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Interception of the endotoxin-induced arterial hyporeactivity to vasoconstrictors

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ABSTRACT

Septic shock is a severe pathophysiologic condition characterized by vasodilation, hypotension, hypoperfusion, tissue hypoxia, multiple organ failure and death. It is unclear what causes the septic vasodilation that may result from general dysfunction of vascular smooth muscles (VSMs) or selective disruption of vasomotor balances in VSMs. The latter could be due to enhanced vasorelaxation and/or depressed vasoconstriction. Understanding these may lead to pharmacological interventions to septic vasodilation. Therefore, we performed studies in isolated and perfused mesenteric arterial rings. A 20-h exposure of the rings to lipopolysaccharide (LPS, 1 μg/ml) led to hyporeactivity to phenylephrine (PE). However, the responses of the LPS-treated rings to high concentrations of KCl (60 mM) and ATP remained comparable to control rings, suggesting that contractility of VSMs is retained. The hyporeactivity was marginally affected by atropine, indomethacin and L-NAME, suggesting that endothelium-dependent vasorelaxation does not play a major role. In addition to PE, the LPS-treated rings were hyporeactive to dopamine, histamine and angiotensin II. They showed intermediate hyporeactivity to the thromboxane-A2 receptor agonist U46619. Little hyporeactivity to endothelin-1 (ET-1), serotonin (5-HT) and vasopressin was found. ET-1-induced vasoconstriction occurred without endothelium, whereas the effect of 5-HT was endothelium dependent. Although rings were hyporeactive to some of the vasopressors, their vasoconstriction effects were significantly potentiated by PE co-application. Taken together, these data suggest that the endotoxin-induced vasodilation may not result from general dysfunction of VSMs, neither from the endothelium-dependent vasorelaxation. The promising vascular response to various vasoconstrictors found in this study warrants further investigations of therapeutic potentials of these agents.

1. Introduction

Approximately, 200,000 people die of severe sepsis or septic shock in the United States each year [2,24]. Septic shock is characterized by excessive vasodilation and vascular hyporeactivity to vasoconstrictors, especially α-adrenergic receptor agonists. The vascular response handicaps therapeutic interventions to vascular tones, leading to uncontrolled hypotension, hypoperfusion, tissue hypoxia, multiple organ failure and death [10,43]. Even the application of antibiotics is hindered by the cascade of events, as the bacterial cell lysis induced by certain antibiotics leads to a release of more septic pathogens such as lipopolysaccharide (LPS, from Gem-negative bacteria) to the blood stream [21]. A treatment with LPS, also known as endotoxins, can produce most the features of the cascade of events in sepsis, including the vascular hyporeactivity to α-adrenergic activators [26,32].

Interruption of the cascade of events in sepsis and endotoxemia relies on restoration or management of vascular responsiveness to vasopressors, while it is unclear how LPS causes the vascular hyporeactivity to vasoconstrictors. Such a vascular response may result from injuries to vascular smooth muscle (VSM) cells or the contractile machineries in the cells leading to dysfunction of VSM contractility; LPS may activate extensively the vasodilatory mechanisms disrupting the balance of vasoconstriction versus vasodilation; alternatively, the regulation of the VSM by certain vasoconstrictors may be impaired.

The understanding of how these potential cellular mechanisms contribute to the vascular hyporeactivity in endotoxemia may lead to novel therapeutic interventions to septic shock. With the belief, we performed these studies in isolated and perfused mesenteric arterial rings. Our results suggest that the vascular hyporeactivity does not seem to result from general injuries to VSM contractility, neither from the enhanced endothelium-dependent vasorelaxation. Remarkably, we found that the regulation of the VSM by certain vasopressors appeared to be disrupted. The disruption of vasoconstriction was selective for several vasoconstrictors. Surprisingly, none of the vasopressors recommended in the current guidelines for management of severe sepsis and septic shock produced effective vasoconstriction in our endotoxin model,

Abbreviations: VSM, vascular smooth muscles; ED, endothelium-denuded; LPS, lipopolysaccharide; PE, phenylephrine.

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while none of the three promising vasopressors, endothelin-1 (ET-1), serotonin (5-HT), and thromboxane A2 receptor agonist) found in our study has been considered for the treatment of septic shock.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents used in this study were purchased from Sigma (Sigma-Aldrich Co. St. Louis, MO) except those described specially. The chemicals were prepared as concentrated stocks in double-distilled water (ddH_2O) or dimethyl sulfoxide (DMSO), and diluted to experimental concentrations immediately before application. The final concentration of DMSO was less than 0.1% known not to affect ring contractility as well as responses to PE.

2.2. Rat mesenteric artery ring preparation and tension measurement

All animal experiments were performed in compliance with approved protocol by the Institutional Animal Care and Use Committees (IACUC) at Georgia State University. Male Sprague–Dawley rats (300–400 g in body weight) were anesthetized by inhalation of saturated isoflurane. Under sterilized condition, mesenteric arteries were dissected free after connective tissues were removed mostly in ice-cold phosphate buffer solution (PBS) (containing in mM: 137.0 NaCl, 2.7 KCl, 10 Na_2HPO_4, 2 KH_2PO_4). The secondary and tertiary mesenteric arteries were cut into 6–8 rings of 3–4 mm in length. The rings were then transferred into the Dulbecco's Modified Eagle Medium (DMEM, Cellgro, Manassas, VA) with 4.5 g/L glucose, 10% fetal bovine serum (FBS) and 2% penicillin–streptomycin (Cellgro, Herndon, VA). The rings were cultured in the DMEM for 20 h in a CO_2 incubator at 37 °C with 5% CO_2. Before culture, 1 μg/ml LPS (*Escherichia coli* 0127:B8) was added into one Petri dish with 3 rings, and another 3 rings in a separate Petri dish without LPS served as sham control. ED rings were also used in which the endothelium was removed by rolling the rings on tweezers for 4–5 times back and forth. The success of endothelium denudation was accepted if the ring lost >80% relaxation to acetylcholine (10^{-6} M) after PE (10^{-4} M) pre-contraction.

