Laboratory Reports

Anti-allodynic effects of oral COX-2 selective inhibitor on postoperative pain in the rat

Tatsuo Yamamoto MD,
Yoshihiko Sakashita MD,
Natsuko Nozaki-Taguchi MD

Purpose: To examine the effect of a cyclooxygenase (COX)-2 inhibitor on the maintenance of mechanical allodynia induced by skin incision (an animal model of postoperative incident pain) in the rat. Also, to compare the effect of a COX-2 inhibitor with that of a nonselective COX-1 and COX-2 inhibitor and B2 receptor antagonist.

Methods: A 1 cm longitudinal skin incision was made in the plantar aspect of the foot. JTE522 (1 – 100 mg·kg⁻¹), a COX-2 inhibitor, indomethacin (1 – 30 mg·kg⁻¹), a nonselective COX-1 and COX-2 inhibitor, or FRI 73657 (10 and 100 mg·kg⁻¹), a bradykinin B2 receptor antagonist, was administered orally five minutes after the end of the surgery. The level of mechanical allodynia was assessed by measuring the frequency of foot withdrawal in response to the application of a 12.5 g on Frey filament at 2, 4, 6, 8 and 24 hr after the drug administration.

Results: Oral administration of JTE522 or indomethacin attenuated the maximum response frequency in a dose-dependent manner at a dose between 1 and 30 mg·kg⁻¹ ($P < 0.05$). Oral FRI 73657, (100 mg·kg⁻¹), had no effect on the maximum response frequency.

Conclusion: These data indicated that a COX-2 inhibitor attenuated the level of mechanical allodynia in the rat model of postoperative pain.

Objectif : Examiner l'effet d'un inhibiteur de la cyclo-oxygénase (COX)-2 sur le maintien d'une allodynie mécanique induite par l'incision cutanée (un modèle animal d'une douleur postopératoire incidente) chez le rat. De plus, comparer l'effet de l'inhibiteur d'une COX-2 avec celui d'un inhibiteur non sélectif de COX-1 et COX-2 et d'un antagoniste du récepteur B2.

Méthode : Une incision cutanée longitudinale de 1 cm a été pratiquée sur la plante du pied. Du JTE522 (1 – 100 mg·kg⁻¹), un inhibiteur de la COX-2, de l'indométhacine (1 – 30 mg·kg⁻¹), un inhibiteur non sélectif de la COX-1 et de la COX-2, ou du FRI 73657 (10 et 100 mg·kg⁻¹), un antagoniste du récepteur de bradykinine B2, a été administré oralement cinq minutes après la fin de l'intervention chirurgicale. Le niveau d'allodynie mécanique a été évalué en mesurant la fréquence du retrait du pied en réaction à l'application d'un filament con Frey de 12,5 g à 2, 4, 6, 8 et 24 h après l'administration du médicament.

Résultats : L'administration orale de JTE522 ou d'indométhacine a diminué la fréquence de réponse maximale d'une manière dépendante de la dose pour des doses entre 1 et 30 mg·kg⁻¹ ($P < 0,05$). Le FRI 73657 oral, (100 mg·kg⁻¹), n'a pas eu d'effet sur la fréquence de réponse maximale.

Conclusion : Ces données indiquent que l'inhibiteur de la COX-2 a diminué le niveau d'allodynie mécanique chez un rat, modèle de douleur postopératoire.

From the Department of Anesthesiology, School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba-shi, Chiba 260, Japan.

Address correspondence to: Tatsuo Yamamoto MD. Phone: 81-43-226-2155; Fax: 81-43-226-2155; E-mail: yamatat@med.m.chiba-u.ac.jp
This study was partly supported by Grant-in-Aid-for Scientific Research (B) 10470314.

Accepted for publication January 16, 2000.

CYCLOOXYGENASE (COX) is an enzyme that catalyzes the conversion of arachidonic acid to prostaglandins. Recently, two different COX forms have been characterized: COX-1 is constitutively expressed, whilst COX-2 is highly inducible in response to inflammatory stimuli.¹ Nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin and indomethacin,² inhibit COX activity and elicit anti-inflammatory and analgesic effects. Most NSAIDs inhibit both COX-1 and COX-2 activity.¹ Recently, COX-2 selective inhibitors have been introduced for clinical use.

