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Cardiac and vascular effects of nitric oxide synthase inhibition in lipopolysaccharide-treated rats

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Abstract

In the present study, intraperitoneal injection of lipopolysaccharide (10 mg/kg) to anaesthetized rats produced a gradual fall in mean arterial pressure in 6 h. Aortic rings from lipopolysaccharide-treated rats showed a significant reduction in the contractile response to vasoconstrictors. Pretreatment with N^G-nitro-L-arginine methyl ester (L-NAME) or aminoguanidine, two nitric oxide synthase (NOS) inhibitors, abolished this vascular hyporeactivity. In ventricular myocytes isolated from lipopolysaccharide-treated rats, both electrically induced Ca^{2+} transients and the intracellular Ca^{2+} response to β-adrenergic stimulation were significantly depressed when compared with those recorded from myocytes from sham control rats. L-NAME and aminoguanidine alone had no effects on electrically stimulated Ca^{2+} transients in ventricular myocytes either from control or lipopolysaccharide-treated rats. However, these two NOS inhibitors augmented the intracellular Ca^{2+} response to β-adrenergic stimulation in myocytes from lipopolysaccharide-treated rats, but not in control myocytes. In addition, 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), an inhibitor of nitric oxide (NO)-sensitive guanylyl cyclase, also reversed the intracellular Ca^{2+} hyporesponsiveness to β-adrenergic stimulation in myocytes from lipopolysaccharide-treated rats. In cardiac myocytes from lipopolysaccharide-rats pretreated with aminoguanidine, the intracellular Ca^{2+} hyporesponsiveness to β-adrenergic stimulation was abolished. However, there still existed a depressed Ca^{2+} response to electrical field stimulation. These data indicate that NO following lipopolysaccharide stimulation contributes to vascular hyporeactivity and the depressed intracellular Ca^{2+} response to β-adrenergic stimulation in lipopolysaccharide-treated rats, but is not responsible for the reduced Ca^{2+} response to electrical stimulation in our experimental conditions.

Keywords: Lipopolysaccharide; Vascular hyporeactivity; Ventricular myocyte; Ca^{2+} transient; β-adrenergic stimulation; Nitric oxide (NO)

1. Introduction

Septic shock induced by gram negative bacterial infection is a major cause of morbidity and mortality in the intensive care unit (Beishuizen et al., 1998). It has been demonstrated that upon exposure to bacterial endotoxin (lipopolysaccharide) or cytokines, an unregulated isoform of nitric oxide synthase (NOS), inducible NOS (iNOS), can be expressed in cardiovascular tissues, including endothelial cells (Balligand et al., 1995), vascular smooth muscle (Chester et al., 1998) and cardiac myocytes (Luss et al., 1995). The subsequent overproduction of nitric oxide (NO) in vessels is believed to lead to vasodilatation, vascular hyporesponsiveness to vasoconstrictors and hypotension during septic shock (Rees et al., 1998). These findings led to the hypothesis that pharmacological inhibition of iNOS might be of therapeutic value for the treatment of septic shock. Indeed, NOS inhibitors have been shown to reverse hypotension in experimental models of septic shock (Meyer et al., 1994; Rees et al., 1998) and patients (Petros et al., 1994). However, in many studies these drugs did not lower the mortality of endotoxic shock (Cobb et al., 1992; Petros et al., 1994). These results might be attributable to the inhibition of endothelial NOS (eNOS), which is constitutively present in the endothelial cells and produces physiological levels of NO, leading to the main-

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tenance of vascular tone and normal blood pressure. It was therefore postulated that selective iNOS inhibitors would have a therapeutic advantage over non-selective NOS inhibitors in the treatment of septic shock (Wright et al., 1992). Aminoguanidine has been described as such a potential candidate. Experiments from both aortic rings (Griffiths et al., 1993) and in vivo animal models (Wu et al., 1995) have shown that aminoguanidine is an inhibitor with higher selectivity for iNOS without impairing the normal production of NO by eNOS. However, Laszlo et al. (1995) showed that aminoguanidine could also inhibit eNOS, at least in the intestinal microvasculature in rats. Moreover, the beneficial effects of iNOS expression have more recently been appreciated (Laubach et al., 1998). iNOS knockout mice exhibited no significant survival advantage over wild-type mice when challenged with lipopolysaccharide (Laubach et al., 1995). It has also been reported that selective inhibition of iNOS activity with 1400 W, the most potent and selective iNOS inhibitor to date, attenuates the circulatory failure induced by endotoxin in the rat, but fails to influence the degree of organ dysfunction (Wray et al., 1998). To clarify this question, we evaluated the selectivity of aminoguanidine for iNOS at different concentrations in isolated rat aorta.

