Activation of silent mechanoreceptive cat C and Aδ sensory neurons and their substance P expression following peripheral inflammation

Guang-Yin Xu *‡, Li-Yen Mae Huang‡§ and Zhi-Qi Zhao *†

*Shanghai Brain Research Institute, †Shanghai Institute of Physiology, Chinese Academy of Sciences, Shanghai 200031, People's Republic of China and ‡Marine Biomedical Institute and §Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77555-1069, USA

1. The effect of inflammation on the excitability and the level of substance P (SP) in cat mechanoreceptive C and Aδ dorsal root ganglion (DRG) neurons were studied *in vivo* using intracellular recording and immunocytochemical techniques.

2. Following injections of carrageenan (Carg) into the cat hindpaw, the percentage of C neurons exhibiting spontaneous activity increased from 7·2 to 20·7% and the percentage of Aδ neurons increased from 6·9 to 18·6%. In contrast to most cells from normal cats, which fired regularly below 10 Hz, many cells from Carg-treated cats fired at higher frequencies or in bursts.

3. Inflammation (Carg treatment) also depolarized membrane potentials, increased membrane input resistance, caused the disappearance of inward rectifying currents and lowered the mean current thresholds of tibial nerve-evoked responses in DRG neurons.

4. With inflammation, the percentage of C or Aδ neurons responding to low threshold mechanoreceptive stimuli increased (C neurons: normal, 13%; inflamed, 41%; Aδ neurons: normal, 13%; inflamed, 39%), while the percentage of C or Aδ neurons responding to high threshold mechanoreceptive stimuli remained unchanged.

5. Some receptive field (RF)-responsive cells were injected with Lucifer Yellow and their SP immunoreactivity was determined. Following Carg treatment, substantially higher percentages of RF-responsive cells were SP positive (C neurons: normal, 35·7%; inflamed, 60%; Aδ neurons: normal, 18·2%; inflamed, 66·7%).

6. These combined increases in the excitability of DRG neurons and SP-containing RF-responsive neurons could lead to sensitization of sensory neurons, thus contributing to the development of hyperalgesia.

Inflammation caused by injection of carrageenan (Carg) into the hindpaw has been used as a model for studying hyperalgesia accompanying inflammatory injuries. Behavioural studies show that thermal and mechanical pain thresholds begin to decline 2 h after Carg injection and hyperalgesia reaches a peak 3–4 h later (Kocher *et al.* 1987; Satoh *et al.* 1992; Traub, 1996; Kilo *et al.* 1997). Since Carg treatment enhances c-fos expression in the spinal cord (Buritova *et al.* 1996), increases the excitability of spinal dorsal horn neurons (Ren *et al.* 1992; Ma & Woolf, 1996) and facilitates synaptic inputs (Baba *et al.* 1999), sensitization of neurons in the central nervous system is thought to contribute to hyperalgesia (Woolf & Thompson, 1991; Ren *et al.* 1992; Treede *et al.* 1992). Because Carg-induced inflammation activates silent (i.e. receptor field-unresponsive) nociceptors and potentiates the activity of primary afferents (Schaible & Schmidt, 1985, 1988), sensitization of sensory neurons also could contribute to hyperalgesia.

Substance P (SP) is an important neuromodulator in primary sensory neurons (Salt & Hill, 1983; Willis & Coggeshall, 1991; Otsuk & Yoshioka, 1993; Iversen, 1998; Cao *et al.* 1998). Peripheral inflammation increases SP expression in the dorsal root ganglion (DRG) (Noguchi *et al.* 1988; Donnerer *et al.* 1992; Galeazza *et al.* 1995; McCarson & Krause, 1995; Neumann *et al.* 1996; McCarson, 1999), and triggers SP release in the spinal dorsal horn (Duggan *et al.* 1987; Oku *et al.* 1987; Schaible *et al.* 1990; Garry & Hargreaves, 1992; Zhao *et al.* 1992). Although both SP expression and

the responsiveness of nociceptors to receptive field (RF) stimuli undergo profound changes during inflammation (Schaible & Schmidt, 1985, 1988), the relationship between SP and RF-responsive neurons in inflamed tissues remains unclear. In normal cats, Leah *et al.* (1985) found that SP-containing C-fibre neurons were not responsive to peripheral noxious stimulation while a majority of DRG C-fibre neurons responding to peripheral stimuli did not contain SP. Lawson *et al.* (1997) showed that all SP-positive cells in normal guinea-pig DRG with identified receptive properties were nociceptive, although not all nociceptors were SP positive. Schmidt and others proposed that many nociceptors in cat or rat peripheral tissues were silent under physiological conditions, but became RF responsive following the induction of inflammation or neuropathy (Schaible & Schmidt, 1988; Habler *et al.* 1988; McMahon & Koltzenburg, 1990; Schmidt *et al.* 1994). It is, therefore, conceivable that the unresponsive DRG C-fibre neurons reported by Leah *et al.* (1985) were SP-containing RF-unresponsive nociceptors. If this is correct, the proportion of SP-containing RF-responsive neurons should increase following inflammation. To determine the properties of DRG neurons and to find the relationship between SP expression and the RF responsiveness of these cells in the inflammatory state, we recorded DRG neurons *in vivo* and determined the SP content in identified RF-responsive DRG cells.