Postoperative pain is a common form of acute pain. There are two types of postoperative pain: pain during rest (rest pain) and pain during function (incident pain). In most studies of postoperative pain, the assessment of pain is performed only during rest.³ This is unfortunate, as differences in efficacy among treatment modalities may appear only when pain is assessed during function (incident pain) compared with rest.³ Recent clinical studies revealed that celecoxib, a COX-2 inhibitor, attenuated the level of dental pain after the removal of two or more molar teeth within one hour after the operation and the potency of the analgesic effect of celecoxib is similar to that of aspirin.⁴ These data suggested that, even when given immediately after surgery, COX-2 inhibition attenuated dental pain after the removal of molar teeth. Dental pain, after the removal of molar teeth, is thought to be rest pain. Systemically administered COX-2 inhibitors have not been used in investigations pertaining to incident pain.⁵ Thus, it is important to determine whether selective COX-2 inhibition alleviates postoperative incident pain.

Recently, a new animal model of postoperative pain has been introduced.⁶ The model involves making a 1-cm longitudinal incision of skin, fascia and muscle of the plantar surface of hindpaw in the rat. The most obvious advantage of this model is that it uses the same painful stimulus as human postoperative pain. This incision causes reproducible, quantifiable mechanical allodynia and non-evoked pain behaviour (rest pain) that parallel the postoperative course of patients.⁶

Mechanical stimulation of a wound, by movement or coughing, produces severe postoperative pain - incident pain. In the clinical situation, inflammatory mediators are released at the site of skin incision and mechanical hyperalgesia/allodynia occurs.⁷ If the level of mechanical hyperalgesia/allodynia is high, patients feel severe incident pain, and if the level of mechanical hyperalgesia/allodynia is low, the patients feel less pain. Thus, mechanical allodynia is one aspect of incident pain. In the present study, to examine whether COX-2 selective

inhibition attenuates the postoperative incident pain, we studied the effects of orally administered JTE522, a COX-2 inhibitor⁸ on mechanical allodynia induced by skin incision and compared the effect of JTE522 with that of indomethacin, a non-selective COX-1 and COX-2 inhibitor.

Two bradykinin receptor subtypes, B1 and B2, have been identified.⁹ When bradykinin was injected into the skin of human subjects, it evoked burning pain which was antagonized by a B2 receptor antagonist.¹⁰ The sensitizing effect of PGE₂ on bradykinin receptor has been reported for cutaneous afferent¹¹ and joint afferent.¹² These data indicate that B2 receptor antagonist may have anti-allodynic effects and may potentiate the effect of COX inhibitors on mechanical allodynia induced by skin incision. Recently, a non-peptide, orally-active B2 receptor antagonist, FR173657, has been reported.¹³ In this study, to determine the role of B2 receptor on the development and maintenance of the postoperative incident pain, the authors also examined the effect of oral FR173657 and the interaction of FR173657 with JTE522 or indomethacin on the mechanical allodynia induced by skin incision.

Methods

The investigations were performed under a protocol approved by the Institutional Animal Care Committee, Chiba University, Chiba, Japan. The animals were treated in accordance with the Ethical Guideline for Investigation of Experimental Pain in Conscious Animals as issued by the International Association for the Study of Pain.¹⁴ Male Sprague-Dawley rats weighing 310 to 370 g were used in this study.

Skin incision surgery was performed under halothane anesthesia. As previously described,⁶ a 1 cm longitudinal incision was made with a number 11 blade through skin and fascia of the plantar aspect of the right foot, starting 0.5 cm from the proximal edge of the heel and extending toward the toes. The plantaris muscle was elevated and incised longitudinally, and the muscle origin and insertion remained intact. After hemostasis with gentle pressure, the skin was apposed with two mattress sutures of 5-0 nylon. After surgery, the animals were allowed to recover in their cages.

Mechanical allodynia was evaluated by measuring the frequency of foot withdrawals in response to non-noxious stimuli. The rats were placed in a clear plastic cage (10 × 20 × 24 cm) on an elevated mesh floor (grid: 10 × 10 mm). To initiate a test, a rat was placed in the box and allowed five to ten minutes to habituate. Frequency of paw withdrawal to repetitive applications of a 12.5 g von Frey filament was tested in an unrestrained rat standing on four legs. In this mea-

surement, a von Frey filament (Stoelting, Wood Dale, IL) was applied to the medial side of the wound near the heel of the right paw where primary hyperalgesia/allodynia occurred.¹⁵ The authors chose a 12.5 g von Frey filament because application of a 12.5 g von Frey filament to the foot pad of normal untreated rats elicited no withdrawal response and application of 20.9 g filament produced paw withdrawal response in about 10% of the animals. During one trial, the von Frey filament was repetitively applied to the right hind-paw (incised paw) ten times with an inter-stimulus interval of five seconds. The number of paw withdrawal responses was counted and expressed as the percent response frequency (response frequency (%)).