Although it has been well established that induction of NO is a central component of the vascular hyporeactivity and hypotension in septic shock, the data supporting the role of NO in myocardial depression associated with septic shock still remain controversial. Many studies have documented myocardial depression following iNOS induction, manifested as either a decrease in basal myocardial contractile function (Brady et al., 1992) or just a reduction in β-adrenergic inotropic responsiveness (Balligand et al., 1993). In an early study (Brady et al., 1992), it was found that ventricular myocytes isolated from guinea pigs after in vivo injection with lipopolysaccharide exhibited reduced cell shortening. This depressed contractility was partially restored by acute treatment with N^G-nitro-L-arginine methyl ester (L-NAME), a commonly used NOS inhibitor. However, some studies failed to demonstrate the mediatory role of NO in lipopolysaccharide-induced cardiac dysfunction (Keller et al., 1995; Toth and Heard, 1997). NOS activity, nitrate/nitrite and cGMP concentrations, indicators of NO production, were not altered in these lipopolysaccharide-treated animals. NOS inhibitors did not reverse the depressed contractility of atrial preparations or ventricular myocytes isolated from endotoxemic guinea pigs in these experiments. Although the cellular mechanisms underlying myocardial dysfunction are unresolved, one possibility is that decreased intracellular free Ca^{2+} ($[Ca^{2+}]_i$) might contribute to myocardial depression in sepsis. Therefore, in our present study, we investigated the intracellular Ca^{2+} responses to both electrical field stimulation and β-adrenergic stimulation in myocytes isolated from lipopolysaccharide-treated rats and examined whether NOS inhibitors had any effect on these responses.

2. Materials and methods

2.1. Animal model and haemodynamic measurements

This study was approved by the Committee on the Use of Live Animals in Teaching and Research of the University of Hong Kong. Male Sprague–Dawley rats (300–350 g) were anaesthetized by intraperitoneal (i.p.) injection of urethane (1.2 g/kg), a rectal probe inserted and body temperature maintained at 37°C. The trachea was cannulated to facilitate respiration. The right femoral artery was cannulated and connected to a blood pressure transducer (MLT1050, ADInstruments). The signals for systemic arterial blood pressure and heart rate were fed into a personal computer via an analogue-digital converter (PowerLab/410, ADInstruments). After a period of stabilization (30 min), either lipopolysaccharide (10 mg/kg) or saline (1 ml/kg) was injected intraperitoneally and haemodynamic parameters were monitored over 6 h.

2.2. Organ bath experiments

Male Sprague–Dawley rats (300–350 g) were injected intraperitoneally with either lipopolysaccharide (10 mg/kg) or vehicle (saline, 1 ml/kg). Four hours later, animals were killed by stunning and subsequent cervical dislocation. Thoracic aortae were rapidly removed and stored in cold Kreb's solution of the following composition (in mM): NaCl 120, $NaHCO_3$ 25, glucose 11.1, KH_2PO_4 1.2, $MgSO_4$ 1.2, KCl 4.5, and $CaCl_2$ 1.25. This solution routinely contained 1 μM propranolol, 0.1 μM rauwolscine and 10 μM indomethacin to prevent β, α_2-adrenergic stimulation and cyclooxygenase product synthesis, respectively. Aortae were cleaned of fat and connective tissues and cut into 3 mm rings. Rings were suspended in organ chambers containing 10 ml Kreb's solution at 37°C, bubbled with 95% O_2 and 5% CO_2. After equilibration under no tension for 15 min, the preparations were passively stretched to an optimal tension of 2 g. Rings were equilibrated for 1 h and the Kreb's solutions were changed every 15 min. Changes in isometric tension were recorded by force transducers (Grass FT03) connected to a data logger (Picolog). Before each experiment, rings were stimulated with 60 mM KCl at least three times until a reproducible contractile response was obtained.

