Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 507 (2005) 29–34

www.elsevier.com/locate/ejphar

A tachykinin NK_1 receptor antagonist attenuates the 4β-phorbol-12-myristate-13-acetate-induced nociceptive behaviour in the rat

Megumi Tsuchiya*, Ayano Sakakibara, Mitsuko Yamamoto

Pfizer Global Research and Development, Nagoya Laboratories, Pfizer Japan Inc., 5-2 Takeyoto, Aichi 470-2393, Japan

Received 29 July 2004; received in revised form 12 November 2004; accepted 15 November 2004
Available online 8 December 2004

Abstract

Antinociceptive effect of a tachykinin NK_1 receptor antagonist ezlopitant [(2*S*,3*S*-*cis*)-2-(diphenylmethyl)-*N*-{(2-methoxy, 5-isopropylphenyl)methyl}-1-azabicyclo[2.2.2]octan-3-amine] was investigated in the 4β-phorbol-12-myristate-13-acetate (PMA)-induced nociceptive test in the rat. Intraplantar injection of PMA-induced paw-licking and flinching behaviour lasted up to 120 min and was accompanied by inflammatory reactions, such as swelling and invasion of granulocytes. Pretreatment with resiniferatoxin [200 μg/kg, subcutaneous (s.c.)] blocked the PMA-induced nociceptive behaviour, suggesting that vanilloid VR_1 receptor-expressing primary sensory neurons play a major role in this response. Subcutaneous pretreatment with ezlopitant (0.3–30 mg/kg) and morphine (0.3–6 mg/kg) caused a dose-dependent inhibition of the behaviour. Ezlopitant (3–30 mg/kg) given subcutaneously after PMA injection also significantly attenuated the behavioural response. When administered intrathecally, ezlopitant and a nonselective glutamate receptor antagonist MK-801 had an inhibitory effect, whereas CJ-12,191, an inactive isomer of ezlopitant, was unaffected. These results suggest that spinal tachykinin NK_1 receptors contribute to processing of ongoing pain associated with peripheral inflammation.

Keywords: Antinociception; Tachykinin NK_1 receptor antagonist; Substance P; Spinal cord; Peripheral inflammation; 4β-Phorbol-12-myristate-13-acetate

1. Introduction

Substance P has been considered to act as an important neurotransmitter implicated in the transmission of nociception. Substance P is contained in small diameter primary sensory fibers and is released into the dorsal horn of the spinal cord following noxious stimulation (Duggan and Hendry, 1986; Tiseo et al., 1990; Ribeiro-da-Silva and Hökfelt, 2000). Substance P preferably binds to and activates tachykinin NK_1 receptor, which is concentrated in laminae I of the spinal dorsal horn in a variety of species including human, monkey, and rat (Ding et al., 1999; Yu et al., 1999; Nakaya et al., 1994; Todd et al., 2002).

Selective tachykinin NK_1 receptor antagonists have been shown to be active in the second phase of the formalin test, used as a model of inflammatory pain (Chapman and Dickenson, 1993; Gonzales et al., 2000; Henry et al., 1999; Rupniak et al., 1996; Traub, 1996; Yamamoto and Yaksh, 1991). In this test, intraplantar injection of formalin solution elicits nociceptive behaviour, such as paw licking and flinching, accompanied by neuronal excitation in the dorsal horn of the spinal cord. It was reported that pretreatment with PD 156982, a tachykinin NK_1 receptor antagonist that poorly penetrates into the central nervous system, had no effect on the nociceptive response (Gonzales et al., 2000). In addition to this, an intrathecal injection of a tachykinin NK_1 receptor antagonists CP-96,345 was reported to suppress the second phase response, but an injection of CP-96,344, an inactive isomer of CP-96,345, had no effect (Henry et al., 1999; Yamamoto and Yaksh, 1991). Thus, it is likely that

* Corresponding author. Tel.: +81 569 74 4539; fax +81 569 74 4656.
E-mail address: megumi.tsuchiya@pfizer.com (M. Tsuchiya).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.11.028

the primary site of analgesic action of tachykinin NK_1 receptor antagonists is in the spinal cord.

