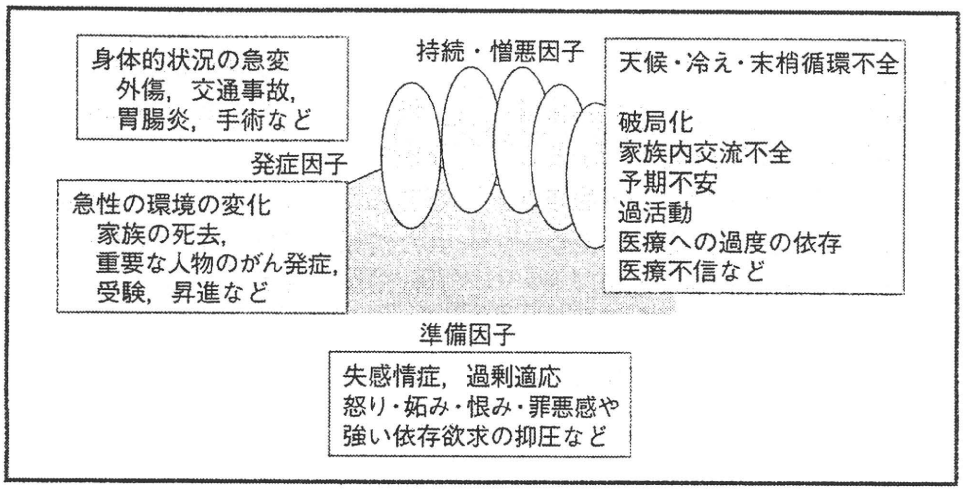

図５　慢性疼痛の症状に関する因子の具体例（文献７より引用改変）

し，患者である娘に自己犠牲を強いる傾向があった．娘が幼少期の虐待の苦悩を母に語っても，母の側に余裕がないために直面化せずに葛藤を無視し，それ以外の対応では娘に正論を迫ることが多く，患者である娘に過剰適応的，自己犠牲的行動特性が固定化していた．その中での過活動に伴う心身の疲弊から，患者の腰痛が発症・持続していた．娘は腰痛が持続する中で，本来の心理的危機感を無意識的に家族に発信していたと理解された．患者の父は患者の慢性疼痛発症後に退職し，自宅で長時間滞在するが，妻には罪悪感があり娘とは疎遠であり，「昭和燃え尽き症候群」の予備軍のような状態であった．患者の母は夫の退職に伴い，長年苦労をかけられた夫に献身的に毎食の世話をすることになり，「主人在宅ストレス症候群」の予備軍となっていた．この症例における実際の語りは，以下のようであり，家族療法を含む段階的心身医学的療法により，患者の情緒的な安定，両親との交流不全の改善，日常生活活動の拡大が得られた．

① 慢性疼痛（腰痛）患者の語り

「背負っているものは，納得したくて深く長く考えるうちに大きくなった．母に対する憎悪，あ，憎悪とかひどいですよね…．」

「母は弱虫です．弱いから，母は自分に頼ったり，我慢させたり，自分が負うべき重荷を背負わせたりしたのかも知れない」

「治るまでの道のりは長いです．あ，でもお盆に両親自分の３人で取っ組み合いの言い争いをしたら，私の痛みは治るかもしれない．」

② 慢性疼痛（腰痛）患者の母の語り

「10年ですよ，先生．この10年のあの子の仕打ち．ずっと無視．あんまり自分がつらいと逆に憎みますよ．今になって愛を求められてもわからない．」

「主人が家でごろごろしていると，ストレスが溜まるんですよ．」

「主人がまた借金をするのではないかが心配なんです．」

③ 慢性疼痛（腰痛）患者の父の語り

「若い頃，外で飲んだり遊んだりした時代は楽しかったです．今はもうできませんね」

「妻には感謝しています．でも，束縛されるのはかないません．」

６．慢性疼痛の症状に関する因子 －Narrative による情報の統合－

慢性疼痛の narrative を傾聴する際に，患者の語る多くのエピソードやそれに対する感情を，慢性疼痛の病態のどのような要因として位

置づけるかに留意しながら傾聴すると有用である．

図5に慢性疼痛の症状に関する因子の具体例を，準備因子，発症因子，持続・増悪因子として記載した[7]．このような理解は，分析的観点となるため，厳密に言うとNBMとはいえない概念になる．しかし，元来分析的である医学・医療に適応している医療スタッフの思考パターンを加味すると，実感的に理解するには有用な統合的理解と考えられる．

おわりに

慢性疼痛医療の困難さと面白さは表裏一体である．その理由は，慢性疼痛そのものの生物医学的病態の複雑さはもとより，慢性疼痛を持つ人間やその周囲の人々の生き様を視野に入れた対応により，患者やその家族の苦悩が救われるからである．また，人は究極の選択を迫られると，場合によっては身体的な痛みよりも孤独・葛藤などの社会的苦痛を避ける傾向があるということも，理解しておくことが重要である．

長年，多くの慢性疼痛患者のnarrativeを大学病院心療内科という現場で心身医学的観点で傾聴する機会がありその蓄積が得られたことは筆者の貴重な体験となっている．思い出される多くの患者が語ってくれた苦悩のプロトタイプをより多くの臨床家に理解していただき，読者の目前の患者の苦悩の理解の一助となり，患者や家族へ治療者として自然な共感が得られるようになれば幸いである．患者が強く求めているのは，積極的な傾聴により理解された実存的な生き様の苦悩についての，治療者の言語的・非言語的受容の姿勢である．

慢性疼痛の背景となるnarrativeを傾聴するなかで，本稿で言及した内容がEBMの概念になじんだ医療スタッフが，narrativeから得られた情報を統合するための考え方のヒントとなれば幸いである．

謝　辞

この総説をまとめるにあたり，船越聖子医師をはじめとして筆者が九州大学病院心療内科における慢性疼痛臨床を行う際に共同治療をしていただき，貴重な示唆をいただきました九州大学病院心療内科の多数の医師・看護師・臨床心理士の皆様方に厚く感謝いたします．

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In vivo patch-clamp analysis of dopaminergic antinociceptive actions on substantia gelatinosa neurons in the spinal cord

Wataru Taniguchi [a,b], Terumasa Nakatsuka [b,*], Nobuyuki Miyazaki [a], Hiroshi Yamada [a], Daisuke Takeda [b], Tsugumi Fujita [c], Eiichi Kumamoto [c], Munehito Yoshida [a]

[a] *Department of Orthopaedic Surgery, Wakayama Medical University, Wakayama 641-810, Japan*
[b] *Pain Research Center, Kansai University of Health Sciences, Kumatori, Osaka 590-0482, Japan*
[c] *Department of Physiology, Faculty of Medicine, Saga University, Saga 849-8501, Japan*

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ABSTRACT

To elucidate the mechanisms of antinociception mediated by the dopaminergic descending pathway in the spinal cord, we investigated the actions of dopamine (DA) on substantia gelatinosa (SG) neurons by *in vivo* whole-cell patch-clamp methods. In the voltage-clamp mode ($V_H = -70$ mV), the application of DA induced outward currents in about 70% of SG neurons tested. DA-induced outward current was observed in the presence of either Na^+ channel blocker, tetrodotoxin (TTX) or a non-NMDA receptor antagonist, CNQX, and was inhibited by either GDP-β-S in the pipette solution or by perfusion of a non-selective K^+ channel blocker, Ba^{2+}. The DA-induced outward currents were mimicked by a selective D2-like receptor agonist, quinpirole and attenuated by a selective D2-like receptor antagonist, sulpiride, indicating that the DA-induced outward current is mediated by G-protein-activated K^+ channels through D2-like receptors. DA significantly suppressed the frequency and amplitude of glutamatergic spontaneous excitatory postsynaptic currents (EPSCs). DA also significantly decreased the frequency of miniature EPSCs in the presence of TTX. These results suggest that DA has both presynaptic and postsynaptic inhibitory actions on synaptic transmission in SG neurons. We showed that DA produced direct inhibitory effects in SG neurons to both noxious and innocuous stimuli to the skin. Furthermore, electrical stimulation of dopaminergic diencephalic spinal neurons (A11), which project to the spinal cord, induced outward current and suppressed the frequency and amplitude of EPSCs. We conclude that the dopaminergic descending pathway has an antinociceptive effect via D2-like receptors on SG neurons in the spinal cord.

1. Introduction

Dopamine (DA) is well known as a neurotransmitter and neuromodulator in the brain, not just as a precursor in the synthesis of other catecholamines. DA receptors are classified into two families: D2-like, incorporating D2 and the closely-related D3 and D4 receptors and D1-like which includes D1 and the closely-related D5 receptor (see reviews [33,50]). DA controls a variety of functions including locomotors activity, cognition, emotion, positive reinforcement, food intake, and endocrine regulation. Compared with the enormous literature devoted to DA actions in the brain, little is known about the role of DA in the spinal cord.

The first-order neurons transmitting pain impulses from the skin are of two types; small-diameter myelinated fibers of the Aδ group that are responsible for a fast or first pain and nonmyelinated C-fibers that give rise to a slow or second, duller, more diffuse type of pain. Noxious information is transmitted through the Aδ- and C-fibers to the superficial dorsal horn, especially the substantia gelatinosa (SG) neurons [26,46,55], as recently reviewed [9]. Descending pathways from the midbrain and brainstem exert an inhibitory effect on dorsal horn transmission, which is subject to various modulatory influences [14]. It has been well established that the descending noradrenergic and serotonergic pathways modulate nociceptive transmission in the spinal dorsal horn (see reviews [32,40,54]). Compared with noradrenaline (NA) and serotonin (5-HT), little is known about the roles of the descending dopaminergic pathway. The periventricular, posterior region (A11) of the hypothalamus is the principle source of descending dopaminergic pathways [10,42,47]. Focal electrical stimulation in the region of the A11 area suppresses the nociceptive responses of neurons in the spinal dorsal horn [12]. These findings strongly suggest that the descending dopaminergic pathway plays an important role in the process of antinociception in the spinal cord. In addition, behavioral studies demonstrate that intrathecal administration of DA induced thermal antinociceptive effects through D2-like receptors when assessed by the tail flick test [3,22]. Whole-cell patch-clamp recordings reveal that DA produces

* Corresponding author. Tel.: +81 72 453 8395; fax: +81 72 453 0276.
E-mail address: nakatsuka@kansai.ac.jp (T. Nakatsuka).

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hyperpolarization and an outward current in a proportion of SG neurons in transverse spinal cord slices [48]. However, it has not been determined whether the excitatory postsynaptic currents (EPSCs) that are inhibited by DA in slice preparations are responsible for pain transmission. Nor have the effects of DA on EPSCs been examined with whole-cell patch-clamp recordings *in vivo*. Even though some SG neurons have been shown to spread their dendrites more than 500 μm rostrocaudally [45], the thicknesses of the transverse spinal cord slices are usually less than 500 μm [55]. Therefore, it is possible that the dendrites of the SG neurons are transected and/or injured in the slice preparations, making it difficult to evaluate the overall effect of DA on the SG neurons. Thus, we sought to investigate the effects of DA on synaptic responses to noxious stimuli and analyze the synaptic actions of DA on SG neurons *in vivo*.

2. Methods

All the experimental procedures involving the use of animals were approved by the Ethics Committee on Animal Experiments, Wakayama Medical University and were in accordance with the UK Animals (Scientific Procedures) Act 1986 and associated guidelines.