METHODS

Animal preparation

Experiments were performed on 62 adult cats of either sex weighing 2–4 kg. Cats were first anaesthetized with an intraperitoneal injection of sodium pentobarbitone (40 mg kg^{-1}). A catheter was then inserted into the superficial vein of the right forelimb for further intravenous application of sodium pentobarbitone. When needed, a dose of 10 mg kg^{-1} sodium pentobarbitone was administered during the course of an experiment. Blood pressure was continuously monitored with a sphygmomanometer in the left carotid artery. The depth of anaesthesia of the animal was judged to be adequate when the pupils were closed and the blood pressure remained stable after strong paw pinches or C-strength electrical stimulation. A cannula was inserted into the trachea to allow artificial respiration and the end-tidal CO_2 level was kept at 3·5–5·0 %. The animal was then placed on a stereotaxic frame and the spinal cord was immobilized with clamps. Gallamine triethiodide (4 mg kg^{-1}, i.v.) was applied to paralyse the animal before experiments. The rectal temperature was maintained at 36–38 °C using a thermostatically regulated heater. Glucose-containing (5 %) saline was infused into the right superficial vein at a rate of 0·1 ml min^{-1}. An experiment was discontinued when the blood pressure dropped below 80 mmHg.

The recording procedure has been described before (Song & Zhao, 1994; Tao *et al.* 1997). Briefly, following a laminectomy of the spinal cord from segments L1 to S1, the cord was transected at the L1–L2 level. Bilateral DRGs at L7 were exposed. The dura and the connective tissue capsule were removed from the ganglion. Agar (2·5%) was poured over the exposed spinal cord and DRGs. A small opening was made at the recorded ganglion and a warm (32–34 °C) Krebs solution was continuously perfused (3–5 ml min^{-1}). The

solution contained (mM): NaCl 117·0, KCl 4·7, MgCl$_2$ 1·2, CaCl$_2$ 2·5, NaH$_2$PO$_4$ 1·2, NaHCO$_3$ 25·0, and glucose 11·5. The pH of the solution was maintained at 7·3–7·4 by bubbling it with 95%O$_2$ and 5%CO$_2$ gas. The tibial or sciatic nerves were dissected out for electrical stimulation. A pool was made with flaps of skin and warm mineral oil was poured over the tibial or sciatic nerve. For induction of peripheral inflammation (Castro-Lopes *et al.* 1994; Tsuruoka *et al.* 1997), a total of 2–3 ml (20–30 mg kg^{-1}) of Carg (3%, Sigma) solution was injected subcutaneously at various locations in the cat hindpaw 2 h before recordings began. In control animals, the same amount of saline (0·9% NaCl) solution was injected instead. After the conclusion of the experiments, the animals were killed with an overdose of sodium pentobarbitone followed by an i.v. injection of 10 ml saturated KCl. All experiments were performed according to the IASP guidelines which were approved by the Research Centre on Animals in Shanghai Brain Research Institute, Chinese Academy of Sciences.

Identification of conduction velocity of DRG neurons

Intracellular recordings were made from the somata of DRGs using a microelectrode filled with 4 M potassium acetate or Lucifer Yellow (30–50 mg ml^{-1} dissolved in 0·5 M LiCl solution). The typical resistance of the potassium acetate electrodes was 30–50 MΩ and of the Lucifer Yellow electrodes 80–160 MΩ. Once a stable recording was established, the tibial nerve was stimulated with 8 mA and 0·5 ms pulses (a supramaximal strength for C-afferent fibre activation) to evoke action potentials in the somata. Axonal conduction velocity (CV) was calculated from the latency of action potentials recorded in the DRG and the distance between the recording electrode at the DRG soma and the stimulating electrode at the tibial nerve. From the CV histogram, we could divide the CVs of neurons into three groups: $> 20·0$ m s^{-1}, $2·0–20·0$ m s^{-1} and $< 2·0$ m s^{-1}, which correspond to Aβ-, Aδ- and C-afferent neurons as reported by others (Schmidt *et al.* 1994; Tao *et al.* 1997). Only Aδ and C neurons were studied.

