ELSEVIER

European Journal of Pharmacology 344 (1998) 115–120

ejp

Evaluation of PD 154075, a tachykinin NK_1 receptor antagonist, in a rat model of postoperative pain

M. Isabel Gonzalez, Mark J. Field, Elizabeth F. Holloman, John Hughes, Ryszard J. Oles, Lakhbir Singh *

Department of Biology, Parke-Davis Neuroscience Research Centre, Cambridge University Forvie Site, Robinson Way, Cambridge CB2 2QB, UK

Received 11 September 1997; revised 18 November 1997; accepted 9 December 1997

Abstract

PD 154075 ([(2-benzofuran)-CH_2OCO]-(*R*)-α-MeTrp-(*S*)-NHCH(CH_3)Ph) is a selective tachykinin NK_1 receptor antagonist. Its effect on development and maintenance of thermal and mechanical hypersensitivity was examined in a rat model of surgical pain. When administered 30 min before surgery, PD 154075 dose-dependently (3–100 mg/kg, s.c.) prevented the development of thermal and mechanical hypersensitivity with respective minimum effective doses of 10 and 30 mg/kg. These antihypersensitivity effects lasted for 72 h. In contrast, the administration of PD 154075 (30 mg/kg, s.c.) after surgery had little or no effect on these nociceptive responses. PD 154075 antagonised thermal hypersensitivity induced by intrathecal administration of substance P, over the same dose range that blocked surgical hypersensitivity. However, it only partially blocked the thermal hypersensitivity induced by the selective NK_2 receptor agonist [βAla^8]neurokinin A-(4–10). Morphine dose-dependently (1–6 mg/kg, s.c.) lengthened isoflurane and pentobarbitone-induced sleeping time in the rat. In contrast, PD 154075 (3–100 mg/kg, s.c.) did not interact with these anaesthetics. It is suggested that tachykinin NK_1 receptor antagonists, such as PD 154075, may possess therapeutic potential as pre-emptive antihypersensitive agents.

Keywords: Development; Maintenance; Thermal hypersensitivity; Mechanical hypersensitivity; (Pre-emptive); Anaesthetic

1. Introduction

The tachykinins, substance P and neurokinin A are co-localised in capsaicin-sensitive neurones that are believed to be nociceptive afferents and are released in response to a variety of noxious stimuli. Three subclasses of tachykinin receptors have been described in the central nervous system (CNS) and periphery: NK_1, NK_2 and NK_3 with substance P, neurokinin A and neurokinin B as their respective preferred endogenous ligands (Maggi et al., 1993). The tachykinin NK_1 receptor is widely distributed in the CNS (Kiyama et al., 1993). In the spinal cord, NK_1 receptor binding sites are widely distributed throughout the dorsal (particularly laminae I and II where substance P containing primary afferent neurones terminate) and ventral horn (Yashpal et al., 1990).

The recent discovery of non-peptide, highly selective tachykinin NK_1 receptor antagonists that penetrate into the CNS after systemic administration has helped to study the role of substance P in nociception. Most studies to date have focused on evaluation of tachykinin NK_1 receptor antagonists in animal models of inflammatory pain. Several studies have reported that selective tachykinin NK_1 receptor antagonists can dose-dependently block the second phase of the formalin response (Seguin et al., 1995; Rupniak et al., 1996; Iyengar et al., 1997). Other studies have shown that tachykinin NK_1 receptor antagonists can also prevent carrageenan-induced hyperalgesia (Yamamoto et al., 1993). There is also evidence that supports a role for the tachykinin NK_1 receptor in peripheral inflammation (Levine et al., 1984). However, the role of the tachykinin NK_1 receptor in other types of pain, including surgical and neuropathic, remains to be clearly established.