Taniguchi et al. (1997) reported that an intraplantar injection of 4β-phorbol-12-myristate-13-acetate (PMA), a protein kinase C (PKC) activator, induces paw licking, and flinching behaviour lasted over 45 min in rats. Souza et al. (2002) also showed that, in mice, a PKC activator phorbol-12,13-didecanoate causes long-lasting paw-licking behaviour (∼120 min), which is completely blocked by coinjection of a PKC inhibitor. Mechanisms for this PKC-dependent nociceptive response were discussed and found to be, at least in part, the development of peripheral inflammation. Based on these previous reports, there appears to be similarities between PMA and formalin tests. Both of them cause spontaneous nociceptive behaviour associated with peripheral inflammation. However, the duration of nociceptive response to PMA is longer than that of the second phase of the formalin test, suggesting that different mechanisms may underlie in these two tests.

Current studies were designed to characterize the PMA-induced response as a novel model of inflammatory pain and examine antinociceptive effects of a tachykinin NK_1 receptor antagonist, ezlopitant, a quinuclidine-based compound with subnanomolar affinities to the human-type tachykinin NK_1 receptors and can penetrate into the central nervous system (Tsuchiya et al., 2002).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (150–200 g, Charles River, Yokohama, Japan) were housed on a 12/12-h light/dark cycle with constant temperature (23±1 °C) and relative humidity (55±15%), given food and water ad libitum. The experiments were carried out according to a protocol approved by the Animal Care and Use Committee (ACUC) at the Nagoya Laboratories of Pfizer Global Research and Development. The ACUC found the model to result in transient and modest pain that was minimized wherever possible and reflects the most refined option available to evaluate the pain-relieving properties of the test compounds.

2.2. 4β-Phorbol-12-myristate-13-acetate (PMA)-induced paw licking and flinching response

PMA-induced paw-licking and flinching behaviours were determined according to the method of Taniguchi et al. (1997). Briefly, the rats were habituated to the observation chamber for at least 60 min prior to the experimental sessions. PMA solution was injected into the plantar surface of the right hindpaw with a volume of 100 μl. After injection, rats were immediately returned to the observation chamber, and their behaviour was observed for 5 s every 30 s for up to 120 min after the injection. The behaviour was scored as '0'=no licking and flinching of the injected paw, or 1=licking or flinching of the injected paw in each 5 s. A pain score was summed in each 10-min block of time.

2.3. Formalin-induced paw-licking and flinching response

The rats were habituated to the observation chamber for at least 60 min prior to the experimental sessions. Formalin (2% v/v in saline) was injected into the plantar surface of the right hindpaw with a volume of 50 μl. After injection, rats were immediately returned to the observation chamber, and the formalin-induced paw-licking and flinching behaviours were scored for 5 s every 30 s for 90 min.

2.4. Histological testing

Animals were killed after termination of the nociceptive response to (120 and 50 min after PMA and formalin injection, respectively, n=2 per group). The skin of the injected paw was removed and immediately fixed in 4% paraformaldehyde, embedded in paraffin and sectioned.

2.5. Reagents

PMA, MK-801, and resiniferatoxin were purchased from Sigma (St. Louis, MO). Morphine hydrochloride was purchased from Shionogi (Osaka, Japan). Formalin was purchased from Wako (Osaka, Japan). Ezlopitant [(2*S*,3*S*-*cis*)-2-(diphenylmethyl)-*N*-{(2-methoxy, 5-isopropylphenyl)methyl}-1-azabicyclo[2.2.2]octan-3-amine] and its (2*R*,3*R*)-isomer CJ-12,191 were synthesized in the Nagoya Laboratories, Pfizer Global Research and Development (Taketoyo, Japan). PMA was dissolved in dimethylsulfoxide at 10 mM and dissolved in physiological saline. Formalin was dissolved in physiological saline at 2% (v/v). Resiniferatoxin was dissolved in ethanol and administered subcutaneously (s.c.) to the rat 7 days prior to the behavioural experiment. For s.c. injection, morphine and ezlopitant were dissolved in physiological saline and given 10 min before the PMA injection. For intrathecal (i.t.) injection, ezlopitant and CJ-12,191 were dissolved in physiological saline and given to the rats just before PMA challenge. The i.t. injection was made in a 10 μl volume, by lumber puncture between L5 and L6, while rats were briefly anesthetized with isoflurane. Penetrations were judged successful if there was a tail flick response.

