Effect of Chronic Inflammation on Dorsal Horn Nociceptive Neurons in Aged Rats

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Kitagawa, Junichi, Kenro Kanda, Miho Sugiura, Yoshiyuki Tsuboi, Akiko Ogawa, Kohei Shimizu, Natsu Koyama, Hiroshi Kamo, Tatsuhisa Watanabe, Ke Ren, and Koichi Iwata. Effect of chronic inflammation on dorsal horn nociceptive neurons in aged rats. J Neurophysiol 93: 3594–3604, 2005. First published January 19, 2005; doi:10.1152/jn.01075.2004. To elucidate the effect of chronic inflammation on spinal nociceptive neurons in the elderly, we compared nociceptive behavior, peripheral inflammatory responses, and spinal dorsal horn neuronal activities between the aged (29–34 mo) and adult (7–12 mo) male rats after injection of complete Freund’s adjuvant (CFA) into the hind paw. Aged rats exhibited a significantly lower mechanical paw withdrawal threshold before inflammation. However, after CFA injection mechanical allodynia developed in both adult and aged rats after CFA injection. The changes of foot temperature and thickness after CFA injection were greater and lasted longer in aged than in adult rats. Sets of 124 wide dynamic range (WDR) neurons (aged: 59, adult: 65) and 26 nociceptive specific (NS) neurons (aged: 13, adult: 13) were recorded from the lumbar spinal dorsal horn. NS neurons from the inflamed adult rats showed significantly higher responses to noxious mechanical stimulation than those in aged rats, whereas WDR neurons from inflamed adult and aged rats were similar. Background activity of WDR neurons from the adult rats increased after CFA, whereas WDR neurons of aged rats and NS neurons from either group were not. The afterdischarge followed by noxious mechanical stimulation was significantly greater for WDR neurons in both adult and aged rats, whereas no significant differences were observed in NS neurons. Two days after CFA injection, Fos expression increased similarly in aged and adult rats. Thus the aged rats showed enhanced peripheral inflammatory responses to CFA injection with only a slight change in dorsal horn neuronal activity. Together with our previous finding that nociceptive neurons in aged rats exhibit hyperexcitability, these results suggest that the dorsal horn nociceptive system becomes sensitized with advancing age and its excitability cannot be further increased by inflammation.

INTRODUCTION

Previous studies have indicated that many physiological properties are different between aged and adult people (Hayflick 1998; Janssens et al. 1999; Ribera-Casado 1999). Specifically, the pain perception mechanism is strongly affected by age (Harkins and Scott 1996; Schludermann and Zubek 1962). It has been reported that the chronic pain conditions are more prevalent in senescent individuals (Brena and Bonica 1970; Ferrell 1991; Harkins et al. 1988) and it is very difficult to relieve chronic pain form senescent patients because of their physical conditions (Corran et al. 1977; Wijeratne et al. 2001). Some animal studies on neuropathic and chronic inflammatory pain revealed that partial peripheral nerve injury and chronic inflammation induce more severe or prolonged hyperalgesia in aged rats as compared with young rats (Kim et al. 1995; Novak et al. 1999). The expression profile of a number of neuropeptides is changed with advancing age in the peripheral and central pain pathways (Goicoechea et al. 1997; Ko et al. 1997).

We have studied physiological properties of nociceptive spinal dorsal horn neurons in aged rats, and reported that nociceptive neurons in the aged rats showed stronger excitability to noxious heat stimulation than that observed in the adult rats (Iwata et al. 2002). The background discharge frequency and the afterdischarges following strong mechanical stimulation of the receptive fields in aged rats were significantly higher compared with those in adult rats. This hyperexcitability of nociceptive neurons may be induced by a deficit in the descending inhibitory modulation system in aged rats, since the density of noradrenergic and serotoninergic immunoreactive fibers in the dorsal horn was reduced in aged rats (Iwata et al. 2002). These findings suggest that the CNS is highly involved in pain modulation with advancing age.

