British Journal of Pharmacology (1997) 120, 1522–1526

Absence of prostaglandin E_2-induced hyperalgesia in NMDA receptor ε subunit knockout mice

*Toshiaki Minami, †Junko Sugatani, ‡Kenji Sakimura, ‡Manabu Abe, #Masayoshi Mishina & [1]†Seiji Ito

*Department of Anesthesiology, Osaka Medical College, Takatsuki 569, †Department of Medical Chemistry, Kansai Medical University, Moriguchi 570, ‡Department of Cellular Neurobiology, Brain Research Institute, Niigata University, Niigata 951, and #Department of Pharmacology, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan

1 We have previously found that intrathecal administration of prostaglandins E_2 (PGE_2) and D_2 (PGD_2) into conscious mice induced hyperalgesia by the hot plate test. The present study investigated the involvement of *N*-methyl-D-aspartate (NMDA) receptor in the prostaglandin-induced hyperalgesia by use of mice lacking NMDA receptor ε1, ε4, or ε1/ε4 subunits.

2 PGE_2 induced hyperalgesia over a wide range of doses from 50 pg to 500 ng kg^{-1} in wild-type mice. But PGE_2 could not induce hyperalgesia in ε1, ε4, or ε1/ε4 subunit knockout mice.

3 The NMDA receptor antagonist D-(−)-2-amino-5-phosphonovaleric acid (D-AP5), the non-NMDA receptor antagonist γ-D-glutamylaminomethyl sulphonic acid (GAMS), and the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME) inhibited the PGE_2-induced hyperalgesia in wild-type mice.

4 PGD_2 induced hyperalgesia at doses of 25 ng to 250 ng kg^{-1} in both wild-type and ε1/ε4 subunit knockout mice. The substance P receptor antagonist CP 96,345 blocked the PGD_2-induced hyperalgesia in wild-type and ε1/ε4 subunit knockout mice.

5 These results demonstrate that the pathways leading to hyperalgesia are different between PGD_2 and PGE_2 and that both ε1 and ε4 subunits of the NMDA receptor are involved in the PGE_2-induced hyperalgesia.

Keywords: Prostaglandins; hyperalgesia; NMDA receptor ε subunit; knockout mice; spinal cord

Introduction

Prostaglandins are formed in virtually all mammalian tissues and involved in various aspects of inflammation including pain (Coleman *et al*., 1990). Recent evidence indicates that prostaglandins are critical for the processing of pain not only by sensitizing the peripheral terminals of primary afferent nociceptors but also by augmenting processing of pain information at the spinal level (Malmberg & Yaksh, 1992a, b; Minami *et al*., 1992; 1994a, c). We previously showed that intrathecal (i.t.) administration of prostaglandin D_2 (PGD_2) and PGE_2 into mice induced hyperalgesia, which was blocked by antagonists for substance P and *N*-methyl-D-aspartate (NMDA) receptors, respectively (Uda *et al*., 1990; Nishihara *et al*., 1995a). The NMDA receptor channel is formed by the ε (NR2) and ζ (NR1) subfamilies (Hollmann & Heinemann, 1994; Mori & Mishina, 1995). Highly active NMDA receptor channels are produced only when the ζ subunit is expressed together with any one of four ε subunits (ε1 – ε4). Because the four ε subunits are distinct in distribution, functional properties, and regulation, the molecular diversity of the ε subunit family is considered to underlie the functional heterogeneity of the NMDA receptor channel (Mishina *et al*., 1993). Although many studies including ours have demonstrated the involvement of glutamate receptors in pain transmission and processing in the spinal cord, these studies have been confined to studies of NMDA versus non-NMDA receptors due to a lack of selective antagonists (Aanonsen & Wilcox, 1987; Urca & Raigorodsky, 1988; Malmberg & Yaksh, 1992a; Minami *et al*., 1994b). To elucidate the physiological significance of ε subunits of the NMDA receptor channel, the gene targeting technique has been employed (Sakimura *et al*., 1995; Ikeda *et al*., 1995; Kutsuwada *et al*., 1996; Ebralidze *et al*., 1996). In the present study to clarify which subtype of the NMDA receptor is involved in the prostaglandin-induced hyperalgesia, we examined the hyperalgesic response in mice lacking NMDA receptor ε1, ε4, or ε1/ε4 subunits.

[1]Author for correspondence at: Department of Medical Chemistry, Kansai Medical University, 10–15 Fumizono, Moriguchi 570, Japan.

