Role of 12-lipoxygenase in volatile anesthetic-induced delayed preconditioning in mice

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Yasu M. Tsutsumi, Hemal H. Patel, Diane Huang, and David M. Roth. Role of 12-lipoxygenase in volatile anesthetic-induced delayed preconditioning in mice. Am J Physiol Heart Circ Physiol 291: H979–H983, 2006. First published April 28, 2006; doi:10.1152/ajpheart.00266.2006.—Delayed cardiac protection mediated by 12-lipoxygenase (12-LO) expression and activity has been linked to opioid receptor stimulation. The role of 12-LO in volatile anesthetic-induced delayed cardiac protection has not been determined. We tested the hypothesis that expression and activity of 12-LO mediate delayed cardiac protection induced by isoflurane in the mouse heart in vivo. Mice were pretreated with 1.4% isoflurane for 30 min and allowed to recover for 1, 12, or 24 h. Immunoblot analysis showed isoflurane significantly enhanced 12-LO protein expression at 12 and 24 h after isoflurane exposure, and this induction of 12-LO was confirmed by immunohistochemistry of whole heart sections at 24 h. The induced protein expression appeared to be localized to intercalated disc regions adjoining adjacent cardiac myocytes. Additionally, mice ± isoflurane (24 h previously) were subjected to 30 min coronary artery occlusion followed by 2 h of reperfusion in the presence and absence of a 12-LO inhibitor. Isoflurane reduced infarct size (27.1 ± 2.2% of the area at risk; n = 8) compared with the control group (44.6 ± 3.6%, n = 8). Baicalein (3 mg/kg), a selective 12-LO inhibitor, blocked the delayed protective effects of isoflurane pretreatment on infarct size (40.6 ± 3.6%, n = 8). These data suggest that 12-LO is an important mediator of isoflurane-induced delayed preconditioning.

immunoblot/immunohistochemistry

BRIEF ischemia leads to a reduction of cardiac injury after a second sustained period of ischemia (16). This protection, termed ischemic preconditioning (IPC), has been described to be a biphasic event. The early phase occurs immediately after the preconditioning stimulus (16), and the late phase (also called delayed cardiac protection or the second window of protection) is seen 12–24 h after the preconditioning stimulus (2). Recently, many studies have shown that volatile anesthetics (12, 26) and opioids (6, 21) also exert biphasic cardiac protection, and many characteristics of these pharmacological preconditioning agents are similar to those observed with IPC (11, 19); however, the common mediators of delayed cardiac protection remain unexplored.

The metabolism of arachidonic acid (AA) has been implicated in delayed cardiac protection. The most well-known metabolic pathway for AA metabolism involves cyclooxygenase (COX), and recent studies have implicated a deleterious effect of inhibiting the inducible COX isofrom, COX-2, in the ischemic myocardium (24). Importantly, AA, which is released during ischemia, can be metabolized via several other enzyme systems (i.e., lipoxygenase and cytochrome P-450) to generate signaling molecules (17), some of which have been detected in ischemic myocardium (14). Experiments in an isolated heart model suggest that IPC stimulates the activity of 12-lipoxygenase (12-LO) to generate 12-hydroxystearoenoic acid (12-HETE), leading to cardiac protection (4). Additionally, it has been reported that 12-LO is a downstream mediator of opioid-induced delayed preconditioning (18). Therefore, we hypothesized that activation and expression of 12-LO are important mediators of isoflurane-induced delayed cardiac protection.

MATERIALS AND METHODS

All animals were treated in compliance with animal use protocols approved by the Veterans Affairs San Diego Healthcare System Institutional Animal Care and Use Committee (San Diego, CA). C57BL/6 male mice (8–10 wk old, 24–26 g body wt) were purchased from Jackson Laboratory (Bar Harbor, ME).