The presence of endothelium was verified by addition of acetylcholine (10^{-5} M) to rings pretreated with phenylephrine (3×10^{-7} M). Rings that did not relax to acetylcholine were excluded. Rings with intact endothelium were rinsed three times and then incubated with either vehicle, 300 μM L-NAME or aminoguanidine (10 μM, 100 μM or 1 mM) for 1 h before a concentration-dependent curve to phenylephrine (10^{-8}–10^{-4} M) was constructed. Tension was expressed as a percentage of the KCl-induced contrac-

tion. In another set of experiments, after incubation with either vehicle or NOS inhibitors, rings from control rats were submaximally contracted with phenylephrine and then the cumulative concentration–response curves to acetylcholine (10^{-8}–10^{-4} M) or calciomycin (A23187) (10^{-8}–10^{-6} M) were obtained. Relaxation was calculated as a percentage of the maximal contraction induced by phenylephrine.

2.3. Isolated myocyte experiments

Male Sprague–Dawley rats were given an intraperitoneal injection of either saline or lipopolysaccharide (10 mg/kg) and killed by stunning and subsequent cervical dislocation 4 h later. In the NOS inhibitor-treated group, aminoguanidine was given 30 min prior to lipopolysaccharide administration. The hearts were rapidly removed, mounted to a Langendorff apparatus and perfused retrogradely at a constant flow (8 ml/min) with modified Eagle's medium (MEM) supplemented with 10 mM $NaHCO_3$, 10 mM Hepes, and 1.25 mM $CaCl_2$ (37°C, 95% O_2/5% CO_2, pH 7.25) for 5 min. Perfusion was then switched to a Ca^{2+}-free MEM solution for 5 min, and a Ca^{2+}-free MEM solution containing 25 U/ml collagenase (type I) and 0.1% bovine serum albumin for a further 35 min. The ventricles were then cut off, minced, and myocytes dissociated by gentle trituration. The supernatant was filtered and centrifuged at 100 g for 1 min. The pellet containing isolated myocytes was washed three times and re-suspended in Ca^{2+}-free MEM solution containing 2% bovine serum albumin. Ca^{2+} concentration in the MEM solution was then gradually increased to 1.25 mM within 35 min.

After isolation, cells were loaded with 5 μM Fura-2 acetoxymethyl ester (Fura-2 AM) by incubation for 30 min at room temperature. Fura-2 was then washed out and cells were transferred into an experimental chamber mounted on the stage of a fluorescence microscope and allowed to adhere to the cover-slip at the bottom of the chamber. The chamber was then perfused (1 ml/min) at room temperature with Kreb's solution containing 1.25 mM Ca^{2+}. Myocytes were stimulated via a pair of platinum electrodes (stimulator Grass S88) at 0.2 Hz, 20% supra-threshold amplitude with 5 ms square wave stimuli. For Fura-2 fluorescence, cells were alternatively illuminated at 340 and 380 nm. The emitted fluorescence was collected by the objective lens, filtered at 510 nm and detected by photomultiplier tube. The 510 nm fluorescence emitted during 340 nm illumination was electronically divided by that emitted during 380 nm illumination to give a fluorescence ratio indicative of the $[Ca^{2+}]_i$. Background fluorescence was determined at the end of the experiment and data corrected accordingly.

After a steady state response to electrical stimulation was obtained in control Kreb's solution, test drugs were perfused until a new steady state was reached. Changes in Ca^{2+} transients were monitored, and average steady state Ca^{2+} transients in different experimental conditions (control or with drugs) were obtained from about 10 cells from at least six different animals. To eliminate beat-to-beat variation in peak amplitude, 10 consecutive Ca^{2+} transients recorded in steady state conditions were averaged to obtain a mean value in each individual cell. To test the involvement of the NO/cGMP pathway, 300 μM L-NAME, 100 μM aminoguanidine or 10 μM 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) was added 10 min prior to β-adrenergic stimulation with 10 nM isoproterenol and was present for the duration of the experiment.