It has recently been suggested that the tachykinin NK_1 receptor is involved in induction but not maintenance of nociception (Ma and Woolf, 1995; Traub, 1996). This implies that the major therapeutic potential of tachykinin NK_1 receptor antagonists would be as pre-emptive analgesic agents in the treatment of surgical pain. Recently, a

* Corresponding author. Tel.: +44-1223-210929; fax: +44-1223-249106; e-mail: singhl@camb.wl.com

rat model of postoperative pain has been described (Brennan et al., 1996). It involves an incision of the skin, fascia and muscle of the plantar aspect of the hindpaw. This leads to an induction of reproducible and quantifiable mechanical (Brennan et al., 1996) and thermal hypersensitivity (Field et al., 1997) lasting several days. PD 154075 is a selective tachykinin NK_1 receptor antagonist. It possesses nanomolar affinity for the human and the guinea pig tachykinin NK_1 receptors (Singh et al., 1997). In the present study we have examined the ability of PD 154075, to block the induction and maintenance of these hypersensitivity states.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (180–220 g), obtained from Bantin and Kingman (Hull) were housed in groups of 6 under a 12 h light/dark cycle (lights on at 7.00) with food and water ad libitum.

2.2. Surgery

Animals were anaesthetised with 2% isoflurane and 1:4 O_2/NO_2 mixture. A 1 cm longitudinal incision was made through the skin and muscle of the plantar aspect of the right hindpaw (Brennan et al., 1996). The wound was closed with two sutures and covered with topical antibiotics. Postoperatively animals were housed on 'Aqua-sorb' bedding consisting of air laid cellulose (Beta Medical and Scientific, Sale). The sutures were removed after 48 h.

2.3. Assessment of thermal and mechanical hypersensitivity

Animals were tested for thermal hypersensitivity in the plantar test and mechanical hypersensitivity with Semmes-Weinstein von Frey hairs as previously described (Field et al., 1997).

2.4. Effect of PD 154075 on substance P and [βAla^8]neurokinin A-(4–10)-induced thermal hypersensitivity

Substance P and [βAla^8]neurokinin A-(4–10) were administered intrathecally in a volume of 10 μl using a 100 μl Hamilton syringe by exposing the spine of the rats under brief isoflurane anaesthesia. Injections were made into the intrathecal space between lumbar region 5–6 with a 10 mm long 27 gauge needle. Penetrations were judged successful if there was a tail flick response. The wound was sealed with an autoclip and rats appeared fully awake within 2–3 min following injection. Paw withdrawal latency baselines were determined prior to drug treatment in the plantar test. PD 154075 or its vehicle was administered s.c. 30 min prior to intrathecal substance P (8 μg/animal) or [βAla^8]neurokinin A-(4–10) (2 μg/animal). Paw withdrawal latencies were determined once again every 10 min from 30 through to 60 min post intrathecal injection.

2.5. Interaction with anaesthetics

The potential for PD 154075 to interact with anaesthetics was investigated by measuring changes in the sleeping time-induced by isoflurane or sodium pentobarbitone. In both cases, the animals received a single s.c. injection of morphine sulphate (1–6 mg/kg) or PD 154075 (3–100 mg/kg) 30 min before induction of anaesthesia. Anaesthesia was induced by sodium pentobarbitone (45 mg/kg, i.p.) or by exposure of the animals for 30 s to a saturated chamber with 5% isoflurane and 1:4 O_2/NO_2 mixture and then further exposure to 2% isoflurane for 4.5 min. The time of recovery of the righting reflex from sodium pentobarbitone or isoflurane was recorded.

2.6. Drugs

PD 154075 ([(2-benzofuran)-CH_2OCO]-(*R*)-α-MeTrp-(*S*)-NHCH(CH_3)Ph) was synthesised at Parke-Davis Neuroscience Research Centre (Cambridge). It was dissolved in poly(ethylene glycol) 200 (PEG-200, Sigma). Substance P was obtained from Sigma and [βAla^8]neurokinin A-(4–10) was obtained from RBI, USA, and dissolved in 0.9% w/v NaCl. PD 154075 was administered s.c. in a volume of 1 ml/kg. The two neuropeptides were administered intrathecally in a volume of 10 μl.

2.7. Statistics

Data obtained for thermal hypersensitivity and for sleeping time were subjected to a one-way-analysis of variance (ANOVA) followed by Dunnett's *t*-test. Data obtained for mechanical hypersensitivity were analysed by an individual Mann-Whitney *t*-test.