The input resistance ($R_{in} = \Delta V_m \Delta I^{-1}$) of a neuron was determined by passing a hyperpolarizing current of 0·05 or 0·1 nA through the microelectrode (i.e. $\Delta I = 0·05$ or 0·1 nA) and recording changes in the membrane potential (ΔV_m). In some experiments, action potentials were evoked by passing depolarizing currents through microelectrodes. An Axoclamp-2A amplifier (Axon Instruments) operated in the bridge mode was used to record spontaneous discharges and evoked responses. Data were stored on computer disks. The frequencies and patterns of spontaneous activities were analysed off-line with SMUP software (provided by Shanghai Medical University, P. R. of China).

Identification of receptive field of neurons

After measuring the CV, we searched for the mechanical RF of the recorded neuron. For noxious mechanical pinch, the skin of the hindlimb was pinched with a serrated forceps of ~2·5 N force for 1–2 s, a strength that produced moderate pain when applied to human skin (Tao *et al.* 1997). For innocuous stimulation, repeated broad 1–2 s strokes of ~0·2 N force were applied to the cutaneous surface of the hindlimb with a camel hair paint brush. According to the responses to the mechanical stimuli, the recorded neurons were categorized into: (1) low threshold mechanical (LTM) neurons which responded to brush stimuli, (2) high threshold mechanical (HTM) neurons which responded to noxious pinch stimuli, and (3) non-responding (silent) neurons which responded neither to brush nor pinch. Since noxious heat was not tested here, the HTM neurons could include polymodal nociceptors that responded to both noxious mechanical and heat stimulation. We adopted the term 'silent

neurons' used by Schaible & Schmidt (1985, 1988) to emphasize the unresponsiveness of these neurons to our RF stimuli. Since the stimuli were not exhaustive, it should be noted that these neurons could well be responsive to stimuli not tested, e.g. heat or vibration.

Some RF-responsive cells were labelled with Lucifer Yellow (LY), which was applied electrophoretically with hyperpolarizing 3–4 nA, 350 ms rectangular pulses at 1 Hz for 3–5 min (Smithson *et al.* 1984; Leah *et al.* 1985; Tao *et al.* 1997). The total currents passed for the LY injection were 3·0–5·3 nA min^{-1}. These values were much lower than those (16–60 nA min^{-1}) used by Scharfman *et al.* (1989), who found that high levels of intracellular dye, including LY, might mask immunoreactivity of transmitters, e.g. GABA. It therefore is unlikely that the SP immunoreactivity in our experiments was affected by LY injections. To ensure that LY was indeed injected into the cell, electrical stimuli were again applied to the same cell after the dye injection. If action potentials could no longer be evoked, the cell was rejected from the study. Initially, we labelled only one cell in each DRG. In later experiments, two neurons at opposite ends of the ganglia were injected. To exclude inappropriately marked cells, the depth and the position of each LY-injected cell was recorded and compared with those obtained in immunocytochemical experiments. Those ganglia that had more than expected LY-labelled cells or had labelled cells at unexpected locations were not included in the analyses.

Following injections of LY, animals were killed with an overdose of sodium pentobarbitone. DRGs were dissected out, fixed with 4% paraformaldehyde for 8–10 h and left overnight in a sucrose (20%)-containing phosphate buffer solution (PBS) at 4°C. Serial frozen sections of 30 μm thickness were then cut and mounted on slides previously coated with gelatin (0·1%, by weight gelatin, in 0·0002 M CrK(SO$_4$)$_2$.12H$_2$O). This section thickness was chosen because it gave the most consistent results. Since the diameters of some small cells were < 30 μm, the shapes of the LY-labelled cells were carefully recorded to minimize the possibilities of false identification of SP labelling. Dye-labelled neurons were located and photographed under a fluorescence microscope with a camera lucida attachment. The location and neuronal profiles were drawn in detail.

Identification of SP immunoreactivity in LY-labelled RF neurons

Sections containing LY-labelled neurons were incubated in 0·3% H$_2$O$_2$ for 10 min to block endogenous peroxidases. After being washed in cold PBS for 30 min, the sections were incubated at 4°C in a PBS solution containing antiserum to SP (Peninsula Lab. Inc., 1:5000), 5% goat serum and 0·1% Triton X-100. Forty-eight hours later, sections were washed with PBS three times for 30 min and incubated in goat anti-rabbit IgG (Vector, 1:200) at 37°C. After 2 h, sections were washed again and incubated in ABC-peroxidase complex (Vector, 1:100) for another 2 h at 37°C. Sections were then washed with Tris-HCl buffer (50 mmol l^{-1}, pH 7·2) and reacted with diaminobenzidine (0·05 mg ml^{-1}) and 0·01% H$_2$O$_2$. The specificity of the antisera were tested by replacing the primary antisera with normal rabbit sera or by omitting the primary or secondary antibodies in the experiment. No immunoreactivity was detected under these conditions. After the immunocytochemical procedure, the sections were air-dried, dehydrated, cleared and coverslipped. The SP-like immunoreactivity (SP-LI) in LY-labelled neurons was evaluated under a microscope.