Immunoblot analysis. Under light anesthesia (pentobarbital sodium; 40 mg/kg ip), mice were randomly divided into groups and received 30 min 100% oxygen in the control group or 1.4% isoflurane [1.0 minimum alveolar concentration (MAC) for mice] (27) by using a pressure-controlled ventilator (TOPO Ventilator, Kent Scientific, Torrington, CT; peak inspiratory pressure: 15 cmH2O, respiratory rate: 100 breaths/min), followed by a recovery period. After 1, 12, and 24 h of recovery ± isoflurane, mice were anesthetized with pentobarbital sodium (80 mg/kg ip), and hearts were excised. Left ventricles were homogenized in lysis buffer containing protease inhibitors and centrifuged at 1,000 rpm for 20 min to remove nuclei and debris. The supernatant was collected, and protein concentration was determined by Bradford assay. Protein was separated by SDS-PAGE 10% polyacrylamide precast gels (Invitrogen, Carlsbad, CA) and transferred to a polyvinylidene difluoride membrane by electroelution. Membranes were blocked in PBS containing 1.5% nonfat dry milk and incubated with 12-LO primary antibody (Cayman Chemical, Ann Arbor, MI) overnight at 4°C. Bound primary antibodies were visualized by using secondary antibodies conjugated with horseradish peroxidase from Santa Cruz Biotech (Santa Cruz, CA) and ECL reagent from Amersham Pharmacia Biotech (Piscataway, NJ). All displayed bands migrated at the appropriate size, as determined by comparison with molecular weight standards (Santa Cruz Biotech).

Immunofluorescence microscopy of the heart. After 24 h of recovery ± isoflurane, hearts were removed and the atrial tissue was dissected away. Ventricular tissue was mounted on a cryostat (−23°C), and 5-μm sections were cut in long axis. Sections were fixed with 2% paraformaldehyde in PBS for 10 min; incubated with

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tetrazolium chloride, 1% (Sigma) for 5 min at 37°C. After over-

was immediately excised and cut into 1.0-mm slices. Each slice of

the heart and the coronary artery was again occluded. The area at risk (AAR) b y2ho freperfusion. After reperfusion, mice were heparinized,

reperfusion. Mice then underwent 30 min index ischemia, followed

Louis, MO), a 12-LO inhibitor (22), 20 min before ischemia-

in Fig. 1. Some mice were given baicalein (3 mg/kg, Sigma, St.

randomly assigned to one of four experimental protocols as described

pad and a lamp, and ECG leads were placed to record heart rate.

Hemodynamics and blood gas analyses (i-STAT PCA, I-STAT, East Windsor, NJ) were performed 20 min after baicalein in a separate
group of mice anesthetized with pentobarbital sodium (80 mg/kg) with cannulation of the right carotid artery with a heparin-filled
catheter (Micro-Cannula; Harvard Apparatus, Holliston, MA).

Statistical analysis. A two-way ANOVA with Bonferroni post hoc
testing was used to determine significant changes in heart rates at each
time point. Statistical analyses of all other data were performed by one-way ANOVA, followed by a Bonferroni post hoc test. All data
are expressed as means ± SE. Statistical significance was defined as

RESULTS

Experimental animals. Experiments were performed in 76 mice. Seven mice died during or shortly after ischemia-reperfusion because of arrhythmias (control; 1; control +
baicalein, 3; isoflurane + baicalein, 3).

Hemodynamics. Heart rates after exposure to oxygen or isoflurane with and without 12-LO inhibition are summarized in Table 1. No significant differences were observed. Admin-
istration of baicalein in saline (3.0 mg/kg) had no significant effect on heart rate, mean arterial pressure, rate-pressure product, arterial pH, arterial P CO 2 , and arterial P O 2 levels compared
with those of untreated control animals, respectively (448 ± 16
vs. 444 ± 21 beats/min, 64 ± 2 vs. 64 ± 4 mmHg, 29.1 ± 1.7
vs. 28.1 ± 0.7 beats·min -1·mmHg -1 , 3, 7.40 ± 0.7 beats
vs. 7.44 ± 0.05 vs. 37 ± 3 vs. 40 ± 2 mmHg, 444 ± 29 vs. 445
± 52 mmHg, respectively; n = 4; P = nonsignificant).

Immunoblot analysis and immunofluorescence microscopy of the heart. Protein levels of 12-LO were increased signifi-
cantly at 12 and 24 h after 1.0 MAC isoflurane (Fig. 2,

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Table 1. Heart rate

<table>
<thead>
<tr>
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<th>Baseline</th>
<th>Treatment (30 min)</th>
<th>Recovery Period (15 min)</th>
<th>Baseline</th>
<th>Ischemia (30 min)</th>
<th>Reperfusion (2 h)</th>
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<tbody>
<tr>
<td>Control</td>
<td>433 ± 14</td>
<td>427 ± 15</td>
<td>437 ± 13</td>
<td>467 ± 11</td>
<td>437 ± 14</td>
<td>442 ± 10</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>438 ± 18</td>
<td>429 ± 17</td>
<td>442 ± 13</td>
<td>467 ± 19</td>
<td>437 ± 16</td>
<td>451 ± 17</td>
</tr>
<tr>
<td>Control + baicalein</td>
<td>452 ± 17</td>
<td>457 ± 18</td>
<td>457 ± 15</td>
<td>465 ± 14</td>
<td>442 ± 10</td>
<td>454 ± 24</td>
</tr>
<tr>
<td>Isoflurane + baicalein</td>
<td>459 ± 12</td>
<td>448 ± 17</td>
<td>456 ± 7</td>
<td>451 ± 16</td>
<td>439 ± 10</td>
<td>455 ± 20</td>
</tr>
</tbody>
</table>