3. Results

3.1. Effect of PD 154075 on substance-P and [βAla^8]neurokinin A-(4–10) induced thermal hypersensitivity

The intrathecal administration of substance P or [βAla^8]neurokinin A-(4–10) induced thermal hypersensitivity which was present 40–60 min post injection (Fig. 1). The s.c. administration of PD 154075 at 30 min before substance P (8 μg/animal) dose dependently (10–100 mg/kg, s.c.) blocked the development of substance P-induced hypersensitivity with a minimum effective dose (MED) of 30 mg/kg (Fig. 1a). The highest dose of 100 mg/kg completely blocked the development of thermal hypersensitivity for the duration of the experiment (Fig.

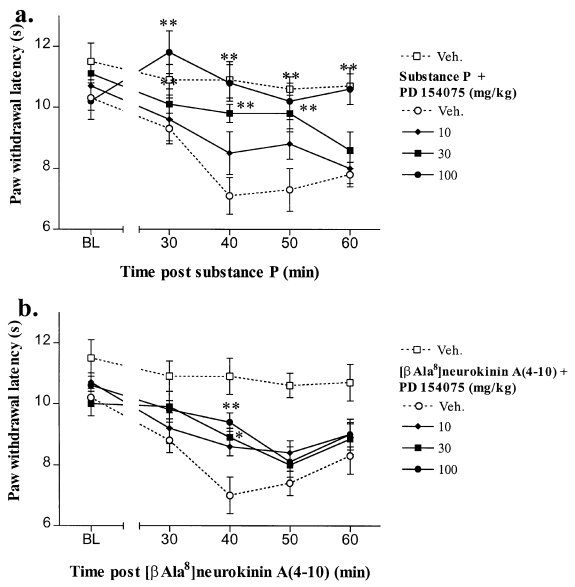

Fig. 1. Effect of PD 154075 on the development of (a) Substance P and (b) [βAla^8]neurokinin A-(4–10) induced thermal hypersensitivity. Thermal hypersensitivity was assessed using the plantar test. Baseline (BL) paw withdrawal latencies were determined prior to drug treatment. PD 154075 or its vehicle was administered s.c. 30 min prior to intrathecal substance P (8 μg/animal) or [βAla^8]neurokinin A-(4–10) (2 μg/animal). Paw withdrawal latencies were determined once again every 10 min from 30 through to 60 min post intrathecal injection. Results are expressed as mean $\pm$ S.E.M. Paw withdrawal latencies of 8–12 animals. $^{*}P < 0.05$, $^{**}P < 0.01$ significantly different from vehicle + agonist group at each time point (one-way ANOVA followed by Dunnett's *t*-test).

1a). In contrast, similar administration of PD 154075 partially blocked [βAla^8]neurokinin A-(4–10)-induced hypersensitivity (Fig. 1b). Furthermore, unlike the antagonism of substance P-induced response, the blockade of [βAla^8]neurokinin A-(4–10)-induced hypersensitivity by PD 154075 was not dose related and was only observed at one time point (40 min post NK_2 agonist) (Fig. 1b).

3.2. Effect of PD 154075 on induction of thermal and mechanical hypersensitivity by surgical injury

The incision of the plantaris muscle-induced thermal and mechanical hypersensitivity lasted at least 3 days in the ipsilateral paw (Fig. 2). No such hypersensitivity was observed in the contralateral paw (Fig. 2). For these studies the same animals were tested for thermal and mechanical hypersensitivity, and 1 h recovery was allowed between tests. A single dose of PD 154075 administered 30 min before surgery dose-dependently (3–100 mg/kg, s.c.) prevented the development of thermal (Fig. 2a) and mechanical hypersensitivity (Fig. 2b) with MED dose of 10 and 30 mg/kg, respectively. The highest dose of PD 154075 (100 mg/kg) blocked the development of mechanical and thermal hypersensitivity for 49 and >72 h, respectively. PD 154075 did not effect either response in the contralateral paw which remained consistent with baseline values (data not shown).

3.3. Effect of PD 154075 on maintenance of thermal and mechanical hypersensitivity induced by surgical injury

Two different groups of animals were used in this experiment, one group for evaluation of thermal and the other for mechanical hypersensitivity. PD 154075 (30 mg/kg, s.c.) did not have any effect on thermal or mechanical hypersensitivity when administered 1 h after surgery (Fig. 3). However, when it was administered 24 h after surgery, it produced a modest but statistically significant blockade of thermal hypersensitivity. It took 2 h for this effect to appear and it lasted at least 5 h (Fig. 3). PD 154075 (30 mg/kg, s.c.) did not have any effect on mechanical hypersensitivity when administered 24 h after surgery (Fig. 3).