Methods

Animals

The mutant mice lacking either the ε1 or ε4 subunit of the NMDA receptor channel were obtained by the gene targeting technique (Sakimura *et al*., 1995; Ikeda *et al*., 1995). The murine ε1 and ε4 subunit genes were localized in different autosomes; chromosomes 16 and 7, respectively (Nagasawa *et al*., 1996). Crossing of the ε1 subunit knockout mouse strain H10 and the ε4 subunit knockout mouse strain G72 yielded the mutant mice lacking both the ε1 and ε4 subunits of the NMDA receptor channel (the ε1/ε4 subunit mutant mice). The genotypes of the mice were determined by Southern blotting analyses of tail DNA as described by Sakimura *et al*. (1995) and Ikeda *et al*. (1995). The ε1, ε4, and ε1/ε4 subunit mutant mice grew and mated normally. The animals were housed under conditions of a 12 h light-dark cycle and a constant temperature of 22 ± 2°C and 60 ± 10% humidity.

Hot plate test

Male mice weighing 20 ± 2 g were used in this study. A 27-gauge stainless-steel needle (0.35 mm, o.d.) attached to a microsyringe was inserted between the L_5 and L_6 vertebrae by a slight modification of the technique of Hylden & Wilcox (1980). The volume of the i.t. injection was 5 μl. Mice were placed on a hot plate maintained at 55°C, and the elapsed time until the mice showed the first avoidance responses (licking the

feet, jumping, or rapidly stamping the paws) was recorded as described previously (Minami *et al*., 1994a). The response time of the mice to the hot plate was measured at 30 min and 10 min after the i.t. injection of PGE_2 and PGD_2, respectively.

The animals were used only for one measurement in each experiment. This study was conducted with the approval of the local ethics committee and in accordance with the guidelines of the Ethics Committee of the International Association for the Study of Pain (Zimmermann, 1983).

Chemicals

PGD_2 and PGE_2 were generous gifts from Ono Central Research Institute (Osaka, Japan). D-(−)-2-Amino-5-phosphonovaleric acid (D-AP5) and γ-D-glutamylaminomethyl sulphonic acid (GAMS) were obtained from Cambridge Research Biochemicals (Cambridge, England). N^{ω}-Nitro-L-arginine methyl ester (L-NAME) was purchased from Sigma (St. Louis, MO, U.S.A.). CP 96,345 (2**S**,3**S**)-*cis*-2-(diphenylmethyl)-N-((2-methoxyphenyl)methyl-1-azabicyclo[2.2.2]octan-3-amine) was a gift from Pfizer Inc. (Groton, CT, U.S.A.). All chemicals were dissolved in sterile saline on the day of experiments and kept on ice until used. All drugs, including saline, were coded to assure blind testing.

Statistics

The statistical analyses were carried out by analysis of variance (ANOVA). Statistical significance ($P<0.05$) was further examined with Duncan's test for multiple comparison.

Results

Effects of i.t. PGE_2 and PGD_2 on hyperalgesia in NMDA receptor ε1/ε4 subunit knockout mice

PGE_2 produced a hyperalgesic action by the hot plate test between 3–30 min after i.t. injection in conscious mice (Uda *et al*., 1990). Because the maximum effect was observed between 15–30 min, here we assessed the hyperalgesic response to PGE_2 at 30 min after i.t. injection. Saline-injected control mice responded to the hot plate with a constant latency (16.0±0.7 s and 15.9±1.2 s, mean±s.e.mean, $n=10$) in wild-type and ε1/ε4 subunit mutant mice, respectively. The PGE_2-induced hyperalgesia was observed in wild-type mice over a range of doses (50 pg–500 ng kg^{-1}) with a maximum effect at 500 ng kg^{-1} (10.2±0.7 s). However, PGE_2 did not show a hyperalgesic effect in ε1/ε4 subunit mutant mice (Figure 1a).

PGD_2 showed a hyperalgesic effect between 3–60 min after i.t. injection and the maximum effect was observed at 10 min. The PGD_2-induced hyperalgesic effect at 10 min was observed at doses of 25 to 250 ng kg^{-1} with a maximum effect at 50 ng kg^{-1} (9.2±0.6 s and 9.8±0.7 s) in wild-type and ε1/ε4 subunit mutant mice, respectively. There was no significant difference in dose dependency of PGD_2 for hyperalgesia between the mutant and wild-type mice (Figure 1b).

