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ß-adrenoceptor stimulation exacerbates detrimental effects of ischemia and reperfusion in isolated guinea pig ventricular myocytes

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ABSTRACT

We investigated whether ß-adrenoceptor stimulation exacerbates detrimental effects of ischemia and reperfusion on electrical and contractile function and on intracellular Ca^{2+} homeostasis in isolated guinea pig ventricular myocytes. Myocytes were exposed to 20 min of simulated ischemia (37 °C) in the absence or presence of isoproterenol (10 nM, applied prior to and during ischemia) and reperfused with Tyrode's solution for 30 min. Unloaded cell shortening, Ca^{2+} transients (fura-2), and cell viability were recorded at 5 min intervals in field-stimulated cells (2 Hz). In experiments using microelectrodes, membrane potentials, contractions, and transmembrane currents also were recorded at 5 min intervals. In the absence of ischemia, 10 nM isoproterenol had little effect on either contractile function or Ca^{2+} homeostasis. In contrast, when cells were exposed to ischemia, isoproterenol increased the size of contractions and Ca^{2+} transients and augmented the increase in diastolic Ca^{2+} concentration during ischemia in field-stimulated myocytes. Exposure to isoproterenol also promoted contractile depression in reperfusion. In voltage clamp experiments, isoproterenol abolished the decrease in the magnitude of L-type Ca^{2+} current caused by ischemia. Isoproterenol also increased the incidence of abnormal contractile activity and induced delayed afterdepolarizations and the arrhythmogenic transient inward current in ischemia. Additionally, the decline in cell viability in ischemia and reperfusion was exacerbated by isoproterenol. This study shows that ß-adrenoceptor stimulation strongly potentiates adverse effects of ischemia and reperfusion on electrical and contractile function. These adverse effects of isoproterenol are likely caused by an increase in intracellular Ca^{2+} accumulation during ischemia.

1. Introduction

Prolonged periods of myocardial ischemia and reperfusion can result in contractile and electrical dysfunction in the heart and can even result in cell death (Kim et al., 2001; Karliner et al., 1989; Saini and Dhalla, 2005). A rise in intracellular Ca^{2+} during ischemia is thought to play an important role in these deleterious effects of myocardial ischemia and reperfusion (Moens et al., 2005). Increased intracellular Ca^{2+} concentrations during ischemia are implicated in post-ischemic contractile depression, a phenomenon known as stunning (Lascano et al., 2002; Bolli and Marban, 1999; Lee and Allen, 1991). Elevated intracellular Ca^{2+} also can induce spontaneous Ca^{2+} release from the sarcoplasmic reticulum, resulting in delayed afterdepolarizations (DADs), induction of the arrhythmogenic transient inward current (I_{Ti}) (Vassalle and Lin, 2004; Karliner et al., 1989), and associated spontaneous contractile activity (Ferrier, 1977; Cordeiro et al., 1994). High levels of intracellular Ca^{2+} also have been implicated in cell death in ischemia and reperfusion (Tomaselli and Zipes, 2004). Thus, intracellular Ca^{2+} overload gives rise to contractile and electrical dysfunction in the setting of myocardial ischemia and reperfusion.

Previous studies have shown that catecholamines, such as adrenaline and noradrenaline, are released during myocardial ischemia and accumulate in interstitial spaces in the heart (Carlsson et al., 1985; Willerson and Buja, 1988; Schomig, 1990; Behonick et al., 2001). It is thought that catecholamines contribute to the pathogenesis of ischemic heart disease (Downing and Chen, 1985; Willerson and Buja, 1988), although the mechanisms by which catecholamines promote ischemia and reperfusion injury are not fully understood. In addition, endogenous catecholamines have been shown to play a role in the induction of arrhythmias in both ischemia and reperfusion (Penny, 1984; Sheridan and Culling, 1985). Penny (1984) showed that endogenous release of catecholamines in intact hearts increased the incidence of ventricular arrhythmias during ischemia and reperfusion. Interestingly, arrhythmias that occur during ischemia can be abolished by myocardial catecholamine depletion and can be reversed by ß-adrenoceptor blockade (Penny et al., 1985; Culling et al., 1987; Clements-Jewery et al., 2002). This suggests that endogenous catecholamines induce arrhythmias in ischemia and reperfusion by

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effects on ß-adrenoceptors, although the underlying mechanisms remain unclear.

