Sarcolemmal K\textsubscript{ATP} channel triggers delayed ischemic preconditioning in rats

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Patel, Hemal H., Eric R. Gross, Jason N. Peart, Anna K. Hsu, and Garrett J. Gross. Sarcolemmal K\textsubscript{ATP} channel triggers delayed ischemic preconditioning in rats. Am J Physiol Heart Circ Physiol 288:H445–H447, 2005; doi:10.1152/ajpheart.00031.2004.—Previous work from our laboratory has shown that the sarcolemmal K\textsubscript{ATP} channel (sK\textsubscript{ATP}) is required as a trigger but not a mediator for delayed IPC-induced infarct size reduction. Because mechanistic differences have been found among \(\delta\)-opioids and that due to ischemic preconditioning (IPC), we determined whether the triggering mechanism of delayed IPC-induced infarct size reduction involves either the sK\textsubscript{ATP} or mK\textsubscript{ATP}. Male Sprague-Dawley rats received either sham surgery or IPC (3- to 5-min cycles of ischemia and reperfusion) 24 h before being subjected to 30 min of ischemia and 2 h of reperfusion. Infarct size was determined and expressed as a percentage of the area at risk, with significance compared with sham reported at \(P \leq 0.001\). A subset of both sham and IPC-treated rats received either the selective sK\textsubscript{ATP} channel antagonist, HMR-1098 (6 mg/kg), or the selective mK\textsubscript{ATP} channel antagonist, 5-hydroxydecanoic acid (5-HD; 30 mg/kg), given 5 min before IPC. Rats subjected to IPC demonstrated a significant reduction in infarct size compared with sham (29.2 ± 4.7 vs. 59.3 ± 2.5\%, respectively; \(P \leq 0.001\)). Prior administration of HMR-1098, but not 5-HD, abolished IPC-induced infarct size reduction (48.8 ± 2.9 and 28.8 ± 4.0\%, respectively; \(P \leq 0.001\)). Furthermore, administration of HMR 24 h after IPC, before index ischemia, did not abrogate IPC-induced infarct size reduction (33.0 ± 5.0 vs. 29.2 ± 4.7\%, respectively; \(P \leq 0.001\)). These data suggest that the sK\textsubscript{ATP} channel is required as a trigger but not a mediator for delayed IPC-induced infarct size reduction in rat hearts.

5-hydroxydecanoic acid; HMR-1098

The protection afforded by ischemic preconditioning (IPC) has been a standard for infarct size reduction against which other pharmacological stimuli are compared, with IPC-induced infarct size reduction found to occur in all species studied (16, 17, 19). Isolated human cardiomyocytes have also been shown to be protected by preconditioning before simulated ischemia (1, 3). IPC-induced infarct size reduction has been found to have a biphasic window: an early phase, which persists for 1–3 h after IPC and a delayed phase reappearing at 24 h (11). Although acute IPC has been extensively studied, little is known about the mechanism responsible for the trigger of the delayed, or late phase, of infarct size reduction produced by ischemia.

ATP-sensitive K\textsuperscript{+} channels (K\textsubscript{ATP}) have been previously found to be an end effector of IPC-induced delayed infarct size reduction, because administration of the nonselective K\textsubscript{ATP} channel antagonist glibenclamide or the selective mitochondrial K\textsubscript{ATP} (mK\textsubscript{ATP}) channel antagonist 5-hydroxydecanoic acid (5-HD) abolishes delayed IPC-induced infarct size reduction when administered the next day (2, 12). However, it is unknown whether the sarcolemmal K\textsubscript{ATP} (sK\textsubscript{ATP}) or mK\textsubscript{ATP} channel is involved in triggering or mediating the protection afforded by delayed IPC. Therefore, this study examined whether pharmacological inhibition of the sK\textsubscript{ATP} or mK\textsubscript{ATP} channel with selective inhibitors given at the time of IPC, or selective sK\textsubscript{ATP} inhibition before index ischemia can abolish IPC-induced infarct size reduction.