The cultured mesenteric artery rings were mounted on a 6-channel force-electricity transducer (i-DAQ-4 Data Acquisition System, Globaltown Microtech, Sarasota, FL) in 3 ml 37 °C water organ bath perfused with Krebs solution (containing in mM: 118.0 NaCl, 25.0 $NaHCO_3$, 3.6 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 11.0 glucose, and 2.5 $CaCl_2$), gassed with 95% O_2 and 5% CO_2. Before the experiment, the rings were given a 0.4 g pre-load and equilibrated for 30 min. The vitality of control rings and septic model rings was confirmed with the vasoconstriction to phenylephrine (PE) dose from 10^{-9} to 10^{-4} M. The hyporeactivity to PE was accepted when the rings lost 80% contractility after LPS treatment in comparison to the sham control. Otherwise, the preparation was considered as a failure and rejected from further studies. Another criterion of variability of the ring preparation was the response to high dose KCl (60 mM) at the end of each experiment, indicating the intact of contractile machinery of VSMs in both control and LPS-treated groups.

2.3. Data analysis

The relationship of contractile force with agent concentration was described using the Hill equation: $f = F_{Max}/[1 + (EC_{50}/x)^h]$, where f is contractile force, F_{Max} is maximum contractile force, x is agent concentration, and h is the Hill coefficient. Data are presented as the means ± S.E. Differences were evaluated using Student t-tests for a pair data and ANOVA for three groups or more. Statistical significance was accepted if $P < 0.05$.

3. Results

3.1. Hyporeactivity of mesenteric arterial rings to phenylephrine (PE) with LPS treatment

LPS is known to cause systemic hypotension and the vascular hyporeactivity to circulating vasoconstrictors, both of which are characteristic symptoms of septic shock. Therefore, endotoxins are ideal for the study of the vascular hyporeactivity although it may not produce all clinical manifestations of sepsis [9]. To achieve reliable vascular hyporeactivity and to demonstrate unambiguously its reversibility, we chose to use a high concentration of LPS (1 μg/ml) as described in our previous studies [37]. An over-night exposure of the isolated mesenteric rings to LPS caused clear hyporeactivity to PE (Fig. 1). In response to a graded increase in PE concentrations, the endothelium-intact (EI) rings cultured without LPS showed a concentration-dependent increase in contractility. An obvious increase in the contractile force was seen with 10^{-7} M PE, and the maximum vasoconstriction was reached at 10^{-5} M. In contrast, rings treated with LPS for the same period as for the sham control failed to respond to PE at 10^{-7} M, and 10^{-5} M PE produced only a transient vasoconstriction with the much smaller amplitude than that of the sham control rings (Fig. 1A). When the contractile force was plotted against PE concentration, the concentration dependence was observed in the control rings, which was described by the Hill equation with EC_{50} 7×10^{-7} M ($h = 1.1$). The force–concentration relationship curve shifted to the right in rings

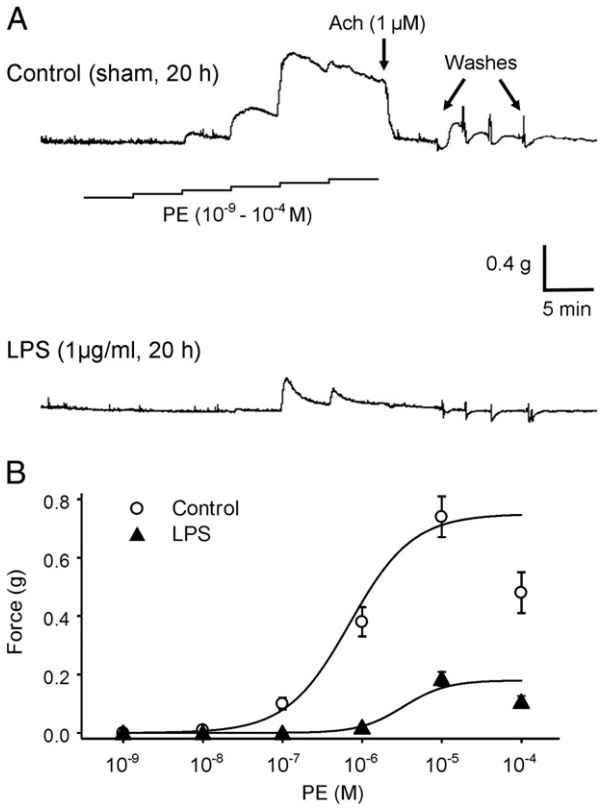

Fig. 1. The hyporeactivity of mesenteric rings to α-adrenergic agonist in endotoxin exposure. A. Concentration-dependent vasoconstrictions are clearly seen in the control ring (upper) with PE (10^{-9} to 10^{-4} M). The same PE exposure had rather weak effect on the mesenteric ring treated with LPS (1 μg/ml) overnight (lower). Both control and LPS-treated rings in each set comparative experiment, cultured in the same condition for 20 h except LPS, and treated in the same way during the experiment. Lower trace is sharing the same label with the upper trace. B. The contractility of the rings is a function of PE concentrations. The relationship of the contractile force vs. PE concentration can be described by the Hill equation with EC_{50} 7×10^{-7} M and h 1.1 for the control group ($n = 12$), and 3.3×10^{-6} M and h 1.6 for LPS treated rings ($n = 12$), respectively. Data are presented as means ± S.E.