The drugs were suspended in carboxymethyl cellulose (CMC) 0.5% solution and administered orally in a volume of 1 ml. For oral administration, a stainless steel tube was inserted through the esophagus to the stomach of restrained animals. The agents used in this study were indomethacin (Research Biochemical, Natick, MA), JTE522 (4-(4-cyclohexyl-2-methyloxazol-5-yl)-2-fluorobenzenesulfonamide; Japan Tobacco Inc, Osaka, Japan), FR173657 ((E)-3-(6-acetamido-3-pyridyl)-N-[N-[2-4-dichloro-3-[(2-methyl-8-quinolinyl)oxymethyl]phenyl]-N-methylaminocarbonyl-methyl]acrylamide, Fujisawa Pharmaceutical Co., Osaka Japan) and carboxymethyl cellulose sodium salt (Wako, Osaka, Japan). Preliminary study revealed that, in the normal untreated rats, all the rats administered 100 mg·kg⁻¹ of indomethacin died within seven days after the drug administration, and rats administered 30 mg·kg⁻¹ of indomethacin showed a normal feeding and a normal weight gain. Thus, 30 mg·kg⁻¹ of indomethacin is the highest dose applied in this study because of its toxicity. Oral administration of 100 mg·kg⁻¹ of JTE522 had no effect on feeding and drinking and did not affect weight gain in the normal untreated rats.

Before skin incision, the right hind-paw (incised paw) was tested to provide the base-line response frequency. Five minutes after the end of skin incision, animals received JTE522 (1, 3, 10, 30 and 100 mg·kg⁻¹), indomethacin (1, 3, 10 and 30 mg·kg⁻¹) or FR173657 (10 and 100 mg·kg⁻¹) administered orally. Response frequency of the right hind-paw was measured at 2, 4, 6, 8 and 24 hr after the drug administration in the JTE522 or indomethacin treated group and at 2, 4 and 6 hr after the drug administration in the FR173657 treated rats. To obtain control data, the vehicle was administered orally. To analyze the interaction of FR173657 with indomethacin or JTE522, 100 mg·kg⁻¹ of FR173657 was co-administered with 10 mg·kg⁻¹ indomethacin or 10 mg·kg⁻¹

JTE522 to separate groups of rats and the response frequency of the right hind paw was measured at 2, 4 and 6 hr after the drug administration. After the observation period, the animals were immediately killed with an overdose of barbiturate.

To analyze the effects of drugs on the response frequency, the maximum response frequency (%) during the first eight hours after the drug administration was used. To analyze dose-dependency, the Kruskal-Wallis test with Dunnett's multiple comparison test was used. To compare the response frequency between groups at each time point, we used Kruskal-Wallis test with Dunnett's multiple comparison test. To analyze the interaction between FR173657 with indomethacin or JTE522, we compared the maximum response frequency (%) between the FR173657 treated group and the FR173657 and JTE522 treated group or between the FR173657 treated group and the FR173657 and indomethacin treated group with the Mann-Whitney rank sum test.

Whenever appropriate, results are expressed as mean ± SEM. Critical values that reached $P < 0.05$ level of significance were considered significant.

Results

Oral administration of indomethacin decreased the level of the maximum response frequency (%) in a dose dependent manner at a dose between 1 and 30 mg·kg⁻¹ (Figure 1, $P < 0.005$ by Kruskal-Wallis test). Oral administration of JTE522 also decreased the level of the maximum response frequency (%) in a dose dependent manner at a dose between 1 and 30 mg·kg⁻¹ (Figure 1, $P < 0.05$). The JTE522 dose response curve showed a limited efficacy, with a plateau effect at a dose of 100 mg·kg⁻¹. At the most effective dose employed in this study (indomethacin: 30 mg·kg⁻¹; JTE522: 30 mg·kg⁻¹), indomethacin and JTE522 decreased the peak effect to 50% of the response frequency. Compared with vehicle treated rats, 30 mg·kg⁻¹ of JTE522 and 30 mg·kg⁻¹ indomethacin decreased the response frequency at two, four and six hours after administration (Figure 1, $P < 0.05$). The level of the maximum response frequency in the 30 mg·kg⁻¹ JTE522 treated rats was not different from those in the 30 mg·kg⁻¹ of indomethacin treated rats (JTE522 treated rats ($n = 8$): $46 \pm 15\%$; indomethacin treated rats ($n = 7$): $60 \pm 14\%$; $P > 0.6$ by Mann Whitney test).

