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Stimulation of prostaglandin E_2-EP3 receptors exacerbates stroke and excitotoxic injury

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Abstract

The effect of PGE_2 EP3 receptors on injury size was investigated following cerebral ischemia and induced excitotoxicity in mice. Treatment with the selective EP3 agonist ONO-AE-248 significantly and dose-dependently increased infarct size in the middle cerebral artery occlusion model. In a separate experiment, pretreatment with ONO-AE-248 exacerbated the lesion caused by *N*-methyl-D-aspartic acid-induced acute excitotoxicity. Conversely, genetic deletion of EP3 provided protection against *N*-methyl-D-aspartic acid-induced toxicity. The results suggest that PGE_2, by stimulating EP3 receptors, can contribute to the toxicity associated with cyclooxygenase and that antagonizing this receptor could be used therapeutically to protect against stroke- and excitotoxicity-induced brain damage.

Keywords: Cerebral ischemia; EP3 receptor agonist; G-protein-coupled receptors; Neurotoxicity; NMDA

1. Introduction

Inflammation has been shown to play a major role in the pathological response and outcome of stroke and other central nervous system disorders (Huang et al., 2006; Lucas et al., 2006). Inflammation is mediated at least in part by prostaglandins (PGs), which are produced through the cyclooxygenase (COX) pathway. PGs are secreted from a variety of cells in response to physiological or pathological insults (Doré et al., 2003; Minghetti, 2004) and mediate a variety of actions via specific membrane-bound receptors to maintain local homeostasis. Prostaglandin E_2 (PGE_2) mainly binds to a family of G-protein-coupled receptors known as EP receptors (Narumiya et al., 1999). The members of the EP receptor family, EP1, EP2, EP3, and EP4, elicit their actions by altering cyclic adenosine monophosphate (cAMP) or intracellular calcium concentrations. EP1 activates phospholipase C and phosphatidylinositol turnover and stimulates the release of intracellular calcium via a G_i-coupled mechanism. EP2 and EP4 both signal through a G_s-coupled mechanism that stimulates adenylyl cyclase and increases intracellular levels of cAMP (Narumiya et al., 1999). The EP3 receptor, which has several isoforms (Bilson et al., 2004), mediates the activation of several signaling pathways, leading to changes in cAMP levels, calcium mobilization, and activation of phospholipase C (Namba et al., 1993; Narumiya et al.,

Abbreviations: cAMP, cyclic adenosine monophosphate; CBF, cerebral blood flow, PG, prostaglandin; MABP, mean arterial blood pressure; MCA, middle cerebral artery; NMDA, *N*-methyl-D-aspartic acid; TTC, 2,3,5-triphenyl-tetrazolium chloride; ICV, intracerebroventricular.

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1999). Of the isoforms identified in mouse, EP3α and EP3β are reported to be coupled to G_i protein, which leads to the inhibition of adenylyl cyclase, whereas EP3γ has both inhibitory and stimulatory effects on adenylyl cyclase and cAMP accumulation (Irie et al., 1994; Sugimoto et al., 1993).

It has been reported that EP3 receptors are expressed in glial cells after intrastriatal injection of quinolic acid in rats (Slawik et al., 2004). This finding implies a direct role for EP3 receptors in various neurodegenerative disorders, such as stroke and Alzheimer disease. In addition, Zacharowski and colleagues (Zacharowski et al., 1999) reported that ONO-AE-248, a selective EP3 agonist, prevented the forskolin-induced increase in cAMP in CHO cell lines. Recently, Yamazaki et al. (2006) showed that EP3 receptor protein expression was significantly elevated in placenta 24 h after ischemia-reperfusion injury. EP3 receptors also have been shown to participate in inflammatory reactions in a mouse model of pleurisy, a model of acute inflammation (Yuhki et al., 2004), and have been reported to trigger pulmonary edema induced by platelet-activating factor in rats (Goggel et al., 2002). In addition, activation of EP3 receptor by PGE_2 in mice inhibits cAMP production in platelets and promotes platelet aggregation (Fabre et al., 2001).

