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Plastic changes in sensory inputs to rat substantia gelatinosa neurons following peripheral inflammation

Terumasa Nakatsuka[a,b], Jin-Soo Park[a], Eiichi Kumamoto[a], Tetsuya Tamaki[b], Megumu Yoshimura[a,*]

[a]*Department of Physiology, Saga Medical School, Nabeshima, Saga 849-8501, Japan*
[b]*Department of Orthopedic Surgery, Wakayama Medical School, Wakayama 640-8516, Japan*

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Abstract

Although hyperalgesia elicited by inflammation has been shown to be partly due to central sensitization, the cellular mechanisms are not clear at the moment. The present study was designed to address this issue using the blind whole-cell patch-clamp technique; glutamatergic primary-afferent inputs to substantia gelatinosa (SG) neurons were compared between spinal cord slices of naive rats and rats inflamed by an intraplantar injection of complete Freund's adjuvant. In naive rats, a large number of SG neurons examined received monosynaptic Aδ- (69% of 41 neurons innervated by A fibers) and/or polysynaptic C- (94% of 36 neurons innervated by C fibers) afferent inputs, and only a few neurons received monosynaptic Aβ inputs (7%). In addition, when examined in neurons which have both of the A- and C-afferent inputs, A afferent-evoked excitatory postsynaptic currents (EPSCs) were larger in amplitude than C afferent-induced ones; a ratio (A/C ratio) of the former to latter amplitude was 1.8 ± 0.1 ($n = 36$). In inflamed rats, a change in the synaptic responses was observed: (1) SG neurons receiving monosynaptic Aδ-afferent inputs decreased in number (to 20% of 30 neurons tested, innervated by A fibers), whereas those having monosynaptic Aβ-afferent inputs increased to 33%, and (2) the A/C ratio decreased to 0.7 ± 0.1 ($n = 33$). These results suggest that after inflammation, a substantial number of Aβ-afferents sprout into the SG from their original location (laminae III–V) and that sensory information that used to be conveyed directly to the SG through Aδ afferents is transmitted there indirectly through interneurons. These reorganizations of sensory pathway may contribute, at least in part, to underlying mechanisms for the development of hyperalgesia due to inflammation.

Keywords: Peripheral inflammation; Hyperalgesia; Spinal dorsal horn; Plasticity; Sprouting; Patch-clamp

1. Introduction

Hypersensitivity has been considered as either increased sensation of pain following a noxious stimulus (hyperalgesia) or the sensation of pain in response to normally innocuous stimuli (allodynia) (see Meyer et al. (1994) for review), and is the common characteristics of peripheral inflammation. Although it has been accepted that peripheral nerves could be sensitized by chemicals that are present at the site of tissue injury and inflammation, resulting in hyperalgesia (see Raja et al. (1988) for review), a change in excitability of neurons in the dorsal horn of the spinal cord may also participate in the generation of hyperalgesia (see Treede et al. (1992) for review). This change in excitability manifests as either the enlargement in size of receptive field (McMahon and Wall, 1984; Hylden et al., 1989), increase in firing rates in response to electrical stimuli (Neugebauer and Schaible, 1990), or a novel occurrence of responses to low-intensity stimuli (Simone et al., 1991; Woolf et al., 1994b); they appear to be mediated by glutamate (Ma and Woolf, 1995a), substance P (Ma and Woolf, 1995b) and opioids (Iadarola et al., 1988; Hylden et al., 1991). These modifications would collectively constitute the phenomenon of central sensitization. Alternatively, the hypersensitivity may be elicited by a change in circuitry in the dorsal horn, in such that afferents innervating mechanoreceptors begin to induce pain. Evidence for this idea has been given by neuroanatomical studies (Woolf et al., 1992; Koerber et al., 1994), and it is unknown whether this reorganization is functional.

A combination of anatomical and physiological techni-

* Corresponding author. Tel.: +81-952-34-2271; fax: +81-952-34-2013; e-mail: yoshimum@post.saga-med.ac.jp

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ques has provided evidence suggesting that neurons within the substantia gelatinosa (SG; lamina II of Rexed) in the dorsal horn form a local interneuronal circuit (Rexed, 1952), which is thought to integrate noxious afferent information from different classes of fibers and thus to modify the output of projection neurons located in both lamina I and deeper regions of the dorsal horn (Kumazawa and Perl, 1978; Light et al., 1979). Neurons located in the SG receive predominantly fine afferent fibers, many of which have conduction velocities (CVs) in a range of those of Aδ- and C-fibers (Rethelyi, 1977; Light and Perl, 1979; Sugiura et al., 1989; Yoshimura and Jessell, 1989; Yoshimura and Jessell, 1990) and respond to noxious stimuli (Woolf and Fitzgerald, 1983). Thus, it is conceivable that the SG neurons play a critical role in the development of hypersensitivity. Reliable analyses of mechanisms for the hypersensitivity, however, had been hampered until recently because of the difficulty in obtaining stable recordings from SG neurons, which are among the smallest cells in the central nervous system. Since the introduction of the slice and hemisection of spinal cord preparations together with glass-microelectrode and patch-clamp recording techniques, precise analyses of electrophysiological and pharmacological properties of synaptic transmissions in SG neurons have become available at the single cell level (Yoshimura and North, 1983; Urban and Randic, 1984; Schneider and Perl, 1988; Yoshimura and Jessell, 1989, 1990; Yoshimura and Nishi, 1993; Baba et al., 1994; Yajiri et al., 1997) (see Yoshimura (1996) for review).

