

# Action of dexmedetomidine on the substantia gelatinosa neurons of the rat spinal cord

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## Abstract

Dexmedetomidine is a highly specific, potent and selective  $\alpha_2$ -adrenoceptor agonist. Although intrathecal and epidural administration of dexmedetomidine has been found to produce analgesia, whether this analgesia results from an effect on spinal cord substantia gelatinosa (SG) neurons remains unclear. Here, we investigated the effects of dexmedetomidine on postsynaptic transmission in SG neurons of rat spinal cord slices using the whole-cell patch-clamp technique. In 92% of the SG neurons examined ( $n = 84$ ), bath-applied dexmedetomidine induced outward currents at  $-70$  mV in a concentration-dependent manner, with the value of effective concentration producing a half-maximal response ( $0.62 \mu\text{M}$ ). The outward currents induced by dexmedetomidine were suppressed by the  $\alpha_2$ -adrenoceptor antagonist yohimbine, but not by prazosin, an  $\alpha_1$ -,  $\alpha_{2B}$ - and  $\alpha_{2C}$ -adrenoceptor antagonist. Moreover, the dexmedetomidine-induced currents were partially suppressed by the  $\alpha_{2C}$ -adrenoceptor antagonist JP-1302, while simultaneous application of JP-1302 and the  $\alpha_{2A}$ -adrenoceptor antagonist BRL44408 abolished the current completely. The action of dexmedetomidine was mimicked by the  $\alpha_{2A}$ -adrenoceptor agonist oxymetazoline. Plots of the current–voltage relationship revealed a reversal potential at around  $-86$  mV. Dexmedetomidine-induced currents were blocked by the addition of GDP- $\beta$ -S [guanosine-5'-*O*-(2-thiodiphosphate)] or  $\text{Cs}^+$  to the pipette solution. These findings suggest that dexmedetomidine hyperpolarizes the membrane potentials of SG neurons by G-protein-mediated activation of  $\text{K}^+$  channels through  $\alpha_{2A}$ - and  $\alpha_{2C}$ -adrenoceptors. This action of dexmedetomidine might contribute, at least in part, to its antinociceptive action in the spinal cord.

## Introduction

$\alpha_2$ -Adrenoceptor agonists mediate a number of physiological phenomena such as antinociception (Yaksh, 1985). They produce an antinociceptive effect by action on the locus ceruleus (Guo *et al.*, 1996) and spinal cord (Yaksh, 1985; Takano & Yaksh, 1991). Clonidine was the first  $\alpha_2$ -adrenoceptor agonist to be introduced clinically. It has analgesic properties when given epidurally or intrathecally in humans. Epidural clonidine was found to be effective in reducing both postoperative pain (Anzai & Nishikawa, 1995) and intractable neuropathic cancer pain (Eisenach *et al.*, 1995). Intrathecal clonidine also decreased postoperative pain (Filos *et al.*, 1994). Dexmedetomidine is another  $\alpha_2$ -adrenoceptor agonist. Intravenous administration of dexmedetomidine has been used clinically for sedation in the intensive care unit (Venn & Grounds, 2001). Meanwhile, several lines of evidence indicate that administration of dexmedetomidine produces spinal analgesia as efficiently as clonidine. Behavioral studies in rats have demonstrated an inhibition of nociceptive responses by intrathecally (Fisher *et al.*, 1991) and epidurally (Asano *et al.*, 2000) administered dexmedetomidine.  $\alpha_2$ -Adrenoceptor agonists are also used in as adjuncts to general anesthesia. The effect of oral clonidine premedication can reduce volatile anesthetic (Ghignone *et al.*, 1987) and opioid (Ghignone *et al.*,

1986) requirements in the perioperative period. Intravenous administration of dexmedetomidine similarly diminishes the volatile anesthetic (Aho *et al.*, 1992) and opioid (But *et al.*, 2006) requirements.

Although both clonidine and dexmedetomidine are  $\alpha_2$ -adrenoceptor agonists, several differences exist between the two. First, the potency of dexmedetomidine is much greater than that of clonidine at maximum efficacy. Intraperitoneal administration of clonidine decreased minimum alveolar concentration, which produces immobility in 50% of subjects exposed to a noxious stimulus, for halothane about 40% in rats, whereas minimum alveolar concentration was decreased about 90% by dexmedetomidine (Maze *et al.*, 1987; Segal *et al.*, 1988). A second difference lies in the relative  $\alpha_2/\alpha_1$  selectivity ratio. The  $\alpha_2/\alpha_1$ -adrenoceptor selectivity ratio of clonidine is 220 : 1 (Virtanen *et al.*, 1988). Accordingly, high doses of clonidine may induce cardiovascular side-effects via the  $\alpha_1$ -adrenoceptor (Virtanen *et al.*, 1988). The  $\alpha_2/\alpha_1$ -adrenoceptor selectivity ratio of dexmedetomidine is 1620 : 1 (Virtanen *et al.*, 1988). As dexmedetomidine is a highly selective  $\alpha_2$ -adrenoceptor agonist (Savola & Virtanen, 1991), dexmedetomidine causes fewer side-effects mediated by  $\alpha_1$ -adrenoceptor activation than clonidine.

Neurons of the superficial dorsal horn, especially lamina II (substantia gelatinosa: SG), are thought to play an important role in modulating nociceptive transmission, because they preferentially receive thin myelinated A $\delta$ - and unmyelinated C-primary afferent fibers, both of which carry nociceptive information from the periphery (Kumazawa & Perl, 1978; Yoshimura & Jessell, 1989). Moreover, binding and immunohistochemical studies show that the highest density of  $\alpha_2$ -adrenoceptors is in the superficial layers of the spinal

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dorsal horn (Roudet *et al.*, 1994; Stone *et al.*, 1998). However, whether dexmedetomidine exerts its effect on SG neurons remains unclear. In the present study, we used whole-cell patch-clamp recording to clarify whether dexmedetomidine has postsynaptic effects on SG neurons and to confirm the involvement of  $\alpha_2$ -adrenoceptors.

