Alterations of Coronary Perfusion Pressure and Cardiac Contraction during Lipopolysaccharide Challenge

J. Tu[1], Q-X. Shan[1], H-F. Jin[1], I.C. Bruce[2], Q. Xia[1]

[1]Department of Physiology, Zhejiang University School of Medicine, Hangzhou; [2]Department of Physiology, The University of Hong Kong, Hong Kong, China

E-mail: xiaqiang@zju.edu.cn

Abstract—In the present study, we used the Langendorff technique to evaluate the involvement of endothelin-1 (ET-1) and nitric oxide (NO) in coronary vasoconstriction and myocardial depression in hearts isolated from lipopolysaccharide (LPS)-treated rats. Coronary perfusion pressure (CPP) increased markedly in hearts from LPS-treated rats. Pretreatment with BQ-123, an ET-1 type A receptor antagonist, significantly reduced the increase in CPP induced by LPS. LPS induced a marked decrease in left ventricular developed pressure, the product of left ventricular developed pressure and heart rate, as well as the maximal rate of rise/fall of left ventricular pressure. Pretreatment with BQ-123 partially reversed the LPS-induced cardiac depression. Administration of BQ-123 and AMG, an inhibitor of iNOS, prior to LPS challenge significantly blocked the negative inotropic effect. These results suggest that ET-1 augments the NO-mediated cardiac contractile depression induced by LPS and the accompanying increase in coronary resistance.

Keywords—Coronary perfusion pressure, contraction, LPS, nitric oxide, endothelin

I. INTRODUCTION

The isolated heart has been used widely as a model to explore the actions of drugs that affect cardiovascular efficiency. The Langendorff technique is a classical means of measuring the contractile status of the myocardium. Coronary perfusion pressure in the Langendorff system is an important parameter that reflects the effects drugs on coronary artery resistance.

Cardiovascular dysfunction induced by lipopolysaccharide (LPS) has long been of great concern. LPS, an integral part of the outer layer of the gram-negative bacterial cell wall, stimulates a systemic inflammatory process and the production of proinflammatory cytokines and vasoactive mediators [1,2]. Endothelin-1 (ET-1) and nitric oxide (NO) are known to markedly increase during LPS challenge [3]. But no direct experimental evidence from the isolated heart (i.e., in the absence of neural and humoral factors) has clarified the interaction between ET-1 and NO in the coronary arterial and cardiac inotropic responses after LPS treatment.

In the present study, we used the Langendorff preparation to investigate the roles of ET-1 and NO in LPS-induced coronary vasoconstriction and cardiac depression.

II. METHODOLOGY

(1) Preparation of isolated rat hearts

All experiments were carried out on male Sprague-Dawley rats (240-300g). Immediately after decapitation, the heart was rapidly excised and rinsed in 4°C Krebs-Henseleit buffer (composition in mM: NaCl 118, NaHCO$_3$ 25, KCl 4.7, MgSO$_4$ 1.2, CaCl$_2$ 1.4, KH$_2$PO$_4$ 1.1, glucose 11, pH 7.4) maintained at 37°C and gassed with 95% O$_2$/5% CO$_2$. Within 1 min the heart was mounted on the Langendorff apparatus, and perfused at a constant flow (8ml·min^{-1}).

(2) Experimental setup and data processing

A. Experimental setup

The coronary perfusion pressure (CPP) was continuously recorded by a pressure transducer connected to a computer. A fluid-filled latex balloon was introduced through the mitral valve into the left ventricle. The balloon was connected via a short plastic tube to another pressure transducer and computer for measurement of left ventricular developed pressure (LVDP), heart rate (HR), and maximal rate of rise/fall of ventricular pressure (+/-dP/dtmax).