The present study was designed to address the question about what are the consequential changes in sensory pathways following peripheral inflammation, by a comparison of glutamatergic excitatory synaptic responses elicited in SG neurons by stimulating primary-afferent fibers both in spinal cord slices, which were obtained from naive rats and from rats inflamed by intraplantar injection of complete Freund's adjuvant.

2. Materials and methods

2.1. Animal model

Male Sprague–Dawley rats (8-week-old) were injected with complete Freund's adjuvant (CFA, Mycobacterium tuberculosis; Sigma, St. Louis, MO) as described previously (Woolf et al., 1994a), 7–10 days prior to record excitatory synaptic responses from SG neurons. Three-hundred microliters of CFA suspended in an oil/saline (1:1) emulsion was injected at a concentration of 0.5 mg/ml into the plantar surface of the left hindpaw of rats under anesthesia (pentobarbital sodium, 45–50 mg/kg, i.p.). This procedure produced a persistent peripheral inflammation in the injected hindpaw that was characterized by edema and mechanical hyperalgesia as described below. As a control, we used rats in which the same volume of saline was injected.

2.2. Inflammation tests

A degree of edema was assessed by measuring the thickness of dorsal-ventral paw by use of calipers at various times following the injection of CFA. Mechanical hyperalgesia was determined using a set of von Frey hairs (0.0045–447 g, Stoelting, USA) over the same time course. The minimum force required to elicit a reproducible flexion withdrawal reflex on each of three applications of von Frey hairs to the hindpaw was measured as a mechanical nociceptive threshold, as described elsewhere (Woolf et al., 1994a). All the experimental procedures involving rats have been approved by the institutional Animal Use and Care Committee.

2.3. Intracellular recording from dorsal root ganglion neurons

L5 dorsal root ganglion (DRG) was isolated from 9-week-old rats together with a proximal dorsal root having a length of 10–15 mm. The DRG was then submerged and superfused at a rate of 5–10 ml/min with Krebs solution equilibrated with 95% O_2 and 5% CO_2 and maintained at 36 ± 1°C. The composition of Krebs solution used was (in mM); NaCl 117, KCl 3.6, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11. Antidromic stimulation (0.1 ms duration) at a central end of the dorsal root was performed with a suction electrode. Intracellular recordings of action potentials (APs) were made from DRG neurons with glass-microelectrodes having a DC tip resistance of 50–80 MΩ, filled with 4 M potassium-acetate. Signals were amplified with a high input-impedance bridge amplifier (Axoclamp 2A, Axon Instruments, Foster City, CA), and were monitored on a digital oscilloscope (VC-11, Nihon Kohden, Japan). Data from neurons with resting membrane potentials more negative than –50 mV and with AP amplitudes larger than 60 mV were included in the present study. The antidromic APs were analyzed with respect to CV, AP duration (APD) and threshold stimulus intensity (TSI) for eliciting an AP. The APD was determined at half of the peak amplitude of AP. Primary-afferent fibers were classified into one of three groups, Aβ-, Aδ- and C-type, on the basis of a combination of values of the axonal CV, APD and TSI.

2.4. Preparation of spinal cord slices

The methods used for obtaining slice preparations of the adult rat spinal cord were similar to those described previously (Yoshimura and Nishi, 1993). Briefly, male Sprague–Dawley rats (9-week-old) were anesthetized with urethane (1.2 g/kg, i.p.) and then a lumbosacral laminectomy was performed. The lumbosacral segments of the spinal cord (L1–S3) with the ventral and dorsal roots intact was removed and placed in cold (1–3°C) Krebs solution pre-equilibrated with 95% O_2 and 5% CO_2. After cutting

all of the ventral and dorsal roots near the root entry zone, except for the left L5 dorsal root, the pia-arachnoid membrane was removed. The spinal cord was mounted on a Vibratome and then a 600 μm-thick transverse slice with the dorsal root attached was cut. After the slice was placed on a nylon mesh in the recording chamber, a titanium electron microscope grid was placed on the top of the slice and held in position by a silver wire loop attached to a micromanipulator. The slice was completely submerged and superfused at a rate of 20–30 ml/min with Krebs solution equilibrated with 95% O_2 and 5% CO_2 and maintained at 36 ± 1°C (Fig. 1). The composition of Krebs solution was the same as that used for intracellular recordings from DRG neurons.

2.5. Patch-clamp recording from SG neurons

Blind whole-cell voltage-clamp recordings were made from SG neurons with a patch-pipette which was made of a thin-walled fiber-filled glass (1.5 mm o.d.). The electrode was filled with a solution having the following composition (in mM): potassium gluconate 135, KCl 5, $CaCl_2$ 0.5, $MgCl_2$ 2, EGTA 5, HEPES 5, Mg-ATP 5, and had a resistance of 8–15 MΩ. Typical seal resistances of the electrodes were 5–20 GΩ. Signals were amplified with a patch-clamp amplifier (Axoclamp 200B, Axon Instruments). Membrane currents were continuously monitored on either digital oscilloscope or pen recorder (OMNILIGHT, San-ei, Japan). Data were low-pass-filtered at 5 kHz, digitized at 333 kHz with an A/D converter, stored and analyzed with a personal computer using pCLAMP data acquisition program (Version 6.0, Axon Instruments). Orthodromic stimulation of the dorsal root to elicit excitatory postsynaptic currents (EPSCs) was performed with the same suction electrode that was used in the experiments of DRG neurons (Fig. 1). The holding potential used was −70 mV at which glycine- and γ-aminobutyric acid-mediated synaptic currents were negligible (Yoshimura and Nishi, 1993). The duration of stimuli used was 0.1 ms throughout the experiments.