Data analyses

Quantitative data (threshold, resting potentials and input resistance) were expressed as means ± S.E.M. and their statistical significance was determined using Student's two-tailed *t* test. The significance of frequency data (e.g. RF, SP-LI, spontaneously active neurons) was determined with the χ^2 test.

RESULTS

Conduction velocity, current threshold and firing patterns

The cat hindpaw started to swell 2 h after Carg injection. The swelling reached a peak around 4 h and persisted for more than 8 h. Intracellular recordings of L7 DRG neurons were conducted during the 2–8 h post-injection period. A total of 452 neurons were recorded from L7 DRGs, 242 of which were from Carg-treated animals and 210 neurons were from control animals. All neurons displayed stable resting potentials ranging from −40 to −75 mV. Using the latency of evoked action potentials, we divided the recorded neurons into Aβ (CV > 20 m s^{-1}), Aδ (CV between 2 and 20 m s^{-1}) and C (CV < 2 m s^{-1}) neurons. Only Aδ and C afferent neurons were studied in detail. There was no significant difference in the frequency distribution histograms of CVs obtained from control and Carg-treated groups.

Current stimulations of the tibial nerve induced action potentials in both control and Carg-treated (inflamed) DRG neurons. Threshold currents for tibial nerve stimulation, defined as the smallest current required to evoke action potentials in the DRG soma, were much lower in Carg-treated than in control cats. Mean threshold currents for nerve stimulation in normal cats were 5·3 ± 0·4 mA ($n = 40$) for C neurons and 3·9 ± 0·3 mA ($n = 23$) for Aδ neurons. After inflammation, mean threshold currents were 3·3 ± 0·5 mA for C neurons ($n = 49$) and 2·1 ± 0·4 mA for Aδ neurons ($n = 54$; $P < 0·05$; Fig. 1).

We then studied the firing patterns of DRG neurons evoked by intracellular current injection. With close to threshold stimuli, the intracellular current injection evoked single
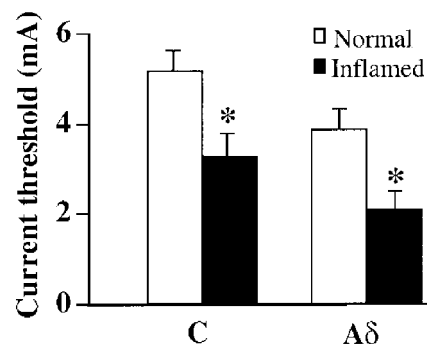

Figure 1. Carrageenan-induced inflammation decreased the current stimulation thresholds of tibial nerves

Under control conditions, the mean current threshold was 5·3 mA for C neurons and 3·9 mA for Aδ neurons. After inflammation, the mean current threshold was 3·3 mA for C neurons and 2·1 mA for Aδ neurons. The changes were significant (* $P < 0·05$).

action potentials in most neurons recorded from normal cats, but evoked multiple spikes in neurons from Carg-treated cats (Fig. 2A). To determine the percentage of cells exhibiting repetitive firings, suprathreshold rectangular current pulses (0·6 nA, 200 ms) were injected into the somata via recording microelectrodes. In the control group, the current injections evoked single action potentials in ∼95% of the cells. Only 5·1% (7/138) of C and 5·6% (4/72) of Aδ neurons fired repetitively. After Carg treatment, the same current stimuli evoked repetitive action potentials in larger percentages of C (32·1% (45/140), $P < 0\cdot001$) and Aδ (17·7% (18/102), $P < 0\cdot01$) neurons (Fig. 2B).

Membrane properties

In addition to changes in the active membrane properties, the resting potential (RP) and membrane input resistance (R_{in}) changed noticeably following the Carg injection. Resting membrane potentials and R_{in}s of various cells were recorded at different time points after Carg treatment (Fig. 3). Since the RPs and R_{in}s of a neuron collected at different time points from normal rats during the course of experiments did not change significantly ($P > 0\cdot05$), the RPs and R_{in}s obtained under these conditions were pooled together and taken as the zero point values (Fig. 3B). Mean

