Blockade by ONO-NT-012, a unique prostanoid analogue, of prostaglandin E₂-induced allodynia in conscious mice

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1 Intrathecal (*i.t.*) administration of prostaglandin E₂ (PGE₂) to conscious mice was reported to induce allodynia, a state of discomfort and pain evoked by innocuous tactile stimuli through prostaglandin E receptor subtype EP₁ and hyperalgesia through prostaglandin E receptor subtypes EP₂ and/or EP₃. In the present study, we investigated the effects of an EP₁ antagonist on these sensory disorders by use of ONO-NT-012 or AH6809.

2 ONO-NT-012 dose-dependently antagonized the PGE₂-induced allodynia but had no effect on the PGE₂-induced hyperalgesia by the hot plate test. On the other hand, AH6809 blocked the PGE₂-induced hyperalgesia at the highest dose examined (50 µg kg⁻¹) but had no effect on the PGE₂-induced allodynia. The *i.t.* injection of AH6809 or ONO-NT-012 alone did not have any effect on the response to noxious or innocuous stimuli.

3 Increasing doses (5 pg kg⁻¹–500 ng kg⁻¹) of ONO-NT-012 produced parallel shifts to the right of the dose-response curves to PGE₂. The Schild plot regression line was linear and the slope was close to unity. The pA₂ value against PGE₂ was calculated to be 9.96.

4 The present study demonstrates that *i.t.* administration of PGE₂ exerts allodynia through EP₁ in the mouse spinal cord and that ONO-NT-012 is a highly potent, simple competitive antagonist for the PGE₂-induced allodynia.

Keywords: ONO-NT-012; EP₁ antagonist; allodynia; hyperalgesia; spinal cord

Introduction

We recently reported that intrathecal (*i.t.*) administration of prostaglandin E₂ (PGE₂) to conscious mice 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, and hyperalgesia by the hot plate test (Minami *et al.*, 1994c).

PGE₂ produces a broad range of biological actions in diverse tissues through its binding to specific receptors on plasma membranes (Coleman *et al.*, 1987; 1989; Campbell, 1990). EP-receptors are pharmacologically divided into at least three subtypes, EP₁, EP₂, and EP₃, and are considered to be coupled to Ca²⁺ mobilization and stimulation and inhibition of adenylate cyclase, respectively. The diversity of PGE₂ actions could be ascribed to EP-receptor subtypes coupled to different signal transduction pathways. We have demonstrated, on the basis of data obtained with 7 synthetic prostanoid analogues, that PGE₂ might exert allodynia through EP₁-receptors and hyperalgesia through EP₂- and/or EP₃-receptors in the mouse spinal cord (Minami *et al.*, 1994b). Confirmation of this conclusion has however been hampered by the lack of selective EP-receptor subtype antagonists. Previously, EP-receptors in isolated tissues that are susceptible to blockade by SC19220 and AH6809 were designated as EP₁-receptors (Coleman *et al.*, 1985). AH6809 was also a prostaglandin D₂-receptor specific blocking agent in human platelets and some cultured cells (Keery & Lumley, 1988; Ito *et al.*, 1990). ONO-NT-012 has recently been reported to be a unique compound with both EP₁/thromboxane A₂-receptor antagonist activity and EP₃-receptor agonist activity (Maruyama *et al.*, 1994). In the present study, we investigated the effects of ONO-NT-012 or AH6809 on the PGE₂-induced allodynia and hyperalgesia.

Methods

Intrathecal administration

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 and a constant temperature of 22 ± 2°C and 60 ± 10% humidity. In order to insert a needle quickly and accurately, we incised the skin on the back (1 cm circumference) and inserted a 27-gauge stainless-steel needled (0.35 mm, o.d.) attached to a microsyringe between L₅ and L₆ vertebrae by the method of Hylden & Wilcox (1980). Drugs in vehicle were injected slowly into the subarachnoid space of conscious mice at 22 ± 2°C. The volume of the *i.t.* injection was 5 µl. It was previously confirmed by use of Commaassie brilliant blue and [³H]-PGE₂ that the injected solution does not extend to the cervical segments (Uda *et al.*, 1990).

Studies on allodynia

Studies on allodynia were carried out essentially according to the method of Yaksh & Harty (1988). The mice were divided into various groups (*n* = 6–8 per group). Control mice were given physiological saline (5 µl). Drug-treatment groups were injected with 5 µl of vehicle containing various doses of test agents. After the *i.t.* injection, each mouse was placed in an individual 13 × 8.5 × 13 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 allodynia 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, and strong efforts to escape.