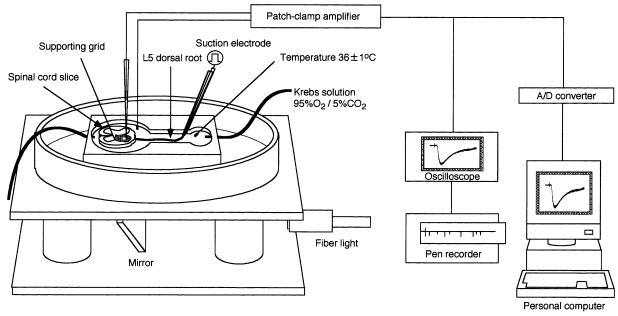

Fig. 1. Schematic diagram of the experimental setup to record excitatory postsynaptic currents (EPSCs) elicited in substantia gelatinosa (SG) neurons in a spinal cord slice by stimulating the dorsal root with a suction electrode. The slice was fixed by using a supporting grid, and superfused with Krebs solution equilibrated with 95% O_2/5% CO_2 and maintained at 36 ± 1°C. The SG was discernible by illuminating the slice from below with a fiber light refracted by mirror. EPSCs amplified with a patch-clamp amplifier were monitored on either oscilloscope or pen recorder; the data were digitized with an A/D converter, and analyzed with a personal computer.

When CVs were calculated from the latency of monosynaptic EPSC from a stimulus artifact and the length of dorsal root, the values became smaller as the stimulus point, activated with a monopolar sliver-wire electrode, moved toward a more proximal site, suggesting that the CV was not the same throughout the dorsal root but decreased in magnitude with approaching the spinal cord (Yoshimura and Jessell, 1989). A CV value was, therefore, determined by stimulating the dorsal root with an electrode positioned at two points at the distal and proximal sites and then by measuring a difference in latencies of those EPSCs and a distance between the stimulated points. The drug used in the present work was 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Tocris Neuramin, Bristol, UK).

2.6. Identification of SG neurons

Neurons were identified as SG neurons based on their locations and morphological features (Yoshimura and Jessell, 1989). Under a dissecting microscope with a magnification of 20–40 and with transmitted illumination, the SG was clearly discernible as a relatively translucent band across the dorsal horn (Fig. 1). However, the border between laminae I and II, and also that between laminae II and III were not determined with certainty; therefore, the patch electrode was inserted at the center of SG under visual control. In some instances, the recorded neurons were further identified by their precise location and feature using cell staining with neurobiotin (0.3% in patch-pipette solution; Vector, USA) as reported previously (Yajiri et al., 1997).

2.7. Statistics

Data are presented as mean ± SEM. Statistical significance was determined as $P < 0.05$ using non-parametric Kolmogorov Smirnov's test. In all cases, n refers to the number of neurons studied.

3. Results

3.1. Inflammation measurements

Intraplantar injection of CFA into the left hindpaw of rats resulted in edema, erythema and sensory hypersensitivity. Fig. 2A,B demonstrate changes in paw thickness (a measure of edema) and in mechanical nociceptive sensitivity, respectively, which were plotted against days after the CFA injection. Before the injection, values of paw thickness and of mechanical withdrawal-threshold averaged to be 5.66 ± 0.06 mm and 59.0 ± 7.4 g (n = 20), respectively. One day after the injection, the thickness of injected paw increased to a

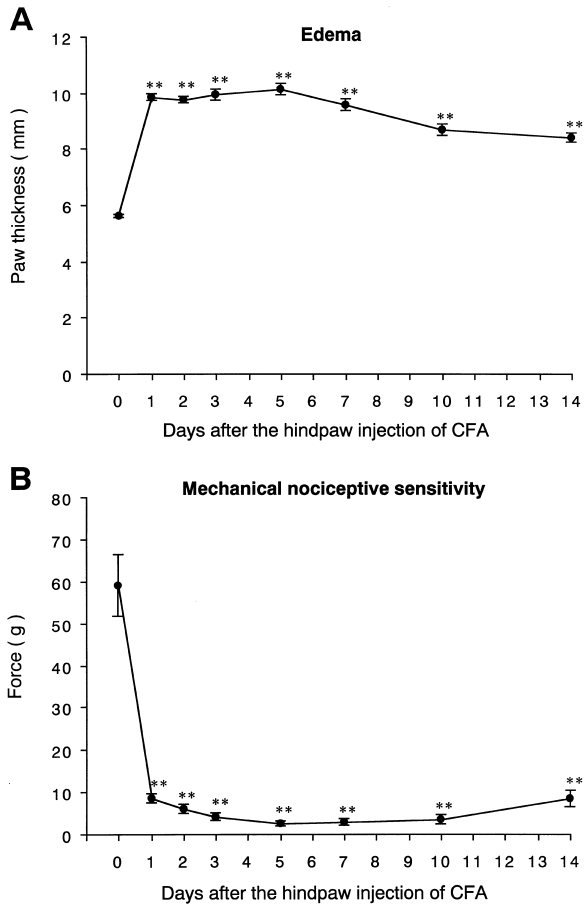

Fig. 2. Time course of inflammation (A) and mechanical hyperalgesia (B) following intraplantar injection of complete Freund's adjuvant (CFA) into the left hindpaw of rats ($n = 20$). The inflammation and hyperalgesia were measured as changes in dorsal-ventral paw thickness and mechanical threshold for eliciting a flexion withdrawal reflex with von Frey hairs, respectively, which were plotted against days after the CFA injection. $^{**}P < 0.01$; this was determined by comparing the thickness and threshold in CFA-injected rats with those in pretreated rats.

