Sex differences in myocardial infarct size are abolished by sarcolemmal K_{ATP} channel blockade in rat

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Johnson, Micah S., Russell L. Moore, and David A. Brown. Sex differences in myocardial infarct size are abolished by sarcolemmal K_{ATP} channel blockade in rat. Am J Physiol Heart Circ Physiol 290: H2644–H2647, 2006. First published February 10, 2006; doi:10.1152/ajpheart.01291.2005.—This study was conducted to examine the relationship between myocardial ATP-sensitive potassium (K_{ATP}) channels and sex differences in myocardial infarct size after in vitro ischemia-reperfusion (I/R). Hearts from adult male and female Sprague-Dawley rats were excised and exposed to an I/R protocol (1 h of ischemia, followed by 2 h of reperfusion) on a modified Langendorff apparatus. Hearts from female rats showed significantly smaller infarct sizes than hearts from males (23 ± 4 vs. 40 ± 5% of the zone at risk, respectively; P < 0.05). Administration of HMR-1098, a sarcolemmal K_{ATP} channel blocker, abolished the sex difference in infarct size (42 ± 4 vs. 45 ± 5% of the zone at risk in hearts from female and male rats, respectively; P = not significant). Further experiments showed that blocking the K_{ATP} channels in ischemia, and not reperfusion, was sufficient to increase infarct size in female rats. These data demonstrate that sarcolemmal K_{ATP} channels are centrally involved in mechanisms that underlie sex differences in the susceptibility of the intact heart to I/R injury.

ISCHEMIC HEART DISEASE is the leading cause of death in both males and females in the industrialized world, with myocardial infarction being the most common manifestation of ischemic heart disease (18). Among humans, premenopausal women have a lower risk of cardiovascular disease than age-matched men, and there is growing evidence that there is a sex-specific difference in the susceptibility of the myocardium to infarction after ischemia in dogs (15), rats (1, 4), mice (10, 11), as well as in humans (19).

The cellular basis for sex differences in the susceptibility of the heart to ischemia-reperfusion (I/R) injury has not been elucidated. However, there has been speculation that that sarcolemmal ATP-sensitive potassium (sarcK_{ATP}) channels are involved. This speculation derives from observations that, relative to males, the protein expression of sarcK_{ATP} channel involved. This speculation derives from observations that, relative to males, the protein expression of sarcK_{ATP} channel is greater in hearts from females (4, 26). Additionally, treatment of heart-derived H9c2 cells with 17β-estradiol elicits a marked increase in the expression of both Kir6.2 and SUR2a subunits and an increase in the resistance of those cells to hypoxia-induced Ca^{2+} overload that, in turn, could be abolished by sarcK_{ATP} channel blockade with HMR-1098 (25). Whereas these observations clearly implicate a role for sarcK_{ATP} channels in the sex-dependent differences in the susceptibility of the heart to I/R injury, pharmacological blockade of sarcK_{ATP} channels in the intact heart has not been performed to confirm their role in sex-dependent cardioprotection. Thus the major objective of this study was to determine whether blocking sarcK_{ATP} channels would abolish the sex difference in myocardial infarct size after I/R.

METHODS

Experimental animals. Adult male and female Sprague-Dawley rats (Harlan) were used in the study (n = 43 animals in total). All animals were provided food (standard rat chow) and water ad libitum. All experiments were conducted with prior approval from the Institutional Animal Care and Use Committee at the University of Colorado at Boulder and in accordance with guidelines established by the American Physiological Society.

Infarct size measurements. The I/R protocol and the measurement of myocardial infarct size employed were identical to the protocol previously described in detail by our laboratory (2, 3, 4). Briefly, hearts were isolated and perfused on a modified Langendorff apparatus and, after 15 min of “baseline” perfusion, were subjected to 1 h of regional ischemia, followed by 2 h of reperfusion. Detailed descriptions and schematics of the I/R protocol used in each of our studies and the times during which HMR-1098 (30 μM) was administered to the hearts can be found in Figs. 1 and 2 and in their respective legends.

Statistical analysis. Infarct sizes between groups were compared using a 2 × 2 ANOVA. Significant main effects of both drug (control vs. HMR-1098) and sex difference were followed by a two-tailed Student’s t-test between groups for comparison. The temporal effect of HMR-1098 administration on infarct size was analyzed by using a one-way ANOVA, followed by Bonferroni-corrected post hoc tests. A P < 0.05 value was chosen to indicate statistical significance.

RESULTS

Infarct size. The mean zone at risk (ZAR) for the experimental groups ranged from 39–44% of the left ventricle, with no significant differences between experimental groups. Hearts from female rats exhibited significantly less I/R damage (Fig. 1) than those of their male counterparts (23 ± 4 vs. 40 ± 5% of the ZAR, P = 0.038). The addition of HMR-1098 during the I/R protocol abolished the sex difference in myocardial infarct size [42 ± 4 vs. 45 ± 5% of the ZAR in hearts from female and male rats, respectively, P = not significant (NS); Fig. 1]. Whereas the presence of HMR-1098 had no effect on infarct size in male hearts exposed to I/R, infarct size increased by 82% in female hearts perfused with HMR-1098 during the 3-h I/R protocol (P = 0.013).

