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Journal of Molecular and Cellular Cardiology 39 (2005) 637–646
Journal of Molecular and Cellular Cardiology
www.elsevier.com/locate/yjmcc

Original article

Reduced effectiveness of HMR 1098 in blocking cardiac sarcolemmal K_{ATP} channels during metabolic stress

R.D. Rainbow [a], R.I. Norman [b], D. Hudman [a], N.W. Davies [a], N.B. Standen [a,*]

[a] *Department of Cell Physiology and Pharmacology, University of Leicester, PO Box 138, Leicester LE1 9HN, UK*
[b] *Department of Cardiovascular Sciences, University of Leicester, PO Box 138, Leicester LE1 9HN, UK*

Received 11 January 2005; received in revised form 3 June 2005; accepted 21 June 2005

Available online 15 August 2005

Abstract

ATP-sensitive K^+ (K_{ATP}) channels are involved in ischemic cardioprotection induced by preconditioning (IPC), though the relative role of sarcolemmal (sK_{ATP}) and mitochondrial ($mitoK_{ATP}$) channels remains controversial. The sK_{ATP}-selective sulphonylthiourea HMR 1098 has often been reported to be without effect on ischemic cardioprotection, suggesting minimal involvement of sK_{ATP}. Since some sulphonylureas show reduced potency under conditions of metabolic stress, we used patch clamp to assess the ability of HMR 1098 to block sK_{ATP} currents of adult rat ventricular myocytes activated by metabolic inhibition (MI, NaCN + iodoacetate). In contrast to the prototype sulphonylurea glibenclamide, HMR 1098 (10 μM) was without effect on sK_{ATP} currents, and also did not inhibit MI-induced action potential shortening. However, HMR 1098 blocked sK_{ATP} current induced by the K_{ATP} opener pinacidil (IC_{50} = 0.36 ± 0.02 μM), and reversed pinacidil-induced action potential shortening. In inside-out patches, block by HMR 1098 was relieved by increasing MgADP concentrations (1–100 μM). HMR 1098 inhibited pinacidil-activated recombinant Kir6.2/SUR2A channels with a similar IC_{50} (0.30 ± 0.04 μM), but was less effective when channels were activated by low intracellular ATP. HMR 1098 displaced binding of the pinacidil analogue [^{3}H]P1075 to native cardiac membranes with a biphasic inhibition curve. Our results show that HMR 1098 becomes a much less effective inhibitor of sK_{ATP} during metabolic stress, and suggest that the lack of effect of HMR 1098 on ischemic cardioprotection reported in some studies may represent loss of block by the drug under these conditions rather than a lack of involvement of sK_{ATP} channels.

Keywords: Cardiac myocytes; HMR 1098; K_{ATP} channel; Preconditioning; Sulphonylurea; Kir6.2/SUR2A; Ischemia

1. Introduction

ATP-sensitive (K_{ATP}) channels of cardiac muscle are involved in the response to metabolic stress, where there is good evidence that they serve a protective function. In particular, they appear to play a central role in the powerful protective effect of ischemic preconditioning (IPC), where a brief period of ischemia followed by reperfusion protects against damage induced by a subsequent prolonged ischemia [1,2]. Initially, this protective effect was ascribed to K_{ATP} channels in the surface membrane (sarcolemmal or sK_{ATP} channels), but later it was suggested that K_{ATP} channels expressed in the mitochondrial inner membrane ($mitoK_{ATP}$) mediate protection [3–5]. The relative roles of sK_{ATP} and $mitoK_{ATP}$ channels in cardioprotection remain controversial and much of the evidence for their involvement has relied on the selectivity of pharmacological agents. In particular the opener diazoxide and blocker 5-hydroxydecanoate (5-HD) have been used to provide evidence for the involvement of $mitoK_{ATP}$ channels [2–5], though the selectivity of these agents for $mitoK_{ATP}$ channels, rather than other mitochondrial processes, has been questioned recently [6–9]. Conversely, the sulphonylthiourea compounds HMR 1883 and its sodium salt HMR 1098 show good selectivity for cardiac sK_{ATP} channels over $mitoK_{ATP}$ as well as pancreatic or vascular K_{ATP} channels [10–12], and have therefore been used to assess the involvement of sK_{ATP} channels in cardioprotection.

Recent molecular work has provided renewed evidence for a role of sK_{ATP} channels in cardioprotection. While the molecular composition of $mitoK_{ATP}$ channels is currently undetermined, Kir6.2 pore-forming subunits and SUR2A sul-

* Corresponding author. Tel.: +44 116 252 3302; fax: +44 116 252 5045.
E-mail address: nbs@le.ac.uk (N.B. Standen).

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doi:10.1016/j.yjmcc.2005.06.017

phonylurea receptor subunits are required for cardiac sK_{ATP} channels [13]. Ischemic protection is abolished in Kir6.2 knockout mice, which lack functional cardiac sK_{ATP} channels [14], and in rat cardiac myocytes transfected with a dominant-negative fragment of SUR2A that downregulates sK_{ATP} expression [15]. In both of these studies protection by preconditioning was also absent, which could reflect the loss of sK_{ATP} channels as a mediator, but might also result from a change in the conditions necessary to induce preconditioning in a situation where sK_{ATP} channels are absent or downregulated. Whatever the explanation, these experiments suggest an essential role for sK_{ATP} in cardioprotection.