2.6. Statistical analysis

Data were analyzed by a one-way analysis of variance (ANOVA) followed by post hoc Bonferroni test for

statistical evaluation, otherwise stated. A P value of <0.05 was accepted as significant. Group results are expressed as mean±S.E.M. from 6 to 12 animals.

3. Results

3.1. Characterization of response induced by intraplantar injection of PMA

An intraplantar injection of PMA (0.3–10 μg) into the hindpaws of rats dose-dependently increased paw-licking and flinching behaviour (Fig. 1A). The response to PMA initiated at 10–20 min postinjection and lasted up to 120 min. An intraplantar injection of formalin (2% v/v) produced typical biphasic paw-licking and flinching behaviour (Fig. 1B). The second phase response was observed 10 to 30 min after formalin injection.

An injection of PMA at the concentration of higher than 3 μg appeared to cause inflammatory reactions, such as swelling and erythema at the site of injection. Correspondingly, light microscopy of paws injected with 3 μg of PMA showed tissue changes typical for acute inflammation, swelling, and invasion of granulocytes (Fig. 2A and B). Based on these results, the concentration of 3 μg was chosen for PMA injections to examine the effects of treatments in all experiments described below. After an injection of 2% formalin also induced accumulation of granulocytes (Fig. 2C).

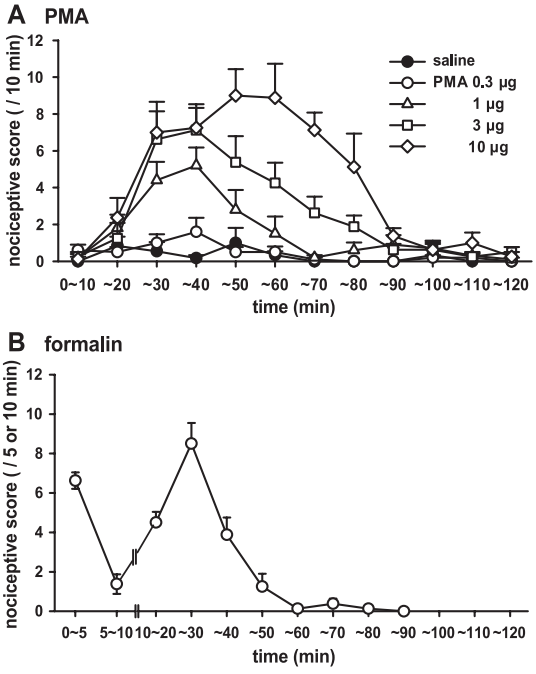

Fig. 1. Paw-licking and flinching response induced by various concentrations of PMA (A) and 2% v/v formalin (B) in rats. The data are expressed as mean ±S.E.M. from 6 to 10 animals.