2.4. Chemicals and reagents

Fura-2 AM was purchased from Molecular Probes (Eugene, OR, USA). Aminoguanidine was from Research Biochemicals (Natick, MA, USA). ODQ was from Tocris Cookson (Bristol, UK). Lipopolysaccharide (*Escherichia coli* Serotype 0111:B4), L-NAME, isoproterenol and all other chemicals were from Sigma (St. Louis, MO, USA).

2.5. Statistical analysis

Data were expressed as mean ± S.E.M. Comparisons were made by unpaired or, where appropriate, paired Student's *t*-test. Comparisons between multiple groups were made by one-way analysis of variance. A $P < 0.05$ was considered significant.

3. Results

3.1. The animal model of septic shock

Both observations of awake rats and haemodynamic measurements in the anaesthetized rats after lipopolysaccharide injection were characteristic of septic shock. Two hours after lipopolysaccharide treatment of the conscious rats destined to provide aortic rings and cardiomyocytes, they started to develop signs of endotoxemia, including piloerection, apathy and diarrhea. In the anaesthetized rats, the blood pressure did not significantly change until 3 h after the endotoxin challenge and gradually fell to 45.0 ± 10.6 mm Hg in 4 h.

3.2. Organ bath experiments

3.2.1. Effects of aminoguanidine and L-NAME on agonist-induced relaxation in aortic rings from sham control rats

The selectivity of NOS inhibitors for eNOS was evaluated by their inhibitory effects on endothelium-dependent

vasodilatation in isolated blood vessels (Griffiths et al., 1993). Following the submaximal precontraction with phenylephrine (3×10^{-7} M), acetylcholine (10^{-8}–10^{-4} M) and A23187 at low concentrations (10^{-8}–3×10^{-7} M) induced concentration-dependent relaxations in aortic rings with endothelium from control rats, while A23187 at high concentration (10^{-7}–10^{-6} M) caused contraction because of its direct effect on vascular smooth muscle. Treatment of aortic rings with 300 μM L-NAME for 1 h resulted in a complete inhibition of the endothelium-dependent relaxations to acetylcholine or A23187. In contrast, 100 μM or 1 mM aminoguanidine produced significant but partial inhibition of relaxations to these two vasodilators while 10 μM aminoguanidine had no effect (Fig. 1a and b).

3.2.2. Effects of aminoguanidine and L-NAME on phenylephrine-induced contraction in aortic rings from lipopolysaccharide-treated rats

In aortic rings removed from lipopolysaccharide-treated rats, the concentration–contraction curve to phenylephrine was significantly shifted to the right and the maximal contraction was significantly decreased (25.1 ± 6.8% vs. 98.7 ± 3.6% in control rings, $P < 0.01$) when compared to that from control rats. This hyporeactivity to phenylephrine was completely abolished by treatment of the rings with either 100 μM aminoguanidine, 1 mM aminoguanidine or 300 μM L-NAME for 1 h, but not by 10 μM aminoguanidine (Fig. 2).

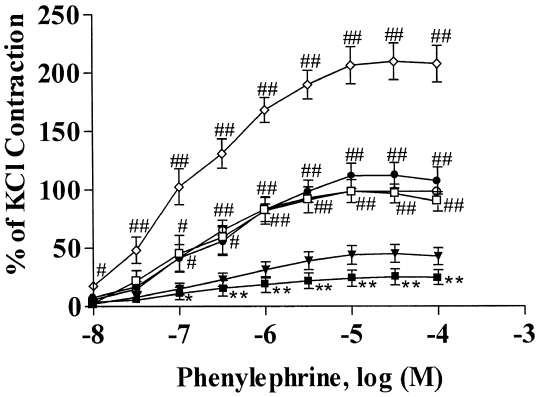

Fig. 2. Effects of L-NAME (300 μM, ●) and aminoguanidine (10 μM, ▼; 100 μM, □ and 1 mM, ◇) on the concentration–contraction curves evoked by phenylephrine in aortic rings from lipopolysaccharide-treated rats (■). Results are expressed as mean ± S.E.M. of 6–8 observations and are shown as percentage of KCl-induced contraction. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with rings from sham control rats (○). $^{\#}P < 0.05$; $^{\#\#}P < 0.01$, statistically significant differences between untreated and NOS inhibitor-treated rings from lipopolysaccharide-treated rats.

