Involvement of neurokinin receptors in the induction but not the maintenance of mechanical allodynia in rat flexor motoneurones

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1. Intrathecal (I.T.) injections of the (tachykinin) NK_1 receptor agonist, substance P methyl ester (SPME; 20 pmol), or the NK_2 receptor agonist, neurokinin A (NKA; 20 pmol), substantially decreased the cutaneous mechanical threshold and markedly enhanced the touch-evoked response of posterior biceps femoris–semitendinosus (PBF–ST) spinal flexor motoneurones in decerebrate–spinal rats. This cutaneous mechanical reflex allodynia was prevented by pretreatment with the NK_1 antagonist RP 67580 (2·28 nmol, I.T.) and the NK_2 antagonist MEN 10376 (0·7 nmol, I.T.), respectively.

2. Electrical stimulation of the sural nerve at C fibre strength or cutaneous application of the irritant, mustard oil, produced prolonged cutaneous mechanical allodynia in PBF–ST motoneurones (15 min and >1 h, respectively). Pretreatment with RP 67580 but not MEN 10376 prevented this, but when RP 67580 was administered 25 min after the application of mustard oil, the established hypersensitivity of the flexor motor reflex was not reversed. The enantiomer of RP 67580, RP 68651 was without effect.

3. Injection of bradykinin (60 μM, 80 μl) into the gastrocnemius muscle increased the cutaneous mechanical hypersensitivity of PBF–ST flexor motoneurones for 40–50 min. MEN 10376, but not RP 67580, prevented this, but only when administered prior to the bradykinin injection.

4. These results suggest that the induction, but not the maintenance, of cutaneous mechanical allodynia in flexor motoneurones is NK receptor dependent, with cutaneous C fibre conditioning inputs acting via NK_1 and muscle C fibre conditioning inputs via NK_2 receptor subtypes.

Activation of C fibre afferents induces a prolonged increase in the excitability of spinal neurones, the phenomenon of central sensitization (Woolf, 1983; Woolf & Wall, 1986), which, in combination with peripheral sensitization (Treede, Meyer, Raja & Campbell, 1992), underlies the development of both allodynia (pain, pain-related behaviour or a flexion withdrawal reflex in response to normally innocuous stimuli) and hyperalgesia (enhanced pain, pain-related behaviour or a flexion withdrawal reflex in response to noxious stimuli) following peripheral tissue injury or inflammation. Although both the induction and the maintenance of central sensitization are NMDA (*N*-methyl-D-aspartate) receptor dependent (Woolf & Thompson, 1991; Ma & Woolf, 1995), glutamate is unlikely to be the only transmitter involved. C fibre afferents evoke slow synaptic potentials in spinal neurones (Yoshimura & Jessell, 1989; Thompson, King & Woolf, 1990) which summate at low frequencies, producing a cumulatively increasing postsynaptic depolarization and action potential 'wind-up' (Price, Hull & Buchwald, 1971; Thompson *et al.* 1990), which may be the trigger for a prolonged heterosynaptic facilitation (Thompson, Woolf & Sivilotti, 1993). Although these changes are substantially reduced by NMDA receptor antagonists (Davies & Lodge, 1987; Dickenson & Sullivan, 1987; Thompson *et al.* 1990), the slowest component of the C fibre-evoked synaptic potential is NMDA receptor independent (Thompson, Gerber, Sivilotti & Woolf, 1992) and there are indications that the tachykinins substance P (SP) and neurokinin A (NKA) are involved.

SP and NKA are present in dorsal root ganglion neurones with fine unmyelinated axons (Ogawa, Kanazawa & Kimura, 1985; McNeill, Westlund & Coggeshall, 1989) and are released in response to a variety of noxious stimuli (Linderoth & Brodin, 1988; Duggan, Hendry, Morton, Hutchison & Zhao, 1988; Duggan, Hope, Jarrott, Schaible & Fleetwood-Walker, 1990). The slow synaptic potentials produced by C fibre afferent inputs can be mimicked by SP and NKA agonists and reduced by their antagonists (Urban & Randic, 1984; Nagy, Miller & Woolf, 1994).

When injected intrathecally, both peptides increase the excitability of spinal flexor motoneurones to noxious stimuli, mimicking C fibre conditioning inputs (Xu, Maggi & Wiesenfeld-Hallin, 1991; Xu, Dalsgaard & Wiesenfeld-Hallin, 1992). SP or NKA, and their more selective analogues, also produce decreased behavioural nociceptive thresholds and these effects can be blocked by their antagonists (Picard, Boucher, Regoli, Gitter, Howbert & Couture, 1993).

Three subclasses of tachykinin receptors have been described in the CNS and periphery: the neurokinin receptors NK_1, NK_2 and NK_3 with SP, NKA and neurokinin B as their respective preferred endogenous ligands (for a review see Maggi, Patacchini, Rovero & Giachetti, 1993). In the spinal cord, NK_1 binding sites are distributed throughout the dorsal and ventral horns, especially in laminae I and outer substantia gelatinosa, and NK_3 binding sites are confined to superficial laminae of the dorsal horn (Yashpal, Dam & Quirion, 1990). The distribution of NK_2 receptors is controversial, though, for while autoradiographic studies reveal the existence of NK_2 binding sites in the spinal cord (Yashpal *et al.* 1990), only a low level of mRNA for NK_2 receptors can be detected (Tsuchida, Shigemoto, Yokota & Nakanishi, 1990).

C fibre afferent inputs of either cutaneous or muscle origin can facilitate the flexion reflex elicited by noxious cutaneous test stimuli in the rat (Woolf, 1983; Woolf & Wall, 1986). The induction of the facilitation following electrical stimulation of cutaneous C fibres has an NK_1 receptor-dependent component (Xu *et al.* 1992; Laird, Hargreaves & Hill, 1993), whereas that produced by muscle C fibres is mainly mediated by NK_2 receptors (Xu *et al.* 1991).

Although NMDA receptor antagonists attenuate the cutaneous mechanical reflex allodynia induced in rats either by C fibre conditioning inputs (Ma & Woolf, 1995) or by intrathecal injections of the glycine antagonist strychnine and the GABA antagonist bicuculline (Yaksh, 1989), nothing is known about the role of neurokinin receptors in this particular manifestation of central sensitization. We have examined, therefore, whether NK_1 and NK_2 receptors are involved in the induction and the maintenance of cutaneous mechanical allodynia in flexor motoneurones.