To evaluate the effects of the agents on allodynia, the values obtained over the 50-min test period were cumulated and expressed as a percentage of the maximum possible

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score. Thus, the maximum possible score for allodynia per animal was 20.

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 by Woolfe & MacDonald (1944). The response time of the mice to the hot plate was measured at 30 min after *i.t.* injection.

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

Drugs

PGE₂ and ONO-NT-012 (5(Z)-7-[(1S, 2S, 3S, 5R)-3-(*trans*- β -styren) sulphonamido-6,6-dimethylbicyclo (3.1.1) hept-2-yl]-5-heptenoic acid) were generous gifts from Ono Central Research Institute (Osaka, Japan). PGE₂ and ONO-NT-012 were stored in ethanol solution at -20°C. For injection, an aliquot of the desired stock PGE₂ or ONO-NT-012 solution was placed in a borosilicate tube and the ethanol was removed by evaporation to dryness under nitrogen gas. Sterile saline was then added to dissolve the PGE₂ or ONO-NT-012. AH6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid) was kindly provided by Glaxo Group Research Ltd. (Hertfordshire, U.K.). AH6809 was dissolved and diluted in 1% NaHCO₃ in 0.9% NaCl (saline). All solutions were 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. IC₅₀ values with 95% confidence limits (95% CL) were calculated by use of a computerized Probit test.

Results

Effect of AH6809 or ONO-NT-012 on PGE₂-evoked allodynia

In order to examine the effect of EP₁-receptor antagonists on the PGE₂-induced allodynia, we used AH6809 and ONO-NT-012. When assessed by a cumulative score during the 50 min period, the allodynia caused by PGE₂, 0.5 $\mu\text{g kg}^{-1}$, was dose-dependently blocked by ONO-NT-012 with IC₅₀ values (95% CL) of 1.04 ng kg^{-1} (477 pg kg^{-1} –2.03 ng kg^{-1}). On the other hand, AH6809 had little effect on the PGE₂-induced allodynia at doses up to 50 $\mu\text{g kg}^{-1}$ (Figure 1). ONO-NT-012 produced parallel and dose-dependent rightward shifts in the dose-response curve to PGE₂ (Figure 2a). A Schild plot analysis was carried out based on these data, by which dose-ratios were calculated at a response intersecting all curves obtained with 5 pg kg^{-1} –0.5 $\mu\text{g kg}^{-1}$ ONO-NT-012 (representing 20% of the maximum possible cumulative score over the 50-min period). As shown in Figure 2b, a Schild plot regression line revealed a linear relationship and the slope was close to unity. The pA₂ value against PGE₂ was 9.96.

These results indicate that ONO-NT-012 is a simple and competitive antagonist of PGE₂-induced allodynia in the spinal cord.

Figure 1 Effect of AH6809 or ONO-NT-012 on prostaglandin E₂ (PGE₂)-induced allodynia. PGE₂ (0.5 $\mu\text{g kg}^{-1}$) was injected simultaneously with various doses of AH6809 or ONO-NT-012 into the subarachnoid space. Assessment of allodynia was made as described under Methods. The score of PGE₂ alone was taken as 100%. The values shown are the mean $\pm$ s.e.mean ($n = 6$). Statistical analyses were carried out by Duncan's test. * $0.01 \leq P < 0.05$, ** $P < 0.01$, as compared with PGE₂-injected group.

Figure 2 Effect of ONO-NT-012 on prostaglandin E₂ (PGE₂)-induced allodynia. (a) Dose-response curves of ONO-NT-012 for the PGE₂-induced allodynia. PGE₂ was injected simultaneously without (○) or with 0.5 $\mu\text{g kg}^{-1}$ (+), 50 ng kg^{-1} (×), 5 ng kg^{-1} (▲), 0.5 ng kg^{-1} (Δ), 50 pg kg^{-1} (■), or 5 pg kg^{-1} (□) ONO-NT-012 into the subarachnoid space. Assessment of allodynia was made as described under Methods. The values shown are the mean $\pm$ s.e. mean ($n = 6-8$). (b) Schild plot analysis for the antagonism of response to PGE₂ by ONO-NT-012. DR, dose-ratio, i.e., the ratio of the dose of the PGE₂ required to produce 20% of the maximum possible cumulative score over the 50-min period of the PGE₂-induced allodynia in the presence of a given dose of ONO-NT-012 to that without ONO-NT-012. The best fit to the line and the correlation coefficient (r) were calculated by linear least squares regression.