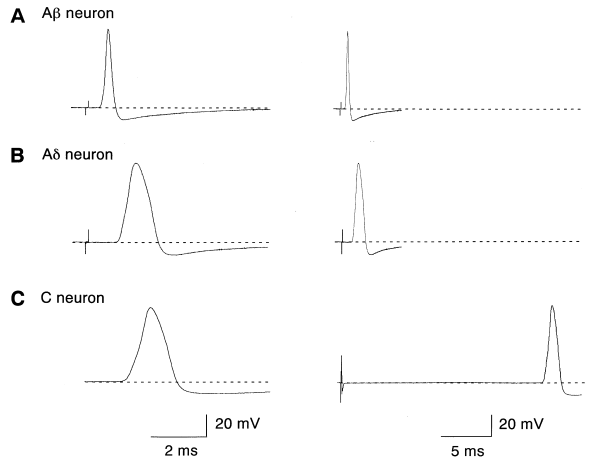

Fig. 3. Action potentials (APs) in three subgroups, Aβ- (A), Aδ- (B) and C-type (C), of dorsal root ganglion neurons obtained from naive rats. In each of them, APs are shown on a fast (left) and slow (right) time base. The APs were elicited by antidromic stimulation of the L5 dorsal root. Conduction velocities of the dorsal roots in (A), (B) and (C) were 16.7, 6.4 and 0.4 m/s, respectively. The dotted line in each of (A), (B) and (C) indicates the resting membrane potential; they were −63, −60 and −67 mV, respectively.

maximal value (9.76–10.11 mm), and then the increment lasted for the next 2 weeks with a tendency to be gradually decreased. When tested by using von Frey hairs (range: 0.028–126 g), the mechanical withdrawal-threshold also exhibited a similar alteration; this markedly decreased to 8.4 ± 0.8 g 1 day after the injection, and then reached a minimum at about 1 week. The mechanical hyperalgesia persisted for more than 2 weeks without a significant reduction, an observation indicating that the inflamed rats continue to perceive more pain sensation than naive rats in response to a mechanical noxious stimulation. Based on these data, DRGs and slice preparations of the spinal cord in inflamed rats for electrophysiological recordings were obtained from rats of 7–10 days after the CFA injection. However, preparations from naive rats were obtained at similar days after an injection of saline instead of CFA.

Table 1
Comparison of electrophysiological properties between DRG neurons obtained from naive and inflamed rats

		RMP (mV)	CV (m/s)	APD (ms)	TSI (μA)
Naive	Aβ neurons	−64 ± 2	20.6 ± 1.1	0.30 ± 0.01	7.6 ± 0.3
	(n = 24)	(51–75)	(14.2–30.2)	(0.22–0.4)	(5.0–9.0)
	Aδ neurons	−65 ± 3	88 ± 0.8	0.55 ± 0.04	14.5 ± 1.7
	(n = 12)	(51–77)	(3.3–13.6)	(0.31–0.67)	(10.0–26.0)
	C neurons	−51, −80	0.5, 0.8	1.21, 1.40	270, 305
	(n = 2)	(51–80)	(0.5–0.8)	(1.21–1.40)	(270–305)
Inflamed	Aβ neurons	−62 ± 2	17.9 ± 0.9	0.26 ± 0.02	7.7 ± 0.5
	(n = 1l)	(52–71)	(14.3–24.5)	(0.20–0.33)	(5.0–10.0)
	Aδ neurons	−65 ± 2	9.0 ± 0.7	0.54 ± 0.08	14.4 ± 1.6
	(n = 7)	(52–70)	(6.4–12.1)	(0.29–0.90)	(11.0–22.0)
	C neurons	65 ± 4	0.6 ± 1.6	1.14 ± 0.10	267 ± 52
	(n = 3)	(57–72)	(0.4–0.7)	(0.94–1.25)	(180–360)

RMP, resting membrane potential; CV, conduction velocity; APD, duration of action potential measured at its half-maximal amplitude; TSI, threshold stimulus intensity. Values are shown as means ± SEM. TSI was determined with a stimulus duration of 0.1 ms. The range of values of each parameter is given in parentheses.