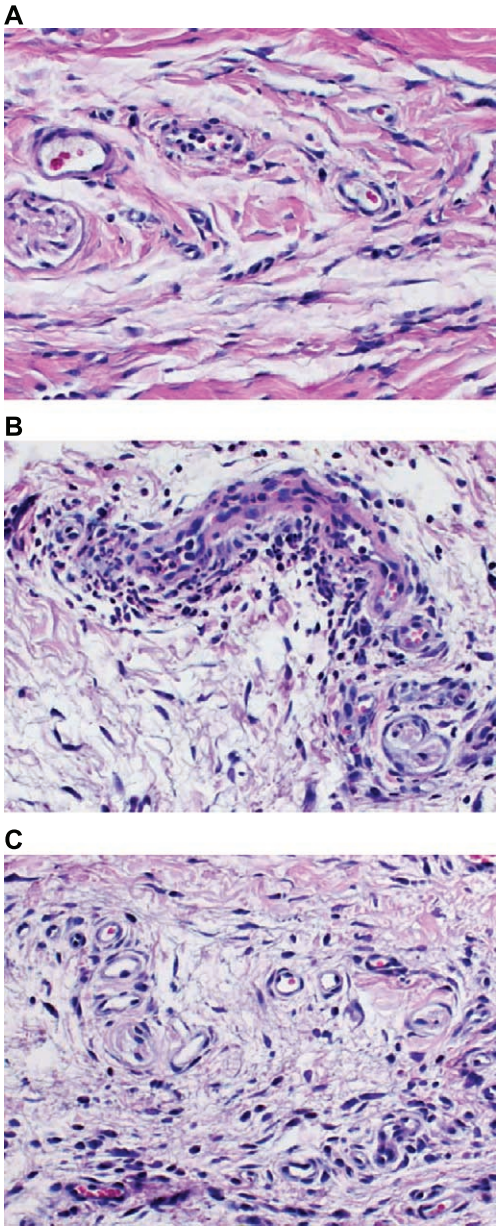

Fig. 2. Microscopic examination of rat hindpaw skin. Saline (A), PMA (3 μg, B), or formalin (2% v/v, C) was injected s.c. into the hindpaw of rats 120 min (saline and PMA) or 50 min (formalin) prior to removal. In (A), there is no reaction, in (B) and (C), invasion of granulocytes and edema can be seen. Magnification, ×20.

3.2. Effects of pretreatment of rats with resiniferatoxin on the PMA-induced nociceptive response

Resiniferatoxin is an ultrapotent capsaicin analog that binds to and activates vanilloid VR_1 receptor (Szallasi and Blumberg, 1989). Rats given a single subcutaneous dose of resiniferatoxin (200 μg/kg) 1 week before testing had no eye-blinking reaction to capsaicin solution (10 μM), indicating desensitization of the capsaicin-sensitive primary sensory neurons. The PMA-induced paw-licking and flinching response was remarkably attenuated by pretreatment with resiniferatoxin (Fig. 3). The cumulative nociceptive score from 0 to 120 min was 56.5±3.2

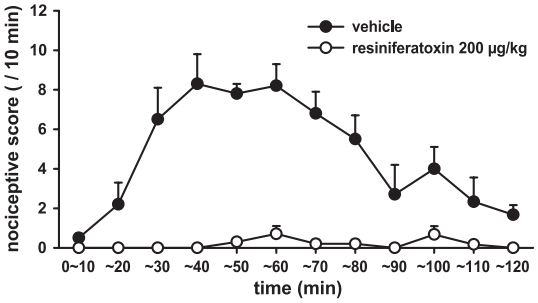

Fig. 3. Effect of pretreatment with resiniferatoxin (200 μg/kg) on PMA (3 μg)-induced paw-licking and flinching response in rats. Resiniferatoxin or vehicle (ethanol) was subcutaneously administered 1 week prior to PMA injection. The data are expressed as mean±S.E.M. from six animals.

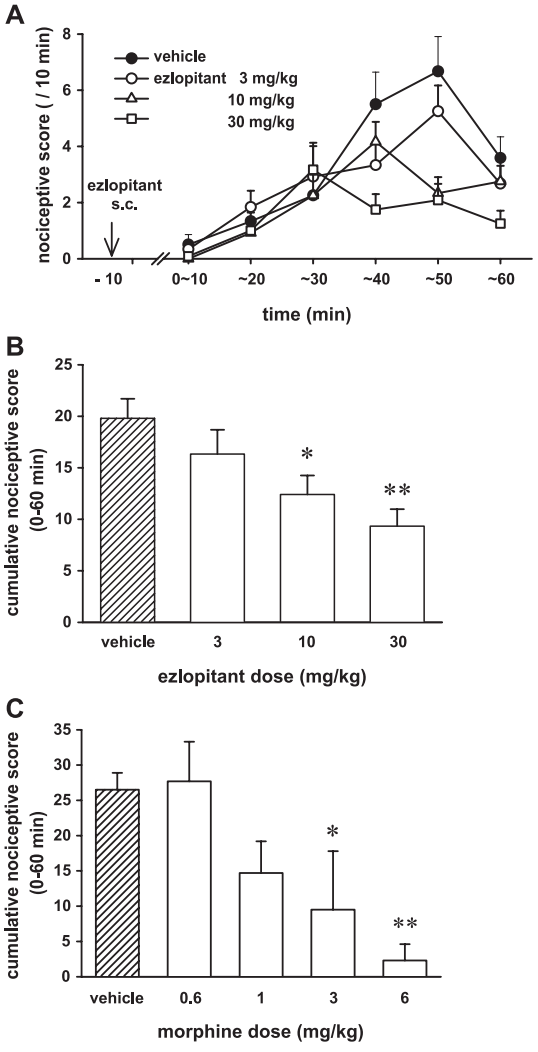

Fig. 4. Effects of ezlopitant (A) and morphine (B) on PMA (3 μg)-induced paw-licking and flinching response in rats. Ezlopitant or morphine was subcutaneously administered 10 min before PMA injection, and nociceptive behaviours were observed for 60 min after PMA injection. The data are expressed as mean±S.E.M. from 8 to 12 animals. *P<0.05 and **P<0.01 versus vehicle-treatment group.