Preliminary results have been published (Ma, Sivilotti, Nagy & Woolf, 1993).

METHODS

Animal preparation

Experiments were performed on adult male Sprague–Dawley rats (200–300 g). Under halothane anaesthesia a carotid artery and the trachea were cannulated. Anaesthesia was then maintained with small doses of Saffan (alphaxalone–alphadolone; Pitman-Moore, Uxbridge, Middlesex, UK). The rats were decerebrated by aspiration of all the cranial contents rostral to the mesencephalon and spinalized via a laminectomy at T3–T5. Immediately prior to the spinal transection an intrathecal catheter (PE10) was implanted with its tip at the lumbar spinal cord, segment L4–L5. The anaesthetic was then discontinued, and the animals paralysed with Flaxedil (gallamine; May & Baker, Dagenham, Essex, UK) and artificially ventilated. Rectal temperature (35·0–36·0 °C) was maintained with a heating pad. The expiratory CO_2 level was continuously monitored and maintained at 3·5–4%. The ECG and heart rate were monitored. Experiments were terminated on those very rare occasions when the heart rate, ECG waveform, rectal temperature and expiratory CO_2 level deviated more than transiently from normal values.

Extracellular recordings of flexor α-motoneurone activity were made as previously described in detail (Woolf & Wall, 1986). Briefly, the nerve to the posterior biceps femoris–semitendinosus muscles (PBF–ST) on one side was exposed in the popliteal fossa, a fine filament dissected free, cut and its proximal end placed on a silver recording electrode. The sural nerve was dissected free and placed in continuity on a pair of silver stimulating electrodes.

Chemicals

Substance P methyl ester ([Met-OMe^{11}]-substance P, SPME) and NKA (Sigma) were dissolved in normal saline. The non-peptide NK_1 receptor antagonist 2-[1-imino-2-(2-methoxy phenyl) ethyl]-7,7 diphenyl-4 perhydroisoindolone-(3aR,7aR) (RP 67580; Rhône-Poulenc Rorer, Vitry-sur-Seine, France) and its inactive enantiomer 2-[1-imino-2-(2-methoxy phenyl) ethyl]-7,7 diphenyl-4 perhydroisoindolone-(3aS,7aS) (RP 68651; Rhône-Poulenc Rorer) were dissolved in 0·11% HCl diluted in normal saline. The NK_2 receptor antagonist [Tyr^5,D-Trp^{6,8,9},Lys^{10}]-NKA (fragment 4–10) (MEN 10376; Menarini Pharmaceuticals, Florence, Italy) was dissolved in 0·35% dimethyl sulphoxide (DMSO; Sigma) diluted in normal saline. Bradykinin (Sigma) was dissolved in Tyrode solution. Allyl-isothiocyanate (mustard oil; Bush Boake Allen, London, UK) was diluted (5%) in mineral oil.

Experimental procedures

Experiments were started 1 h after spinalization, to allow for recovery from the anaesthetic and stabilization of the excitability of the preparation. Spikes were counted with a pulse integrator, and the spike shape monitored continuously on an analog-delay line. To determine the excitability of the flexion reflex, as described previously (Sivilotti & Woolf, 1994), measurements were made of the following. (1) Spontaneous activity (for 10 s). (2) The mechanical threshold of the flexion reflex (tested with nylon monofilament von Frey hairs of 8·0, 18·6, 46·1, 71·5, 82·3, 91·1, 228·3, 573·3 and 705·6 mN). The value of the lowest force von Frey hair, which evoked a consistent discharge on each occasion when applied three times to the plantar surface of any one of the middle three toes, was taken as the cutaneous mechanical threshold of the flexion reflex. (3) The total discharge elicited by standard touch stimuli (8 light touches applied with the flat surface of the experimenter's thumb 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 every 4 s (Sivilotti & Woolf, 1994; Ma & Woolf, 1995). The test protocol was applied at 5 min intervals during the course of the experiment.

Prior to any conditioning stimulus or drug administration, baseline values were obtained by repeating the protocol at 5 min intervals for 30 min. Three types of conditioning stimuli were used: (1) electrical stimuli at a strength sufficient to activate C fibres (5 mA, 0·5 ms, 1 Hz) applied to the sural nerve for 20 s; (2) mustard oil applied to an approximately 4 mm^2 patch of skin

on the dorsum of the foot; and (3) bradykinin (60 μM, 80 μl) injected into the gastrocnemius–soleus muscle. All neurokinin agonists, antagonists and vehicles were injected intrathecally in a volume of 10 μl, followed by 10 μl of normal saline to flush the catheter. The antagonists or their vehicles were administered 15 min prior to the conditioning stimuli or the agonists (Xu *et al.* 1991; Laird *et al.* 1993). Only one antagonist was tested in each animal.

Data analysis

The touch-evoked response was analysed as the total number of spikes evoked during 32 s by eight touches, minus the extrapolated spontaneous activity in the same period. Statistical analysis was performed both on the peak changes in threshold or spike discharge using Student's unpaired t tests (if S.D.s were not equal, then Welch's t test was used) or on normalized data expressed as percentages of the baseline calculated from the three readings prior to any conditioning stimulus. In the latter case the differences between groups were tested with one-way ANOVA, followed by the least significant difference test for multiple comparisons. All data are illustrated as means ± S.E.M. and $P < 0.05$ was considered significant.

RESULTS

In the decerebrate–spinal rat preparation without conditioning stimuli, the threshold and the very small touch-evoked response were relatively stable (< 15% variation for the threshold, < 30% for the touch-evoked response) over an 80 min recording period ($n = 5$). Based on published data, two doses of the NK_1 antagonist RP 67580 or the NK_2 antagonist MEN 10376 were examined to find a dose which effectively and selectively antagonized agonist activity (see below), but did not affect the basal flexion reflex. At 2·28 nmol (Chapman & Dickenson, 1993), RP 67580 had no detectable effect on the mechanical threshold and the touch-evoked response but at 22·8 nmol it blocked the flexion reflex responsiveness over 40 min ($n = 2$). The enantiomer, RP 68651 (2·28 nmol) did not affect the baseline reflex. Intrathecal injection of 0·7 nmol MEN 10376 (Xu *et al.* 1991; Maggi *et al.* 1993) had no effect on the basal threshold or touch-evoked response. Of three animals injected with 2·1 nmol

