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Tachykinin NK_1 receptor antagonist RP67580 attenuates progressive hypersensitivity of flexor reflex during experimental inflammation in rats

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Abstract

We have now examined whether the tachykinin NK_1 receptor is involved in mediating progressive hypersensitivity of spinal flexor motoneurons induced by repeated peripheral stimulation of inflamed tissue in decerebrate-spinal rats. The mechanical threshold of spinal flexor motoneurons was significantly decreased, and the touch- and pinch-evoked responses significantly increased, 48 h after intra-plantar injection of 100 μl complete Freund's adjuvant. The threshold was further progressively decreased and the touch- and pinch-evoked responses increased over the 80 min testing period. Subcutaneous injection of the tachykinin NK_1 receptor antagonist RP67580 (2-[1-imino-2-(2-methoxy phenyl) ethyl]-7,7 diphenyl-4 perhydroisoindolone-(3*aR*,7*aR*)) (20 min prior to the beginning of the test) at 1 mg and 10 mg/kg significantly attenuated the progressive decrease of mechanical withdrawal threshold, and the progressive increase of the touch- and pinch-evoked responses. The inactive enantiomer RP68651 (2-[1-imino-2-(2-methoxy phenyl) ethyl]-7,7 diphenyl-4 perhydroisoindolone-(3*aS*,7*aS*)) at 1 mg and 10 mg/kg had no significant effect. The present results indicate that substance P and its preferred tachykinin NK_1 receptor are involved in mediating progressive hypersensitivity during inflammation.

Keywords: Hyperalgesia; Allodynia; Pain; Tachykinin; Flexor reflex

1. Introduction

Peripheral tissue injury or inflammation induces behavioural allodynia and hyperalgesia. Both peripheral sensitization (increased sensitivity of peripheral nociceptors; for review, see Levine and Taiwo, 1994) and central sensitization (increased excitability of spinal neurons) (Woolf, 1983; Wall and Woolf, 1984; Woolf and Wall, 1986) contribute to the generation of the pain hypersensitivity. It is well demonstrated experimentally that innocuous stimuli can evoke pain sensation in allodynic area (Simone et al., 1989; Torebjork et al., 1992) and that central sensitization can only be induced by C-fibre afferent input in normal animals (Woolf, 1983; Wall and Woolf, 1984; Woolf and Wall, 1986), but it is less clear what effect pain-evoking, normally innocuous stimuli to the allodynic and hyperalgesic areas have on the sensitivity of the respective spinal neurons. We have recently reported a novel phenomenon that repeated normally innocuous tactile stimuli induced a progressive increase in the sensitivity of withdrawal flexion reflex in inflamed rats, progressive tactile hypersensitivity (Ma and Woolf, 1996). Unlike the transient increase in excitability, which lasts a few minutes, of spinal neurons induced by a 20-s C-fibre conditioning stimulus at 1 Hz, the sensitivity increase in progressive tactile hyperalgesia happens gradually and progressively over a relatively long testing period and has a long after-effect. Aβ-fibre afferents have been demonstrated to contribute to the generation of progressive tactile hypersensitivity (Ma and Woolf, 1996).

Following inflammation, a number of neurochemical changes occur in both the dorsal root ganglion cells and the spinal cord neurons (Iadarola et al., 1988; Noguchi et al., 1988, 1991, 1992; Kuraishi et al., 1989; Hanesch et al., 1993; Mapp et al., 1993; Stucky et al., 1993; Garry and Hargreaves, 1992). One feature of these changes is the increased synthesis and release of substance P (Hanesch et al., 1993; Garry and Hargreaves, 1992; Mapp et al., 1993). The change in substance P coincides with the finding that NGF is essential for the normal function of small dorsal root ganglion cells which synthesize substance P and conduct nociceptive messages (Johnson et al., 1980; Ruit et al., 1992; Crowley et al., 1994; Lindsay and Harmar,

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1989; Lindsay et al., 1989). Moreover, anti-NGF serum attenuates the hyperalgesia, the increase in substance P and CGRP (calcitonin-gene-related peptide) content in nerve and dorsal root ganglia, and the c-*fos* expression following inflammation (Donnerer et al., 1992; Woolf et al., 1994a). It is conceivable that some of these neurochemical changes during inflammation also contribute to the phenomenon of progressive tactile hypersensitivity.