It is known that peripheral inflammation induces various changes in nociceptive neurons in young adult rats. The responsiveness of medullary dorsal horn nociceptive neurons to mechanical and heat stimuli, background activity, afterdischarge, and receptive fields is significantly enhanced after inflammation (Iwata et al. 1999). It seems that the changes we have seen in the aged rats without inflammation were similar to those reported for adult rats with peripheral inflammation. Inflammation, especially chronic inflammation, prevails among the elderly. The chronic inflammation is known to produce a variety of changes in both the peripheral and central nervous systems. The physiological responses to chronic inflammation may be different between aged and adult rats.
However, the peripheral and central mechanisms of changes in pain pathways with advancing age were unknown.

Thus the present study was undertaken to examine changes in the CNS induced by peripheral inflammation with advancing age. We compared nocifensive behavior, peripheral inflammation, and alterations in activity of spinal dorsal horn nociceptive neurons between the aged and adult rats after hind paw inflammation.

**Methods**

The experiments were performed on male Fischer 344/DuCrj rats in the 2 age groups: 29–34 mo old (aged, neuronal recording experiments: 244 ± 11 g, n = 13) and 7–12 mo old (adult, neuronal recording experiments: 232 ± 9 g, n = 15). The rats were raised under pathogen-free conditions and fed without restriction. They were housed 3 per cage and maintained on a 12:12 light:dark schedule (lights on at 0600 h) at 22°C. The study was approved by the Animal Experimentation Committee at Nihon University School of Dentistry and at the Tokyo Metropolitan Institute of Gerontology. The animals were treated according to the guidelines of the International Association for the Study of Pain (Zimmermann 1983).

**Complete Freund’s adjuvant (CFA) injection**

Aged and adult rats were anesthetized with pentobarbital Na [50 mg/kg, administered intraperitoneally (ip)]. The inflammatory agent CFA was suspended in an oil/saline (1:1) emulsion and a volume of 0.05 ml was injected subcutaneously into the left hind paw.

**Behavioral test**

In daily sessions, rats (aged: 245 ± 6 g, n = 6, adult: 228 ± 4 g, n = 5) were trained to stay in the plastic cage during mechanical stimulation of the hind paw with von Frey filaments (Stoelting, Wood Dale, IL). The maximum intensity used in this study was 28.2 g for behavioral testing. One day before CFA injection, von Frey hair mechanical stimulation was applied to the hind paw and the escape threshold was measured in aged and adult rats. After CFA injection, the mechanical escape threshold was measured every day. Quantitative mechanical stimuli were applied to the hind paw in ascending and descending orders to evaluate the escape threshold. Each von Frey filament was applied 5 times. When rats showed one escape response to a filament, the bending force of that filament was defined as the escape threshold intensity. The median threshold intensity was calculated from the values after 2 ascending trials and one descending trial.

**Measurement of the paw temperature and thickness**

The paw temperature and thickness were measured in naïve aged and adult rats for 3 days before CFA injection to obtain consistent baseline data. The paw temperature and thickness were measured daily after CFA injection into the left hind paw (age: 215 ± 9 g, n = 5, adult: 283 ± 5 g, n = 5).

**Measurement of the Paw Temperature.** The experiments were conducted under a thermoneutral condition (room temperature 26 ± 0.5°C). Rats were anesthetized with pentobarbital Na (50 mg/kg, ip). At 30 min after the pentobarbital injection, rats were gently held and placed on the platform. The skin temperature was measured by a computer-assisted infrared thermograph (Thermotracer TH3100ME, NEC-Sanei Instruments, Tokyo, Japan). The area of thermographic recording covered the whole hind paw region.

**Measurement of the Paw Thickness.** Paw thickness was measured just after the measurement of paw temperature. The dorsoventral thickness of the middle portion of the hind paw was measured using a micrometer caliper, as illustrated in Fig. 3C.