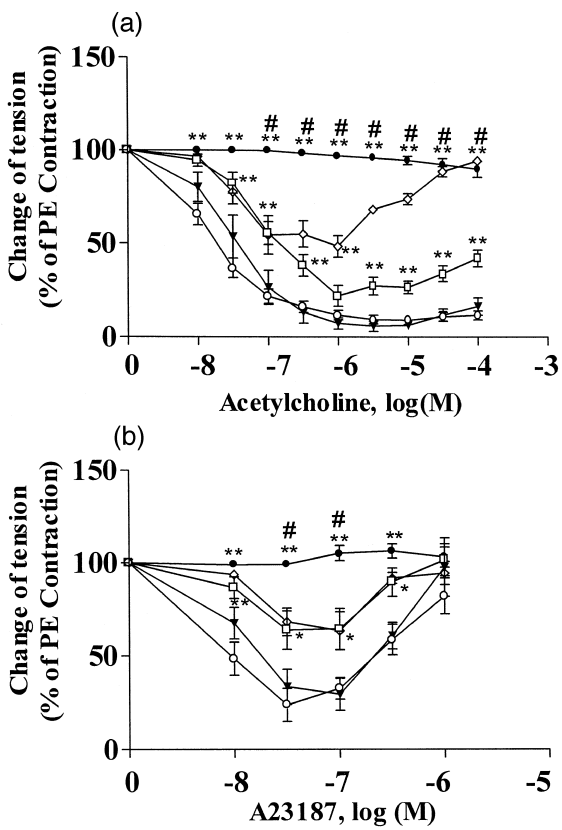

Fig. 1. Effects of L-NAME (300 μM, ●) and aminoguanidine (10 μM, ▼; 100 μM, □ and 1 mM, ◇) on the endothelium-dependent relaxation curves induced by acetylcholine (a) and A23187 (b) in aortic rings from sham control rats (○). Results are presented as mean ± S.E.M. of 6–8 observations and are expressed as percentage of the contraction induced by phenylephrine (PE). $^{*}P < 0.05$, $^{**}P < 0.01$, statistically significant differences between the control and NOS inhibitors-treated rings. $^{\#}P < 0.05$, statistically significant difference between the relaxation inhibited by 300 μM L-NAME and that inhibited by 100 μM aminoguanidine.

3.3. Isolated myocyte experiments

3.3.1. $[Ca^{2+}]_i$ responses in myocytes from control and lipopolysaccharide-treated rats

In the electrically stimulated single ventricular myocytes from control rats, β-adrenergic stimulation with isoproterenol (10 nM) induced a significant elevation in the amplitude of $[Ca^{2+}]_i$ transients. In contrast, in myocytes isolated from lipopolysaccharide-treated rats, both the amplitude of $[Ca^{2+}]_i$ transients induced by electrical field stimulation (0.45 ± 0.02, $n = 51$ vs. 0.61 ± 0.02, $n = 48$ of control myocytes, $P < 0.01$, Fig. 3a) and intracellular Ca^{2+} response to β-adrenergic stimulation with isoproterenol (Fig. 3b) were significantly depressed when compared to those obtained in myocytes from control rats.

3.3.2. Effects of inhibition of NOS and guanylyl cyclase on $[Ca^{2+}]_i$ responses in myocytes from lipopolysaccharide-treated rats

To determine whether endogenous NO in myocytes directly modulates $[Ca^{2+}]_i$ responses, we examined the effects of the NOS inhibitors, L-NAME (300 μM) and aminoguanidine (100 μM), on $[Ca^{2+}]_i$ responses to electrical and β-adrenergic stimulation in cardiac myocytes