To elucidate the difference in the mechanisms of hyperalgesia induced by PGE_2 and PGD_2, we examined the effects of various agents on the PGE_2- and PGD_2-induced hyperalgesia. As shown in Figure 2, the NMDA receptor antagonist D-AP5 (50 μg kg^{-1}), the non-NMDA receptor antagonist GAMS (50 μg kg^{-1}), and the nitric oxide (NO) synthase inhibitor L-NAME (50 ng kg^{-1}) attenuated the hyperalgesia induced by PGE_2 (500 ng kg^{-1}) in wild-type mice. Uda *et al*. (1990) have previously shown that the PGE_2-induced hyperalgesia was not inhibited by a substance P receptor antagonist. On the other hand, the PGD_2 (50 ng kg^{-1})-induced hyperalgesia was blocked by the substance P receptor antagonist CP 96,345 (500 ng kg^{-1}), but not blocked by L-NAME (50 ng kg^{-1}), in both wild-type and ε1/ε4 subunit mutant mice (Figure 3). Be-

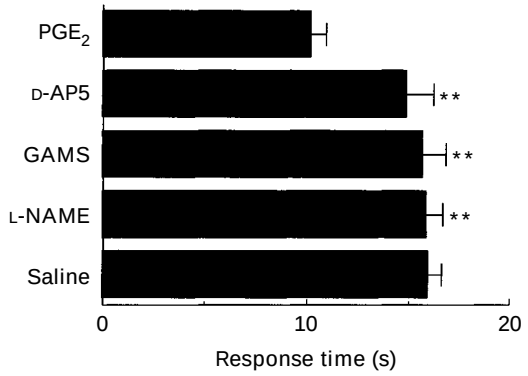

Figure 2 Effect of various agents on PGE_2-induced hyperalgesia in wild-type mice. PGE_2 (500 ng kg^{-1}) was injected alone or simultaneously with D-AP5 (50 μg kg^{-1}), GAMS (50 μg kg^{-1}), or L-NAME (50 ng kg^{-1}) into the subarachnoid space of wild-type mice. The time until the mice showed the first avoidance response to the hot plate test (55°C) was measured 30 min after i.t. injection. Each column represents the mean±s.e.mean of responses in ten mice. Statistical analyses were carried out by Duncan's test. $^{**}P<0.01$, as compared with the PGE_2-injected control group.

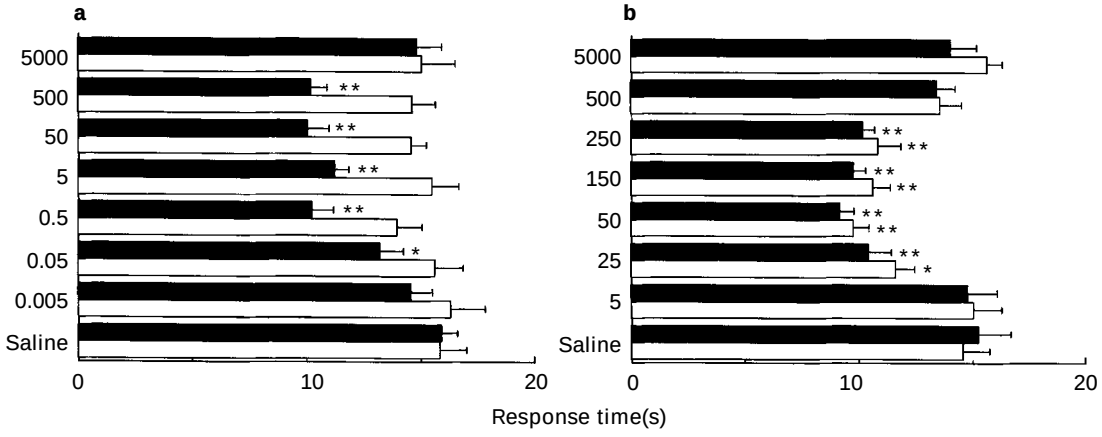

Figure 1 Dose dependency for the effects of i.t. injection of PGE_2 and PGD_2 on hyperalgesia in wild-type and ε1/ε4 subunit mutant mice. Various doses of PGE_2 (a) or PGD_2 (b) were injected into the subarachnoid space of wild-type (solid columns) and ε1/ε4 subunit mutant (open columns) mice. The time until the mice showed the first avoidance response to the hot plate test (55°C) was measured 30 min (PGE_2) or 10 min (PGD_2) after i.t. injection. Each column represents the mean±s.e.mean of responses in ten mice. Statistical analyses were carried out by Duncan's test. $^{*}P<0.05$, $^{**}P<0.01$, as compared with the saline-injected control group.

