ELSEVIER

European Journal of Pharmacology 315 (1996) 115–118

ejp

Short communication

Trypsin alters ATP sensitivity of K_{ATP} channels in control and hypertrophied myocytes

Vandana H. Sodder *, Lisa D. Bowie, John S. Cameron

Department of Biological Sciences, Wellesley College, Wellesley, MA 02181, USA

Received 20 August 1996; accepted 23 August 1996

Abstract

Increased ATP-sensitive potassium (K_{ATP}) channel activity in cardiac muscle during hypertension and myocardial hypertrophy may be induced by the release of endogenous proteases, altering inhibitory binding sites for intracellular ATP. To test this hypothesis, we studied the effects of trypsin (1.5 mg/ml) on channel sensitivity to ATP in myocytes from control (WKY) and spontaneously hypertensive rats (SHR). Trypsin increased channel activity in 63% of membrane patches from WKY rats, but in only 29% from SHR. Pre-treatment with trypsin decreased sensitivity to the inhibitory effects of ATP in both groups. These results support the possibility that K_{ATP} channel modification during chronic metabolic stress is caused by intracellular proteolysis, which decreases sensitivity to $[ATP]_i$.

Keywords: K^+ channel, ATP-sensitive; Hypertension; Myocardial hypertrophy; Trypsin; Single-channel recording

1. Introduction

The activation of adenosine 5′-triphosphate-sensitive potassium (K_{ATP}) channels is inhibited by physiological concentrations of intracellular ATP. In cardiac muscle, these channels are opened in ischemia and hypoxia, contributing to the shortening of action potential duration characteristic of those conditions (Venkatesh et al., 1991). This result of K_{ATP} activation could be perceived as contributing to tissue damage, loss of cellular K^+ and failure of action potential duration to recover upon reperfusion. Other evidence suggests, however, that K_{ATP} channels play a beneficial role in adaptation to acute or chronic hypoxia and in myocardial hypertrophy. Cameron et al. (1988) reported that K_{ATP} channels in hypertrophied hearts were less sensitive to the inhibitory effects of ATP than those in control cells, suggesting that the current was more readily activated in metabolically compromised tissues. Increased channel activity in the heart could serve to maintain resting potential and reduce Ca^{2+} influx and contractile activity, thus preserving high energy phosphate levels.

Any enhanced activation of cardiac K_{ATP} channels in animal models of hypertension and myocardial hypertrophy could reflect an intrinsic alteration of the channel itself, or might occur through the influence of extrinsic modulators (Cameron and Baghdady, 1994). It has been suggested that endogenous proteases, released as oxygen levels fall, can remove inhibitory binding sites or 'inactivation sequences' from channel proteins (Mayorga-Wark et al., 1993). To explore this possibility, previous investigators have studied the effects of trypsin on K_{ATP} channels in normal muscle cells. The sensitivity of the K_{ATP} channel to ATP was significantly reduced after trypsin treatment in guinea pig and rabbit myocytes (Deutsch and Weiss, 1994) and in pancreatic β-cells (Proks and Ashcroft, 1993). To date, there have been no studies of the effects of trypsin on K_{ATP} channels from ischemic or metabolically compromised hearts. The purpose of the present study, then, was to examine the effects of trypsin on channel activity and ATP sensitivity in control and hypertrophied cardiac myocytes. Our goal was to clarify the mechanism underlying enhanced K_{ATP} channel activity in hypertension and left ventricular enlargement.

2. Materials and methods

Cardiac ventricular myocytes were isolated from spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) control rats as previously described (Ciampolillo et al., 1992), using a 50–50 mixture of collagenase C-0130 and

* Corresponding author. Tel.: (1-617) 283-3352; Fax: (1-617) 283-3642; e-mail: vsodder@wellesley.edu

PII S0014-2999(96)00686-3

C-1085 (Sigma). Cells were studied within 48 h of isolation. SHR rats have been shown to have an increased heart to body mass ratio (Cameron et al., 1990), with enlarged ventricular myocytes.