3.4. Lack of interaction of PD 154075 with anaesthetics

Isoflurane induced anaesthesia in control rats lasting (mean $\pm$ S.E.M.) 2.91 $\pm$ 0.29 min. The s.c. administration

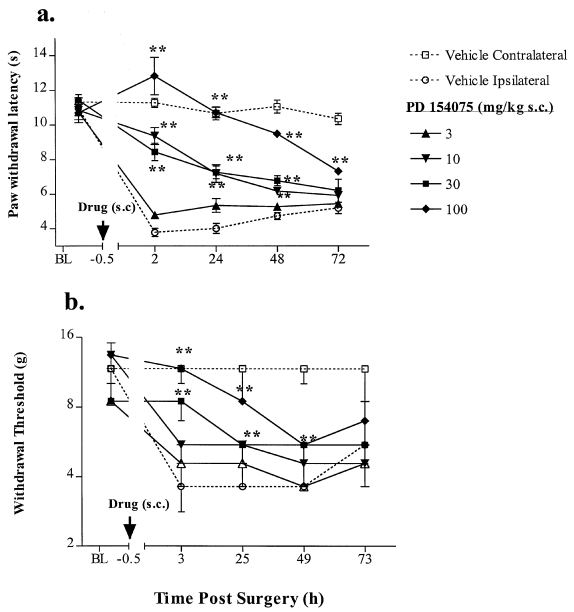

Fig. 2. Effect of PD 154075 on development of thermal and mechanical hypersensitivity in the rat postoperative pain model. Thermal hypersensitivity was assessed by determining paw withdrawal latencies using the plantar test (a). Mechanical hypersensitivity was assessed by measuring the force required in grams to elicit paw withdrawal using von Frey hair filaments (b). Baseline (BL) measurements were taken before surgery. Animals received a single s.c. injection of PD 154075 (3–100 mg/kg) 30 min before surgery. Paw withdrawal latencies and withdrawal thresholds were re-assessed at various times after surgery. Results are expressed as mean $\pm$ S.E.M. for thermal hypersensitivity and as median $\pm$ 1st and 3rd quartiles for mechanical hypersensitivity. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different from ipsilateral paw of vehicle treated animals (ANOVA followed by Dunnett's *t*-test for thermal hypersensitivity and Mann-Whitney *t*-test for mechanical hypersensitivity; $n = 6$–11).