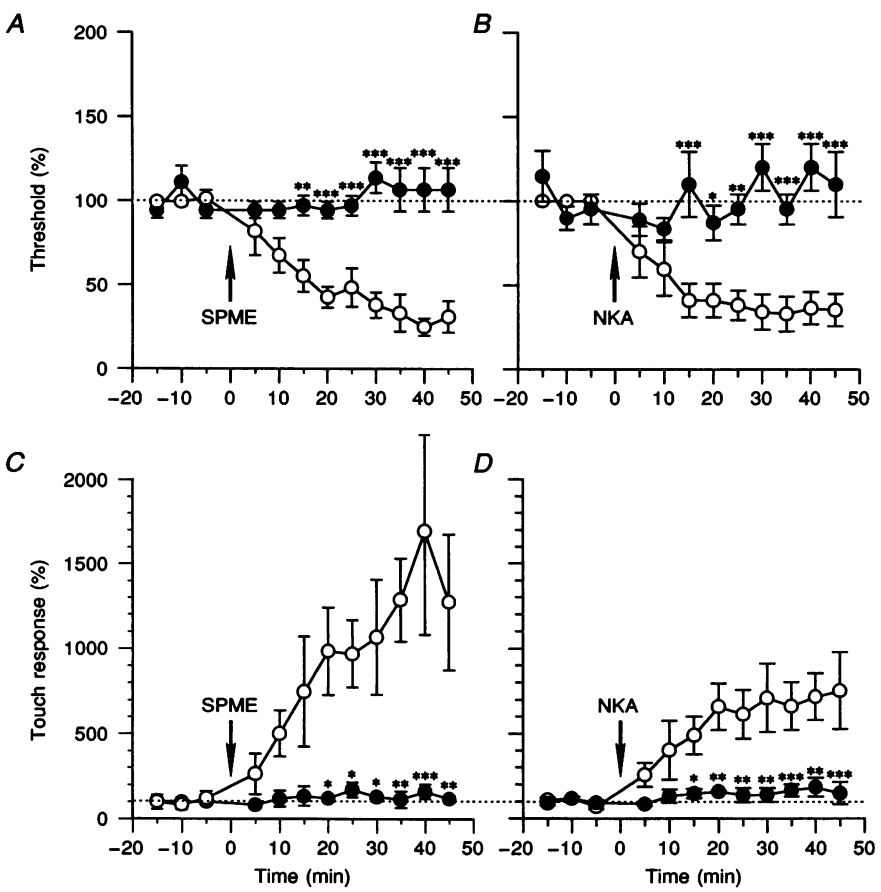

Figure 1. Effects on cutaneous mechanical threshold and touch-evoked response of flexor motoneurones following the intrathecal injection of substance P methyl ester or neurokinin A

Decrease in the cutaneous mechanical threshold and enhancement of the touch-evoked response of flexor motoneurones following the I.T. injection of 20 pmol substance P methyl ester (SPME) (*A* and *C*) or 20 pmol neurokinin A (NKA; *B* and *D*), and the effects of NK_1 and NK_2 receptor antagonists on these changes. RP 67580 (2·28 nmol; *A* and *C*) and MEN 10376 (0·7 nmol; *B* and *D*) were injected I.T. 15 min before SPME or NKA. ○, vehicle; ●, drug. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared with the vehicle control.

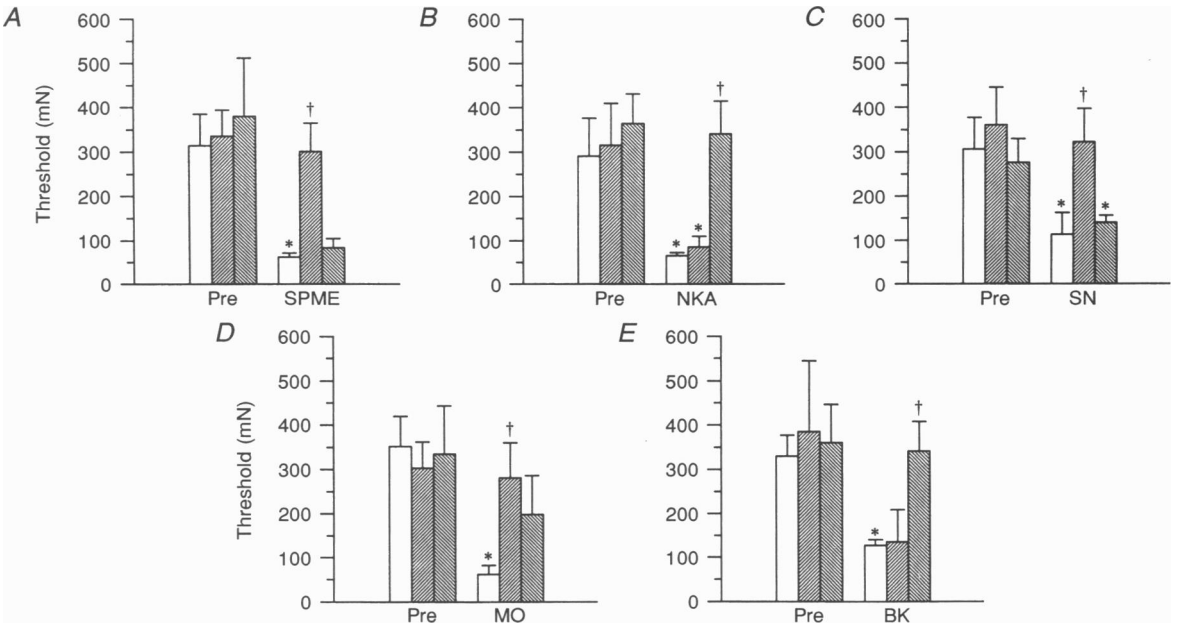

Figure 2. Effects of SPME, NKA, electrical stimulus to sural nerve, mustard oil or bradykinin on cutaneous mechanical thresholds of flexor motoneurones

Comparison of pre- (Pre) and post-treatment cutaneous mechanical thresholds of flexor motoneurones indicating the effect of the following treatments: 20 pmol SPME I.T. (*A*), 20 pmol NKA I.T (*B*), electrical stimulation of the sural nerve (SN) at C fibre strength (5 mA, 0·5 ms and 1 Hz for 20 s; *C*), cutaneous application of mustard oil (*D*) or intramuscular injection of bradykinin (BK, 60 μM, 80 μl; *E*). In each case the effects of the NK_1 receptor antagonist RP 67580 (2·28 nmol, ▨) or the NK_2 antagonist MEN 10376 (0·7 nmol, ▧) on these changes are shown. In *A*, *B* and *C*, □ represents vehicle. In *C* and *D*, 2·28 nmol RP 68651, the inactive enantiomer of RP 67580, was used as control (□) instead of vehicle. * $P < 0.05$, compared with pretreatment; † $P < 0.05$, compared with vehicle or enantiomer control.