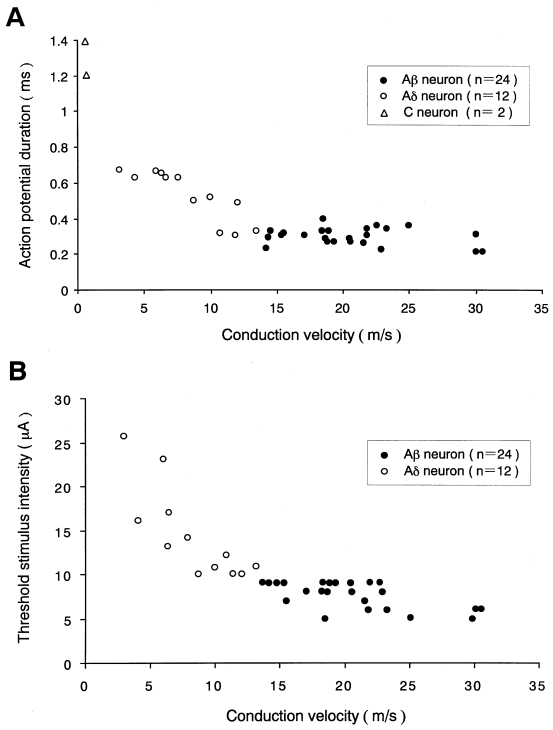

Fig. 4. Action potential duration (A; APD) and threshold stimulus intensity (B; TSI) measured in dorsal root ganglion (DRG) neurons in naive rats, which were plotted against conduction velocity (CV) of the dorsal root. The APD was estimated at half of the peak amplitude of action potential. The DRG neurons were classified into three subgroups, Aβ-(●), Aδ-(○) and C-type (△), based on values of the APD, TSI and CV. A distinction between the Aβ- and Aδ-types was arbitrarily made based on the two relationships shown in (A) and (B). In (B), data of C cells were not shown, because their TSI values (270, 305 μA) were much larger than those of Aβ and Aδ neurons.

3.2. Antidromic action potentials to DRG neurons

Intracellular recordings were made from 38 and 21 DRG neurons, respectively, in naive and inflamed rats, in any cases of which spontaneous firings were not observed. Fig. 3 shows antidromic APs elicited in DRG neurons obtained from naive rats by stimulating the dorsal root. There was a variation in the latency of AP, APD and TSI among the DRG neurons. Neurons, judged to be fast-conducting from the AP latency, had a brief APD, whereas slow-conducting ones had a relatively broad APD (Table 1), an observation consistent with those reported by Harper and Lawson (1985b) and also by Waddell and Lawson (1990). Fig. 4A,B demonstrate the values of APD and TSI, respectively, each of which was plotted against CV values. DRG neurons were divided into three subgroups, Aβ-, Aδ- and C-types, based on a combination of the values of CV, APD and TSI. A similar classification was done also for DRG neurons obtained from inflamed rats. After examining the active properties, a resting membrane potential was determined by a sudden withdrawal of microelectrode from the DRG neuron. Table 1 summarizes values of resting membrane potential, CV, APD and TSI of DRG neurons in naive and inflamed rats. There was no significant difference in each of their parameters between the two groups.

3.3. Synaptic responses elicited in SG neurons in response to dorsal root stimulation

Whole-cell patch-clamp recordings could be obtained from slices maintained in vitro for more than 10 h and stable recordings were made from single SG neurons for up to 4 h. All of the SG neurons examined exhibited spontaneous EPSCs which were completely blocked by a non-*N*-methyl-D-aspartate receptor antagonist, CNQX (10 μM). Their mean frequency and amplitude in slices obtained from naive and inflamed rats were, respectively, 18.2 ± 4.1 Hz; 9.1 ± 2.8 pA and 21.8 ± 2.0 Hz; 10.6 ± 2.2 pA; they were not significantly different from each other ($P > 0.1$ in both frequency and amplitude; not shown). In addition, no significant differences in passive membrane properties such as resting membrane potential and input resistance were detected between SG neurons obtained from naive and inflamed rats.

Single stimuli applied to the dorsal root, with the same suction electrode used for the experiments in DRG neurons, evoked a mono- and/or polysynaptic EPSC in SG neurons in slices obtained from naive and inflamed rats, all of which were blocked by CNQX (10 μM). In naive rats, stimuli of intensities less than 10 μA did not evoke any EPSCs in the majority of SG neurons examined (37 out of 41) while higher-intensity stimuli to be more than 10 μA but less than 36 μA elicited EPSCs having a short (less than 2.1 ms) latency in those neurons. EPSCs in 69% of the 41 neurons seemed to be monosynaptic in origin, because (1) when the dorsal root was stimulated at a frequency of 0.2 Hz with an intensity just above the threshold, the EPSCs had a constant latency, and (2) with repetitive stimuli at 20–50 Hz, the constancy was maintained and no failures were observed (Fig. 5A). In 22% of the neurons tested, EPSCs

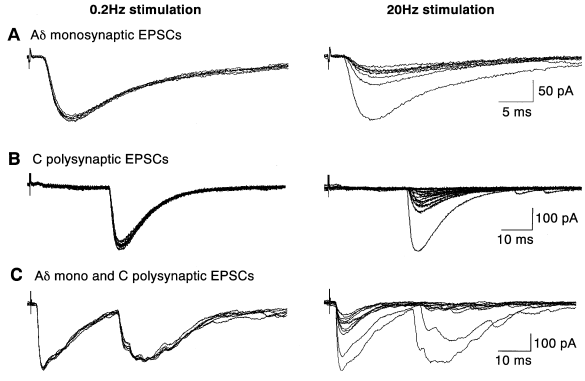

Fig. 5. Various patterns of EPSCs elicited by stimulating the dorsal root at a frequency of 0.2 Hz (left) or 20 Hz (right) in slices obtained from naive rats. (A,C) Monosynaptic Aδ-afferent EPSCs, (B,C) polysynaptic C-afferent EPSCs; in (C), both of the monosynaptic Aδ- and polysynaptic C-afferent EPSCs were observed in the same neuron. Stimulus intensities used in (A), (B) and (C) were 12, 320 and 260 μA, respectively. Holding potential = − 70 mV.