## Materials and methods

This study was approved by the Animal Research Committee of Niigata University Graduate School of Medical and Dental Sciences in Niigata, Japan. All efforts were made to minimize the number of animals used.

### Spinal cord slice preparation

Male Wistar rats (4–8 weeks old) were anesthetized with urethane (1.5 g/kg, i.p.). The lumbosacral segment of the spinal cord was removed as described previously (Yoshimura & Nishi, 1993; Kohno *et al.*, 2003) and placed in a preoxygenated ice-cold (1–3°C) sucrose-substituted Krebs solution containing (in mM): KCl 6.4, MgSO<sub>4</sub> 4.1, NaHCO<sub>3</sub> 26, NaH<sub>2</sub>PO<sub>4</sub> 1.3, glucose 10 and sucrose 252, bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub> (Wang *et al.*, 1999). Transverse spinal cord slices (500  $\mu$ m) were cut on a vibrating microslicer. The slices were placed on a nylon mesh in a recording chamber, and then perfused at a rate of 10 ml/min with normal Krebs solution containing (in mM): NaCl 117, KCl 3.6, CaCl<sub>2</sub> 2.5, MgCl<sub>2</sub> 1.2, Na<sub>2</sub>HPO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25, glucose 11, at 36  $\pm$  1°C for at least 1 h prior to recordings.

### Electrophysiological recordings

The lamina II was clearly discernible as a relatively translucent band across the dorsal horn under a dissecting microscope with transmitted illumination. Blind whole-cell patch-clamp recordings were made from SG neurons in voltage clamp mode using patch-pipette electrodes with a resistance of 5–10 M $\Omega$  (Yoshimura & Nishi, 1993; Kohno *et al.*, 2003). Two pipette solutions were used. The first solution contained (in mM): potassium gluconate 135, KCl 5, CaCl<sub>2</sub> 0.5, MgCl<sub>2</sub> 2, EGTA 5, HEPES 5 and ATP-Mg 5 (pH 7.2). Guanosine-5'-*O*-(2-thiodiphosphate) (GDP- $\beta$ -S, 2 mM) was used as a GTP-binding protein blocker when necessary. The second solution contained (in mM): Cs<sub>2</sub>SO<sub>4</sub> 110, tetraethylammonium (TEA) 5, CaCl<sub>2</sub> 0.5, MgCl<sub>2</sub> 2, EGTA 5, HEPES 5 and ATP-Mg 5 (pH 7.2). Cs and TEA were used as K<sup>+</sup> channel blockers. Membrane currents were amplified using an Axopatch 200B amplifier (Molecular Devices). Signals were filtered at 2 kHz and digitized at 5 kHz. Data were collected and analysed using pClamp9.0 software (Molecular Devices).

### Application of drugs

Drugs were applied by superfusion without alteration of the perfusion rate or temperature. The drugs used were dexmedetomidine (provided by Abbott Japan), prazosin, yohimbine, tetrodotoxin (TTX) (Wako, Japan), oxymetazoline, GDP- $\beta$ -S, acridin-9-yl-[4-(4-methylpiperazin-1-yl)-phenyl]amine (JP-1302) (Sigma-Aldrich, USA), 2-[[4,5-dihydro-1H-imidazol-2-yl)methyl]-2,3-dihydro-1-methyl-1H-isoindole maleate (BRL44408) (Tocris Bioscience, USA).

### Data analysis

Numerical data are expressed as means  $\pm$  SEM. Statistical significance was assessed as  $P < 0.05$  using the paired Student's *t*-test. In all

cases, *n* refers to the number of neurons studied. Relative peak amplitude was calculated as an amplitude of dexmedetomidine-induced current in the presence of the drugs, divided by that of currents produced by dexmedetomidine alone. The continuous curve for the concentration–response relationship of dexmedetomidine was drawn according to the following Hill equation:  $y = 1.3/[1 + (EC_{50}/x)^b]$ , where *x* is the dexmedetomidine concentration and *b* is the Hill coefficient.

## Results

### Dexmedetomidine induces currents in dorsal horn neurons

The resting membrane potentials in the SG neurons were  $-65.8 \pm 0.8$  mV ( $n = 17$ ). In 92% of the SG neurons examined ( $n = 84$ ), bath-applied dexmedetomidine induced outward currents at  $-70$  mV. As shown in Fig. 1A, outward currents exhibited a clear dose-dependency on dexmedetomidine perfused at the surface of the spinal cord. The onset of the responses became slow and recovery was delayed with increasing concentrations of dexmedetomidine. Fig. 1B shows a concentration–response curve of the dexmedetomidine-induced outward currents. Analysis of the curve gave 0.62  $\mu$ M as the effective concentration producing a half-maximal response ( $EC_{50}$ ) with a Hill coefficient of 1.34. A higher concentration of dexmedetomidine produced a long duration at a holding potential of  $-70$  mV (Fig. 1A) and it was difficult to be applied repeatedly. Consequently, we used a low concentration of dexmedetomidine (0.03  $\mu$ M) in order to reproduce responses when it was repeatedly applied. In addition, the dexmedetomidine (0.03  $\mu$ M)-induced outward currents could not be observed at a holding potential of  $-70$  mV, as shown in Fig. 1B. Therefore, we recorded the currents at a holding potential of  $-40$  mV (Fig. 1C and D). To confirm that the dexmedetomidine-induced currents were postsynaptic effects, we examined the currents in the presence of TTX to remove any possible influence of  $\alpha_2$ -adrenoceptors on presynaptic terminals. We compared peak amplitude elicited by dexmedetomidine (0.03  $\mu$ M) in the absence and presence of TTX (1  $\mu$ M). TTX had no significant effect on the amplitude of dexmedetomidine (Fig. 1C and D,  $96.2 \pm 1.3\%$  of control,  $P = 0.91$ ,  $n = 5$ ).