METHODS

The experimental procedures and protocols used in this study were reviewed and approved by the Animal Care and Use Committee of the Medical College of Wisconsin and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Pharmacological agents. The agents used for this study included the selective sK\textsubscript{ATP} channel antagonist HMR-1098 (Aventis) or the selective mK\textsubscript{ATP} channel antagonist 5-hydroxydecanoic acid (5-HD; RBI). Both agents were dissolved in water. A drop of 1 N sodium hydroxide was also added to the HMR-1098 solution to ensure solubility. Doses of HMR-1098 (6 mg/kg) and 5-HD (10 mg/kg) were selected based on previous studies conducted in the delayed infarct size model by our laboratory and others (4, 6, 14). The doses selected for this study were previously shown to block infarct size reduction produced by putative sK\textsubscript{ATP} and mK\textsubscript{ATP} channel openers selectively when given 5 min before the K\textsubscript{ATP} channel openers, 24 h before index ischemia (6).

Infarct size studies. Male Sprague-Dawley rats (230–335 g) were obtained from Harlan and subjected to an in vivo model of ischemia and reperfusion. Rats underwent primary anesthesia with an intraperitoneal injection of pentobarbital sodium (10 mg/kg) and were orally intubated and ventilated with room air. The right jugular vein was exposed and a catheter inserted for drug administration. A pericardiotomy was then performed, followed by adjustment of the left atrial appendage to locate the left coronary artery. Rats were then separated into eight groups (\(n = 5–7\) per group), with the first group consisting of untreated sham rats. A second group of rats were subjected to IPC that consisted of three cycles of 5 min of ischemia, followed by 5 min of reperfusion. In the next two groups, one group received HMR-1098 (6 mg/kg) and the other group received 5-HD (10 mg/kg) 5 min before IPC. Another group received HMR 24 h after IPC, 10 min before index ischemia. Additional groups received either HMR-1098 or 5-HD alone. After these interventions, the catheter was removed from the right jugular vein and the chest was closed. Each group was allowed to recover for 24 h. Rats then underwent 30 min index ischemia, followed by 2 h of reperfusion with assessment of infarct size as previously described (15). Hemodynamics, including heart rate, mean arterial pressure, and rate pressure product, were quantified during baseline, 15 min into ischemia and at 2 h of reperfusion and compared with untreated sham rats for each group.

Statistical measurements. All values were denoted as means ± SE, and the data were analyzed for statistical significance by Prism software. A two-way ANOVA for time and treatment with Bonfer...
roni’s correction for multiplicity was used to determine significant changes in hemodynamics at the three time points measured. Statistical significance for changes in infarct size was determined by performing a one-way ANOVA with Bonferroni’s correction for multiplicity. Values significantly different from untreated sham were indicated (P value of <0.001).

RESULTS

Hemodynamics. Heart rate, mean arterial pressure, and rate pressure product were quantified during baseline, 15 min into ischemia and at 2 h of reperfusion and compared with untreated sham rats for each group (Table 1).

<table>
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<th></th>
<th>Baseline</th>
<th>15 min Ischemia</th>
<th>2 h Reperfusion</th>
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<tr>
<td></td>
<td>HR</td>
<td>MAP</td>
<td>RPP</td>
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<tr>
<td>Sham</td>
<td>346 ± 13</td>
<td>110 ± 6</td>
<td>46 ± 2</td>
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<td>IPC</td>
<td>374 ± 7</td>
<td>97 ± 8</td>
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<tr>
<td>5-HD (10 mg/kg) + IPC</td>
<td>374 ± 20</td>
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<td>HMR (T, 6 mg/kg)</td>
<td>398 ± 21</td>
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<tr>
<td>5-HD (10 mg/kg)</td>
<td>341 ± 16</td>
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<td>IPC + HMR (6 mg/kg)</td>
<td>364 ± 10</td>
<td>123 ± 5</td>
<td>54 ± 3</td>
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<tr>
<td></td>
<td>380 ± 5</td>
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Values are means ± SE; n, no. of animals. T, trigger; drug was administered 24 h before index ischemia; E, end effector; drug was administered 10 min before index ischemia; IPC: ischemic preconditioning; 5-HD, 5-hydroxydecanoic acid; HR, heart rate; MAP, mean arterial pressure; RPP, rate pressure product. Drug doses are listed in the chronological order of intervention. *P < 0.001, significant differences in means ± SE vs. vehicle.

