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Antinociceptive effects of AS1069562, the (+)-isomer of indeloxazine, on spinal hypersensitivity induced by intrathecal injection of prostaglandin in mice: Comparison with duloxetine and amitriptyline

Nobuhito Murai*, Mina Tsukamoto, Seiji Tamura, Toshiaki Aoki, Nobuya Matsuoka

Pharmacology Research Laboratories, Drug Discovery Research, Astellas Pharma Inc., 21 Miyukigaoka, Tsukuba-shi, Ibaraki 305-8585, Japan

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ABSTRACT

The (+)-isomer of indeloxazine AS1069562 exerts multiple pharmacological actions including the inhibition of serotonin (5-HT) and norepinephrine reuptake and analgesia in experimental animal pain models. Here, we evaluated the antinociceptive effects of AS1069562 and the antidepressants duloxetine and amitriptyline in mouse models of prostaglandin-induced spinal hypersensitivity. Prostaglandin E_2 (PGE_2) and $F_{2\alpha}$ ($PGF_{2\alpha}$) were intrathecally administered to induce spinal hypersensitivity, causing tactile allodynia in mice. Allodynia induced by $PGF_{2\alpha}$ but not by PGE_2 was suppressed by desensitization of C-fibers with systemic pretreatment with resiniferatoxin. C-fiber hyperexcitability might therefore play a role in allodynia induced by $PGF_{2\alpha}$ but not PGE_2. In the PGE_2-induced allodynia model, AS1069562 and duloxetine significantly suppressed allodynia, whereas amitriptyline did not. In the $PGF_{2\alpha}$-induced allodynia model, AS1069562 and amitriptyline significantly ameliorated allodynia, whereas duloxetine did not. To demonstrate the broad effects of AS1069562 compared to duloxetine, additional studies were conducted to elucidate other target mechanisms of AS1069562 beyond 5-HT and norepinephrine reuptake inhibition. AS1069562 exhibited affinity for both 5-HT_{1A} and 5-HT_3 receptors, and the analgesic effect of AS1069562 on $PGF_{2\alpha}$-induced allodynia was significantly blocked by the 5-HT_{1A} receptor antagonist (S)–WAY100135 and the 5-HT_3 receptor agonist SR57227. Taken together, these results indicate that AS1069562 inhibits both C-fiber- and non-C-fiber-dependent prostaglandin-induced allodynia, while duloxetine inhibits only non-C-fiber-triggered allodynia, and amitriptyline inhibits only C-fiber-triggered allodynia. These broad antinociceptive effects of AS1069562 may be due not only to 5-HT and norepinephrine reuptake inhibition but also to its effects on 5-HT receptors such as 5-HT_{1A} and 5-HT_3 receptors.

1. Introduction

Antidepressants such as the serotonin (5-HT) and norepinephrine reuptake inhibitor duloxetine and the tricyclic antidepressant amitriptyline are currently used to treat chronic pain, such as neuropathic pain. The analgesic effects of these antidepressants are based on the enhancement of the serotonergic and noradrenergic descending inhibition system (Thor et al., 2007), which presynaptically inhibit glutamate release at the spinal cord (Yoshimura and Furue, 2006).

Indeloxazine is a cerebral metabolic enhancer with multiple pharmacological effects, such as 5-HT and norepinephrine reuptake inhibition (Yamamoto, 1990). AS1069562 is the optical (+)-isomer of indeloxazine and also exerts inhibitory effects against 5-HT and norepinephrine reuptake (Shimizu-Sasamata et al., 1993). We recently found that AS1069562 significantly improved both mechanical allodynia and thermal hyperalgesia in a rat model of chronic constriction injury (CCI)-induced neuropathic pain. In contrast, duloxetine only induced a significant amelioration of mechanical allodynia, while amitriptyline significantly ameliorated thermal hyperalgesia. Further, AS1069562 increased the ratio of both 5-HT and norepinephrine to their metabolites contents in the rat spinal cord, while duloxetine slightly increased only the ratio of 5-HT to its metabolite contents (Murai et al., 2014). Although these results imply that AS1069562 may have distinct spinal analgesic mechanisms compared with duloxetine and amitriptyline, no such mechanism has been elucidated.

Mouse models of intrathecal (i.t.) prostaglandin-induced allodynia are useful in investigating spinal antinociceptive mechanisms of

* Corresponding author. Tel.: +81 29 863 7174; fax: +81 29 856 2515.
E-mail address: nobuhito.murai@astellas.com (N. Murai).

