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Regulatory Peptides 139 (2007) 141 – 145
REGULATORY PEPTIDES
www.elsevier.com/locate/regpep

Role of endogenous nitric oxide in classic preconditioning in rat hearts

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Received 13 June 2006; received in revised form 15 September 2006; accepted 3 October 2006
Available online 22 December 2006

Abstract

Ischemic preconditioning (IPC) protects the heart against subsequent sustained ischemia reperfusion (RP). Despite many triggers and signaling pathways, which seem to be involved in IPC, the IPC-mechanisms remain a controversial issue. One of them is endogenous production of nitric oxide (NO). To assess the role of NO in IPC and its relation with glycogen and glycolysis, the effects of inhibiting NO synthase with L-NAME (50 μM) were examined in IPC rat hearts perfused with medium containing 10 mM glucose. Left ventricular developed pressure-rate product (RPP) and end diastolic pressure (EDP), lactate and glycogen contents, and cell viability were measured. Global ischemia (25 min) was followed by 30 min RP. IPC consisted in one cycle of 3 min ischemia–5 min RP. IPC reduced EDP and improved RP recovery of RPP. L-NAME had no effects on the non-IPC group but abolished these effects of IPC. IPC reduced ischemic decrease of glycogen and the acceleration of glycolysis, and improved cell viability. L-NAME did not affect these effects of IPC. The results suggest that NO is ineffective on the noxious effects of ischemia-RP in non-IPC hearts and on the effects of IPC on cell viability, glycogenolysis and glycolysis whereas it is only involved in functional protection.

Keywords: Ischemia; *N*-nitro-L-arginine methyl ester; Glycogenolysis; Glycolysis

1. Introduction

Brief intermittent periods of ischemia protect the heart against subsequent prolonged ischemia reperfusion (RP), reducing infarct size, contractile dysfunction and the incidence of arrhythmias [1,2].This phenomenon, known as ischemic preconditioning (IPC) exhibits two phases of enhanced tolerance to ischemia: "classical" IPC which develops within minutes after the ischemic stress and lasts less than 3 h and the "second window" of protection which is characterized by a slow onset and a duration of up to 72 h.

Although many endogenously released agents including adenosine, catecholamines, bradykinin, opioid peptides, and nitric oxide (NO) have been implicated in the cardioprotective response [1–5], the intracellular signaling pathway is not well characterized [2]. Furthermore, despite a body of evidence ascribing a pivotal role to the mitochondrial ATP-sensitive potassium channel in the signal transduction pathway activated in response to IPC [6–9], not all authors coincide about the relevance of this mechanism [10–15].

Interestingly, despite being demonstrated that endogenous NO is involved in the myocardial adaptation to the ischemic insult, its role in the protective effects of "classic" IPC remains a controversial issue [16,17]. It should also be mentioned in this respect that, aside from the effects of NO already found on the activity of the ATP-sensitive K channel [6,9,18], several authors have reported convincing data showing that during ischemia NO affects glucose uptake and lactate production [19–21] accompanied by a decreased rate of glycogen breakdown [19]. Since it is well-known that under oxygen limited conditions either glycolytic flux and glycogenolysis are involved in the myocytes survival [22,23], it may be inferred that the effects of NO on the ischemic-RP heart as well as the mechanisms of IPC, might be exerted through these metabolic pathways. On these bases, it seemed interesting to further examine the role of endogenous NO in the protective effects of IPC, by means of the non-specific inhibitor of the nitric oxide synthase, *N*-nitro-L-arginine methyl ester (L-NAME). In order to meet this goal, the effects of L-NAME on postischemic functional recovery and cell viability were tested in IPC hearts in relation with glycogen content and lactate production.

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0167-0115/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.regpep.2006.10.015

2. Materials and methods

2.1. Experimental protocol

The investigation was conducted in accordance with the Guide for Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1985) and the Argentine Law No. 14346 concerning animal protection. Wistar female rats weighing 250–350 g, maintained on a 12-h dark–light cycle, fed *ad libitum* were anesthetized with diethylether and heparin (250 IU) was injected into the jugular vein. The hearts were rapidly isolated and cooled in ice-cold saline until contractions stopped. Thereafter the hearts were mounted on a modified Langendorff apparatus and isovolumically perfused at a constant pressure of 70 mm Hg with a non-recirculating Krebs–Ringer bicarbonate solution (in mM: NaCl 120, $NaHCO_3$ 25, KCl 4.8, $MgSO_4$ 1.33, KPO_4H_2 1.2, $CaCl_2$ 1.6, Na_2EDTA 0.02 and glucose 10). The perfusate was gassed with 95% O_2–5% CO_2 (pH 7.4). The oxygenator and the water-jacketed organ-bath were kept at 37 °C by means of a Lauda thermocirculator.