### Pharmacological analysis of dexmedetomidine-induced responses

We examined the effects on dexmedetomidine-induced responses of the antagonists prazosin (2  $\mu$ M) and yohimbine (4  $\mu$ M), as compared with those of previous studies (prazosin 0.5–2  $\mu$ M, yohimbine 1–4  $\mu$ M) (Baba *et al.*, 2000; Kawasaki *et al.*, 2003; Sonohata *et al.*, 2004). The dexmedetomidine-induced outward currents were attenuated by the application of the  $\alpha_2$ -adrenoceptor antagonist yohimbine (4  $\mu$ M), while the  $\alpha_1$ -adrenoceptor antagonist prazosin (2  $\mu$ M) had no significant effect (Fig. 2A). Figure 2B shows a summary of the suppressive effects of each adrenergic antagonist. We next examined the effects on dexmedetomidine-induced responses using a lower concentration of prazosin (0.2  $\mu$ M) and yohimbine (0.4  $\mu$ M). However, these antagonists did not have any effect on the dexmedetomidine-induced outward currents (prazosin,  $93.3 \pm 4.1\%$  of control,  $P = 0.71$ ; yohimbine,  $91.0 \pm 7.4\%$  of control,  $P = 0.88$ ,  $n = 5$ ). These results suggested that the dexmedetomidine-induced outward current was mediated by  $\alpha_2$ -adrenoceptors.

Application of a high concentration of dexmedetomidine (3  $\mu$ M) produced an outward current of long duration. Superfusing yohimbine (4  $\mu$ M) alone accelerated the recovery of outward currents to baseline level (Fig. 2C and D), confirming that the dexmedetomidine-induced

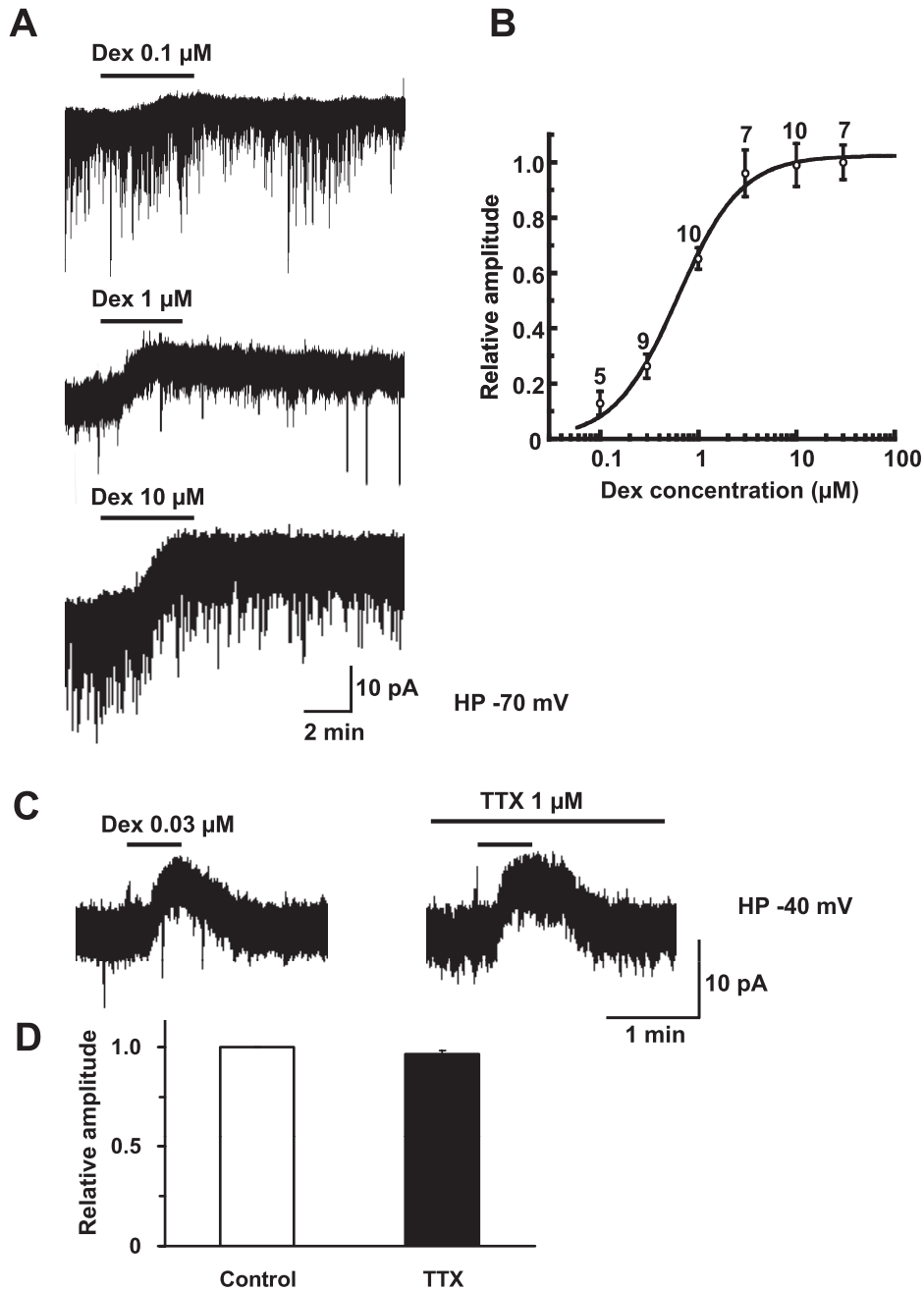


FIG. 1. Dexmedetomidine induces an outward current in a concentration-dependent manner in SG neurons. (A) Outward currents induced by dexmedetomidine (0.1, 1 and 10 μM). Duration of drug superfusion is shown by horizontal bars above chart recordings. Holding potential = -70 mV. (B) Relative peak amplitude was calculated as an amplitude of the dexmedetomidine (0.1–30 μM)-induced current divided by that of the current produced by dexmedetomidine (30 μM). The continuous curve was drawn according to the Hill plot with an EC<sub>50</sub> value of 0.62 μM (95% confidence interval, 0.51–0.77 μM) and a Hill coefficient of 1.34. The number next to each point denotes the number of neurons examined. Holding potential = -70 mV. (C) Outward currents elicited by dexmedetomidine (0.03 μM) in the absence and presence of TTX (1 μM). These currents were obtained from the same neuron (n = 5). Holding potential = -40 mV. (D) Relative peak amplitude was calculated as an amplitude of dexmedetomidine-induced current in the presence of TTX (5.3 ± 1.3 pA) divided by that of currents produced by dexmedetomidine (5.6 ± 1.4 pA) without TTX.