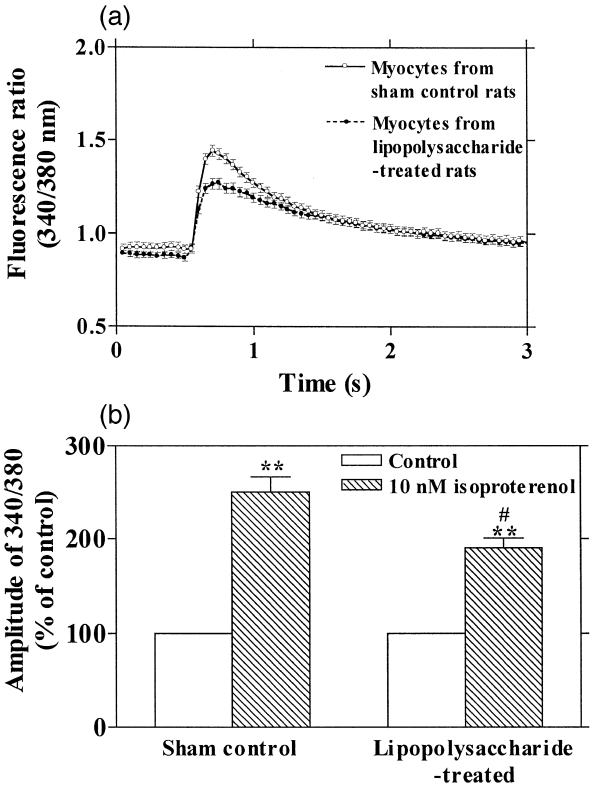

Fig. 3. Comparisons between $[Ca^{2+}]_i$ responses to electrical field stimulation (a) and β-adrenergic stimulation with 10 nM isoproterenol (b) of ventricular myocytes freshly isolated from control and lipopolysaccharide-treated rats. Data are mean ± S.E.M. of 10–12 cells and normalized as percentage of the amplitude of Ca^{2+} transients induced by electrical stimulation only in (b). $^{**}P < 0.01$, compared with electrically stimulated Ca^{2+} transients. $^{\#}P < 0.05$, compared with the sham control myocytes.

isolated from control and lipopolysaccharide-treated rats. L-NAME or aminoguanidine alone had no effect on $[Ca^{2+}]_i$ responses to electrical stimulation in myocytes isolated from either control or lipopolysaccharide-treated rats (data not shown). After pretreatment with L-NAME or aminoguanidine for 10 min, the depressed $[Ca^{2+}]_i$ responses to β-adrenergic stimulation in myocytes from lipopolysaccharide-treated rats were completely reversed (Fig. 4). However, these two NOS inhibitors did not alter the $[Ca^{2+}]_i$ responses to β-adrenergic stimulation in control myocytes (data not shown). In addition, ODQ (10 μM), an inhibitor of NO-sensitive guanylyl cyclase, also reversed the intracellular Ca^{2+} hyporesponsiveness to β-adrenergic stimulation in myocytes from lipopolysaccharide-treated rats (Fig. 4), but not the reduced electrically stimulated $[Ca^{2+}]_i$ transients.

3.3.3. $[Ca^{2+}]_i$ responses in myocytes from lipopolysaccharide-rats pretreated with aminoguanidine

To assess the effects of lipopolysaccharide on electrically stimulated $[Ca^{2+}]_i$ transients and the $[Ca^{2+}]_i$ response to β-adrenergic stimulation when rats are pretreated

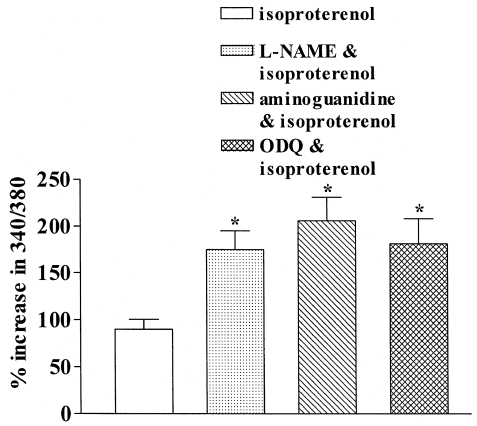

Fig. 4. Effects of 300 μM L-NAME, 100 μM aminoguanidine and 10 μM ODQ on $[Ca^{2+}]_i$ responses to β-adrenergic stimulation with 10 nM isoproterenol of ventricular myocytes freshly isolated from lipopolysaccharide-treated rats. Data are the mean ± S.E.M. of responses obtained in 11–15 cells and are expressed as percentage increase in fluorescence ratio following the treatment. $^{*}P < 0.05$, compared with isoproterenol only.

with the NOS inhibitor, we examined the $[Ca^{2+}]_i$ responses in cardiac myocytes from the rats receiving