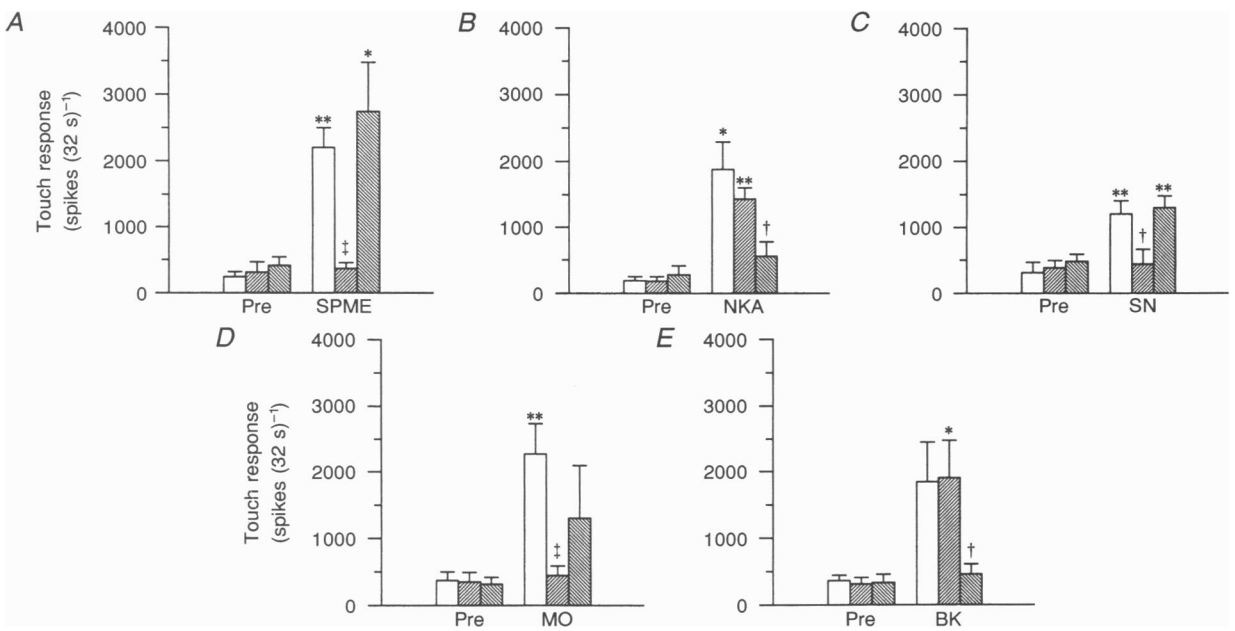

Figure 3. Effects of SPME, NKA, electrical stimulus to sural nerve, mustard oil or bradykinin on touch-evoked responses of flexor motoneurones

Peak pre- (Pre) and post-treatment touch-evoked responses of flexor motoneurones indicating the effect of the treatments as detailed in Fig. 2 legend. * $P < 0.05$, ** $P < 0.01$, compared with the pretreatment; † $P < 0.05$, ‡ $P < 0.01$, compared with the vehicle or the enantiomer control. ▨, RP 67580; ▧, MEN 10376; □, control.

MEN 10376, there was no effect on the baseline reflex in two but a marked reduction in responsiveness in one. No change in body temperature, expiratory CO_2 level, heart rate or ECG waveform was detected following intrathecal injection of RP 67580, RP 68651 or MEN 10376 at any of the dose levels.

Effects of intrathecal SPME on the flexion reflex

The mechanical threshold and the touch-evoked response were marginally affected by a 7 pmol intrathecal injection of SPME in two rats. Intrathecal injection of 20 pmol SPME decreased the cutaneous mechanical threshold of the flexion reflex by > 60% (Figs 1*A* and 2*A*), an effect that lasted over 1 h ($n = 5$). The touch-evoked response increased 8-fold following injection of 20 pmol SPME (Figs 1*C* and 3*A*). Intrathecal injection of 100 pmol SPME caused a more than 10-fold increase in the spontaneous activity of the spinal flexor motoneurones ($n = 3$).

Pretreatment with RP 67580 (2·28 nmol, I.T., $n = 4$) prevented the threshold decrease (Figs 1*A* and 2*A*) and the enhancement of the touch-evoked response produced by 20 pmol SPME (Figs 1*C* and 3*A*), while pretreatment with MEN 10376 (0·7 nmol, I.T., $n = 4$) had no significant effect (Figs 2*A* and 3*A*).

Effects of intrathecal NKA on the flexion reflex

NKA at a dose of 7 pmol had a marginal effect on the threshold and the touch-evoked response of the spinal flexor motoneurones ($n = 2$), but 20 pmol ($n = 4$) produced a prolonged decrease in threshold (Figs 1*B* and 2*B*) and an enhancement of the touch-evoked response (Figs 1*D* and 3*B*). NKA at 100 pmol produced a more than 10-fold increase in spontaneous activity ($n = 2$).