The capacity of C-fibre afferents to evoke slow synaptic potentials in some spinal neurons of the rat (Yoshimura and Jessell, 1989; Thompson et al., 1990) has been well established. The slow potentials may summate to produce a cumulatively increasing postsynaptic depolarization and action potential wind-up (Price et al., 1971), which has been implicated as the underlying mechanism of C-fibre afferent-induced central sensitization. It has been well documented that these slow synaptic potentials and wind-up are mediated by NMDA and tachykinin receptors (Thompson et al., 1990, 1992; Nagy et al., 1993). The NMDA receptor antagonist MK801 and the tachykinin NK_1 receptor antagonist RP67580 can block the mechanical allodynia induced by the conditioning C-fibre stimulation (Ma and Woolf, 1995a,b). While a transiently increased release of glutamate and tachykinins is responsible for the hyperexcitability of spinal neurons evoked by a short period of electrical stimulation of C-fibre afferents, it seems that at least a continuous increase of glutamate and tachykinin release may be needed to fully account for the long-lasting behavioural hyperalgesia and allodynia following tissue injury or inflammation.

Since the maintenance of central sensitization does not involve the tachykinin receptors (Ma and Woolf, 1995a), it appears paradoxical that substance P and tachykinin NK_1 receptors are up-regulated in primary afferents (Hanesch et al., 1993; Mapp et al., 1993; Woolf et al., 1994a; Garry and Hargreaves, 1992) and spinal cord (Schafer et al., 1993; Stucky et al., 1993) respectively during inflammation. Our recent study, however, indicates that inflammation not only evokes hyperalgesia and allodynia, but also results in a state which enables low-intensity peripheral stimuli to induce further hyperresponsiveness of spinal neurons, progressive hypersensitivity (Ma and Woolf, 1996). The aim of the present study has been to investigate whether the tachykinin NK_1 receptors and substance P are responsible for the generation of progressive hypersensitivity of spinal flexor motoneurons during experimental inflammation.

2. Materials and methods

2.1. Animal preparation

All experiments were performed on adult male Sprague-Dawley rats (200–300 g). Inflammation was induced 48 h prior to the electrophysiological recording by an intra-plantar injection of complete Freund's adjuvant (Sigma) into the left hind paw, 50 μl into the paw and 10 μl into each toe, under fluothane (ICI) anaesthesia. The animal preparation for electrophysiological recording has been described in detail before (Wall and Woolf, 1984; Woolf and Wall, 1986; Ma and Woolf, 1996). Briefly, cannulation of one carotid artery and the trachea was made under fluothane anaesthesia, and anaesthesia was then maintained by small doses (0.2–0.3 ml) of Saffan (alphaxalone/alphadalone, Pitman-Moor). The rats were decerebrated by aspiration of all the cranial contents rostral to the mesencephalon and spinalized via a laminectomy at T3–T5. The anaesthetic was then discontinued, and the animals paralysed with Flaxedil (gallamine) and artificially ventilated. The nerve to the posterior biceps femoris/semitendinosus muscles was exposed in the popliteal fossa, a very fine terminal branch dissected free in the muscle and cut, and the central end placed on a silver recording electrode. The sural nerve was dissected free and placed in continuity on a pair of silver stimulating electrodes. Rectal temperature, expired pCO_2, heart rate and ECG (electrocardiogram) were monitored.

2.2. Recordings

Recordings were started no earlier than 1 h after spinalization. Single units were detected by using a conventional window discriminator. Spikes were counted with a pulse integrator, and the spike shape monitored continuously by an analog delay line. To monitor the excitability of the flexor reflex, as described in detail previously (Sivilotti and Woolf, 1994; Woolf et al., 1994b; Ma and Woolf, 1996), measurements were made of: (1) spontaneous activity (for 10 s); (2) the mechanical threshold, which was recorded as the lowest force of von Frey hairs evoking a consistent discharge on each occasion when applied three times to the plantar surface of any one of the middle three toes; (3) the total discharge elicited by eight light touch stimuli to the plantar surface of the foot, each touch lasting 2 s and moving from the middle position of the foot to the distal foot pads, applied once every 4 s; (4) the total discharge elicited by a standard test pinch applied with a pair of calibrated forceps with a 13×1 mm retangular surface attached to each blade, sprung at 210 g, on the three middle toes of the hindpaw for 2 s; and (5) the total discharge evoked by a train of Aβ-fibre strength stimuli (100 μA, 50 μs, 10 Hz) applied to the sural nerve. Spike counts were integrated over the stimulation period for the sural Aβ stimuli and over the stimulation period plus 2 s for touch and pinch stimuli, to include any afterdischarge. The test protocol was applied at 5-min intervals during the entire course of the experiment.