2.1. Preparation

The methods used for the *in vivo* patch-clamp recording were similar to those described previously [13,36,44]. Male Sprague–Dawley rats (5–7 weeks of age, 150–250 g) were anesthetized with urethane (1.2–1.5 g kg^{-1}, intraperitoneal). Artificial ventilation of the pneumothorax was not performed as the rats could be maintained in good condition without artificial ventilation by supplying oxygen through a nose cone (Fig. 1) [44]. If a withdrawal reflex appeared, then a supplemental dose of urethane was given during surgery and the data collection period. A heating pad was placed beneath the rat to maintain its body temperature. The lumbar spinal cord was exposed at the level from L3 to L5 by a thoracolumbar laminectomy at the level from Th12 to L2 and the rat was placed in a stereotaxic apparatus (Model STS-B & SR-5R-HT, Narishige, Tokyo, Japan). Under a binocular microscope with 8× to 40× magnification, the dura was cut and removed. Then, the dorsal root that enters the spinal cord above the level of recording sites was gently shifted bilaterally, using a small glass retractor, to expose Lissauer's tract so that a recording electrode could be advanced into the SG from the surface of the spinal cord. The pia-arachnoid membrane was removed using microforceps to make a window large enough to allow the patch electrode to enter the spinal cord. The surface of the spinal cord was irrigated with 95% O_2 5% CO_2-equilibrated Krebs solution (10–15 ml min^{-1}) (mM: NaCl 117, KCl 3.6, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, glucose 11, and $NaHCO_3$ 25) through glass pipettes at 36.5 ± 0.5 °C. At the end of the experiments, the rats were given an overdose of urethane and then killed by exsanguination.

2.2. Patch-clamp recordings

The patch electrodes were pulled from thin-walled borosilicate glass capillaries (o.d. 1.5 mm) using a p-97 puller (Sutter Instrument, Novato, CA, USA), and were filled with a patch-pipette solution composed of the following (mM): potassium gluconate 135, KCl 5, $CaCl_2$ 0.5, $MgCl_2$ 2, EGTA 5, ATP-Mg 5 and Hepes-KOH 5; pH 7.2. When necessary, guanosine-5′-*O*-(2-thiodiphosphate) (GDP-β-S) was added at a concentration of 2 mM to the patch-pipette solution. The electrode with a resistance of 8–12 MΩ was advanced at an angle of 30–45 degrees into the SG through the window in the pia-arachnoid membrane using a micromanipulator

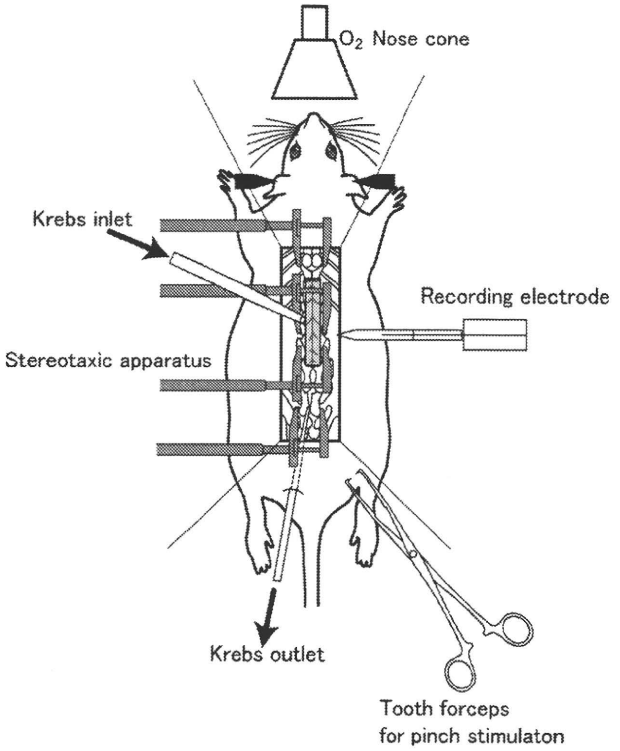

Fig. 1. Schematic diagrams of *in vivo* rat preparation. Whole-cell patch-clamp recordings were done while supplying oxygen to a urethane-anesthetized rat through a nose cone. The lumbar spinal cord was exposed at the level from L3 to L5 by a thoraco-lumbar laminectomy at the level from Th12 to L2, then the rat was placed and fixed in a stereotaxic apparatus. The surface of the spinal cord was irrigated with 95% O_2 5% CO_2-equilibrated Krebs solution from inlet to outlet. Drugs were dissolved in Krebs solution and applied by perfusion. The noxious and innocuous mechanical stimuli were applied to the receptive field of the ipsilateral hindlimb with toothed forceps and air puffs.

(Model MWS-32S, Narishige, Tokyo, Japan). A giga-ohm seal (resistance of at least 10 GΩ) was then formed with neurons at a depth of 30–150 μm. Membrane potentials were held at −70 mV in voltage-clamp mode. After making a giga-ohm seal, the membrane patch was ruptured by a brief period of more negative pressure, thus resulting in a whole cell configuration. Signals were collected using an Axopatch 200B amplifier in conjunction with a Digidata 1440A A/D converter (Molecular Devices, Sunnyvale, CA, USA) and stored on a personal computer using pCLAMP 10 data acquisition program (Molecular Devices, Sunnyvale, CA, USA). They were analyzed using Mini Analysis 6.0 software (Synaptosoft, Fort Lee, NJ, USA) and pCLAMP 10 data acquisition program.

2.3. Stimulation protocols

The noxious and innocuous mechanical stimuli were applied to the receptive field of the ipsilateral hindlimb using toothed forceps or air puffs (Pressure system IIe, Toohey Company, Fairfield, NJ, USA), respectively. To keep a fixed strength noxious stimulation, the toothed forceps was clamped during skin pinching.

2.4. Electrical stimulation of A11

The methods used for electrical stimulation of the brain during *in vivo* patch-clamp recording on SG neurons were similar to those described previously [23]. A burr hole was made in the skull, and the 26 gauge (0.46 mm outer diameter (OD)) guide cannula was inserted 2 mm above the A11 and fixed to the skull by dental cement. A concentric bipolar stimulating electrode (0.2 mm OD; model IMB-9002; Inter Medical Company, Nagoya, Japan) was inserted

through this guide cannula. The stimulating sites were aimed at A11 (stereotaxic coordinates: 3.0–3.5 mm posterior to the bregma and 0.5 mm lateral, 8.0 mm ventral to the dura) [37]. The stimulating electrode was equipped with stoppers to extend 2 mm beyond the tip of the guide cannula. The electrical stimulation was performed with rectangular pulses (duration, 100 μs; intensity, 100 μA; frequency, 10 Hz).

2.5. Drug application

Drugs were dissolved in Krebs solution and applied by perfusion via a three-way stopcock without any change in the perfusion rate or the temperature. The time necessary for the solution to flow from the stopcock to the surface of the spinal cord was approximately 10 s. The drugs used in this study were DA, SKF38393, sulpiride, tetrodotoxin (TTX) (Wako, Osaka, Japan), quinpirole, barium chloride dihydrate, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and GDP-β-S (Sigma, St Louis, MO, USA). Sulpiride and CNQX were first dissolved in dimethyl sulfoxide (DMSO) at 1000 times the concentrations to be used. DA, TTX, barium chloride dihydrate, SKF38393 and quinpirole were first dissolved in distilled water at 1000 times the concentrations to be used. These drugs were diluted to the final concentration in Krebs solution immediately before use.

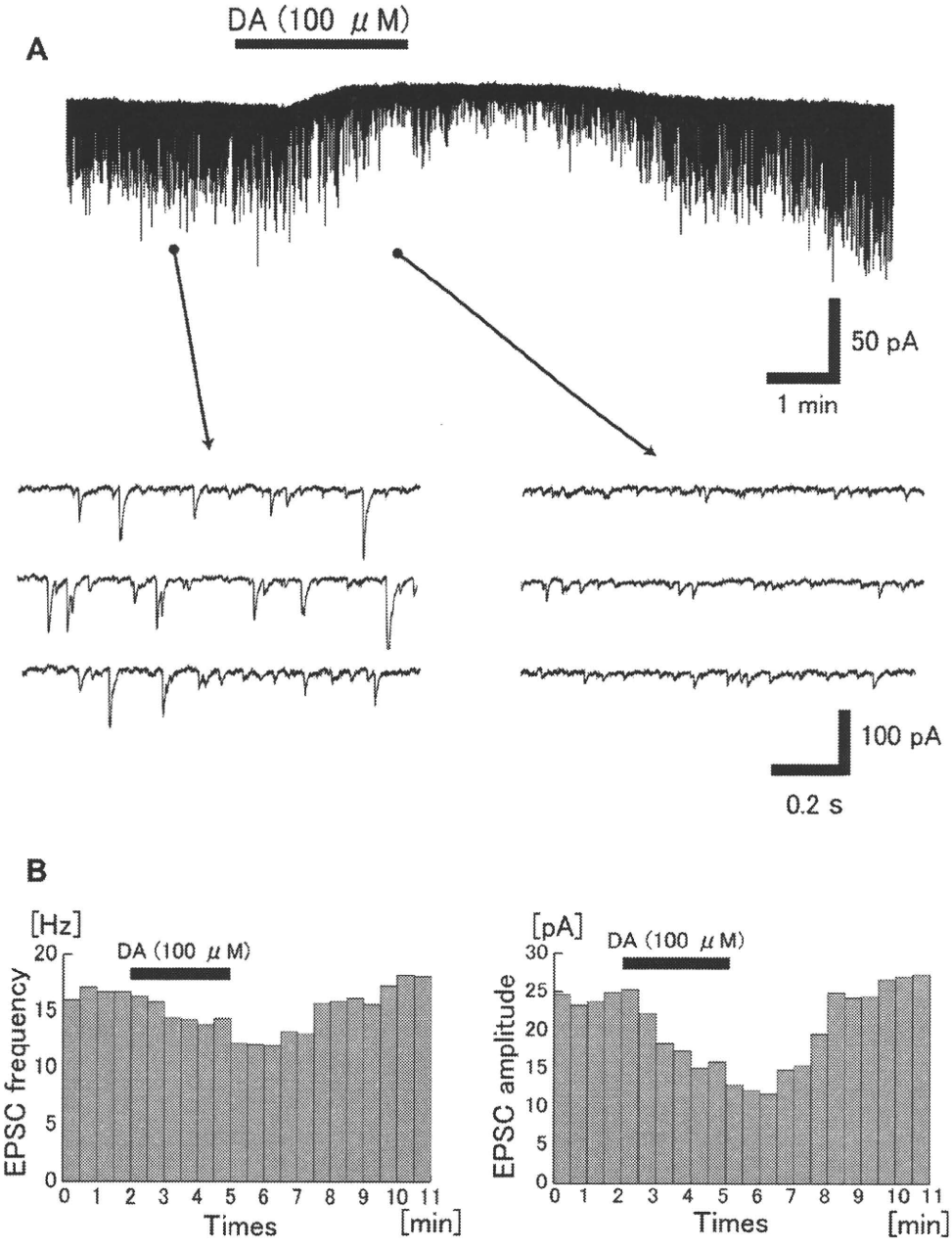

Fig. 2. DA induces an outward current and decreases the frequency and amplitude of EPSCs in SG neurons. (A) In voltage-clamp mode (V_H = −70 mV), bath application of DA (100 μM) produced an outward current in 155 out of 219 (70.8%) neurons recorded. The neuronal activity in this figure shows a typical response to DA. In this and subsequent figures, a bar shown above the recording indicates the period during which drugs were superfused. The three traces, shown in an expanded scale in time, indicate that the frequency and amplitude of EPSCs are clearly reduced during DA perfusion compared with those of the controls. (B) The frequency and amplitude of EPSCs following the application of DA (100 μM), plotted against time. Each bar indicates data calculated from the EPSCs measured for 30 s. (Same neuron as in Fig. A).