Pretreatment with MEN 10376 (0·7 nmol, I.T., $n = 4$) prevented both the threshold decrease (Figs 1*B* and 2*B*) and the enhancement of the touch-evoked response (Figs 1*D*

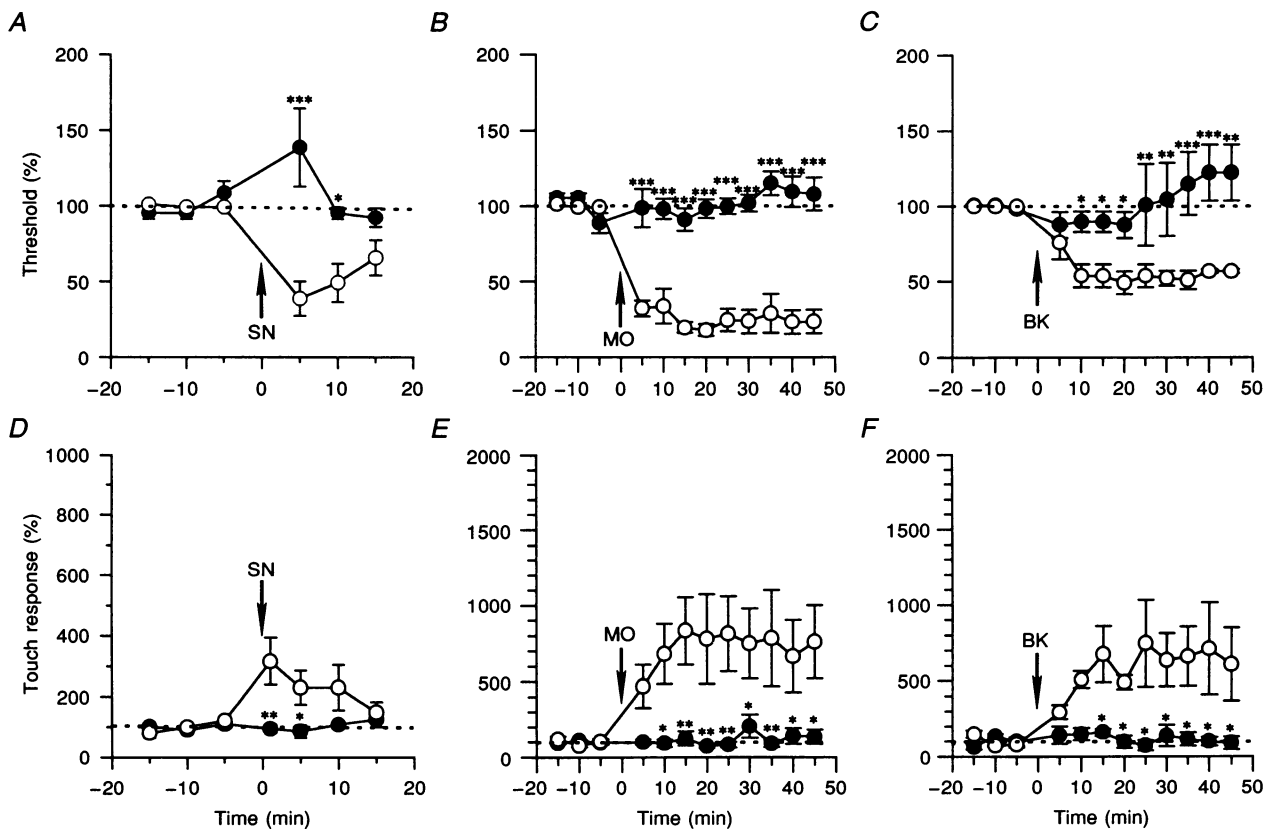

Figure 4. Effects of electrical stimulus to sural nerve, mustard oil or bradykinin on cutaneous mechanical threshold and touch-evoked response of flexor motoneurones

Decrease in the cutaneous mechanical threshold and facilitation of the touch-evoked response of flexor motoneurones following: electrical stimulation of the sural nerve (SN) at C fibre strength (5 mA, 0·5 ms and 1 Hz for 20 s; *A* and *D*), cutaneous application of mustard oil (*B* and *E*) or intramuscular injection of bradykinin (BK, 60 μM, 80 μl; *C* and *F*), and the effects of the NK_1 receptor antagonist RP 67580 (2·28 nmol; *A*, *B*, *D* and *E*) or the NK_2 antagonist MEN 10376 (0·7 nmol; *C* and *F*) on these changes. RP 67580, RP 68651, MEN 10376 and their vehicle were injected I.T. 15 min before the conditioning stimuli. In *A*, *B*, *D* and *E*, ● represents RP 67580 and ○, RP 68651; in *C* and *F*, ● represents MEN 10376 and ○, vehicle control; * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$, compared with the enantiomer or vehicle controls, as appropriate.

and 3*B*) produced by 20 pmol NKA but pretreatment with RP 67580 (2·28 nmol, I.T., $n = 4$) did not have this effect (Figs 2*B* and 3*B*).

Effects of RP 67580 and MEN 10376 on the facilitation of the flexion reflex elicited by sural nerve conditioning stimulation

A sural nerve C fibre strength conditioning stimulus (CS) (1 Hz, 20 s) decreased the cutaneous mechanical threshold of the flexion reflex and markedly enhanced the touch-evoked response ($n = 9$) for 10–15 min. Pretreatment with the NK_1 receptor antagonist RP 67580 (2·28 nmol, I.T., $n = 5$) completely prevented these changes (Figs 2*C*, 3*C*, 4*A* and *D*), whereas its inactive enantiomer RP 68651 (2·28 nmol, I.T., $n = 6$) had no significant effect (Figs 2*C*, 3*C*, 4*A* and *D*). Intrathecal injection of the vehicle for RP 67580 also had no effect on the sural CS-induced facilitation of the flexion reflex.

Pretreatment with intrathecal MEN 10376 (0·7 nmol, I.T., $n = 4$) did not prevent the threshold decrease following the sural nerve CS (Fig. 2*C*), and only slightly attenuated the effects of the sural nerve CS on the touch-evoked response (Fig. 3*C*).

Effects of RP 67580 and MEN 10376 on the facilitation of the flexion reflex elicited by mustard oil

Cutaneous application of mustard oil produced a prolonged period of facilitation of the flexion reflex, lasting over 1 h. Pretreatment with 2·28 nmol RP 67580 ($n = 5$) attenuated both the threshold decrease (Figs 2*D* and 4*B*) and the enhancement of the touch-evoked response (Figs 3*D* and 4*E*), while its inactive enantiomer RP 68651 (2·28 nmol, I.T., $n = 5$) had no such effect (Figs 2*D*, 3*D*, 4*B* and *E*). Pretreatment with MEN 10376 (0·7 nmol, I.T., $n = 4$) did not prevent the mustard oil from producing a threshold decrease (Fig. 2*D*) or an enhancement of the touch-evoked response (Fig. 3*D*). Vehicle injections were without effect.