**Recording from dorsal horn nociceptive neurons**

**Animal preparation.** Because the paw temperature and paw thickness peaked at 1–2 days after CFA injection into the hind paw, the recording and Fos protein immunohistochemical experiments were done at 2 days after CFA injection. Two days after CFA injection, rats (aged: 250 ± 9 g, n = 7, adult: 233 ± 8 g, n = 8) were introduced for extracellular recording experiments. Naïve rats without CFA injection (aged: 237 ± 9 g, n = 6, adult: 231 ± 10 g, n = 7) were used for recording experiments as controls. Animals were anesthetized with pentobarbital Na (50 mg/kg, ip) and the trachea and left external jugular veins were cannulated to allow artificial respiration and intravenous (iv) administration of drugs, respectively. Anesthesia was maintained with halothane (2–3%) mixed with air during surgery. The rats were mounted on a stereotaxic frame, the L3–6 spinal cord was exposed, and a mineral oil pool was made with the skin flaps surrounding the laminectomy. After surgery, anesthesia was maintained throughout the experiment by continuous inhalation of halothane (1–2%) mixed with oxygen. During recording sessions, rats were immobilized with pancuronium bromide (1 mg · kg⁻¹ · h⁻¹, iv) and ventilated artificially. The expired CO₂ concentration was monitored and maintained between 3.0 and 4.0%. Rectal temperature was maintained at 37–38°C by a thermostatically controlled heating pad (ATB-1100, Nihon Kohden, Tokyo, Japan) and the electrocardiogram was monitored. If the heart rate increased after mechanical or thermal stimulation of the receptive fields, the percentage of halothane was increased (2–3%).

**Stimulation and recording.** Enamel-coated tungsten microelectrodes (impedance = 10–12 MΩ, 1,000 Hz) were advanced into the spinal dorsal horn at the L4 to L5 levels in 2-μm steps. Spinal dorsal horn neurons were searched for by applying mechanical stimulation (pressure or brush) to the skin or the hip and leg regions. When a single neuron was isolated, the responses to mechanical stimulation of the foot were carefully examined and the receptive field was mapped. Then, graded mechanical stimuli using von Frey filaments and pinch with small arterial clip were applied to the most sensitive areas of the receptive fields for 5 s. Each neuron was classified either as 1) a wide dynamic range (WDR) neuron that responded to both nonnoxious and noxious mechanical stimuli and increased its firing frequency as stimulus intensity increased, or as 2) a nociceptive-specific (NS) neuron that responded exclusively to noxious mechanical stimulation of the receptive fields. After characterization with mechanical stimuli, responses to thermal stimuli were further examined by heating the most sensitive area of the mechanical receptive fields. Before application of the thermal stimulus to the receptive field, the surface temperature was adapted to 38°C for 180 s. The skin heating ranged from 42 to 50°C and lasted 10 s. The rate of temperature change was set at 10°C/s. The thermal stimuli were applied every 190 s to avoid sensitization of peripheral nociceptors (Beitel and Dubner 1976). The tip of the thermal probe was 10 mm in diameter. Neuronal activity was fed into a computer disk for subsequent analysis. After evaluating the response properties of spinal dorsal horn neurons, lesions were made at the recording site by passing negative DC of 10 μA for 10 s for histological identification of the recording site. Two to 3 recording sites were chosen for making lesions, where nociceptive neurons were encountered.

**Histological confirmation of the recording site.** At the conclusion of the experiment, rats were overdosed with sodium pentobarbital (100 mg/kg) and perfused transcardially with 50 ml 0.01 M PBS (pH 7.4) followed by 10% formalin in 0.1 M phosphate buffer. The spinal cord was removed, placed in cold fixative for a few days, and then transferred to cold phosphate-buffered 30% sucrose for 48 h.
Serial sections (thickness 50 μm) were cut along the path of the electrode penetration. The sections were counterstained with Thionin for identification of the recording sites. Camera lucida tracings of the recording sites were drawn at 400 × magnification with a drawing tube.

**ANALYSIS OF NEURONAL ACTIVITIES.** The waveform of single neuronal activities was analyzed off-line. The waveform of each neuron was identified using Spike2 microcomputer software (CED, Cambridge, UK). Peristimulus time histograms (bin-width = 1 s) were generated in response to each stimulus. Background discharges were first recorded for 10 s before application of the mechanical or thermal stimulus and they were subtracted from the neuronal responses during analysis. Stimulus–response (S-R) functions of each nociceptive neuron were obtained in response to the quantitative mechanical (1.2, 5.4, 15.1, 28.2, and 75.8 g) and pinch or thermal (44–50°C) stimuli. The mechanical or thermal stimulation of the receptive fields was considered to have induced an effect when the peak firing frequency at 5 s after mechanical and 10 s (one trial for each neuron with 180-s intervals) after thermal stimulation differed from the mean background discharge rate by ±2SD. The receptive fields of all neurons were drawn to scale on standard diagrams of a rat leg. Areas of the receptive fields were calculated using image-analysis software (National Institutes of Health Image 1.61).