2.6. Statistical analysis

All numerical data were expressed as the mean ± S.E.M. Statistical significance was determined as $P < 0.05$ using Student's t test and Student's paired t test. In electrophysiological data, n refers to the number of neurons studied. The membrane potentials were not corrected for the liquid junction potential between the Krebs and patch-pipette solutions.

3. Results

An animal preparation could be maintained in a stable condition for over 10 h, which was comparable to that maintained in previous experiments using an artificial ventilator. Whole-cell patch-clamp recordings were made from 272 SG neurons. All neurons studied had membrane potentials more negative than −50 mV. The membrane potentials were −61.7 ± 0.5 mV ($n = 166$). All SG neurons tested exhibited EPSCs at a holding potential (V_H) of −70 mV where no inhibitory postsynaptic currents (IPSCs) were observed, because the reversal potential for IPSCs was near −70 mV [57].

3.1. Pre- and postsynaptic DA actions in SG neurons

In the voltage-clamp mode, perfusion with DA (100 μM) induced a clear outward current in 155 out of 219 (70.8%) neurons recorded (Fig. 2A). The average amplitude of the DA-induced outward currents was 19.5 ± 1.6 pA ($n = 64$). In 19 of 219 neurons tested, an inward current was observed (data not shown). When DA was applied repeatedly at 5-min intervals, it produced similar responses with similar amplitudes (Fig. 3A). Furthermore, an outward current did not show any attenuation during the 10 min continuous application of DA (Fig. 3B). During the simultaneous application of TTX (1 μM), a Na^+ channel blocker, DA (100 μM) also induced outward currents (13.3 ± 2.3 pA; $n = 10$) (Fig. 3C). All of the neurons examined exhibited miniature EPSCs (mEPSCs) that were completely blocked by a non-NMDA receptor antagonist, CNQX (10 μM), and in its presence DA generated an outward current ($n = 5$) (Fig. 3D). DA sometimes decreased the frequency and amplitude of EPSCs (65.2 ± 6.3% of the control frequency and 75.1 ± 3.2% of the control amplitude; $n = 19$, Fig. 2B). The frequency and amplitude of EPSCs were suppressed by the application of TTX (73.1 ± 10.0% of the control frequency and 74.9 ± 5.6% of control amplitude; $n = 10$) within 30 s, suggesting that a substantial amount of EPSCs initiated by action potentials from interneurons or primary afferents were included under these *in vivo* conditions. Moreover, in the presence of TTX, DA significantly decreased the frequency of mEPSCs compared with that of TTX alone (73.6 ± 9.4%; $n = 10$, $P < 0.05$).

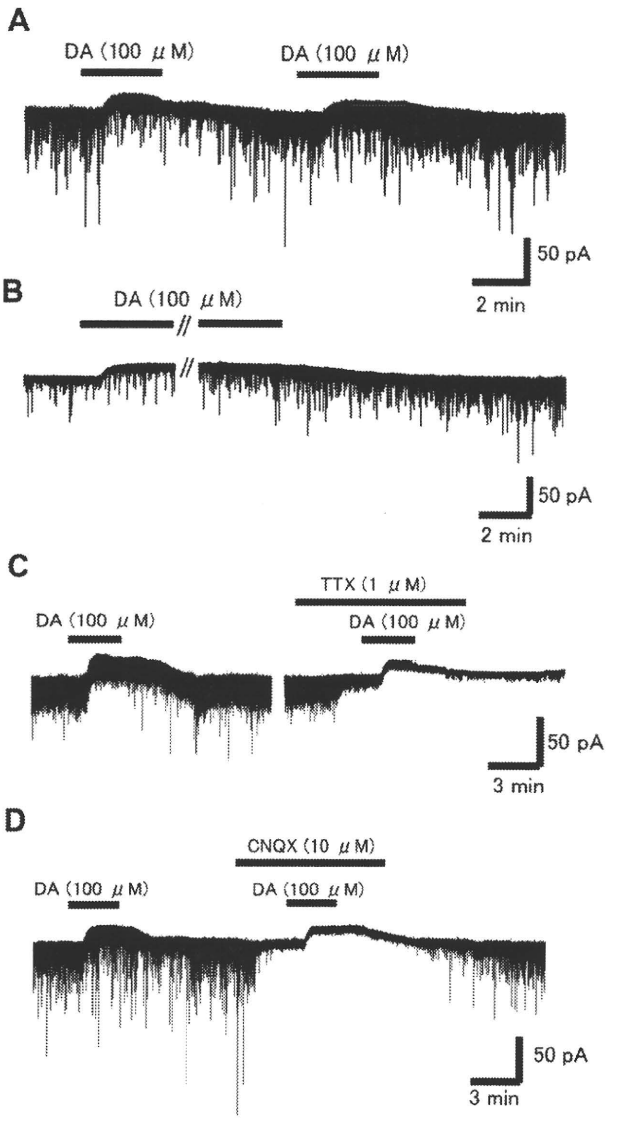

Fig. 3. Characterization of the DA-induced outward currents. (A) When DA (100 μM) was repeatedly applied at 5-min intervals, it produced similar outward currents. (B) DA (100 μM) application for 10 min induced an outward current without desensitization. (C) During the simultaneous application of TTX (1 μM), DA (100 μM) also induced outward currents (13.3 ± 2.3 pA; $n = 10$). (D) In the presence of CNQX (10 μM), DA (100 μM) induced an outward current without any decrease in the amplitude.

3.2. Pharmacological analysis of the DA-induced responses

Bath application of the K^+ channel blocker Ba^{2+} (1 mM) alone induced a small inward current (Fig. 4A), which seems to be due to inhibition of K^+ channels [55]. The average of the DA-induced outward currents in the presence of Ba^{2+} was 6.3 ± 1.8 pA ($n = 8$) and it significantly decreased to 34.7 ± 7.8% of the controls (18.9 ± 3.3 pA; $n = 8$, $P < 0.05$) (Fig. 4A). To examine the involvement of G-proteins in the DA-induced outward current, GDP-β-S (2 mM), a non-hydrolysable analogue of GDP that competitively inhibits G-proteins, was added to the pipette solution. When DA (100 μM) was applied just after establishing the whole-cell configuration with pipettes containing potassium gluconate and GDP-β-S, an outward current was clearly observed (19.4 ± 2.5 pA; $n = 8$). When DA was again applied 30 min later, it was significantly suppressed (4.3 ± 1.6 pA, 22.3 ± 8.9% of the control amplitude; $n = 8$, $P < 0.01$) (Fig. 4B). These findings suggested that the DA-induced outward current was mediated by K^+ channels through activation of G-proteins.

3.3. Effects of DA receptor agonists and antagonists

We further examined which subtype of DA receptors is involved in the DA-induced outward currents by using DA receptor agonists and antagonists. SKF38393 (100 μM), a D1-like receptor agonist, induces little or no outward current (1.9 ± 0.6 pA; $n = 9$) (Fig. 5B), while quinpirole (100 μM), a D2-like receptor agonist, produced a clear outward current (15.7 ± 2.0 pA; $n = 9$) (Fig. 5A). The SKF38393-induced outward current was significantly lower than either the DA- or quinpirole-induced outward current ($P < 0.01$). There was no significant difference between DA-induced outward current and that of quinpirole ($P > 0.05$) (Fig. 5D). When a D2-like receptor antagonist, sulpiride (30 μM), was administrated 5 min prior to the DA application, the average amplitude of the

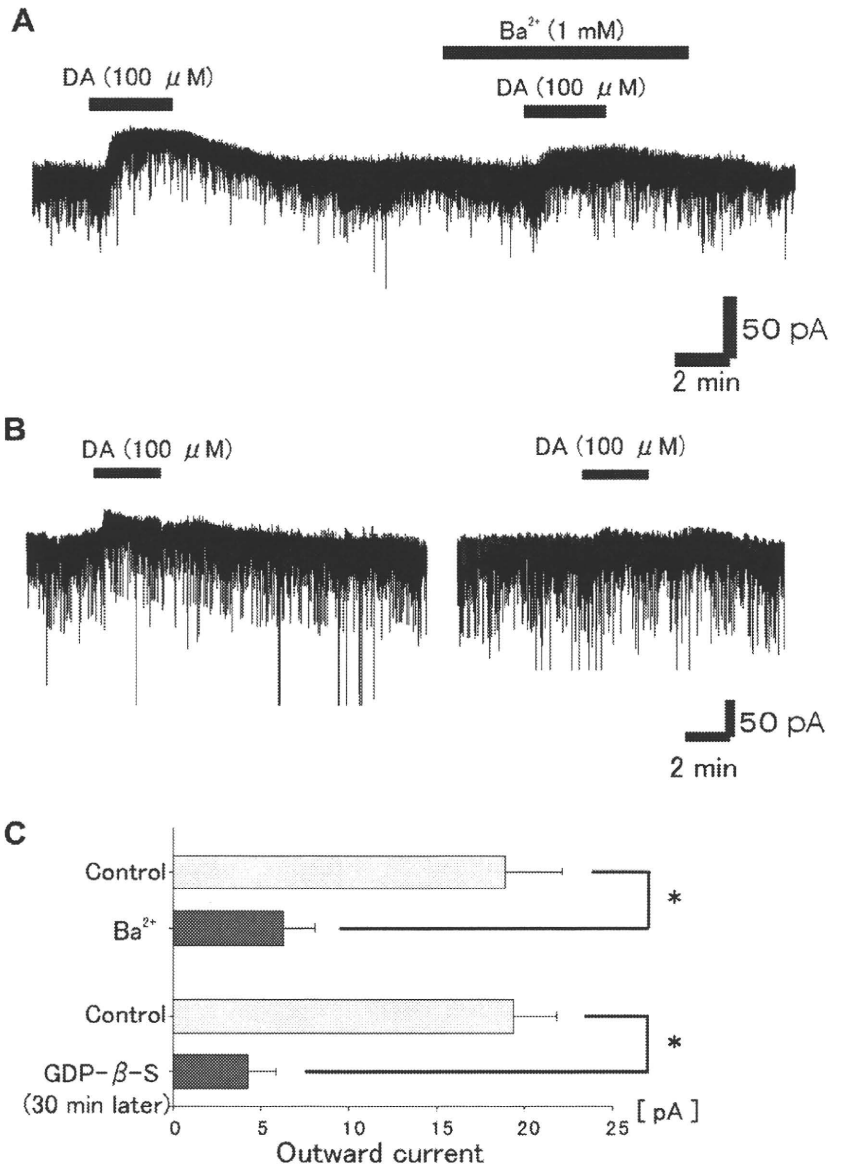

Fig. 4. Inhibition of DA-induced outward current by Ba^{2+} and GDP-β-S. (A) DA (100 μM) was administrated in the absence and presence of Ba^{2+} (1 mM). The outward current was significantly reduced in the presence of Ba^{2+} (34.7 ± 7.8% of the controls; $n = 8$; $P < 0.05$). (B) An outward current was recorded with potassium gluconate pipette solution containing GDP-β-S. DA produced an outward current just after establishing whole-cell recording, but it was markedly attenuated when DA was again applied 30 min later (22.3 ± 8.9% of control amplitude; $n = 8$; $P < 0.05$). (C) Summary of DA-induced outward current in the presence of Ba^{2+} or intracellular injection of GDP-β-S, relative to the control. In this and subsequent figures, horizontal lines accompanied by bars indicate S.E.M.; statistical significance ($P < 0.05$) between data shown by bars is indicated by an asterisk (*); n.s.: not significant.