When RP 67580 (2·28 nmol, I.T., $n = 5$) was given 25 min after the cutaneous application of mustard oil, when the facilitation of the flexion reflex was fully developed, neither

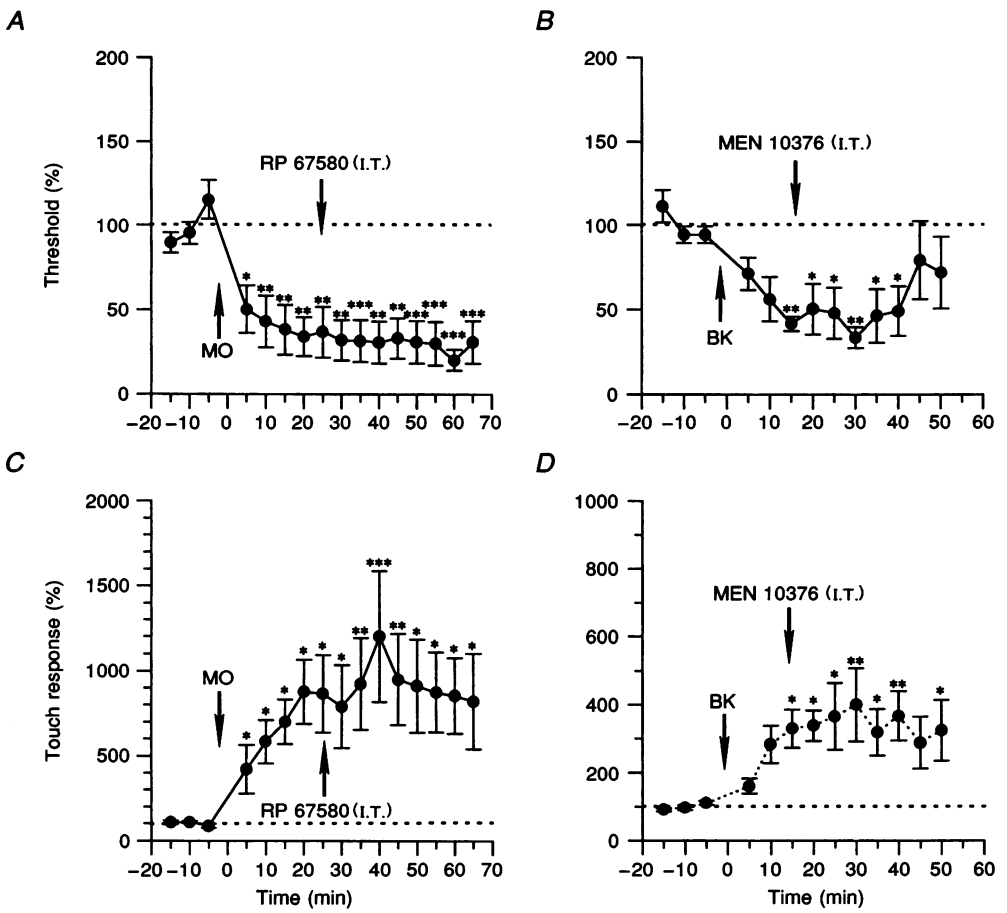

Figure 5. Effects of RP 67580 and MEN 10376 on the maintenance of the facilitation of flexor motoneurones following mustard oil or bradykinin

The effects of RP 67580 (2·28 nmol) and MEN 10376 (0·7 nmol) on the maintenance of the facilitation of flexor motoneurones following the cutaneous application of mustard oil (MO) and intramuscular bradykinin (BK), respectively. RP 67580 and MEN 10376 were injected I.T. when the facilitation had fully developed. * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$, compared with the three baseline values.

the threshold decrease nor the enhanced touch-evoked response was reversed (Fig. 5*A* and *C*).

Effects of RP 67580 and MEN 10376 on the facilitation of flexion reflex elicited by intramuscular bradykinin

Injection of 80 μl of 60 μM bradykinin into the gastrocnemius–soleus muscle ($n = 5$) produced a threshold decrease (Figs 2*E* and 4*C*) and an enhancement of the touch-evoked response (Figs 3*E* and 4*F*), which lasted 40–50 min. Pretreatment with intrathecal 2·28 nmol RP 67580 ($n = 5$) did not significantly affect these changes (Figs 2*E* and 3*E*) but pretreatment with MEN 10376 (0·7 nmol, I.T., $n = 4$) completely prevented the intramuscular bradykinin from producing a threshold decrease (Figs 2*E* and 4*C*) or an enhancement of the touch-evoked response (Figs 3*E* and 4*F*).

When 0·7 nmol ($n = 5$) MEN 10376 was injected 15 min after the intramuscular injection of bradykinin, neither the established decreased threshold nor the enhanced touch-evoked response was reversed (Fig. 5*B* and *D*).

DISCUSSION

Although allodynia has long been recognized in humans to constitute a part of the postinjury pain hypersensitivity syndrome, most animal studies on changes in sensitivity in the somatosensory system have targeted hyperalgesia, possibly because of a lack of suitable models for monitoring altered responses to innocuous inputs. Nociceptive reactions or escape behaviours in response to innocuous stimuli have been induced, however, in rats by intrathecal injections of high doses of morphine (Woolf, 1981), the glycine antagonist strychnine or the GABA antagonist bicuculline (Yaksh, 1989). Cutaneous C fibre afferents are also able to induce a substantial reduction of nociceptive thresholds and an enhancement of the normally absent touch-evoked response of spinal flexor motoneurones (Sivilotti & Woolf, 1994) as well as producing Aβ fibre-mediated pain in humans (Torebjörk, Lundberg & LaMotte, 1992). Intramuscular bradykinin, which activates fine afferents (Mense & Meyer, 1985), can also now be seen to produce cutaneous mechanical reflex allodynia in the rat. Change of the high threshold flexion withdrawal reflex to a low threshold reflex, with an accompanying marked enhancement of touch-evoked responses, while similar to such alterations in dorsal horn neurones or sensation in humans, should not be considered to be equivalent, as the mechanisms involved may not be identical.

