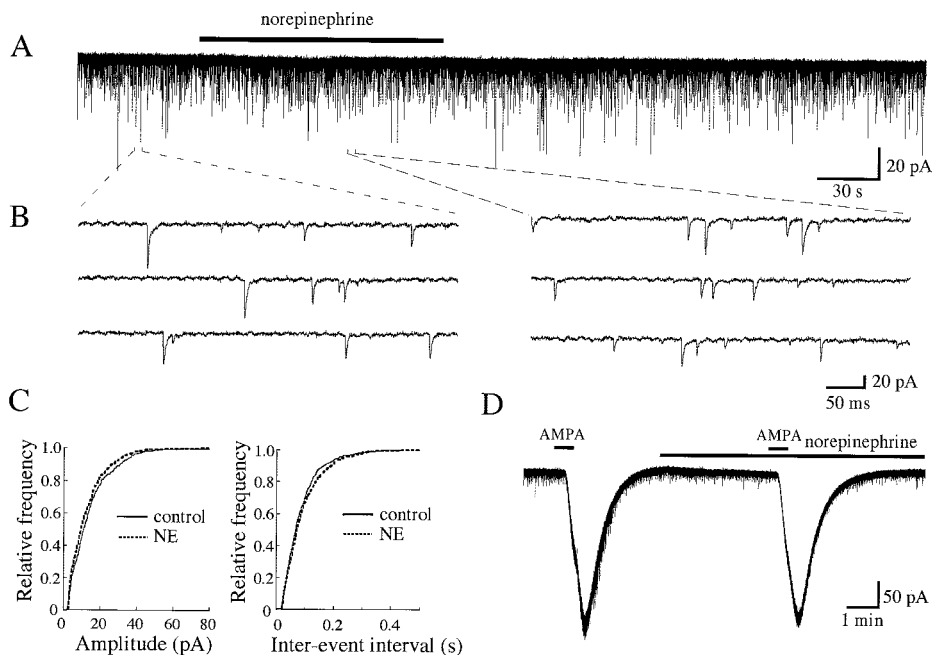
**Fig. 6.** Effect of norepinephrine (50  $\mu\text{M}$ ) on A $\delta$ -fiber and C-fiber excitatory postsynaptic currents (EPSCs) evoked in substantia gelatinosa neurons in the presence of an  $\alpha_2$  adrenoceptor antagonist, yohimbine (1  $\mu\text{M}$ ). (A and B) A $\delta$ -fiber and C-fiber EPSCs, respectively, in the control (left) and in the presence of norepinephrine together with yohimbine (right; where control EPSC is superimposed for comparison). Note that norepinephrine had no effects on both EPSCs in the presence of yohimbine. Each of the records is an average of 12 traces of EPSCs;  $V_H = -70$  mV.

frequency were unaffected by norepinephrine; they were  $98 \pm 7\%$  ( $P > 0.05$ ;  $n = 10$ ) and  $102 \pm 1\%$  ( $P > 0.05$ ;  $n = 10$ ) of control ( $17 \pm 3$  pA and  $9 \pm 2$  Hz), respectively, as reported by Baba *et al.*<sup>23</sup> When examined for cumulative distributions of the amplitude and interevent interval of mEPSC, they were also unaffected by norepinephrine, as seen from figure 7C. To determine any effect of norepinephrine on the sensitivity of SG neurons to L-glutamate, we examined whether an AMPA

(10  $\mu\text{M}$ ) response is affected by norepinephrine (50  $\mu\text{M}$ ). As seen in figure 7D, norepinephrine did not affect the peak amplitude of the AMPA response ( $88 \pm 5\%$  of control,  $n = 12$ ;  $P > 0.05$ ).

**Discussion**

The current study demonstrated that norepinephrine inhibits glutamatergic excitatory transmission to SG neu-



**Fig. 7.** Norepinephrine (50  $\mu\text{M}$ ) did not affect a sensitivity of substantia gelatinosa neurons to AMPA. (A) Chart recording of miniature excitatory postsynaptic currents (mEPSCs) in the absence and presence of norepinephrine. (B) mEPSCs, shown in an expanded time scale, in the absence (left) and presence (right) of norepinephrine for a period indicated by a mark shown below the chart recording in A. (C) Cumulative distributions of the amplitude (left) and interevent interval (right) of mEPSCs before (continuous line) and during (dotted line) 1 min after the application of norepinephrine (NE), which were obtained by analyzing 645 and 702 mEPSC events, respectively. Norepinephrine had no effect on their distributions ( $P > 0.05$ ; Kolmogorov-Smirnov test). (D) AMPA (10  $\mu\text{M}$ ) responses, obtained by its superfusion for 30 s, in the absence and presence of norepinephrine.  $V_H = -70$  mV.