Infarct size studies. For each group, the area at risk compared with total left ventricular weight was calculated. No significant differences were seen between groups (data not shown). Rats subjected to IPC demonstrated a significant reduction in IS compared with untreated sham (Fig. 1; 29.2 ± 4.7 vs. 59.3 ± 2.5%, respectively; P < 0.001). Prior administration of HMR-1098, but not 5-HD, abolished IPC-induced IS reduction (Fig. 1; 48.8 ± 2.9 and 28.8 ± 4.0%, respectively, P < 0.001). Furthermore, administration of HMR 24 h after IPC, before index ischemia, did not abrogate IPC-induced infarct size reduction (33.0 ± 5.0 vs. 29.2 ± 4.7%, respectively; P ≤ 0.001). Administration of either HMR-1098 or 5-HD 24 h before index ischemia or HMR 10 min before index ischemia had no effect on infarct size when administered alone (Fig. 1; 56.8 ± 1.5, 58.0 ± 2.0 and 59.4 ± 2.2%, respectively).

DISCUSSION

This is the first study to examine the role of K_ATP channels in the triggering mechanism of delayed IPC. Our findings demonstrate that the triggering of delayed IPC is sensitive to sK_ATP channel blockade; however, it is insensitive to mK_ATP channel blockade. Previous studies (2, 12) have found a role in the mitochondrial K_ATP channel as a mediator/end effector. In combination with our findings, the
sK_{ATP} channel is a trigger, whereas the mK_{ATP} channel is an end effector of delayed IPC in rats (see Fig. 2).

Additional studies have also addressed the question of whether K_{ATP} channels are involved as a trigger of delayed infarct size reduction produced by heat shock (8), opioids (4, 14), or K_{ATP} channel openers (6). Administration of either glibenclamide or 5-HD before heat shock failed to abolish delayed heat shock-induced infarct size reduction (8); however, administration of HMR-1098 or glibenclamide, but not 5-HD, just before the δ-opioid agonist, SNC-121, was able to abolish delayed δ-opioid-induced infarct size reduction (14). Administration of either 5-HD or HMR-1098 could also abolish κ-opioid-induced delayed infarct size reduction produced by U50,488H (4). The delayed infarct size reduction afforded by the K_{ATP} channel openers, P-1075 and diazoxide, could also be abolished by prior administration of either HMR-1098 or 5-HD (6). These data, together with our present findings, would suggest that the triggering mechanism involved in delayed infarct size reduction may vary due to the stimulus involved; IPC, heat shock, opioids, or K_{ATP} channel openers.

Acute IPC-induced infarct size reduction is absent in a knockout mouse model with the sK_{ATP} channel subunit Kir6.2 deleted, although the knockout mouse has functional mK_{ATP} channels (19). This knockout mouse also shows diminished IPC-induced changes in myocardial energetics (7) and showed attenuated cardioprotective effects afforded by diazoxide (18). For acute IPC-induced infarct size reduction, the mK_{ATP}, but not the sK_{ATP}, appears to be required for protection, which indicates that delayed IPC occurs through a different temporal K_{ATP} channel-dependent mechanism compared with acute IPC (5).

Our findings are not without potential limitations, including the possibility that 5-HD may have nonspecific sites of action, such as the electron transport chain, as previously suggested (9, 10). In addition, our findings are also limited because both HMR-1098 and 5-HD were administered as a bolus before IPC, instead of infusing HMR-1098 or 5-HD for the duration of IPC. This protocol was selected to allow for a direct comparison between these findings and previous studies of opioids and K_{ATP} channel openers that used the same dose and method of administration of HMR-1098 and 5-HD (4, 6, 14). This method of administration should adequately block IPC because the half-life for 5-HD was reported previously to be 7 min in dogs and the half life of HMR is between 60–90 min (13, and H. Goegerlein, unpublished observation).

In summary, our findings implicate a role for the sK_{ATP}, but not the mK_{ATP} channel, as a trigger for IPC-induced delayed infarct size reduction in rats. These findings further extend our understanding of the mechanism of infarct size reduction involving IPC and K_{ATP} channels.

GRANTS
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REFERENCES