There is good evidence that HMR 1098 is an effective blocker of sK_{ATP} [10,12,16], however HMR 1098 has often been reported not to block the cardioprotective effects of IPC or of other preconditioning stimuli in a variety of preparations. For example, HMR1098 did not prevent the reduction in infarct size induced by IPC or bradykinin in rabbit hearts exposed to subsequent ischemia [17,18], or the protective effects of IPC or opioids in rat hearts [19–21]. Similarly, HMR 1098 did not prevent the protective effect of IPC in an isolated human myocardial tissue model [22]. We considered, therefore, whether the lack of effect of HMR 1098 on cardioprotection reported in some studies might result from a reduction in the effectiveness of the sulphonylurea under conditions of metabolic stress or ischemia, rather than indicating a lack of involvement of sK_{ATP} channels. The classical sulphonylurea glibenclamide has been shown to partially lose its potency in blocking sK_{ATP} channels under conditions of metabolic inhibition (MI) [23,24], and we have shown that this effect is much more marked for the newer sulphonylurea glimepiride and suggested that this may account for its lack of effect on protection by IPC [25,26]. In the present study we have, therefore, investigated the ability of HMR 1098 to block sK_{ATP} channels of native cardiac myocytes or cloned Kir6.2/SUR2A channels when these channels are activated either by MI or by the K_{ATP} channel opener pinacidil. Our results show that the effectiveness of HMR 1098 in blocking either sK_{ATP} current or action potential shortening was greatly reduced under conditions of MI. These findings suggest that a lack of effectiveness of HMR 1098 in preventing protective effects of IPC or of other cardioprotective stimuli on damage during subsequent ischemia does not necessarily rule out the involvement of sK_{ATP} channels.

2. Methods

2.1. Isolation of rat ventricular myocytes

Adult male Wistar rats (300–400 g) were killed by cervical dislocation. The care and sacrifice of animals conformed to the requirements of the UK Animals (Scientific Procedures) Act 1986. The heart was rapidly removed and perfused using the Langendorff technique with collagenase (type I, Sigma) and protease (type XV, Sigma) solution as described previously [27]. Myocytes were then mechanically dispersed in a shaking water bath followed by washing in Tyrode solution. Typically, there was a 70–90% yield of quiescent, rod-shaped cells. Cells were stored at room temperature in Tyrode for a maximum of 24 h.

2.2. HEK 293 cells expressing Kir6.2/SUR2A

HEK 293 cells stably expressing the cardiac sarcolemmal K_{ATP} channel subunits Kir6.2 and SUR2A were a kind gift from Dr. Andrew Tinker. These cells were maintained in Minimal Eagles Medium with Earle's salts supplemented with 10% foetal calf serum, 10 mM L-glutamine and the antibiotics Zeocin (364 μg ml^{-1}) and G418 (727 μg ml^{-1}) for selection purposes. Cells were used between passages 12 and 24.

2.3. Solutions

Isolated ventricular myocytes were maintained in normal Tyrode solution containing (in mM): NaCl 135, KCl 6, NaH_2PO_4 0.33, Na-pyruvate 5, glucose 10, $MgCl_2$ 1, $CaCl_2$ 2, and *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulphonic acid] (HEPES) 10, titrated to pH 7.4 with NaOH. The internal solution for whole-cell recording contained, in mM, KCl 140, $MgCl_2$ 1, BAPTA 10, HEPES 10, ATP 2, ADP 0.1 and GTP 0.1, titrated to pH 7.2 with KOH. For action potential recordings the BAPTA was replaced with 5 mM EGTA. MI Tyrode contained 2 mM NaCN and 1 mM iodoacetic acid in substrate-free Tyrode solution (without glucose or pyruvate). Cyanide and iodoacetic acid were added on the day of use. The pipette solution for outside-out patches contained, in mM, K^+ 140 (30 KOH and 110 KCl), $MgCl_2$ 1.2, $CaCl_2$ 1, EGTA 10 and HEPES 5 titrated to pH 7.2 with KOH. The same solution with the addition of ATP, ADP and HMR 1098 as indicated in the text and adjusted to pH 7.4, was used to perfuse the cytoplasmic face of inside-out patches. The bath solution for outside-out patches (which was also used as the pipette solution for inside-out patches) contained, in mM, KCl 140, $MgCl_2$ 1.2, $CaCl_2$ 2.6 and HEPES 5, pH 7.4. Other compounds were added to the solutions as indicated in the text.

2.4. Electrophysiology

For whole-cell recording from rat ventricular myocytes, electrodes were made from thin-walled borosilicate glass, and had resistances of 3–6 MΩ when filled with internal solution. Currents from HEK 293 cells were recorded from inside-out and outside-out patches using thick-walled glass electrodes with resistances of 6–10 MΩ. Membrane voltage was controlled and current recorded using an Axopatch 200A amplifier (Axon Instruments). Currents were filtered at 2 kHz and analogue signals were digitised at 10 kHz using a Digidata 1322 interface (Axon Instruments). Recordings were acquired using pCLAMP 9.0 and analysed using pCLAMP 9.0 (Axon instruments), Excel XP (Microsoft) and SigmaPlot 8 (SPSS). Whole-cell currents were recorded from rat ventricular myo-