DA-induced outward currents was 5.4 ± 1.9 pA, $n = 6$ (Fig. 5C), 34.1 ± 10.0% of that in the absence of sulpiride ($P < 0.05$).

3.4. Effects of DA on the responses to noxious stimuli

All 33 SG neurons examined responded to noxious (pinch) or innocuous (air puff) mechanical stimuli. In the voltage-clamp mode, either pinch or air stimuli applied to the ipsilateral hindlimb elicited a barrage of EPSCs; these disappeared within 1 s after the stimuli were terminated (Fig. 6A and B). The limb point most sensitive to stimulation was different for each cell tested. Stimulating the contralateral hindlimb did not elicit any synaptic responses (data not shown). The peak amplitudes were not determined, because multiple summations resulting from the high-frequency bursting of EPSCs made it difficult to obtain an accurate estimation. So, we investigated the change of frequency and area surrounded by the baseline and border of EPSCs (Fig. 7A). It was considered significant if EPSCs decreased or increased by more than 10%. The pinch stimulus initially produced large and summated EPSCs that were followed by a barrage of EPSCs, and again large EPSCs at the end of the stimulus (Fig. 6A). When DA (100 μM) was applied to the surface of the spinal cord, the area of the evoked EPSCs decreased in 4 of 6 neurons tested (74.6 ± 7.5% of the control; $n = 6$, $P < 0.05$) (Fig. 7B), although the amplitudes of the large EPSCs at the beginning and end of stimulation were not affected (Fig. 6A). Similarly, the frequency of EPSCs decreased in 4 of 6 neurons tested (78.0 ± 8.0% of the control; $n = 6$, $P < 0.05$) (Fig. 7B). The barrage of EPSCs induced by pinch again appeared after the washing-out of DA. The area of the evoked EPSCs by innocuous (air puff) mechanical stimuli decreased as much as with the pinch stimuli (Fig. 7B). The area of the evoked EPSCs after an air puff decreased in 14 of 18 neurons tested (70.9 ± 11.0% of the control; $n = 18$, $P < 0.01$) and the frequency of EPSCs after an air puff decreased in 11 of 18 neurons tested (80.6 ± 6.3% of the control; $n = 18$, $P < 0.01$)

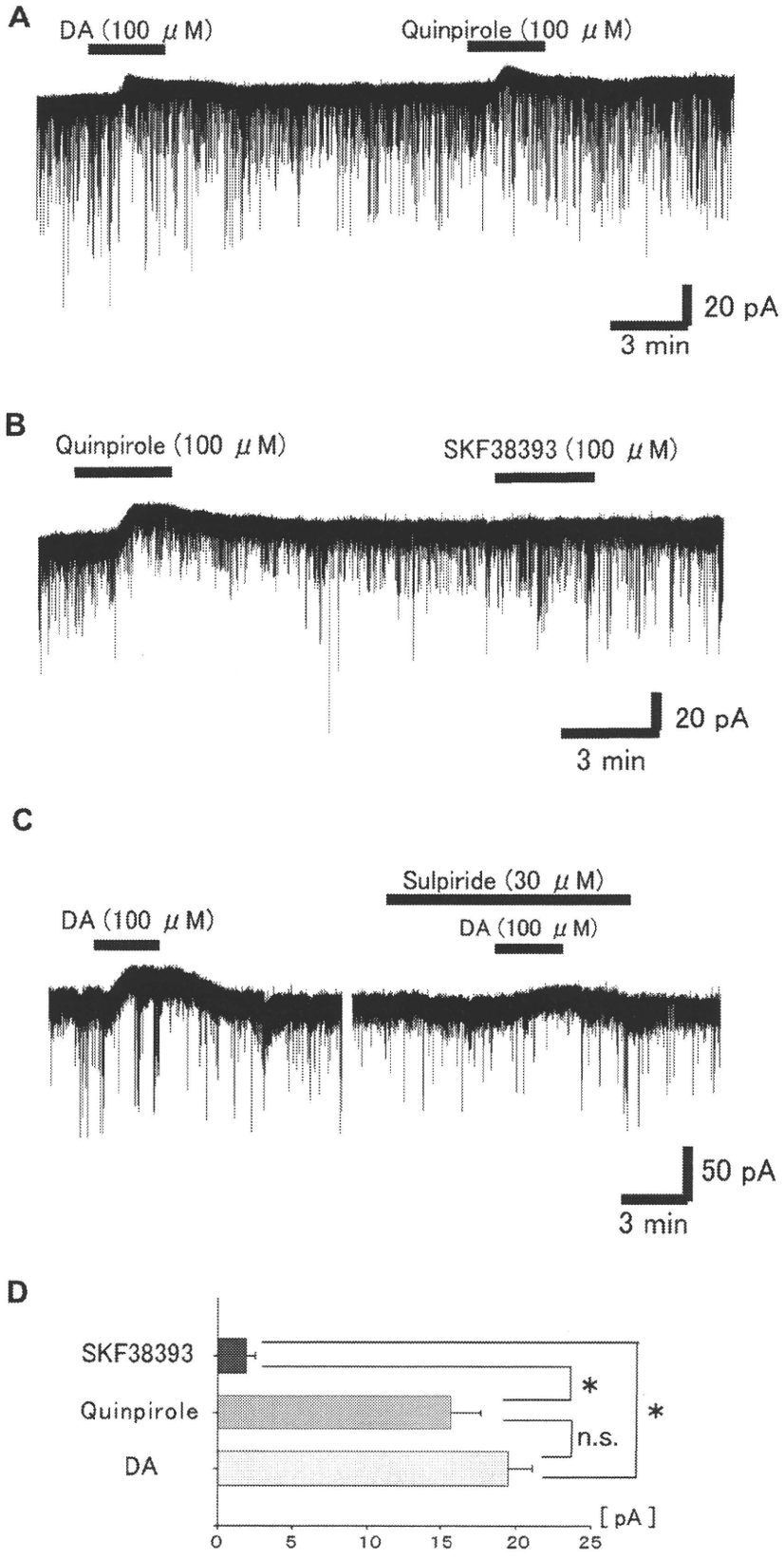

Fig. 5. Effect of DA receptor agonists and antagonists on the DA-induced outward currents. (A) Quinpirole (100 μM), a D2-like receptor agonist, mimicked the DA-induced outward current. (B) SKF 38393 (100 μM), a D1-like receptor agonist, induced no outward current, whereas quinpirole (100 μM) induced an outward current in the same neuron. (C) In the presence of a D2-like receptor antagonist, sulpiride (30 μM), a DA-induced outward current was markedly attenuated (34.1 ± 10.0% of that in the absence of sulpiride; $n = 6$; $P < 0.05$). (D) An SKF38393-induced outward current was zero or very low (1.9 ± 1.7 pA; $n = 9$). In contrast, quinpirole-induced outward current amplitude was 15.7 ± 3.9 pA ($n = 9$) which was similar to DA-induced outward currents (19.5 ± 12.4 pA; $n = 64$). The SKF38393-induced outward current was significantly lower than a DA- or quinpirole-induced outward current ($P < 0.01$). There was no significant difference between a DA-induced outward current and that of quinpirole.

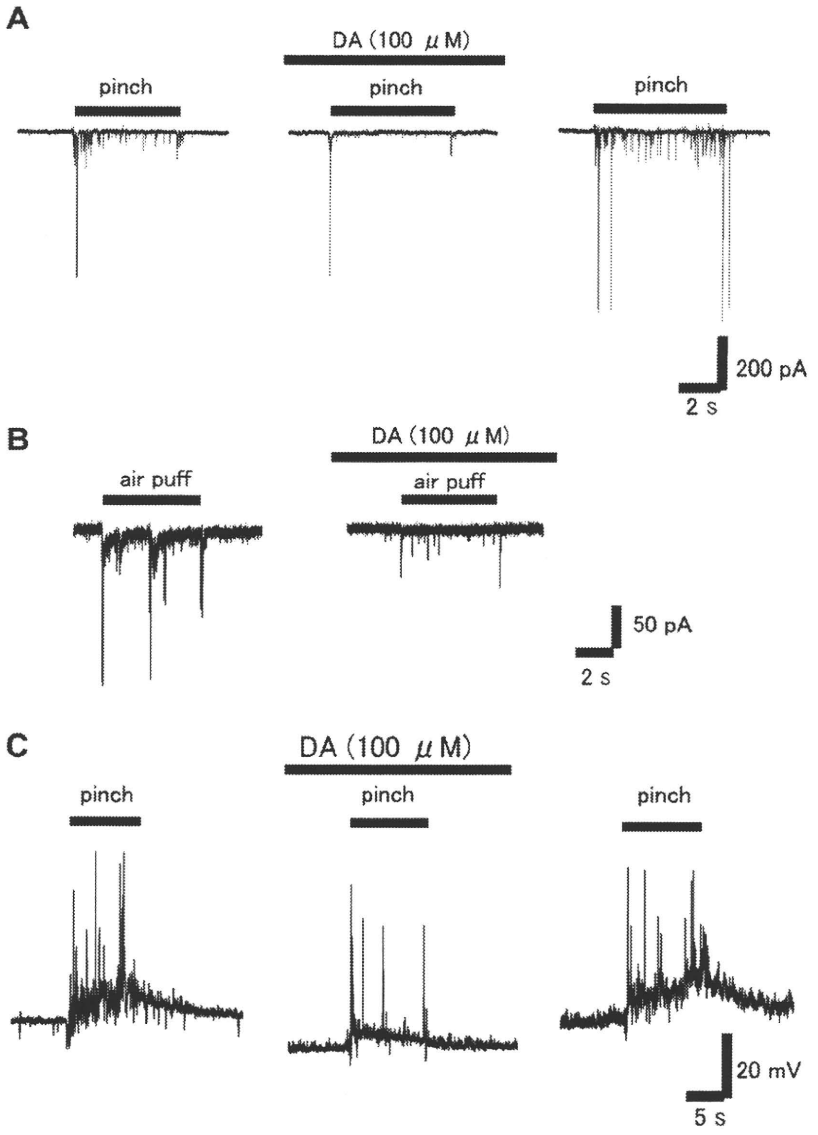

Fig. 6. Reduction of pinch and air puff evoked firing in SG neurons by DA. (A) EPSCs elicited by pinching in voltage-clamp mode (V_H = −70 mV) in the control (left). DA (100 μM) suppressed repeated EPSCs during pinching in a reversible manner without affecting amplitude of large EPSCs evoked at the beginning and end of pinch stimulus (middle and right). (B) EPSCs elicited by air puff in voltage-clamp mode (V_H = −70 mV) in the control (left). DA (100 μM) suppressed repeated EPSCs during air puff (right). (C) Pinch stimuli applied to the ipsilateral hindlimb produced a barrage of EPSPs accompanied by action potentials under a current-clamp condition (left). DA (100 μM) hyperpolarized membrane of SG neuron and inhibited the action potentials in a reversible manner (middle and right).

