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Inhibition of nociceptin-induced allodynia in conscious mice by prostaglandin D_2

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1 We recently showed that intrathecal administration of nociceptin induced allodynia by innocuous tactile stimuli and hyperalgesia by noxious thermal stimuli in conscious mice. In the present study, we examined the effect of prostaglandins on nociceptin-induced allodynia and hyperalgesia.

2 Prostaglandin D_2 (PGD_2) blocked the allodynia induced by nociceptin in a dose-dependent manner with an IC_{50} of 26 ng kg^{-1}, but did not affect the nociceptin-induced hyperalgesia at doses up to 500 ng kg^{-1}. BW 245C (an agonist for PGD (DP) receptor) blocked the allodynia with an IC_{50} of 83 ng kg^{-1}.

3 The blockade of nociceptin-induced allodynia by PGD_2 was reversed by the potent and selective DP-receptor antagonist BW A868C in a dose-dependent manner with an ED_{50} of 42.8 ng kg^{-1}.

4 Glycine (500 ng kg^{-1}) almost completely blocked the nociceptin-induced allodynia. A synergistic effect on the inhibition of nociceptin-evoked allodynia was observed between glycine and PGD_2 at below effective doses.

5 Dibutyryl cyclic AMP, but not dibutyryl cyclic GMP, blocked the nociceptin-induced allodynia with an IC_{50} of 2.9 μg kg^{-1}.

6 PGE_2, $PGF_{2\alpha}$, butaprost (an EP_2 agonist) and cicaprost (a PGI receptor agonist) did not affect the nociceptin-induced allodynia.

7 These results demonstrate that PGD_2 inhibits the nociceptin-evoked allodynia through DP receptors in the spinal cord and that glycine may be involved in this inhibition.

Keywords: Nociceptin; prostaglandin D_2; allodynia; hyperalgesia; spinal cord; glycine

Introduction

The dorsal horn of the spinal cord is an important site for pain transmission and many substances are involved in the modulation of incoming pain information (Yaksh & Aimone, 1989). Recent studies from our laboratory and others have demonstrated that prostaglandins are critical for the processing of pain not only by sensitizing the peripheral terminals of primary afferent nociceptors but also by augmenting the processing of pain information at the spinal level. For example, intrathecal (i.t.) administration of acetylsalicyclic acid, indomethacin, and other non-steroidal anti-inflammatory drugs produce analgesia (Malmberg & Yaksh, 1992a,b), and abolish the enhanced excitability of dorsal horn convergent neurones to both noxious and innocuous mechanical stimuli after ischaemia (Gelgor & Mitchell, 1995). Conversely, i.t. injection of prostaglandin D_2 (PGD_2) and prostaglandin E_2 (PGE_2) induced hyperalgesic effects to the noxious stimuli (Taiwo & Levine, 1986; Uda *et al*., 1990; Minami *et al*., 1994a). In addition, i.t. administration of PGE_2 and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) induced allodynia, a state of discomfort and pain, evoked by innocuous tactile stimuli; the mice showed squeaking, biting, and scratching movements in response to low-threshold stimuli (Minami *et al*., 1992; 1994c). PGD_2 is the most prominent prostaglandin synthesized in the central nervous system of mammals (Abdel-Halim *et al*., 1977; Shimizu *et al*., 1979). PGD_2 is involved in the modulation of important neural functions such as thermoregulation and sleep-wake regulation (Ito *et al*., 1989; Shimizu & Wolfe, 1990; Hayaishi, 1991). Whereas PGD_2 itself induces hyperalgesia, it can block the PGE_2-evoked allodynia through DP receptors in the spinal cord. Therefore, we suggested that endogenous PGD_2 may play an inhibitory role in the appearance of allodynia under physiological conditions (Minami *et al*., 1996).

