行政院國家科學委員會專題研究計畫成果報告

內源性氧化氮調控兒茶酚胺對大鼠心肌肌凝蛋白基因表達之效應

Modulation of Catecholamine Effect on Rat Cardiac Myosin Gene Expression by Endogenous Nitric Oxide

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一、中文摘要

氧化氮在內生性心臟肥大上扮演重要控制角色。在本研究裡,我們使用已建立之新生鼠心肌細胞培養系統探討α_1-腎上腺刺激對氧化氮合成酶及氧化氮產生之效應,和偵測內源性氧化氮是否能調控因兒茶酚胺誘發增加之乙型心肌肌凝蛋白重鏈基因的表現。兒茶酚胺誘發氧化氮產生呈劑量和時間正比例之效應。北方氏轉漬雜交反應顯示兒茶酚胺刺激內皮性氧化氮合成酶之基因表現增加達 1.9 倍,但對誘發性和神精性氧化氮合成酶之基因表現則無增加效應。同時添加氧化氮合成酶抑制劑（L-NAME）或氧化氮清除劑（PTIO）明顯加強因兒茶酚胺誘發增加之乙型心肌肌凝蛋白重鏈基因（β-MyHC）和迅早期基因（c-fos）的 mRNA 表現程度;相反地、添加氧化氮生成劑（SNAP 或 SIN-1）則呈抑制效應。進一步使用氯徵素乙醯基轉移酶分析法偵測內源性氧化氮調控因兒茶酚胺誘發增加之乙型心肌肌凝蛋白重鏈基因的啟動子活性,結果顯示:L-NAME 和 PTIO 增強因兒茶酚胺誘發增加之乙型心肌肌凝蛋白重鏈基因的啟動子活性;相反地、SNAP 或 SIN-1 則抑制增加效應。總之、在心肌細胞內兒茶酚胺能誘發內皮性氧化氮合成酶基因表現和氧化氮產生之增加; 內源性氧化氮與兒茶酚胺誘發肌凝蛋白重鏈基因表現之調節有關。

關鍵詞：
氧化氮,氧化氮合成酶,兒茶酚胺,肌凝蛋白重鏈。

二、英文摘要

Abstract

Nitric oxide(NO) has been implicated in endogenous control of myocardial hypertrophy. In this study, we investigate the effects of α_1-adrenergic stimulation on NO synthase(NOS) gene expression and NO production in cultured neonatal rat cardiac myocytes and determine whether modulation of endogenous NO production alters the hypertrophy-related β -MyHC gene expression induced by norepinephrine(NE). NE caused a significant increase in the production of nitrite, a stable metabolite of NO in a dose- and time-dependent manner. Northern blotting hybridization revealed that NE(1 μ M) stimulated endothelial NOS(eNOS) gene expression by 1.9 fold-increase(compared with the control group). However, NE has no detectable effect on inducible NOS(iNOS) or neuronal NOS(nNOS) gene expression. Simultaneous incubation with NOS inhibitor, N^G-nitro-L-arginine methyl ester(L-NAME; 100 μM) and NO scavenger,2-phenyl-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide(PTIO; 10 μ M) significantly augmented NE-induced increases in c-fos and β -MyHC mRNA level. In contrast, NO donor, S-nitroso-N-acetyl-D,L-penicillamine(SNAP; 10 μ M) and 3-morpholino-sydnonimine(SIN-1; 10 μM) completely suppressed the NE-stimulated increases in c-fos and β -MyHC mRNA level.We further analyze NO modulation of NE-stimulated increases in the promoter

activity of β -MyHC gene by chloramphenical acetyltransferase(CAT) assay. L-NAME and PTIO enhanced the NE-stimulated increase in β -MyHC promoter activity. Contrarily, NO donor, SNAP and SIN-1, inhibited the increment of β -MyHC promoter activity induced by NE. In conclusion, NE induces eNOS gene expression and NO production in cardiac myocytes. NO is involved in the regulation of NE-stimulated β -MyHC gene expression.