2.3. Chemicals

The non-peptide tachykinin NK_1 receptor antagonist 2-[1-imino-2-(2-methoxy phenyl) ethyl]-7,7 diphenyl-4

perhydroisoindolone-(3*aR*,7*aR*) (RP67580; Rhône-Poulenc Rorer, Vitry-sur-Seine, France) and its inactive enantiomer 2-[1-imino-2-(2-methoxy phenyl) ethyl]-7,7 diphenyl-4 perhydroisoindolone-(3*aS*,7*aS*) (RP68651; Rhône-Poulenc Rorer) were dissolved in 0.11% HCl diluted in normal saline. All the injections were given 20 min prior to the testing.

2.4. Data analysis

The touch-, pinch- and Aβ afferent-evoked responses were analyzed as the total number of spikes recorded, minus the extrapolated spontaneous activity in the same period, and in the case of the electrical stimulation, the contribution of the stimulus artefacts. Statistical analysis was performed both on the baseline and the peak changes in threshold or spike discharge or on data expressed as percentages of the baseline calculated from the first three readings. In the former case, the differences between the baselines and peak changes of control and complete Freund's adjuvant groups were analyzed by using unpaired *t*-test (if S.D. values are not equal, then Welch's *t*-test was used), and the effects of different drug treatments on the baseline and peak change of complete Freund's adjuvant inflamed animals were tested by one-way ANOVA (analysis of variances), followed by Tukey's test for multiple comparisons. In the latter case the differences between groups were tested with one-way ANOVA or non-parametric ANOVA in case of unequal S.D. values, followed by Tukey's test for multiple comparisons. All data are illustrated as means ± S.E.M. and $P < 0.05$ was considered significant.

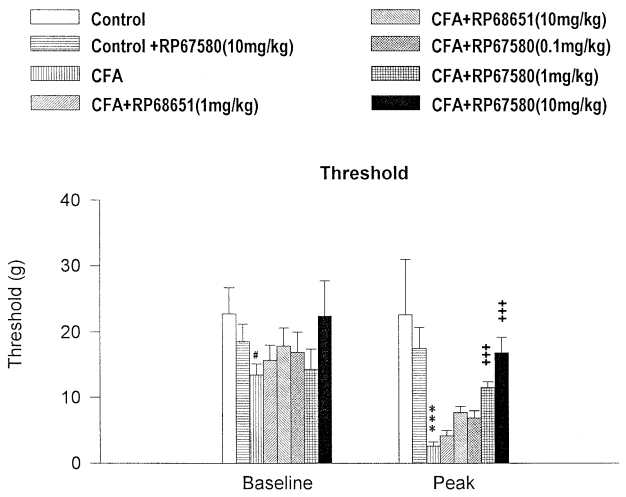

Fig. 1. The effect of the tachykinin NK_1 receptor antagonist RP67580 on the baseline of and the progressive reduction in the mechanical threshold during the 80-min testing period. The mechanical threshold was stable in control animals during the testing period; 10 mg/kg RP67580 (s.c.) had significant effect on neither the baseline nor the peak reduction of the mechanical threshold in control animals. The baseline threshold in inflamed animals was significantly lower than that of control, but was not significantly affected by any of the drug treatments. RP67580 at 10 mg/kg significantly attenuated the peak reduction in threshold during the 80-min testing period. $^{\#}$ $P < 0.05$, compared with the baseline of control. *** $P < 0.001$, compared with its own baseline. $^{+++}$ $P < 0.001$, compared with the peak of the complete Freund's adjuvant (CFA) group.