Single-channel K^+ currents were recorded at room temperature from inside-out membrane patches (Hamill et al., 1981; Ciampolillo et al., 1992) clamped at a range of holding potentials. Cells were placed in a tissue bath containing (in mM): 140 KCl, 5 HEPES and 1 EGTA (pH 7.4, free Ca^{2+} < 0.1 μM); recording electrodes were filled with the same high K^+ solution. Trypsin (1.5 mg/ml, type IX; Sigma) or ATP (1–2 mM; Sigma) were administered to the 'intracellular' side of the patch for 2 min prior to recordings. In some experiments, trypsin was followed by ATP (at 2 min) in the continued presence of the enzyme.

Open-state probability (P_o) was taken as the primary indicator of channel activity; it was calculated by measuring the times t_j, spent at current levels corresponding to $j = 0,1,2,3...N$ channels open. P_o was then given by the equation:

$$\left(\sum_{j=1}^{N} t_j \right) / TN$$

where T was the total observation time (5 s) and N was the number of active channels in the patch. For each patch, P_o was determined at several different holding potentials before and after drug administration. The results reported were obtained in patches from at least 3 different cell preparations for each rat type and drug combination studied. Analysis of variance (ANOVA) for repeated measures with post-hoc Fisher PLSD (protected least significant differences) and Scheffe F-tests were used to determine statistical significance ($P < 0.05$) among group means; results were expressed as mean ± S.E.

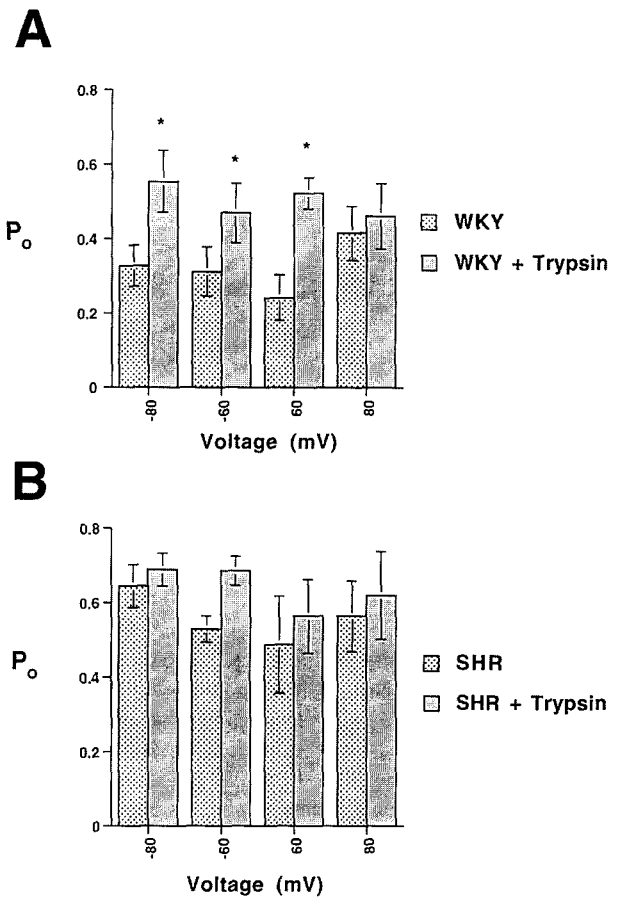

Fig. 1. Effect of trypsin on K_{ATP} channel open-state probability (P_o). Inside-out patches from ventricular myocytes were incubated with trypsin (1.5 mg/ml) for 2 min and channel activity was subsequently recorded at a series of holding potentials. (A) In myocytes from WKY hearts, administration of trypsin to the intracellular surface of the membrane significantly increased P_o at most voltages ($n \geq 7$, $^* P < 0.05$). (B) In myocytes from hypertensive rat hearts (SHR), trypsin did not significantly increase P_o at any potential ($n \geq 4$).

