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AICAR Preconditioning Prevents Postischemic Leukocyte Rolling and Adhesion: Role of K_{ATP} Channels and Heme Oxygenase

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Abstract

Objective—We previously demonstrated that pharmacologic activation of AMP-activated protein kinase (AMPK) with 5-aminoimidazole-4-carboxamide 1- β -D-ribofuranoside (AICAR) 24 hours prior to (AICAR preconditioning; AICAR-PC) ischemia/reperfusion (I/R) prevents postischemic leukocyte-endothelial cell adhesive interactions (LEI) by a mechanism initiated by endothelial nitric oxide synthase (eNOS)-dependent NO production during the period of AICAR-PC. The major aim of this study was to examine the role of ATP-sensitive potassium (K_{ATP}) channels and heme oxygenase as mediators of the antiadhesive effects of AICAR-PC during I/R 24 hours later.

Methods—Intravital fluorescence microscopy was used to quantify LEI in the small intestine of AICAR-preconditioned C57BL/6J mice treated with K_{ATP} channel or heme oxygenase inhibitors during I/R 24 hours after AICAR-PC in separate experiments.

Results—I/R induced marked increases in LEI relative to sham control mice, proadhesive responses that were prevented by AICAR-PC 24 hours prior to I/R. The effects of AICAR-PC to prevent postischemic LEI were abolished by K_{ATP} channel or heme oxygenase inhibition during I/R.

Discussion—Our results indicate that the antiadhesive effects of AICAR-PC are mediated by K_{ATP} channel- and heme oxygenase-dependent mechanisms during I/R.

Keywords

ischemia; reperfusion; leukocyte rolling and adhesion; AMPK; preconditioning; K_{ATP} channels; heme oxygenase

A wide variety of diverse agents and stimuli (e.g., short bouts of ischemia, ethanol ingestion at low to moderate levels, treatment with adenosine receptor agonists, reactive oxygen species

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(ROS), opioids, calcitonin gene-related peptide (CGRP), bradykinin, or nitric oxide (NO) donors) have been shown to induce the development of a protective anti-inflammatory phenotype in postcapillary venules, such that these vessels fail to support adhesion molecule expression, leukocyte rolling and adhesion, and increased vascular permeability when the small bowel is subsequently exposed to prolonged I/R [8,9,24,25,36,37,50]. The agents and stimuli that induce development of such protected states are referred to as preconditioning stimuli. The cellular mechanisms involved in preconditioning are often operationally subdivided into three categories: triggers or initiators, downstream signaling cascades, and mediators or effectors of the preconditioned states. Triggers or initiators of preconditioning refer to chemical signals that are released and/or ion channels that are activated in response to exposure to preconditioning stimuli. These inaugurating events, in turn, activate downstream signaling cascades that include a variety of kinases that function to sequentially phosphorylate proteins involved in the activation of normally dormant stress-responsive transcription factors. Transcriptional upregulation of protective genes culminates in the synthesis of new proteins that serve as mediators or effectors of the beneficial actions of preconditioning during I/R 24 hours later. Most of the agents that induce the development of preconditioned states do so by a triggering mechanism that involves the formation of NO by endothelial nitric oxide synthase (eNOS).

We recently demonstrated that preconditioning with 5-aminoimidazole-4-carboxamide 1- β -D-ribofuranoside (AICAR), an activator of the ubiquitously expressed heterotrimeric serine/threonine kinase AMP-activated protein kinase (AMPK), triggers entrance into a anti-inflammatory phenotype (a phenomenon termed AICAR preconditioning; AICAR-PC) by an eNOS-dependent mechanism [12]. Interestingly, the glucose-lowering agent, metformin, has been shown to activate AMPK and prevent the signaling of inflammatory cytokines through nuclear factor kappa B and tumor necrosis factor α [18]. This study also demonstrated that adhesion molecule expression was reduced by metformin or AICAR administration, a protective effect that was abrogated by the addition of a siRNA directed toward AMPK α 1. These activities have previously been attributed to eNOS by others [1,49]. A more recent study presented evidence of metformin's cardioprotective potential against I/R and its effect on AMPK activation and eNOS phosphorylation [3]. However, little is known about the effector mechanisms that are activated during I/R 24 hours after AMPK-dependent eNOS activation to mediate the reductions in postischemic leukocyte rolling and adhesion.