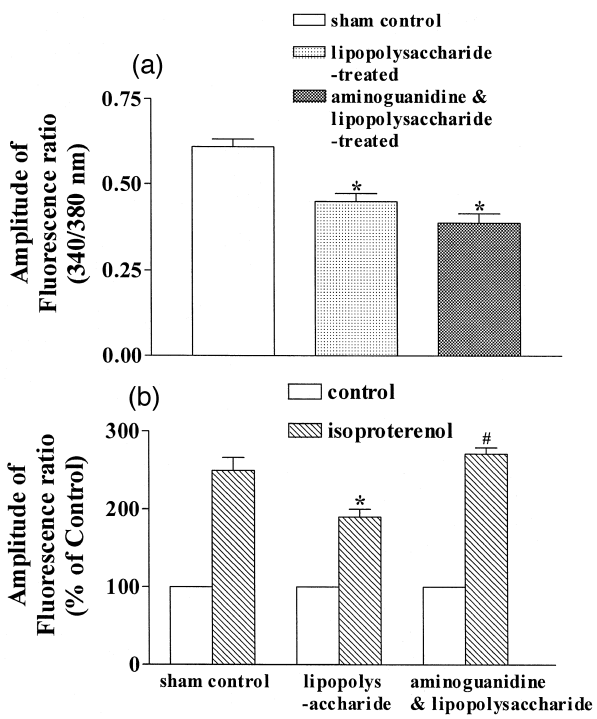

Fig. 5. Comparisons between $[Ca^{2+}]_i$ responses to electrical field stimulation (a) and β-adrenergic stimulation with 10 nM isoproterenol (b) of ventricular myocytes freshly isolated from control, lipopolysaccharide (10 mg/kg, i.p.)-treated rats or lipopolysaccharide-rats pretreated with aminoguanidine (50 mg/kg, i.p.) (aminoguanidine and lipopolysaccharide-treated). Data are mean ± S.E.M. of 10–12 cells and expressed as the amplitude of the electrically induced Ca^{2+} transients in (a) and percentage of the amplitude of Ca^{2+} transients induced by electrical stimulation only in (b), respectively. $^{*}P < 0.05$, compared with sham control myocytes. $^{\#}P < 0.05$, compared with the lipopolysaccharide-treated group.

aminoguanidine (50 mg/kg, i.p.), given 30 min prior to lipopolysaccharide administration. In our preliminary study, this dose of aminoguanidine has been shown to reduce lipopolysaccharide-induced accumulation of NO in the serum and heart to values that were not significantly different from control (data not shown). In these cells, the intracellular Ca^{2+} hyporesponsiveness to β-adrenergic stimulation was abolished (Fig. 5b). However, there still existed a depressed response to the electrical field stimulation (Fig. 5a).

4. Discussion

Our study confirms that the intraperitoneal injection of bacterial endotoxin (lipopolysaccharide) caused a fall in mean aterial pressure in a rat model of septic shock. Aortic rings from these lipopolysaccharide-treated rats showed a reduced contractile response to the vasoconstrictor, phenylephrine. After treatment with the NOS inhibitors, L-NAME or aminoguanidine, the hyporeactivity to vasoconstrictors was completely reversed. These data indicate that the production of NO following lipopolysaccharide stimulation is responsible for the vascular hyporeactivity seen in lipopolysaccharide-treated rats. In contrast to the report that aminoguanidine had no effect on eNOS (Griffiths et al., 1993), we demonstrate here that aminoguanidine partly inhibits the endothelium-dependent relaxation to vasodilators in vitro, which indicates its ability to inhibit eNOS. However, at the doses examined here, the potent ability of aminoguanidine to inhibit lipopolysaccharide-induced NO with less effect on endothelium-dependent vasodilatation still makes it a relatively selective iNOS inhibitor when compared to L-NAME, which caused a complete inhibition of agonist-induced release of NO.