rons in the spinal dorsal horn from the periphery. It was revealed here for the first time that both of A $\delta$  and C primary afferent transmission are depressed by norepinephrine, although Travagli and Williams<sup>24</sup> had not examined the action of norepinephrine on each of their transmissions to SG neurons of the spinal trigeminal nucleus. Both actions of norepinephrine were due to the activation of  $\alpha_2$  adrenoceptors, as reported for evoked EPSCs in spinal trigeminal SG neurons.<sup>24</sup> This result may be consistent with the observations that an antinociceptive effect of either intrathecally administered norepinephrine or electrical stimulation of sites near the A7 cell group and also of the periaqueductal gray is due to the activation of  $\alpha_2$  adrenoceptors.<sup>4,14,16,17</sup> Although the  $\alpha_2$  adrenoceptors are subdivided into  $\alpha_{2A}$ ,  $\alpha_{2B}$ , and  $\alpha_{2C}$  receptors,<sup>34</sup> the  $\alpha_2$  action in the current study appears to be due to the activation of the  $\alpha_{2A}$  type because an  $\alpha_{2A}$  adrenoceptor agonist, oxymetazoline,<sup>34</sup> reduced the A $\delta$  and C afferent transmission. This idea is supported by a report of Stone *et al.*<sup>35</sup> which demonstrated that a mouse expressing a point mutation in the  $\alpha_{2A}$  receptor was without  $\alpha_2$  agonist-mediated spinal analgesia in the tail-flick test. Although the activation of  $\beta$  adrenoceptors is known to enhance neurotransmitter release in many types of neurons, including sympathetic ganglion neurons,<sup>36</sup> this was not the case in SG neurons because isoproterenol did not affect the transmission.

Since norepinephrine did not affect mEPSC amplitude and the sensitivity of SG neurons to AMPA, its actions on evoked EPSCs were presynaptic in origin. Since these actions were examined for monosynaptic transmission, the norepinephrine actions are suggested to be due to the activation of  $\alpha_2$ , possibly  $\alpha_{2A}$ , adrenoceptors existing in primary afferent central terminals. Consistent with this idea, an inhibition by norepinephrine of the release of L-glutamate from rat spinal cord synaptosomes is mediated by  $\alpha_2$  adrenoceptors,<sup>37</sup> and the  $\alpha_{2A}$  adrenoceptor is expressed in primary afferent terminals in the rat.<sup>20</sup> The action on C-fiber transmission in the current study may be consistent with the observation that capsaicin-induced release of L-glutamate from spinal cord synaptosomes is reduced by the activation of the  $\alpha_{2A}$  adrenoceptor<sup>38</sup> because capsaicin is known to excite C fibers. Although a cellular mechanism for the norepinephrine action was not examined here, this would be due to an inhibition of voltage-gated Ca<sup>2+</sup> channels existing in nerve terminals because norepinephrine reduces Ca<sup>2+</sup>-channel currents in many types of neurons, including dorsal root ganglion neurons.<sup>39</sup> There was a dissociation between the actions of norepinephrine on evoked EPSC and mEPSC in that the former was presynaptically depressed, while mEPSC frequency was unaffected, an observation different from that in spinal trigeminal SG neurons.<sup>24</sup> One explanation for this discrepancy may be that norepinephrine acts differentially on evoked and spontaneous transmitter release mechanisms in the spi-

nal dorsal horn, resulting in a distinct action on their EPSCs, because each of the releases in the SG is suggested to be mediated by different types of Ca<sup>2+</sup> channels.<sup>40</sup> Alternatively, mEPSCs in spinal cord SG neurons may be produced by inputs not only from primary afferent fiber axon but also from interneuron axon, the former terminals having more  $\alpha_2$  adrenoceptors than the latter ones, where mEPSCs mainly originate from interneuron terminals. This idea may be consistent with the observation that many  $\alpha_{2A}$  adrenoceptors are colocalized in the spinal dorsal horn with neuropeptides, which are contained in primary afferent fibers.<sup>20</sup> A similar discrepancy between spontaneous and evoked transmission has been seen in the actions of a  $\mu$ -opioid receptor agonist<sup>31</sup> and anandamide<sup>28</sup> in spinal cord SG neurons. Although Pan *et al.*<sup>41</sup> have very recently reported a clonidine-induced decrease in mEPSC frequency in SG neurons, a discrepancy between this and the current study may be due to the fact that different SG neurons were tested because they examined neurons in the outer layer of SG, while we investigated neurons located at the center of SG. The possibility cannot be ruled out that SG neurons exhibiting no effect of norepinephrine on mEPSCs in the current study (where the blind patch clamp technique was used) had located in the inner layer of SG because visually identified neurons in the inner layer of SG appeared to be without actions of clonidine on mEPSCs.<sup>41</sup>

The current study revealed for the first time that norepinephrine inhibits A $\delta$ -fiber transmission more effectively than C-fiber transmission. There are two possible explanations for this result. One is that  $\alpha_2$  adrenoceptors are more densely expressed in A $\delta$ -fiber than C-fiber terminals in the spinal dorsal horn. The other is that there is a different type of  $\alpha_2$  adrenoceptors in each of the A $\delta$ -fiber and C-fiber terminals, although oxymetazoline could not discriminate between them in the extent of inhibition. This remains to be examined by using other agents regarding subtypes of  $\alpha_2$  adrenoceptors. The action of norepinephrine was the same as that of anandamide<sup>28</sup> but different from those of baclofen<sup>25</sup> and nociceptin<sup>27</sup> in that A $\delta$ -fiber transmission was more sensitive than C-fiber transmission. It is suggested that norepinephrine as well as anandamide may inhibit fast-conducting transmission more potently than slow-conducting pain transmission.

In conclusion, the current study provides a cellular basis for the antinociceptive action of norepinephrine through a mechanism in primary afferent terminals at the spinal cord level. Although norepinephrine may contribute to a prolongation of analgesia through its vasoconstrictive action in spinal anesthesia, the current finding of the inhibition of excitatory transmission supports its role as an important negative modulator of pain transmission to SG neurons together with a norepinephrine-induced hyperpolarization<sup>21,22</sup> and enhancement of inhibitory transmission.<sup>23</sup>



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