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Nitric Oxide Modulates Calcium Current in Cardiac Myocytes but not in Intact Atrial Tissues

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ABSTRACT

The aim of the present study was to know whether exogenously administered nitric oxide (NO) may differently modulate muscle mechanics between heart and aorta. We used PIANO method to generate NO. In isolated rat atrial tissues, neither heart rate nor contractility was affected by PIANO (STZ, 30 ~100 μM). Only high concentration (100 μM) of 8-bromo cyclic GMP slightly depressed cardiac contractility. However, the same concentrations of 8-Br cGMP and PIANO significantly relaxed the rat thoracic aorta contracted with phenylephrine (0.1 μM). In isolated rabbit cardiac atrial myocytes, the amplitude of calcium currents were decreased in the whole voltage range by the presence of streptozotocin, which was further potentiated by UV light. Calcium currents were also decreased in those preparations treated with bradykinin, nitroprusside and 8-Br cGMP. These findings suggest that exogenous NO may modulate calcium current in cardiac myocyte. However, it remains why this does not affect myocardial contractility and heart rate. We concluded that NO may differently regulate calcium signal between aorta and heart muscle.

Key Words: Photorelaxation, Rabbit cardiac atrial myocyte, Nitric oxide, Contractility, Rat aorta, Rat atrium

INTRODUCTION

Recently, Chung and Chang (1994) reported that a so called photo-induced adequate nitric oxide (PIANO) system, in which a NO- or NO_2-carrying molecule is photoactivated to release a potent relaxing substance, NO. Thus the PIANO system can be exploited to investigate the role of NO in various physiological processes in which NO is suggested as a mediator. Thus far, PIANO relaxes blood vessels (Chang *et al.*, 1993a, 1993b), trachea (Chang *et al.*, 1993a), rabbit corpus cavernosum (Chung and Chang, 1994), human uterus (Lee and Chang, 1995) and rat gastric fundus (Chang 1995). Although low concentrations of NO clearly have cardioprotective effects in ischemic reperfused hearts, it is reported that physiological concentrations of exogenous NO does not acutely depress the inotropic state of the rat heart (Weyrich *et al.*, 1994). However, high concentrations of NO are thought to depress cardiac contractility during the late stages of septic shock (Brady *et al.*, 1992). Furthermore, functional differences exist between the modulatory role of cGMP in the control of myocardial contractility and smooth muscle tone also reported (Baumner and Nawrath, 1995). In the present study, we tested whether the PIANO-mediated release of NO can effectively depress cardiac

contractility in isolated rat atrial muscle as well as inhibits calcium current in rabbit isolated cardiac myocytes.

MATERIALS AND METHODS

Materials

Streptozotocin (STZ) phenylephrine HCL (PE), indomethacin, Mg-ATP, TEA and carbachol HCl were obtained from Sigma Chemical Co. (St. Louis, MO). Collagenase was obtained from Yakult (Tokyo, Japan).

Rabbit single atrial cell preparation

Single atrial cells of the rabbit were isolated by Chang *et al.* (1994). In brief, the heart was perfused with low Ca^{2+}-tyrode solution (30~50 mM Ca^{2+}) containing collagenase (4 mg/50 ml) for 15~20 min by using Langendorff perfusion system. Atrial tissue was dissected out and mechanically agitated to disperse the cells and then stored in low Cl^-, high K^+ medium in the refrigerator. During experiments, cells were transferred on the inverted microscope (Olympus, CK-2) and superfused (1 ml/min) at 37℃. The solution to superfuse atrial cells contained (in mM): NaCl, 140; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1; NaH_2PO_4, 0.33; glucose, 5; HEPES, 5; pH 7.4. The internal solution of the patch electrode normally contained (in mM): Cs-aspartate, 2.5; di-Tris-creatine phosphate, 2.5; di-sodium-creatine phosphate, 2.5; $MgCl_2$, 1; TEA-Cl, 20; EGTA, 5; pH 7.4.

Measurement of Ca^{2+} current

The cells were voltage-clamped by using a whole-cell patch clamp apparatus (EPC-7, Germany). Glass electrodes with resistance of 2-3 mega ohm were used. The Ca^{2+} current was recorded during various depolarizations for 200 ms from holding potential of -40 mV using the whole cell voltage clamp technique. Voltage sensitive transient outward current was blocked by the application of 2 mM 4-aminopyridine to the bath solution and replacement of K^+ with Cs and TEA in the pipette solution. The data were recorded on a pulse code modulator data recorder for analysis.