current was mediated by α<sub>2</sub>-adrenoceptors. Furthermore, to examine the involvement of α<sub>2A</sub>- and α<sub>2C</sub>-adrenoceptors on the dexmedetomidine-induced current, we used α<sub>2A</sub>- and α<sub>2C</sub>-adrenoceptor subtype-preferring antagonists (Fig. 2E and F). Application of the α<sub>2C</sub>-adrenoceptor antagonist JP-1302 (5 μM) partially suppressed the dexmedetomidine (0.03 μM)-induced current. In addition, it was markedly abolished when dexmedetomidine was again applied in the presence of JP-1302 (5 μM) and the α<sub>2A</sub>-adrenoceptor antagonist

BRL44408 (5 μM). Relative peak amplitude was reduced to 3.3 ± 0.3 pA in the presence of JP-1302 and to 0.1 ± 0.1 pA in the presence of JP-1302 plus BRL44408 (dexmedetomidine alone, 6.9 ± 0.6 pA, Fig. 2F). We next used oxymatazoline, an α<sub>2A</sub>-adrenoceptor agonist (Miyazaki *et al.*, 1998; Kawasaki *et al.*, 2003). In nine of ten neurons, oxymatazoline (10 μM) induced an outward current similar to that of dexmedetomidine (Fig. 2G) with a peak amplitude of 10.2 ± 1.0 pA (n = 9). This result suggested that α<sub>2A</sub>-adrenoceptors