(Figs. 6B, 7B). In the current-clamp mode, pinch stimuli elicited a barrage of excitatory postsynaptic potentials (EPSPs), some of which were accompanied by an action potential (Fig. 6C). The evoked EPSPs disappeared within 1 s after the stimulation, and did not show any desensitization of the responses. Perfusion with DA caused a slight hyperpolarization of the membrane and suppressed action potentials in a reversible manner (Fig. 6C). Evaluation by frequency and area was also used in the current-clamp mode. DA decreased the area of action potentials or EPSPs during pinching the skin in all the neurons tested (42.7 ± 6.9% of the control; n = 9, P < 0.01) and the frequency of action potentials or EPSPs with pinch decreased in 7 of 9 neurons tested (70.2 ± 12.4% of the control; n = 9, P < 0.05) (Fig. 7B).

3.5. Effect of electrical stimulation of A11

The region of A11 is the principle source of descending dopaminergic pathways. We investigated whether antinociceptive actions were induced in SG neurons by focal electrical stimulation (ES) of A11. Schematic diagrams of A11-ES are shown in Fig. 8A. A11-ES induced outward currents in 36 of 50 (72%) neurons recorded (Fig. 8B), with an average amplitude of 7.5 ± 1.6 pA (n = 36). Moreover, A11-ES decreased the frequency and amplitude of EPSCs (Fig. 8C). Our definition of significance was if the frequency or amplitude of EPSCs decreased by more than 10% compared with those of the control. The frequency was decreased in 39 of 46 (84.7%) neurons (average of 61.9 ± 3.0%, n = 39, of the controls) by A11-ES. The amplitude was decreased in 24 of 46 (52.2%) neurons (average 82.2 ± 1.5%, n = 24, of the control) by A11-ES. We also examined whether the D2-like antagonist, sulpiride, blocked these A11-ES effects (Fig. 8D). In the presence of sulpiride (100 μM), the A11-ES induced outward current was blocked, and its average amplitude was only 1.6 ± 0.6 pA (n = 16). The frequency and amplitude by A11-ES under sulpiride were 91.3 ± 4.7% and 98.7 ± 3.3% (n = 17), respectively, of the controls. These results were significantly lower than that in the absence of sulpiride (P < 0.01).

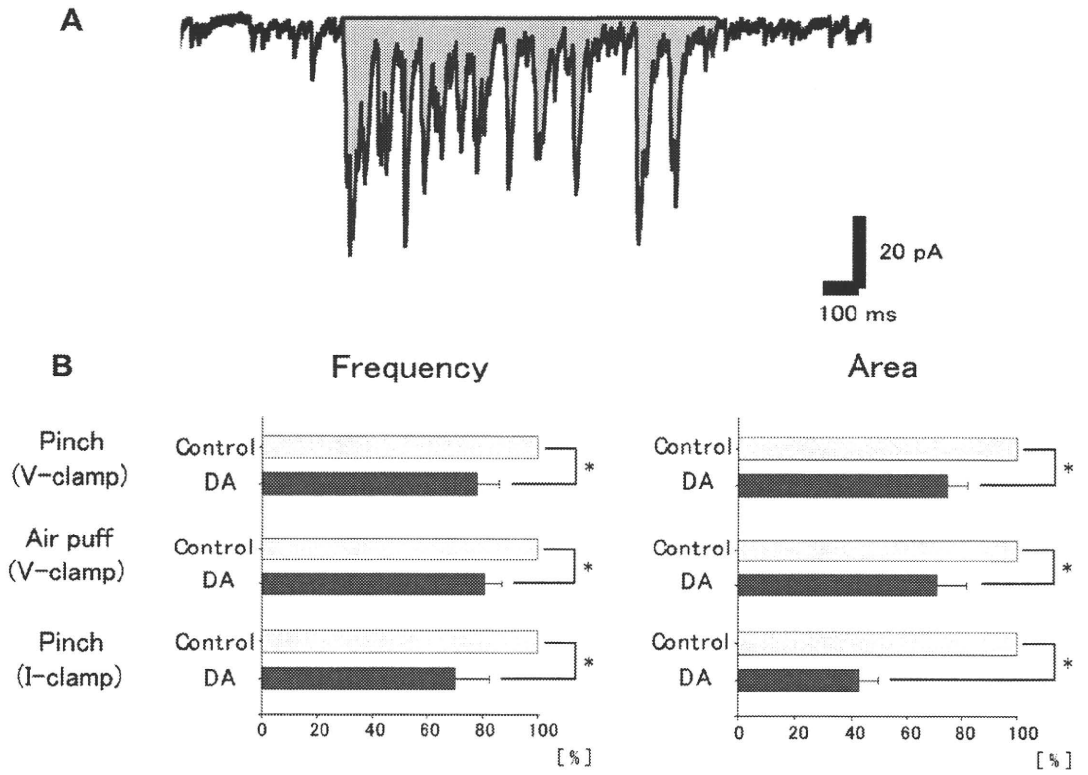

Fig. 7. Analysis of the responses to the noxious and innocuous stimuli. (A) Schematic diagrams of area. Analysis of area surrounded by the baseline and border of EPSCs was done by using software (clampfit10). (B) Analysis about the frequency and area of EPSCs, EPSPs and action potentials, which were induced by noxious and innocuous stimuli to the ipsilateral hindlimb. The frequency and area significantly decreased during DA perfusion compared with the controls in the absence of DA. These results were showed when either noxious or innocuous stimuli to the skin were given.

4. Discussion

The descending inhibitory system of nociceptive information is important for the control of excessive pain. Compared with the large quantity of literature on NA and 5-HT as the descending inhibitory systems, there is little on the analgesic effect of DA. Several reports have described the direct actions of supraspinal DA on nociceptive responses in regions of the brain [39], such as the striatum [1,31,38] and in review, [19], the basal ganglia [17], the nucleus accumbens [25,49], the anterior insular cortex [8], the anterior cingulated cortex [30] and the ventrolateral orbital cortex [41]. However, there have been no pharmacological studies using agonists and antagonists for DA receptor subtypes of the superficial dorsal horn in whole-cell patch-clamp recordings under *in vivo* conditions. In the present study, the effects of DA on SG neurons were tested using the *in vivo* patch-clamp recording technique that enabled us to examine the effect of drugs on synaptic responses evoked by noxious (pinch) and innocuous (air puff) stimuli applied to the skin. In addition, we investigated whether antinociceptive actions were induced in SG neurons by A11-ES.

4.1. Pre- and postsynaptic actions and character of D2-like receptors in SG neurons

The application of DA induced outward currents or hyperpolarization in about 70% of SG neurons tested in the present study. The DA-induced outward current is observed in the presence of TTX (Fig. 3C). DA also significantly decreases the frequency of mEPSCs in the presence of TTX. Furthermore, mEPSCs are completely blocked by CNQX (Fig. 3D), but the DA-induced outward current is observed in the presence of CNQX. These results suggest that DA has both presynaptic and postsynaptic actions to inhibit synaptic transmission in SG neurons. The DA-induced outward current was completely blocked by GDP-β-S in the pipette solution (Fig. 4B). The perfusion of a non-selective K^+ channel blocker, Ba^{2+}, also inhibited the outward currents induced by DA (Fig. 4A). Since these DA-induced outward currents were mimicked by a selective D2-like receptor agonist, quinpirole, and attenuated by a selective D2-like receptor antagonist, sulpiride (Fig. 5A, 5C), it is suggested that the DA-induced outward current is mediated by G-protein-activated K^+ channels through D2-like receptors. Thus, DA acts directly on D2-like receptors in the postsynaptic membrane of SG neurons. The DA-induced outward currents and hyperpolarization were induced by D2-like receptor-mediated activation of K^+ channels, which have been reported in various neurons other than SG neurons, such as the substantia nigra pars compacta [27], striatal neurons [18,51], ventral tegmental area [34] and pituitary cells [7,11]. A previous study has shown that glutamatergic fibers that terminate onto ventral tegmental dopaminergic neurons possess D2-like receptors, activation of which inhibits glutamate release by reducing Ca^{2+} influx [24]. Similarly, these presynaptic D2-like receptors activate glutamate release onto postsynaptic neurons. However, further investigations will be required to clarify the role of presynaptic dopaminergic receptors in the spinal dorsal horn.

In the present study, an inward current was observed in 19 neurons of 219 recorded SG neurons (9%). D1-like receptor-mediated postsynaptic excitation by inhibiting K^+ conductance or activating cation conductance has been reported in striatal cholinergic interneurons [2]. Although an autoradiographic study has demonstrated that both D1-like and D2-like receptors are densely localized in the superficial laminae of the spinal dorsal horn [28], the effect of D1-like receptor agonist on membrane currents was not detected in the present study. Thus, further investigations will