resting potentials recorded 4–6 h after the injection were more depolarized (C neurons: normal, $-53\cdot0 \pm 1\cdot8$ mV ($n = 40$); inflamed, $-45\cdot0 \pm 2\cdot6$ mV ($n = 29$), $P < 0\cdot05$; Aδ neurons: normal, $-58\cdot1 \pm 1\cdot9$ mV ($n = 23$); inflamed, $-51\cdot8 \pm 2\cdot3$ mV ($n = 23$), $P < 0\cdot05$; Fig. 3A). The R_{in}s were significantly higher following Carg treatment. The average R_{in} of Aδ neurons increased from $59\cdot0 \pm 12\cdot1$ MΩ ($n = 23$) in normal cats to $71\cdot9 \pm 9\cdot8$ MΩ ($n = 20$) ($P < 0\cdot05$) 2–4 h after injection and to $72\cdot5 \pm 10\cdot0$ MΩ ($n = 23$) ($P < 0\cdot05$) 4–6 h after injection. Increases in the R_{in}s of C neurons lasted up to 8 h after Carg treatment (normal: $72\cdot0 \pm 9\cdot2$ MΩ ($n = 40$), inflamed at 2–4 h: $89\cdot0 \pm 9\cdot5$ MΩ ($n = 20$), $P < 0\cdot05$; at 4–6 h: $95\cdot0 \pm 0\cdot6$ MΩ ($n = 29$), $P < 0\cdot05$; at 6–8 h: $118\cdot0 \pm 14\cdot9$ MΩ ($n = 22$), $P < 0\cdot05$; Fig. 3B).

The membrane conductances of C neurons were also examined under current-clamp conditions. In the normal group (6 cats), injection of hyperpolarizing currents into LTM ($n = 6$) and HTM C ($n = 6$) neurons generated transient hyperpolarizations which decayed to steady-state levels within 25 ms (Fig. 4A). The voltage–current relationship exhibited a distinct inward rectification (Fig. 4B). In the Carg-treated group (8 cats), however, hyperpolarizing current stimuli produced steady electrotonic potentials 2–6 h after
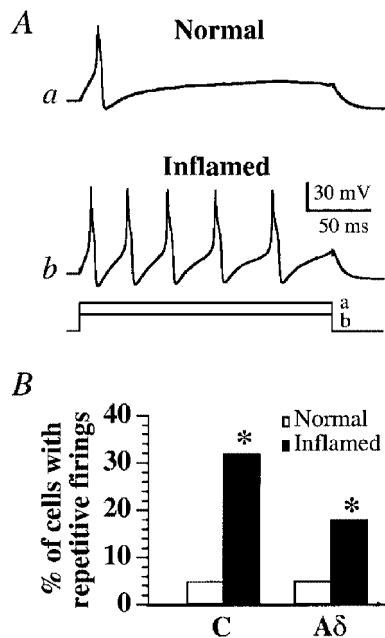

Figure 2. Inflammation changed the waveforms of the evoked responses of C and Aδ neurons

Intracellular current stimulation of a C neuron at an intensity of 0·32 nA induced single action potentials in a normal cat (Aa) while a weaker stimulus intensity (0·22 nA) could evoke repetitive firings in a C neuron from a Carg-treated cat (Ab). The bottom traces in A indicate the duration and the magnitude of the intracellular currents injected into these two cells. B, bar graph showing the percentages of C and Aδ neurons that exhibited repetitive discharges evoked by suprathreshold rectangular current pulses (0·6 nA, 200 ms). A significantly higher percentage (* $P < 0\cdot05$) of C and Aδ neurons in Carg-treated cats fire repetitively.
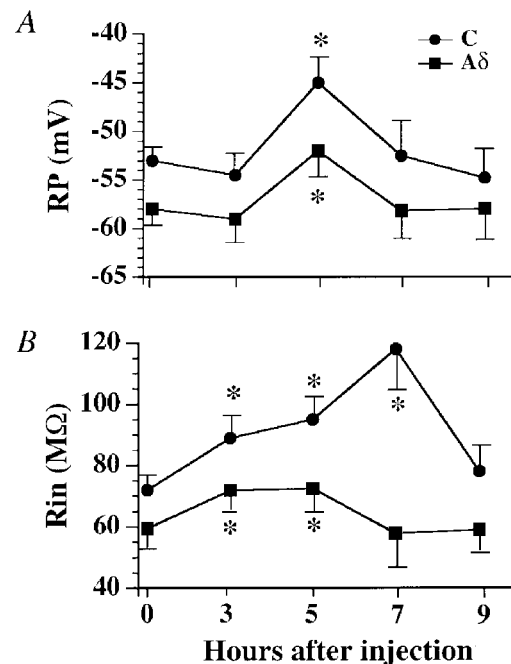

Figure 3. The changes in resting membrane potentials (RP) and input resistances (R_{in}) of Aδ and C neurons after the injection of Carg

A, RPs became significantly depolarized 4–6 h after Carg injections (* $P < 0\cdot05$). B, R_{in}s were significantly elevated (* $P < 0\cdot05$) between 2 and 6 h for Aδ neurons and 2 and 8 h for C neurons following the Carg treatment. The values at 0 h indicate the mean values for RPs or R_{in}s obtained from normal cats. The values at 3, 5, 7 and 9 h were the mean RPs or R_{in}s obtained from tested cells measured between 2 and 4 h, 4 and 6 h, 6 and 8 h, and 8 and 10 h after inflammation, respectively.