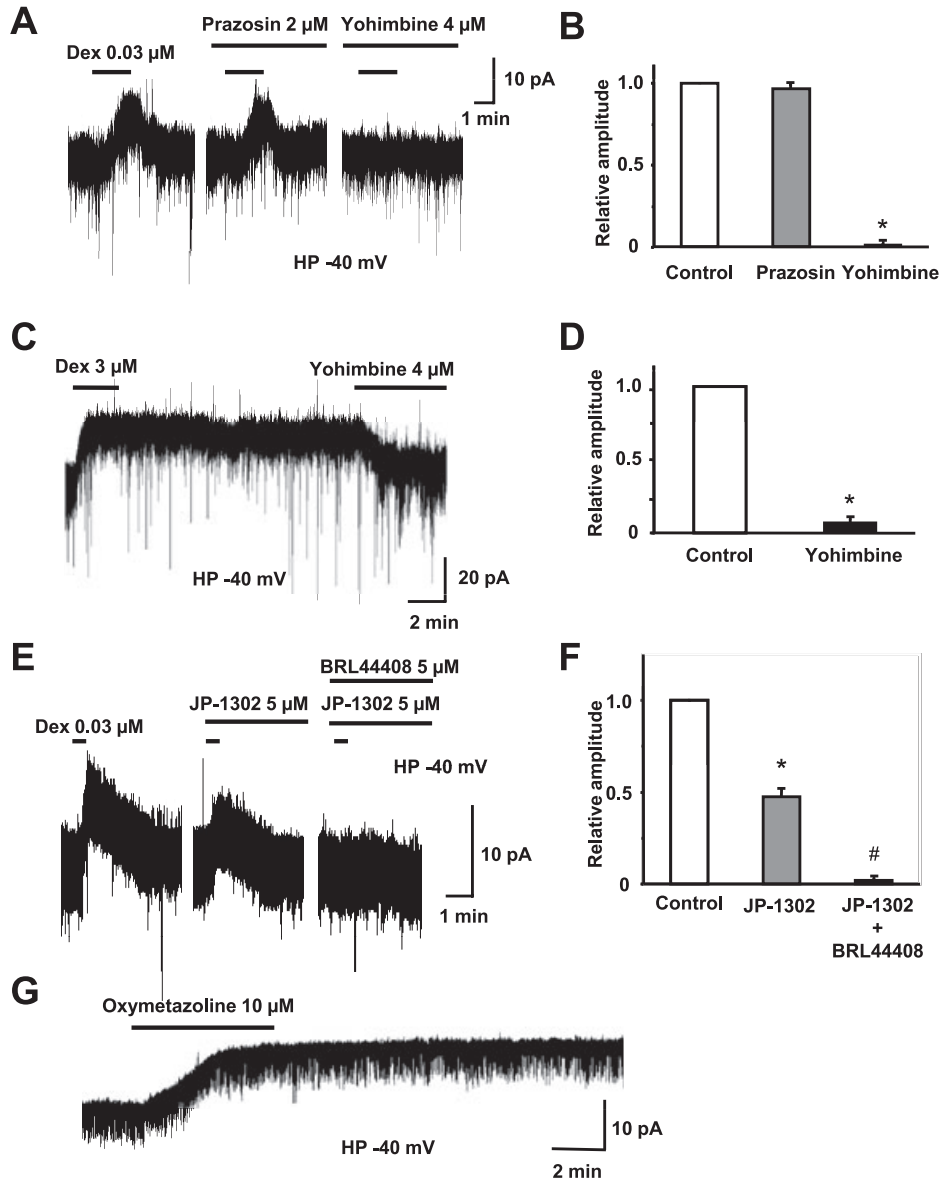


FIG. 2. Dexmedetomidine-induced currents are mediated by  $\alpha_2$ - ( $\alpha_{2A}$ - and  $\alpha_{2C}$ -) but not by  $\alpha_1$ -adrenoceptors. (A) Prazosin (2  $\mu$ M), an  $\alpha_1$ -adrenoceptor antagonist, did not affect the amplitude of the outward current induced by dexmedetomidine (0.03  $\mu$ M), whereas the currents were suppressed by application of yohimbine (4  $\mu$ M), an  $\alpha_2$ -adrenoceptor antagonist. These currents were obtained from the same neuron ( $n = 6$ ). Holding potential = -40 mV. (B) Relative peak amplitude was calculated as an amplitude of dexmedetomidine-induced current in the presence of prazosin ( $8.7 \pm 2.2$  pA) or yohimbine ( $0.1 \pm 0.1$  pA) divided by that of currents produced by dexmedetomidine ( $9.3 \pm 2.6$  pA) alone.  $*P < 0.05$ , control vs. yohimbine. (C) Dexmedetomidine (3  $\mu$ M) produced an outward current of long duration. A persistent outward current after washout of dexmedetomidine was completely reduced in amplitude by yohimbine (4  $\mu$ M). Holding potential = -40 mV. (D) The dexmedetomidine-induced outward currents in the presence of yohimbine were significantly smaller than control ( $*P < 0.05$ ,  $n = 10$ ). (E) Application of JP-1302 (5  $\mu$ M) partially blocked the dexmedetomidine (0.03  $\mu$ M)-induced current, and simultaneous application of JP-1302 (5  $\mu$ M) and BRL44408 (5  $\mu$ M) abolished the current completely. These currents were obtained from the same neuron ( $n = 6$ ). Holding potential = -40 mV. (F) Relative peak amplitude was calculated as an amplitude of dexmedetomidine-induced current in the presence of JP-1302 ( $3.3 \pm 0.3$  pA) or JP-1302 plus BRL44408 ( $0.1 \pm 0.1$  pA) divided by that of currents produced by dexmedetomidine ( $6.9 \pm 0.6$  pA) alone.  $*P < 0.05$ , control vs. JP-1302.  $\#P < 0.05$ , control vs. JP-1302 plus BRL44408. (G) Dexmedetomidine-induced outward current was mimicked by the  $\alpha_{2A}$ -adrenoceptor agonist oxymetazoline (10  $\mu$ M) on SG neurons. Holding potential = -40 mV.

were present and activatable on SG neurons. These findings indicated that both  $\alpha_{2A}$ - and  $\alpha_{2C}$ -adrenoceptors were involved in the dexmedetomidine-induced current.

#### Dexmedetomidine activates $K^+$ channels through the activation of G-proteins on SG neurons

We next investigated what kinds of channels mediate the dexmedetomidine current. Figure 3A shows the dexmedetomidine

(3  $\mu$ M)-induced currents recorded at different holding potentials. Plots of the current-voltage relationship revealed a reversal potential at around -86 mV (Fig. 3B), which is slightly different from the equilibrium potential (-97 mV) of  $K^+$ , as calculated from the Nernst equation using  $K^+$  concentrations ( $[K^+]_o$ , 3.6 mM;  $[K^+]_i$ , 140 mM) of the solutions. This slight difference might be considered to reflect a liquid junction potential (9–10 mV) existing between the Krebs and patch-pipette solutions (Yajiri *et al.*, 1997). Superfusing dexmedetomidine opens  $K^+$  channels on postsynaptic

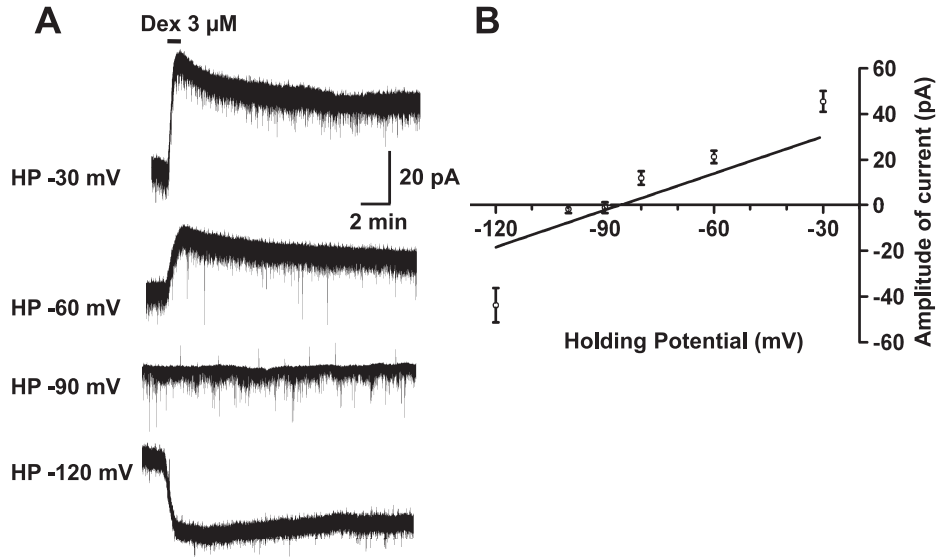


FIG. 3. (A) Dexmedetomidine-induced currents show voltage dependency. (B) Relationship between holding potential and current amplitude. The regression equation was  $y = 0.901x + 77.1$  [ $R^2 = 0.92$ ,  $P = 0.002$ ,  $y =$  amplitude of current (pA),  $x =$  holding potential (mV)].

SG neurons, generating an outward current at holding potentials below  $-90$  mV.

To determine whether G-proteins are responsible for the dexmedetomidine-induced current, GDP- $\beta$ -S (2 mM), a non-hydrolysable analog of GDP that competitively inhibits G-proteins, was used in the pipette solution to prevent the postsynaptic activation of  $\alpha_2$ -adrenoceptors. When dexmedetomidine (3  $\mu$ M) was applied shortly after establishing the whole-cell configuration, an outward current was

observed (Fig. 4A). This outward current was completely abolished when dexmedetomidine was again applied 15 min later (Fig. 4A,  $n = 5$ ). We next used the pipette solution containing  $\text{Cs}^+$  and TEA to inhibit the postsynaptic effect of  $\text{K}^+$  channels. Dexmedetomidine (3  $\mu$ M)-induced outward current was recorded just after establishing the whole-cell configuration (Fig. 4B). However, this current was abolished after the second application of dexmedetomidine, which was performed more than 5 min later (Fig. 4B,  $n = 7$ ). These findings