3. Results

ATP-sensitive K^+ channel activity was characterized in cardiac myocytes from WKY and SHR rats prior to the addition of trypsin or ATP. Current-voltage (I-V) curves for both groups showed no significant difference in conductance between control vs. hypertrophied myocytes (60 ± 1 vs. 61 ± 1 pS, respectively). Over a range of holding potentials, however, channel activity (P_o) was greater in patches from SHR rats relative to WKY as previously reported (Ciampolillo et al., 1992; Fig. 1).

3.1. Effect of trypsin on P_o of K_{ATP} channels

Channel activity was recorded in cardiac myocytes from SHR and WKY rats at a number of holding potentials in the presence of trypsin (1.5 mg/ml). In the WKY group, channel activity was increased in the majority of experiments (12 of 19 patches, 63%). The overall increase was statistically significant over a range of holding potentials; at −80 mV, for example, P_o increased from 0.328 ± 0.055 to 0.554 ± 0.083 ($P < 0.05$; Fig. 1A). In the SHR group, trypsin consistently increased P_o and overall channel activity at most holding potentials, but in fewer than half of the experiments (4 of 14 patches, 29%). The overall increase in activity was not statistically significant (Fig. 1B). There was no significant difference in control channel activity between patches that responded to trypsin administration and those that did not.

3.2. Modification by trypsin of channel ATP sensitivity

To test the effect of trypsin on ATP sensitivity of K_{ATP} channels, membrane patches were treated with the enzyme and subsequently exposed to ATP at concentrations which abolished most channel activity (Cameron et al., 1988; Fig. 2). Six of 9 patches (67%) from WKY myocytes showed a decreased sensitivity to 2 mM ATP; channel activity was

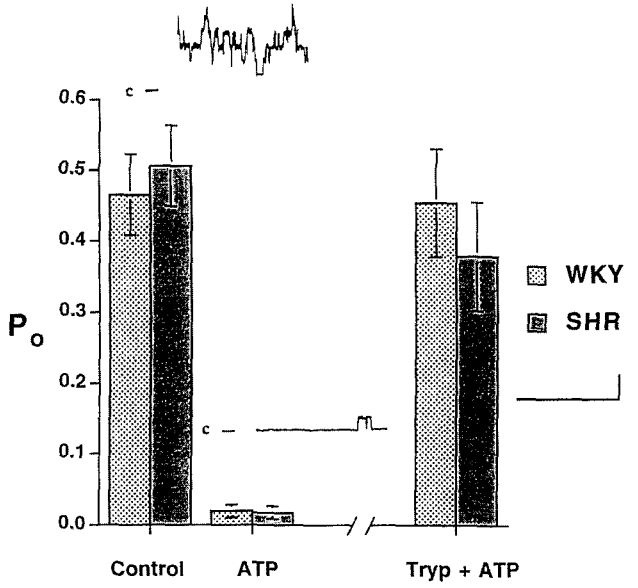

Fig. 2. Effect of trypsin on K_{ATP} channel sensitivity to ATP-induced inhibition. At 2.0 mM ATP, channel open-state probability (P_o) in cells from both WKY and SHR hearts (holding potential = −60 mV) was markedly decreased relative to control (left; $n \geq 8$). However, pre-treatment with trypsin (1.5 mg/ml) prevented the ATP-induced inhibition of channel activity in both WKY ($n = 5$) and SHR ($n = 7$) patches (right). Insets show channel activity in a WKY patch before and after administration of ATP (baseline at c–; horizontal scale, 1 s; vertical, 4 pA).

not significantly reduced by the addition of ATP after trypsin proteolysis (Fig. 2). At a holding potential of −80 mV, for example, overall P_o prior to ATP was 0.508 ± 0.164 vs. 0.510 ± 0.097 after administration of the nucleotide. In the SHR myocytes, however, ATP sensitivity was eliminated in only 3 of the 8 patches examined (38%). The overall decrease in P_o with ATP was still not significant, however (at −80 mV, for example, P_o decreased from 0.492 ± 0.072 to 0.419 ± 0.073 after treatment with ATP).