Carg. The depolarizing sags were completely absent and the voltage–current curve was linear in both LTM ($n = 8$) and HTM C ($n = 4$) neurons. With increasing R_{in}s in these neurons, the same current stimuli would produce larger hyperpolarizations and rebounding action potentials.

Spontaneous activity

In normal cats, spontaneous discharges were observed in 7·2 % (10/138) of C neurons and 6·9 % (5/72) of Aδ neurons. In Carg-treated cats, a significantly higher percentage of C neurons (20·7 % (29/140), $P < 0·005$) and of Aδ neurons (18·6 % (19/102), $P < 0·05$) were spontaneously active (Fig. 5A). Furthermore, the Carg treatment altered the firing patterns of neurons. Three spontaneous firing patterns, i.e. regular, irregular and burst firing, were observed in DRG neurons (Table 1). In normal cats, most spontaneously active cells fired regularly at low frequencies (Fig. 5B, Table 1). In Carg-treated cats, more cells fired spontaneously at high frequencies (> 10 Hz; Fig. 5Ca) or in bursts (Fig. 5Cb; Table 1).

To determine the origin of the spontaneous activity of DRG neurons in Carg-treated cats, neuronal activity was measured following sectioning of nerves at proximal and/or distal ends of the ganglia (C neuron, $n = 6$; Aδ neuron, $n = 2$; Fig. 6). Transection of dorsal roots did not affect the spontaneous activity of DRG neurons (Fig. 6B, arrow a). On the other hand, the activity of the neurons ceased completely within 15–20 s after the tibial or sciatic nerve was sectioned (Fig. 6B, arrow b). Since the resting potentials of recorded cells remained stable for > 30 min after transection, and intracellular current stimulation still elicited spikes in these cells, sectioning of nerve fibres did not abolish the intrinsic excitability of the cells. Thus, the spontaneous discharge of DRG neurons in Carg-treated cats appears to initiate at peripheral nerve terminals or/and axons.

Mechanical receptive fields and SP immunoreactivity

While > 90 % of the spontaneously active cells were mechanical RF responsive in both normal and Carg-treated cats, most (> 75 %) of the RF-responsive cells did not fire spontaneously. After Carg treatment, the percentages of RF-responsive C neurons increased from 32·6 % (45/138 cells) to 58·6 % (82/140 cells; $P < 0·05$) and RF-responsive

Figure 4. Loss of inward rectification in electrotonic potentials in Carg-treated cat DRG neurons

A, typical electrotonic potentials recorded from C neurons in normal and Carg-treated cats. The hyperpolarizing current steps of 200 ms duration used to evoke the responses are shown at the top. *B*, steady-state voltage–current curves from data given in *A*. (The arrows in *A* indicate the time points at which the measurements were taken.) Note the electrotonic potentials exhibit inward rectification at hyperpolarizing potentials in the normal cat but not in the Carg-treated cat.
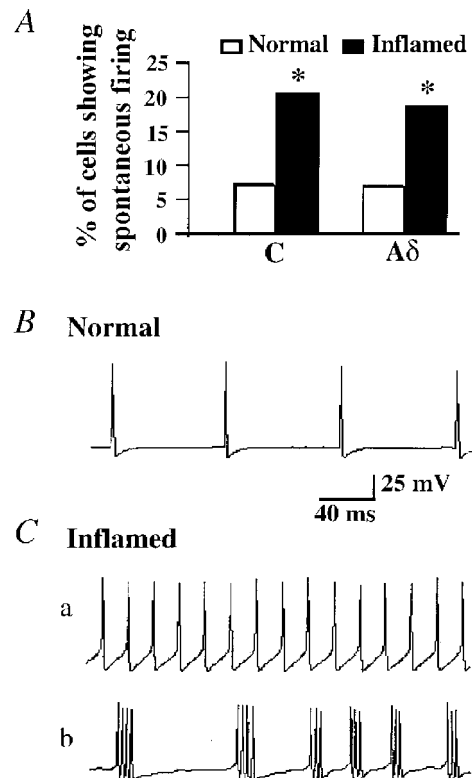
Figure 5. Inflammation changed the occurrence and patterns of the spontaneous firing of DRG neurons

A, the percentage of C and Aδ cells exhibiting spontaneous firing increased significantly (*$P < 0·05$) following Carg treatment. *B*, an Aδ neuron recorded from the normal cat exhibited regular spontaneous firings. *C*, examples of spontaneous firing patterns recorded from DRG neurons in Carg-treated cats. Aδ neurons recorded from the Carg-treated cat fired at high frequency (80 Hz) (a) or in bursts (b).