and 2.2±0.5 for vehicle- and resiniferatoxin-treated group, respectively (P<0.01, n=6 per group).

3.3. Effects of prophylactic administration of ezlopitant and morphine on PMA-induced nociceptive response

Subcutaneous pretreatment with ezlopitant (3–30 mg/kg) dose-dependently attenuated paw-licking and flinching behaviour after induced by intraplantar injection of PMA (Fig. 4A). The highest dose of ezlopitant (30 mg/kg) inhibited the cumulative nociceptive score from 0 to 60 min after PMA injection by 46% (P<0.01). Subcutaneous pretreatment with morphine (0.6–6 mg/kg) dose-dependently inhibited the behavioural response to PMA (Fig. 4B).

3.4. Effects of therapeutic administration of ezlopitant on PMA-induced nociceptive response

The effect of a subcutaneous administration of ezlopitant (3–30 mg/kg) on the PMA-induced response was examined after the development of nociceptive response (30 min after PMA challenge). The injection of either vehicle or ezlopitant spontaneously reduced the PMA-induced response during a 30–40 min period (Fig. 5A). Afterward, the nociceptive behaviour was recovered in the vehicle-

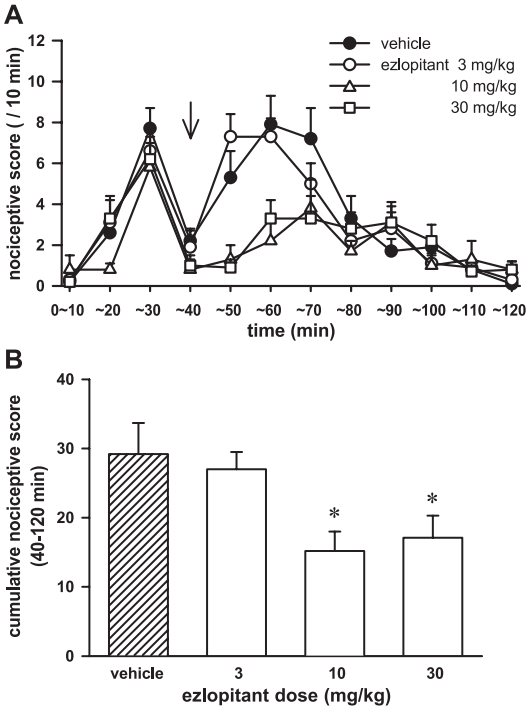

Fig. 5. Effects of therapeutic administration of ezlopitant on PMA (3 μg)-induced paw-licking and flinching response in rats. Ezlopitant was subcutaneously administered 30 min after PMA injection (indicated by an arrow), and nociceptive behaviours were observed for 90 min (120 min after PMA injection). The data are expressed as mean±S.E.M. licking and flinching scores for individual intervals (A) and cumulative scores from 40 to 120 min period (B) from nine animals. *P<0.05 versus vehicle-treatment group.