**Fos protein immunohistochemistry**

At 2 days after the CFA injection, rats (aged: 223 ± 3 g, n = 5, adult: 283 ± 7 g, n = 5) were anesthetized with sodium pentobarbital (80 mg/kg, ip) and perfused through the aorta with 500 ml 0.02 M phosphate-buffered saline (PBS, pH 7.4) followed by 500 ml 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The L4–5 spinal cords were removed and postfixed in the same fixative for 3 days at 4°C. The tissues were then transferred to 30% sucrose (wt/vol) in PBS for several days for cryoprotection. Sections (thickness 30 μm) were cut with a freezing microtome and every 4th section was collected in PBS. Free-floating tissue sections were rinsed in PBS, 10% normal goat serum in PBS for 1 h, and then incubated in rabbit anti-Fos (1:10,000) for 24 h at room temperature. Subsequently, the sections were incubated in biotinylated goat anti-rabbit IgG (1:200; Vector Labs, Burlingame, CA) for 1 h at 37°C. After washing, the sections were incubated in peroxidase-conjugated avidin–biotin complex (1:100; ABC, Vector Labs) for 4 h at 37°C. After washing in 0.05 M Tris Buffer (TB), the sections were incubated in 0.035% 3,3’-diaminobenzidine-tetra HCl (DAB, Sigma), 0.2% nickel ammonium sulfate, and 0.05% peroxide in 0.05 M TB (pH 7.4). After washing in PBS, the sections were serially mounted on gelatin-coated slides, dehydrated in alcohols, and coverslipped. The number of Fos protein-like immunoreactive (LI) cells in the L4–5 spinal dorsal horn was analyzed.

**Statistical analysis**

Statistical analysis was performed by using ANOVA followed by the Dunnett test. Student’s t-test or Mann–Whitney U test was also used as appropriate. Differences were considered significant at P < 0.05. Results are presented as means ± SE.

**RESULTS**

**Nocifensive behavior**

Before CFA injection, the paw withdrawal threshold was significantly lower in the aged rats than in adult rats, as illustrated in Fig. 1A (aged, ipsi: 4.89 ± 0.24 g, contra: 4.89 ± 0.29 g) and Fig. 1B (adult, ipsi: 8.93 ± 0.33 g, contra: 9.27 ± 0.56 g) (P < 0.01). After CFA injection into one hind paw, the mechanical withdrawal threshold became significantly lower in both aged and adult rats (aged, ipsi: 3.27 ± 0.30 g, contra: 6.11 ± 0.25 g, adult, ipsi: 5.60 ± 0.21 g, contra: 14.61 ± 0.33 g at 1 day after CFA injection). The reduction of the threshold persisted during the observation period. We also observed a slight increase in paw withdrawal threshold on the contralateral side to CFA injection in both aged and adult rats, as illustrated in Fig. 1. The paw withdrawal threshold of the inflamed paw was not significantly different between the aged and adult rats after CFA injection, except at 1 day after CFA injection (Fig. 1). Because the paw withdrawal threshold of the adult rats was significantly higher than that of the aged rats, these results suggest that the nocifensive behavior of the adult rats was more strongly affected by inflammation.

**Changes in paw temperature and thickness after CFA injection**

After an injection of CFA into the hind paw, the surface temperature of the injected paw was significantly increased in all rats (Fig. 2). The increase in surface temperature started at the injection site and spread gradually to include the whole paw. Although the peak increase in paw temperature was similar in the aged and adult rats (n = 5 per group), the time course of the temperature change was different. The significant increase in the paw surface temperature lasted for 5 days in the aged group (Fig. 2A), whereas it lasted for only 3 days in the adult rat group (Fig. 2B). There was a slight change in the paw temperature on the contralateral side in the aged rats.