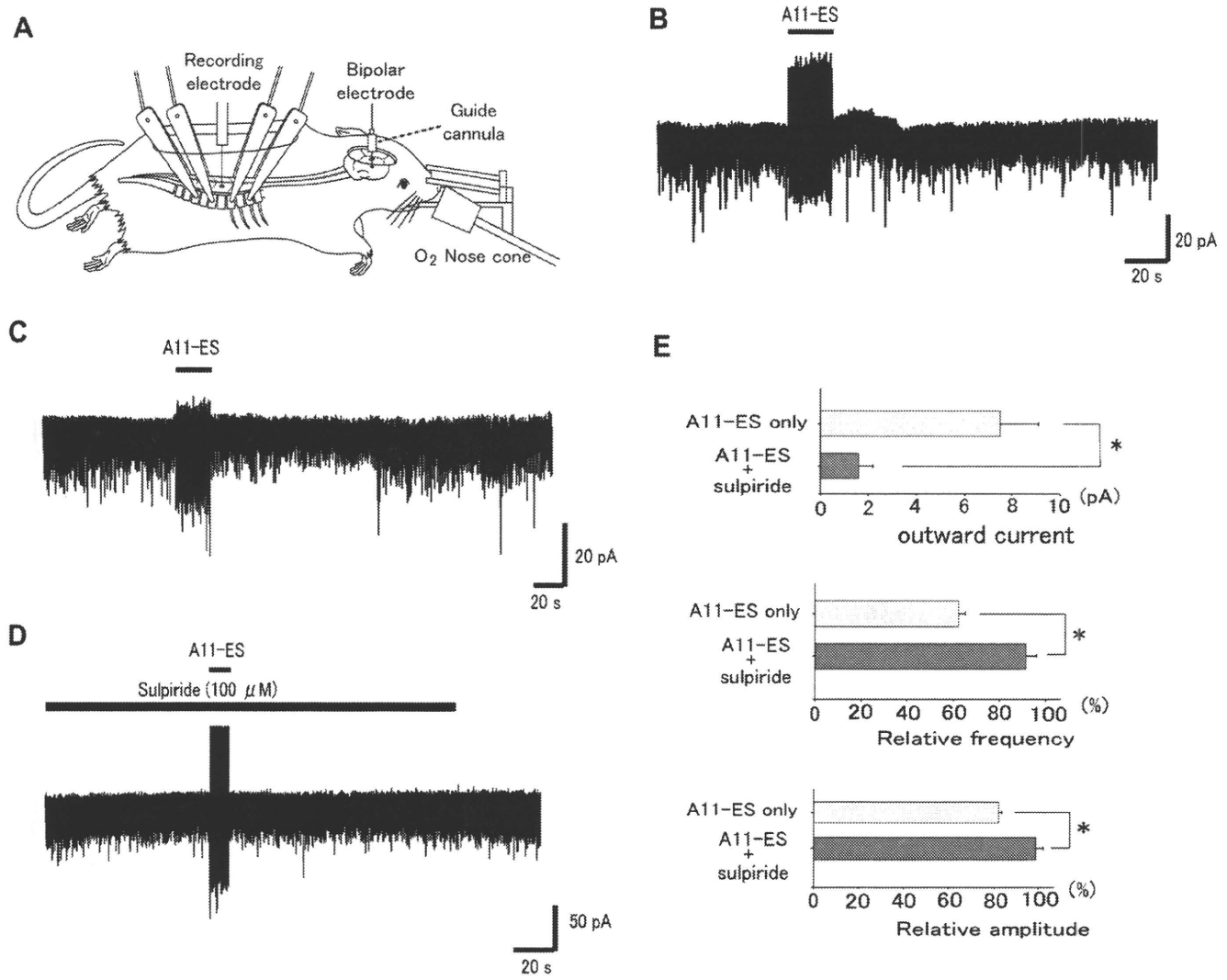

Fig. 8. A11-ES induced antinociceptive effects through activation of D2-like receptor in SG neurons. (A) Schematic diagrams of A11-ES during *in vivo* patch-clamp recordings on SG neurons. (B) A11-ES induced outward currents immediately in 36 of 50 (72%) neurons recorded. The average amplitude was 7.5 ± 1.6 pA; $n = 36$. (C) A11-ES decreased the frequency and amplitude of EPSCs. The frequency was decreased in 39 of 46 (84.7%) neurons (61.9 ± 3.0% of the control; $n = 39$). The amplitude was decreased in 24 of 46 (52.2%) neurons (82.2 ± 1.5% of the control; $n = 24$). (D) In the presence of sulpiride (100 μM), A11-ES induced outward current was blocked, and its average amplitude was only 1.6 ± 0.6 pA ($n = 16$). The frequency and amplitude by A11-ES under sulpiride ($n = 17$) were 91.3 ± 4.7% and 98.7 ± 3.3%, respectively, of the control. These results were significantly lower than those in the absence of sulpiride ($P < 0.01$).

be required to clarify the role of D1-like receptors in the spinal dorsal horn.

4.2. DA produced analgesic effects in SG neurons for both noxious and innocuous stimuli to the skin

In this study, the pinch-evoked action potentials and EPSPs were attenuated in frequency and area by the application of DA, probably due to the hyperpolarization of membrane (Figs. 6C, 7B). In the voltage-clamp mode, the pinch-induced EPSCs were also markedly attenuated in frequency and area by the application of DA (Fig. 7B). The EPSCs evoked by an innocuous (air puff) mechanical stimuli were decreased in frequency and area by the application of DA, as with the pinch stimuli (Fig. 7B). This shows that DA has inhibitory effects in the spinal cord to both noxious and innocuous stimuli to the skin. As shown in Fig. 2A, spontaneous EPSCs are observed in a substantial number of SG neurons *in vivo*, while spontaneous EPSCs are hardly recorded in slice preparations [56]. Both the frequency and amplitude of EPSCs observed in the *in vivo* preparations are markedly depressed by DA (Fig. 2B). These results may be due to the membrane hyperpolarization in interneurons or presynaptic depression of transmitter release by DA acting at the terminals of the primary afferents. It is suggested that the DA-induced blockade of noxious transmission is at least partly mediated by the actions of DA on the presynaptic site, suppressing the evoked EPSCs, thereby reducing the number of action potentials in SG neurons during pinching.

4.3. DA in the dorsal horn is projected from dopaminergic neurons in the region of A11

Previous studies have demonstrated that the intrathecal administration of DA and/or D2-like agonist have attenuated pain related behavior which was induced by thermal stimuli [3,22,29]. Similarly, intrathecal administration of DA induces mechanical antinociception when assessed by withdrawal thresholds [48]. This antinociceptive action of DA agonists is selectively blocked by D2-like antagonists, but not by D1-like antagonists [3,29]. Furthermore, it was previously reported that intrathecal administration of not only D2-like receptor agonists but also high doses of a D1-like

receptor agonist produced the analgesic effect in carrageenan induced chronic inflammation [15,16], or haloperidol-induced dopamine-supersensitivity [4]. Thus, D2-like receptors within the spinal dorsal horn may play a significant regulatory role in a variety of pain sensations and D1-like receptors could have some of an analgesic action affecting morbidity after chronic inflammation or dopamine-supersensitivity.

The dopaminergic A11 neurons in the periventricular, posterior region of the hypothalamus are a major source of descending dopaminergic projections to the spinal cord [5,20,43]. Focal electrical stimulation of A11 neurons produce an antinociceptive effect in the spinal dorsal horn, and which is reversed by the spinal administration of a D2-like receptor antagonist [12,52]. A D2-like agonist administered onto A11 neurons produces attenuation of neuropathic hypersensitivity, and this effect is reversed by intrathecal administration of an α_2-adrenoceptor antagonist or a 5-HT_{1A} receptor antagonist but not by a D2-like antagonist [52]. These findings suggest that activation of the dopamine D2 receptor in A11 neurons may selectively suppress neuropathic hypersensitivity, owing to mechanisms that involve descending noradrenergic pathways acting on spinal α_2-adrenoceptor, and 5-HT_{1A} receptor. This action is probably different from the antinociception induced by electrical stimulation of A11 neurons, because our results confirm a direct inhibition of SG neurons in the spinal cord by activation of dopamine D2-like receptors.

Dopaminergic innervation of the spinal cord is largely derived from the brain. A previous study reported that there are no dopaminergic cell bodies in the spinal cord and that only fibers and terminals are immunoreactive for DA [21]. Therefore, the potential origin of endogenous DA appears to be from dopaminergic neurons in the region of A11. On the other hand, it has been reported that there is a small population of DRG neurons that exhibits a clear DA-immunoreactivity [53]. However, in that report, the amount of DA in DRG is about 10-fold lower than the levels found in dorsal horn of spinal cord. Moreover, in another recent report, tyrosine hydroxylase (TH) was expressed in small DRG neurons in the adult mouse, but aromatic amino acid decarboxylase was rare and did not colocalize with TH [6]. In addition, our previous study showed that stimulating a dorsal root either singly or repetitively does not evoke a slow current in SG neurons [35]. Therefore, it is possible that DA is not released from the central terminal of primary afferents innervating SG neurons. Although the possibility cannot be completely excluded that DA is released from the peripheral nerve terminals, it is reasonable to consider that most of dorsal horn DA arises from projections of dopaminergic neurons in the region of A11. Since we demonstrate that A11-ES induced antinociceptive effects in SG neurons are similar to those following perfusion of DA in the spinal cord, it is suggested that DA in the dorsal horn originates from dopaminergic neurons projected from the A11 region as a descending inhibitory system.

In conclusion, the present study using the *in vivo* patch-clamp technique indicates that the antinociceptive effects of DA originating from the descending dopaminergic pathway are mediated by the actions on both presynaptic and postsynaptic sites, reducing glutamate release onto SG neurons. The postsynaptic SG neurons induce hyperpolarizations by interacting with D2-like receptors and G-protein-mediated activation of K^+ channels. These findings suggest that the dopaminergic descending pathway has an antinociceptive effect and it may be of use in the understanding of intractable pain.

5. Conflict of interest statement

There are no conflicts of interest regarding this study, for any of the authors.

Acknowledgements

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今月のテーマ 神経因性疼痛

脊髄刺激による鎮痛効果とメカニズム

Clinical application and cellular mechanism for spinal cord stimulation-induced analgesia

中塚 映政
NAKATSUKA Terumasa

・鎮痛薬や神経ブロック治療に対して抵抗性の難治性慢性疼痛に対して脊髄電気刺激による鎮痛法が臨床応用されているが，その鎮痛機構は未だ不明である．
・後根付き成熟ラット脊髄横断薄切片の膠様質ニューロンにパッチクランプ法を適用して，脊髄電気刺激による鎮痛機構を検討した．
・後根の反復性電気刺激により膠様質ニューロンに緩徐なシナプス応答は観察されなかったが，脊髄後角を局所的に反復性電気刺激すると約30％の膠様質ニューロンにおいて緩徐な抑制性シナプス後電流が発生した．
・電気刺激によって脊髄介在ニューロンあるいは下行性抑制性神経線維終末から遊離される内因性のソマトスタチンによってGIRKチャネルが活性化し，膠様質ニューロンの膜が過分極することが明らかとなった．本機序が脊髄電気刺激による鎮痛機構に関与することが推察された．

KEY WORDS 脊髄刺激，鎮痛，ソマトスタチン，パッチクランプ法，抑制性シナプス後電流

はじめに

脊髄電気刺激による鎮痛法（脊髄電気刺激療法）は，薬物療法・理学療法・神経ブロック治療・手術に対して抵抗性の難治性慢性疼痛，特に神経因性疼痛に対して臨床応用されている．神経因性疼痛とは，神経系を含む組織の損傷後，傷が治癒したにもかかわらず発生する慢性で激しい痛みである．その発症には神経系の可塑的変化が関与しており，CRPS（Complex regional pain syndrome），幻肢痛，帯状疱疹後神経痛，脊髄損傷後神経痛，腕神経叢引抜き損傷，癌性疼痛，糖尿病性神経障害，HIV関連神経因性疼痛，三叉神経痛など多彩な病態を呈する．また，脊髄電気刺激療法は神経因性疼痛だけでなく，バージャー病や狭心痛などの虚血性疼痛やFailed Back Surgery Syndromeによる腰下肢痛にも応用されている．このように，脊髄電気刺激療法は非ステロイド性消炎鎮痛剤（NSAIDs）やモルヒネなどの鎮痛薬が無効な症例に対して有効であることから，その作用機序は従来の鎮痛薬とはまったく異なることが示唆されてきた．

脊髄電気刺激療法による鎮痛機構を明らかにするために，Meyersonらは脊髄電気刺激療法を施行している患者の脳脊髄液を採取し，モノアミン

関西医療大学保健医療学部疼痛医学分野 教授
Address/NAKATSUKA T：Pain Research Center, Kansai University of Health Sciences, OSAKA 590-0482

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をはじめ，サブスタンスP，ソマトスタチン，コレシストキニン，ニューロテンシンなど種々の神経ペプチド含有量を測定した[1]．採取した脳脊髄液において脊髄電気刺激によって有意に増加している物質はサブスタンスPだけであり，脊髄電気刺激によって遊離する物質は少量でかつ急速に代謝されるために脳脊髄液の採取によって脊髄電気刺激に関連する物質を同定することは困難であると結論づけられた．彼らはマイクロダイアリシス法を用いて実験を続け，脊髄電気刺激によって脊髄後角におけるグルタミン酸などの興奮性アミノ酸の遊離が減少する一方，GABAの遊離が増加することを示した[2]．一方，馬場らは脊髄スライス標本を用いて脊髄膠様質ニューロンにパッチクランプ法を適用し，脊髄電気刺激によってGABAの遊離が増加するだけでなく，脊髄膠様質ニューロンにおいて緩徐な抑制性シナプス後電位が発生することを単一細胞レベルで明らかにした[3]．残念ながら，末梢からの痛み情報を伝達するAδ線維ならびにC線維が入力する脊髄膠様質ニューロンにおいて緩徐な抑制性応答が発生することは脊髄電気刺激による鎮痛機構にとってきわめて重要であると考えられたが，その発生機序はまったく不明であった．今回，われわれは成熟ラットの脊髄スライス標本を用いて，脊髄後角の膠様質ニューロンからパッチクランプ記録を行い，脊髄電気刺激によって発生する緩徐な抑制性応答を詳細に解析した．