Tension recording

The strips were suspended with a glass capillary rod to force transducer (FT03, Grass) on one end, and fixed with silk ties to a metallic support on the opposite end. The physiologic solution was gassed with 95% O_2-5% CO_2 and had the following composition (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.18; KH_2PO_4, 1.18; $NaHCO_3$, 24.9; glucose, 10 and EDTA, 0.03. The aortic strips and rat left and right atrial tissues were equilibrated at 1g resting tension for 90 minutes prior to drug addition. Isometric tension was induced using a submaximal concentration of PE (0.1 μM) for rat aorta or NE (10 μM) for rat heart and was recorded on a Grass physiograph (model 79E, Grass Instruments, Quincy, MA) using a force displacement transducer. For left atrial tissues, electrical field stimulation was applied through the experiment.

Measurement of PIANO-mediated relaxation

PIANO-mediated relaxation was measured as described previously (Chung and Chang, 1994). In brief, after reaching a plateau of contraction with PE, tissues were exposed to UV light (1-15s) using a long wavelength UV lamp (366 nm, Mineralight UV GL 58, San Gabriel, CA) in the presence or absence of streptozotocin (STZ, 100 μM). UV lamp was placed 5-6cm distance from the muscle chamber. To investigate the influence of PIANO on cardiac contractility and chronotropic effect, STZ was added after stabilizing the tissues which usually takes around 60 min. Cardiac muscles were then subjected to UV irradiation as indicated.

RESULTS

PIANO-mediated responses in rat aorta and atrial muscle

As shown in Fig. 1, after STZ treatment, rat aortic strips were rapidly relaxed upon UV irradiation which was dependent on exposure time and distance to UV light. While in rat heart muscles, the same concentration of STZ was without effect both on contractility and

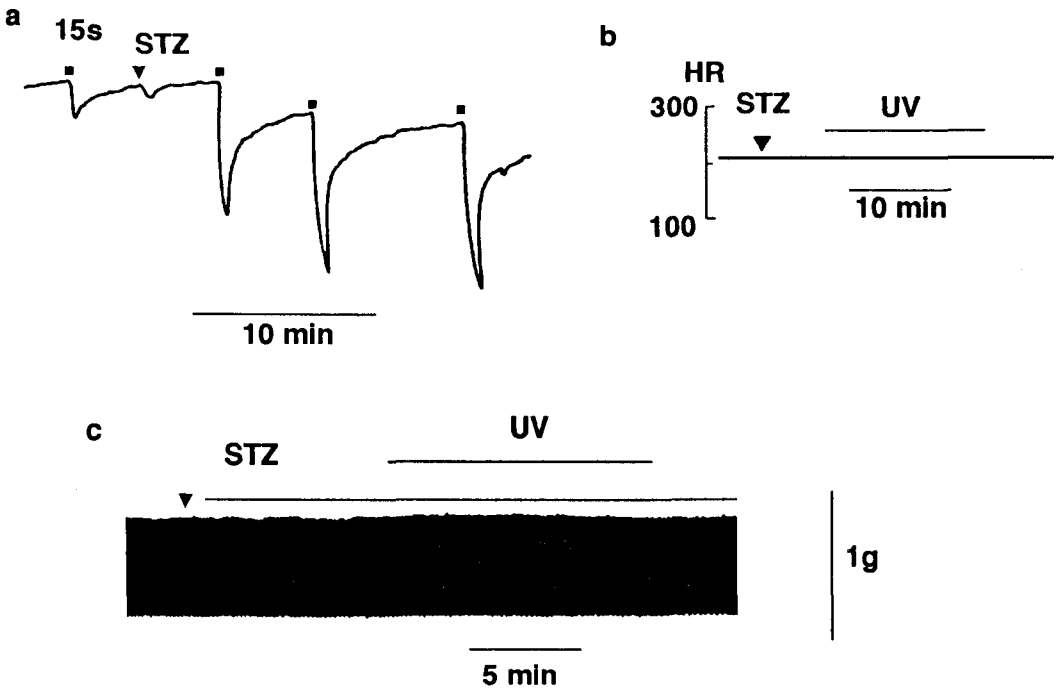

Fig. 1. Effects of PIANO on myocardial contractility, chronotropic action (b, c) and vascular smooth muscle relaxation (a). PIANO was utilized with UV light exposure in the presence of STZ.

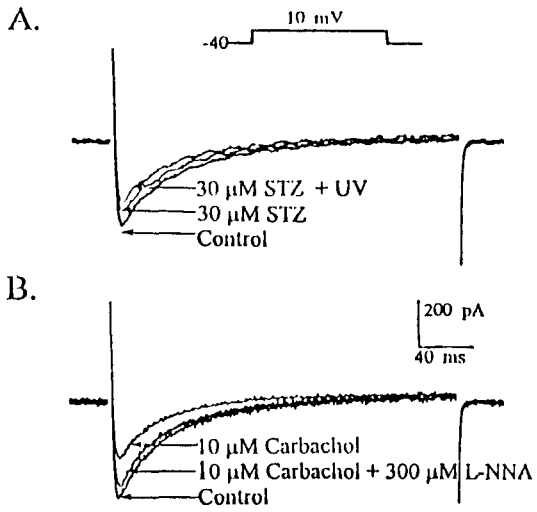

Fig. 2. Effects of PIANO on the Ca^{2+} current in cardiac myocytes. Ca^{2+} current was decreased by STZ alone, which was further potentiated by UV irradiation (A). NO syntase inhibitor, L-NNA, partially blocked the effects of carbachol on the Ca^{2+} current (B)

heart rate (Fig. 1).