Opioid receptors are distributed throughout the central and peripheral nervous systems and opioid drugs are powerful pharmacological substances widely used in mammals for their analgesic properties. Recently, the heptadecapeptide called nociceptin/orphanin FQ (hereafter nociceptin) has been identified as an endogenous ligand of the opioid-like receptor ORL_1 or ROR-C (Meunier *et al*., 1995; Reinscheid *et al*., 1995; Okuda-Ashitaka *et al*., 1996). Different from the other endogenous opioids which produce analgesic effects, i.t. administration of nociceptin induces both hyperalgesia and allodynia (Okuda-Ashitaka *et al*., 1996; Hara *et al*., 1997). We also showed that nociceptin-evoked pain responses were blocked by a simultaneous i.t. injection of glycine. Furthermore, we demonstrated that the glutamate-nitric oxide system might be involved in the nociceptin-evoked allodynia (Hara *et al*., 1997), in a manner similar to that induced by PGE_2 (Minami *et al*., 1994b). This raised the possibility that the nociceptin-induced allodynia may be mediated by a pathway common to the PGE_2-induced one. To examine this possibility, here we studied the effect of PGD_2 on the nociceptin-induced pain transmission by simultaneous administration of prostaglandins and nociceptin into conscious mice.

Methods

Studies on allodynia

Male ddY-mice weighing 22 ± 2 g were used in this study. The animals were housed under conditions of a 12 h light-dark cycle, a constant temperature of $22 \pm 2^{\circ}$C, and $60 \pm 10\%$ humidity. 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 and drugs in vehicle were injected slowly into the subarachnoid space of conscious mice by a slight modification of the method of Hylden and Wilcox (1980).

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Studies on allodynia were carried out as described previously (Okuda-Ashitaka *et al*., 1996). The mice were divided into various groups ($n=6$/group). Drug-treatment groups were injected with 5 μl of vehicle containing various doses of test agents. Control mice were given physiological saline (5 μl). After the i.t. injection, each mouse was placed in an individual $13\times8.5\times13$ cm Plexiglas enclosure with wood chips on the floor and observed. Allodynia was assessed once every 5 min over a 50 min period by light stroking of the flank of the mice with a paintbrush. The allodynic response was ranked as follows: 0, no response; 1, mild squeaking with attempts to move away from the stroking probe; 2, vigorous squeaking evoked by the stroking probe, biting at the probe, or strong efforts to escape. The maximum possible score for allodynia of 6 mice was $2\times6=12$ in any 5 min period and was taken as 100%. To evaluate the effect of agents on allodynia, we assessed the effect at the maximal score of allodynia obtained 10 min after i.t. injection of 2.5 ng kg^{-1} nociceptin. The animals were used only for one experiment.

Hot plate test

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 (Okuda-Ashitaka *et al*., 1996). The response time of the mice to the hot plate was measured at 10 and 15 min, the points of the maximal hyperalgesic effect obtained with PGD_2 and nociceptin, respectively. The animals were used only for one measurement.

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

The following prostanoids were generous gifts: PGD_2, PGE_2, and $PGF_{2\alpha}$ from Ono Central Research Institute (Osaka, Japan); BW 245C (5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropylamino) hydantoin; Whittle *et al*., 1983) and BW A868C (3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino; hydantoin) Giles *et al*., 1989) from Dr P. M. Raper of Wellcome Research Laboratories (U.K.); cicaprost and ZK 110841 (9-chloro-15-cyclohexyl-11,15-dihydroxypentano-5,13-prostadi-enoic acid; Thierauch *et al*., 1988; Ito *et al*., 1990) from Dr K.-H. Thierauch (Schering AG, Berlin, Germany); butaprost from Dr P.J. Gardiner of Bayer (U.K.). Nociceptin and substance P were obtained from Peptide Institute (Osaka, Japan). Dibutyryl adenosine 3′:5′-cyclic monophosphate (db-cyclicAMP) and dibutyryl cyclic GMP (db-cyclicGMP) were purchased from Sigma (St. Louis, MO, U.S.A.). All chemicals were dissolved in sterile saline on the day of the experiments and kept on ice until used. All drugs, including saline, were coded to assure blind testing.

Statistics

Data for hyperalgesia were analysed by parametric ANOVA and statistical significance ($P<0.05$) was further examined by Duncan's test. Data for allodynia were analysed by non-parametric ANOVA and statistical significance ($P<0.05$) was further examined by Williams' test for multiple comparison. IC_{50} values with 95% confidence limits (95% CL) were calculated by use of the computer programme of Probit test.