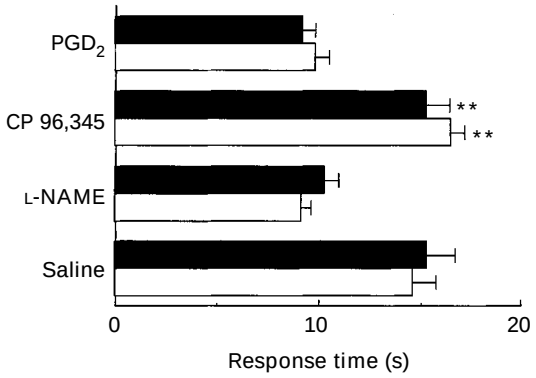

Figure 3 Effects of the substance P receptor antagonist CP 96,345 on PGD_2-induced hyperalgesia in wild-type and $\varepsilon1/\varepsilon4$ subunit mutant mice. PGD_2 (50 ng kg^{-1}) was injected alone or simultaneously with CP 96,345 (500 ng kg^{-1}) or L-NAME (50 ng kg^{-1}) into the subarachnoid space of wild-type (solid columns) and $\varepsilon1/\varepsilon4$ subunit mutant (open columns) mice. The time until the mice showed the first avoidance response to the hot plate test (55°C) was measured 10 min after i.t. injection. Each column represents the mean ± s.e.mean of responses in ten mice. Statistical analyses were carried out by Duncan's test. $^{**}P<0.01$, as compared with the PGD_2-injected control group.

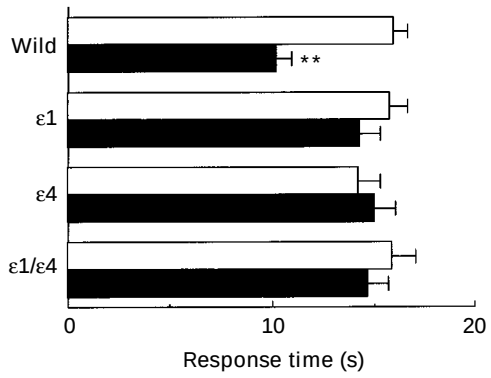

Figure 4 Effects of PGE_2 on hyperalgesia in wild-type, ε1, ε4, or ε1/ε4 subunit mutant mice. PGE_2 (500 ng kg^{-1}, solid columns) or saline (open columns) was injected in wild-type, ε1, ε4, or ε1/ε4 subunit mutant mice. The time until the mice showed the first avoidance response to the hot plate test (55°C) was measured 30 min after i.t. injection. Each column represents the mean ± s.e.mean of responses in ten mice. $^{**}P<0.01$, as compared with the saline-injected control group.

cause animals receiving simultaneous injections of D-AP5 or GAMS with PGD_2 displayed hind-leg muscle flaccidity, the mice did not respond to the hot plate by 20 s.

Induction of hyperalgesia by PGE_2 in ε1 and ε4 subunit mutant mice

To assess which subtype of the NMDA receptor channel is involved in the PGE_2-induced hyperalgesia, we examined the induction of hyperalgesia by PGE_2 in ε1 or ε4 subunit mutant mice in addition to $\varepsilon1/\varepsilon4$ double mutant mice. Figure 4 clearly demonstrates that PGE_2 (500 ng kg^{-1}) lost its hyperalgesic effect in either ε1, ε4 or $\varepsilon1/\varepsilon4$ subunit mutant mice.

