Sarcolemmal $K_{\text{ATP}}$ channel triggers delayed ischemic preconditioning in rats

Hemal H. Patel,* Eric R. Gross,* Jason N. Peart, Anna K. Hsu, and Garrett J. Gross

Medical College of Wisconsin, Department of Pharmacology and Toxicology, Milwaukee, Wisconsin

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Patel, Hemal H., Eric R. Gross, Jason N. Peart, Anna K. Hsu, and Garrett J. Gross. Sarcolemmal $K_{\text{ATP}}$ 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 $K_{\text{ATP}}$ channel (s$K_{\text{ATP}}$) is required as a trigger for delayed cardioprotection upon exogenous opioid administration. We also established that the mitochondrial $K_{\text{ATP}}$ (m$K_{\text{ATP}}$) channel is not required for triggering delayed opioid-induced infarct size reduction. Because mechanistic differences have been found among opioid and that due to ischemic preconditioning (IPC), we determined whether the triggering mechanism of delayed IPC-induced infarct size reduction involves either the s$K_{\text{ATP}}$ or m$K_{\text{ATP}}$. 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 s$K_{\text{ATP}}$ channel antagonist, HMR-1098 (6 mg/kg), or the selective m$K_{\text{ATP}}$ channel antagonist, 5-hydroxydecanoic acid (5-HD; 10 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 s$K_{\text{ATP}}$ 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

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Report

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$^+$ channels (K$_{\text{ATP}}$) have been previously found to be an end effector of IPC-induced delayed infarct size reduction, because administration of the nonselective K$_{\text{ATP}}$ channel antagonist glibenclamide or the selective mitochondrial K$_{\text{ATP}}$ (mK$_{\text{ATP}}$) 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$_{\text{ATP}}$ (sK$_{\text{ATP}}$) or mK$_{\text{ATP}}$ channel is involved in triggering or mediating the protection afforded by delayed IPC. Therefore, this study examined whether pharmacological inhibition of the sK$_{\text{ATP}}$ or mK$_{\text{ATP}}$ channel with selective inhibitors given at the time of IPC, or selective sK$_{\text{ATP}}$ 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$_{\text{ATP}}$ channel antagonist HMR-1098 (Aventis) or the selective mK$_{\text{ATP}}$ 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$_{\text{ATP}}$ and mK$_{\text{ATP}}$ channel openers selectively when given 5 min before the K$_{\text{ATP}}$ 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 Bonferroni correction was used.
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). Significant differences were found for mean arterial pressure and rate pressure product for some groups compared with sham.

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 KA_{ATP} channels in the triggering mechanism of delayed IPC. Our findings demonstrate that the triggering of delayed IPC is sensitive to sKA_{ATP} channel blockade; however, it is insensitive to mKA_{ATP} channel blockade. Previous studies (2, 12) have found a role for the mKA_{ATP} channel as an end effector of delayed IPC, which would suggest that in combination with our findings, the
sK\textsubscript{ATP} channel is a trigger, whereas the mK\textsubscript{ATP} channel is an end effector of delayed IPC in rats (see Fig. 2).

Additional studies have also addressed the question of whether K\textsubscript{ATP} channels are involved as a trigger of delayed infarct size reduction produced by heat shock (8), opioids (4, 14), or K\textsubscript{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\textsubscript{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\textsubscript{ATP} channel openers.

Acute IPC-induced infarct size reduction is absent in a knockout mouse model with the sK\textsubscript{ATP} channel subunit Kir6.2 deleted, although the knockout mouse has functional mK\textsubscript{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\textsubscript{ATP}, but not the sK\textsubscript{ATP}, appears to be required for protection, which indicates that delayed IPC occurs through a different temporal K\textsubscript{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 ischemia. In addition, our findings are also limited because both HMR-1098 and 5-HD were administered as a bolus before ischemia.  

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