Results

Blockade of nociceptin-induced allodynia by PGD_2

When tactile stimuli were applied to the flank every 5 min after i.t. injection of 2.5 ng kg^{-1} nociceptin, prominent allodynic responses such as vocalization, biting and escape from the probe, were observed in all mice and continued over the 50 min experimental period (Figure 1a). The i.t. administration of saline had no effect on allodynia. There was no difference in behavioural responses to tactile stimuli between control mice (no i.t. injection), mice injected with saline and those injected with nociceptin.

We examined the effect of prostaglandins on the nociceptin-induced allodynia. Simultaneous injection of PGD_2 (500 ng kg^{-1}) almost completely blocked the nociceptin (2.5 ng kg^{-1})-induced allodynia over the 50 min experimental period (Figure 1a). Figure 1b shows the dose-dependence of the inhibitory effect of this prostaglandin on nociceptin-induced allodynia. The allodynia caused by nociceptin was dose-dependently blocked by PGD_2 with an IC_{50} value (95% CL) of 26 ng kg^{-1} (2.82 ng kg^{-1} – 116 ng kg^{-1}). However, other prostaglandins, PGE_2, $PGF_{2\alpha}$, butaprost (an agonist for EP_2 subtype of PGE receptor) and cicaprost (a PGI receptor agonist), did not affect the nociceptin-induced allodynia at doses up to 500 ng kg^{-1} (Figure 1b).

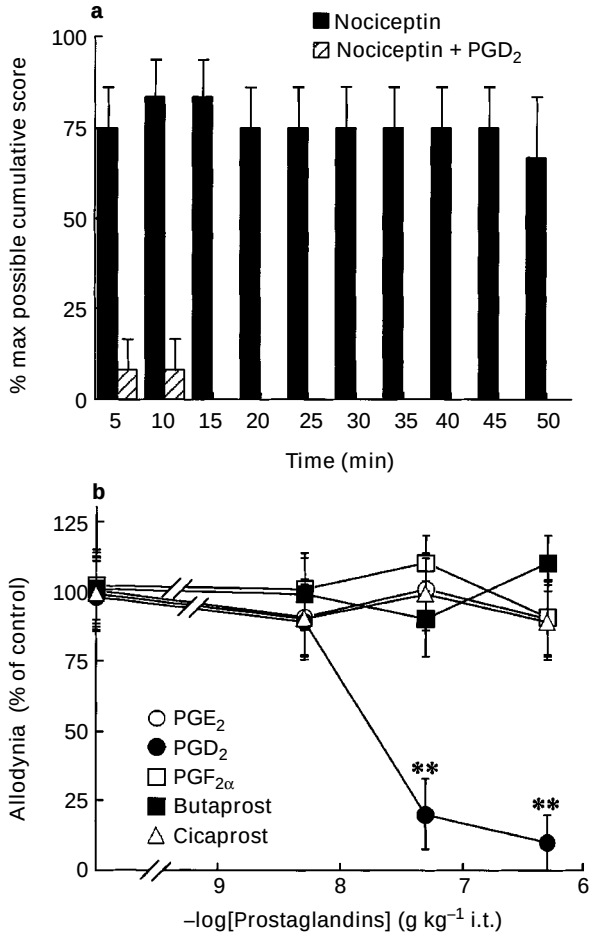

Figure 1 Effect of PGD_2 on nociceptin-induced allodynia. (a) Time course: nociceptin (2.5 ng kg^{-1}) was injected i.t. into conscious mice without or with 500 ng $kg^{-1}PGD_2$. Each column represents the % of the maximum possible cumulative score of 6 mice evaluated every 5 min. Allodynia was assessed as described under Methods. (b) Dose-dependence: nociceptin (2.5 ng kg^{-1}) was injected simultaneously with various doses of PGD_2, PGE_2, butaprost, $PGF_{2\alpha}$, or cicaprost into the subarachnoid space of conscious mice. Assessment of the nociceptin-induced allodynia was made 10 min after i.t. injection and the score of nociceptin alone (83.3% of the maximum possible cumulative score) was taken as 100%. The values shown are the mean ($n=6$); vertical lines indicate s.e.mean. Statistical analyses were carried out by Williams' test. $^{**}P<0.01$, as compared with the nociceptin-injected group.

