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Zaltoprofen, a non-steroidal anti-inflammatory drug, inhibits bradykinin-induced pain responses without blocking bradykinin receptors

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Abstract

Zaltoprofen, a preferential COX-2 inhibitor, exhibited a potent inhibitory action on the nociceptive responses induced by a retrograde infusion of bradykinin into the right common carotid artery in rats. However, other COX-2 preferential inhibitors such as meloxicam and etodolac did not exhibit any apparent action, and also, preferential COX-1 inhibitors mofezolac and indomethacin, COX-1 and COX-2 inhibitor loxoprofen sodium showed a weak effect. These non-steroidal anti-inflammatory drugs (NSAIDs) except for zaltoprofen, strongly inhibited an acetic acid-induced writhing response related to PGs based on COX-1, at lower doses. Zaltoprofen had a moderate inhibitory effect compared with those of the above-mentioned NSAIDs. These results suggest that the inhibitory effect of zaltoprofen on bradykinin-induced nociceptive responses is not explainable by the inhibition of cyclooxygenase (COX). So, we examined the inhibitory effect of zaltoprofen on bradykinin-induced nociceptive responses by performing several in vitro experiments. Zaltoprofen did not bind to B_1 and B_2 receptors in a radio-ligand binding assay. In the cultured dorsal root ganglion cells of mature mice, zaltoprofen completely inhibited the bradykinin-induced increase of $[Ca^{2+}]_i$, which was inhibited by B_2 antagonist D-Arg-[Hyp^3, Thi^{5,8}, D-Phe^7]-bradykinin, but not by B_1 antagonist. In addition, the inhibition of zaltoprofen on the increase of $[Ca^{2+}]_i$ was observed even under extracellular Ca^{2+}-free conditions. The above results suggest that zaltoprofen produces an analgesic action on bradykinin-induced nociceptive responses by blocking the B_2 receptor-mediated pathway in the primary sensory neurons. Taken together, these results suggest that zaltoprofen may serve as a potent and superior analgesic for the treatment of pain.

Keywords: Zaltoprofen; Bradykinin; Acetic acid; NSAIDs; Nociceptive response; Dorsal root ganglion

1. Introduction

A subset of non-steroidal anti-inflammatory drugs (NSAIDs) has recently been reported to demonstrate a diverse pharmacological effect that is not related to the inhibitory activity of cyclooxygenases (COXs). Zaltoprofen ((+/−)-2-(10,11-dihydro-10-oxo-dibenzo[*b*, *f*]thiepin-2-yl)-propionic acid) is an NSAID with powerful anti-inflammatory effects as well as an analgesic action on inflammatory pain (Tsurumi et al., 1986a; Kameyama et al., 1987). Zaltoprofen selectively inhibits PGE_2 production at inflammatory sites exhibiting an anti-inflammatory effect (ED_{50}, 1–5 mg/kg, p.o.) with a good safety margin (UD_{50}, 50–100 mg/kg, p.o.) (Tsurumi et al., 1986a,b; Nishioka et al., 2000; Kunigami et al., 2001). In addition, zaltoprofen was shown to inhibit bradykinin-induced nociceptive responses more potently than indomethacin whereas diclofenac sodium and ibuprofen were not (Kameyama et al., 1987). We have also shown that zaltoprofen inhibits effects on bradykinin-induced 12-lipoxygenase (LOX) activation in vitro (Tang et al., 2005).

Recently, research on the mechanism of pain development at polymodal nociceptors has advanced, clarifying that bradykinin is an algesic or sensitizing substance (Premkumar and Ahern, 2000; Mizumura et al., 1990). There are B_1 and B_2 bradykinin receptors; the former is inducible, while the latter is constitutive. It has been reported that bradykinin-induced nociceptive responses are produced through the Gq protein-coupled B_2 receptor and a number of different signal transduction pathways, including phospholipase C (PLC), phospholipase A_2, LOX, COX and others, in dorsal root ganglion (DRG) cells (Chuang et al., 2004; Vanegas and Schaible, 2001; Shin et al., 2002; Tang et al., 2004). In addition,

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it has been reported that stimulation of the B_2 receptor sensitizes vanilloid receptor 1 (VR-1) via protein kinase C (PKC) (Sugiura et al., 2002).

Bradykinin is a potent endogenous algesic substance, and many studies have demonstrated its association with pain in humans. For example, the injection of bradykinin causes muscular hyperalgesia in humans (Graven-Nielsen and Mense, 2001; Mork et al., 2003). In addition, several studies have proven that pain and the production of bradykinin at the inflamed sites increase over time following third molar extraction (Hargreaves et al., 1993), that blood bradykinin concentration increases with the development of muscle pain following excessive weight training (Blais et al., 1999), and that bradykinin concentration increases in the joint cavities of patients with rheumatoid arthritis (Sharma and Buchanan, 1994). Thus, there is a large body of evidence regarding the role of bradykinin in the development of pain associated with various diseases.

NSAIDs were classified according to their chemical structure or their selective inhibition of COX-1 and COX-2. Zaltoprofen is a propionic acid derivative with a tri-cyclic formula. In the present study, we examined whether zaltoprofen and other types of NSAIDs have a similar analgesic action on bradykinin- and acetic acid-induced nociceptive responses, and found that only zaltoprofen inhibited bradykinin-induced nociceptive responses. Thus, we examined the inhibitory effect of zaltoprofen on bradykinin-induced nociceptive responses in several in vitro experiments using a radio-ligand binding assay and cultured mouse dorsal root ganglion cells.

