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Norepinephrine-induced Sustained Myocardial Adaptation to Ischemia is Dependent on α_1-Adrenoceptors and Protein Synthesis

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X. Meng, J. C. Cleveland Jr, R. T. Rowland, M. B. Mitchell, J. M. Brown, A. Banerjee and A. H. Harken. Norepinephrine-induced Sustained Myocardial Adaptation to Ischemia is Dependent on α_1-Adrenoceptors and Protein Synthesis. *Journal of Molecular and Cellular Cardiology* (1996) 28, 2017–2025. The authors have shown that stimulation of cardiac α_1-adrenoceptors confers immediate cardioprotection in the isolated rat heart against post-ischemic dysfunction, and have recently demonstrated that *in vivo* treatment of rats with norepinephrine (NE) induces cardiac heat shock protein 72 and myocardial adaptation to ischemia 24 h after treatment. To characterize the delayed myocardial adaptive response induced by NE further, the present study examined its time course and effects of adrenoceptor antagonism and protein synthesis inhibition on this adaptive response during optimal myocardial protection.

Rats were treated with NE (3.1 μmol/kg, i.p.) or normal saline (0.4 ml, i.p.), and hearts isolated at 2, 4, 24, 72 and 168 h after injection. Isolated hearts were subjected to 25 min of normothermic global ischemia and 40 min of reperfusion by the Langendorff technique, and left ventricular developed pressure (LVDP) was assessed. There was no difference in baseline LVDP among groups. Post-ischemic LVDP recovered to 44.7 ± 2.1 mmHg in pooled saline control. LVDP was significantly improved in hearts isolated at 4, 24 and 72 h after injection of NE (66.3 ± 3.8, 68.6 ± 2.7 and 72.6 ± 8.3 mmHg, respectively, $P<0.05$ *v* control) but not in hearts isolated at 2 or 168 h. Effects of antecedent adrenoceptor antagonism and protein synthesis inhibition were examined in hearts isolated at 72 h after NE treatment. Prazosin pretreatment (2.4 μmol/kg, i.p.) abolished the delayed myocardial adaptive response induced by NE at 72 h (post-ischemic LVDP 48.3 ± 6.1 mmHg, $P>0.05$ *v* control) while propranolol pretreatment (3.4 μmol/kg, i.p.) had no effect (post-ischemic LVDP 67.3 ± 3.7 mmHg, $P<0.05$ *v* control). Cycloheximide pretreatment (3.6 μmol/kg, i.p.) also abolished the beneficial effect of NE at 72 h (post-ischemic LVDP 50.2 ± 6.0 mmHg, $P>0.05$ *v* control).

In conclusion, administration of NE to rats can induce delayed and sustained cardioprotection against post-ischemic myocardial dysfunction. NE-induced myocardial adaptation to ischemia at 72 h is mediated by α_1-adrenoceptors and appears to require protein synthesis.

Key Words: Norepinephrine; Cycloheximide; α_1-Adrenoceptors; Ischemia and reperfusion; Myocardial contractility; Rat.

Introduction

The induction of myocardial adaptation to ischemia, 24 h or more after systemic stress, has been repeatedly reported since the late 1980s. Increased myocardial resistance to ischemia can be induced at 24 h after an exposure to endotoxin (Brown *et al.*, 1989; Maulik *et al.*, 1995) or cytokines (Brown *et al.*, 1990; Nogae *et al.*, 1995). Furthermore, hyperthermia (Currie *et al.*, 1988; Karmazyn *et*

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al., 1990), forced restraint (Meerson *et al.*, 1992), hypoxia (Meerson *et al.*, 1992; Tajima *et al.*, 1994), and cardiac ischemia (Marber *et al.*, 1993; Kuzuya *et al.*, 1993, Baxter *et al.*, 1994) have also been shown to induce delayed cardioprotection against ischemia/reperfusion injuries. In intact animals, these stresses promote the activities of various mechanisms including central reflex and release of humoral factors. These endogenous neurohumoral factors can subsequently activate a set of intrinsic mechanisms permuted by the specificity of a stressor. Induction of a conserved adaptive response by endogenous neurohumoral factors could be a therapeutically accessible alternative to these extreme stresses.

