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Two-pore K^+ channels, NO and metabolic inhibition

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Abstract

Ischemic preconditioning is a potent endogenous mechanism protecting many organs from the devastating effects of prolonged ischemia. In the heart, NO is one mediator of this myoprotective response thought to involve activation of the K_{ATP} channel. Ischemic preconditioning is known to be induced by metabolic inhibition using sodium cyanide (NaCN) in single cardiomyocytes. In the present study, we show for the first time that the end effector channel activated by NaCN has been incorrectly identified. The channel activated is not K_{ATP} but instead belongs to the relatively new family of two-pore domain potassium channels (K2P). Further when activated by metabolic ischemia, the amplitude of K2P current is directly modulated by activators and inhibitors of the NO pathway.

Keywords: Ischemic preconditioning; Ion channel; NO

Since the initial report by Murry et al. [1], ischemic preconditioning has been recognized as a potent endogenous mechanism of myoprotection. In an attempt to investigate the mechanism of this protection, Liu et al. [2] developed a single cell model of metabolic ischemia. Upon exposure to NaCN in glucose-free solution an outward current is induced (Fig. 1A) in isolated ventricular myocytes (Materials and methods). The amplitude of this current increases and the time taken for the appearance of this current decreases when the preparation is first exposed to preconditioning agents [3]. In the initial study and subsequently [2,4], the current activated by metabolic ischemia was identified as I_{KATP} because it declined when glibenclamide, a blocker of the K_{ATP} channel, was applied at the peak of the response. However, even in the absence of this channel blocker, the current declines on its own (Fig. 1A). Further when glibenclamide is included throughout the exposure to NaCN there is no effect on the amplitude of the response (inset of Fig. 2A). This result led us to question whether the channel activated in preconditioning had been correctly identified. In 1996 a new class of ion channels was identified [5]. These channels were K^+-specific, also insensitive to classic K^+ channel blockers like Ba^{2+} and Cs^+ but instead were blocked by either Zn^{2+} or Quinidine [6]. Fig. 2A demonstrates that the current activated by metabolic ischemia is not blocked by glibenclamide but is blocked by Zn^{2+}. Since glibenclamide is a poor blocker of K_{ATP} channels in acidotic conditions we asked whether the current activated by metabolic ischemia might still be K_{ATP} and that this channel might also be blocked by Zn^{2+} or Quinidine. Fig. 2B and D show our results. I_{KATP} was activated by pinacidil together with a low concentration of intracellular ATP (0.1 mM). This current is identified as I_{KATP} by its sensitivity to glibenclamide [7]. It is however unaffected by either Zn^{2+} or quinidine. There are at least 15 members in the K2P family, all of which are K^+-specific [6,8,9]. The current activated by NaCN is K^+-specific (Fig. 1C–E). A number of these channels such as TALK-1 and TALK-2 are directly activated by nitric oxide (NO) [10]. Given the importance of NO to

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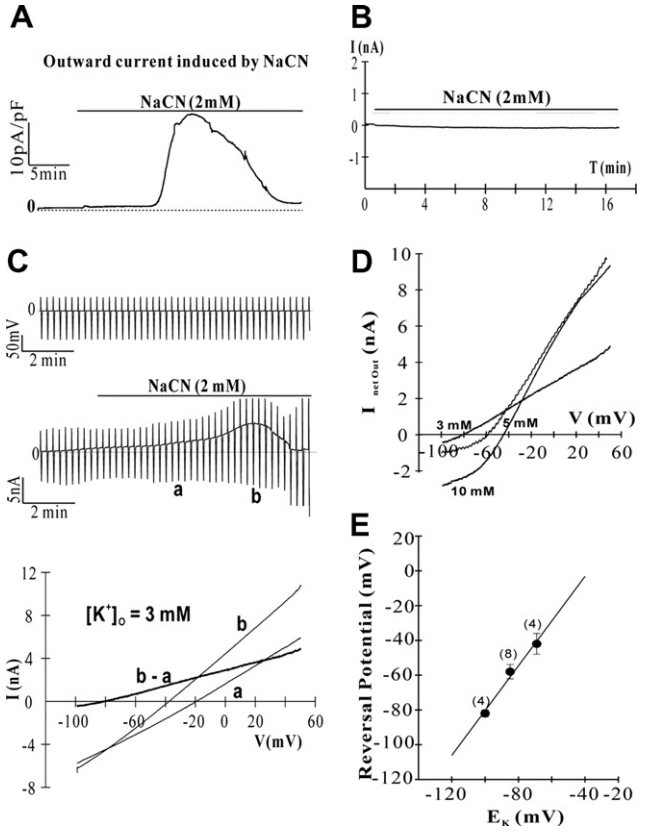