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analgesics, as their evaluation in a pure allodynic state facilitates the identification of active sites in the pain pathway. Intrathecal injection of prostaglandin E_2 (PGE_2) or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) induce tactile allodynia, a clinically important manifestation of neuropathic pain, by facilitating glutamate release from presynaptic terminals at the spinal cord dorsal horn via prostaglandin E receptor 1 (EP1) and prostaglandin F receptor (FP), respectively (Minami et al., 1992, 1994a, 1994b; Muratani et al., 2003; Tsukamoto et al., 2010).

In this study, first, we confirmed the dose-dependent induction of tactile allodynia including their time course via i.t. administration of PGE_2 and $PGF_{2\alpha}$ in mice. In addition, to investigate the dependence of C-fiber hyperexcitability in these spinal hypersensitivity models, we examined the effects of resiniferatoxin (RTX), an ultrapotent analog of capsaicin. After these validative experiments, we assessed the antinociceptive effects of AS1069562, duloxetine, and amitriptyline in these models to determine the different spinal antinociceptive mechanisms of these drugs. Further, to determine whether or not AS1069562 exerts its activity via different mechanisms from its 5-HT and norepinephrine reuptake inhibition, we investigated the possible involvement of cross activity for 5-HT receptor subtypes in the antinociceptive effect using spinal pharmacological tools, a 5-HT_{1A} receptor antagonist and a 5-HT_3 receptor agonist. The results of this study provided novel mechanistic aspects of AS1069562 different from a selective 5-HT and norepinephrine reuptake inhibitor, duloxetine.

2. Materials and methods

2.1. Drugs

(R)-2-[(1 H-inden-7-yloxy)methyl]morpholine monobenzenesulfonate (AS1069562) and duloxetine were synthesized at Astellas Pharma Inc. (Ibaraki, Japan). Hydrochloride salts of AS1069562 and duloxetine were used as AS1069562 and duloxetine, respectively. Amitriptyline, RTX, PGE_2, and $PGF_{2\alpha}$ were purchased from Sigma-Aldrich (St. Louis, MO, USA). The 5-HT_{1A} receptor antagonist (S)–WAY100135 and 5-HT_3 receptor agonist SR57227 were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). For in vivo studies, AS1069562, duloxetine, and amitriptyline were suspended in distilled water and administered at an oral (p.o.) dose of 10 ml/kg. AS1069562 and duloxetine were administered at 3, 10, and 30 mg/kg, while amitriptyline was administered at 10, 30, and 100 mg/kg as previously reported (Katoh et al., 1995; Shimizu-Sasamata et al., 1993). RTX was suspended in solution of 10% EtOH, 10% Tween 80, and 80% saline and was administered at a subcutaneous (s.c.) dose of 0.001 mg/10 ml/kg. (S)–WAY100135 and SR57227 were suspended in saline and administered at an i.t. dose of 2 nmol/5 μl/animal. PGE_2 and $PGF_{2\alpha}$ were dissolved in ethanol, and an aliquot of the desired solution was evaporated under nitrogen gas to remove the ethanol. PGE_2 and $PGF_{2\alpha}$ were then dissolved in saline and administered at an i.t. volume of 5 μl/animal. PGE_2 was administered at 1, 10, and 100 ng/animal, and $PGF_{2\alpha}$ at 100, 300, and 1000 ng/animal to investigate dose dependency. PGE_2 was administered at 10 ng/animal and $PGF_{2\alpha}$ at 300 ng/animal for drug evaluation. Drug concentrations were calculated in terms of free base.

2.2. Animals

Male imprinting control region (ICR) mice (18–22 g; Japan SLC, Hamamatsu, Japan) were used for in vivo experiments. Animals were group-housed under a 12-h light-dark cycle (light on: 7:30–19:30) at room temperature (23 ± 1 °C) with free access to food and water. All animal experimental procedures were approved by the Committee for Animal Experiments of Astellas Pharma Inc., and all efforts were made to minimize the number of animals used as well as their suffering.