Table 1. Number of DRG cells with various spontaneous firing patterns in normal and Carg-treated cats

Cell type	Total	Regular[a] < 10 Hz	10–30 Hz	30–50 Hz	>50 Hz	Bursting[b]	Irregular[c]
C cells							
Normal	10	7	2	—	—	1	—
Inflamed	28	10	5	4	2	4	3
Aδ cells							
Normal	5	4	1	—	—	—	—
Inflamed	19	5	2	4	1	5	2

[a] Regular: cells fire with constant interspike intervals. [b] Bursting: cells fire with bursts of 3–5 action potentials. [c] Irregular: cells fire with variable interspike intervals.

Aδ neurons increased from 34·7% (25/72 cells) to 54·9% (56/102 cells) ($P < 0.05$; Fig. 7B). Thus, inflammation activated 26·0% of RF-unresponsive C neurons and 20·2% of RF-unresponsive Aδ neurons. To determine whether inflammation altered the nature of the responsiveness of neurons, percentages of LTM and HTM neurons in control and Carg-injected cats were analysed (Fig. 7A and C). Among the 138 C neurons recorded from normal cats, 18 (13·0%) were LTM neurons and 27 (19·6%) were HTM neurons (Fig. 7C). Of the 72 recorded Aδ neurons, 9 (12·5%) were LTM neurons and 16 (22·2%) were HTM neurons. After inflammation, 57/140 recorded C neurons (40·7%) were LTM neurons and 25/140 neurons (17·9%) were HTM neurons. Of the Aδ neurons 40/102 (39%) were LTM

Figure 6. Spontaneous activity initiated at peripheral afferent terminals

A, schematic diagram illustrating the locations of the section. B, after the central terminals of the recorded DRG C neuron in the Carg-treated cat were sectioned (arrow a) there was no change in the spontaneous activity. However, when the peripheral nerves were sectioned (arrow b) the spontaneous firing ceased completely.

Figure 7. Inflammation changed the characteristics and responsiveness of peripheral mechanical receptive fields of C and Aδ neurons

A, examples of action potentials evoked by both brush (a) and pinch (b) stimuli in a C neuron of a Carg-treated cat. This neuron is a LTM type. B, the percentages of C and Aδ neurons that had RFs increased significantly (* $P < 0.05$) after inflammation. C, the RF-responsive neurons were divided into LTM and HTM neurons. Following Carg treatment, the percentages of HTM C and Aδ neurons did not change. On the other hand, the percentages of LTM C and Aδ neurons were significantly higher (* $P < 0.05$).

neurons and 16/102 (15·7%) were HTM neurons. Thus, inflammation did not alter the percentage of the HTM neurons ($P > 0·05$), but significantly increased the percentage of the LTM neurons ($P < 0·01$; Fig. 7).

Among the cells which were found to have RFs, 100 cells were injected with LY. These ganglia were then fixed and reacted for SP (Fig. 8*A* and *B*). For C neurons, 35·7% of neurons (15/42) from control cats and 60·0% (21/35) of the neurons from the Carg-treated cats were SP-LI positive. For Aδ neurons, only 18·2% (2/11) of the neurons from control cats, but 66·7% of neurons (8/12 cells) from the Carg-treated cats were SP-LI positive. Thus, the number of SP-LI-positive C and Aδ cells that responded to the stimulation of RFs increased significantly ($P < 0·05$ for both types of C and Aδ neurons) following Carg-induced inflammation (Fig. 8*C*).

