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Short communication

Attenuation of endothelium-dependent hyperpolarizing factor by bacterial lipopolysaccharides

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Abstract

Endothelium-dependent hyperpolarizing factor (EDHF) is an important contributor to agonist-induced vascular dilation. Recent studies suggest that bacterial lipopolysaccharides attenuate endothelium-dependent dilation. Whether or not this effect is mediated through inhibition of EDHF is not known. We studied the in vitro influence of *Escherichia coli* lipopolysaccharides on endothelium-dependent smooth muscle dilation and hyperpolarization in porcine coronary arteries. Endothelium-intact porcine coronary arterial rings were examined after 20 h of incubation with either saline or *E. coli* lipopolysaccharides (100 μg/ml). Endothelium-dependent dilation elicited by increasing concentrations of bradykinin was significantly attenuated by lipopolysaccharides. Baseline values of smooth muscle membrane potential were not influenced by lipopolysaccharides. However, lipopolysaccharides significantly attenuated bradykinin-induced smooth muscle membrane hyperpolarization. Our results suggest that attenuation of EDHF is an important mechanism through which lipopolysaccharides influence vascular dilation in severe sepsis.

Keywords: Endotoxin; EDHF (endothelium-dependent hyperpolarizing factor); Myocardium; Nitric oxide (NO)

1. Introduction

It is well established that the endothelium, by releasing numerous autacoids actively participates in the regulation of vascular tone. One of these recently characterised autacoids is nitric oxide (NO) which is synthesized by a group of hemoproteins known as nitric oxide synthases (Knowles and Moncada, 1994). Several agonists such as acetylcholine and bradykinin induce vascular dilation by activating the endothelial isoform of nitric oxide synthase and thus increase the release of NO which, in turn, acts on smooth muscle cells, leading to elevation of smooth muscle cGMP concentration and relaxation (Knowles and Moncada, 1994).

Many investigators have reported that, in addition to the release of NO, agonist-induced dilation is mediated through the release of endothelium-dependent hyperpolarizing factor (EDHF) which elicits smooth muscle relaxation by activating membrane K^+ channels which, in turn, results in hyperpolarization of smooth muscle membrane potential (Komori and Vanhoutte, 1990). More recently, EDHF has been identified to be a P450-dependent epoxyeicosatrienoic acid metabolite (Campbell et al., 1996). The contribution of EDHF to endothelium-dependent dilation varies considerably, depending on the species, vessel and agonist. It is estimated that EDHF is responsible for up to 40% of endothelium-dependent relaxation in rat vessels (Chen and Suzuki, 1989).

Depressed cardiac contractility as a result of maldistribution of myocardial blood flow is believed to be an important cause of high mortality in patients in septic shock (Goldfarb et al., 1986; Cunnion et al., 1986). Maldistribution of myocardial blood flow in septic shock has been blamed on attenuation of endothelium-dependent dilatory mechanisms through direct and/or indirect effects of lipopolysaccharides on endothelial cells (Parker and Adams, 1993; Parker et al., 1991). The exact mechanisms through which lipopolysaccharides attenuate endothelium-dependent dilation, however, remain unidentified. One possible mechanism is the down-regulation of mRNA expression of endothelial nitric oxide synthase isoform (MacNaul and Hutchinson, 1993). Another likely mechanism is

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the attenuation of EDHF. However, the effect of lipopolysaccharides on the EDHF pathway has not been investigated. The aim of this study was, therefore, to assess whether or not the EDHF dilatory pathway in coronary arteries is influenced by lipopolysaccharide exposure. We used bradykinin which is a well characterized stimulator of EDHF release in porcine coronary arteries.

2. Material and methods

2.1. Surgical procedure

Fresh porcine hearts were obtained from a nearby slaughterhouse immediately after the animals were killed. They were placed in cold physiological salt solution (Krebs-Ringer buffer, see below) previously bubbled with a 95% O_2/5% CO_2 mixture and transported to the laboratory.