The paw thickness, a measure of inflammation-induced edema, peaked at 1 day after CFA injection and lasted for more...
than 6 days in both aged and adult rats, as illustrated in Fig. 3A (aged, 1 day: 7.23 ± 0.15 mm, 6 days: 6.29 ± 0.18 mm, n = 5) and Fig. 3B (adult, 1 day: 6.85 ± 0.06 mm, 6 days: 5.69 ± 0.13 mm, n = 5). However, the increase in paw thickness was significantly greater in the aged rats at 2- and 4- to 6-day time points compared with that of the adult rats (Fig. 3). We could
not observe any significant effect of CFA injection on the contralateral hind paw (as illustrated in Fig. 3).

Spatial distribution of nociceptive neurons in the spinal dorsal horn

A total of 150 nociceptive neurons in the spinal dorsal horn (65 WDR and 13 NS neurons in 15 adult rats; 59 WDR and 13 NS neurons in 13 aged rats) were analyzed (Table 1). Most nociceptive neurons were distributed in the superficial and deep laminae of the L4–5 spinal dorsal horn, as illustrated in Fig. 4. We did not observe differences in distribution of nociceptive neurons in the aged and adult rats. Consistent with the literature, WDR neurons were encountered in the superficial and deep laminae and NS neurons were mainly distributed in the superficial laminae of the spinal dorsal horn (Fig. 4).

Mechanical and thermal responses

Figure 5 illustrates a typical example of a WDR neuron recorded from the superficial lamina of the L5 spinal dorsal horn in a 32-mo-old rat after CFA injection. This neuron increased firing frequency with graded mechanical stimulation of the hind paw (Fig. 5A). High-frequency afterdischarges were elicited after pinch stimulus of the center of the receptive fields (Fig. 5A, right). This neuron also responded to heating of the receptive field (Fig. 5B). The firing frequency was increased after an increase in stimulus temperature in a graded manner. The recording site of this neuron is illustrated in Fig. 5C. The lesion site is indicated by the arrow. The receptive field of this neuron covered the ventral and dorsal surface of the hind paw (Fig. 5D).

The mechanical responses of WDR and NS neurons are summarized in Fig. 6. All nociceptive neurons increased firing frequency after the graded increase in mechanical stimulus intensity. It is interesting that NS neurons in the adult rats exhibited greater responses after CFA treatment to strong mechanical stimulation (28.2 g, 75.8 g, and pinch stimuli in Fig. 6C). However, the responses of NS neurons in the aged rats did not show an increased response to mechanical stimulation after inflammation (Fig. 6A). The response of WDR neurons to mechanical stimulation was similar in the aged and

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FIG. 4. Illustration of the recording sites of wide dynamic range (WDR) and nociceptive-specific (NS) neurons in the aged (A: naïve, B: inflammation) and adult rats (C: naïve, D: inflammation).
adult groups and there was no significant change after inflammation (Fig. 6, B and D).

The relationship between responses and intensity of heat stimulation is shown in Fig. 7. The inflammation did not have any effect on heat responses of WDR neurons in the aged rats, whereas there was a significant effect on responses in the adult rats after graded heat stimulation of the receptive fields.

**Background activity and afterdischarge**

In the naïve aged rats, the background activity of WDR neurons (2.99 ± 0.74, n = 35 (open column in Fig. 8A) was significantly higher than that of the adult rats (1.17 ± 0.31, n = 33) (open column in Fig. 8C; P < 0.05). The background activity of WDR neurons was significantly higher in the adult rats (3.24 ± 0.84, n = 32) (P < 0.01) compared with that in

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**FIG. 5.** Response properties of a typical nociceptive neuron in the dorsal horn of a 32-mo-old rat. A: PST histograms of neuronal discharges to graded mechanical stimulation of the low threshold area of a WDR neuron. After pinching of the receptive field, high-frequency afterdischarges were observed. B: PST histograms of neuronal discharges to graded heat stimulation of the low-threshold area of the receptive field of a WDR neuron. C: photomicrograph of the recording site in the dorsal horn. Lesion site is indicated by the arrow. D: low- (solid area) and high-threshold (shaded area) areas of the receptive fields indicated by the arrows.