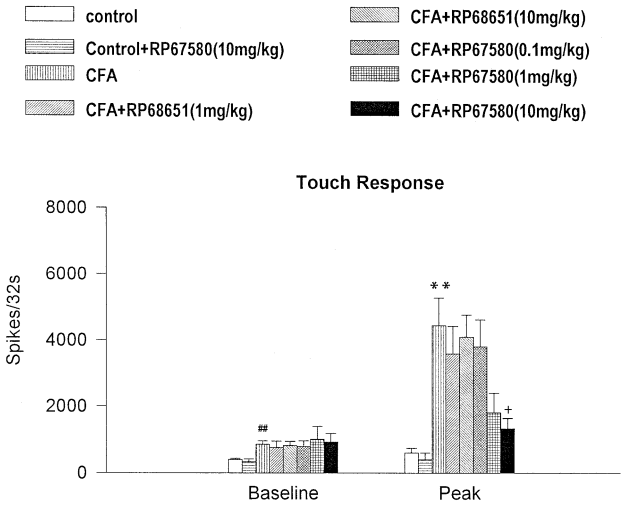

Fig. 2. Effects of RP67580 on the baseline and the peak of the touch-evoked response during the 80-min testing period. The touch-evoked response was stable in control and not significantly affected by RP67580. The baseline touch-evoked response in the complete Freund's adjuvant group was significantly higher than that of control, but was not significantly attenuated by any of the drug treatments. RP67580 at doses of 1 and 10 mg/kg significantly reduced the peak of the touch-evoked response during the 80-min testing period. $^{\#\#}$ $P < 0.01$, compared with baseline of the control. ** $P < 0.01$, compared with it own baseline. $^{+}$ $P < 0.05$, compared with the peak of the complete Freund's adjuvant (CFA) group.

3. Results

Thirty-nine animals were divided into eight groups. Group 1 was normal control, group 2 was normal control + RP67580 (10 mg/kg), group 3 was complete Freund's adjuvant inflamed animals, group 4 was complete Freund's adjuvant + RP68651 (1 mg/kg), group 5 was complete Freund's adjuvant + RP68651 (10 mg/kg), group 6 was complete Freund's adjuvant + RP67580 (0.1 mg/kg), group 7 was complete Freund's adjuvant + RP67580 (1 mg/kg) and group 8 was complete Freund's adjuvant + RP67580 (10 mg/kg). The baseline mechanical threshold in group 3 (complete Freund's adjuvant) was significantly lower than that of group 1 (normal control), and touch- and pinch-evoked responses were significantly higher. RP67580 at a dose of 10 mg/kg did not have a significant effect on the baseline mechanical threshold, touch- and pinch-evoked responses in normal animals (group 2, normal control + 10 mg/kg RP67580), nor did RP68651 at doses of 1 and 10 mg/kg and RP67580 at doses of 0.1, 1 and 10 mg/kg on those of complete Freund's adjuvant inflamed animals (groups 4–8) (Figs. 1–3).

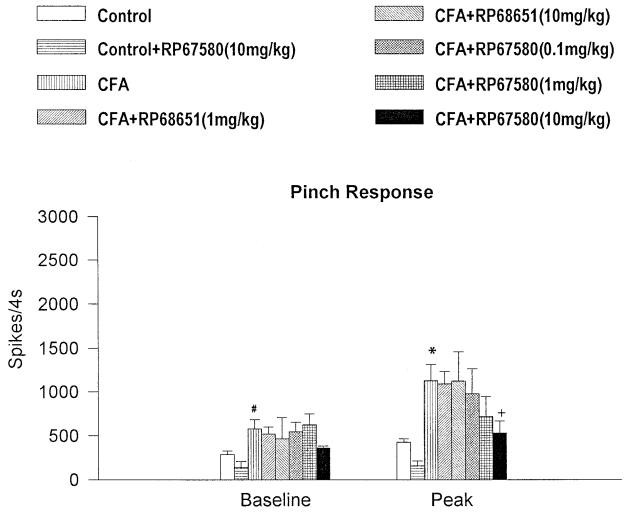

Fig. 3. Effects of RP67580 on the baseline and the peak of the pinch-evoked response during the 80-min testing period. The pinch-evoked response had no significant changes in control and was not significantly affected by RP67580. The baseline pinch-evoked response in the complete Freund's adjuvant group was significantly higher than that of control, but was not significantly attenuated by any of the drug treatments. RP67580 at 10 mg/kg significantly reduced the peak of the pinch-evoked response during the 80-min testing period. $^{\#}$ $P < 0.05$, compared with baseline of the control. * $P < 0.05$, compared with it own baseline. $^{+}$ $P < 0.05$, compared with the peak of the complete Freund's adjuvant (CFA) group.

While the mechanical threshold, touch- and pinch-evoked responses were fairly stable during the 80 min period of repeated testing in normal control animals with and without pre-treatment of 10 mg/kg of the tachykinin NK_1 receptor antagonist RP67580 (groups 1 and 2) (Figs. 1–3), the touch- and pinch-evoked responses were significantly and progressively increased, and the threshold was significantly reduced in complete Freund's adjuvant inflamed animals (group 3) (Figs. 1–4). The inactive enantiomer of RP67580, RP68651 at doses of 1 and 10 mg/kg, had no significant effect on the progressive increase of the touch- and pinch-evoked responses, and the progressive decrease of mechanical threshold in inflamed animals (groups 4 and 5) (Figs. 1–3).