Oral administration of FR173657 had no effect on the maximum response frequency compared with vehicle treated group (Figure 2). Coadministration of 100 mg·kg⁻¹ of FR173657 with 10 mg·kg⁻¹ of indomethacin had no effect on the maximum response

FIGURE 1 Orally administered JTE522 and indomethacin on the response frequency.
Upper graph: Time course of 30 mg·kg⁻¹ JTE522 (n = 10) and 30 mg·kg⁻¹ indomethacin (n = 8). For comparison, a time course of the vehicle (0.5% carboxymethyl cellulose (CMC)) treated group (n = 10) is presented. Mean ± SEM. The abscissa shows time after the drug administration (hours), and the ordinate shows the response frequency (%). * *P* < 0.05 compared with 0.5 % CMC treated rats at each time point.
Lower graph: Dose response curves for the effect of JTE522 and indomethacin. Mean ± SEM of seven to ten rats. The abscissa shows the log dose (mg·kg⁻¹), and the ordinate shows the maximum response frequency. * *P* < 0.05 compared with 0.5 % CMC treated rats.

frequency compared with the 10 mg·kg⁻¹ of indomethacin treated group (FR173657 + indomethacin treated rats (n = 6): 75 ± 16%; indomethacin treated rats (n = 7) 87 ± 6%; Figure 3, *P* > 0.1). On the other hand, co-administration of 100 mg·kg⁻¹ FR173657 with 10 mg·kg⁻¹ JTE522 increased

FIGURE 2 Oral 100 mg·kg⁻¹ (n = 8) and 10 mg·kg⁻¹ (n = 8) FR173657 on the response frequency (mean ± SEM). For comparison, a time course of the vehicle (0.5% carboxymethyl cellulose (CMC)) treated group (n = 10) is presented. The abscissa shows time after the drug administration (hr), and the ordinate shows the response frequency. FR173657 had no effect on the response frequency.

the maximum response frequency compared with the 10 mg·kg⁻¹ JTE522 treated group (FR173657 + JTE522 treated rats (n = 6): 93 ± 3%; JTE522 treated rats (n = 10) 56 ± 9%; Figure 3, *P* < 0.05).

Discussion

In this study, the authors demonstrated that either indomethacin, a non-selective COX-1 and COX-2 inhibitor, or JTE522, a selective COX-2 inhibitor, partly attenuated the level of mechanical allodynia induced by skin incision in the rat in a dose-dependent manner. This anti-allodynic effect of indomethacin or JTE522 lasted six hours after drug administration. Oral FR173657, a B2 receptor antagonist, had no effect on mechanical allodynia induced by skin incision at the doses administered in this study. Moreover, there was no synergistic interaction between a B2 receptor antagonist and COX inhibitors on the mechanical allodynia induced by skin incision.

Orally administered JTE522 or indomethacin has been shown to attenuate edema induced by carageenan injection for more than eight hours after administration⁸ and oral FR173657 decreased the edema induced by carageenan injection during the first four hours after administration.¹³ Thus, in the present study, the authors measured the response frequency at 2, 4, 6, 8 and 24 hr

FIGURE 3 Coadministration of 100 mg·kg⁻¹ FR17365 with 10 mg·kg⁻¹ JTE522 or 10 mg·kg⁻¹ indomethacin on the response frequency (mean ± SEM). The abscissa shows the time after the drug administration (hr), and the ordinate shows the response frequency. There is no interaction between FR173657 with JTE522 or indomethacin.

after the oral administration of JTE522 or indomethacin and at 2, 4, and 6 hr after the oral administration of FR173657. In the present study, anti-allodynic effect of indomethacin or JTE522 lasted only six hours. Moreover, a B2 receptor antagonist had no effect on mechanical allodynia. This suggested that, during inflammation, the role of COX and a B2 receptors in the maintenance of allodynia is different from that in the maintenance of edema.