2.2. Coronary artery ring contractility

Left anterior descending and circumflex arteries were dissected and cleaned of adherent connective tissue. They were cut into rings (4 mm in length) which were suspended in 25 μl organ baths filled with warmed modified Krebs-Ringer bicarbonate solution (composition in mM: 118.3 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 11.1 glucose and 0.01 indomethacin). The rings were suspended by means of two stainless steel stirrups. One of the stirrups was anchored at the bottom of the organ chamber and the other was connected to a force transducer (Grass Model FT03) to record changes in isometric force. The rings were stretched over 60 min to a baseline tension of 6 g which produces maximum active tension in response to 120 mM KCl stimulation. Vessel length was then measured with a micrometer. After 60 min of equilibration, the rings were contracted with prostaglandin $F_{2\alpha}$ (5×10^{-6} M) to elicit tension equivalent to 60–80% of maximum. Bradykinin concentration-dilation relationships were then constructed by adding stock solutions of bradykinin directly to the organ bath. We examined three groups of vessels. Group 1 vessels (control, $n = 8$ obtained from 6 different pigs) were incubated over a 20 h period in dishes containing minimal essential medium (MEM) and were maintained at 37°C in a cell culture incubator at a final CO_2 concentration of 5%. Group 2 (control + L-NAME, $n = 7$ obtained from 5 different pigs) vessels were treated as the control group except that N^G-nitro-L-arginine methyl ester (L-NAME, selective inhibitor of nitric oxide synthases) was added into the organ bath 15 min before the dilatory response to bradykinin was assessed. The final concentration of N^G-nitro-L-arginine methyl ester in the organ bath was 0.1 mM. Group 3 vessels (lipopolysaccharides, $n = 10$ obtained from 7 different pigs) were incubated with MEM containing 100 μg/ml *Escherichia coli* endotoxin (Sigma Serotype 055:b5). At the end of the experiments, the vessels were blotted dry and then desiccated overnight at 50°C. Tension was expressed as g per mg dry weight.

2.3. Smooth muscle membrane potential measurement

Porcine corollary arteries were dissected as above and cut into 1 cm vessel segments. They were examined after 20 h of incubation with MEM (control) or MEM containing 100 μg/ml of *E. coli* endotoxin (lipopolysaccharides). At the end of the incubation period, the arterial rings were cut transversely and laid flat in a glass well containing Krebs-Ringer bicarbonate buffer with the endothelial surface facing upward. In order to control for the effect of stretching on membrane potential, vessel length was adjusted to that measured at a baseline tension of 6 g (see above). Smooth muscle cells were impaled through the endothelial cell layer with microelectrodes (WPI) ranging from 40 to 80 MΩ of resistance. Membrane voltage was amplified with a Grass P-16 microelectrode amplifier. Data were acquired and recorded with a data acquisition system (DataQ Instruments, Akron, OH, USA). Resting membrane potentials were recorded after impalement. For each impaled cell, bradykinin (for a final concentration of 2×10^{-7} M) was then added to the bath and smooth muscle membrane hyperpolarization was recorded. The above protocol was carried out in a limited number of impalements in the presence of 0.1 mM N^G-nitro-L-arginine methyl ester. The data were digitized, analyzed with DataQ software and expressed as absolute change in membrane potential in response to bradykinin. Absolute changes in membrane potential were measured from baseline to peak negative deflection.

2.4. Data analysis

The data are shown as means ± S.E.M. The control and lipopolysaccharide groups were compared in terms of force or changes in membrane potential by two-way analysis of variance and the Student *t*-test. *P* values less than 0.05 were considered significant.

3. Results

3.1. Bradykinin-induced dilation

The response of control coronary arteries to 120 mM KCl and 5×10^{-6} M prostaglandin $F_{2\alpha}$ was 1.86 ± 0.24 and 1.17 ± 0.26 g/mg dry weight, respectively. Incubation with *E. coli* lipopolysaccharides did not influence coronary arterial response to 120 mM KCl (1.75 ± 0.25 g/mg) and 5×10^{-6} M prostaglandin $F_{2\alpha}$ (0.97 ± 0.18 g/mg). Fig. 1A illustrates the dilatory effects of bradykinin on prostaglandin $F_{2\alpha}$-preconstricted vessels in the control,