方　　法

1．脊髄横断スライスの作製

成熟Sprague-Dawley系雄性ラットをウレタン腹腔内投与（1.2～1.5g/kg）によって深麻酔を施行した後，腰仙部レベルの椎弓切除を行った．脊髄を摘出した後，直ちに95% O_2－5% CO_2で飽和し，かつ冷却した人工脳脊髄液（2～4℃）を浸したシャーレに移し，実体顕微鏡下に硬膜を除去した後，左L5以外のすべての後根および前根を切除した．くも膜および軟膜を除去した後，浅い溝を形成したブロック状の寒天上に置き固定した．マイクロスライサを用いて，厚さ650μmの左L5後根付き脊髄横断スライスを作製した．切り出したスライスを記録用チャンバ内に移し，95% O_2－5% CO_2で飽和した人工脳脊髄液を5～10ml/minの速度で灌流した．人工脳脊髄液の組成は，NaCl 117mM, KCl 3.6mM, NaH_2PO_4 1.2 mM, $CaCl_2$ 2.5mM, $MgCl_2$ 1.2mM, $NaHCO_3$ 25 mM, glucose 11mMであった．

2．細胞内記録

脊髄膠様質はスライス下部から透過光で観察すると，後角の背側表層に位置する比較的明るい帯として観察することができる．実体顕微鏡の観察下（20～40倍）に記録電極を盲目的に膠様質に刺入して，単一の膠様質ニューロンからパッチクランプ法を用いて電気応答を記録した．記録用電極には，potassium gluconate 135mM, KCl 5 mM, $CaCl_2$ 0.5mM, $MgCl_2$ 2 mM, EGTA 5 mM, ATP-Mg 5 mM, HEPES 5 mMを充填した先端電極抵抗5～10MΩの微小ガラス電極を用いた．得られた記録電流はパッチクランプ増幅器（Molecular Devices社製，Axopatch 200B）により増幅し，データ記録および解析用ソフト（Molecular Devices社製，pClamp 8）を用いて，記録ならびに解析した．データはすべてmean±SEで表わした．検定はStudent's paired t testで行い，$P<0.05$を有意とした．括弧内のnは記録したニューロンの数である．

使用した薬剤について，somatostatin, cyclo (7-aminoheptanoyl-Phe-D-Trp-Lys-Thr[Bzl]) (cyclo-somatostatin), tertiapin-Q, endomorphin-1, naloxone hydrochloride, D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2 (CTAP), CGP 35348, methiothepin, 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX), 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX), DL-2-amino-5-phosphonopentanoic acid (AP5), GDP-β-Sはsigma社から，yohimbine hydrochlorideとsulpirideは和光純薬，CGP 52432は

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結　　果

脊髄膠様質ニューロンにホールセル・パッチクランプ法を適用し，膜電位を－50mV に固定して，保持膜電流の変化に着目した（図 1 A）．単極の刺激電極を用いて，脊髄後角を局所的に20 Hz で20回（刺激強度：0.3～1.0mA，刺激持続時間：0.4ms）反復刺激すると，記録した289ニューロンのうち，94ニューロンにおいて，緩徐な抑制性シナプス後電流（slow inhibitory postsynaptic current：slow IPSC）が観察された（図 1 B）．脊髄電気刺激によって生じた slow IPSC の平均振幅は62±4 pA，平均持続時間は59±4 s であった（n＝73）．また，脊髄電気刺激を 5 ～10分間隔で行っても，slow IPSC の振幅に変化はなかった．過去にも報告したように[9]，吸引刺激電極を用いて後根を刺激（強度：0.5mA，持続時間：

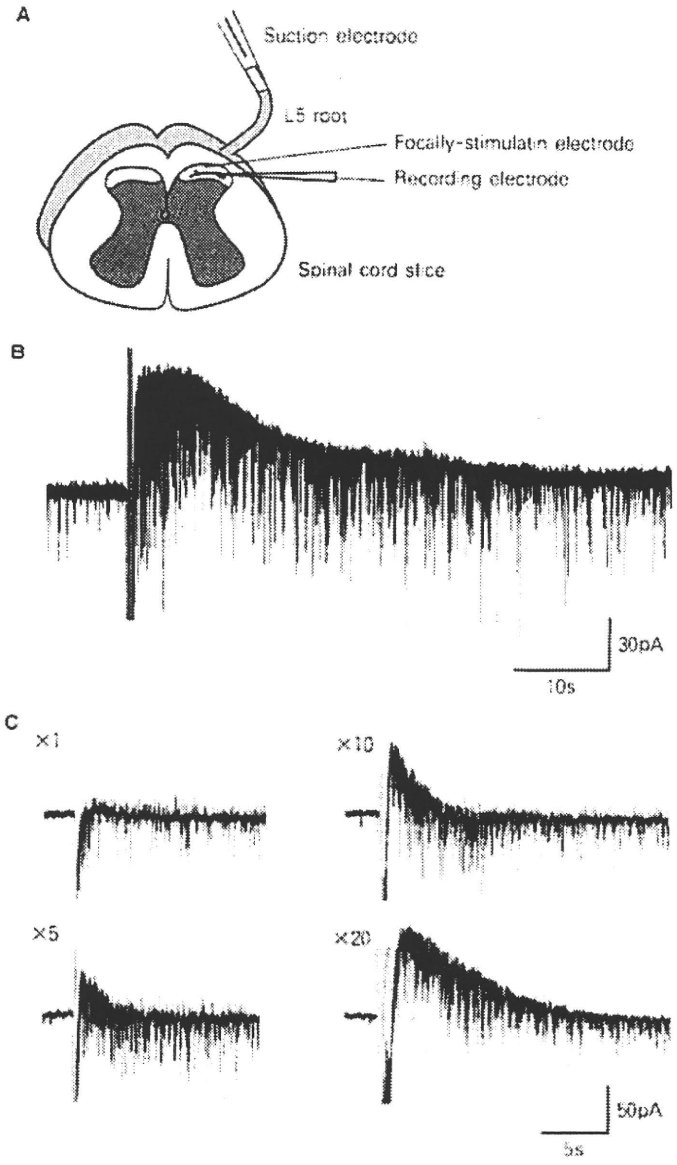

図 1　脊髄電気刺激によって誘起される抑制性シナプス応答

(A) L5後根付き脊髄横断スライス標本．

(B) 膜電位を－50mV に固定して，単極の刺激電極を用いて脊髄後角を局所的に20Hz で20回（刺激強度：0.3～1.0mA，刺激持続時間：0.4ms）反復刺激すると，緩徐な抑制性シナプス後電流（slow inhibitory postsynaptic current：slow IPSC）が観察される．

(C) 脊髄刺激回数を増やすと slow IPSC の振幅ならびに持続時間は増加する．

0.1ms）するとAδ線維誘起興奮性シナプス後電流（excitatory postsynaptic current：EPSC）あるいはC線維誘起EPSCが観察されたが，同様の刺激条件で後根を20Hzで20回反復刺激しても緩徐なシナプス応答は観察されなかった（データ記載なし）．

図1Cのように，脊髄刺激回数を増やすとslow IPSCの振幅ならびに持続時間は増加した．また，slow IPSCは細胞外カルシウムイオン濃度に依存していた（図2A）．無カルシウム溶液中におけるslow IPSCの平均振幅は15±6 pAで，同一記録ニューロンにおける通常の人工脳脊髄液中の

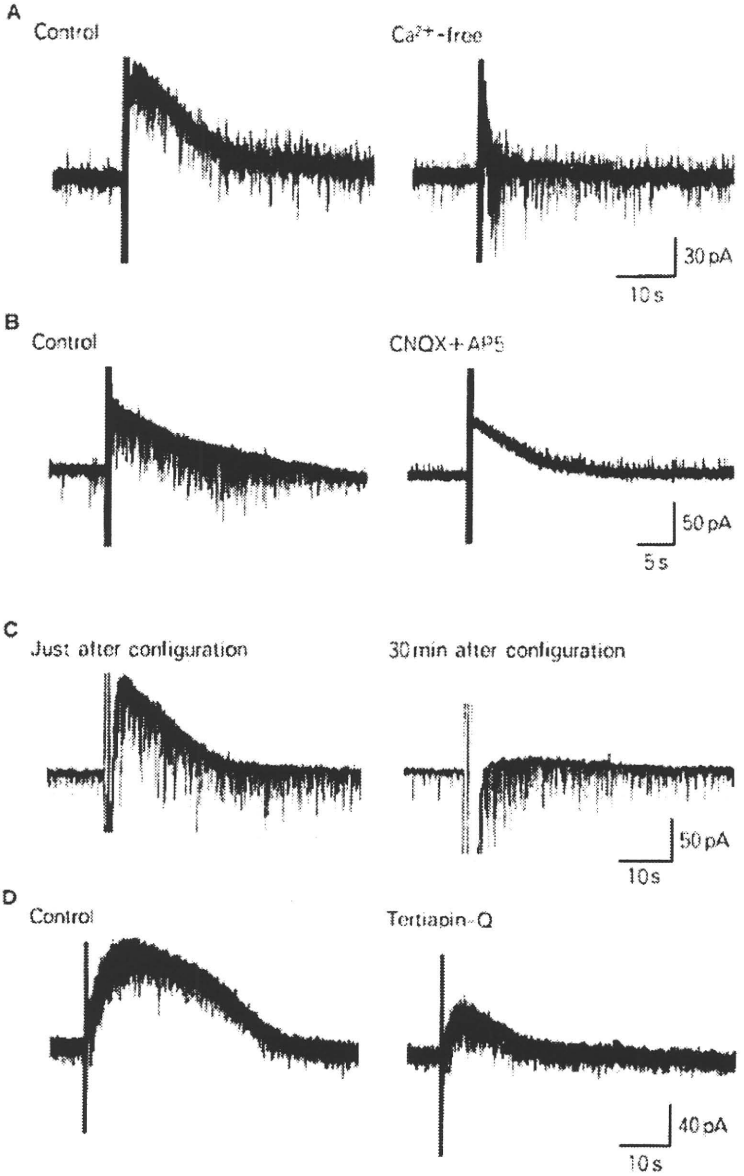

図2　脊髄電気刺激によって誘起される抑制性シナプス応答の特性

（A）無カルシウム溶液中においてslow IPSCの振幅は減少する．

（B）slow IPSCの振幅はグルタミン酸受容体阻害薬であるCNQXとAP5存在下で変化しない．

（C）記録電極内にG蛋白質の活性を阻害するGDP-β-Sを加えることによってslow IPSCの振幅は減少する．

（D）G蛋白共役型内向き整流性K^+チャネル（G-protein-coupled, inwardly-rectifying K^+チャネル：GIRKチャネル）の阻害薬であるtertiapin-Q存在下においてslow IPSCの振幅は減少する．