When given two hours after skin incision, JTE522 attenuated the level of mechanical allodynia. COX-2 is induced by inflammatory stimuli at the site of inflammation. Thus, immediately after surgery, the level of COX-2 at the site of skin incision may be low and, therefore, the site of action of JTE522 given two hours

after skin incision may not be at the site of skin incision. In normal conditions, COX-2-like immunoreactivity has been found in the superficial dorsal horn of the spinal cord (Lamina I and II) and around the central canal (Lamina X) and COX-2-like immunoreactivity was not observed in dorsal root ganglion cell bodies.¹⁶ It is possible that oral COX-2 inhibitor acts, at least in part, at the dorsal horn of the spinal cord two hours after skin incision. Willingale *et al.*¹⁶ reported that wind-up of a spinal nociceptive reflex evoked by electrical stimulation of the sural nerve at C-fibre strength was inhibited dose-dependently by *in vivo* indomethacin or SC58125, a COX-2 selective inhibitor. This suggested that, when the spinal cord neurons are rendered hyperexcitable, COX-2 plays an important role in spinal nociceptive transmission. Recently, we have found that intrathecal JTE522 attenuated the level of mechanical allodynia induced by skin incision in a dose dependent manner four hours after the skin incision.¹⁷

Surprisingly, the maximum response frequency in the rats treated with the most effective dose of indomethacin (30 mg·kg⁻¹) is almost the same as that in the rats treated with the most effective dose of JTE522 (30 mg·kg⁻¹). It has been reported that the IC₅₀ values for the inhibition of sheep COX-2 by JTE522 and indomethacin were 0.64 μM and 12 μM and that those for COX-1 were >100 μM and 0.27 μM, respectively.⁸ If COX-1 plays an important role in the development and maintenance of mechanical allodynia induced by skin incision, the potency of indomethacin on mechanical allodynia should be much higher than that of JTE522. Thus, the authors' data suggested that COX-2 plays a more important role in the development and maintenance of mechanical allodynia induced by skin incision than COX-1. Although JTE522 only partly attenuated the level of mechanical allodynia even at the most effective dose, the authors' data suggested that orally administered COX-2 selective inhibitor may alleviate the postoperative incident pain in a clinical condition.

In the present study, administration of 100 mg·kg⁻¹ and 10 mg·kg⁻¹ FR173657 had no effect on mechanical allodynia. It is possible that these doses were too small to produce an anti-allodynic effect. Oral FR173657 was reported to attenuate the level of paw edema induced by carageenan injection in a dose-dependent manner at a dose between 1 and 10 mg·kg⁻¹.¹³ Thus, we believe that 100 mg·kg⁻¹ FR173657 is a sufficient dose to inhibit the inflammatory response. It has been reported that, in cutaneous polymodal receptors, mechanical sensitivity cannot be sensitized by bradykinin but heat sensitivity can be.^{10,18} Thus, the contribution of bradykinin to the peripheral

mechanical sensitization may be small and B2 receptor antagonist may have no effect on the mechanical allodynia induced by skin incision.

The role of prostaglandins on the sensitization of the bradykinin response is not clear. It has been reported that a sensitizing effect of PGE₂ on the bradykinin response was observed at a concentration which by itself did not induce excitation.^{18,19} On the other hand, Lang *et al.*²⁰ reported that a sensitizing effect of PGE₂ on the bradykinin response of nociceptors was not observed in rat skin-nerve preparations. We could not find any interaction of indomethacin with FR173657 on the maintenance of mechanical allodynia induced by skin incision. Coadministration of FR173657 with JTE522 attenuated the effect of JTE522 on the level of mechanical allodynia. Also, we do not know why FR173657 produced such a negative effect on the anti-allodynic effect of JTE522 in this study.

It has been assumed that primary hyperalgesia/allodynia is mediated by an increase in peripheral excitability and that the secondary hyperalgesia/allodynia is mediated by an increase in central excitability.²¹ COX inhibitors produce anti-inflammatory effect mainly by the peripheral mechanisms.¹ Thus, to assess the effect of COX inhibitors on the level of mechanical allodynia, we applied a von-Frey filament to the medial side of the wound where primary hyperalgesia/allodynia occurred.

In conclusion, the study demonstrated that orally administered COX-2 inhibitor attenuated incident pain induced by skin incision in the rat, and the potency of COX-2 inhibitor on the incident pain is similar to that of indomethacin, a COX-1 and COX-2 non-selective inhibitor. B2 receptor did not play an important role in the maintenance of incident postoperative pain.

Acknowledgment

The authors wish to thank Professor Takashi Nishino, Department of Anesthesiology, School of Medicine, Chiba University for his generous support of the study.