RP67580 at a dose of 0.1 mg/kg did not significantly attenuate the progressive hypersensitivity of flexor motoneurons in complete Freund's adjuvant inflamed animals (group 6). At 1 mg/kg RP67580 significantly attenuated the progressive increase of the touch- and pinch-evoked responses, and significantly attenuated the progressive reduction of mechanical threshold in inflamed animals (Figs. 1–4). At a dose of 10 mg/kg it almost completely prevented the progressive increase of the touch- and pinch-evoked responses, and the progressive decrease of the mechanical threshold (Figs. 1–4).

4. Discussion

Substance P has long been proposed as a primary afferent neurotransmitter for nociception. It exists in dorsal root ganglion neurons with fine unmyelinated afferents (Hökfelt et al., 1975; McNeill et al., 1989), and is released in response to a variety of noxious stimuli (Yaksh et al., 1980; Kuraishi et al., 1983; Duggan et al., 1988). Behavioural studies have also shown that intrathecal injection of substance P and its more selective analogues, produces decreased nociceptive thresholds, and these effects can be blocked by their respective antagonists (Cridland and Henry, 1986; Picard et al., 1993). Since substance P receptor antagonists do not block the direct postsynaptic action potential responses to transient high-threshold afferent inputs (Chapman and Dickenson, 1993), nor do they alter the baseline reflex response to noxious or innocuous stimuli (Xu et al., 1992; Ma and Woolf, 1995a), substance P is more likely to be involved in mediating gain control of nociceptive transmission. Following inflammation, the substance P or preprotachykinin mRNA content in dorsal root ganglia (Donaldson et al., 1992; Noguchi et al., 1988; Smith et al., 1992; Mapp et al., 1993) as well as in the affected nerve (Donnerer et al., 1992; Woolf et al., 1994a), and the proportion of substance P-mRNA containing dorsal root ganglion cell (Hanesch et al., 1993) are increased, suggesting a possible role of substance P in the develop-

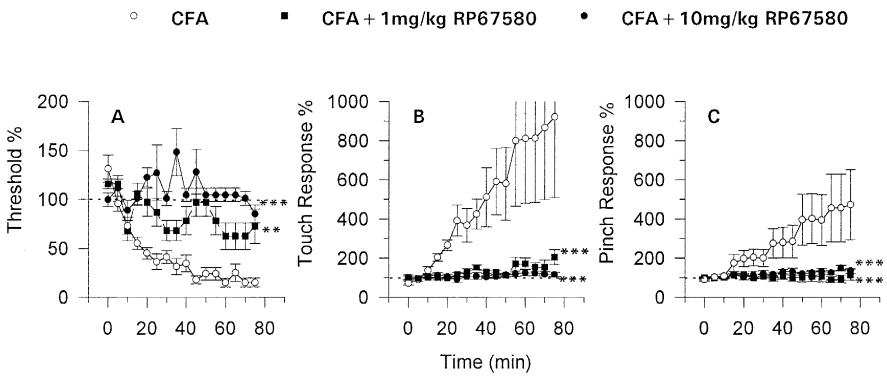

Fig. 4. The effects of RP67580 on the progressive decrease of mechanical threshold, and the progressive increase of the touch- and pinch-evoked responses in the inflamed animals during the 80-min testing period. RP67580 at doses of 1 and 10 mg/kg significantly attenuated the progressive hypersensitivity. ** $P < 0.01$, *** $P < 0.001$, compared with control.

ment of inflammatory hyperalgesia. The increased substance P may mediate (1) the induction, (2) the maintenance of central sensitization and (3) the phenomenon of progressive tactile hypersensitivity (Ma and Woolf, 1996).

C-fibre afferent-induced slow synaptic potentials and excitability changes of spinal flexor motoneurons can be mimicked by substance P and blocked by substance P receptor antagonists (DeKoninck and Henry, 1991; Ma and Woolf, 1995a; Murase and Randic, 1984; Nagy et al., 1993, 1994; Urban and Randic, 1984; Wiesenfeld-Hallin, 1986; Woolf and Wiesenfeld-Hallin, 1986; Xu et al., 1990), suggesting that substance P and its preferred tachykinin NK_1 receptor are involved in the induction of central sensitization. The up-regulation of substance P following inflammation, however, seems unlikely to mediate the induction of central sensitization, since the C-fibre conditioning stimuli are sufficient to induce hyperexcitability well before the upregulation of substance P happens (Xu et al., 1992; Woolf et al., 1994b; Sivilotti and Woolf, 1994; Ma and Woolf, 1995a,b). Therefore, a role in the maintenance or the progressive tactile hypersensitivity (Ma and Woolf, 1996) should be considered for the up-regulation of substance P during inflammation.