2.3. Binding assay

Binding assays were conducted using rat cerebral cortex (5.0 mg of protein) for the 5-HT_{1A} receptor, rat whole brain (5.0 mg of protein) for the 5-HT_{1B} receptor, human recombinant 5-HT_{2B} receptor (7.0 μg of protein; Perkin Elmer, Boston, MA, USA), and human recombinant 5-HT_3 receptor (2.5 μg of protein; Perkin Elmer). For the 5-HT_{1A} receptor binding assay, [Propyl-2,3-ring-1,2,3-^{3}H]-8-hydroxy-DPAT (0.47 nM; Perkin Elmer) and competitor (5-HT hydrochloride; Sigma-Aldrich) were incubated at 37 °C for 30 min in 50 mM Tris-HCl buffer (pH 7.4). For the 5-HT_{1B} receptor binding assay, [^{125}I]iodo-(±)-cyanopindolol (0.01 nM; Perkin Elmer) and competitor (5-HT hydrochloride; Sigma-Aldrich) were incubated at 25 °C for 60 min in 50 mM Tris-HCl buffer (pH 7.4). For the 5-HT_{2B} receptor binding assay, 2-(+)-[^{125}I]iodo-lysergic acid diethylamide (0.27 nM; Perkin Elmer) and competitor (5-HT hydrochloride; Sigma-Aldrich) were incubated at 37 °C for 30 min in 50 mM Tris-HCl buffer (pH 7.4) containing 4 mM $CaCl_2$. For the 5-HT_3 receptor binding assay, [N-methyl-^{3}H]–GR65630 (0.48 nM; Perkin Elmer) and competitor (MDL72222, 5-HT_3 receptor antagonist; Sigma-Aldrich) were incubated at 25 °C for 60 min in 50 mM Tris-HCl buffer (pH 7.4) containing 5 mM $MgCl_2$ and 1 mM EDTA. To determine non-specific binding, 100 μM 5-HT hydrochloride for 5-HT_{1A}, 5-HT_{1B}, or 5-HT_{2B} receptor, or 100 μM MDL72222 for 5-HT_3 receptor was used. Incubated mixtures were filtered using a cell harvester. The filter paper was rinsed three times with 50 mM Tris-HCl buffer (pH 7.4), and the radioactivity of paper was determined via scintillation counting. Specific binding was defined as a portion of total binding, which was replaced by 100 μM 5-HT hydrochloride for the 5-HT_{1A}, 5-HT_{1B}, or 5-HT_{2B} receptor, or MDL72222 for 5-HT_3 receptor. Dissociation constant (K_d) and binding site density (B_{max}) were calculated via Scatchard analysis. IC_{50} values were calculated from the non-linear regression analysis. Values of the apparent equilibrium dissociation constant of inhibitors (K_i) were calculated using the method of Cheng and Prusoff (1973).

2.4. Intrathecal prostaglandin-induced allodynia model in mice

The prostaglandin-induced spinal hypersensitivity model was induced as previously described (Tsukamoto et al., 2010). Mice were randomly divided into various groups, with eight animals per group. A 30-gauge stainless steel needle attached to a microsyringe was inserted between the L5 and L6 vertebrae of conscious mice, and a prostaglandin (PGE_2 or $PGF_{2\alpha}$ in saline at a volume of 5 μl) was slowly administered via i.t. injection into the subarachnoid space (Hylden and Wilcox, 1980). Saline was used as a vehicle control of PGE_2 and $PGF_{2\alpha}$. Tactile hypersensitivity was assessed 5, 10, 15, and 30 min after i.t. injection of prostaglandin by lightly stroking the flank of the mice with a paintbrush. The allodynia response was ranked as 0 (no response), 1 (avoidance), or 2 (vigorous squeaking or strong avoidance), with scores expressed as a percentage of the maximum possible cumulative score for all time points. To confirm the dose-dependent induction of tactile allodynia, the following doses were administered: PGE_2 at 1, 10, and 100 ng/animal and $PGF_{2\alpha}$ at 100, 300, and 1000 ng/animal. Doses of prostaglandins (i.t.) that produced a submaximal allodynic response (PGE_2: 10 ng/animal; $PGF_{2\alpha}$: 300 ng/animal) were selected. To investigate the involvement of C-fiber in prostaglandin-induced hypersensitivity, we examined desensitization of C-fibers with RTX. RTX (0.001 mg/kg s.c.) was administered for 24 h prior to prostaglandin injection, thereby desensitizing C-fibers. A solution of 10% EtOH, 10% Tween 80, and 80% saline was used as a vehicle control for RTX. To assess their analgesic effects on prostaglandin-induced spinal