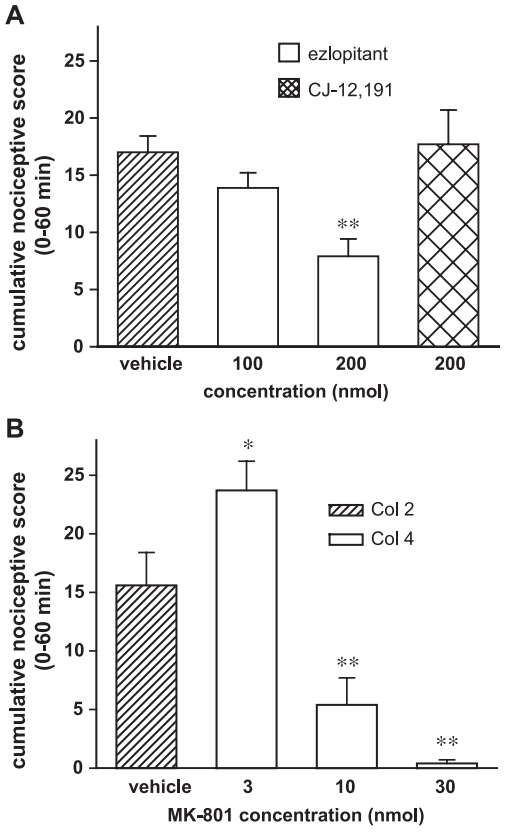

Fig. 6. Effects of intrathecal administration of ezlopitant and its inactive isomer CJ-12,191 and MK-801 on PMA-induced licking and flinching response in rats.

treated group, while the response was continuously inhibited by treatment with ezlopitant at 10 and 30 mg/kg. The cumulative nociceptive score from 40 to 120 min was reduced by 48% and 41% by treatment with ezlopitant at 10 and 30 mg/kg, respectively ($P<0.05$; Fig. 5B).

3.5. Effects of intrathecal administration of ezlopitant and MK-801 on the PMA-induced nociceptive response

An intrathecal injection of ezlopitant at concentrations of 100 and 200 nmol attenuated the PMA-induced response observed for 60 min (Fig. 6A). In a side-by-side experiment, intrathecal administration of a 200 nmol of CJ-12,191, an inactive isomer of ezlopitant (Tsuchiya et al., 2002), had no effect. The observed state of rats after receiving ezlopitant or CJ-12,191 was indistinguishable from that of vehicle-treated animals in that there was no observable sedation or motor impairment. Intrathecal injection of MK-801 (3–30 nmol), a nonselective glutamate receptor antagonist, also reduced the nociceptive response in a concentration dependent manner (Fig. 6B).

4. Discussion

In this study, we demonstrated that intraplantar injection of PMA, a PKC activator, caused spontaneous nociceptive behaviour that lasted over 90 min. The development of nocicepetive response to PMA was associated with the formation of edema and erythema in the injected paws. The cellular inflammatory reaction at the site of the PMA injection was observed in our histological studies. Thus, the present results suggest that the nociceptive behaviour in response to PMA is consistent with noxious input due to the formation of peripheral inflammation.

It was also found that the PMA-induced response was completely blocked by pretreatment with resiniferatoxin. It is well known that treatment with sufficient doses of capsaicin or other vanilloid VR_1 receptor agonists like resiniferatoxin desensitizes capsaicin-sensitive neurons (Szallasi and Blumberg, 1989, 1999; Szallasi et al., 1989). Using the immunohistochemical technique, Tominaga et al. (1998) showed that most (~85%) of the substance P-immunoreactive cells contained for vanilloid VR_1 in lumber dorsal root ganglion. Thus, substance P could play a pivotal role in conveying noxious input evoked by PMA injection from peripheral to the spinal cord. In contrast, the second phase of the formalin test is only partially blocked by systemic resiniferatoxin pretreatment in rats (Wismer et al., 2003). Capsaicin-sensitive fibers may not be the only set of fibers activated by formalin injection, and mechanistic differences may underlie in transmission of nociception caused by PMA and formalin injection. The duration of nociceptive behaviour induced by PMA was much longer than that of the second phase of the formalin test. It is believed that continuous noxious inputs from peripheral tissue can develop hypersensitivity in the spinal cord dorsal horn neurons, so-called “central sensitization“ (Ji et al., 2003). The continuous and long-lasting noxious inputs due to peripheral inflammation elicited by PMA injection may develop central sensitization that may contribute to the maintenance of nociception. Under this condition, the tachykinin NK_1 receptor may play a key role in the nociceptive processing since subcutaneous pretreatments with ezlopitant significantly blocked the nociceptive behaviour induced by a PMA injection. In addition, therapeutic treatment with ezlopitant attenuated the subsequent nociceptive behaviour induced by a PMA injection to the same extent as the pretreatment did, supporting our idea that tachykinin NK_1 receptors are activated continuously to transmit ongoing nociceptive signaling associated with peripheral inflammation.