Data are means ± SE (in beats/min). Mice were randomly exposed to 1.4% isoflurane for 30 min (day 1). After a 24-h recovery period, mice were subjected to 30 min of ischemia followed by 2 h of reperfusion (day 2).
reduced IS after 2 h of reperfusion (27.1 ± 2.2% of the AAR; n = 8) compared with control mice (44.6 ± 3.6%, n = 8; Fig. 3). Baicalein significantly attenuated the protective effects of isoflurane treatment with respect to IS (40.6 ± 3.6%, n = 8; Fig. 3).

DISCUSSION

Our laboratory has previously demonstrated that isoflurane acutely reduces irreversible ischemic injury in mice (27). The results of the current investigation indicate that isoflurane has a long memory period and protects the myocardium when administered 24 h before coronary artery occlusion in mice in vivo. This is the first study to demonstrate that isoflurane induces the expression of 12-LO after 12–24 h. We show that the delayed cardiac protection afforded by isoflurane is blocked by baicalein, an inhibitor of 12-LO. These data suggest that 12-LO is an important downstream mediator of volatile anesthetic-induced delayed cardiac protection.

Studies examining the ability of volatile anesthetics to produce delayed cardiac protection have produced conflicting results. Isoflurane produces delayed cardiac protection when administered 24 h before coronary artery occlusion and reperfusion in rabbit hearts in vivo (26), and sevoflurane produces delayed cardiac protection in the rat (15). In contrast, isoflurane did not produce delayed cardiac protection in dogs (10). These data suggest that delayed cardiac protection conferred by volatile anesthetics may differ among species. We show that isoflurane can produce delayed cardiac protection in the mouse. Variations in experimental design (i.e., isoflurane exposure time, method of...
volatile anesthetic exposure, and extent of ischemic insult) may account for differences between previous findings.

Our findings should be interpreted within the constraints of potential limitations. We acknowledge that baikalein, a bioactive herbal flavonoid, may have multiple pharmacological actions in addition to inhibition of 12-LO [i.e., reactive oxygen species scavenger (23), anti-inflammation (9), and inhibition of inducible nitric oxide (5)] that could affect delayed preconditioning in our model. Additionally, we measured only heart rate as a hemodynamic parameter after isoflurane. We showed previously that isoflurane exposure in a similar mouse protocol does not significantly alter hemodynamics or blood gases (27). Therefore, the reductions in myocardial IS produced by isoflurane most likely were not a result of changes in hemodynamic determinants of myocardial oxygen consumption.

AA metabolism can result in a variety of products that include prostaglandins, leukotrienes, and HETEs derived from COX and lipoxygenase. Ischemic and pharmacological preconditioning are characterized by complex signal transduction cascades in which COX-2 (13, 24, 26) and lipoxygenase (18, 25) have been implicated. The second window of ischemic and pharmacological preconditioning was abolished by selective COX-2 inhibition (13, 24, 26). However, no studies have examined the role of lipoxygenase in anesthetic-induced delayed preconditioning.

12-LO is a member of a family of lipoxygenases and has been shown to be induced by ischemic or hypoxic stress (1, 3, 20). Previous studies have observed an accumulation of 12-LO metabolites, including 12-HETE, after IPC, and subsequent lipoxygenase inhibition blocks the protective effects of IPC (4, 25). Additionally, 12-LO knockout mice show an impaired IPC response (7). A recent study in the rat demonstrates that pretreatment with SNC-121, a δ-opioid agonist, enhances the expression of 12-LO mRNA after 12 h and protein expression after 24 h. Opioid pretreatment was associated with increased conversion of AA to 12-HETE (18). 12-LO inhibitors block the conversion of AA to 12-HETE and attenuate the delayed protective effects of SNC-121 in terms of IS reduction (18). Consistent with these previous studies, our data show isoflurane induces 12-LO expression over 24 h, and 12-LO inhibition blocks isoflurane-induced delayed cardiac protection. These data suggest an important role for 12-LO in anesthetic-induced delayed cardiac protection.

GRANTS

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