Many of the different preconditioning stimuli that have been studied to date appear to induce protection by K_{ATP} channel-dependent mechanisms. Most of this work is consistent with the concept that such preconditioning stimuli trigger entrance into a preconditioned state by activating surface (i.e., plasmalemmal) K_{ATP} (sK_{ATP}) channels while mitochondrial K_{ATP} ($mitoK_{ATP}$) channels serve as effectors that mediate protection during I/R 24 hours after the preconditioning stimulus is applied [31,32]. In light of these observations, we determined whether K_{ATP} channels serve as triggers versus effectors in AICAR-PC as the first goal of this study. Additionally, several lines of evidence suggested the possibility that heme oxygenase may serve as an effector of AICAR-PC. For example, AICAR-PC is triggered by the formation of eNOS-derived NO [12] and heme oxygenase activity is upregulated by NO donors [29]. In addition, the catalytic activity of heme oxygenase produces metabolites that exhibit powerful antiadhesive and antioxidant effects [14,20,30,42,43,46]. Thus, we hypothesized that this protein may serve as an important effector of AICAR-PC, mediating the antiadhesive actions of antecedent treatment with AICAR during I/R 24 hours later.

MATERIALS AND METHODS

Animals

Wild-type male C57BL/6J mice (six to seven weeks of age) were obtained from the Jackson Laboratories (Bar Harbor, Maine, USA). All mice were maintained on standard mouse chow and water ad libitum with 12-hour light-dark cycles, and used at 8–10 weeks of age. The experimental procedures have been described previously [25] and were performed according to the criteria outlined in the National Institutes of Health guidelines and were approved by the University of Missouri–Columbia Institutional Animal Care and Use Committee.

AICAR Preconditioning, Surgical Procedures, and Induction of I/R

Mice were preconditioned with the AMPK agonist, 5-aminoimidazole-4-carboxamide 1- β -D-ribofuranoside (AICAR; Sigma, St. Louis, MO, USA) (100 mg/kg, 0.5 mL) by intraperitoneal (i.p.) injection 24 hours prior to the induction of I/R in C57BL/6J mice. Twenty-four hours later, the mice were anesthetized initially by intramuscular (i.m.) injection of a mixture of ketamine (150 mg/kg body weight) and xylazine (7.5 mg/kg body weight). After attaining a surgical plane of anesthesia, a midline abdominal incision was performed and the superior mesenteric artery (SMA) was occluded with a microvascular clip for 0 (sham) or 45 minutes. After these procedures, the right carotid artery was cannulated and systemic arterial pressure was measured with a Statham P23A pressure transducer (Gould, Valley View, OH, USA) connected to the carotid artery catheter. Systemic blood pressure was recorded continuously with a personal computer (Power Macintosh 8600; Apple Cupertino, CA, USA) equipped with an analog-to-digital converter (MP 100; Biopac Systems, Goleta, CA, USA). The left jugular vein was cannulated for the administration of carboxyfluorescein diacetate, succinimidyl ester (CFDASE; Molecular Probes, Eugene, Oregon, USA), a fluorescent dye that labels leukocytes [40]. CFDASE was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 5 mg/mL, divided into 25- μ L aliquots, and stored in light-tight containers at -20°C until use. During the preparation and storage of CFDASE, care was taken to minimize light exposure. After the 45-minute ischemic period, the clip was gently removed and leukocytes were labeled with CFDASE by intravenous (i.v.) administration of the fluorochrome solution (250 $\mu\text{g/mL}$ saline) at a rate of 20 $\mu\text{L/min}$ for five minutes. The sham group had an equivalent 45-minute period without occlusion of the SMA prior to CFDASE administration. Leukocyte/endothelial cell adhesive interactions were observed over minutes 30–40 and 60–70 of reperfusion, or at equivalent time points in sham groups, via intravital fluorescence microscopy.