Effect of DP agonists and a DP antagonist on nociceptin-evoked allodynia

To clarify whether the blockade of the nociceptin-induced allodynia by PGD_2 was mediated by a DP receptor, we examined the effect of two DP-receptor agonists BW 245C and ZK 110841 and the selective and potent DP-receptor antagonist BW A868C on the allodynia. The allodynia caused by nociceptin was dose-dependently blocked by BW 245C with an IC_{50} value (95% CL) of 83 ng kg^{-1} (10.3 ng kg^{-1}–2.2 μg kg^{-1}), but was not affected by ZK 110841 at doses up to 500 ng kg^{-1} (Figure 2). The dose-dependence of the inhibitory effect of BW 245C on allodynia was similar to that of PGD_2. As shown in Figure 3, the blockade by 500 ng kg^{-1} PGD_2 of the nociceptin-induced allodynia was reversed by BW A868C in a dose-dependent manner with an ED_{50} value of 42.8 ng kg^{-1}. Although hyperalgesia induced by PGD_2 has been shown to be mediated by substance P (Uda *et al*., 1990), the nociceptin-induced allodynia in our study was not blocked by substance P (Figure 2).

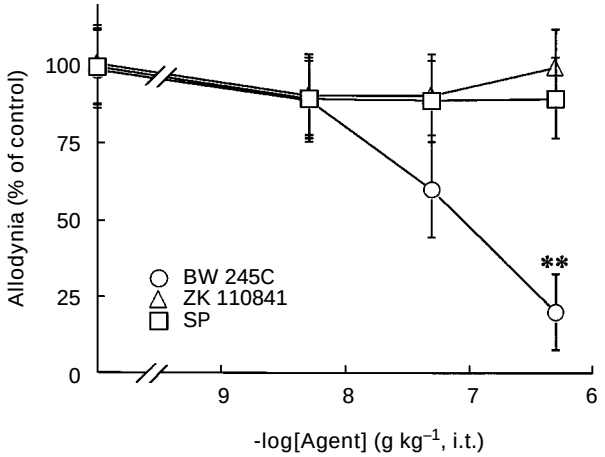

Figure 2 Effect of DP agonists and substance P on the nociceptin-induced allodynia. Nociceptin (2.5 ng kg^{-1}) was injected simultaneously with the indicated doses of BW 245C, ZK 110841, or substance P (SP) into the subarachnoid space. Assessment of allodynia was made at 10 min after i.t. injection. See details for allodynia in the legend of Figure 1b. $^{**}P<0.01$, as compared with the nociceptin-injected group.

Synergistic effect of glycine and PGD_2 on inhibition of nociceptin-induced allodynia

We recently showed that i.t. glycine attenuated the nociceptin-induced allodynia (Hara *et al*., 1997). As shown in Figure 4, glycine alone almost completely blocked the nociceptin-evoked allodynia at 500 ng kg^{-1}. To clarify the site of action of PGD_2, we examined the effect of glycine on the dose-response curve of PGD_2 for inhibition of the nociceptin-evoked allodynia. PGD_2 did not significantly affect the nociceptin-induced allodynia at doses below 5 ng kg^{-1} (Figures 1b and 4). Neither did glycine significantly affect the nociceptin-induced allodynia at doses below 5 ng kg^{-1} (Figure 4). However, PGD_2 blocked the nociceptin-evoked allodynia in the presence of 5 ng kg^{-1} glycine in a dose-dependent manner (5 pg–5 ng kg^{-1}) and the extent of inhibition by a combination of 5 ng kg^{-1} PGD_2 and 5 ng kg^{-1} glycine was similar to that by 500 ng kg^{-1} PGD_2 or 500 ng kg^{-1} glycine alone (Figures 1b and 4). A similar synergistic effect was observed with the combination of PGD_2 (5 pg–5 ng kg^{-1}) and 0.5 ng kg^{-1} glycine, and 5 ng kg^{-1} PGD_2 and 50 ng kg^{-1} glycine (Figure 4). These results demonstrate that glycine may be involved in the inhibition of nociceptin-evoked allodynia by PGD_2.

Effect of db-cyclicAMP and db-cyclicGMP on nociceptin-evoked allodynia

The DP receptor is coupled to stimulation of adenylate cyclase. Hence, we examined the effect of db-cyclicAMP and db-cyclicGMP, membrane-permeable analogues of cyclicAMP and cyclicGMP, on the nociceptin-induced allodynia. As shown in Figure 5, db-cyclicAMP, but not db-cyclicGMP, dose-dependently blocked the nociceptin-induced allodynia with an IC_{50} value (95% CL) of 2.92 μg kg^{-1} (31.6 ng kg^{-1}–68 μg kg^{-1}). Neither db-cyclicAMP nor db-cyclicGMP induced allodynia alone at a dose of 50 μg kg^{-1} (data not shown).