It is well documented that different forms of stress including toxins, physical stress and ischemia can evoke the release of endogenous NE, both systemically and locally in the heart (Jones and Yelich, 1987; Schomig *et al.*, 1987). Catecholamines can induce immediate cardioprotection against cardiac dysfunction (Kitakaze *et al.*, 1991; Banerjee *et al.*, 1993; Mitchell *et al.*, 1995; Hu and Nattel, 1995) and myocardial infarction (Thornton *et al.*, 1993; Bankwala *et al.*, 1994; Tsuchida *et al.*, 1994) resulting from myocardial ischemia/reperfusion. The receptor mechanisms can be mediated either directly by the noradrenergic α_1 pathway or by ancillary metabolite pathways. While it seems possible that endogenous NE, released in response to stress, may promote an immediate adaptive response, little is known about induction of delayed and sustained myocardial adaptation by the sympathetic neurotransmitter.

NE regulates cardiac gene expression (Lee *et al.*, 1988; Kolbeck-Ruhmkorff and Zimmer, 1995; Venkataraman *et al.*, 1995) and promotes specific phenotypic programs (Waspe *et al.*, 1990). Indeed, several cardiac genes contain conserved sequences for the regulation by α_1-adrenoceptor mediated pathways. Additionally, α_1-adrenoceptors can stimulate cardiac protein synthesis at the translational level (Fuller *et al.*, 1990). It was hypothesized, therefore, that the sympathetic response to stress could be involved in the induction of delayed myocardial adaptation. The authors have found that injection of a stressful dose of NE to the rat induces the expression of cardiac c-*fos* and heat shock protein 70 genes (Meng *et al.*, 1996). Interestingly, administration of NE to the rat also induces protection against post-ischemic contractile dysfunction in hearts isolated 24 h later (Meng *et al.*, 1996). The purposes of this study were: (1) to examine the time course of NE-induced delayed myocardial adaptation; (2) to identify the adrenoceptor subtype dependence of peak myocardial adaptation; and (3) to examine the requirement of protein synthesis in the acquisition of delayed myocardial adaptation.

Materials and Methods

Animals and agents

Male Sprague–Dawley rats, body weight 320–350 g, were acclimated in a quarantine room and fed Purina Chow and water *ad libitum* for 2 weeks before the experiments. All experiments were approved by the Animal Care and Research Committee, University of Colorado Health Sciences Center, and the investigation conforms with the *Guide for the care and use of laboratory animals* published by the US National Institute of Health (NIH publication No. 85–23, revised 1985).

NE bitartrate, prazosin hydrochloride, propranolol hydrochloride and cycloheximide were obtained from Sigma Chemical Co. (St Louis, MO, USA).

Experimental protocols

The experimental protocols are shown in Table 1. To examine the time course of NE-induced cardiac adaptation, 42 rats were divided into two groups and treated with either normal saline or NE. The groups were treated serially, except that control animals were interspersed throughout the study. Thirty-two rats were treated with NE (bitartrate salt dissolved in normal saline, 3.1 μmol/kg, i.p.) and sacrificed at 2, 4, 24, 72 and 168 h, respectively, after treatment. Control rats ($n=12$) were treated with normal saline (0.4 ml, i.p.) and sacrificed at 2, 4, 24, 72 and 168 h after treatment.

A group of animals were treated with prazosin (hydrochloride salt dissolved in 5% ethanol, 2.4 μmol/kg, i.p.) alone and another group with propranolol (hydrochloride salt dissolved in normal saline, 3.4 μmol/kg, i.p.) alone. They were sacrificed at 72 h after treatment, serving as adrenoceptor antagonist controls. Prazosin was given to a group of rats 1 h prior to administration of NE, and the animals were sacrificed at 72 h after NE treatment. Another group of rats were pretreated with propranolol 1 h prior to administration of NE and sacrificed at 72 h after NE treatment.

To examine the effects of protein synthesis inhibition on the baseline cardiac contractility and