Keywords:
Nitric Oxide, Nitric oxide synthase, Catecholamine, Myosin heavy chain.

三、Introduction

Nitric oxide synthase(NOSs) that make nitric oxide(NO) are now known to be present in cardiac myocytes and play an important role on the regulation of cardiac contractile function. In isolated cardiac myocyte preparation, it has been demonstrated that the depression in myocyte contractile responsiveness to β -adrenergic agonist correlated with an increase in myocyte generation of inducible NOS(iNOS)-derived nitric oxide in response to lipopolysaccharide and specific cytokines.

In the heart, endothelial NOS(eNOS) may also modulate the neurohumoral control of cardiac function. The positive inotropic response to β -adrenergic agonists is enhanced by inhibition of cardiac eNOS both in cultured cardiac myocytes and animal models. Ikeda et al. observed that both the α -adrenergic agonists and angiotensin II enhance inducible nitric oxide synthase expression in rat cardiac myocytes. Thus, nitric oxide, in addition to its vasodilating properties, has now been considered to be an important modulator of cardiac contractility in response to autonomic nervous system agonists. We have previously shown that catecholamines and angiotensin II exert positive inotropic and chronotropic effects on cardiac myocytes resulting in myocyte hypertrophy and myosin gene expression. However, it remains less will characterized that catecholamine effect on cardiac myosin gene expression is modulated by nitric oxide. In this study, we investigate whether modulation of endogenous NO production alters the cardiac myosin gene expression in response to exogenous catecholamine in culture system.

四、Results

Effects of Norepinephrine on nitrite production

We first investigated the effect of norepinephrine(NE) on NO production by cardiac myocyte. NO secreted by cells is rapidly decomposed to the more stable products nitrite and nitrate, treatment of cardiac myocyte with NE caused marked accumulation of nitrite in the culture medium in a time-dependent manner(Fig. 1). The levels of nitrite increased significantly at 6h and continued to increase for at least 24h after exposure to NE. As shown in Figure 2, incubation of NE for 24h,increased nitrite production in a dose-dependent manner(0.25-10 μ M). A23187(Ca^{+2} Inophore) and Interleukin-1 β (IL-1 β)-stimulated NO production were used as positive control.

Effects of L-NAME and PTIO on NE-induced nitrite production

As shown in Figure 3 simultaneous incubation with either the NOS inhibitor, L-NAME(100 μ M), or NO scavenger,PTIO(10 μ M),for 24h inhibited NE-induced nitrite production by cardiac myocytes.

Effects of NE on NOSs mRNA expression

Since NE significantly induced nitrite production by cardiac myocytes, we determined which isoform of NOSs was expressed by identification of transcriptions for NOS isoenzyme. As shown in Table 1, addition of 1 μ M NE to cardiac myocytes for 24h clearly induced eNOS mRNA expression analyzed by reverse transcriptase-polymerase chain reaction(RT-PCR), while no

detectable iNOS or nNOS gene transcripts were induced by NE.

NO modulates NE-induced c-fos and β-MyHC gene expression.

As shown in Table 2., simultaneous incubation with either the L-NAME (100 μM)or PTIO(10 μM) for 24h significantly augmented the NE-stimulated increase in c-fos and β-MyHC mRNA level. In contrast, the increase in c-fos and β-MyHC mRNA level in response to NE were markedly inhibited by SNAP(10 μM) and SIN-1 (10 μM). Using CAT assay, we further analyze the regulation of the promoter activity of β-MyHC gene by NO. The change of NE-stimulated promoter activity of β-MyHC gene in response to the NOS inhibitor, NO scavenger and NO donor corresponds to their respective mRNA levels. As shown in Figure 4, L-NAME and PTIO potentiated the NE-stimulated promoter activity of β-MyHC gene. In contrast, SIN-1 and SNAP significantly suppressed its promoter activity induced by NE.