2. Materials and methods

2.1. Animals

Eight-week-old male Wistar rats (Charles River Japan Inc.) and 5–8-week-old male ICR mice (Charles River Japan Inc.) were housed under conditions of constant temperature (23 ± 2 °C) and constant humidity (55 ± 10%) for at least 5 days, and only healthy animals were used for the study. All procedures used in the present study were approved by the Research Laboratories Animal Care Committee in Nippon Chemiphar Co. Ltd. and by the Tokai University Animal Care Committee, and were in compliance with the ethical guidelines of the International Association for the Study of Pain (Zimmermann, 1983).

2.2. Drugs

Zaltoprofen ((+/−)-2-(10,11-dihydro-10-oxo-dibenzo[*b*, *f*]thiepin-2-yl)-propionic acid), meloxicam and celecoxib were synthesized in the laboratory of Nippon Chemiphar Co. Ltd. We used crystals of loxoprofen sodium, etodolac and mofezolac extracted from marketed products (Loxonin tablets, Sankyo; Hypen tablets, Nippon Shinyaku; Disopain tablets, Yoshitomi, respectively). Indomethacin and diclofenac sodium were purchased from Sigma–Aldrich, Japan. The test compounds were suspended or dissolved in a 1% methylcellulose (Shin-Etsu Chemical) solution for a rat in vivo study. All compounds were suspended in a 0.2% carboxy methylcellulose solution for the study in mice. Bradykinin (Peptide Institute Inc.) was dissolved in physiological saline to be diluted for use in the bradykinin-induced pain study. In intracellular calcium measurements using DRG, bradykinin (Sigma–Aldrich) was dissolved in distilled water to 1 mg/2 mL, and the solution was divided into 200 μL to be frozen and stored. The bradykinin solutions were diluted with Na^+-rich solution (modified HEPES Ringer solution) before use. Lys-[des-Arg^9, Leu^8]-bradykinin, a bradykinin B1 antagonist and D-Arg-[Hyp^3, Thi^{5,8}, D-Phe^7]-bradykinin, a bradykinin B2 antagonist, were purchased from Sigma–Aldrich. 0.5 mL/100 g body weight was orally administered in the rat in vivo study. In the mouse study, 0.1 mL/10 g body weight was orally administered.

2.3. Bradykinin-induced nociceptive responses in rats

A total of six to eight male Wistar rats per group were used. Under ether anesthesia, a polyethylene cannula (Intramedic, PE-50, i.d. = 0.58 mm, o.d. = 0.965 mm) filled with physiological saline containing heparin was inserted retrogradely toward the heart into the right common carotid artery. The opposite end of the cannula was pulled out from the back of the neck and was capped. After the rats awoke from anesthesia, 0.4–0.6 μg of bradykinin solution (2 μg/mL) were infused through the rubber cap of the cannula. The rats which clearly exhibited the three different pseudo pain reactions ((1) bending of the right forelimb, (2) turning of the head to the right and (3) increase in motor activity) within 30 s after the injection of bradykinin were selected. Bradykinin was injected every 15 min, and the animals were observed up to 120 min after the oral administration of the test compound. Test compound was assessed the analgesic effect to be present when two out of the three reactions were inhibited. The effects of test compounds were investigated in blinded manner.

2.4. Acetic acid-induced writhing test in mice

A total of 10 male ICR mice (5-week-old) per group were used. An acetic acid (0.6%) solution at 0.1 mL/10 g b.w. was intraperitoneally administered to count the incidence of writhing after administration for 20 min. The test compound was orally administered at 30 min before the administration of the acetic acid. Meloxicam was administered at 60 min before. The percentage of inhibition was calculated as follows:

$$\text{Percentage of inhibition} = 100 \times \frac{A - B}{A},$$

where A is the incidence of writhing in the control group and B is the incidence of writhing in the test compound-treated group.

2.5. Receptor binding assay

2.5.1. B_1 membranes preparation

Membranes were prepared from human HS729 (rhabdomyosacroma) cells that were treated with 0.1 μg/mL phorbol 12-myristate 13-acetate (PMA) for 4–6 h to enhance the number of bradykinin B_1 receptors.

The cells in the plate were washed two to three times with 20 mL Dulbecco's phosphate buffered saline (DPBS). To this, 10 mL TEM buffer (25 mM Tris–HCl, 1 mM EDTA, 6 mM $MgCl_2$ with 100 μM PMSF, 1 μM pepstatin A and 1 μM leupeptin) was added, and the cells were scraped off. The cells were transferred to a 50 mL centrifuge bottle, and the cell suspension was kept on ice. These steps were repeated until all the cells were harvested. Units of 30 mL of cells were homogenized at a time using 10 strokes with a motor-driven Teflon pestle (Potter Elvehjem Tissue Grinder, Wheaton). The homogenate was centrifuged at 800 × *g* for 10 min at 4 °C. The supernatant was transferred to centrifuge tubes, the cell pellets were collected, and this process was repeated. The supernatant was combined and centrifuged at 100,000 × *g* (L8-M Ultracentrifuge, Beckman) for 45 min at 4 °C. The pellets were collected and resuspended in the TEM buffer. This was recentrifuged at 100,000 × *g* for 45 min at 4 °C. The pellets were collected, and 1 mL aliquots of the membrane preparation were frozen in the TEM buffer and stored at −80 °C. The protein concentration was determined using the Pierce BCA protein assay.

2.5.2. B_2 membrane preparation

Ilea tissues from adult male Duncan Hartley-derived guinea pigs were homogenized in 0.32 M sucrose using a polytron at setting 5 for 20 s. The homogenate was centrifuged at 500 × *g* for 10 min at 4 °C. Subsequently, the supernatant was spun at 100,000 × *g* (L8-M Ultracentrifuge, Beckman) for 45 min at 4 °C. The pellets were stored at −80 °C until use.