Table 2
Comparison of primary-afferent inputs to SG neurons between spinal cord slices of naive and inflamed rats

	A-fiber evoked EPSCs				C-fiber evoked EPSCs			A and C responses	
	Aβ-mono	Aβ-poly	Aδ-mono	Aδ-poly		Mono	Poly		
Naive	3	1	28	9	Naïve	2	34	Naive	35
(n = 41)	(7%)	(2%)	(69%)	(22%)	(n = 36)	(6%)	(94%)	(n = 43)	(81%)
Inflamed	10	2	6	12	Inflamed	2	31	Inflamed	20
(n = 30)	(33%)	(7%)	(20%)	(40%)	(n = 33)	(6%)	(94%)	(n = 43)	(47%)

The numbers in A and C responses inicate those of SG nuerons in which both A- and C-fiber evoked ESPCs were observed.

showed a variable latency and failures with repetitive stimuli (not shown), indicating their polysynaptic origin. The average of CV values, calculated from a difference in onset time between these EPSCs evoked by stimulating the dorsal root at two points (see Section 2.4), was 8.0 ± 3.4 m/s with a range from 4.4 to 13.2 m/s, values being within those for Aδ-afferent fibers obtained from the experiment in DRG neurons. In addition, the stimulus intensities were consistent with those for Aδ fibers of dorsal roots, as seen in Table 1.

The observation that EPSCs in the remaining neurons (n = 4) were elicited by stimuli with an intensity (less than 10 μA) insufficient to activate Aδ afferents suggests an innervation by Aβ afferents. EPSCs observed in three of those neurons appeared to be monosynaptic, as assessed by its short (less than 0.7 ms) and constant latency as described above, while the remaining one neuron seemed to receive polysynaptic inputs (Table 2). Consistent with an idea of the activation of Aβ afferents, CV values, estimated from the onset of monosynaptic EPSC, averaged to be 20.1 ± 2.1 m/s, a value being in the range of those for Aβ fibers (Table 1). Such a monosynaptic Aβ input on SG neurons may be consistent with anatomical data by Woolf et al. (1992), that 8.4% of sural Aβ fibers penetrated into the inner lamina II, although a possibility cannot be ruled out that the recordings were made from large dendrites of neurons whose cell bodies exist in deeper laminae (Willis and Coggeshall, 1991). As described above, we confirmed in some neurons that the soma of a neuron into which neurobiotin was injected through a patch-pipette is located in the SG.

In addition to the A-afferent responses, EPSCs having a long latency, presumably evoked by C-afferents, were observed in 36 neurons. Higher-stimulus intensity, which was sufficient to recruit C- as well as A-afferents, produced a long-latency EPSC which was either with (Fig. 5C) or without (Fig. 5B) a preceding A afferent-evoked EPSC. Only two (6%) of the neurons tested seemed to receive a direct input, since the latency remained constant when the dorsal root was repetitively stimulated at a frequency of 20 Hz. The values of CV estimated by a method similar to that described above, averaged to be 0.34 ± 0.03 m/s; this was within the range of those of C-afferent fibers (Table 1). In the remaining 34 neurons, the latency was variable and failures were observed with a repetitive stimulation at 10–20 Hz (Fig. 5B,C).

Different from those in naive rats, SG neurons in slices obtained from inflamed rats received a large number of monosynaptic Aβ-afferent inputs. Single stimuli with a strength sufficient to activate Aβ afferents elicited an EPSC in 12 neurons (40% of 30 neurons tested, innervated by A fibers). Ten out of the 12 neurons appeared to receive a direct input, since the latency of EPSC was short (less than 0.8 ms) and no failures were observed when the dorsal root was stimulated repetitively at 20 Hz, as seen in Fig. 6A. In the remaining two neurons, the latency was variable and many failures were observed by such a repetitive stimulation, indicating polysynaptic origin.

However, neurons showing Aδ-afferent EPSCs were decreased in number in inflamed (n = 18; 60% of the neurons innervated by A fibers) compared with naive (91%) rats. Six out of the 18 neurons exhibited a monosynaptic Aδ-EPSC (not shown) while the remaining neurons received polysynaptic inputs (Fig. 6B). C-afferent inputs exhibiting mono- and polysynaptic responses, however, did not differ in proportion between preparations in naive and inflamed rats, when tested in 33 SG neurons obtained from inflamed rats. In inflamed rats, most of the C-afferent synaptic responses (n = 31) were polysynaptic in origin as shown in Fig. 6C and monosynaptic EPSCs were observed in only two neurons. Table 2 summarizes the numbers of neurons having A and C afferent-evoked EPSCs in slices obtained from naive and inflamed rats.