Effects of PIANO on Ca^{2+} current in rabbit isolated cardiac myocyte

Fig. 2 shows that Ca^{2+} current was decreased by 30 mM streptozotocin which has nitric oxide group (A). UV irradiation (PIANO) further potentiated the effect of STZ. While carbachol (10 μM) decreased the Ca^{2+} current. N^G-nitro-L-arginine (L-NNA, 300 μM), the concentration that inhibits NO synthase, partially blocked the effect of carbachol on the Ca^{2+} current (B).

Effects of SNP on the current-voltage relationship of the Ca^{2+} current

Fig. 3 shows the effect of SNP on the current-voltage relationship of the Ca^{2+} current in rabbit cardiac myocytes. Ca^{2+} currents were shown in normal Tyrode solution (A) and after application of 5 μM SNP (B). It is noted that the current-voltage relationship was not affected by the SNP but the amplitude of the Ca^{2+}

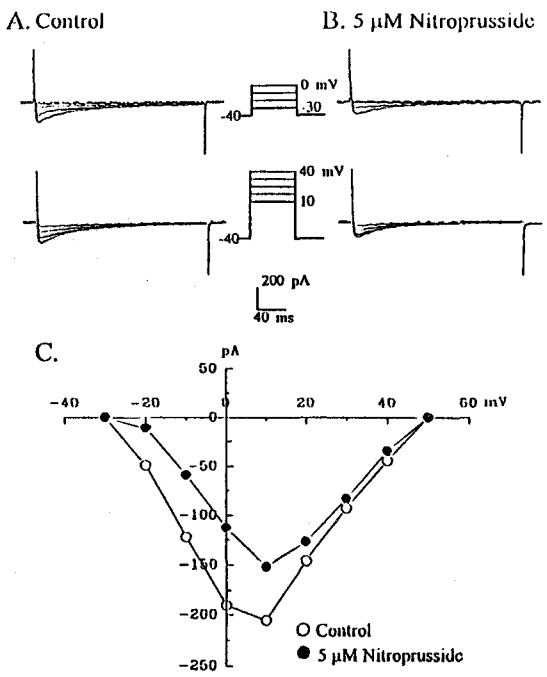

Fig. 3. Effects of SNP on the current-voltage relationship of the Ca^{2+} current.

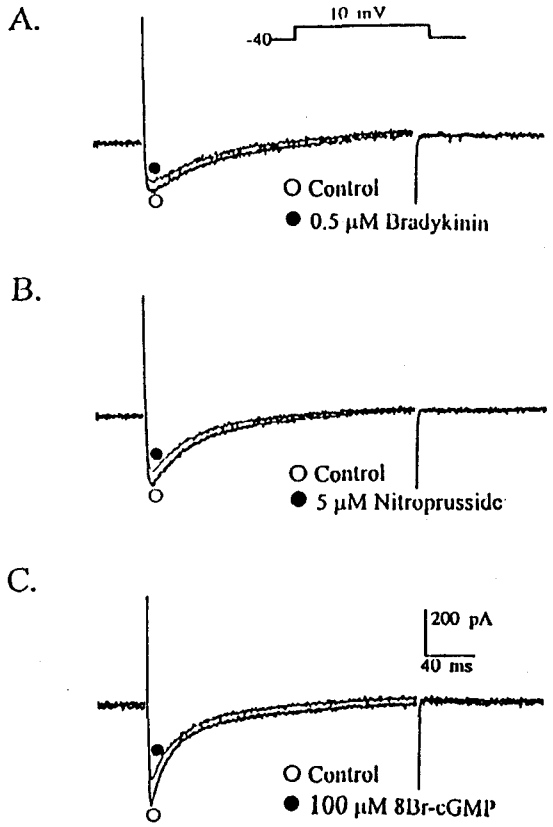

Fig. 4. Effects of bradykinin, sodium nitroprusside and 8-bromo cyclic GMP on the Ca^{2+} current in isolated single cardiac atrial myocyte of rabbit.

current was only decreased (C).