Discussion

There is considerable evidence to implicate activation of NMDA receptors and subsequent NO production in the mechanisms that underlie thermal hyperalgesia and, like other regions of the central nervous system such as hippocampus and neocortex, long-term, use-dependent changes are suggested to be involved in central mechanisms associated with hypersensitivity in the spinal cord (Meller & Gebhart, 1993). We previously showed that i.t. injection of PGE_2 induced thermal hyperalgesia, which was blocked by NMDA antagonists and L-NAME (Nishihara *et al*., 1995a; Minami *et al*., 1996). In the present study, we confirmed that the PGE_2-induced hyperalgesia is mediated through the glutamate receptor-NO system in wild-type mice and provided direct evidence that both ε1 and ε4 subunits of NMDA receptor are involved in the PGE_2-induced hyperalgesia. While the ε1 subunit mRNA is found in all regions of the gray matter except for the lamina 2, the ε4 subunit mRNA is faintly expressed in mouse spinal cord at postnatal day 21 (Watanabe *et al*., 1994). Interestingly, the ε4 subunit mRNA is distributed widely in the spinal cord during embryonic and early postnatal periods. Comparing $\varepsilon1/\zeta1$ and $\varepsilon4/\zeta1$ heteromeric NMDA receptor channels, the $\varepsilon1/\zeta1$ channel is more sensitive to Mg^{2+} block than the $\varepsilon4/\zeta1$ channel (Mishina *et al*., 1993). The $\varepsilon1/\zeta1$ channel, but not $\varepsilon4/\zeta1$ channel, is positively modulated by treatment with a protein kinase C activator (Mori *et al*., 1993). Because the ε1 and ε4 subunits are thus distinct in distribution, properties and regulation, Mishina *et al*. (1993) proposed that ε1 subunit as well as ε2 subunit plays an important role in synaptic plasticity and that ε4 subunit may mediate synaptic transmission at early stages of development. Consistent with this notion, recent studies have demonstrated that the NMDA-receptor channel currents and long-term potentiation are significantly reduced at the hippocampal CA1 synapses in ε1 subunit mutant mice and that the mutant mice exhibit moderate deficiency in spatial learning (Sakimura *et al*., 1995). On the other hand, whereas the ε4 subunit mutant mice showed a reduced spontaneous locomotor activity, no obvious deficit in motor activity and anxiety tests was observed in the mutant mice (Ikeda *et al*., 1995). We demonstrated here that the PGE_2-induced hyperalgesia was completely abolished in ε4 subunit mutant mice and that the ε4 subunit may also play a role in neural functions in the central nervous system.

Activation of primary afferent C fibres by noxious stimuli gives rise to spinal release of the excitatory amino acids, glutamate and aspartate, and neuropeptides including substance P and these mediators may facilitate the cascade of nociceptive processing (Malmberg & Yaksh, 1995). Previous studies on pain transmission suggested mutual interactions between substance P and glutamate, and prostaglandins. The NMDA receptor activation evokes the spinal release of PGE_2 (Sorkin, 1993). Substance P elicits a time- and dose-dependent release of PGD_2 and PGE_2 from rat spinal cord astrocytes (Marriott *et al*., 1991). Non-steroidal anti-inflammatory drugs administered intrathecally blocked the excessive sensitivity to pain induced by the activation of spinal glutamate and substance P receptors and by subcutaneous injection of formalin (Malmberg & Yaksh, 1992a, b). These observations suggested that the effects of non-steroidal anti-inflammatory drugs are probably mediated by inhibition of prostaglandin synthesis in the spinal cord. Conversely, PGE_2 could enhance the release of glutamate from rat spinal cord synaptomoses (Nishihara *et al*., 1995b) and the release of substance P from isolated neurones of the avian dorsal root ganglion (Nicol *et al*., 1992). Taken together with the present study, these results suggest that PGE_2 may enhance the release of glutamate from C fibres induced by thermal stimulation, which acts on two classes of ionotropic glutamate receptor, NMDA- and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-types, on the same postsynaptic membranes in wild-type mice. Depolarization produced by activation of the AMPA receptor removes the Mg^{2+} block on NMDA receptors, allowing for NMDA receptor activation, an influx of Ca^{2+}, NO and prostaglandin production. Alterations in these biochemical cascades and circuits in the spinal cord will evoke a long-lasting facilitation of spinal pain processing by noxious stimuli.

Substance P is found in high concentrations in small diameter primary afferent axons which terminate in the superficial