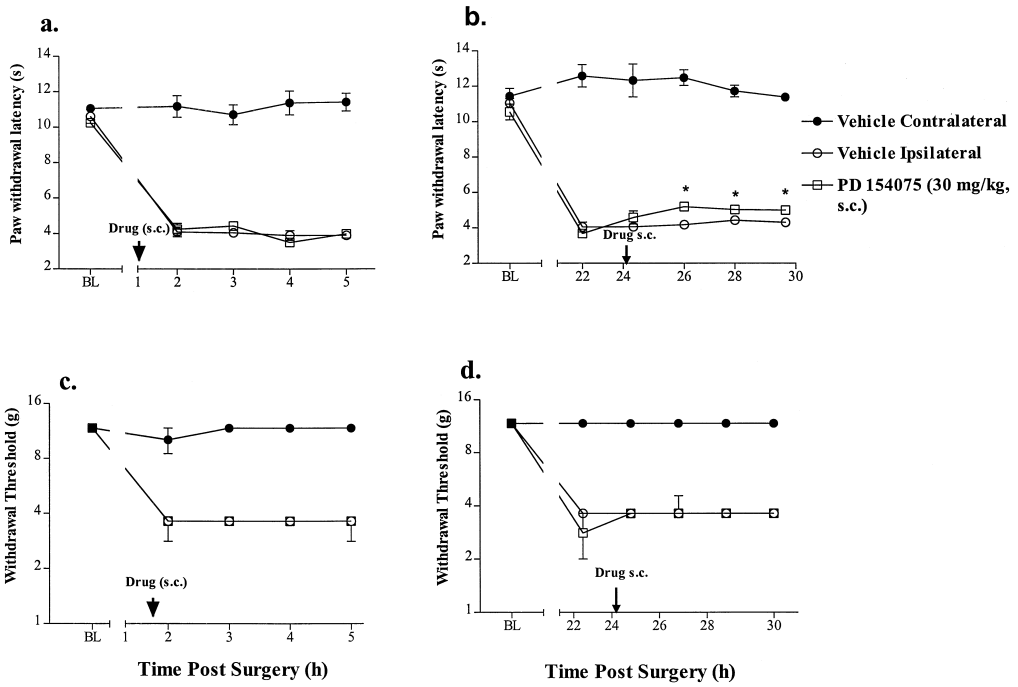

Fig. 3. Effect of PD 154075 on the maintenance of thermal and mechanical hypersensitivity in the rat postoperative pain model. Thermal hypersensitivity was assessed by determining paw withdrawal latencies using the plantar test (a, b). Mechanical hypersensitivity was assessed by measuring the force required in grams to elicit paw withdrawal using von Frey hair filaments (c,d). Baseline (BL) measurements were taken before surgery. Animals received a single s.c. injection of PD 154075 (30 mg/kg), 60 min or 24 h after surgery. Paw withdrawal latencies and withdrawal thresholds were re-assessed at various times after surgery. Results are expressed as mean ± S.E.M. for thermal hypersensitivity and as median ± 1st and 3rd quartiles for mechanical hypersensitivity. $^{*}P < 0.05$ significantly different from ipsilateral paw of vehicle treated animals (unpaired *t*-test; $n = 6–11$).

of morphine 30 min before isoflurane dose-dependently lengthened the sleeping time. The respective sleeping times for 1, 3 and 6 mg/kg morphine were: 2.79 ± 0.65, 9.94 ± 0.78** and 15.95 ± 3.00** min ($^{**}P < 0.01$ vs. controls, ANOVA followed by Dunnett's *t*-test). In contrast, similar administration of PD 154075 did not affect isoflurane-induced sleeping time with respective sleeping times of 2.36 ± 0.32, 2.41 ± 0.2, 3.56 ± 0.45 and 3.14 ± 0.78 min for 3, 10, 30 and 100 mg/kg PD 154075.

Sodium pentobarbitone induced anaesthesia in control rats lasting 63.4 ± 4.0 min. The s.c. administration of morphine 30 min before sodium pentobarbitone dose-dependently lengthened the sleeping time with respective values of 77.7 ± 5.9, 80.1 ± 10.0 and 115.6 ± 6.8** min for 1, 3 and 6 mg/kg morphine ($^{**}P < 0.01$ vs. controls, ANOVA followed by Dunnett's *t*-test). In contrast, similar administration of PD 154075 did not affect pentobarbitone induced sleeping time with respective values of 74.1 ± 2.0, 70.3 ± 5.1 and 68.9 ± 5.7 min for 10, 30 and 100 mg/kg PD 154075.

4. Discussion

The results of the present study suggest that the tachykinin NK_1 receptor plays an important role during the induction of thermal and mechanical hypersensitivity in a rat model of postoperative pain. This is consistent with a previous study that reached a similar conclusion using animal models of inflammatory pain (Traub, 1996). However, the tachykinin NK_1 receptor appears to have little or no role during the maintenance of these nociceptive responses. It is noticeable that high doses of PD 154075 were required for the prevention of thermal and mechanical hypersensitivity. The reason for this is likely to be the low affinity of PD 154075 for the rat tachykinin NK_1 receptor (Singh et al., 1997). It is possible that at these high doses PD 154075 loses selectivity and the antihyperalgesic and antihypersensitive effects do not involve the tachykinin NK_1 receptor. However, the results of the present study show that PD 154075 can dose-dependently block tachykinin NK_1 receptor mediated hypersensitivity in the rat. Moreover, this antagonism was complete and required the same doses as those used in the surgical pain model. The results presented here further show that PD 154075 can only partially and transiently block the NK_2 receptor mediated hypersensitivity. Previously, radioligand binding studies have shown that PD 154075 is a highly selective tachykinin NK_1 receptor antagonist (Singh et al., 1997). Taken together, these observations suggest that the tachykinin NK_1 receptor is likely to be the predominant site of action mediating the antihyperalgesic and antihypersensitive effects of PD 154075.