**FIG. 6.** Stimulus–response functions of responses to graded mechanical stimuli in the aged (A: NS, B: WDR) and adult (C: NS, D: WDR) rats, Pl, pinch. Note that the mechanical response was significantly larger in NS neurons after the inflammation. BG activity: background activity in this and following figures. *,#P < 0.05 (t-test).
 naïve adult rats, but not in the aged rats (3.75 ± 0.94, n = 24) compared with aged naïve rats (P = 0.55; Fig. 8, A and C). The background activity of NS neurons in the aged rats was slightly higher than that in the adult naïve rats compared with aged naïve rats (aged: 0.43 ± 0.38, n = 4, adult: 0.24 ± 0.18, n = 9, P = 0.83; Fig. 8, B and D). Although there was a trend for an increase in background activity of NS neurons in adult and aged rats, the increase was not significant for either age group (aged: 0.43 ± 0.38, n = 4, adult: 0.24 ± 0.18, n = 9, P = 0.83; Fig. 8, B and D).

The afterdischarge of WDR neurons was significantly higher in the inflamed aged rats compared with that in naïve aged rats (naïve: n = 35, inflamed: n = 24, P < 0.01), but not significantly higher in inflamed adult rats compared with that in naïve adult rats (naïve: n = 33, inflamed: n = 32, P = 1.00) (Fig. 9, A and C) and there was no difference between the 2 age groups. Compared with adult naïve rats, the afterdischarges of NS neurons were generally higher in the aged naïve rats, but inflammation did not induce significant increases in afterdischarges in either group (Fig. 9, B and D).

Receptive field property

In the present study, the receptive fields of WDR neurons were designated as low- and high-threshold areas according to their responses to mechanical stimuli, as illustrated in Fig. 5D. The low-threshold areas were activated by both noxious and innocuous mechanical stimuli, whereas the high-threshold areas were activated only by noxious stimulation. In general, the low-threshold areas were surrounded by the high-threshold areas. Figure 10 illustrates the effect of CFA injection on receptive fields size of WDR neurons (A) and NS neurons (B). We observed that the high-threshold portion of the receptive fields of WDR neurons was significantly larger in the aged naïve rats than that in adult naïve rats (P < 0.01, Fig. 10A). However, the receptive fields of WDR neurons were significantly larger in adult inflamed rats than in naïve adult, but not in aged rats (Fig. 10A). There was no difference in the low-threshold areas of the receptive fields between the aged and adult rats in both naïve and inflamed rats. For NS neurons, the receptive field was significantly larger only in the adult inflamed rats than that in naïve adult (P < 0.05, Fig. 10B). However, the receptive field of inflamed aged rats was at a similar size as that in the aged naïve rats (Fig. 10B).

Expression of the Fos protein–LI cells

Two days after CFA injection into the hind paw, Fos protein–LI cells were observed in the superficial spinal dorsal horn of the aged and adult rats ipsilateral to the injection site, as illustrated in Fig. 11. Most Fos protein–LI cells were distributed in the superficial laminae of the dorsal horn and

FIG. 7. Stimulus–response functions of the WDR neurons after graded heat stimuli in the aged and adult rats. Note that the heat response was significantly greater in adult rats after inflammation. **P < 0.01; *P < 0.05 (t-test); #P < 0.05 (Mann–Whitney U test), aged vs. adult rats with inflammation.

FIG. 8. Mean background activity of WDR (A: aged, C: adult) and NS neurons (B: aged, D: adult). Open column indicates the naïve rat’s data and the solid column indicates the data from the inflamed rats. Note that only in adult WDR neurons showed a significant increase in background activity after the CFA injection. **P < 0.01 (t-test); *P < 0.05 (Mann–Whitney U test), WDR neurons in aged vs. adult rats.
sparse in the deep laminae. The Fos protein–LI cells were restricted in the medial portion of the superficial dorsal horn, whereas those in deep laminae were widely distributed (Fig. 11, A and B). The distribution pattern of Fos protein–LI cells to the aged and adult rats was similar. As illustrated in Fig. 11C, the number of Fos protein–LI cells on the ipsilateral side to CFA injection was slightly larger in the aged rats than that in adult in the superficial and deep dorsal horn (laminae I–II, aged: 8.02 ± 0.57, adult: 6.37 ± 0.85; laminae III–V, aged: 2.37 ± 0.68, adult: 2.30 ± 0.79, n = 5 in each group, P > 0.05), but the difference did not reach a level of significance.