4. Discussion

The present study indicates that K_{ATP} channels in excised, inside-out membrane patches from WKY ventricular myocytes exhibit increased activity after tryptic digestion. These findings are in general agreement with those of Furukawa et al. (1993) and Proks and Ashcroft (1993), who showed that trypsin reactivated K_{ATP} channels (after run-down) in ventricular myocytes and pancreatic β-cells, respectively. The enzyme has also been shown to activate muscarinic K^+ channels in atrial myocytes (Kirsch and Brown, 1989). In none of these studies did trypsin alter single-channel conductance.

A similar increase in activity was *not* observed in channels from SHR myocytes after trypsin treatment. Prior to drug administration, overall K_{ATP} channel activity may be enhanced in SHR myocytes relative to WKY (Ciampolillo et al., 1992; Fig. 1). One interpretation of this observation is that K_{ATP} channels in the heart are intrinsically altered during long-term metabolic stress, promoting their activation and providing a counter-regulatory mechanism to enhance cardiac muscle relaxation and distensibility. These chronically altered channels may not be subject to further stimulation by trypsin.

In WKY, the addition of trypsin to the intracellular surface of membrane patches resulted in a greatly reduced sensitivity of K_{ATP} channels to the inhibitory effects of ATP. A similar effect has been reported in pancreatic β-cells (Proks and Ashcroft, 1993) and in guinea pig ventricular myocytes (Deutsch and Weiss, 1994), where sensitivity of K_{ATP} channels to ATP was significantly reduced in the presence of trypsin. This suggests an *extrinsic* alteration in channel structure that in some way influences (or eliminates) the binding site for ATP. In support of the possibility that such a mechanism could be at work in hypertension and myocardial hypertrophy is our previous report that channel sensitivity to ATP is diminished in hypertrophied myocytes (Cameron et al., 1988). In addition, the present observation that trypsin did not significantly alter K_{ATP} channel sensitivity to ATP in SHR myocytes suggests that these channels had already been modified in vivo, and could not be further affected by the enzyme. Decreased sensitivity to ATP inhibition in hypertrophied heart muscle could provide a mechanism for enhanced activity of K_{ATP} channels, even when high energy phosphates are not significantly depleted.

In contrast to studies cited above, other groups have reported that trypsin does not affect, or affects only slightly, the ATP sensitivity of K_{ATP} channels from normal ventricular myocytes (Fan and Makielski, 1993; Furukawa et al., 1993; Nichols and Lopatin, 1993). Trypsin did reduce or abolish sensitivity to the inhibitory effects of glibenclamide, however (Nichols and Lopatin, 1993; Deutsch and Weiss, 1994). The source of the variability in previous studies is unclear, but may reflect the complex series of interactions that apparently underlie K_{ATP} channel regulation in different tissues and species (Findlay, 1994). Proks and Ashcroft (1993) postulate that large molecules, such as trypsin, may be restricted in terms of access to the 'intracellular' surface of inside-out patches that are drawn up into a microelectrode. Variations in experimental protocol may also alter results, including the presence or absence of intracellular Mg^{2+} and/or Ca^{2+} during the application of trypsin (Deutsch and Weiss, 1994; Proks and Ashcroft, 1993).