laminae of dorsal horn (Hökfelt *et al*., 1975) and is released into the dorsal horn upon noxious stimulation (Otsuka & Konishi, 1976; Duggan *et al*., 1987). In the dorsal horn, substance P preferentially excites nociceptive neurones and this excitation is blocked by the substance P receptor antagonist, CP 96,345 (De Koninck & Henry, 1991). Substance P, ad ministered by intrathecal injection, facilitates spinal nociceptive reflex function and CP 96,345 blocks the substance P-induced facilitation and nociceptive responses to noxious thermal and chemical stimuli (Yashpal *et al*., 1993). Consistent with the localization of the ε1 subunit mRNA mentioned above, it has recently been shown that substance P receptors are expressed in spinothalamic tract neurones in laminae I and III-V and they are monosynaptic targets for peptidergic C afferents (Marshall *et al*., 1996). On the basis that substance P and glutamate coexist in the terminals of the C fibres, it has been postulated that substance P can activate nociceptive-specific dorsal neurones, possibly by a mechanism involving glutamate and the NMDA receptor (De Biasi & Rustioni, 1988; Urban *et al*., 1994). In fact, several studies have suggested that substance P and excitatory amino acids interact synergistically to induce central stabilization through substance P and NMDA receptors (Xu *et al*., 1992). Both substance P and NMDA receptor antagonists attenuate or block the hypersensitivity of spinal neurones evoked by noxious stimulation and the combined administration of these antagonists produced an enhanced inhibitory effect which suggests a synergistic interaction between these two types of antagonist. On the other hand, previous studies have suggested that substance P is neither necessary nor sufficient to elicit pain, and cannot be a traditional neurotransmitter of nociception (Bossut *et al*., 1988). Thus some controversy exists about the exact role of substance P in pain transmission in the spinal cord. It is difficult to distinguish a direct postsynaptic action from polysynaptically mediated events by use of receptor agonists and antagonists. Although the involvement of non-NMDA receptors in the PGD_2-induced hyperalgesia remains unknown, the present study clearly demonstrated that the PGD_2-evoked hyperalgesia is mediated by substance P (Figure 3) and that the deprivation of ε1 and ε4 subunits of NMDA receptors does not affect the PGD_2-evoked hyperalgesia (Figure 1b). The present study shows that the gene targeting technique provides a valuable tool for investigation of pain transmission and central hypersensitivity associated with chronic pain at the molecular levels.

We thank Ms Rie Natsume of Niigata University and members of Department of Medical Chemistry, Kansai Medical University for animal care. This work was supported in part by Grants-in-Aid for Scientific Research on Priority Areas and Encouragement of Young Scientists (06671243) from the Ministry of Education, Science, and Culture of Japan, and by grants from the Science Research Promotion Fund of the Japan Private School Promotion Foundation, the Japan Medical Association and the Ono Medical Research Foundation.

References

AANONSEN, L.M. & WILCOX, C.L. (1987). Nociceptive action of excitatory amino acids in the mouse: effects of spinally administered opioids, phencyclidine and σ agonists. *J. Pharmacol. Exp. Ther*., **243,** 9 – 19.

BOSSUT, D., FRENK, H. & MAYER, D.J. (1988). Is substance P a primary afferent neurotransmitter for nociceptive input? IV. 2-Amino-5-phosphonovalerate (APV) and [D-Pro^2, D-Trp^{7,9}]-substance P exert different effects on behaviors induced by intrathecal substance P, strychnine and kainic acid. *Brain Res*., **455,** 247 – 253.

COLEMAN, R.A., KENNEDY, I., HUMPHREY, P.P.A., BUNCE, K. & LUMLEY, P. (1990). Prostanoids and their receptors. In *Comprehensive Medical Chemistry*, Vol. 3. ed. Hansch, C., Sammes, P.G., Taylor, J.B. & Emmett, J.C. pp. 643 – 714. Oxford: Pergamon Press.

DE BIASI, S. & RUSTIONI, A. (1988). Glutamate and substance P coexist in primary afferent terminals in the superficial laminae of spinal cord. *Proc. Natl. Acad. Sci. U.S.A*., **85,** 7820 – 7824.

DE KONINCK, Y. & HENRY, J.L. (1991). Substance P-mediated slow excitatory postsynaptic potential elicited in dorsal horn neurons in vivo by noxious stimulation. *Proc. Natl. Acad. Sci. U.S.A*., **88,** 11344 – 11348.

DUGGAN, A.W., MORTON, C.R., ZHAO, Z.Q. & HENDRY, I.A. (1987). Noxious heating of the skin releases immunoreactive substance P in the substantia gelatinosa of the cat: a study with antibody microprobes. *Brain Res*., **403,** 345 – 349.

EBRALIDZE, A.K., ROSSI, D.J., TONEGAWA, S. & SLATER, N.T. (1996). Modification of NMDA receptor channels and synaptic transmission by targeted disruption of the NR2C gene. *J. Neurosci*., **16,** 5014 – 5025.