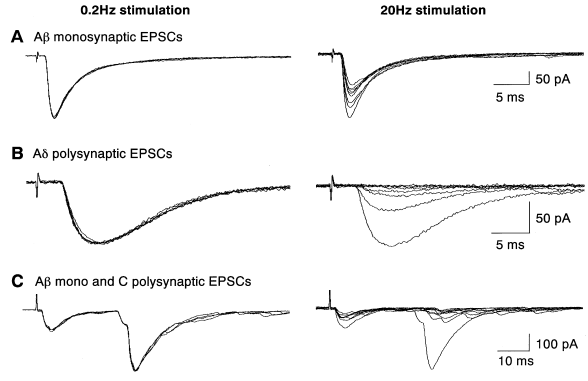

Fig. 6. Various patterns of EPSCs elicited by stimulating the dorsal root at a frequency of 0.2 Hz (left) or 20 Hz (right) in slices obtained from inflamed rats. (A,C) Monosynaptic Aβ-, (B) polysynaptic Aδ-, and (C) polysynaptic C-afferent EPSCs; in (C), both of the monosynaptic Aβ- and polysynaptic C-afferent EPSCs were observed in the same neuron. Stimulus intensities used in (A), (B) and (C) were 8, 19 and 340 μA, respectively. Holding potential = −70 mV.

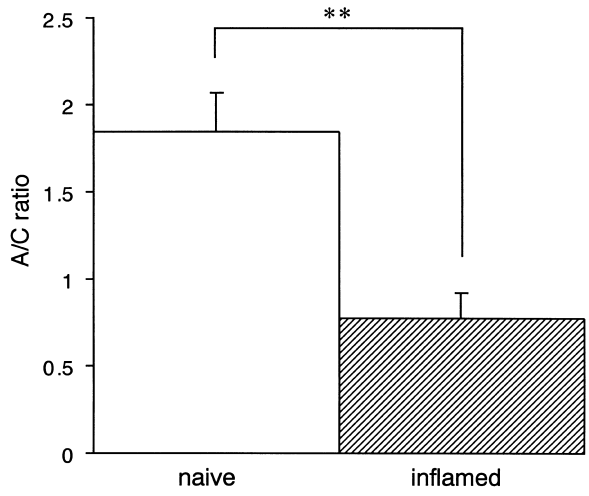

Fig. 7. Ratio (A/C ratio) of the peak amplitude of EPSC evoked by stimulating A afferents to that of C afferent-induced one in neurons exhibiting both of these EPSCs; these were obtained in naive (open column; $n = 36$) and inflamed rats (hatched column; $n = 33$). Stimulus intensities used were those of suprathreshold to activate C afferents. Holding potential = − 70 mV $^{**}P < 0.01$.

To clarify a correlation between A and C afferent-evoked synaptic responses, the peak amplitudes of EPSCs were measured in such neurons exhibiting both A- and C-afferent responses as those shown in Table 2, Figs. 5C and 6C, regardless of their mono- or polysynaptic origin. Fig. 7 demonstrates a ratio (A/C ratio) of the peak amplitude of EPSC evoked by stimulating A afferents to that of C afferent-induced one. The A/C ratios in naive and inflamed rats were 1.8 ± 0.1 ($n = 36$) and 0.7 ± 0.1 ($n = 33$), respectively; these were significantly different from each other ($P < 0.01$).

4. Discussion

The present study examined glutamatergic excitatory synaptic responses elicited in SG neurons, by stimulating the dorsal root in slice preparations obtained from naive and inflamed rats, and it was revealed that SG neurons in inflamed rats receive more direct Aβ-afferent inputs compared to those in naive rats, which is accompanied by a decrease in direct Aδ-afferent inputs and to some extent by an increase in polysynaptic Aδ-afferent inputs (Table 2). In addition to such a change in afferent inputs to the SG, the contribution of A- and C-afferent inputs to the synaptic responses in SG neurons was altered in proportion after inflammation.

Anatomical and physiological studies concerning central termination of primary afferent fibers have provided evidence suggesting that most of terminals of Aβ-afferent fibers locate in laminae III–V of the dorsal horn while only a few of them exist at the most ventral part of lamina II (Woolf et al., 1992), and that many of SG neurons receive Aδ- and C-afferent inputs (Rethelyi, 1977; Light and Perl, 1979; Sugiura et al., 1989; Yoshimura and Jessell, 1989, 1990). The present study using naive rats demonstrated that only a small number of SG neurons examined receive monosynaptic inputs from Aβ-afferent fibers while the majority of them receive monosynaptic inputs from Aδ afferents, an observation being consistent with those in previous studies. Most of SG neurons tested, however, did not receive directly C-afferent inputs (Table 2), where a repetitive stimulation at 20 Hz was used to identify whether the synaptic responses are mono- or polysynaptic in origin. According to our previous study where a stimulation having a lower frequency such as 5–10 Hz was used for the identification, a substantial number of SG neurons received direct C-afferent inputs (Yoshimura and Jessell, 1989). In addition, an AP propagation along C fibers has been shown not to be constant, rather to be slowed by repetitive stimulation (unpublished observations). Therefore, the frequency parameter such as 20 Hz used in the present study may have been too high for C fibers and thus the number of neurons exhibiting monosynaptic C-afferent responses may have been underestimated. This issue was not further examined.