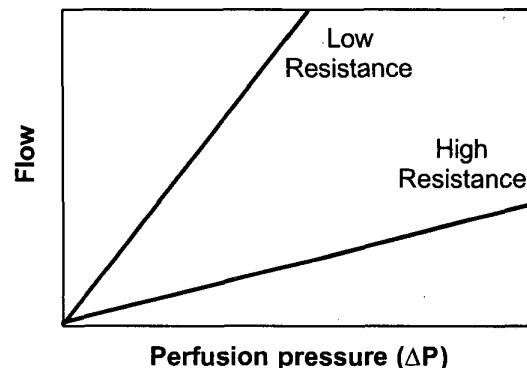*B. The principle of coronary perfusion pressure measurement*

Fig 1. Relationships between pressure, flow and vascular resistance

In the present study the heart was perfused at a constant flow of 8ml min^{-1} and the CPP in the perfusion tubing was continuously recorded. Under the constant flow, the pressure reflects the resistance of the coronary artery.

C. Experimental protocols

Rats received a bolus intraperitoneal injection of LPS (10mg kg^{-1}). After 4h, the animals were killed and the hearts

rapidly excised for *ex vivo* perfusion by the Langendorff technique (LPS group).

In experiments examining the effects of endothelin, the selective ET-1 type A receptor antagonist BQ-123 (0.8×10^{-6} mol kg^{-1}) was given 15 min before the LPS injection and 4h later the animals were killed for isolated heart perfusion (BQ-123+LPS group).

To investigate the effects of nitric oxide, aminoguanidine (AMG) (100mg kg^{-1}), an inhibitor of inducible nitric oxide synthase (iNOS), was given immediately before injection of BQ-123. 15 min later LPS was administered. The hearts were removed for isolated perfusion 4h after LPS challenge (AMG+BQ-123+LPS group)

Separate sets of hearts from rats not exposed to LPS served as controls.

D. Data analysis and statistics

Data were expressed as mean±SD. Comparisons between multiple groups were made by one-way ANOVA followed by the Student-Newman-Keuls *post hoc* test. $P < 0.05$ was considered statistically significant.

III. RESULTS

A. Involvement of endothelin-1 in LPS-induced coronary vasoconstriction

Exposure to LPS caused a marked rise in CPP compared with controls (n=8). This response was maintained over a prolonged period (at least 60 min). The vasoconstriction following LPS exposure was abolished by pretreatment with BQ-123 (0.8×10^{-6} mol kg^{-1}, n=7), while BQ-123 had no significant effect in control hearts. In addition, there was no significant change in CPP in the AMG+BQ-123+LPS group *vs* the BQ-123+LPS group (Fig. 2).
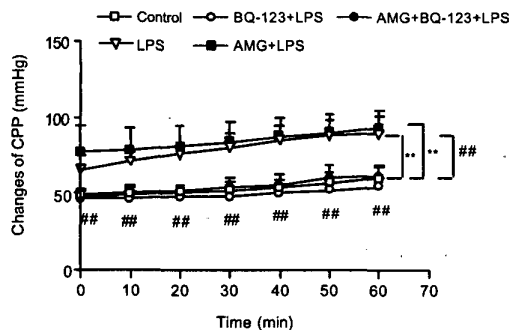
Fig. 2. The effect of BQ-123 with or without AMG on the increase of CPP induced by LPS. Data expressed as mean±SD, n=7-8. **p<0.01, LPS and AMG+LPS groups vs control; ##p<0.01, BQ-123+LPS and AMG+BQ-123+LPS groups vs LPS group

B. Effects of ET-1 and NO on the contraction of isolated rat hearts following LPS challenge

LPS challenge depressed cardiac contractile function. LVDP, the product of LVDP and HR (LVDP×HR) and ±dP/dtmax decreased markedly. Treatment with BQ-123 alone prior to LPS administration partially reversed the inhibitory action of LPS on cardiac contraction. BQ-123 in the presence of AMG (100mg kg^{-1}) significantly blocked the cardiac depression induced by LPS exposure (Fig. 3.).
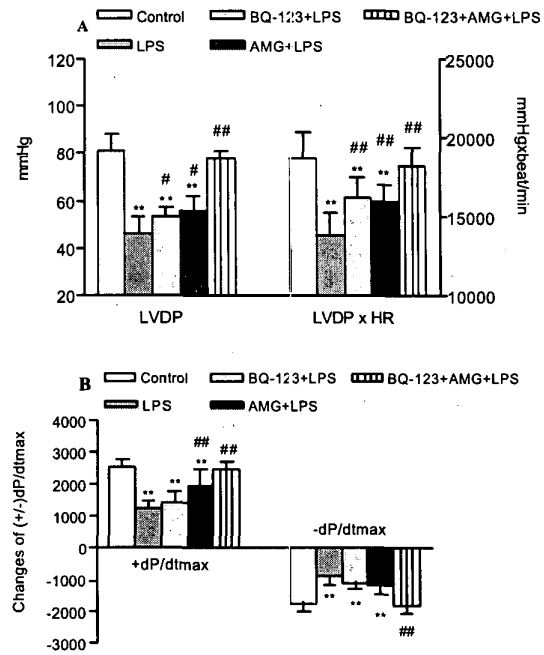
Fig. 3. Effect of BQ-123 with or without AMG on LPS-induced cardiac depression. Data expressed as mean±SD, n=7-8. **p<0.01 vs control; ##p<0.01 vs LPS group