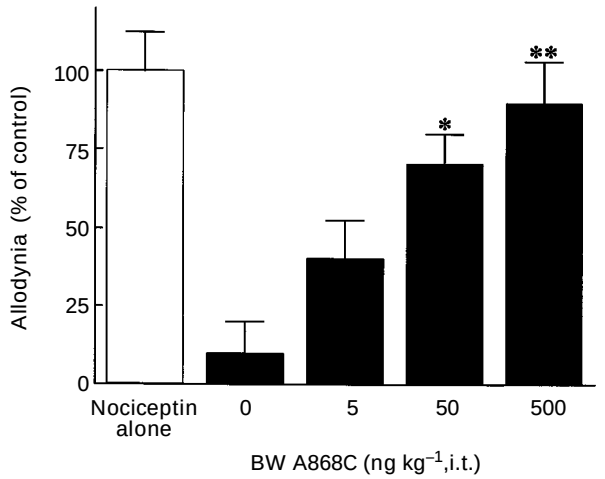

Figure 3 Effect of DP antagonist on PGD_2-induced inhibition of the allodynia evoked by nociceptin. Nociceptin (2.5 ng kg^{-1}) was injected i.t. simultaneously without or with 500 ng kg^{-1} PGD_2 and indicated doses of BW A868C. See details for allodynia in the legend of Figure 1b. $^{*}P<0.05$, $^{**}P<0.01$, as compared with the nociceptin- and PGD_2-injected group.

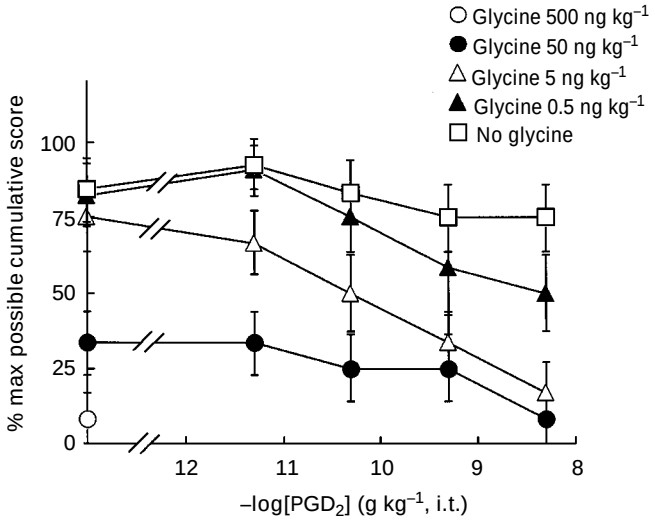

Figure 4 Synergistic effect of PGD_2 and glycine on inhibition of nociceptin-induced allodynia. Nociceptin (2.5 ng kg^{-1}) was injected (i.t.) simultaneously with the indicated doses of PGD_2 and 0, 0.5, 5 or 50 ng kg^{-1} of glycine. High dose (500 ng kg^{-1}) glycine almost completely inhibited the nociceptin-evoked allodynia by itself. Assessment of the nociceptin-induced allodynia was made 10 min after the i.t. injection. The values shown are the mean and vertical lines indicate s.e.mean ($n=6$).

Effect of PGD_2 on nociceptin-evoked hyperalgesia

The hyperalgesic effect of nociceptin was assessed by the hot plate (55°C) test. There was no significant difference in the latency period between mice that had not received an i.t. injection (14.4±0.4 s, mean±s.e.mean, $n=10$) and those treated with i.t. saline (14.5±1.0 s, at 15 min). Consistent with our recent findings (Hara *et al.*, 1997), administration of 50 pg kg^{-1} nociceptin shortened the response latency to 8.2±0.5 s 15 min after i.t. injection, demonstrating that the experimental mice developed increased sensitivity to the thermal stimuli after i.t. nociceptin. Although PGD_2 itself induced hyperalgesia within a narrow dose range of 25–250 ng kg^{-1}, it did not affect the nociceptin-evoked hyperalgesia at doses up to 500 ng kg^{-1} (Figure 6).

