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Bradykinin induces vascular contraction after hemorrhagic shock in rats

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ARTICLE INFO

Article history:
Received 12 April 2014
Received in revised form
12 June 2014
Accepted 18 June 2014
Available online 24 June 2014

Keywords:
Bradykinin
Shock
Vascular reactivity
Connexin
PKC

ABSTRACT

Background: Bradykinin (BK) has many biological effects in inflammation, allergy, and septic shock. Studies have shown that low doses of BK can induce vascular relaxation and high doses can induce vascular contraction in many pathophysiological conditions, but the role and mechanisms that high doses of BK have on vascular contraction in hemorrhagic shock are not clear.

Methods: With hemorrhagic-shock rats and hypoxia-treated superior mesenteric artery (SMA), we investigated the role and mechanisms of high doses of BK-induced vascular contraction in hemorrhagic shock.

Results: High doses of BK (500–50,000 ng/kg *in vivo* or 10^{-10} to 10^{-5} mol/L *in vitro*) dose dependently induced vascular contraction of SMA and increased the vascular calcium sensitivity in normal and hemorrhagic-shock rats. Less than 10^{-10} mol/L of BK induced vascular dilation BK-induced increase of vascular contractile response and calcium sensitivity was reduced by denudation of the endothelium, 18α-glycyrrhetic acid (an inhibitor of myoendothelial gap junction) and connexin 43 antisense oligodeoxynucleotide. Further studies found that high concentrations of BK-induced vascular contraction in hemorrhagic shock was closely related to the activation of Rho A–Rho kinase pathway and Protein Kinase C (PKC) α and ε.

Conclusions: High doses of BK can induce vascular contraction in hemorrhagic shock condition, which is endothelium and myoendothelial gap junction dependent. Cx43-mediated activation of Rho A–Rho kinase and Protein Kinase C (PKC) pathway plays a very important role in this process. This finding provided a new angle of view to the biological role of BK in other pathophysiological conditions such as hemorrhagic shock or hypoxia.

1. Introduction

Bradykinin (BK) is a member of the kinin family. BK is a vasoactive peptide, which participates in numerous pathophysiological events, and has antihypertensive, antithrombogenic, antiproliferative, and antifibrogenic effects [1]. Studies have shown that BK is a key mediator that participates in vascular leakage, inflammation, and septic shock [1–4]. Arvidsson *et al.* [5] found that inhalation challenge with BK causes bronchial plasma exudation in humans. Several studies have shown that BK, as a vasoactive peptide, has biphasic effects on the vascular contractile function. BK can

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http://dx.doi.org/10.1016/j.jss.2014.06.033

induce vasodilation at lower concentrations ($\leq 10^{-12}$ mol/L) and induce vasoconstriction at higher concentrations ($>10^{-10}$ mol/L) [6,7]. The role and mechanisms that lower concentrations of BK induce vascular dilation is basically clear. Studies have demonstrated that BK-induced vasodilation at lower concentrations is possibly related to nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing factors via its B1 receptors in vascular endothelial cells (VECs) [8,9]. While the role and mechanisms that higher concentrations of BK induce vascular contraction in hemorrhagic shock are not known.

In health, the blood level of BK is relatively low ($0.2–7.1 \times 10^{-12}$ mol/L) [1–4]. Septic shock or endotoxin stimulation can greatly increase the blood level of BK [10,11]. For example, Niles *et al.* [10] found that endotoxin infusion in nonanesthetized monkeys could significantly increase the blood level of BK, and that the concentration of BK in blood could reach 5×10^{-9} to 11×10^{-9} mol/L 15–120 min after endotoxin infusion. However, what effects do high concentrations of BK have on vascular contractile response in hemorrhagic shock and its mechanisms are not known. It is important to elucidate this issue for profoundly understanding the biological effects of BK and its potential application value. Our previous study demonstrated that the myoendothelial gap junction (MEGJ) participates in the regulation of vascular reactivity after shock [12]. Gap junction protein connexin 37 (Cx37) and connexin 43 (Cx43) mediate endothelium-dependent vascular contraction. Whether high concentrations of BK-induced vascular contraction are related to the MEGJ and their mechanisms needs further investigation.