**DISCUSSION**

The present study showed that mechanical paw withdrawal threshold was significantly lower in aged rats than that in adult rats before CFA. The structure difference of the paw skin between aged and adult rats has been reported previously (Delp et al. 1998; Jung et al. 1997; Oku et al. 1992; Tzaphlidou and Zervakis 2004). The accumulation of fat under the paw skin was less in the aged than in adult rats. The skin is also much thinner in the aged than in adult rats. These phenomena suggest that the structure difference in paw skin between aged and adult rats may contribute to the decrement of mechanical paw withdrawal threshold in the aged rat.

After CFA injection, the difference in mechanical thresholds between the inflamed and noninflamed paws was apparently greater in adult rats than that in aged rats. The magnitude of nocifensive reflex activity has been known to depend on sensitization of the peripheral nociceptors and central sensitization of noxious pathways (Hylden et al. 1989; Schaible and Schmidt 1988). Although present data suggest that the peripheral inflammation produced a stronger effect on nocifensive behavior in adult rats compared with aged rats, one should be cautious about this conclusion because the contralateral paw of the aged rats showed a reduced baseline threshold. A ceiling effect may have prevented a further reduction in threshold in the aged rats.
A significant increase in the paw temperature and thickness was observed in both aged and adult rats after CFA injection. However, judged from the degree of hyperthermia and swelling, the inflammatory reaction to CFA in the aged rats was significantly stronger and longer lasting compared with that in the adult rats. These differences in response to CFA in the aged and adult rats may be explained by alterations in autonomic function in the aged rats. It is well known that the peripheral autonomic functions, such as temperature control and circulation, do not work well in the aged human (D’Esposito et al. 2003; Kenney and Armstrong 1996). It is highly probable that the differences in inflammatory responses in aged and adult rats are a result of differences in peripheral circulation system. The peripheral circulation is significantly affected by age and the blood flow is much slower in the aged rats compared with that in the adult (Khalil and Merhi 2000). The slower blood flow rate may produce a delay of healing of the peripheral inflammation in the aged rats, resulting in a longer-lasting inflammation. The longer-lasting peripheral inflammation as observed in the aged rats may produce stronger sensitization of peripheral nociceptors. The sensitized nociceptors have been known to produce a barrage of action potentials in primary afferent fibers, which are conveyed to spinal dorsal horn neurons (Hylden et al. 1989; Schaible and Schmidt 1988; Schaible et al. 1991).

Peripheral inflammation produces an extensive increase in the excitability of primary afferent small-diameter nerve fibers, leading to a significant increase in the activity of nociceptive neurons in the CNS of young adult rats (Hylden et al. 1989; Iwata et al. 1999; Schaible and Schmidt 1988; Schaible et al. 1991). After peripheral inflammation, dorsal horn nociceptive neurons also showed an expansion of the receptive fields that was explained by the hyperexcitability of dorsal horn neurons (Hylden et al. 1989; Iwata et al. 1999). We observed similar results in the adult rats in the present and previous reports (Iwata et al. 1999, 2002). The nociceptive neurons in the adult rats increased their background activity and both mechanical and heat responses after peripheral inflammation. The increment of the excitability of nociceptive neurons in the adult rats, as observed after CFA injection, may be a result of sensitization of primary afferent neurons as well as altered dorsal horn processing. The activity of C-fiber primary afferents seems to be scarcely affected by age (Sato et al. 1985). The conduction velocity of C-fiber afferents was not significantly changed with advancing age. This suggests that the hyperexcitability of peripheral nociceptors could be relayed to dorsal horn nociceptive neurons in the aged rats similar to that observed in the adult rats. Accordingly, dorsal horn nociceptive neurons in aged rats may show similar excitability after peripheral inflammation, such as that observed in the adult rats. In fact, Fos protein expression in aged and adult dorsal horn neurons after CFA injection was not significantly different (Fig. 11).