Effects of bradykinin, SNP and 8-Br-cGMP on the Ca^{2+} current of single cardiac myocyte of rabbit

As shown in figure 4, Ca^{2+} currents were affected by bradykinin (BK), sodium nitroprusside (NP) and 8-bromo cyclic GMP (8 Br-cGMP) in single atrial cells of the rabbit. Ca^{2+} current was recorded by depolarizing pulse to+ 10 mV for 200 ms from the holding potential of -40 mV. Each of 0.5 μM BK (A), 5 μM NP (B) and 100 μM 8Br-cGMP (C) decreased the amplitude of Ca^{2+} currents.

DISCUSSION

Biological studies of "caged NO compounds", i.e., compounds that are stable in oxygen-containing solutions until photolyzed by UV irradiations, whereupon they release NO, have recently been used as research tool for delivering NO (Pou *et al.*, 1994; Markings *et al.*, 1994; Chamulitrat *et al.*, 1994; Matthew *et al.*, 1994). PIANO-induced relaxation appears to be a suitable model to study the effect of NO-mediated relaxation of smooth muscle (Chang *et al.*, 1993a; Chang *et al.*, 1993b; Chung and Chang, 1994, Lee and Chang, 1995; Chang 1995).

Very few studies exist of the inotropic effects of NO *in vivo*. Lefer *et al.* (1993) reported that SPM-5185 at 500 μmol/L attenuated myocardial necrosis in ischemic/reperfused dog hearts. The NO donor was infused directly into the coronary circulation, and no evidence of reduced cardiac contractility was observed. Many evi-

dences indicate that NO may be important for maintaining normal cardiac contractility (Hasebe *et al.*, 1993). However others (Fort and Lewis, 1991; Weyrich, 1995) insisted that NO does not elicit a physiologically significant acute negative inotropic effect on the whole heart. The purpose of the present study was to investigate whether 1) PIANO also effectively modulates responses in cardiac motility 2) if so, are there any differences in response to NO between cardiac muscle and vascular smooth muscle? The present study confirmed that NO is released by PIANO and is effective in the relaxation of vascular smooth muscle. The prominent feature of the present study is that cardiac muscle, but not cardiac cell was almost resistant to PIANO. These result suggests that NO may have different sensitivity between cardiac muscle and vascular smooth muscle, which was supported the recent investigation of others (Baumner and Nawrath, 1995) that functional differences exists between the modulatory role of cGMP, final effector molecule of NO, in the control of myocardial contractility and smooth muscle tone. So that NO may have more sensitive action in vascular smooth muscle than in cardiac muscle. Furthermore, physiological concentration of exogenous NO does not acutely depress the inotropic state of rat heart was also reported (Weyrich *et al.*, 1994) and that NO donors to alter arrythmias induced by acute myocardial ishemia or reperfusion in rats was recently reported (Barnes and Coker, 1995). While 8-Br cGMP slightly depressed cardiac contractility only above 100 μM, which is the concentration that relaxes vascular smooth muscle competely (data not shown). However, based on the results obtained from PIANO experiment in cardiac myocyte and tissue, it is unclear why PIANO is effective in isolated myocyte but not tissues.

In conclusion, PIANO decreased Ca^{2+} current in cardiac cells and relaxed vascular smooth muscle but had no effect on cardiac contractility and heart rates. Further study is needed to clarify this discrepancy of NO action between vascular smooth muscle and heart muscle.

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=국문요약=

심근세포 및 혈관 평활근에 대한 Nitric Oxide 작용의 민감성의 차이

경상대학교 의과대학 약리학교실, 생리학교실 및 심혈관 연구소

박춘옥 · 강영진 · 이희영 · 장기철

본 연구의 목적은 외부에서 nitric oxide (NO)를 투여 하였을때 심근 수축력, 심박동수의 변화 및 혈관 평활근에 대한 효과를 비교함으로서 NO에 대한 이들 장기의 민감도가 서로 같은지 또는 상이한지를 알아보고자 하였다. 본 실험에서는 PIANO 방법에 의한 근장력의 변화와 아울러 심근에서의 Ca^{2+} current를 측정하였다. 랫트의 심방근에 대한 PIANO (STZ, 100 μM)는 심근수축력 및 심박동수에 전혀 변화를 주지 않았지만 혈관 평활근에서는 강한 이완 작용을 나타내었다. 한편, 8-Br-cGMP도 고농도 (100 μM)에서만 심근 수축력을 억제하였다. 토끼의 심방근 세포에서 Whole cell voltage patch clamp를 사용시 bradykinin, SNP, 8-Br-cGMP 및 PIANO는 Ca^{2+} current를 억제하였다. 이러한 사실은 외부에서 공급되는 NO에 대한 심근과 혈관 평활근의 반응에는 민감도의 차이가 있음을 암시하며 더 나아가 심근의 경우에도 NO 반응에는 종 (species)간의 차이와 동일 종이라 하더라도 세포(cell)와 장기(tissue)에 차이가 있을 가능성을 제시하였다.