Although SP has long been proposed as a primary afferent neurotransmitter for nociception, recent studies indicate that it is more likely to be involved in mediating gain control of nociceptive transmission. SP antagonists, for example, do not block the direct postsynaptic action potential response to transient high-threshold afferent inputs (Chapman & Dickenson, 1993), nor do they alter the baseline reflex response to noxious or innocuous stimuli. Attenuation of the afferent C fibre-evoked slow synaptic potentials by NK antagonists (Nagy *et al.* 1994) may be expected, though, to prevent central sensitization, if the summation of the slow potentials is the key initiating trigger for its induction (Thompson *et al.* 1993). Compatible with this is the finding that NK_1 receptors participate in increasing the excitability of spinal flexor motoneurones to noxious electrical test stimuli following electrical cutaneous C afferent fibre conditioning stimuli (an indirect measure of reflex hyperalgesia) (Xu *et al.* 1992). The present finding that the NK_1 antagonist RP 67580 prevented both the threshold decrease and the enhancement of the touch-evoked response following cutaneous C fibre conditioning suggests that the induction of mechanical allodynia in flexor motoneurones by cutaneous afferents is also NK_1 receptor dependent. This implies that similar mechanisms operate to produce flexor reflex allodynia and hyperalgesia. Although RP 67580 and its enantiomer RP 68651 have similar calcium channel-blocking effects at high doses (but RP 68651 does not bind to the NK_1 receptor) (Ruspniak *et al.* 1993), our results show that RP 67580 is acting as an NK_1 receptor antagonist.

Our results, showing that NK_2 receptors are involved in the induction of cutaneous mechanical allodynia by muscle but not skin C fibre conditioning inputs, are in agreement with the finding that NK_2 but not NK_1 receptor antagonism can prevent the electrical activation of muscle C afferents from producing reflex hypersensitivity to intense electrical test cutaneous inputs (Xu *et al.* 1991). It is not clear what underlies the different effects of NK_1 and NK_2 receptor antagonists on cutaneous and muscle C fibre-evoked facilitations. Besides a different distribution of NK_1 and NK_2 receptors (Yashpal *et al.* 1990), a differential release of SP and NKA following noxious mechanical and thermal stimuli has also been demonstrated (Duggan *et al.* 1988, 1990). SP release is short-lasting, relatively focal and usually limited to the site of termination of unmyelinated primary afferents, whereas release of NKA persists and is detectable throughout the dorsal horn. There is also a marked difference in the site of termination of cutaneous and muscle C fibres. Cutaneous C fibres terminate principally in laminae I and II (Molander & Grant, 1986) while muscle C fibres terminate in laminae I and V, but not II (Molander & Grant, 1987).

While a role for tachykinins seems clear for the induction of central sensitization, its maintenance does not depend upon NK_1 or NK_2 receptor activation. This is in contrast with the NMDA receptor which is involved in both the induction and the maintenance of hyperalgesic and allodynic reflex hypersensitivity (Woolf & Thompson, 1991; Ma & Woolf, 1995). For inflammatory hypersensitivity, the situation is similar: the NMDA antagonist MK-801 attenuates the hyperaesthesia induced by carrageenan, but the NK_1 antagonist CP-96345 has no effect (Yamamoto, Shimoyama

& Mizuguchi, 1993). Neurokinins increase the inward calcium current induced by the activation of NMDA receptors (Rusin, Ryu & Randic, 1992) and enhance the release of glutamate and aspartate (Kangrga & Randic, 1990). These processes, together with the contribution to slow synaptic potentials, may underlie the induction of an abnormal state of hyperexcitability after C afferent inputs. Persistent changes in excitability, in contrast, appear not be contingent on an on-going release of tachykinins.

Chapman, V. & Dickenson, A. H. (1993). The effect of intrathecal administration of RP 67580, a potent neurokinin 1 antagonist on nociceptive transmission in the rat spinal cord. *Neuroscience Letters* **157**, 149–152.

Davies, S. N. & Lodge, D. (1987). Evidence for involvement of *N*-methylaspartate receptors in 'wind-up' of class 2 neurones in the dorsal horn of the rat. *Brain Research* **424**, 402–406.

Dickenson, A. H. & Sullivan, A. F. (1987). Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurones following C fibre stimulation. *Neuropharmacology* **26**, 1235–1238.

Duggan, A. W., Hendry, I. A., Morton, C. R., Hutchison, W. D. & Zhao, Z. Q. (1988). Cutaneous stimuli releasing immunoreactive substance P in the dorsal horn of the cat. *Brain Research* **451**, 261–273.

Duggan, A. W., Hope, P. J., Jarrott, B., Schaible, H.-G. & Fleetwood-Walker, S. M. (1990). Release, spread and persistence of immunoreactive neurokinin A in the dorsal horn of the cat following noxious cutaneous stimulation. Studies with antibody microprobes. *Neuroscience* **35**, 195–202.

Kangrga, I. & Randic, M. (1990). Tachykinins and calcitonin gene-related peptide enhance release of endogenous glutamate and aspartate from the rat spinal dorsal horn slice. *Journal of Neuroscience* **10**, 2026–2038.

Laird, J. M. A., Hargreaves, R. J. & Hill, R. G. (1993). Effects of RP 67580, a non-peptide neurokinin 1 receptor antagonist, on facilitation of a nociceptive spinal flexion reflex in the rat. *British Journal of Pharmacology* **109**, 713–718.

Linderoth, B. & Brodin, E. (1988). Tachykinin release from rat spinal cord in vitro and in vivo in response to various stimuli. *Regulatory Peptides* **21**, 129–140.

Ma, Q.-P., Sivilotti, L. G., Nagy, I. & Woolf, C. J. (1993). Tachykinin receptor involvement in C-fiber evoked slow synaptic potentials in vitro and central sensitization in vivo. *Society for Neuroscience Abstracts* **19**, 522.

Ma, Q.-P. & Woolf, C. J. (1995). Touch-evoked hypersensitivity of the flexion withdrawal reflex induced by noxious stimuli is mediated by *N*-methyl-D-aspartate receptors – implications for the treatment of mechanical allodynia. *Pain* **61**, 383–390.

McNeill, D. L., Westlund, K. N. & Coggeshall, R. E. (1989). Peptide immunoreactivity of unmyelinated primary afferent axons in rat lumbar dorsal roots. *Journal of Histochemistry and Cytochemistry* **37**, 1047–1052.

Maggi, C. A., Patacchini, R., Rovero, P. & Giachetti, A. (1993). Tachykinin receptors and tachykinin receptor antagonists. *Journal of Autonomic Pharmacology* **13**, 23–93.

Mense, S. & Meyer, H. (1985). Different types of slowly conducting afferent units in cat skeletal muscle and tendon. *Journal of Physiology* **363**, 403–417.