We previously reported that in mice, the EP1 receptor plays a toxic role in transient cerebral ischemia and excitotoxicity models (Ahmad et al., 2006a), a finding further substantiated by Kawano et al. (2006), and that EP2 and EP4 receptor activation is protective in *N*-methyl-D-aspartic acid (NMDA)-induced excitotoxic lesions (Ahmad et al., 2005, 2006b). We also have evaluated the effects of the drug 1-OH-PGE1, which stimulates EP4 and to a lesser extent EP3, and found it to be neuroprotective in transient ischemia (Ahmad et al., 2006c) and oxidative stress after β-amyloid exposure in mouse primary cultured neurons (Echeverria et al., 2005).

Because previous studies have shown that the EP3 receptor significantly affects outcomes after ischemia-reperfusion injury in peripheral organs (Martin et al., 2005; Yamazaki et al., 2006; Zacharowski et al., 1999), our goal in this study was to determine the role of EP3 in brain injury induced by ischemia-reperfusion injury. Therefore, we investigated the effects of the EP3 agonist ONO-AE-248 [Ki estimated at 10000, 3700, 7.5, and 4200 for EP1, EP2, EP3, and EP4, respectively (Kiriyama et al., 1997; Narumiya and FitzGerald, 2001; Suzawa et al., 2000)] on middle cerebral artery occlusion (MCAO)-induced infarct volume, relative cerebral blood flow (CBF), mean arterial blood pressure (MABP), and other physiological parameters. Furthermore, since excitotoxicity is involved in the resulting injury caused by ischemia-reperfusion, we went on to determine the effect of ONO-AE-248 on lesion size after NMDA-induced toxicity. To confirm our results, we compared the NMDA-induced lesion volume in wildtype (WT) mice and in mice with a genetic deletion of the EP3 receptor.

2. Materials and methods

2.1. Animals and drugs

Studies were carried out on 8- to 10-week-old male C57BL/6 mice weighing 25 to 30 g obtained from Charles River Laboratories, Inc (Wilmington, MA). EP3 receptor knockout ($EP3^{-/-}$) mice were provided by Shuh Narumiya, University of Kyoto, Japan, and genotypes were confirmed by PCR. All animal experiments were carried out in accordance with the guidelines of the NIH and were approved by the Johns Hopkins University Animal Care and Use Committee. The animals were allowed free access to water and food before and after surgery. ONO-AE-248 was kindly donated by ONO Pharmaceuticals (Osaka, Japan).

2.2. Assessment of EP3 receptor protein expression in mouse brain

To address whether EP3 is present in mouse cortex and striatum, homogenates of the corticostriatal region of mouse brains were analyzed by Western blot, as described previously (Ahmad et al., 2006c). Protein concentrations were quantified by BCA assay (Pierce, Rockford, IL). Electrophoresis was performed on 12% polyacrylamide gels (Invitrogen, Carlsbad, CA), and proteins were transferred to nitrocellulose membrane (BIO-RAD, Hercules, CA). Blots were stained with Ponceau S Solution (Sigma, St. Louis, MO) to verify that equal amounts of protein were loaded into each lane. Membranes were blocked for 1 h at room temperature with 5% skim milk in phosphate-buffered saline with 0.1% Tween 20 before being incubated at 4 °C overnight with rabbit EP3 polyclonal antibody (1:500; Cayman, Ann Arbor, MI). Blots were washed and incubated with secondary antibody for 1 h at room temperature and then developed with ECL (Amersham Biosciences, Piscataway, NJ).

2.3. Stereotactic injection

Mice were anesthetized with 3.0% halothane and maintained with 1.0–1.5% continuous flow of halothane in oxygen-enriched air. Then the mice were mounted on a stereotactic frame and injected with 0.2 μl of different doses of ONO-AE-248 (0.5 nmol, 2.5 nmol, or 5.0 nmol) or vehicle (DMSO) via a 1-μl Hamilton syringe (Reno, NV) into the right lateral ventricle as described previously (Ahmad et al., 2006a). After the injection, the needle was retracted slowly, the hole was plugged with bone wax, and the wound was sutured. The mice were then either transferred to another setup for the MCAO procedure or left in place for NMDA injection (see below).

2.4. MCAO and reperfusion

During the MCAO procedure (Fig. 1, Illustration A), mouse rectal temperature was monitored and maintained at