This is the first report describing the effects of trypsin on K_{ATP} channel activity in hypertrophied ventricular myocytes. Similar to K_{ATP} channels in myocytes subjected to severe metabolic inhibition (Deutsch and Weiss, 1994), channels in SHR myocytes are less sensitive to the inhibitory effects of ATP (Cameron et al., 1988), while showing more overall activity than their counterparts in control cells. The present data indicate that trypsin does

not further activate the K_{ATP} channel in SHR heart, nor does it incrementally decrease the sensitivity of the channel to ATP, as in control. These results support the hypothesis that channel sensitivity to ATP inhibition is reduced during long-term metabolic stress, enhancing channel activity, and that the alteration results from partial digestion of cytosolic channel proteins by endogenous proteases.

Acknowledgements

This work was supported by grants from the National Institutes of Health (HL 50097 and DIR-8900754), and the National Science Foundation (DCB-9005911). We also acknowledge the contribution of Deborah L. Acker to Fig. 2.

References

Cameron, J.S. and R. Baghdady, 1994, Role of ATP-sensitive potassium channels in long term adaptation to metabolic stress, Cardiovasc. Res. 28, 788.

Cameron, J.S., S. Kimura, D.A. Jackson-Burns, D.B. Smith and A.L. Bassett, 1988, ATP-sensitive K^+ channels are altered in hypertrophied ventricular myocytes, Am. J. Physiol. (Heart. Circ. Physiol.) 255, H1254.

Cameron, J.S., C.R. Swigart, G.S. Shin, D. Katz and A.L. Bassett, 1990, Enhanced susceptibility to histamine-induced cardiac arrhythmias in spontaneously hypertensive rats, J. Cardiovasc. Pharmacol. 15, 626.

Ciampolillo, F., D.E. Tung and J.S. Cameron, 1992, Effects of diazoxide and glyburide on ATP-sensitive K^+ channels from spontaneously hypertensive rats, J. Pharmacol. Exp. Ther. 260, 254.

Deutsch, N. and J.N. Weiss, 1994, Effects of trypsin on cardiac ATP-sensitive K^+ channels, Am. J. Physiol. (Heart Circ. Physiol.) 266, H614.

Fan, Z. and J.C. Makielski, 1993, Intracellular H^+ and Ca^{++} modulation of trypsin-modified ATP-sensitive K^+ channels in rabbit ventricular myocytes, Circ. Res. 72, 715.

Findlay, I., 1994, Interactive regulation of the ATP-sensitive potassium channel of cardiac muscle, J. Cardiovasc. Pharmacol. 24 (Suppl. 4), S6.

Furukawa, T., Z. Fan, T. Sawanbori and M. Hiraoka, 1993, Modification of the adenosine 5′-phosphate sensitive K^+ channel by trypsin in guinea pig ventricular myocytes, J. Physiol. 466, 707.

Hamill, O.P., A. Martz, E. Neher, B. Sakmann and F.J. Sigworth, 1981, Improved patch-clamp techniques for high resolution current recordings from cells and cell-free membrane patches, Pflügers Arch. 391, 85.

Kirsch, G.E. and A.M. Brown, 1989, Trypsin activation of atrial muscarinic K^+ channels, Am. J. Physiol. (Heart Circ. Physiol.) 257, H334.

Mayorga-Wark, O., J. Constantin, W.P. Dubinsky and S.G. Schultz, 1993, Effects of a Shaker K^+ channel peptide and trypsin on a K^+ channel in *Necturus* enterocytes, Am. J. Physiol. (Cell Physiol.) 265, C541.

Nichols, C.G. and A.N. Lopatin,1993, Trypsin and a-chymotrypsin abolishes glibenclamide sensitivity of K_{ATP} channels in rat ventricular myocytes, Pflügers Arch. 422, 617.

Proks, P. and F.M. Ashcroft, 1993, Modification of potassium-ATP channels in pancreatic β-cells by trypsin, Pflügers Arch. Eur. J. Physiol. 424, 63.

Venkatesh, N., S.T. Lamp and J.N. Weiss, 1991, Sulfonylureas, ATP sensitive K^+ channels, and cellular K^+ loss during hypoxia, ischemia and metabolic inhibition in mammalian ventricle, Circ. Res. 69, 623.