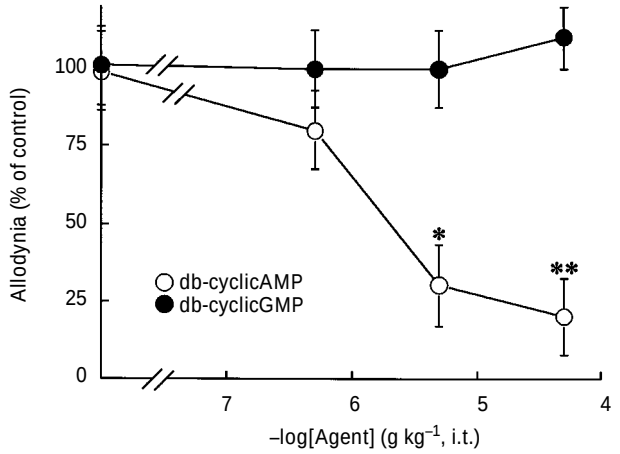

Figure 5 Effect of db-cyclicAMP and db-cyclicGMP on the nociceptin-induced allodynia. Nociceptin (2.5 ng kg^{-1}) was injected (i.t.) simultaneously with the indicated doses of db-cyclicAMP or db-cyclicGMP. See details for allodynia in the legend of Figure 1b. $^*P<0.05$, $^{**}P<0.01$, as compared with the nociceptin-injected group.

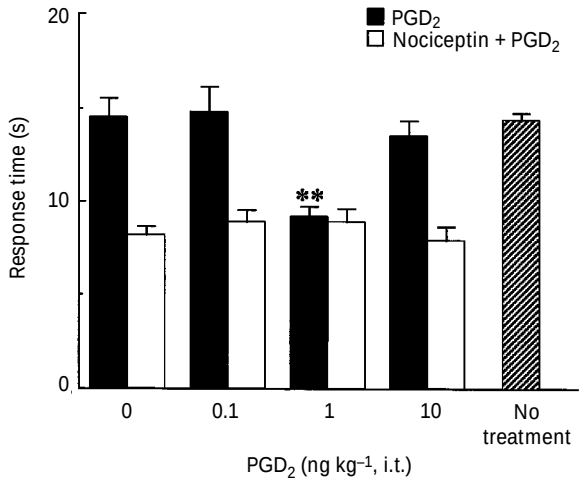

Figure 6 Effect of PGD_2 on nociceptin-induced hyperalgesia. Indicated doses of PGD_2 were injected alone or simultaneously with nociceptin 50 pg kg^{-1} into the subarachnoid space. No treatment represents the latency period of mice which had not received an i.t. injection. Columns for PGD_2 and for nociceptin + PGD_2 at 0 represent latency periods for i.t. injections of saline and nociceptin, alone, respectively. Hyperalgesia was assessed as described under Methods. The values shown are the mean±s.e.mean ($n=10$). Statistical analyses were carried out by Duncan's test. $^{**}P<0.01$, as compared with saline-injected group.

Discussion

We previously showed that i.t. administration of PGE_2 induced allodynia, which was blocked by simultaneous injection of PGD_2 (Minami *et al.*, 1996). We also demonstrated that i.t. nociceptin induced allodynia in conscious mice (Okuda-Ashitaka *et al.*, 1996; Hara *et al.*, 1997). In the present study, we have extended previous studies and demonstrated that the allodynia evoked by nociceptin is dose-dependently blocked by PGD_2. Several lines of evidence indicate that PGD_2 may block the nociceptin-induced allodynia through DP receptors. (1) The blockade of the nociceptin-induced allodynia by PGD_2 was selective in two ways. Firstly, PGD_2 inhibited the nociceptin-induced allodynia, but not hyperalgesia (Figures 1 and 6). Secondly, the DP receptor, PGI receptor and EP_2 subtype of the PGE receptor have been classified as relaxant prostanoid receptors; they preferentially activate adenylate cyclase and produce relaxation in smooth muscle cells (Coleman *et al.*, 1994). Among the prostaglandins examined, only PGD_2 blocked the nociceptin-induced allodynia (Figure 1b). (2) The nociceptin-induced allodynia was blocked by the DP-receptor agonist BW 245C. The blockade of nociceptin-induced allodynia by PGD_2 was dose-dependently reversed by the selective DP-receptor antagonist BW A868C (Figures 2 and 3). Although ZK 110841 has been shown to be a DP-receptor agonist (Thierauch *et al.*, 1988; Ito *et al.*, 1990), it did not block the nociceptin-induced allodynia (Figure 2). Because ZK 110841 has an 11,15-dihydroxy group as does PGE_2, it is an analogue of PGE_2, rather than PGD_2, and can induce allodynia itself, probably by crossreacting with EP receptors (Minami *et al.*, 1996). These results are consistent with those found previously, that PGD_2 inhibits PGE_2-induced allodynia (Minami *et al.*, 1996). Because the nociceptin-induced allodynia was not affected by a 15 min pretreatment with indomethacin (1 μg/mouse, i.t.) (Minami, T., unpublished results), it is unlikely that the nociceptin-induced allodynia is mediated by PGE_2. Therefore, the present study demonstrates that PGD_2 may regulate the induction of allodynia not only by PGE_2 but also by nociceptin.