In conclusion, NE induces eNOS gene expression that makes NO production in cardiac myocytes. NOS inhibitor, L-NAME , and NO scavenger, PTIO, augmented NE-stimulated β-MyHC gene expression. In contrast, NO donor, SIN-1 or SNAP , significantly inhibits β-MyHC gene expression induced by NE.

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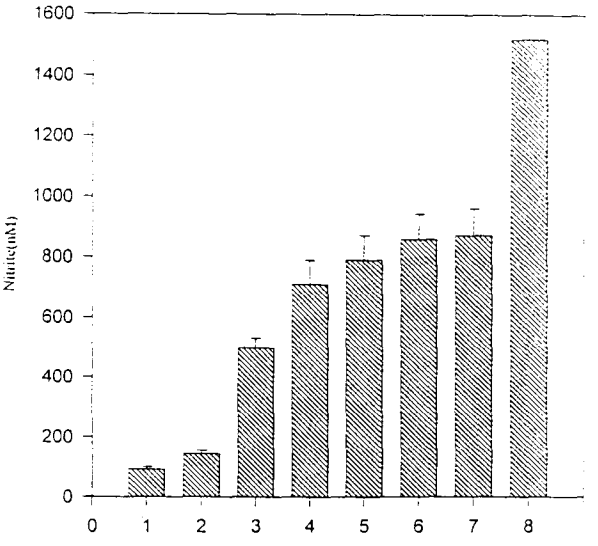

Fig. 2. Dose-dependent effects of Norepinephrine on nitrite production by cardiac myocytes. Cardiac myocytes were exposed to Norepinephrine for 24h. Nitrite accumulation in the culture medium was measured, and the values were normalized to the protein content per dish. Data are means ± S.E.M. of five samples. 1:Control, 2:0.25 μM NE, 3:0.5 μM NE, 4:0.75μM NE, 5:1μM NE, 6:10μM NE, 7:A23187, 8:IL-1 β.

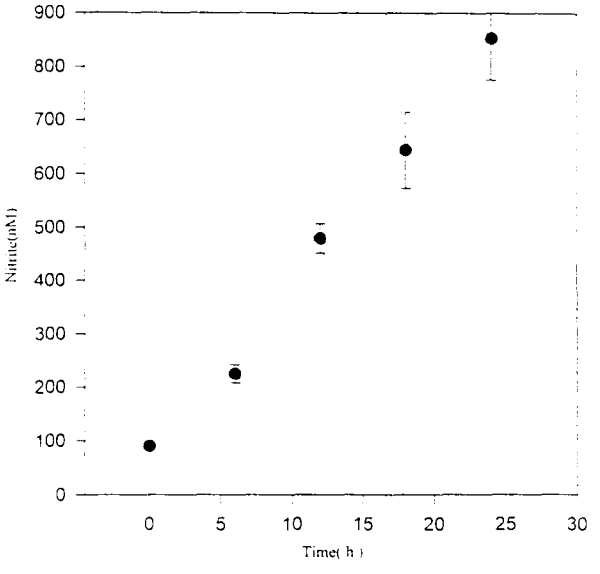

Fig. 1 Effects of Norepinephrine on nitrite production by cultured neonatal rat cardiac myocytes. Cardiac myocytes were exposed to Norepinephrine(1μM) for 24h. Nitrite accumulation in the culture medium was measured, and the values were normalized to the protein content per dish. Data are means ± S.E.M. of five samples.