Data pertaining to the effect of HMR-1098 perfusion on infarct size during different time points in the protocol are presented in Fig. 2. Acute administration of HMR-1098 for 5 min before the ischemic period increased infarct size from...
To the contrary, infarct size, after the addition of HMR-1098 during the reperfusion period only, was not different from that of control hearts (23 ± 3% of the ZAR; P = NS). Infarct size after 3 h of HMR-1098 administration was not statistically different from infarct size when HMR-1098 was administered immediately before ischemia (42 ± 4 vs. 52 ± 3% of the ZAR, respectively; P = NS).

**DISCUSSION**

The majority, but not all (16, 24), of the studies performed on a wide variety of experimental animal models provide strong evidence that myocardium from females, relative to males, is intrinsically more resistant to I/R-induced tissue injury and infarction (1, 4, 7, 10, 15, 19). The data in this study corroborate these findings (Fig. 1). The reasons for the lack of complete consensus on this issue are not known, but the basis for the disparate findings may be related to the interpretive complexities associated with the use of different animals models and analytical methodologies. Additionally, it is apparent that the cellular basis for sex differences in the susceptibility of the heart to infarction is probably quite complex and to date has eluded clear description. To begin to address this issue, this study was conducted to determine the potential contribution of sarcKATP channels in sex-specific resistance to myocardial infarction.

In our earlier work (4), we found that the cardioprotection associated with female sex was accompanied by a greater protein expression of the sarcKATP channel subunits. The present study provides the first demonstration in the intact heart that blockade of sarcKATP channels during in vitro I/R abrogates the sex difference in myocardial infarct size and implicates sarcKATP channels as being required mediators of enhanced protection in female rats. Previous studies from Jovanovic’s laboratory (26) on isolated cell systems have shown that cardiocytes from female hearts expressed a greater pinacidil-induced outward current and were more resistant to cellular calcium overload than cells from male hearts. A subsequent study by this group (25) indicated that 17-β estradiol increased sarcKATP channel expression (and is protective against hypoxia reoxygenation-induced cell injury) in a heart-derived H9c2 cell line and that KATP channel antagonist abolished the protection afforded by 17-β estradiol. The data presented herein provide a crucial extension of these studies in that they clearly demonstrate that the sarcKATP channel-dependent cardioprotection in females is present in the whole heart and that the temporal
involvement of sarc\(\text{K}_{\text{ATP}}\) channels in the cardioprotective process is most important during the ischemic, rather than the reperfusion, phase of I/R stress.

Blocking the sarc\(\text{K}_{\text{ATP}}\) channel population in male rats had no effect on myocardial infarct size (Fig. 1). These data are consistent with a number of studies indicating that sarc\(\text{K}_{\text{ATP}}\) channel blockade during an I/R protocol does not alter infarct size in hearts from male rats (13, 21) and support our evidence that a sex-specific difference in \(\text{K}_{\text{ATP}}\) channel-induced protection from myocardial infarction is present. It is also noteworthy that in nonpreconditioned hearts from both male (21, 27) and female (2) rats, it has been demonstrated that the putative mitochondrial \(\text{K}_{\text{ATP}}\) channel antagonist 5-hydroxydecanoate (5-HD) has no effect on infarct size, suggesting that mitochondrial \(\text{K}_{\text{ATP}}\) channels do not play a central role in the sex-dependent differences in the susceptibility of the heart to myocardial infarction. This also indicates that the intrinsic, sex-dependent resistance of the heart to I/R injury is not analogous to a broad array of acquired cardioprotection models, almost all of which have been shown to be 5-HD sensitive (5, 8, 9, 14, 21–23). One notable exception is cardioprotection acquired by long-term exercise, which has recently been shown to be HMR-1098 sensitive and 5-HD insensitive (2); interestingly, this study was conducted on female rats.

Although the exact mechanisms whereby \(\text{K}_{\text{ATP}}\) channels may provide protection in female, but not male, hearts require further experimentation, our results are intriguing in light of a very recent study by Bae and Zhang (1). These investigators found that the smaller infarct size in female hearts was related to an increased activity of protein kinases B (also called Akt) and C. Analogous to our findings (Fig. 1), inhibition of these kinases before ischemia resulted in expanded infarct size in female, but not male, hearts. Given the fact that protein kinases have been widely shown to influence the activity of myocardial \(\text{K}_{\text{ATP}}\) channels (for review articles, see Refs. 6, 17, 20, and 28), an interesting sex-specific cascade of events incorporating receptor activation, second messenger signaling, \(\text{K}_{\text{ATP}}\) channel phosphorylation, and, ultimately, cardioprotection may be slowly emerging. Obviously, future experiments will be needed to elucidate the specific sex differences in cellular strategies of cardioprotection.

In summary, we have shown that inhibition of sarc\(\text{K}_{\text{ATP}}\) channels during ischemia abolishes the sex difference in infarct size, providing evidence that hearts from males and females may respond to ischemia via very different mechanisms. Given the prevalence of studies examining both paradigms of preconditioning and intrinsic protection from infarction, our hope is that further attention will be given to the sex of animals studied to further explore sex differences in the susceptibility of the myocardium to I/R injury.

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