Intravital Fluorescence Microscopy

The mice were positioned on a 20 $\times$ 30 cm acrylic plastic board in a manner that allowed a selected section of small intestine to be exteriorized and placed carefully and gently over a glass slide covering a 4 $\times$ 3 cm hole centered in the Plexiglas. The exposed small intestine was superfused with warmed (37°C) bicarbonate-buffered saline (BBS; pH 7.4) at 1.5 mL/min, using a peristaltic pump (Model M312; Gilson, Middleton, WI, USA). The exteriorized region of the small bowel was covered with BBS-soaked gauze to minimize tissue dehydration, temperature changes, and the influence of respiratory movements. The superfusate was maintained at $37\pm0.5^{\circ}\text{C}$ by pumping the solution through a heat exchanger warmed by a constant temperature circulator (Model 1130; VWR, West Chester, PA, USA). Body temperature of the mouse was maintained between 36.5 and 37.5°C by use of a thermostatically controlled heat lamp. The Plexiglas board was mounted on the stage of an inverted microscope (Diaphot TMD-EF; Nikon, Melville, NY, USA), and the intestinal microcirculation was observed through a 20X objective lens. Fluorescence images of the microcirculation (excitation wavelength, 420–490 nm; emission wavelength, 520 nm) were detected with a charge-coupled device (CCD) camera (XC-77; Hamamatsu Photonics, Bridgewater, NJ, USA), a CCD camera control unit (C2400; Hamamatsu Photonics), and an intensifier head (M4314; Hamamatsu

Photonics) attached to the camera. Micro-fluorographs were projected on a television monitor (PVM-1953MD; Sony, New York, NJ, USA) and recorded on DVD using a DVD video recorder (DMR-E50; Panasonic, Secaucus, NY, USA) for off-line quantification of measured variables during playback of the recorded images. A video time-date generator (WJ810; Panasonic) displayed the stop-watch function onto the monitor.

The intravital microscopic measurements described below were obtained over minutes 30–40 and 60–70 of reperfusion or at equivalent time points in the sham control groups, as described below. The intestinal segment was scanned from the oral to aboral section, and 10 single, unbranched venules (20–50 μ m in diameter, 100 μ m in length) were observed for at least 30 seconds. Leukocyte-endothelial cell interactions (the numbers of rolling and firmly adherent leukocytes) were quantified in each of the 10 venules, followed by calculation of the mean value, which was used in the statistical analysis of the data. Circulating leukocytes were considered to be firmly adherent if they did not move along, or detach from, the venular wall for at least 30 seconds. Rolling cells are defined as cells crossing an imaginary line in the microvessel at a velocity that is significantly lower than centerline velocity; their numbers were expressed as rolling cells per minute. The numbers of rolling or adherent leukocytes were normalized by expressing each as the number of cells per square millimeter of vessel area.

Experimental Protocol

Figure 1 illustrates the general design of the experimental protocols for the study. Drug doses were selected based on previous experiments in our laboratory and reports in the literature [5,10,11,14,33,37].

Group 1: Sham—As a time control for the effects of experimental duration, mice in this group ($n=9$) received an i.p. injection of 0.5 mL saline, which was used as a vehicle for AICAR and other pharmacologic agents in groups outlined below. Twenty-four hours later, the superior mesenteric artery was exposed but not subjected to occlusion, with leukocyte/endothelial cell adhesive interactions quantified at time points comparable to those described for mice subjected to 45 minutes of intestinal ischemia, followed by 70 minutes of reperfusion (Group 2, below).

Group 2: I/R Alone—Mice in this group ($n=7$) were treated as described for Group 1 above, except that I/R was induced 24 hours after the i.p. injection of saline vehicle on Day 1. Leukocyte rolling and adhesion were quantified during minutes 30–40 and 60–70 of reperfusion following 45 minutes of ischemia on Day 2.

Group 3: AICAR-PC+I/R—To confirm that AMPK activation with AICAR-PC reduces leukocyte rolling and adhesion, mice in this group ($n=6$) were treated with AICAR (Sigma) (100 mg/kg, 0.5 mL, i.p. injection) on Day 1. Twenty-four hours later (Day 2), the intestine was exposed to I/R and leukocyte rolling and adhesion were quantified, as described above.