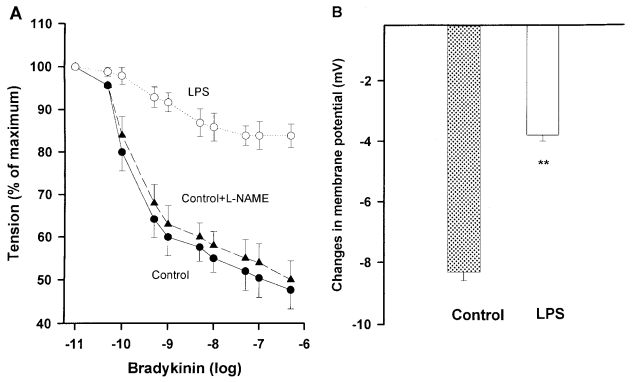

Fig. 1. (A) Dilatory response to increasing concentrations of bradykinin in preconstricted porcine coronary arteries of control and lipopolysaccharide (LPS) groups. Note the significant attenuation of bradykinin-induced dilation in the lipopolysaccharide group. (B) Mean values of smooth muscle membrane hyperpolarization in response to 2×10^{-7} M bradykinin in control and lipopolysaccharide (LPS) vessels. ** $P<0.01$ compared with control vessels.

control-L-NAME and lipopolysaccharide groups. Bradykinin induced progressive dilation of control coronary arteries at concentrations exceeding 5×10^{-11} M. Preincubation with 0.1 mM of N^{G}-nitro-L-arginine methyl ester had no significant effect on the dilatory response to bradykinin. *E. coli* lipopolysaccharides resulted in significant attenuation of bradykinin-induced dilation at concentrations greater than 10^{-11} M (Fig. 1A).

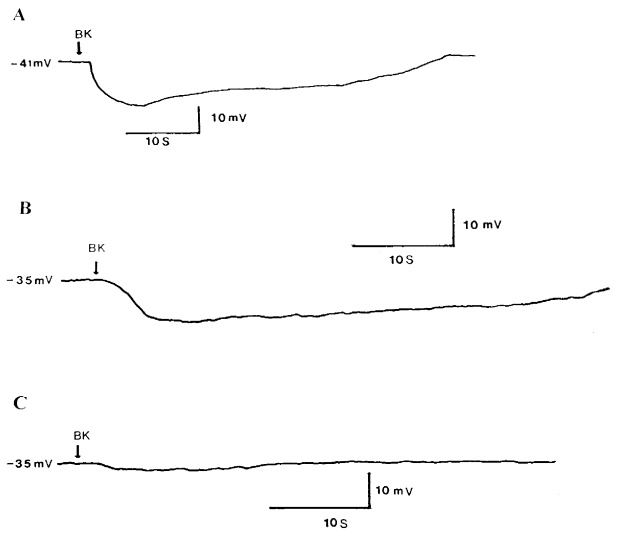

Fig. 2. (A) Representative tracing of smooth muscle membrane potential in response to bradykinin (BK) in a control porcine coronary artery. The coronary artery was incubated for 20 h in MEM and then exposed to bradykinin. (B) Influence of N^{G}-nitro-L-arginine methyl ester (L-NAME) on bradykinin-induced membrane hyperpolarization in a control coronary artery. The artery was preincubated with 0.1 mM of L-NAME and then exposed to bradykinin (2×10^{-7} M). Notice that bradykinin-induced membrane hyperpolarization was not influenced by L-NAME. (C) Influence of lipopolysaccharides on bradykinin-induced membrane hyperpolarization in porcine coronary arteries. Note that bradykinin failed to induce smooth muscle membrane hyperpolarization in a vessel preincubated for 20 h with MEM containing 100 μg/ml of *E. coli* lipopolysaccharides.

3.2. Changes in membrane hyperpolarization

Fig. 2A depicts a typical endothelium-dependent hyperpolarization in response to bradykinin (2×10^{-7} M). Resting vascular smooth muscle membrane potentials in control vessels were typically between −40 and −50 mV (the mean value of 18 measurements, obtained from 6 different pigs, was −44.8 mV). Bradykinin elicited transient membrane hyperpolarization in control vessels which lasted approximately 60 s (Fig. 2A). The reduction of membrane potential by bradykinin in the control vessels averaged −8.4 mV ($n=10$ cells from 5 different pigs). Bradykinin-induced membrane hyperpolarization was resistant to L-NAME ($n=5$, Fig. 2B). Incubation with lipopolysaccharides did not alter the resting membrane potential of smooth muscle cells (mean value of 18 measurements, obtained from 6 different pigs, was −45.8 mV) but resulted in significant attenuation of bradykinin-induced hyperpolarization (Fig. 2C). The absolute change in membrane potential when bradykinin was applied to lipopolysaccharides-incubated vessel segments was −3.8 mV ($n=10$ cells obtained from 5 pigs, $P<0.01$ compared with control vessels, Fig. 1B).