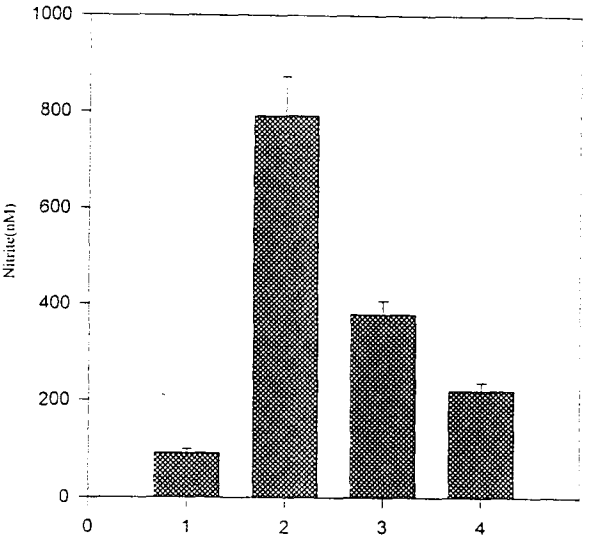

Fig. 3 Effects of Norepinephrine , L-NAME and PTIO on nitrite production by cultured neonatal rat cardiac myocytes. L-NAME and SNAP was added 1h. before the addition of Norepinephrine. Nitrite accumulation in the culture medium was measured, and the values were normalized to the protein content per dish. Data are means ± S.E.M. of five samples.1: Control, 2:Norepinephrine, 3:L-NAME + NE, 4:PTIO +NE

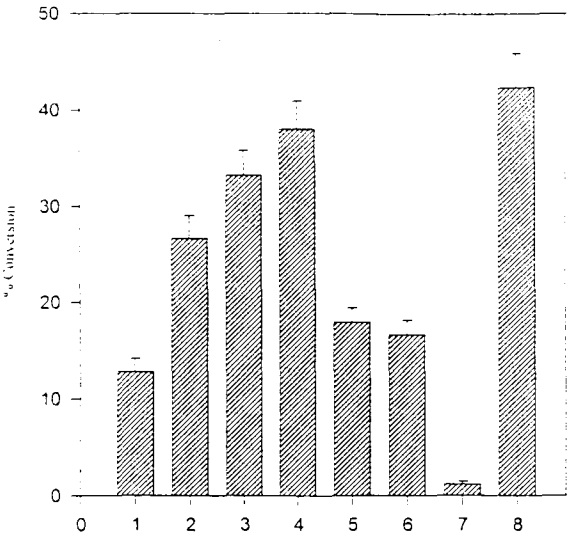

Fig. 4. Effects of Norepinephrine , L-NAME and PTIO on MyHC promoter activity in cardiac myocytes. The cultured cardiac myocytes were transfected with CAT fusion genes of MyHC promoter by Calcium phosphate precipitation. Cultured cardiac myocytes were exposed to Norepinephrine for 24h in the presence or absence of L-NAME or PTIO, applied 1h before NE addition. Results derived from the autoradiograms of 4 separate experiments were analyzed by scanning densitometer and expressed as mean ± S.E.M. 1:Control, 2: Norepinephrine, 3:L-NAME + NE, 4:PTIO +NE, 5:SNAP+NE, 6:SIN-1+NE, 7:CAT3,8:CAT2. CAT2 and CAT3 are shown as positive and negative control.

Table 1. Effect of Norepinephrine on NOSs mRNA level in cardiac myocyte

Treatment	mRNA/18S RNA (Relative to Control)		
	eNOS	iNOS	nNOS
Control	1.0	1.0	1.0
Norepinephrine	1.94(*)	1.02	0.99

Table 2. Effect of NOS inhibitor, NOS scavenger and NO donor on NE-induced increase c-fos and β-MyHC mRNA level in cardiac myocyte.

Treatment	mRNA/18S RNA (Relative to Control)	
	β-MyHC	c-Fos
Control	1.00	1.00
NE	2.56(* *)	12.34(* *)
L-NAME+NE	2.73(* * *)	13.14(* * *)
PTIO+NE	3.14(* * *)	14.22(* * *)
SNAP+NE	2.13(*)	11.46(* *)
SIN-1+NE	2.07(*)	10.87(* *)