平均振幅（54±4 pA, n＝4）と比較して有意に減少した．一方，脊髄電気刺激によって発生したslow IPSCはグルタミン酸受容体阻害薬により影響を受けなかった（図2B）．CNQX（10μM）とAP5（50μM）存在下におけるslow IPSCの平均振幅は48±8 pAで，同一記録ニューロンでのCNQXならびにAP5非存在下における平均振幅（48±8 pA, n＝4）と比較して有意な変化は認めなかった．

slow IPSCの発生に細胞膜G蛋白質が関与するかどうかを調べるために，G蛋白質の活性を阻害するGDP-β-S（2 mM）を加えた記録電極を使用した（図2C）．パッチクランプ記録直後，脊髄電気刺激によって発生したslow IPSCの平均振幅は62±13 pAであったが，記録30分後には5±3 pA（n＝4）となり，記録電極内にGDP-β-Sを加えることによってslow IPSCの平均振幅は有意に減少した．また，非選択的K^+チャネルの阻害薬であるBa^{2+}存在下に実験を行った（データ記載なし）．Ba^{2+}（0.5mM）存在下におけるslow IPSCの平均振幅は14±2 pAで，同一記録ニューロンでのBa^{2+}非存在下における平均振幅（47±7 pA, n＝6）と比較して有意に減少した．G蛋白共役型内向き整流性K^+チャネル（G-protein-coupled, inwardly-rectifying K^+チャネル：GIRKチャネル）の阻害薬であるtertiapin-Q（0.1μM）存在下におけるslow IPSCの平均振幅は16±3 pAで，同一記録ニューロンでのtertiapin-Q非存在下における平均振幅（59±15 pA, n＝5）と比較して有意に減少した（図2D）．

脊髄電気刺激によって発生したslow IPSCがどの神経伝達物質を介する応答であるかを同定するために，種々の神経伝達物質阻害薬を用いて実験を行った．これまでに，脊髄膠様質ニューロンではオピオイド，アデノシン，ノルアドレナリン，セロトニン，ドーパミンなどさまざまな神経伝達物質によって外向き電流が誘起されることが知られている．まず，オピオイド受容体阻害薬を用いて実験を行った（データ記載なし），μ-オピオイド受容体阻害薬であるCTAP（1 μM）存在下におけるslow IPSCの平均振幅は40±13 pAで，同一記録ニューロンでのCTAP非存在下における平均振幅（42±14 pA, n＝4）と比較して有意な変化は認めなかった．さらに，非選択的オピオイド受容体阻害薬であるnaloxone（1 μM）存在下におけるslow IPSCの平均振幅は45±5 pAで，同一記録ニューロンでのCTAP非存在下における平均振幅（52±5 pA, n＝11）と比較して有意な変化は認めなかった．脊髄電気刺激によって発生するslow IPSCは，オピオイド受容体阻害薬と同様に，アデノシンA_1受容体阻害薬（DPCPX），$GABA_B$受容体阻害薬（CGP35348, CGP52432），α_2アドレナリン受容体阻害薬（yohimbine），5-HT_1受容体阻害薬（WAY100635），5-HT_{1A}受容体阻害薬（methiothepin），ドーパミンD2ライク受容体阻害薬（sulpiride）により影響を受けなかった．

近年，神経ペプチドであるソマトスタチンはG蛋白共役型K^+チャネルを介して脊髄膠様質ニューロンの膜を過分極することが報告されている[5)6)]．そこで，われわれはソマトスタチン（1 μM）を灌流投与したところ，記録した13ニューロンのうち，5ニューロンにおいて外向き電流が観察された（図3A）．また，ソマトスタチンにて外向き電流が発生したすべてのニューロンにおいて，脊髄電気刺激によってslow IPSCが発生した．興味深いことに，ソマトスタチンによって外向き電流が発生している間に誘起されたslow IPSCの平均振幅は16±3 pAで，外向き電流が発生していない状況下での平均振幅（68±15 pA, n＝5）と比較して有意に減少した（図3A）．さらに，また，非選択的ソマトスタチン受容体阻害薬であるcyclo-somatostatin存在下に実験を行った（図3B）．cyclo-somatostatin（5 μM）存在下におけるslow IPSCの平均振幅は39±11 pAで，同一記録ニューロンでのcyclo-somatostatin非存在下における平均振幅（69±13 pA, n＝6）と比較して有意に減少していた．

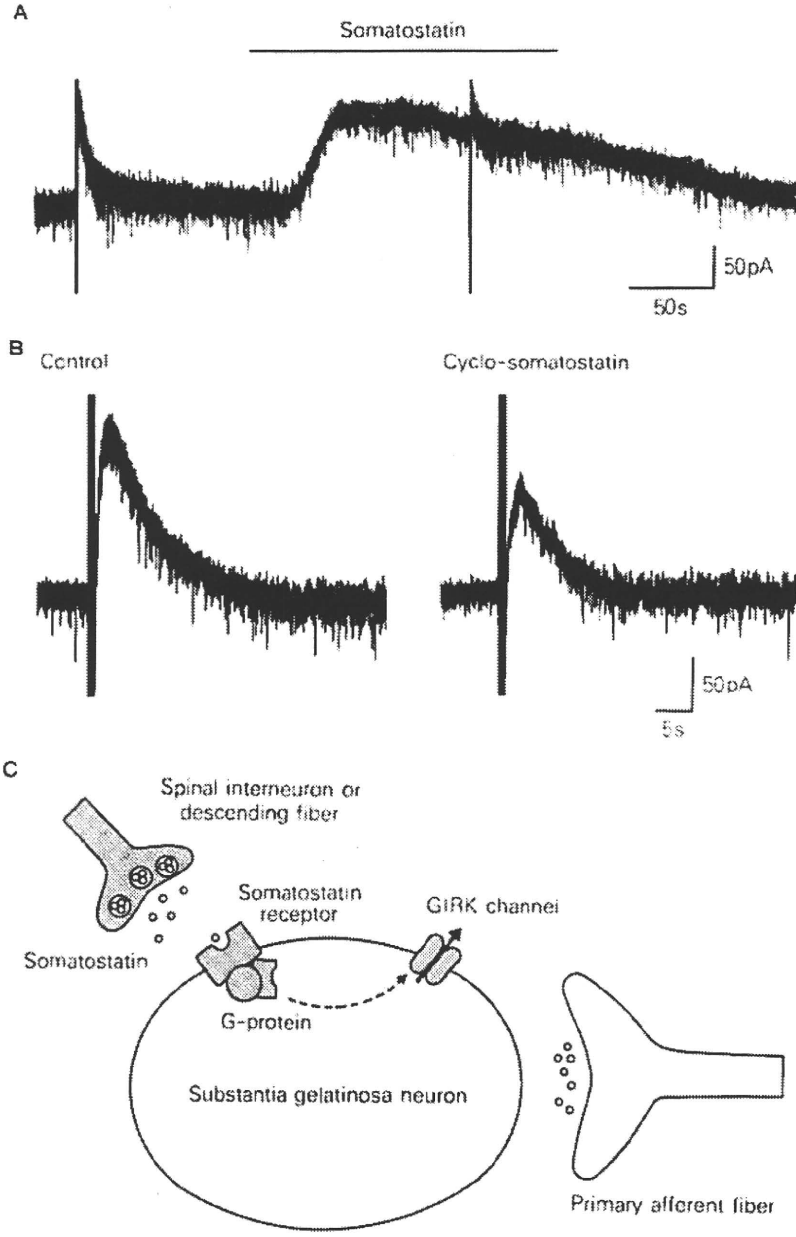

図3　抑制性シナプス応答の発生におけるソマトスタチンの関与

(A) ソマトスタチンを灌流投与によって外向き電流が発生する．ソマトスタチンによって外向き電流が発生している間に誘起されたslow IPSCの振幅は，外向き電流が発生していない状況下での振幅と比較して有意に減少する．

(B) 非選択的ソマトスタチン受容体阻害薬であるcyclo-somatostatin存在下において，slow IPSCの振幅は減少する．

(C) 脊髄電気刺激によって脊髄介在ニューロンあるいは下行性抑制性神経線維終末から遊離される内因性のソマトスタチンによってGIRKチャネルが活性化し，膠様質ニューロンの膜が過分極する．

考　　察

今回，脊髄膠様質ニューロンにおいて，後根を電気刺激しても緩徐なシナプス応答は観察されなかったが，脊髄を局所的に電気刺激するとslow IPSCが観察された．G蛋白質の活性を阻害するGDP-β-Sによってslow IPSCは阻害されること

から，slow IPSCの発生にはG蛋白質の活性化が関与している．また，slow IPSCの振幅が非選択的K^+チャネルの阻害薬であるBa^{2+}あるいはGIRKチャネルの阻害薬であるtertiapin-Qによって抑制されることによって，その発生にはK^+チャネルが関与していることが証明された．一方，slow IPSCの振幅はソマトスタチンによって外向き電流が発生している間に減少し，ソマトスタチン受容体阻害薬によって有意に減少させる．以上の結果から，脊髄電気刺激によって遊離するソマトスタチンが脊髄膠様質ニューロンのソマトスタチン受容体を活性化し，その結果，G蛋白質を介してGIRKチャネルを活性化することが明らかになった（図3C）．

GIRKチャネルは中枢神経系に広く存在し，主に，G蛋白質を介する緩徐な抑制性シナプス応答に関与していることが知られている．GIRKチャネルをコードする遺伝子にはGIRK1-4あるいはKir3.1-3.4とよばれる4種類が存在し，中枢神経系のGIRKチャネルはGIRK1-3を含む4量体で構成されている．脳ではGIRK1-3がいずれも発現しているが，脊髄ではGIRK1とGIRK2だけが後角表層，特に膠様質に限局して発現している[7]．また，GIRK1あるいはGIRK2ノックアウトマウスでは，痛覚過敏を呈することから，GIRKチャネルは鎮痛に関与していることが示唆された[7]．しかしながら，脊髄内のGIRKチャネルがどのような内因性の神経伝達物質によって，どのように機能的に作用するかは不明であった．本研究によって脊髄内のGIRKチャネルは痛みの制御に重要な役割を果たしていることが明らかになった．

ソマトスタチンは当初，視床下部の神経内分泌ホルモンとして発見され，脳下垂体からの成長ホルモン分泌を抑制することが知られている．最近，ソマトスタチンは中枢神経系に広く存在し，G蛋白質共役型ソマトスタチン受容体に結合して多彩な生理作用を呈する．脊髄腔へのソマトスタチン投与は鎮痛作用を呈することや，脊髄後角ニューロンにおける発火がソマトスタチンによって抑制されることから，ソマトスタチンは脊髄レベルでは痛覚伝達機構を制御する[8][9]．さらに，近年，ソマトスタチンは一部の膠様質ニューロンに作用して膜を過分極することが示された[5][6]．ソマトスタチン含有ニューロンやソマトスタチン免疫反応陽性の神経終末が後角表層に豊富に存在する[10][11]．これらソマトスタチン免疫反応陽性の神経終末は，視床下部からの下行性線維だけでなく[12]，一次求心性線維や脊髄介在ニューロンに由来すると考えられる[10]．しかしながら，本研究で後根を電気刺激しても緩徐なシナプス応答は観察されなかったので，脊髄電気刺激によって遊離するソマトスタチンは一次求心性線維ではなく，脊髄内介在ニューロンあるいは下行性線維に由来することが示唆された．

結　　語

脊髄電気刺激によって脊髄介在ニューロンあるいは下行性抑制性神経線維終末から遊離される内因性のソマトスタチンによってGIRKチャネルが活性化し，膠様質ニューロンの膜が過分極することが明らかとなった．したがって，本機序が脊髄電気刺激による鎮痛機構に関与することが推察された．

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