4. Discussion

The main findings of this study are the following: (1) bradykinin-induced dilation in coronary arteries was significantly attenuated by lipopolysaccharide exposure; (2) lipopolysaccharides had no significant effect on baseline vascular smooth muscle membrane potential; and (3) bradykinin-induced membrane hyperpolarization was significantly attenuated by lipopolysaccharides.

4.1. The nature of EDHF

Endothelium-dependent vascular smooth muscle hyperpolarization has been described previously in response to a variety of vasoactive agonists such as acetylcholine and bradykinin (Cohen and Vanhoutte, 1995). The nature of this hyperpolarization is still under investigation since neither NO nor products of cyclo-oxygenase pathways fully explain the phenomenon (Cohen and Vanhoutte, 1995). There is recent evidence indicating that EDHF represents a family of epoxyeicosatrienoic acid metabolites (Campbell et al., 1996). In bovine coronary arteries, release of epoxyeicosatrienoic acid metabolites from the endothelium is stimulated by methacholine, and these products cause smooth muscle hyperpolarization and relaxation by activating Ca^{2+}-activated K^{+} channels (Campbell et al., 1996). Epoxyeicosatrienoic acid metabolite released from the vascular endothelium is inhibited by blockers of cytochrome P450 mono-oxygenase (Campbell et al., 1996). Despite progress made in elucidating the nature of EDHF,

the degree to which it contributes to the regulation of vascular tone remains unclear. In rat, porcine, and guinea-pig coronary arteries, EDHF accounts for a large proportion of maximal relaxation in response to acetylcholine or bradykinin (Parkington et al., 1995; Myers et al., 1992; Chen and Suzuki, 1989). Estimates of as much as 40% of endothelium-dependent dilation have been made in rat vessels (Chen and Suzuki, 1989).

Previous studies indicate that lipopolysaccharide significantly influences the endothelium-dependent dilator response to agonists such as acetylcholine and bradykinin (Wylam et al., 1990; Baxter, 1995). These compounds are known to activate the endothelial isoform of nitric oxide synthase and lead to enhanced NO release from the endothelium. In addition to releasing NO, endothelium-dependent dilators have also been implicated in activating the release of EDHF in a variety of blood vessels. Our findings suggest that attenuation of endothelium-dependent dilation by lipopolysaccharides may be mediated through inhibition of EDHF, but the degree to which EDHF attenuation contributes to impairment of bradykinin-induced dilation after lipopolysaccharide exposure remains unclear because we did not measure arterial tension and smooth muscle membrane potential simultaneously. We speculate that several mechanisms may be involved in reducing the contribution of EDHF following exposure to lipopolysaccharides. One possible mechanism may be derangement of bradykinin receptors located on the endothelial surface. For instance, Spitzer et al. (1989) reported that prolonged exposure to lipopolysaccharides leads to a decrease in the number of endothelial bradykinin receptors. It is also possible that lipopolysaccharide influences the synthesis and release of EDHF in endothelial cells. This may occur via a decrease in the availability of arachidonic acid, endogenous inhibition of cytochrome P450 monooxygenase and/or a decline in the enzyme level because of down-regulation of transcription, translation or increased protein degradation. These possibilities remain speculative since little is known about the influence of lipopolysaccharides on gene expression and regulation of endothelial cytochrome P450 monooxygenase. The possible influence of lipopolysaccharides on this enzyme may be mediated through a direct effect of lipopolysaccharides or through the release of proinflammatory cytokines such as tumor necrosis factor-α, interleukins or interferon-γ. These cytokines are known to directly impair bradykinin-induced dilation in various blood vessels (Greenberg et al., 1993). Finally, it is possible that lipopolysaccharides may not influence the release of EDHF from endothelial cells but may impair smooth muscle responsiveness to EDHF. This would be achieved through alteration in the kinetics of Ca^{2+}-activated K^+ channels which are involved in mediating smooth muscle hyperpolarization in response to EDHF. The fact that baseline membrane potential of smooth muscle cells was not influenced by lipopolysaccharide does not rule out this possibility.

Septic shock, which is associated with elevated endotoxin concentrations, represents a major cause of morbidity and mortality in intensive care units. An important characteristic of septic shock is depressed cardiac function which has been attributed to maldistribution of the coronary blood flow and impaired microvascular regulation (Goldfarb et al., 1986; Cunnion et al., 1986). Our results suggest that in addition to the inhibition of NO release (Kuo et al., 1992), septic shock may also impair coronary blood flow by attenuating the release of EDHF.

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