The maintenance of central sensitization evoked by peripheral application of irritant chemicals does not depend upon tachykinin NK_1 receptor activation, since the tachykinin NK_1 receptor antagonist RP67580 does not reverse the established central sensitization evoked by local application of irritant chemicals such as mustard oil (Ma and Woolf, 1995a). For inflammatory hypersensitivity, post-treatment of the tachykinin NK_1 receptor antagonist CP-96345 or RP67580 has no significant effect on the hyperaesthesia induced by carrageenin (Yamamoto et al., 1993; Traub, 1996). The present results that subcutaneous injection of RP67580 at doses of 0.1–10 mg/kg did not inhibit the baseline flexion reflex, confirmed these previous findings that the maintenance of hyperalgesia does not involve a continuous release of substance P. The concept of non-involvement of substance P in the maintenance of central sensitization contrasts with the findings of a significant increase of substance P in the nerve and dorsal root ganglia following inflammation (Donnerer et al., 1992, 1993; Woolf et al., 1994a; Donaldson et al., 1992; Noguchi et al., 1988; Smith et al., 1992; Mapp et al., 1993). The present results that the tachykinin NK_1 receptor antagonist RP67580 attenuated the progressive hypersensitivity induced by repeated touch and pinch stimuli in inflamed animals, indicate that at least increased substance P during peripheral inflammation may be responsible for the progressive hypersensitivity.

In the present study, we tested repeatedly both normally innocuous touch stimulus and non-damaging noxious pinch stimulus, so that it is not clear which of the two stimuli, touch or pinch or both, is responsible for the generation of progressive hypersensitivity. Non-damaging pinch stimulus per se does not produce progressive hypersensitivity in normal decerebrate-spinal rats (Wall and Woolf, 1984; Woolf and Thompson, 1991; Sivilotti and Woolf, 1994; Woolf et al., 1994b), and we have reported that touch stimuli induce progressive tactile hypersensitivity in awake inflamed animals and that Aβ-fibre afferents contribute to the generation of progressive tactile hypersensitivity (Ma and Woolf, 1996). Therefore, in the present study, the normally innocuous touch stimuli are at least partly responsible for the progressive hypersensitivity and the tachykinin NK_1 receptors responsible for the touch-evoked progressive tactile hypersensitivity. Whether repeated pinch stimulus can induce progressive hypersensitivity or the tachykinin NK_1 receptor mediates a pinch-evoked progressive hypersensitivity is not clear from the present study.

Our present results suggest a role for the tachykinin NK_1 receptors and substance P in the generation of progressive tactile hyperalgesia, but the cellular mechanism is not clear. An Aβ-fibre afferent-mediated central component has been demonstrated in our previous study (Ma and Woolf, 1996), so that a substance P-mediated facilitation of spinal neurons may be responsible. Both the normally responsive and silent C-fibre nociceptors may be activated by pinch and touch stimuli under inflammation (Schaible and Schmidt, 1985), which will contribute to the peripheral component of the progressive tactile hypersensitivity.

The discovery of the phenomenon of central sensitization has led to the concept of pre-emptive analgesia (for review, see Woolf and Chong, 1993). Clinical studies have demonstrated that preoperative opioids (Katz et al., 1992, 1994; Richmond et al., 1993) can decrease postoperative pain, analgesic requirements and wound hyperalgesia more effectively than the same treatment postoperatively. The previous findings from others' and our laboratory that tachykinin NK_1 receptor antagonists prevent the development of central sensitization but do not reverse the established central sensitization (Ma and Woolf, 1995a; Traub, 1996; Yamamoto et al., 1993), indicate that tachykinin NK_1 receptor antagonists would be candidates for preemptive treatment of acute pain rather than for the treatment of on-going pain.

Since most injury and inflammation induced hypersensitivity states will arise before any pre-emptive medication can be given, a treatment capable of eliminating established hypersensitivity would be therapeutically important. Our present results suggest that though tachykinin NK_1 receptor antagonists do not reverse the established central sensitization, they do prevent the further development of central sensitization induced by low intensity stimuli under inflammatory conditions. It can be expected that if the reinforcement of hyperalgesia by low intensity stimuli is stopped, the hyperalgesia may disappear earlier.

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