Fig. 1. Characterization of the K^+ selective outward induced by NaCN from isolated guinea pig ventricular myocytes. (A) Sample trace of a large outward current induced by extracellular application of sodium cyanide (NaCN, 2 mM). The $[K^+]_o$ was 5.4 mM and the patch pipette $[K^+]$ was 150 mM. Note a relatively slow onset phase and sustained phase in the NaCN-induced current, and that the current can decay on its own. Similar results were observed in all of the cells ($n = 18$) studied in the same conditions. The cells were held at 0 mV and experiments were performed at 22 °C. (B) The outward current induced by NaCN did not appear when both external and pipette K^+ were absent. The external K^+ was absent without substitute and the pipette K^+ was substituted with L-aspartic acid, and the pH was adjusted with Trizma Base. Similar results were observed in all of the ($n = 6$) cells studied in the same conditions. (C) A typical recording of NaCN-induced outward current measured in which voltage ramps were applied to obtain the current–voltage relationship. The $[K^+]_o$ was 3 mM and the pipette $[K^+]$ was 150 mM. The cells were held at 0 mV and were subject to hyperpolarizing ramps from +50 mV to −100 mV (2 s duration) with a frequency of 0.1 Hz (top). The corresponding I/V relationship of NaCN-induced current (middle), which is constructed by subtraction of the I/V curve measured at the base (a) from that measured at the peak (b) (bottom). (D) The I/V curves of the NaCN-induced current in three different $[K^+]_o$ were plotted using the same protocol shown in (C). (E) The linear relationship between averaged reversal potentials and equilibrium potential of K^+ (E_K) calculated with Nernst equation at different $[K^+]_o$, suggested that the current induced by NaCN induced is K^+ selective. Note that our measured reversal potentials are not the same as E_K (possibly due to the large intracellular K^+ loss induced by the NaCN-induced current). The numbers in parentheses indicate the cells studied.

preconditioning, we examined the effects of an activator (L-arginine, 400 μM) and an inhibitor (L-NAME, 200 μM) of the NO pathway on the NaCN-induced current. The results are provided in Fig. 2C. Activating the NO pathway increases the NaCN-induced current while inhibiting the pathway reduces the current. These results (summarized