Group 4: Glibenclamide+AICAR-PC+I/R—To determine if AMPK activation with AICAR-PC requires K_{ATP} channels as initiators, the nonspecific K_{ATP} channel inhibitor, glibenclamide, was given prior to AICAR-PC. Mice ($n=6$) were treated with glibenclamide (Sigma) (6 mg/kg, 0.3 mL, i.p. injection) on Day 1, 10 minutes prior to AICAR-PC (Sigma) (100 mg/kg, 0.5 mL, i.p. injection). Twenty-four hours later (Day 2), the intestine was exposed to I/R and leukocyte rolling and adhesion were quantified, as described above.

Group 5: 5-HD+AICAR-PC+I/R—To determine if AMPK activation with AICAR-PC requires mito- K_{ATP} channels as an initiator, on Day 1, the specific mito K_{ATP} channel inhibitor, 5-hydroxydecanoic acid (5-HD), was given prior to AICAR-PC. Mice ($n=8$) were treated with

5-HD (Sigma) (10 mg/kg, 0.3 mL, i.p. injection) on Day 1, 10 minutes prior to AICAR-PC (Sigma) (100 mg/kg, 0.5 mL, i.p. injection). Twenty-four hours later (Day 2), the intestine was exposed to I/R and leukocyte rolling and adhesion were quantified, as described above.

Group 6: HMR-1098+AICAR-PC+I/R—To determine if AMPK activation with AICAR-PC requires sK_{ATP} channels as an initiator, on Day 1, the specific sK_{ATP} channel inhibitor, HMR-1098, was given prior to AICAR-PC. Mice ($n=9$) were treated with HMR-1098 (a generous gift from Garrett Gross, Medical College of Wisconsin Milwaukee, WI, USA) (6 mg/kg, 0.3 mL, i.p. injection) on Day 1, 10 minutes prior to AICAR-PC (Sigma) (100 mg/kg, 0.5 mL, i.p. injection). Twenty-four hours later (Day 2), the intestine was exposed to I/R and leukocyte rolling and adhesion were quantified, as described above.

Group 7: AICAR-PC+5-HD+I/R—To determine if AMPK activation with AICAR-PC requires mitoK_{ATP} channels as an effector, on Day 2, the specific mitoK_{ATP} channel inhibitor, 5-hydroxydecanoic acid (5-HD), was given just prior to I/R. Mice ($n=6$) were treated with AICAR (Sigma) (100 mg/kg, 0.5 mL, i.p. injection) on Day 1. Twenty-four hours later (Day 2), 5-HD (Sigma) (10 mg/kg, 0.3 mL, i.p. injection) was administered to mice 10 minutes prior to the induction of I/R and leukocyte rolling and adhesion were quantified, as described above.

Group 8: AICAR-PC+SnPP+I/R—To determine if AMPK activation with AICAR-PC requires heme oxygenase (HO) as an effector, on Day 2, the specific HO inhibitor, tin protoporphyrin-IX (SnPP), was given prior to I/R. On Day 1, mice ($n=6$) were treated with AICAR (Sigma) (100 mg/kg, 0.5 mL, i.p. injection). Twenty-four hours later (Day 2), SnPP (Porphyrin Products, Logan, Utah, USA) (50 mg/kg, 0.3 mL, i.p. injection, as described by Tulis et al. [42]) was administered one hour prior to I/R, followed by leukocyte rolling and adhesion quantification, as described above.

Group 9: AICAR-PC+CuPP+ I/R—To control for any nonspecific effects of protoporphyrins, the inactive copper protoporphyrin-IX (CuPP), was used on Day 2, 24 hours after AICAR-PC. On Day 1, mice ($n=6$) were treated with AICAR (Sigma) (100 mg/kg, 0.5 mL, i.p. injection). Twenty-four hours later (Day 2), CuPP (Porphyrin Products) (50 mg/kg, 0.3 mL, i.p. injection) was administered one hour prior to I/R, followed by leukocyte rolling and adhesion quantification, as described above.

Group 10: Hemin-PC+I/R—To determine if HO-1 induction with Hemin-PC is a sufficient stimulus to cause late phase protection against I/R, mice ($n=6$) were treated with hemin (Sigma) (50 mg/kg, 0.5 mL, i.p. injection) on Day 1. Twenty-four hours later (Day 2), intestinal I/R was induced and leukocyte rolling and adhesion were quantified, as described above.