HÖKFELT, T., KELLERTH, J.-O., NILSSON, G. & PERNOW, B. (1975). Substance P: localization in the central nervous system and in some primary sensory neurons. *Science*, **190,** 889 – 891.

HOLLMANN, M. & HEINEMANN, S. (1994). Cloned glutamate receptors. *Annu. Rev. Neurosci*., **17,** 31 – 108.

HYLDEN, J.K.L. & WILCOX, G. (1980). Intrathecal morphine in mice: a new technique. *Eur. J. Pharmacol*., **67,** 313 – 316.

IKEDA, K., ARAKI, K., TAKAYAMA, C., INOUE, Y., YAGI, T., AIZAWA, S. & MISHINA, M. (1995). Reduced spontaneous activity of mice defective in the ε4 subunit of the NMDA receptor channel. *Mol. Brain Res*., **33,** 61 – 71.

KUTSUWADA, T., SAKIMURA, K., MANABE, T., TAKAYAMA, C., KATAKURA, N., KISHIYA, E., NATSUME, R., WATANABE, M., INOUE, Y., YAGI, T., AIZAWA, S., ARAKAWA, M., TAKAHASHI, T., NAKAMURA, Y., MORI, H. & MISHINA, M. (1996). Impairment of suckling response, trigeminal neuronal pattern formation, and hippocampal LTD in NMDA receptor ε2 subunit mutant mice. *Neuron*, **16,** 333 – 344.

MALMBERG, A.B. & YAKSH, T.L. (1992a). Hyperalgesia mediated by spinal glutamate or substance P receptor blocked by spinal cyclooxygenase inhibition. *Science*, **257,** 1276 – 1279.

MALMBERG, A.B. & YAKSH, T.L. (1992b). Antinociceptive actions of spinal nonsteroidal anti-inflammatory agents on the formalin test in the rat. *J. Pharmacol. Exp. Ther*., **263,** 136 – 146.

MALMBERG, A.B. & YAKSH, T.L. (1995). The effect of morphine on formalin-evoked behaviour and spinal release of excitatory amino acids and prostaglandin E_2 using microdialysis in conscious rats. *Br. J. Pharmacol*., **114,** 1069 – 1075.

MARRIOTT, D.R., WILKIN, G.P. & WOOD, J.N. (1991). Substance P-induced release of prostaglandins from astrocytes: regional specialisation and correlation with phosphoinositol metabolism. *J. Neurochem*., **56,** 259 – 265.

MARSHALL, G.E., SHEHAB, S.A.S., SPIKE, R.C. & TODD, A.J. (1996). Neurokinin-1 receptors on lumbar spinothalamic neurons in the rat. *Neuroscience*, **72,** 255 – 263.

MELLER, S.T. & GEBHART, G.F. (1993). Nitric oxide (NO) and nociceptive processing in the spinal cord. *Pain*, **52,** 127 – 136.

MINAMI, T., UDA, R., HORIGUCHI, S., ITO, S., HYODO, M. & HAYAISHI, O. (1992). Allodynia evoked by intrathecal administration of prostaglandin $F_{2\alpha}$ to conscious mice. *Pain*, **50,** 223 – 229.

MINAMI, T., NISHIHARA, I., UDA, R., ITO, S., HYODO, M. & HAYAISHI, O. (1994a). Characterization of EP-receptor subtypes involved in allodynia and hyperalgesia induced by intrathecal administration of prostaglandin E_2 to mice. *Br. J. Pharmacol*., **112,** 735 – 740.

MINAMI, T., NISHIHARA, I., UDA, R., ITO, S., HYODO, M. & HAYAISHI, O. (1994b). Involvement of glutamate receptors in allodynia induced by prostaglandins E_2 and $F_{2\alpha}$ injected to conscious mice. *Pain*, **57,** 225 – 231.

MINAMI, T., UDA, R., HORIGUCHI, S., ITO, S., HYODO, M. & HAYAISHI, O. (1994c). Allodynia evoked by intrathecal administration of prostaglandin E_2 to conscious mice. *Pain*, **57,** 217–223.

MINAMI, T., SAKAI, M., HARA, N., ONAKA, M., MORI, H. & ITO, S. (1996). Nitric oxide mediates hyperalgesia induced by intrathecal administration of prostaglandin E_2 in conscious mice. *Pain Res.*, **11,** 63–70.