The MEGJ is a type of membrane channel enabling communication between endothelial cells and smooth muscle cells [13,14]. The MEGJ is constructed by two connexins, and is formed by the oligomerization of six connexins [15]. More than 20 isoforms have been identified in mammals [15,16]. Expression of these isoforms is specific to tissues and cells. Basic research has demonstrated that the connexins between vascular smooth muscle cells (VSMCs) and VECs are mainly Cx37, Cx40, Cx43, Cx45, and Cx46 [12,17,18]. Our previous studies demonstrated that the MEGJ takes part in the regulation of the vascular contractile response after shock, and that the main types that participate in the regulation of vascular reactivity are Cx37, Cx40, and Cx43. The mechanism is possibly related to calcium sensitivity regulatory system in VSMCs [12]. Nussberger *et al.* [19] demonstrated that BK is considered to be a key mediator in vascular leakage because it disrupts the interendothelial junction and integrin extracellular matrix complexes. Thus, we hypothesized that the MEGJ and its connexins might participate in the regulation of high concentrations of BK-induced vascular contractile response in hemorrhagic shock. The mechanism is possibly related to calcium sensitivity regulatory system in VSMCs.

To test this hypothesis, with hemorrhagic shock rats and superior mesenteric arteries (SMAs) as representative and hypoxia-treated VSMCs, we investigated the role of high concentrations of BK on vascular contraction in hemorrhagic shock and the relationship with MEGJ and its connexins, as well as the role of Rho A-Rho kinase, and Protein Kinase C (PKC) pathway in this process.

2. Materials and methods

The study protocol was approved by the Research Council and Animal Care and Use Committee of the Research Institute of Surgery, Daping Hospital, Third Military Medical University (Chongqing, China). Animal experiments were conducted in accordance with the Laboratory Animal Use and Care Guide issued by the US National Institutes of Health (NIH Publication, eighth Edition, 2011). Efforts were made to minimize animal suffering and to reduce the number of animals used.

2.1. *Animal management and preparation of blood vessels*

2.1.1. *Animal management*

Sprague–Dawley rats (200–250 g) were purchased from the Animal Center of Research Institute of Surgery, the Third Military Medical University and housed under controlled conditions (22°C, 55%–65% humidity and 12 h light–dark cycle) and fed a standard rat pellet diet and water *ad libitum* before the experiment. On the day of experiment, rats were first anesthetized with sodium pentobarbital (30 mg/kg body weight, intraperitoneally). Sodium pentobarbital was then added until the rats had no response to a needle stimulus, but the total amount of sodium pentobarbital was ≤50 mg/kg. The right femoral arteries and veins were catheterized with polyethylene catheters for monitoring the mean arterial pressure (MAP) and administration of heparin. After the completion of catheterization, rats were stabilized for 10 min. Rats were then exsanguinated from the right femoral arterial catheter until the MAP was ≤40 mm Hg, and maintained according to the time of the study design (10 min, 30 min, 1 h, or 2 h).

2.1.2. *Preparation of blood vessels*

SMAs were obtained from normal and shock rats. After removing connective tissue, they were cut into 2–3-mm long arterial rings and prepared as endothelium-intact and denuded arteries for measurement of the contractile response to BK or Ca^{2+} and other experiments. According to the experimental requirement, SMA rings from normal rats received hypoxia treatment or not [20]. Briefly, the hypoxia procedure involved SMA rings being placed into a hypoxia culture compartment, 95% N_2 and 5% CO_2 being bubbled through at 10 L/min for 15 min, and the SMA rings being allowed to equilibrate for 10 min. This procedure was repeated thrice until the O_2 concentration was <0.2%, and then maintained for 120 min.

2.2. *Experimental protocol*

2.2.1. *Effects of high concentrations of BK on the vascular contractile response in hemorrhagic-shock rats and its relationship to the endothelium and MEGJ*

2.2.1.1. *In vitro.* Seventy-two SMA rings from hemorrhagic shock or normal control rats were divided randomly into five groups: normal control and shock groups (MAP at 40 mm Hg for 10 min, 30 min, 1 h, and 2 h). Normal control group and shock 2-h group were further divided into endothelium-intact group, endothelium-denuded group, and groups of effects of the MEGJ inhibitor 18α-glycyrrhetic acid (18α-GA) ($n = 8$/gp).