Statistical Analysis

The data were analyzed with standard statistical analysis, such as analysis of variance (ANOVA) with Scheffe's (post-hoc) test for multiple comparisons. All values are expressed as the means $\pm$ standard error of the mean (SEM). Statistical significances were defined at $P<0.05$.

RESULTS

Figure 2 illustrates the effects of coincident administration of AICAR with the various K_{ATP} channel inhibitors 24 hours prior to I/R on postischemic leukocyte rolling and adhesion. I/R markedly increased leukocyte rolling (Figure 2, upper panel) and adhesion (Figure 2, lower panel), compared to sham (i.e., no ischemia) control mice. As we demonstrated previously [11], AICAR-PC completely prevented the postischemic increases in leukocyte rolling and

adhesion in response to I/R. When the dual K_{ATP} channel antagonist (glibenclamide) was administered coincident with AICAR, it blocked the protective effects of AICAR-PC on leukocyte rolling and had no significant effect on leukocyte adhesion. Neither the selective surface K_{ATP} channel inhibitor (HMR-1098) nor the selective mitochondrial K_{ATP} channel inhibitor (5-HD) exerted significant effects on AICAR-PC to inhibit the reduction in postischemic leukocyte rolling or adhesion, although there was a tendency for increased leukocyte rolling. These results suggest that in contrast to most other preconditioning stimuli, the antiadhesive effects of AICAR-PC are not triggered on Day 1 by a surface K_{ATP} channel-dependent mechanism. On the other hand, treatment with the mito K_{ATP} channel inhibitor, 5-HD, during I/R 24 hours after AICAR-PC abrogated the antiadhesive effects of this AMPK agonist to prevent leukocyte rolling and adhesion, indicating that mito K_{ATP} channels may serve as a mediator of these anti-inflammatory effects on Day 2.

The data depicted in Figure 3 show the effects of HO inhibition during I/R on AICAR-PC and demonstrate that the antiadhesive actions of antecedent AICAR can be mimicked by preconditioning with hemin, a HO inducer. Treatment with SnPP, a potent inhibitor of HO, completely abolished the effects of antecedent AICAR to prevent postischemic leukocyte rolling and adhesion when administered during I/R 24 hours after AICAR-PC (Figure 3). Treatment with CuPP, a protoporphyrin that exhibits no inhibitory activity toward HO, was ineffective in preventing the antiadhesive effects of AICAR-PC when administered during I/R on Day 2. Interestingly, preconditioning with hemin, in lieu of AICAR, effectively reduced postischemic leukocyte rolling and adhesion. These observations suggest that HO is an important end effector of AICAR-PC.

DISCUSSION

AMPK is a ubiquitously expressed heterotrimeric serine/threonine kinase that is often referred to as a metabolic “master switch” due to its high sensitivity to changes in the AMP:ATP ratios and its centralized role in both short- and long-term metabolic signaling pathways [4,6,16, 23]. The ability of AMPK activation to protect cellular energy levels and maintain the integrity of the mitochondrial membrane potential makes it an essential prosurvival signaling mediator in protecting cells and tissues from I/R injury [21]. In this regard, we recently demonstrated that an anti-inflammatory state could be induced by preconditioning with the AMPK activator, AICAR [11,12], such that postcapillary venules fail to support increased leukocyte rolling and adhesion during I/R 24 hours later. AICAR is first metabolized to ZMP, an AMP analog, which initially activates AMPK by binding to its gamma subunit [34]. This allosteric activation increases both AMPK activity and its affinity for upstream AMPK kinases, which in turn, phosphorylate AMPK at threonine-172 on the activation loop of the alpha subunit to further activate the enzyme. This latter process is responsible for the majority of AMPK activity [20, 35,38,47,48].