It is possible that deleterious effects of catecholamines in ischemia and reperfusion are mediated, at least in part, by direct effects on ß-adrenoceptors in ventricular myocytes. It is well established that catecholamines act on ß-adrenoceptors in myocytes to increase intracellular Ca^{2+} and thereby increase positive inotropy in the heart (Bohm et al., 1992; Vatner et al., 1999; Homcy et al., 1991). Therefore, direct effects of ß-adrenoceptor agonists on individual ventricular myocytes may exacerbate the adverse effects of ischemia and reperfusion by increasing intracellular Ca^{2+} levels. However, little is known about the impact of ß-adrenoceptor stimulation on Ca^{2+} homeostasis in ventricular myocytes in the setting of ischemia and reperfusion. The goal of this study was to evaluate the contribution of ß-adrenoceptor stimulation to detrimental effects of myocardial ischemia and reperfusion at the level of the individual cardiac myocyte. The specific objectives were: 1) to determine whether ß-adrenoceptor stimulation alters electrical and contractile responses during simulated ischemia and reperfusion in isolated guinea pig ventricular myocytes; 2) to determine whether ß-adrenoceptor stimulation affects cell survival in ischemia and reperfusion; and 3) to determine whether these effects of ß-adrenoceptor stimulation are mediated by changes in intracellular Ca^{2+} homeostasis. Studies were conducted in a well-characterized cellular model of simulated ischemia and reperfusion developed in this laboratory (Cordeiro et al., 1994; Louch et al., 2002; MacDonald and Howlett, 2008).

2. Materials and methods

2.1. Animals and cell isolation

Experiments used ventricular myocytes isolated from male albino guinea pigs (300–375 g, Charles River, Saint-Constant, QC). Experiments conformed to the guidelines in the Canadian Council on Animal Care *Guide to the Care and Use of Experimental Animals* (CCAC, Ottawa, Ontario: Volume 1, 2nd edition, 1993; Volume 2, 1984) and the Dalhousie University Committee on Animal Care approved all animal protocols. Myocytes were isolated as described in detail previously (Ferrier and Howlett, 1995). Briefly, guinea pigs were anesthetized with sodium pentobarbital (120 mg/kg, CDMV, St. Hyacinthe, QC) co-administered with 3.3 UI/g i.p. heparin (Organon Teknika, Scarborough, ON). The aorta was cannulated *in situ* and the heart was perfused with Ca^{2+}-free oxygenated (100% O_2, Praxair, Halifax, NS) solution of the following composition (in mM): 120.5 NaCl, 4 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 10 HEPES, 12 glucose, pH to 7.4 with NaOH at 37 °C for 7–8 min at a rate of 12–20 ml/min with a peristaltic pump (Fred A. Dungey Inc., Agincourt ON). After 5–10 min, the heart was subjected to enzymatic digestion with the buffer solution described above with the addition of collagenase (16–23 mg/50 ml, Worthington Type II) and protease (4 mg/50 ml, Sigma Type XIV) for 5 min. Following digestion, the ventricles were removed, minced and stored at room temperature in a high K^+ buffer of the following composition (in mM): 80 KOH, 30 KCl, 3 $MgSO_4$, 50 glutamic acid, 30 KH_2PO_4, 20 taurine, 0.5 EGTA, 10 HEPES, 10 glucose, pH to 7.4 with KOH. The cell suspension was filtered with a 225 µm polyethylene filter.

2.2. Ischemia and reperfusion experiments

Aliquots of cell suspension were placed in a chamber mounted on the stage of an inverted microscope (Olympus IMT-2, Tokyo, Japan). The experimental chamber was treated with 1 ml of natural mouse laminin for 20 min (Invitrogen, Burlington, ON) prior to experiments. Laminin (1 mg) was dissolved in 100 ml of Medium 199 and frozen in 1 ml aliquots at −20 °C until use. Myocytes were superfused at a rate of 6 ml/min by gravity with a 37 °C oxygenated (95% O_2, 5% CO_2, Praxair, Halifax, NS) normal Tyrode's solution containing (in mM): 129 NaCl, 20 $NaHCO_3$, 0.9 NaH_2PO_4, 4 KCl, 0.5 $MgSO_4$, 0.5 $CaCl_2$, and 5.5 glucose, pH 7.4. After 20–30 min, the Ca^{2+} concentration was increased to 2.5 mM. Experiments were performed on rod-shaped, striated cells with no obvious membrane blebs and no contractile activity in the absence of stimulation.