DISCUSSION

After Carg-induced inflammation in the cat hindpaw, DRG neurons undergo the following changes: (1) firing frequencies are enhanced, (2) more cells become responsive to receptive field stimulation, and (3) an increasing number of RF-responsive cells display SP immunoreactivity. Since changes in the resting membrane potential and membrane-input resistance of DRG cells coincide with the time course of the behavioural effects of Carg (Kocher *et al.* 1987; Traub, 1996; Tsuruoka *et al.* 1997) and c-fos expression in the dorsal horn following Carg treatment (Buritova *et al.* 1996), the above plastic changes in the membrane characteristics of DRG neurons are likely to underlie the development of hyper-algesia. Because spontaneous discharges of DRG neurons in Carg-treated cats ceased immediately and completely after the tibial nerve was sectioned (Fig. 6), the increased activity of DRG neurons we observed depends on peripheral inputs. We also demonstrate that more LTM C and Aδ DRG cells respond to receptive field stimulation in Carg-treated cats than those in normal cats (Fig. 7). Although the exact mechanism underlying this increase is unclear, inflammatory mediators (e.g. histamine, serotonin, bradykinin, prosta-glandin E$_2$, SP and nerve growth factors), which are released from peripheral tissue, macrophages and lymphocytes following tissue damage, are likely to be responsible for this change. These mediators are known to excite and/or sensitize peripheral terminals (Schaible & Schmidt, 1988; Lembeck *et al.* 1992; Otsuka & Yoshioka, 1993) and promote spontaneous activity in DRG neurons (Schaible & Schmidt, 1985). Under long-term inflammatory conditions (> 2 days), others found that the duration of DRG neuron action potentials are shortened (Djouhri & Lawson, 1999) and A-type K$^+$ currents are suppressed (Yoshimura & de Groat, 1999). The mechanisms underlying the short-term changes (2–8 h) in membrane properties of DRG neurons studied here may differ from those studied before. The excitability of DRGs is nevertheless enhanced. As DRG neurons become more depolarized (Fig. 3*A*), and susceptible to intracellular

current stimulation (Fig. 2*B*) in inflammatory states, the sensitivity of neurons to peripheral inputs would be further augmented and thus, in turn, lead to sensitization of DRG neurons.

It is interesting that inflammation results in a reduction of hyperpolarization-activated inward rectifier and an increase in R_{in} (Fig. 4). Since the change in R_{in} lasts only up to 6 h
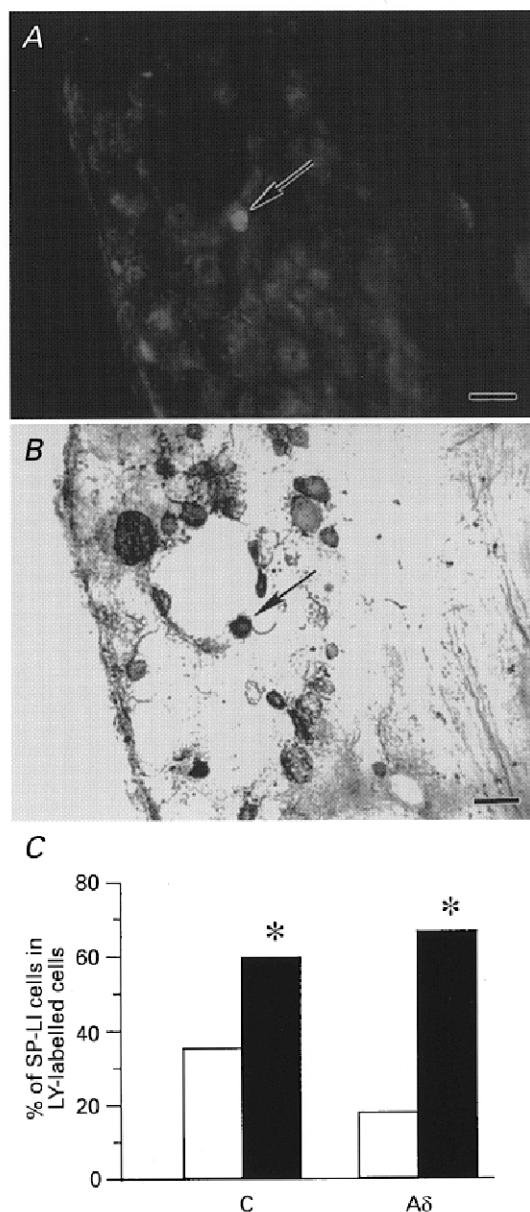

Figure 8. Substance P expression in RF-responsive cells increased with inflammation

A, a LTM C neuron from a cat with inflamed cells was labelled with LY (indicated by arrow). *B*, the same neuron exhibited SP immunoreactivity (indicated by arrow). Scale bars in *A* and *B*, 50 μm. *C*, bar graph showing that a significantly higher percentage of LY-labelled C and Aδ DRG neurons were SP immunoreactive after inflammation (* $P < 0·05$ for both types of neurons). □, normal; ■, inflamed cells.

These changes could lead to the activation of silent mechanoreceptive cat C and Aδ sensory neurons and thus may underlie the development of hyperalgesia.

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Corresponding author

Zhi-Qi Zhao: Shanghai Institute of Physiology, Chinese Academy of Sciences, 320 Yueyang Road, Shanghai 200031, People's Republic of China.

Email: zhao@sunm.shcnc.ac.cn