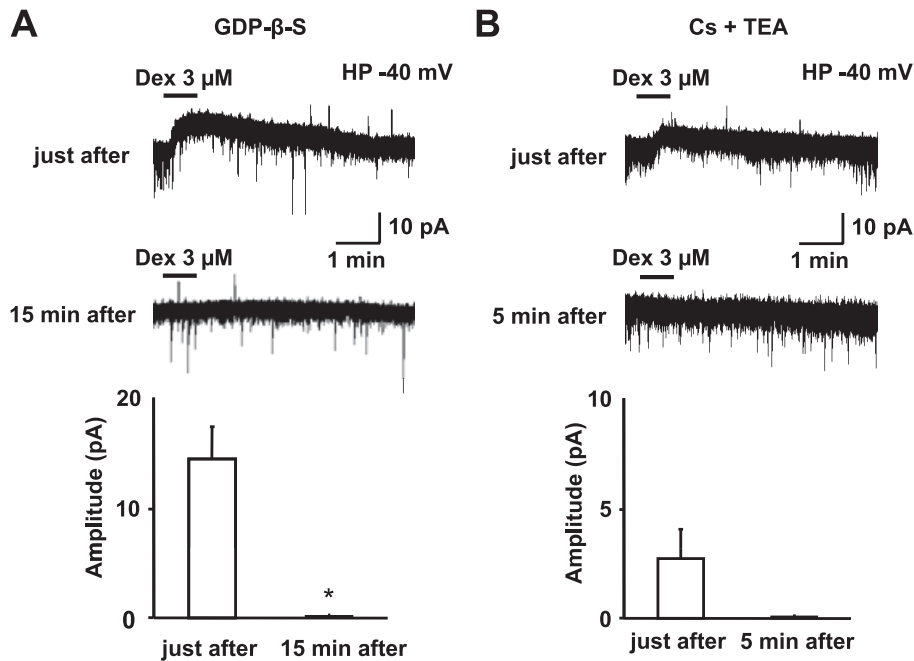


FIG. 4. Inhibition of dexmedetomidine-induced current by GDP- $\beta$ -S or  $\text{Cs}^+$ /TEA. (A) The dexmedetomidine (3  $\mu$ M)-induced outward current was examined with K-gluconate pipette solution containing GDP- $\beta$ -S (2 mM). Dexmedetomidine produced an outward current just after establishing the whole-cell configuration, but this was markedly abolished when dexmedetomidine was again applied 15 min later ( $*P < 0.05$ ,  $n = 5$ ). Holding potential =  $-40$  mV. (B) Dexmedetomidine (3  $\mu$ M) induced an outward current just after establishing whole-cell recording with  $\text{Cs}^+$  and TEA-containing pipette. When dexmedetomidine was again applied 5 min later, the current was completely abolished ( $n = 7$ ). Holding potential =  $-40$  mV.

suggested that the dexmedetomidine-induced outward current was mediated by  $K^+$  channels through the activation of G-proteins.

#### Effects of dexmedetomidine on spontaneous excitatory postsynaptic currents

We next examined the effects of dexmedetomidine on spontaneous excitatory postsynaptic currents (sEPSCs) in SG neurons (Fig. 5A). Amplitude and inter-event interval distributions were not changed by dexmedetomidine (Fig. 5B; amplitude,  $P > 0.05$ , Kolmogorov–Smirnov test; inter-event interval,  $P > 0.05$ ). These data indicate that dexmedetomidine does not affect glutamate release from presynaptic terminals.

#### Discussion

Intrathecal dexmedetomidine is a highly potent antinociceptive agent in animals (Fisher *et al.*, 1991). Lipophilicity may affect the analgesic potency of intrathecally administered agents (Eisenach *et al.*, 1994).

Highly lipophilic agents bind to spinal cord more efficiently than poorly lipophilic drugs. Dexmedetomidine is more lipophilic than clonidine (Savola *et al.*, 1986). Eisenach *et al.* (1994) showed that intrathecal administration of dexmedetomidine at a dose of 100  $\mu\text{g}$  produced an antinociceptive effect that was first evident at 30 min and lasted for 90 min after the injection. As these authors demonstrated in a separate set of experiments in the same study, intrathecal administration of dexmedetomidine at this dose resulted in cerebrospinal fluid concentrations ranging from 300 to 3000 ng/ml (nearly 1–10  $\mu\text{M}$ ) during the period 30–90 min after injection. These results are consistent with the concentrations of dexmedetomidine that induced outward currents in the present study (Fig. 1B). Taken together, these data suggest that the cerebrospinal fluid concentration of dexmedetomidine required for antinociception is 1–10  $\mu\text{M}$ .

Behavioral studies in rats have demonstrated that intrathecal administration of dexmedetomidine produces dose-dependent antinociception (Fisher *et al.*, 1991). Low doses of dexmedetomidine produced a transient period of antinociception. On the other hand, profound antinociception was prolonged by high doses of dexmede-