Figure 3 Effect of AH6809 or ONO-NT-012 on prostaglandin E₂ (PGE₂)-induced hyperalgesia. PGE₂ (0.5 µg kg⁻¹) was injected simultaneously with various doses of AH6809 or ONO-NT-012 into the subarachnoid space. The time until the mice showed the first avoidance response to the hot plate test (55°C) was measured at 30 min after *i.t.* injection. Each column represents the mean ± s.e. mean of responses in 10 mice. Statistical analyses were carried out by Duncan's test. ***P* < 0.01, as compared with the PGE₂-injected group.

Effect of AH6809 or ONO-NT-012 on PGE₂-evoked hyperalgesia

As reported previously (Uda *et al.*, 1990), PGE₂-induced hyperalgesia was observed between 3 and 30 min after *i.t.* injection of PGE₂. In the present study, the response time of mice to the hot plate (55°C) was measured at 30 min after *i.t.* injection of PGE₂ or vehicle so that the effect of PGE₂-induced allodynia would be minimized. There was no significant difference in the latency period between the saline control (14.7 ± 1.1 s, mean ± s.e. mean) and the untreated control (15.4 ± 0.9 s). As reported previously (Minami *et al.*, 1994c), PGE₂ produced its hyperalgesic action over a wide range of doses from 50 pg kg⁻¹ to 0.5 µg kg⁻¹ with two apparent hyperalgesic peaks at 0.5 ng kg⁻¹ (11.0 ± 0.7 s) and 0.5 µg kg⁻¹ (8.8 ± 0.4 s). The hyperalgesia caused by 0.5 µg kg⁻¹ of PGE₂ was not blocked by ONO-NT-012 at all. On the other hand, the hyperalgesia caused by 0.5 µg kg⁻¹ of PGE₂ was blocked by AH6809 at the highest dose examined (50 µg kg⁻¹) (Figure 3).

The *i.t.* injection of ONO-NT-012 or AH6809 alone did not have any effects on the response to noxious or innocuous stimuli.

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Discussion

We recently reported that the *i.t.* administration of PGE₂ to conscious mice induced allodynia and hyperalgesia (Uda *et al.*, 1990; Minami *et al.*, 1994a, c) and that PGE₂ might exert allodynia through the EP₁-receptor and hyperalgesia through EP₂- and/or EP₃-receptors in the mouse spinal cord (Minami *et al.*, 1994b). In the present study, the effects of ONO-NT-012 (a novel EP₁/thromboxane A₂-receptor antagonist and EP₃-receptor agonist) on two pain models of PGE₂-induced allodynia and hyperalgesia were tested and compared with those of AH6809. The PGE₂-induced allodynia was dose-dependently blocked by ONO-NT-012, but not by AH6809 (Figure 1). On the other hand, the PGE₂-induced hyperalgesia assessed by the hot plate test was blocked by AH6809, but not by ONO-NT-012 (Figure 3). The pA₂ value of ONO-NT-012 against PGE₂ was 9.96 (Figure 2b) *i.e.* much smaller than that reported previously with longitudinal smooth muscle of guinea-pig ileum (pA₂ = 5.7) (Maruyama *et al.*, 1994). EP-receptors in isolated tissues that are susceptible to blockade by SC19220 and AH6809 were designated as EP₁-receptors (Coleman *et al.*, 1985). Recently the EP₁-receptor was cloned from the mouse lung cDNA library but AH6809 showed only weak inhibition of binding of [³H]-PGE₂ to membranes prepared from EP₁-receptor cDNA-transfected CHO cells (Watabe *et al.*, 1993). Therefore it was suggested that there may be another form of EP₁-receptor besides one sensitive to AH6809 or that the action of AH6809 is species-specific with little effect on the mouse receptor.

In conclusion, the present study demonstrates that ONO-NT-012 is a potent, competitive, and selective antagonist for PGE₂-induced allodynia. In addition to conventional nociceptive tests such as the tail-flick test, formalin test, and hot plate test, allodynia induced by *i.t.* injection of prostaglandins provides a new model of pain when testing the efficacy of analgesic agents in animals.

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