4.1. The change in synaptic inputs following inflammation is not due to an alteration in primary afferent neurons

Three subgroups of rat DRG neurons have usually been defined on the basis of a discontinuity in the histogram of CV values of their axons. However, a clear distinction has rarely been identified except for the study of Harper and Lawson (1985a) where CV values of Aα- and Aβ-fibers were larger than 14 m/s while those of Aδ- and C-fibers were less than 8 m/s. A considerable overlap in CV values of the axons of low- and high-threshold mechanoreceptors occurs around 10–15 m/s, a region of CVs in which Aβ- and Aδ-cells are arbitrarily distinguished in many studies. However, it has been shown that monophasic APs having a short duration are typical to low-threshold mechanoreceptor cells while biphasic ones having a shoulder on the falling phase to be longer in duration characterize high-threshold mechanoreceptors (Ritter and Mendell, 1992). A combination of two parameters, axonal CV and APD, and an objective statistical analysis have provided a subdivision of Aβ and Aδ neurons that may better reflect their transductional functions (Villiere and McLachlan, 1996). In our experiments, the three subgroups were divided on the basis of a combination of axonal CV, APD and TSI, values of which were determined by stimulating the dorsal root with the same suction electrode through the experiment. Each value of the three parameters and of resting membrane potential was not significantly different between preparations obtained from naive and inflamed rats. It is, therefore, unlikely that the changes in synaptic inputs following inflammation in this work are due to a change in characteristics of DRG neurons or an activation of distinct populations of primary afferent fibers. In support of this idea, Okuse et al. (1997) have

reported that the level of the transcript encoding the tetrodotoxin-resistant sodium channel in DRG neurons was not altered in inflamed rats.

4.2. Hyperalgesia following inflammation is likely due to a redistribution of central afferent terminals

The changes in synaptic responses following inflammation revealed in this study suggest that some of Aδ-afferent pathways change plastically to transmit nociceptive information to the SG, through an interneuron instead of a direct innervation and that Aβ afferents sprout into the SG from deeper layers in the spinal cord. If the former is the case, a decrease in direct Aδ-afferent inputs and an increase in polysynaptic Aδ-afferent inputs revealed in the present study may have been due to an arborization of Aδ-afferents, a part of which terminals make synaptic contacts with interneurons resulting in polysynaptic Aδ-afferent inputs; however, this arborization causes a conduction block of direct Aδ-afferent inputs (because of a decrease in the size of the axon; see Horch et al., 1976). A possibility cannot be ruled out that a tonic inhibition by opioids plays a role in the decrease in direct Aδ-responses, because Iadarola et al. (1988) have demonstrated a large increase in the level of both peptide dynorphin and preprodynorphin mRNAs following CFA-induced hindpaw inflammation, and opioids are known to inhibit excitatory synaptic transmissions in the SG (see North, 1993, for review).

Neuroanatomical studies have demonstrated a reorganization of Aβ afferents to lamina II following an injury of peripheral nerves (Woolf et al., 1992; Koerber et al., 1994). The present study is the first evidence showing that such a reorganization may be functional. A redistribution of central terminals of myelinated afferents such as the sprouting of Aβ-afferent fibers may result in an increase of receptive field sizes in the dorsal horn. Dubner (1991) has proposed that an expansion of receptive fields will lead to a great number of neurons activated by a stimulus applied to a hyperalgesic zone, compared to that of neurons responding to the same stimulus applied to a normal zone. The increase in neural activity would be ultimately perceived as a more intense pain. In our observations, most of SG neurons which came to receive Aβ-afferent inputs following inflammation had nociceptive C-afferent inputs as seen in Fig. 6C, and the ratio of the amplitude of A afferent-evoked EPSC to that of the C afferent-evoked one was reduced in inflamed compared with naive rats. Following peripheral inflammation, SG neurons having high-threshold nociceptive inputs of Aδ- and C-afferents may have come to receive a low-threshold input of Aβ afferents.

An expansion of Aβ afferents to the SG may be a reflection of plasticity of the dorsal horn in response to changes in stimulus input following peripheral inflammation. There are some possibilities that may explain such a plasticity. One is that neurotrophic factors may induce a redistribution of central terminals of myelinated afferents, because they are known to promote the neurite outgrowth and survival of sensory neurons, resulting in an alteration in circuit architecture and function (see Lewin and Barde, 1996, for review). Cho et al. (1997) have demonstrated an increased expression of brain-derived neurotrophic factor (BDNF)-immunoreactive terminals in the dorsal horn following peripheral inflammation elicited by an intraplantar injection of CFA into the rat paws; this enhancement was most obvious at 2–4 days following inflammation. Since Cohen-Cory and Fraser (1995) have demonstrated that the onset of a BDNF-induced sprouting of tadpole optic axons in vivo was within 2 h, it is possible that a sprouting of primary afferents occurs during the inflammation. In support of this idea, there is an enhancement in axonal regeneration of adult rat sensory neurons during 1 day in culture in a BDNF-containing medium (Lindsay, 1988). Another possibility is that immediately early genes such as c-fos play an important role in eliciting the central plasticity associated with periperal inflammation, since some of immediate early genes are known to encode transcriptional factors controlling the expression of downstream genes, which may lead to nerve sprouting (see Doyle et al., 1997, for review). Hunt et al. (1987) have found molecular consequences of the expression of immediately early gene c-fos in the superficial layers of the rat dorsal horn following a noxious stimulation in the periphery.

In conclusion, the present study provides electrophysiological evidence that persistent peripheral inflammation may result in a redistribution of central terminals of myelinated afferents in the SG. Such a plastic change in sensory circuitry in the dorsal horn may attribute, at least in part, to underlying mechanisms for the induction of hyperalgesia following inflammation observed in behavioral animals and clinical studies.

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