Thus it seems that nociceptive information fully reaches the spinal cord after inflammation in both aged and adult rats. However, we did not find significant effects of inflammation on responsibility of dorsal horn nociceptive neurons to noxious mechanical and heat stimuli, and their background discharge and the receptive field size in the aged rats, whereas inflammation induced significant increases in activity in adult rats. One explanation for these observations seems to be the altered sensitivity of dorsal horn neurons to inflammation-induced afferent inputs in the aged rats. It has been reported that the synaptic density of spinal neurons is significantly reduced in aged rats compared with that in young rats (Chen et al. 1997; Santer et al. 2002). It is possible that the reduction of synaptic density in dorsal horn neurons may produce changes in sensitivity of dorsal horn neurons to primary afferent inputs. We noticed that noxious stimulus–evoked responses tended to be higher, and background activity and receptive fields were significantly larger, in the noninflamed aged rats compared with those in the noninflamed adult rats. Thus the effect of inflammation seems to have been occluded in the aged rats. In other words, because nociceptive neurons of the aged rats already exhibited hyperexcitability, inflammation could not produce a further increase in excitability. It is likely that age-related and inflammation-induced changes are underlined by common mechanisms.

The involvement of the descending modulator system could be considered as an alternative mechanism to explain the effect of inflammation on dorsal horn nociceptive neuronal activity in the aged rats. The descending system has been known as a powerful pathway to modulate pain transmission in adult rats (Carpenter et al. 1965; Ren and Dubner 1996a; Ren and Ruda 1996b). It has been reported that an extensive increase in excitability of rostral ventromedial medulla neurons is induced after hind paw inflammation (Miki et al. 2002). This enhancement of function of the descending modulatory system may induce an effect on nociceptive transmission in the spinal dorsal horn. We previously reported that descending inhibition was reduced in the aged rats (Iwata et al. 2002). The descending serotonergic and adrenergic systems were significantly impaired in the aged rats and the spinal block did not affect nociceptive responses in aged rats. If there is a dysfunction of the descending system in the aged rats, the spinal dorsal horn

**FIG. 11.** Fos protein expression in dorsal horn neurons of the aged and adult rats at 2 days after CFA injection into the hind paw. A and B: photomicrographs of L4 dorsal horn ipsilateral to the CFA injection in the aged (A) and adult (B) rats. A large number of Fos protein-LI cells were observed in the superficial laminae of the dorsal horn in aged and adult rats. C: mean number of Fos-LI cells. Number of Fos-LI cells was not significantly different in the aged and adult rats ($P > 0.05$, t-test).
neuronal activity should be significantly increased after peripheral inflammation. However, we observed only a slight change in heat and mechanical responses in the aged rats, whereas dorsal horn nociceptive neurons in the adult rats showed a significant increase in activity after inflammation. These data suggest that the aged rats lack the ability to further increase dorsal horn neuronal activity after peripheral inflammation. The afterdischarges are strongly modulated by the descending pathways. The descending system inhibits the long-lasting afterdischarges produced by strong peripheral stimulation (Robinson et al. 2002). The modulation of afterdischarges should be a good indicator of the function of the descending modulation system. It is likely that a deficit of the descending system in the aged rats resulted in a significant increment of the afterdischarges of dorsal horn neurons after peripheral inflammation.

Relatively small changes in pain threshold for aged human and fairly large alterations in response properties of nociceptive neurons in the aged rat spinal cord suggest that degenerative alterations and reorganization are taking place in the pain-processing system with advancing age. Therefore the descending and ascending systems as well as the peripheral nervous system in the aged rats deserves further study to elucidate the pain system in the aged. This may lead to new ways for treatment of pain in the elderly.

Although the effect of aging on dorsal horn nociceptive neurons in the inflamed rats was discussed in the present study, it should be cautioned that the sex differences and sensitivity to anesthetics used in aged rats may contribute to the difference in the responsiveness of dorsal horn nociceptive neurons of the inflamed aged rats.

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