Our earlier work indicated that AICAR-PC involves both the AMPK $\alpha 1$ and AMPK $\alpha 2$ isoforms and is triggered by eNOS activation [11,12]. However, little is known about the effector mechanisms that are activated during I/R 24 hours after exposure to this preconditioning stimulus. The fact that mito K_{ATP} channels have been implicated as an important mediator for most forms of preconditioning, while treatment with K_{ATP} channel agonists prevent leukocyte infiltration when administered during reperfusion after prolonged ischemia [2,29,30,43,46], led us to first evaluate their role as an effector of AICAR-PC. Administration of the mito K_{ATP} channel inhibitor, 5-HD, during I/R 24 hours after AICAR-PC effectively abolished the antiadhesive effects that are induced by this preconditioning stimulus (Figure 2).

Because strong evidence exists to support the notion that K_{ATP} channels play a dual role in many forms of preconditioning, with sK_{ATP} channels subserving a trigger function in the initiation of a protected state while $mitoK_{ATP}$ channels serve as a mediator during I/R 24 hours later, we also evaluated the role of K_{ATP} channels as initiators for AICAR-PC. Treatment with either the sK_{ATP} channel (HMR-1098) or the $mitoK_{ATP}$ channel (5-HD) blockers coincident with AICAR-PC failed to prevent the protective reduction in postischemic leukocyte adhesion, though a modest increase in I/R-induced leukocyte rolling was observed. The dual K_{ATP} channel blocker, glibenclamide, was able to prevent the anti-inflammatory effects of AICAR-PC on leukocyte rolling but was without effect on leukocyte adhesion when coadministered on Day 1. Our results are consistent with the concept that AICAR prevents leukocyte adhesion by a mechanism independent of K_{ATP} channel activation on Day 1 (Figure 2). Indeed, higher AICAR doses have been shown to inhibit, not promote, K_{ATP} channel activity in murine pancreatic islet cells [45]. Thus, a unique finding of the present study is the apparent lack of dependence of AICAR-PC on a sK_{ATP} channel-dependent triggering mechanism.

We hypothesized that HO might serve as an effector of AICAR-PC for the following reasons. First, HO activity is exquisitely sensitive to upregulation by NO donors [27] and NO appears to play an important role in initiating the effects of AICAR-PC [11]. Second, cAMP, a downstream signaling molecule that is produced in response to adenosine A_2 -receptor activation, increases HO-1 mRNA, protein, and activity [17], and produces a preconditioned phenotype in the small intestine [26]. Adenosine A_2 receptor activation is another important trigger for AICAR-PC in the heart [7]. Third, induction of HO-1 suppresses P-selectin expression and leukocyte adhesion induced by hydrogen peroxide or I/R in the small intestine [19,44], inflammatory processes which are also prevented by AICAR-PC. Fourth, HO-1-derived CO also inhibits the expression of proinflammatory cytokines, as does AMPK activation [13,39]. Fifth, hemin-induced HO-1 expression exerts infarct-sparing effects in the setting of myocardial I/R [15], while the protective effects of ischemic-PC against I/R could be inhibited by pharmacologic inhibition of HO-1 with zinc protoporphyrin (ZnPP) or by prevention of its expression using siRNA approaches [22]. Moreover, HO-1 activity appears to be particularly rich in postcapillary venules of the small intestine [19]. Finally, and perhaps most importantly, the reaction products of HO-1-catalyzed heme degradation exert powerful antiadhesive and antioxidant effects [28,39,41]. Interestingly, hydrogen peroxide-induced leukocyte adhesion is inhibited by bilirubin, but not CO, whereas adhesive responses to lipopolysaccharide are attenuated by CO [20,33]. Although the mechanisms that account for these differential antiadhesive responses to the reaction products of HO activity are not known, they do indicate that the relative importance of each may depend on the nature of the initiating inflammatory stimulus.