We demonstrated that an intrathecal injection of ezlopitant stereoselectively inhibited the PMA-induced response as effectively as subcutaneous administration. Therefore, it is likely that the tachykinin NK_1 receptor expressing in the spinal cord is the predominant site of action mediating the antinociceptive effects of ezlopitant. Interestingly, an intrathecal injection of MK-801, a nonselective antagonist of glutamate receptors completely blocked the PMA-induced nociceptive behaviour, whereas ezlopitant partially inhibited the response. Excitatory amino acids (i.e., glutamate, aspartate) and neuropeptides (i.e., substance P, calcitnin

gene-related peptide) are two major neurotransmitters in the central nervous system. Glutamate functions as a fast, short-acting neurotransmitter through ligand-gated ion channels, such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA), whereas substance P functions as a slow, long-lasting neurotransmitter by activating G-protein-coupled tachykinin NK_1 receptors (Gerber and Randi, 1989; Yoshimura and Jessell, 1990; Urban and Randic, 1984). Studies conducted by using the rat spinal cord preparation have revealed that substance P enhances neuronal excitation or the postsynaptic neurons induced by NMDA (Rusin et al., 1993; Urban et al., 1994). On the basis of these findings, we propose that the tachykinin NK_1 receptor may participate in the process of nociceptive signaling by modifying excitation of postsynaptic neurons in the spinal cord.

In summary, injection of PMA causes persistent spontaneous nociceptive behaviour that is associated with the development of peripheral inflammation, and a tachykinin NK_1 receptor antagonist ezlopitant has analgesic activity in this test. Present results suggest that spinal tachykinin NK_1 receptors are continuously activated by noxious input due to peripheral inflammation and contribute to processing of pain sensation.

References

Chapman, V., Dickenson, A.H., 1993. The effect of intrathecal administration of RP67580, a potent neurokinin 1 antagonist on nociceptive transmission in the rat spinal cord. Neurosci. Lett. 157, 149–152.

Ding, Y.-Q., Zheng, H.-X., Wang, D.-S., Xu, J.-Q., Gong, L.-W., Lu, Y., Qin, B.-Z., Shi, J., Li, H.-L., Li, J.-S., Shigemoto, R., Kaneo, T., Mizuno, N., 1999. The distribution of substance P receptor (NK1)-like immunoreactive neurons in the newborn and adult human spinal cord. Neurosci. Lett. 266, 133–136.

Duggan, A.W., Hendry, I.A., 1986. Laminar localization of the sites of release of immunoreactive substance P in the dorsal horn with antibody-coated microelectrodes. Neurosci. Lett. 68, 134–140.

Gerber, G., Randi, M., 1989. Excitatory amino acid-mediated components of synaptically evoked input from dorsal roots to deep dorsal horn neurons in the rat spinal cord slice. Neurosci. Lett. 106, 211–219.

Gonzales, M.I., Field, M.J., Hughes, J., Singh, L., 2000. Evaluation of selective NK_1 receptor antagonist CI-1021 in animal models of inflammatory and neuropathic pain. J. Pharmacol. Exp. Ther. 294, 444–450.

Henry, J.L., Yashpal, K., Pitcher, G.M., Chabot, J.-G., Coderre, T.J., 1999. Evidence for tonic activation of NK-1 receptors furing the second phase of the formalin test in the rat. J. Neurosci. 19, 6588–6598.

Ji, R.-R., Kohno, T., Moore, A.K., Woolf, C.J., 2003. Central sensitization and LTP: do pain and memory share similar mechanisms? Trends Neurosci. 26, 696–705.

Nakaya, Y., Kaneko, T., Shigemoto, R., Nakanishi, S., Mizuno, N., 1994. Immunohistochemical localization of substance P receptor in the central nervous system of the adult rat. J. Comp. Neurol. 347, 249–274.

Ribeiro-da-Silva, A., Hökfelt, T., 2000. Neuroanatomical localisation of substance P in the CNS and sensory neurons. Neuropeptides 34, 256–271.

Rupniak, N.M.J., Carlson, E., Boyce, S., Webb, J.K., Hill, R.G., 1996. Enantioselective inhibition of the formalin paw late phase by the NK_1 receptor antagonist L-733060 in gerbils. Pain 67, 189–195.