Myocytes were visualized with a closed circuit video camera (Pulnix America Inc., San Jose, CA) and cell images were displayed on a TV monitor (Hitachi Denshi, Tokyo, Japan). Myocytes were superfused for 15 min with normal Tyrode's solution followed by 20 min of "ischemic" Tyrode's solution (Ferrier et al., 1985). The composition of the ischemic solution (in mM) was: 123 NaCl, 6 $NaHCO_3$, 0.9 NaH_2PO_4, 8 KCl, 0.5 $MgSO_4$, 20 Na-lactate, and 2.5 $CaCl_2$ gassed with 90% N_2, 10% CO_2, pH 6.8 (Praxair, Halifax, NS). A 90% N_2, 10% CO_2 gas phase was directed over the chamber during simulated ischemia and was removed upon reperfusion. Cells were reperfused with normal Tyrode's solution for up to 30 min. To examine effects of ß-adrenoceptor stimulation on responses to ischemia and reperfusion, isoproterenol (10 or 100 nM) was added to the ischemic Tyrode's solution. Isoproterenol (10 nM) also was added to the normal Tyrode's solution 5 min after the start of the experiment to examine effect of isoproterenol on responses in the absence of ischemia.

The pO_2 values of the experimental solutions were measured during ischemia and reperfusion with an i-STAT portable clinical analyzer (Abbot Diagnostics, Loveland, CO). The pO_2 was 634.7 ± 6.8 mm Hg (n = 4) in normal Tyrode's solution, declined to 70.7 ± 4.2 mm Hg within the first 5 min of exposure to ischemic solution and remained low throughout ischemia. The decline of nearly 90% in pO_2 was even greater than the 75–80% decrease in pO_2 reported in other cellular models of ischemia and reperfusion (Lu et al., 2005; Maddaford et al., 1999). The pO_2 recovered to pre-ischemic levels within 2 min of reperfusion.

2.3. Contractile and electrical recordings

Cardiomyocytes were field-stimulated with platinum electrodes. Stimuli were 3 millisecond pulses delivered by a Pulsar 6i (Frederick Haers & Co., Brunswick, ME) and a Grass SD9 stimulator (Grass Medical Instruments, Quincy, MA) at two times the threshold value. Cells were continuously stimulated throughout the protocol with trains of ten pulses at a frequency of 2 Hz, followed by a delay of 2.5 s to observe and record spontaneous activity. Contractions were measured as unloaded cell shortening with a video edge detector (Crescent Electronics, Sandy, UT) at a sampling rate of 120 Hz. The analog signals for cell shortening were converted to digital signals (Digidata 1322A, Molecular Devices, Foster City, CA) and stored on a computer for analysis. Contractions were recorded for a period of 20 s at 5 min intervals throughout the experiment with an additional recording made 1 min into reperfusion. Contraction amplitudes were measured as the difference between systolic and diastolic cell length upon stimulation. An average of the last three contractions of a train of ten stimulated beats was used to measure contraction amplitude. The incidences of aftercontractions (an extra beat following a stimulated beat) and spontaneous beats (beats observed without stimulation) (Lukas and Ferrier, 1989) in the absence and presence of isoproterenol were recorded. Cell viability also was measured at each experimental time point. Cells that were spherical in shape, formed membrane blebs, and developed irreversible hypercontracture were tested with trypan blue. Cells that took up trypan blue were considered not viable. All recordings and measurements were made with AxoScope 8.1 and Clampfit 8.2 software (Molecular Devices, Foster City, CA).

In some experiments, myocytes were impaled with high-resistance microelectrodes (18–25 MΩ) filled with 2.7 M KCl. Action potentials were recorded at 5 minute intervals throughout an experiment with an Axoclamp-2A amplifier (Molecular Devices, Foster City, CA). Transmembrane currents also were recorded at 5 minute intervals by switching between conventional recording and voltage clamp modes (discontinuous-single voltage clamp, 6–8 kHz). Voltage clamp protocols were generated with Clampex 8.2 software (Molecular Devices, Foster City,