We recently showed that i.t. glycine blocked the nociceptin-induced allodynia, suggesting that nociceptin may inhibit the release of glycine from presynaptic terminals or modulate the responsiveness of postsynaptic neurones to glycine (Hara *et al.*, 1997). Consistent with this notion, synergistic inhibition was observed between PGD_2 and glycine (Figure 4). Intrathecal db-cyclicAMP as well as PGD_2 blocked the nociceptin-induced allodynia (Figure 5). There are several possible explanations for this blockade. Like other opioid peptides, nociceptin is known to decrease forskolin-induced cyclicAMP accumulation (Meunier *et al.*, 1995; Reinscheid *et al.*, 1995; Okuda-Ashitaka *et al.*, 1996). PGD_2 and db-cyclicAMP may counter the intracellular effects of nociceptin mediated by cyclicAMP. In addition to inhibition of cyclicAMP formation, nociceptin has been recently shown to activate K^+ channels (Matthes *et al.*, 1996; Vaughan & Christie, 1996), inhibit voltage-gated Ca^{2+} channel currents (Knoflach *et al.*, 1996; Connor *et al.*, 1996), and inhibit tachykinin release from peripheral sensory neurones (Giuliani & Maggi, 1996) and K^+-evoked glutamate release from rat cerebrocortical slices (Nicol *et al.*, 1996). It is conceivable that the combination of Ca^{2+} channel inhibition and hyperpolarization resulting from K^+ channel activation by nociceptin may underlie the mechanism of inhibition of glycine release, which may be reversed by PGD_2. Studies on the measurement of the glycine level in the cerebrospinal fluid may be beyond the scope of the present study.

In situ hybridization studies visualized the cells that synthesize nociceptin precursor mRNA in neurones of the tract of Lissauer, an intraspinal inhibitory pathway in the dorsal horn and laminae I-III of dorsal horn of the spinal cord (Houtani *et al.*, 1996; Okuda-Ashitaka *et al.*, 1996). Immunohistochemical studies revealed that nociceptin-immunoreactivity is abundant in nerve terminals in the superficial dorsal horn and suggested that, upon release, nociceptin could presynaptically regulate

transmitter release from primary afferent neurones that express nociceptin receptors (Riedl *et al*., 1996). These results are consistent with the above-mentioned notion that nociceptin may alter neurotransmission in sensory systems. DP receptors were shown to be dense in the substantia gelatinosa by autoradiographic studies (Watanabe *et al*., 1986). PGD_2 formation is regulated by two isozymes of PGD synthase: the spleen-type isozyme located in satellite and Schwann cells and the brain-type isozyme confined to small B_1 neurones in the dorsal root ganglion (Vesin *et al*., 1995). Since prostaglandins act in the vicinity of their sites of formation according to an autocrine or paracrine process, dorsal root ganglion neurones of the subclass B_1 that possess PGD synthase of the brain type could release PGD_2 from their perikarya or axon preterminals. Probably PGD_2 of neuronal origin would be involved in cell-cell communication at the level of the preterminal part of the axons. Further experiments are needed to elucidate the exact role of PGD_2 in pain transmission in mutant mice lacking PGD synthase or DP receptors.

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