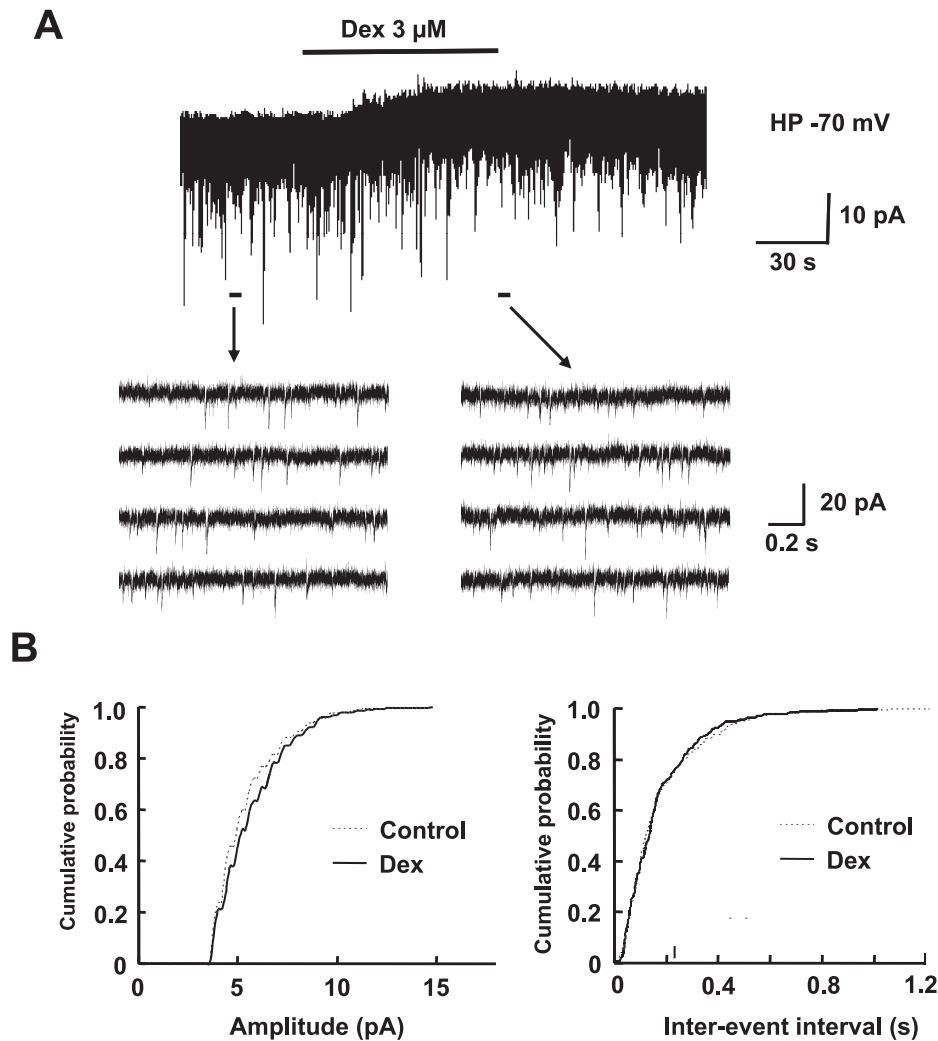


FIG. 5. Dexmedetomidine dose not affect the frequency or amplitude of spontaneous EPSCs in SG neurons. (A) Continuous chart recording of sEPSCs before and during the application of dexmedetomidine (3  $\mu\text{M}$ ) (upper trace). Four consecutive traces of sEPSCs for a period indicated by a short bar below the chart recording are shown on an expanded time scale (lower traces). Holding potential = -70 mV. (B) Cumulative distributions of the amplitude (left) and the inter-event interval (right) of sEPSCs before (dashed line) and during the application of dexmedetomidine (continuous line). Dexmedetomidine had no significant effect on the amplitude ( $P > 0.05$ ; Kolmogorov–Smirnov test) or the inter-event interval distribution ( $P > 0.05$ ).

tomidine. For example, a transient current was evoked by a low concentration of dexmedetomidine (0.03  $\mu\text{M}$ ) in the present study (Fig. 2A). By contrast, a high concentration of dexmedetomidine (3  $\mu\text{M}$ ) produced a current with a long duration (Fig. 2C). The durations of dexmedetomidine (3  $\mu\text{M}$ )-induced currents were longer than 30 min in most neurons without desensitization. The higher the concentration of dexmedetomidine, the longer the duration tended to be. The durations of dexmedetomidine-induced outward currents were much longer than those induced by noradrenaline (Sonohata *et al.*, 2004). This long duration may be caused by the high affinity of dexmedetomidine. A radioligand binding study showed that the binding affinity (pKi) value of dexmedetomidine was higher than that of noradrenaline for the  $\alpha_2$ -adrenoceptor (Jasper *et al.*, 1998). Alternatively, the long-term effect may be due to a slow rate of diffusion of dexmedetomidine out of the spinal cord slice (Koga *et al.*, 2005). This issue remains to be established.

As mentioned above, superficial dorsal horn neurons are important for processing nociceptive information from primary afferent fibers. Binding and immunohistochemical studies have suggested that the highest density of  $\alpha_2$ -adrenoceptors exist in the superficial layers of the spinal dorsal horn (Roudet *et al.*, 1994; Stone *et al.*, 1998). In addition, the binding sites of the  $\alpha_{2A}$ -adrenoceptors in the superficial dorsal horn were dramatically reduced in number after neonatal capsaicin treatment or dorsal rhizotomy, whereas  $\alpha_{2C}$ -adrenoceptors were not significantly reduced by either of these treatments. This finding suggests that  $\alpha_{2C}$ -adrenoceptors exist in postsynaptic sites (Stone *et al.*, 1998; Olave & Maxwell, 2002), and that  $\alpha_{2A}$ -adrenoceptors are located mainly in presynaptic sites (Stone *et al.*, 1998; Kawasaki *et al.*, 2003) of the dorsal horn. By contrast, few  $\alpha_{2B}$ -adrenoceptors were seen in the spinal dorsal horn by *in situ* hybridization studies of the distribution of  $\alpha_{2B}$  mRNA (Nicholas *et al.*, 1993; Shi *et al.*, 1999). Furthermore, an  $\alpha_2$ -adrenoceptor agonist did not change spinal antinociception in  $\alpha_{2B}$ -adrenoceptor knockout mice, suggesting that  $\alpha_{2B}$ -adrenoceptors do not participate in the antinociceptive effect in the spinal cord (Fairbanks *et al.*, 2002). However, these immunohistochemical studies showed a dramatic, but incomplete, reduction in  $\alpha_{2A}$ -adrenoceptor immunoreactivity. This result raised the question of whether  $\alpha_{2A}$ -adrenoceptors were also located on postsynaptic SG neurons, in addition to  $\alpha_{2C}$ -adrenoceptors. We used prazosin as the  $\alpha_1$ -adrenoceptor antagonist, but prazosin acts not only as an  $\alpha_1$ -adrenoceptor, but also has some properties of  $\alpha_{2B}$ - and  $\alpha_{2C}$ -adrenoceptor antagonists (Bylund, 1988; MacDonald *et al.*, 1997). The binding inhibition coefficients (Ki) of prazosin were 2750, 108 and 98 nM for the  $\alpha_{2A}$ -,  $\alpha_{2B}$ - and  $\alpha_{2C}$ -adrenoceptor, respectively (Marjamaki *et al.*, 1993). Nevertheless, our study showed that dexmedetomidine-induced currents were not blocked by the  $\alpha_{2B}$ - and  $\alpha_{2C}$ -adrenoceptor antagonistic action of prazosin in SG neurons (Fig. 2A and B). Therefore, highly selective  $\alpha_{2A}$ - or  $\alpha_{2C}$ -adrenoceptor antagonists were superfused to investigate this question. BRL44408 was noted as a selective  $\alpha_{2A}$ -adrenoceptor antagonist having Ki of 4, 174 and 187 nM for the  $\alpha_{2A}$ -,  $\alpha_{2B}$ - and  $\alpha_{2C}$ -adrenoceptor, respectively (Bylund *et al.*, 1994). JP-1302 was recently described as a novel, highly selective  $\alpha_{2C}$ -adrenoceptor antagonist (Sallinen *et al.*, 2007). The Ki were 1500, 2200 and 16 nM for the  $\alpha_{2A}$ -,  $\alpha_{2B}$ - and  $\alpha_{2C}$ -adrenoceptor, respectively. Application of JP-1302 partially suppressed the dexmedetomidine-induced current, and simultaneous application of JP-1302 and BRL44408 abolished the amplitude of the current completely (Fig. 2E and F). Our findings clarify the involvement of both  $\alpha_{2A}$ - and  $\alpha_{2C}$ -adrenoceptors in the dexmedetomidine-induced current. Furthermore, we showed that the dexmedetomidine-induced current seen in postsynaptic SG neurons was mimicked by oxymetazoline, an  $\alpha_{2A}$ -adrenoceptor agonist