Molander, C. & Grant, G. (1986). Laminar distribution and somatotopic organization of primary afferent fibers from hindlimb nerves in the dorsal horn. A study by transganglionic transport of horseradish peroxidase in the rat. *Neuroscience* **19**, 297–312.

Molander, C. & Grant, G. (1987). Spinal cord projections from hindlimb muscle nerves in the rat studied by transganglionic transport of horseradish peroxidase, wheat germ agglutinin conjugated horseradish peroxidase, or horseradish peroxidase with dimethylsulfoxide. *Journal of Comparative Neurology* **260**, 246–255.

Nagy, I., Miller, B. A. & Woolf, C. J. (1994). NK1 and NK2 receptors contribute to C-fibre evoked slow potentials in the rat spinal cord. *NeuroReport* **5**, 2105–2108.

Ogawa, T., Kanazawa, I. & Kimura, S. (1985). Regional distribution of substance P, neurokinin A and neurokinin B in the rat spinal cord, nerve roots and dorsal root ganglia and the effects of dorsal root section or spinal transection. *Brain Research* **359**, 151–157.

Picard, P., Boucher, S., Regoli, D., Gitter, B. D., Howbert, J. J. & Couture, R. (1993). Use of non-peptide tachykinin receptor antagonists to substantiate the involvement of NK_1 and NK_2 receptors in a spinal nociceptive reflex in the rat. *European Journal of Pharmacology* **232**, 255–261.

Price, D. D., Hull, C. D. & Buchwald, N. A. (1971). Intracellular responses of dorsal horn cells to cutaneous and sural nerve A and C fibre stimulation. *Experimental Neurology* **33**, 291–309.

Rusin, K. I., Ryu, P. D. & Randic, M. (1992). Modulation of excitatory amino acid responses in rat dorsal horn neurons by tachykinins. *Journal of Neurophysiology* **68**, 265–286.

Ruspniak, N. M. J., Boyce, S., Williams, A. R., Cook, G., Longmore, J., Seabrook, G. R., Caeser, M., Iversen, S. D. & Hill, R. G. (1993). Antinociceptive activity of NK1 receptor antagonists: non-specific effects of racemic RP 67580. *British Journal of Pharmacology* **110**, 1607–1613.

Sivilotti, L. G. & Woolf, C. J. (1994). The contribution of $GABA_A$ and glycine receptors to central sensitization: disinhibition and touch-evoked allodynia in the spinal cord. *Journal of Neurophysiology* **72**, 169–179.

Thompson, S. W. N., Gerber, G., Sivilotti, L. G. & Woolf, C. J. (1992). Long duration ventral root potentials in the neonatal rat spinal cord in vitro; the effects of ionotropic and metabotropic excitatory amino acid receptor antagonists. *Brain Research* **595**, 87–97.

Thompson, S. W. N., King, A. E. & Woolf, C. J. (1990). Activity-dependent changes in rat ventral horn neurones in vitro: summation of prolonged afferent evoked postsynaptic depolarizations produce a D-APV sensitive windup. *European Journal of Neuroscience* **2**, 638–649.

Thompson, S. W. N., Woolf, C. J. & Sivilotti, L. G. (1993). Small caliber afferents produce a heterosynaptic facilitation of the synaptic responses evoked by primary afferent A fibres in the neonatal rat spinal cord in vitro. *Journal of Neurophysiology* **69**, 2116–2128.

Torebjörk, H. E., Lundberg, L. E. R. & LaMotte, R. H. (1992). Central changes in processing of mechanoreceptive input in capsaicin-induced secondary hyperalgesia in humans. *Journal of Physiology* **448**, 765–780.

Treede, R.-D., Meyer, R. A., Raja, S. N. & Campbell, J. N. (1992). Peripheral and central mechanisms of cutaneous hyperalgesia. *Progress in Neurobiology* **38**, 397–421.

Tsuchida, K., Shigemoto, R., Yokota, Y. & Nakanishi, S. (1990). Tissue distribution and quantification of the mRNA for three rat tachykinin receptors. *European Journal of Biochemistry* **193**, 751–757.

URBAN, L. & RANDIC, M. (1984). Slow excitatory transmission in rat dorsal horn: possible mediation by peptides. *Brain Research* **290**, 336–341.

WOOLF, C. J. (1981). Intrathecal high dose morphine produces hyperalgesia in the rat. *Brain Research* **209**, 491–495.

WOOLF, C. J. (1983). Evidence for a central component of post-injury pain hypersensitivity. *Nature* **306**, 686–688.

WOOLF, C. J. & THOMPSON, S. W. N. (1991). The induction and maintenance of central sensitization is dependent on *N*-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states. *Pain* **44**, 293–299.

WOOLF, C. J. & WALL, P. D. (1986). The relative effectiveness of C primary afferent fibres of different origins in evoking a prolonged facilitation of the flexor reflex in the rat. *Journal of Neuroscience* **6**, 1433–1443.

XU, X.-J., DALSGAARD, C.-J. & WIESENFELD-HALLIN, Z. (1992). Intrathecal CP-96,345 blocks reflex facilitation induced in rats by substance P and C-fiber-conditioning stimulation. *European Journal of Pharmacology* **216**, 337–344.

XU, X.-J., MAGGI, C. M. & WIESENFELD-HALLIN, Z. (1991). On the role of NK_2 tachykinin receptors in the mediation of spinal reflex excitability in the rat. *Neuroscience* **44**, 483–490.

YAKSH, T. L. (1989). Behavioural and autonomic correlates of the tactile evoked allodynia produced by spinal glycine inhibition: effect of modulatory receptor systems and excitatory amino acid antagonists. *Pain* **37**, 111–123.

YAMAMOTO, T., SHIMOYAMA, N. & MIZUGUCHI, T. (1993). The effects of morphine, MK-801, an NMDA antagonist, and CP-96345, an NK1 antagonist, on the hyperesthesia evoked by carrageenan injection in the rat paw. *Anesthesiology* **78**, 124–133.

YASHPAL, K., DAM, T. C. & QUIRION, R. (1990). Quantitative autoradiographic distribution of multiple neurokinin binding sites in rat spinal cord. *Brain Research* **506**, 259–266.

YOSHIMURA, M. & JESSELL, T. M. (1989). Primary afferent evoked synaptic responses and slow potential generation in rat substantia gelatinosa neurons in vitro. *Journal of Neurophysiology* **62**, 96–108.

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