The long duration of action of PD 154075 observed following pre-treatment is inconsistent with its pharmacokinetic profile. Thus, it has been shown that the half life of PD 154075 is around 4–6 h in the rat (Singh et al., 1997). This suggests that PD 154075 cannot be present in animals for 3 days. Similar long duration of action has previously been observed with a different class of compounds when administered before surgery (Field et al., 1997). It has been suggested that the input from primary afferent fibres during and up to 1 h post surgery, appears to be the major stimulus for the induction of mechanical and thermal hypersensitivity (Field et al., 1997). The input from primary afferents after this time appears to be insufficient for the induction but adequate to maintain these nociceptive responses. It would appear that the induction phase is vital in the generation of hypersensitivity. The prevention of this phase can lead to prolonged antihypersensitive actions.

The induction and maintenance of dorsal horn hypersensitivity has been shown to be dependent on activation of the *N*-methyl-D-aspartic acid (NMDA) receptor complex (Woolf and Thompson, 1991). It has been suggested that the tachykinin NK_1 receptor influences nociception via modulation of the NMDA receptor (Urban et al., 1994). Thus, it has been shown that stimulation of the tachykinin NK_1 receptor can facilitate the activation of NMDA receptors by removing the Mg^{2+} antagonism either by depolarisation of the postsynaptic membrane or by reducing the voltage dependence of the block. Other studies have shown that activation of the tachykinin NK_1 receptor can increase the release of excitatory amino acid neurotransmitters from primary sensory afferents (Kangrga and Randic, 1990). The relatively weak effect of PD 154075 on maintenance of nociceptive responses imply that the tachykinin NK_1 receptor has little or no influence on the NMDA receptor after it has been activated.

It should be noted that PD 154075 completely prevented induction of both thermal and mechanical hypersensitivity. It has been previously reported that morphine is more effective at blocking thermal hyperalgesia than allodynia in a model of surgical pain (Field et al., 1997). Allodynia is the main symptom of surgical pain and is thought to be mediated by large diameter, low threshold Aβ fibres (for review see Woolf and Chong, 1993). These Aβ fibres are activated by innocuous stimuli and terminate in deeper layers of the dorsal horn. This region of the dorsal horn has much lower density of μ-opioid receptors compared with the superficial laminae which receive inputs from small diameter high threshold sensory neurones responsible for mediation of hyperalgesia (Atweh and Kuhar, 1977; Stevens et al., 1991). Furthermore, it has been shown that morphine has poor activity at blocking A-fibre inputs into the dorsal horn (Dickenson and Sullivan, 1986). Unlike μ-opioid receptors, tachykinin NK_1 binding sites are not only distributed in laminae I and II but also X, and various loci in the ventral horns (Yashpal et al., 1990). Furthermore, tachykinin NK_1 receptors are also found in the peripheral nervous system where they are involved in inflammation (Levine et al., 1984). It has been shown that following systemic administration, PD 154075 can penetrate into the CNS (Singh et al., 1997). PD 154075 is a functional antagonist at both peripheral and central tachykinin NK_1 receptors (Boyle et al., 1994). Therefore, it is possible that antagonism of both peripheral and central tachykinin NK_1 receptors contribute towards the antihypersensitive actions of PD 154075.

In conclusion, the results of the present study suggest that tachykinin NK_1 receptor antagonists such as PD 154075 may possess pre-emptive antihyperalgesic and antihypersensitive actions. It should be noted that mechanical hypersensitivity is almost unavoidable following surgery (e.g. clothes touching skin, breathing, coughing, movement of joints). Thus, the ability of PD 154075 to block mechanical hypersensitivity represents a potential advantage over μ-opioid receptor agonists. The apparent lack of interaction of PD 154075 with anaesthetics may also prove to be beneficial in a preventative treatment of surgical pain. As PD 154075 possesses higher affinity for the human compared with the rat tachykinin NK_1 receptor (Singh et al., 1997), it is possible that much lower doses may show efficacy in man. However, it remains to be seen whether blocking part of the nociceptive transmission carried by substance P is sufficient to prevent surgical pain in man.

References

Atweh, S.E., Kuhar, M.J., 1977. Autoradiographic localisation of opiate receptors in the rat brain. I. Spinal cord and lower medulla. Brain Res. 124, 53–68.