On the cellular level, myocardial contractility is basically dependent on Ca^{2+} handling in cardiac myocytes and myofilament sensitivity to Ca^{2+}. It has been shown that NO inhibits the β-adrenergic agent-stimulated L-type Ca^{2+} channel current (I_{Ca}) in ventricular myocytes (Mery et al., 1993; Wahler and Dollinger, 1995). A decrease in I_{Ca} in ventricular myocytes from endotoxemic guinea pigs has also been reported (Zhong et al., 1997). Accordingly, we hypothesized that an alteration in the intracellular Ca^{2+} homeostasis associated with NO might contribute to the depressed cardiac contractile dysfunction in septic shock. To address this hypothesis, we observed the Ca^{2+} transients in response to electrical field-stimulation and β-adrenergic stimulation in single ventricular myocytes isolated from lipopolysaccharide-treated rats. Our data showed that both Ca^{2+} transients induced by electrical field-stimulation and the intracellular Ca^{2+} response to β-adrenergic stimulation with isoproterenol were significantly decreased in these cells. The intracellular Ca^{2+} hyporesponsiveness to β-adrenergic stimulation was reversed by the NOS inhibitors, L-NAME and aminoguanidine, and the NO-sensitive guanylyl cyclase inhibitor, ODQ. These results suggest that NO produced in myocytes isolated from lipopolysaccharide-treated rats may be involved, through a cGMP-dependent pathway, in the intracellular Ca^{2+} hyporesponsiveness to β-adrenergic stimulation.

However, NOS inhibition failed to affect the decreased basal Ca^{2+} transients in the myocytes from lipopolysaccharide-treated rats in the absence of isoproterenol in the present study, which suggested that NO was not involved in this phenomenon or that it had induced long lasting or irreversible changes within the cells prior to their isolation. To differentiate these possibilities, we further assessed whether the NOS inhibitor-insensitive effect of lipopolysaccharide treatment on ventricular Ca^{2+} signals induced by electrical-field stimulation persist when rats are pretreated with aminoguanidine before lipopolysaccharide injection. Our results showed that in these cells, the intracellular Ca^{2+} hyporesponsiveness to β-adrenergic stimulation was abolished. However, there still existed a depressed response to electrical field stimulation. This confirms that the overproduction of NO by ventricular myocytes is not responsible for the reduced Ca^{2+} response to electrical field stimulation in lipopolysaccharide-treated rats and indicates that myocardial depression in septic shock cannot be explained solely by the NO-cGMP-mediated signaling pathway. This observation contradicts earlier reports that NO donors and authentic NO attenuate contractility (Brady et al., 1993) and NOS inhibitors reverse the depressed basal contractile function of myocytes isolated from lipopolysaccharide-treated guinea pigs (Brady et al., 1992). Differences in species and experimental conditions could be reasons for the differences in our results from the previous studies. However, our present data are consistent with findings reported by several groups (Toth and Heard, 1997; Klabunde and Coston, 1995). Klabunde and Coston (1995) found that administration of NOS inhibitors (N^G-nitro-L-arginine and aminoguanidine) prior to endotoxin did not prevent lipopolysaccharide-induced myocardial depression. These results suggest that cardiac depression during endotoxemia is not caused by NOS activation and increased NO production. Indeed, it has been reported that lipopolysaccharide can also activate NO-independent soluble guanylate cyclase (Wu et al., 1998) and other enzyme systems in addition to iNOS, such as cyclooxygenase-2 (Wu, 1995) and haem oxygenase-1 (Yet et al., 1997). In addition, since Lefer and Martin (1970) provided evidence of the existence of a circulating myocardial depressant factor in 1970, a variety of substances with negative inotropic effects have been described. But their chemical structures and exact mechanisms of action are still unknown. The effect of the cadiodepressant factor seems to be due to the blockage of the Ca^{2+} current, which is found in both adult and neonatal cardiac myocytes (Hallstrom et al., 1991). Recent find-

ings provided evidence that adrenomedullin might also play a major role in the lipopolysaccharide-induced decrease in blood pressure (Mazzocchi et al., 2000). Taken together, it is most likely that a series of factors, including NO, with different mechanisms contribute to the cardiodepression in septic shock.

In summary, the present data suggest that the production of NO is involved in the vascular hyporeactivity to vasoconstrictors and the depressed intracellular Ca^{2+} response to β-adrenergic stimulation in cardiac myocytes from lipopolysaccharide-treated rats, but is not responsible for the reduced Ca^{2+} response to electrical field stimulation in our experimental conditions. Our study supports previous reports indicating that NO is not the only factor responsible for septic shock. However, the other factors involved and the cellular mechanisms remain to be identified.

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