MISHINA, M., MORI, H., ARAKI, K., KUSHIYA, E., MEGURO, H., KUTSUWADA, T., KASHIWABUCHI, N., IKEDA, K., NAGASAWA, M., YAMAZAKI, M., MASAKI, H., YAMAKURA, T., MORITA, T. & SAKIMURA, K. (1993). Molecular and functional diversity of the NMDA receptor channel. *Ann. New York Acad. Sci.*, **707,** 136–152.

MORI, H. & MISHINA, M. (1995). Structure and function of the NMDA receptor channel. *Neuropharmacology*, **34,** 1219–1237.

MORI, H., YAMAKURA, T., MASAKI, H. & MISHINA, M. (1993). Involvement of the carboxyl-terminal region in modulation by TPA of the NMDA receptor channel. *NeuroReport*, **4,** 519–522.

NAGASAWA, M., SAKIMURA, K., MORI, K.J., BEDELL, M.A., COPELAND, N.G., JENKINS, N.A. & MISHINA, M. (1996). Gene structure and chromosomal localization of the mouse NMDA receptor channel subunits. *Mol. Brain Res.*, **36,** 1–11.

NICOL, G.D., KLINGBERG, D.K. & VASKO, M.R. (1992). Prostaglandin E_2 increases calcium conductance and stimulates release of substance P in avian sensory neurons. *J. Neurosci.*, **12,** 1917–1927.

NISHIHARA, I., MINAMI, T., UDA, R., ITO, S., HYODO, M. & HAYAISHI, O. (1995a). Effect of NMDA receptor antagonists on prostaglandin E_2-induced hyperalgesia in conscious mice. *Brain Res.*, **677,** 138–144.

NISHIHARA, I., MINAMI, T., WATANABE, Y., ITO, S. & HAYAISHI, O. (1995b). Prostaglandin E_2 stimulates glutamate release from synaptosomes of rat spinal cord. *Neurosci. Lett.*, **196,** 57–60.

OTSUKA, M. & KONISHI, S. (1976). Release of substance P-like immunoreactivity from isolated spinal cord of newborn rat. *Nature*, **264,** 83–84.

SAKIMURA, K., KUTSUWADA, T., ITO, I., MANABE, T., TAKAYAMA, C., KUSHIYA, E., YAGI, T., AIZAWA, S., INOUE, Y., SUGIYAMA, H. & MISHINA, M. (1995). Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor ε1 subunit. *Nature*, **373,** 151–155.

SORKIN, L.S. (1993). It ketorolac blocks NMDA-evoked spinal release of prostaglandin E2 (PGE2) and thromboxane B2 (TXB2). *Anesthesiology*, **79,** A909.

UDA, R., HORIGUCHI, S., ITO, S., HYODO, M. & HAYAISHI, O. (1990). Nociceptive effects induced by intrathecal administration of prostaglandin D_2, E_2, or $F_{2\alpha}$ to conscious mice. *Brain Res.*, **510,** 26–32.

URBAN, L., THOMPSON, S.W.N. & DRAY, A. (1994). Modulation of spinal excitability: co-operation between neurokinin and excitatory amino acid neurotransmitters. *Trends Neurosci.*, **17,** 432–438.

URCA, G. & RAIGORODSKY, G. (1988). Behavioral classification of excitatory amino acid receptors in mouse spinal cord. *Eur. J. Pharmacol.*, **153,** 211–220.

WATANABE, M., MISHINA, M. & INOUE, Y. (1994). Distinct spatiotemporal distributions of the N-methyl-D-aspartate receptor channel subunit mRNAs in the mouse cervical cord. *J. Comp. Neurol.*, **345,** 314–319.

XU, X.-J., DALSGAARD, C.-J. & WIESENFELD-HALLIN, Z. (1992). Spinal substance P and N-methyl-D-aspartate receptors are coactivated in the induction of central sensitization of the nociceptive flexor reflex. *Neuroscience*, **51,** 641–648.

YASHPAL, K., RADHAKRISHNAN, V., CODERRE, T.J. & HENRY, J.L. (1993). CP-96,345, but not its steroisomer, CP-96,344, blocks the nociceptive responses to intrathecally administered substance P and to noxious thermal and chemical stimuli in the rat. *Neuroscience*, **52,** 1039–1047.

ZIMMERMANN, M. (1983). Ethical guidelines for investigations of experimental pain in conscious animals. *Pain*, **16,** 109–110.

(Received November 18, 1996
Received January 2, 1997
Accepted January 9, 1997)