Boyle, S., Guard, S., Higginbottom, M., Horwell, D.C., Howson, W., McKnight, A.T., Martin, K., Pritchard, M.C., O'Toole, J., Raphy, J., Rees, D.C., Roberts, E., Watling, K.J., Woodruff, G.N., Hughes, J., 1994. Functional design of high affinity tachykinin NK_1 receptor antagonists. Bio. Med. Chem. 2, 357–370.

Brennan, T.J., Vandermeulen, E.P., Gebhart, G.F., 1996. Characterization of a rat model of incisional pain. Pain 64, 493–501.

Dickenson, A.H., Sullivan, A.F., 1986. Electrophysiological studies on the effects of intrathecal morphine on nociceptive neurones in the rat dorsal horn. Pain 24, 211–222.

Field, M.J., Holloman, E.F., McCleary, S., Hughes, J., Singh, L., 1997. Evaluation of gabapentin and *S*-(+)-3-isobutylgaba in a rat model of postoperative pain. J. Pharmacol. Exp. Ther. 282, 1242–1246.

Iyengar, S., Hipskind, P.A., Gehlert, D.R., Schober, D., Lobb, K.L., Nixon, J.A., Helton, D.R., Kallman, M.J., Boucher, S., Couture, R., Li, D.L., Simmons, R.M.A., 1997. LY303870, a centrally active neurokinin-1 antagonist with a long duration of action. J. Pharmacol. Exp. Ther. 280, 774–785.

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. J. Neurosci. 10, 2026–2038.

Kiyama, H., Maeno, H., Tohyama, M., 1993. Substance P receptor (NK-1) in the central nervous system: Possible functions from a morphological aspect. Regul. Pept. 46, 114–123.

Levine, J.D., Clark, R., Devor, M., Helms, C., Moskovitz, M.A., Basbaum, A.I., 1984. Intraneuronal substance P contributes to the severity of experimental arthritis. Science 226, 547–549.

Ma, Q.-P., Woolf, C.J., 1995. Involvement of neurokinin receptors in the induction but not the maintenance of mechanical allodynia in rat flexor motoneurones. J. Physiol. 486, 769–777.

Maggi, C.A., Patacchini, R., Rovero, P., Giachetti, A., 1993. Tachykinin receptors and tachykinin receptor antagonists. J. Autonom. Pharmacol. 13, 23–93.

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-733,060 in gerbils. Pain 67, 189–195.

Seguin, L., Le Marouille-Girardon, S., Millan, M.J., 1995. Antinociceptive profiles of non-peptidergic neurokinin$_1$ and neurokinin$_2$ receptor antagonists: A comparison to other classes of antinociceptive agent. Pain 61, 325–343.

Singh, L., Field, M.J., Hughes, J., Kuo, B.-S., Suman-Chauhan, N., Tuladhar, B.R., Wright, D.S., Naylor, R.J., 1997. The tachykinin NK_1 receptor antagonist PD 154075 blocks cisplatin-induced delayed emesis in the ferret. Eur. J. Pharmacol. 321, 209–216.

Stevens, C.W., Lacey, C.B., Miller, K.E., Elde, R.P., Seybold, V.S., 1991. Biochemical characterization and regional quantification of μ, δ and κ opioid binding sites in rat spinal cord. Brain Res. 550, 77–85.

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.

Urban, L., Thompson, S.W.N., Dray, A., 1994. Modulation of spinal excitability: Co-operation between neurokinin and excitatory amino acid neurotransmitters. Trends Neurol. Sci. 17, 432–438.

Woolf, C.J., Chong, M.-S., 1993. Preemptive analgesia-treating postoperative pain by preventing the establishment of central sensitization. Anesth. Analg. 77, 362–379.

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.

Yamamoto, T., Shimoyama, N., Mizuguchi, T., 1993. Effects of intrathecal FK888, a novel dipeptide NK_1 receptor antagonist, on the formalin test in the rat. Neurosci. Lett. 161, 57–59.

Yashpal, K., Dam, T.C., Quirion, R., 1990. Quantitative autoradiographic distribution of multiple neurokinin binding sites in rat spinal cord. Brain Res. 506, 259–266.