(Fig. 2G). The Ki were 6, 3150 and 180 nM for the  $\alpha_{2A}$ -,  $\alpha_{2B}$ - and  $\alpha_{2C}$ -adrenoceptor, respectively (Marjamaki *et al.*, 1993). Taking these data together, it is likely that dexmedetomidine activates both  $\alpha_{2A}$ - and  $\alpha_{2C}$ -adrenoceptors on postsynaptic SG neurons and induces currents. As we did not examine the application of an  $\alpha_{2C}$ -adrenoceptor agonist, we could not conclude whether there were  $\alpha_{2C}$ -adrenoceptors in postsynaptic SG neurons. Further studies are required with highly selective  $\alpha_{2A}$ - or  $\alpha_{2C}$ -adrenoceptor agonists and antagonists.

We showed that activation of  $\alpha_2$ -adrenoceptors with dexmedetomidine resulted in outward currents that were mediated by activation of  $\text{K}^+$  channels through the activation of G-proteins (Figs 3 and 4). These findings suggest that dexmedetomidine hyperpolarizes the membrane potentials of SG neurons by opening G-protein-coupled inwardly rectifying potassium (GIRK) channels. The GIRK1 and GIRK2 subunits are concentrated in lamina II of the mouse spinal cord (Marker *et al.*, 2005). As the antinociceptive effect of clonidine was reduced in GIRK2-knockout mice (Mitrovic *et al.*, 2003), it is possible that dexmedetomidine exerts its effects on GIRK channels similarly to clonidine.

We focussed on the postsynaptic effects of dexmedetomidine in SG neurons. However,  $\alpha_2$ -adrenoceptors are also localized on primary afferent terminals (Stone *et al.*, 1998). Noradrenaline acts on  $\alpha_2$ -adrenoceptors on postsynaptic (Sonohata *et al.*, 2004) and presynaptic (Kawasaki *et al.*, 2003) SG neurons, and produces an antinociceptive effect. Kawasaki *et al.* (2003) showed that noradrenaline, clonidine and oxymetazoline inhibited the peak amplitudes of monosynaptically evoked A $\delta$ - and C-fiber EPSCs, while miniature EPSC (mEPSC) amplitude and frequency was unaffected by noradrenaline. Pan *et al.* (2002) reported that the peak amplitude of evoked EPSCs was attenuated by clonidine in the outer zone of SG. Furthermore, clonidine significantly decreased the frequency of mEPSCs. A discrepancy between this and the current study may be due to the fact that different SG neurons were tested; Pan *et al.* 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 noradrenaline on mEPSCs (Kawasaki *et al.*, 2003) or no effect of dexmedetomidine on sEPSCs 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 (Pan *et al.*, 2002). Li & Zhuo (2001) showed that clonidine inhibited glutamate-mediated evoked EPSCs. However, clonidine preferentially depressed polysynaptic but not monosynaptic A $\delta$ -fiber-evoked field potentials in superficial spinal dorsal horn (Ruscheweyh & Sandkuhler, 2000). Moreover,  $\alpha_2$ -adrenoceptor agonists depressed the NMDA receptor-mediated excitatory postsynaptic potential on A- and C-primary afferent fibers (Faber *et al.*, 1998). Our results showed that dexmedetomidine did not affect glutamate release from presynaptic terminals. Whether dexmedetomidine exhibits a similar presynaptic action on these currents needs to be established.

In conclusion, the present study suggests that dexmedetomidine hyperpolarizes the membrane potentials of spinal dorsal horn neurons via G-protein-mediated activation of  $\text{K}^+$  channels through  $\alpha_2$ -adrenoceptors. This action of dexmedetomidine might contribute, at least in part, to its antinociceptive action in the spinal cord.

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## Abbreviations

BRL44408, 2-[(4,5-dihydro-1H-imidazol-2-yl)methyl]-2,3-dihydro-1-methyl-1H-isoindole maleate; EPSCs, excitatory postsynaptic currents; GDP- $\beta$ -S, guanosine-5'-O-(2-thiodiphosphate); GIRK, G-protein-coupled inwardly rectifying potassium; JP-1302, acridin-9-yl-[4-(4-methylpiperazin-1-yl)-phenyl]amine; Ki, binding inhibition coefficient; pKi, binding affinity; SG, substantia gelatinosa; TEA, tetraethylammonium; TTX, tetrodotoxin.

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