IV. DISCUSSION

The isolated heart is a classical experimental preparation used to define the alterations of inotropic properties under pathological conditions without the complicating influences of autonomic innervation and circulating hormones. In the present study, according to the relationship *Pressure ∝ flow × resistance*, we used isolated rat hearts perfused at a constant flow by the Langendorff technique to determine alterations in the coronary arterial response of hearts that had experienced LPS challenge. We found that administration of LPS caused a sustained increase in coronary perfusion pressure, which was significantly attenuated by administration of the ET-1 type A receptor-selective antagonist BQ-123. It is known that ET-1 is a novel endothelium-derived vasoconstrictive peptide. Bacterial endotoxin and cytokine mediators released in response to endotoxin induce ET-1 gene expression and

stimulate ET-1 protein secretion [4]. In a number of species, LPS increases circulating ET-1 levels 2- to 10-fold in vivo [5]. The actions of ET-1 are mediated by ET_A and ET_B receptors. ET_A is the principal constrictor receptor, whereas ET_B has mixed actions: direct constriction and nitric oxide-dependent vasodilation. ET_A receptors are the predominant receptors in the coronary arteries, accounting for > 85% of all ET receptors [6]. Hori and his co-workers also observed that rat coronary vasculature expresses the ET_A receptor subtype [7]. Thus we used BQ-123, a highly specific ET_A receptor antagonist to probe the effect of ET-1 in rat coronary arteries. It has been demonstrated that ET-1 inhibits cytokine-induced iNOS mRNA expression [8] and dose-dependently inhibits cGMP production by the nitric oxide donor sodium nitroprusside [9]. Together, these lines of evidence indicate that the increase in coronary perfusion pressure induced by LPS exposure is due to a pronounced release of ET-1.

Many studies have pointed out that LPS activates iNOS, which contributes to the myocardial depression accompanying sepsis [10] [11]. Cardiac iNOS expression increases several hours after exposure to LPS, with a peak after 6h [12]. In the present study, these effects were significantly blocked by AMG, indicating that nitric oxide mediated the cardiac depression. The intracellular mechanism involves the stimulation of soluble guanylate cyclase to increase intracellular levels of cGMP with a subsequent reduction in intracellular calcium. Brady and his colleagues showed that the stable cGMP analogue, 8-bromoguanosine 3',5'-cyclic monophosphate, caused a concentration-related reduction in myocyte contractility and this effect was reversed by methylene blue, an inhibitor of guanylate cyclase [13]. These findings further confirm that NO acts by elevating intracellular levels of cGMP.

In the present study, we observed that pretreatment with BQ-123 partially reversed the negative inotropic effect induced by exposure to LPS. We have shown that LPS treatment induces the production of ET-1, leading to a marked and long lasting coronary vasoconstriction. Hence, the coronary resistance increased, which may cause myocardial ischemia and so depress cardiac function. Thus we predict that ameliorating the blood supply of coronary arteries would help to reverse the depression of cardiac contractility induced by LPS. In addition, the NO-mediated vasodilatation is somewhat endothelium-dependent [14], so a pronounced coronary resistance develops following endothelial injury. Also, because ET-1 is able to inhibit iNOS mRNA expression, we confirm that ET-1 causes coronary vasoconstriction as well as augmenting cardiac depression.

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