Rusin, K.I., Bleakman, D., Chard, P.S., Randic, M., Miller, R.J., 1993. Tachykinins potentiate *N*-methyl-D-aspartate responses in acutely isolated neurons from the dorsal horn. J. Neurochem. 60, 952–960.

Souza, A.L.S., Moreira, F.A., Almeida, K.R., Bertollo, C.M., Costa, K.A., Coelho, M.M., 2002. In vivo evidence for a role of protein kinase C in peripheral nociceptive processing. Br. J. Pharmacol. 135, 239–247.

Szallasi, A., Blumberg, P.M., 1989. Resiniferatoxin, a phorbol-related diterpene, acts as an ultrapotent analog of capsaicin, the irritant constituent in red pepper. Neuroscience 30, 515–520.

Szallasi, A., Blumberg, P.M., 1999. Vanilloid (capsaicin) receptors and mechanisms. Pharmacol. Rev. 51, 159–211.

Szallasi, A., Joo, F., Blumberg, P.M., 1989. Duration of desensitization and ultrastructural changes in dorsal root ganglia in rats treated with resiniferatoxin, an ultrapotent capsaicin analog. Brain Res. 503, 68–72.

Taniguchi, K., Shinjo, K., Mizutani, M., Shimada, K., Ishikawa, T., Menniti, F.S., Nagahisa, A., 1997. Antinociceptive activity of CP-101,606, an NMDA receptor NR2B subunit antagonist. Br. J. Pharmacol. 122, 809–812.

Tiseo, P.J., Adler, M.W., Liu-Chen, L.Y., 1990. Differential release of substance P and somatostatin in the rat spinal cord in response to noxious cold and heat: effect of dynorphin A(1–17). J. Pharmacol. Exp. Ther. 252, 539–545.

Todd, A.J., Puskár, Z., Spike, R.C., Hughes, C., Watt, C., Forrest, L., 2002. Projection neurons in lamina I of rat spinal cord with the neurokinin 1 receptor are selectively innervated by substance P-containing afferents and respond to noxious stimulation. J. Neurosci. 22, 4103–4113.

Tominaga, M., Caterina, M.J., Malmberg, A.B., Rosen, T.A., Gilbert, H., Skinner, K., Raumann, B.E., Basbaum, A.I., Julius, D., 1998. The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 21, 531–543.

Traub, R.J., 1996. The spinal contribution of substance P to the generation and maintenance of inflammatory hyperalgesia in the rat. Pain 67, 151–161.

Tsuchiya, M., Fujiwara, Y., Kanai, Y., Mizutani, M., Shimada, K., Suga, O., Ueda, S., Watson, J.W., Nagahisa, A., 2002. Anti-emetic activity of the novel nonpeptide tachykinin NK_1 receptor antagonist ezlopitant (CJ-11,974) against acute and delayed cisplatin-induced emesis in the ferret. Pharmacology 66, 144–152.

Urban, L., Randic, M., 1984. Slow excitatory transmission in rat dorsal horn: possible mediation by peptides. Brain Res. 290, 336–341.

Urban, L., Naeem, S., Patel, I.A., Dray, A., 1994. Tachykinin induced regulation of excitatory amino acid responses in the rat spinal cord in vitro. Neurosci. Lett. 168, 185–188.

Wismer, C.T., Faltynek, C.R., Jarvis, M.F., McGaraughty, S., 2003. Distinct neurochemical mechanisms are activated following administration of different P2X receptor agonists into the hindpaw of a rat. Brain Res. 965, 187–193.

Yamamoto, T., Yaksh, T.L., 1991. Stereospecific effects of a nonpeptidic NK1 selective antagonist, CP-96,345: antinociception in the absence of motor dysfunction. Life Sci. 49, 1955–1963.

Yoshimura, M., Jessell, T., 1990. Amino acid-mediated EPSPs at primary afferent synapses with substantia gelatinosa neurons in the rat spinal cord. J. Physiol. 430, 315–335.

Yu, X.H., Zhang, E.-T., Craig, A.D., Shigemoto, R., Ribeiro-da-Silva, A., De Koninck, Y., 1999. NK-1 receptor immunoreactivity in distinct morphological types of lamina I neurons of the primate spinal cord. J. Neurosci. 19, 3545–3555.