After a 30 min equilibration period, the hearts were subjected to 25 min global ischemia and 30 min RP. Ischemia was started by completely shutting off perfusate flow. IPC was elicited by 3 min ischemia followed by 5 min of RP before sustained ischemia. L-NAME 50 μM (Sigma) was administered 5 min before IPC. To determine the effect of L-NAME in the non-IPC control group, the inhibitor was administered 13 min before sustained ischemia. The drug was added directly into the perfusate to obtain final concentration and remained until the end of sustained ischemia. The concentration of L-NAME used in this study which has been shown to decrease NO accumulation during ischemia in rat hearts [24] decreased coronary flow from 10.8±1.37 to 7.6±0.4 mL/min, $p<0.05$ without altering mechanical parameters before testing ischemia (data not shown). Only hearts with left ventricular developed pressure (LVDP)>60 mm Hg and heart rate>200 beats/min at the end of the equilibration period were included.

2.2. Measurement of heart function

For the measurement of the ventricular pressures, the left atrium was removed and a latex balloon connected to a pressure transducer was inserted through the mitral valve into the left ventricle. The volume of the balloon was adjusted to obtain left ventricular end diastolic pressure (LVEDP) of 10 mm Hg. Values for the LVDP, peak rates of contraction (+dP/dt) and relaxation (−dP/dt) were obtained using a digital data acquisition system. Heart rate was measured by means of a heart rate counter triggered by the LVDP pulse. Rate-pressure product (RPP) was determine by multiplying heart rate and LVDP.

2.3. Measurement of cell viability

At the end of the RP period and after removal of connective tissue, the hearts were frozen and cut into 6–8 slices of approximately 0.8 mm up to 1.0 mm of thickness. Following defrosting, the slices were incubated at room temperature with 1% triphenyltetrazolium chloride (TTC) in phosphate buffer (100 mM, pH 7.4) for 90 min and fixed in 10% formaldehyde solution to distinguish clearly stained viable tissue and unstained necrotic tissue. The areas of viable tissue were determined by computer morphometry (Scion Image B 4). The risk area was the sum of the total ventricular area minus cavities. The cellular viability was calculated and presented as percentage of risk area. A close correlation between myocardial infarct size determined histologically and by the TTC technique has been reported [25].

2.4. Biochemical assays

Additional hearts treated according to above protocols were removed and immediately frozen between two blocks of ice at −21 °C just before the onset of sustained ischemia and at the end of the 25 min ischemia for determination of tissue glycogen and lactate. A sample of approximately 60 mg of wet tissue was used to determine the dry to wet ratio and to calculate the total dry weight (g) of the heart.

Glycogen, in ~200 mg of frozen ventricular tissue, was determined by the method of Walaas and Walaas [26] with the use of α-glucosidase. Glycogen values were expressed as μg/100 mg dry weight. Lactate was extracted from ~100 mg of frozen ventricular tissue into 6% ice-cold perchloric acid and measured enzymatically. Lactate values were expressed as μM/gram dry weight.

2.5. Statistical analysis

Values represent the mean±SEM. Changes of the ventricular contractile functions were statistically compared using a two

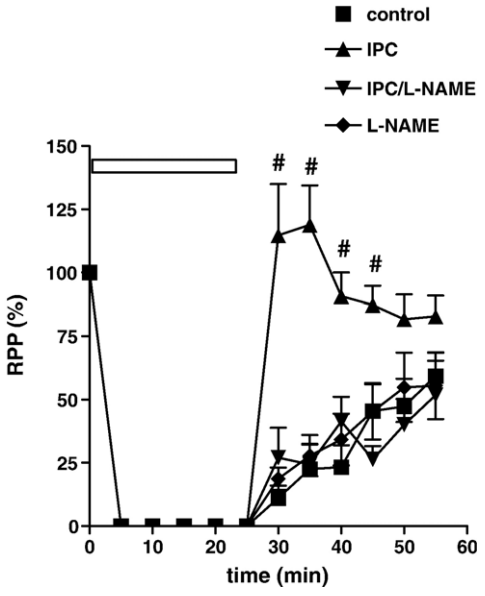

Fig. 1. Changes in rate-pressure product (RPP) due to ischemia reperfusion. Values are expressed as percentages of respective basal values recorded before the onset of the 25 min ischemia. IPC, preconditioned hearts; L-NAME, 50 μM L-NAME treated hearts. Values are the average of 8 hearts±SEM in each group. #: $p<0.01$ vs all other groups. The bar indicates the ischemic period.