In light of these considerations, we sought to determine whether treatment with SnPP, an HO inhibitor, would prevent the anti-inflammatory actions of AICAR-PC, which it did when administered during I/R 24 hours later (Figure 3). Importantly, administration of CuPP just prior to I/R, which demonstrates no inhibitory activity toward HO, failed to prevent the anti-inflammatory actions of antecedent AICAR (Figure 3). This latter result indicates that possible nonspecific effects due to the protoporphyrin moiety do not account for the ability of SnPP to prevent AICAR-PC. Finally, treatment with hemin, an agent known to induce the expression of HO-1, mimicked the antiadhesive actions of antecedent AICAR and prevented postischemic leukocyte rolling and adhesion when administered 24 hours prior to I/R (Figure 3). Similar results have been reported by Hayashi et al. [20], who demonstrated that increased leukocyte-endothelial cell adhesive interactions in the mesentery provoked by a hemorrhagic shock/resuscitation protocol were prevented by hemin-induced HO expression. While our studies do not shed light on the HO isoform involved in AICAR-PC, it is likely that induced expression of HO-1 plays a major role. Support for this notion is provided by our observation that SnPP treatment in the absence of I/R does not influence baseline leukocyte rolling or adhesion when

measured 1 or 24 hours after administration of this HO inhibitor (unpublished observations), suggesting that constitutively expressed HO-2 does not play a role in baseline leukocyte adhesion.

CONCLUSIONS

Although the results of our study indicate that HO and mitoK_{ATP} channels both play important roles as effectors of the anti-inflammatory phenotype elicited by antecedent AICAR treatment (Figures 2 and 3), it is not clear whether these mediators work in concert or separately to limit postischemic leukocyte rolling and adhesion. It is tempting to speculate that because independent blockade of either effector during I/R completely prevented the beneficial antiadhesive actions of AICAR-PC, HO and mitoK_{ATP} channel activity may be coupled as sequential signaling elements to mediate these effects. However, it is also possible that both function independently to influence leukocyte rolling and adhesion, but the additive effects of both are required to limit postischemic adhesive responses. This latter concept implies that multiple effector activities may be required to reach a threshold for the development of the antiadhesive effects of AICAR-PC. Clearly, much additional work will be required to address these possibilities. Nevertheless, our results clearly indicate that both HO and mitoK_{ATP} channels serve as important effectors of AICAR-PC during I/R 24 hours later.

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Figure 1.

Schematic illustration of the experimental protocols assigned to each group. The numbers at the top of the diagram refer to minutes in the time line for the protocol on Days 1 and 2 (24 hours between both 0s). Hatched bars indicate when the 10-minute video recordings were obtained in the protocol. Solid bars depict the 45-minute period of ischemia. Triangles illustrate when administration of saline vehicle or drugs was accomplished in the protocol time line. I/R, ischemia and reperfusion. See the text for further details.

Figure 2.

Role of K_{ATP} channels in preconditioning with AICAR (AICAR-PC) 24 hours prior to ischemia/reperfusion (I/R) on postischemic leukocyte rolling (upper panel) and adhesion (lower panel) determined after 30 and 60 minutes of reperfusion in wild-type C57BL/6J mice. The nonspecific K_{ATP} channel inhibitor, glibenclamide (Glib), as well as the specific surface K_{ATP} channel inhibitor, HMR-1098, and the mitochondrial K_{ATP} channel-specific inhibitor, 5-HD, were administered either 10 minutes prior to preconditioning with AICAR on Day 1 or 10 minutes prior to I/R on Day 2. Open and solid bars represent data obtained at minutes 30–40 and 60–70 of reperfusion, respectively. * denotes values statistically different from control

($P < 0.05$). # denotes values statistically different from I/R ($P < 0.05$). ϕ denotes values statistically different from AICAR-PC+I/R.

Figure 3.

Role of heme oxygenase (HO) in preconditioning with AICAR (AICAR-PC) 24 hours prior to ischemia/reperfusion (I/R) on postischemic leukocyte rolling (upper panel) and adhesion (lower panel) determined after 30 and 60 minutes of reperfusion in wild-type C57BL/6J mice. The HO inhibitor, SnPP, or the inactive protoporphyrin, CuPP, was administered one hour prior to I/R on Day 2, 24 hours after preconditioning with AICAR on Day 1. The HO-1 inducer, hemin, was given on Day 1 in place of AICAR-PC to examine its ability to induce a protective phenotype during I/R 24 hours later on Day 2. Open and solid bars represent data obtained at minutes 30–40 and 60–70 of reperfusion, respectively. * denotes values statistically different

from control ($P<0.05$). # denotes values statistically different from I/R ($P<0.05$). ϕ denotes values statistically different from AICAR-PC I/R.