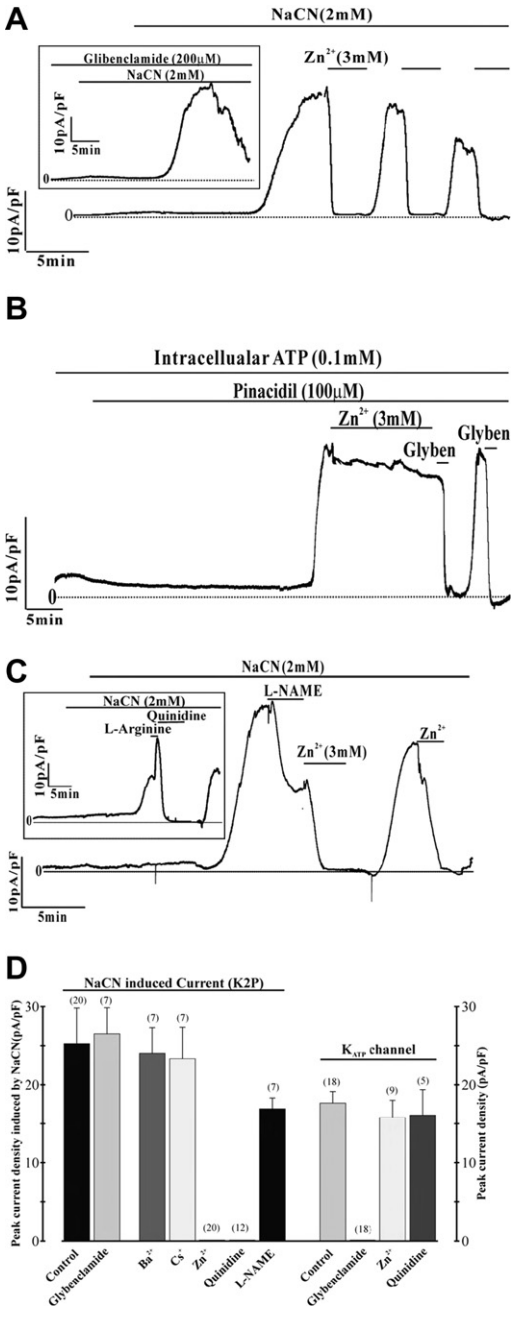

Fig. 2. The NaCN-induced current from guinea pig ventricular myocytes is not IK_{ATP} nor IK_1. (A) The outward current induced by NaCN can be reversibly abolished by Zn^{2+} (3 mM). (Inset) Glibenclamide (200 μM) cannot prevent the appearance of the NaCN induced current. (B) The IK_{ATP} activated by pinacidil (100 μM) together with low intracellular ATP (0.1 mM) cannot be blocked by Zn^{2+} (3 mM), but is abolished by glibenclamide. (C) NO-regulation of the NaCN-induced current. Note the NaCN-induced current is reduced by the NOS inhibitor, L-NAME (200 μM), and the NaCN-induced current is reactivated after washout of Zn^{2+}. (Inset) A typical current trace showing that L-arginine (400 μM) can additionally activate an outward current which is sensitive to Quinidine (1 mM). (D) In summary, the average results suggest that the NaCN-induced current is not blocked by either the K_{ATP} channel blocker (glibenclamide) or classical K^+ channel blockers (1 mM Ba^{2+} or 1 mM Cs^+), it is sensitive to typical K2P channel blockers (both Zn^{2+} and Quinidine) and modulated by NO. In contrast, classical IK_{ATP} is completely blocked by glibenclamide and unaffected by typical K2P channel blockers. All the cells were held at 0 mV. $P < 0.05$ was considered statistically significant by unpaired Student's t-test.

in Fig. 2D) suggest that there is constitutive NO production in guinea pig ventricular myocytes and that the NaCN-induced current is a K2P channel that is modulated by NO.

In summary, the current initiated by metabolic ischemia is not mediated by the K_{ATP} channel. Instead, an NO-sensitive member of the K2P family is implicated. It is well known that the K2P channels help to regulate cell volume [11]. It is possible that their role in volume regulation plays a key role in the protection they afford from prolonged ischemia where cell swelling can induce apoptosis [9]. Finally, with the identification of a novel sarcolemmal channel involved in preconditioning, there is a new therapeutic target to investigate. Other activators of the K2P channels should have the potential to induce preconditioning.

Materials and methods

Single ventricular cells were enzymatically isolated from adult male guinea pig hearts as described in Gao et al. [12]. An Axopatch 1D amplifier (Axon Instruments Inc) and the classical whole-cell patch clamp technique were employed to observe cell membrane current [13]. The pipette solution contained (in mM): K-aspartic acid 125; KCl 15; KOH 10; $MgCl_2$ 1; Hepes 10; EGTA 11; Mg-ATP 1 (pH 7.2). The external Tyrode solution contained (in mM): NaCl 137.7; NaOH 2.3; KCl 4; $MgCl_2$ 1; Hepes 5; $CaCl_2$ 1; $CdCl_2$ 1; Glucose 10 (pH 7.4). Sodium cyanide (NaCN) was dissolved in Tyrode solution without glucose and prepared at the target concentration to simulate metabolic ischemia [3]. All patch clamp data were digitized by the data acquisition program pClamp8 (Axon Instruments Inc) for later analysis. Cell capacitance was obtained for each cell and currents were normalized to cell capacitances. NaCN, glibenclamide, collagenase (type II) and other reagents were obtained from Sigma Chemical (St. Louis, MO).

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