References

- 1 Vane JR, Botting RM. Mechanism of action of nonsteroidal anti-inflammatory drugs. *Am J Med* 1998; 104: 2S-8S.
- 2 Vane JR. Inhibitor of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature New Biology* 1971; 231: 232-5.
- 3 Kehlet H. Postoperative pain relief – what is the issue? (Editorial) *Br J Anaesth* 1994; 72: 375-8.
- 4 Lane NE. Pain management in osteoarthritis: the role of COX-2 inhibitors. *J Rheumatol* 1997; 24(Suppl49): 20-4.
- 5 Mitchell JA, Warner TD. Cyclo-oxygenase-2: pharmacology, physiology, biochemistry and relevance to NSAID therapy. *Br J Pharmacol* 1999; 128: 1121-32.
- 6 Brennan TJ, Vandermeulen EP, Gebhart GF. Characterization of a rat model of incisional pain. *Pain* 1996; 64: 493-501.
- 7 Hargreaves KM, Swift JQ, Roszkowski MT, Bowles W, Garry MG, Jackson DL. Pharmacology of peripheral neuropeptide and inflammatory mediator release. *Oral Surg Oral Med Oral Pathol* 1994; 78: 503-10.
- 8 Matsushita M, Masaki M, Yagi Y, Tanaka T, Wakitani K. Pharmacological profile of JTE-522, a novel prostaglandin H synthase-2 inhibitor, in rats. *Inflamm Res* 1997; 46: 461-6.
- 9 Rang HP, Perkins MN. The role of B₁ and B₂ bradykinin receptors in inflammatory pain. In: Borsook D (Ed.). *Molecular Neurobiology of Pain. Progress in Pain Research and Management*, Vol. 9. Seattle: IASP press, 1997: 221-37.
- 10 Manning DC, Raja SN, Meyer RA, Campbell JN. Pain and hyperalgesia after intradermal injection of bradykinin in humans. *Clin Pharmacol Ther* 1991; 50: 721-9.
- 11 Chahl LA, Iggo A. The effects of bradykinin and prostaglandin E₁ on rat cutaneous afferent nerve activity. *Br J Pharmacol* 1977; 59: 343-7.
- 12 Schaible H-G, Schmidt RF. Excitation and sensitization of fine articular afferents from cat's knee joint by prostaglandin E₂. *J Physiol (Lond)* 1988; 403: 91-104.
- 13 Asano M, Inamura N, Hatori C, *et al.* The identification of an orally active, nonpeptide bradykinin B₂ receptor antagonist, FR173657. *Br J Pharmacol* 1997; 120: 617-24.
- 14 Zimmerman M. Ethical guidelines for investigations of experimental pain in conscious animals (Editorial). *Pain* 1983; 16: 109-10.
- 15 Zahn PK, Brennan TJ. Primary and secondary hyperalgesia in a rat model for human postoperative pain. *Anesthesiology* 1999; 90: 863-72.
- 16 Willingale HL, Gardiner NJ, McLymont N, Giblett S, Grubb BD. Prostanoids synthesized by cyclo-oxygenase isoforms in rat spinal cord and their contribution to the development of neuronal hyperexcitability. *Br J Pharmacol* 1997; 122: 1593-604.
- 17 Yamamoto T, Sakashita Y. The role of the spinal opioid receptor Like1 receptor, the NK-1 receptor, and cyclooxygenase -2 in maintaining postoperative pain in the rat. *Anesth Analg* 1999; 89: 1203-8.
- 18 Mizunuma K, Kumazawa T. Modification of nociceptor responses by inflammatory mediators and second messengers implicated in their action – a study in canine testicular polymodal receptors. In: Kumazawa T,

- Kruger L, Mizumura K (Eds.). Progress in Brain Research, Vol 113. The Polymodal Receptor: a Gateway to Pathological Pain. Amsterdam: Elsevier, 1996: 115-41.
- 19 Mizumura K, Sato J, Kumazawa T. Effects of prostaglandins and other putative chemical intermediaries on the activity of canine testicular polymodal receptors studied in vitro. *Pflügers Arch* 1987; 408: 565-72.
- 20 Lang E, Novak A, Reeh PW, Handwerker HO. Chemosensitivity of fine afferents from rat skin in vitro. *J Neurophysiol* 1990; 63: 887-901.
- 21 Simone DA, Sorkin LS, Oh U, et al. Neurogenic hyperalgesia: central neural correlates in responses of spinothalamic tract neurons. *J Neurophysiol* 1991; 66: 228-46.