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Nitric oxide modulation of endothelium-derived hyperpolarizing factor in agonist-induced depressor responses in anesthetized rats

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ABSTRACT

Vasodilators, such as prostacyclin, nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF), released from the vascular endothelium are important in the maintenance of systemic blood pressure. Some studies have shown that NO affects EDHF-induced vasodilator responses in isolated perfused blood vessel segments. However, the effects of NO on EDHF-mediated dilation, and their contribution to systemic blood pressure, have not been clarified. Therefore, in the present study we investigated the mechanisms underlying acetylcholine- and bradykinin-induced depressor responses, as well as the interaction between NO and EDHF, by measuring systemic blood pressure in anesthetized rats. In the presence of indomethacin and N^G-nitro-L-arginine (L-NA; an NO synthase inhibitor), apamin plus charybdotoxin significantly inhibited depressor responses to acetylcholine and bradykinin, whereas glibenclamide, iberiotoxin, quinacrine, catalase, and combination of ouabain plus $BaCl_2$ failed to inhibit EDHF-induced depressor responses. 4-Aminopyridine significantly inhibited depressor responses to acetylcholine, but not to bradykinin. In the presence of indomethacin and L-NA, carbenoxolone, a gap junction inhibitor, significantly inhibited depressor responses to agonists. L-NA alone significantly potentiated agonist-induced depressor responses. In contrast, infusion of sodium nitroprusside, an NO donor, or 8-br-cGMP significantly inhibited depressor responses to agonist. The findings of the present study raise the possibility that agonist-induced depressor responses are elicited by propagation of endothelial hyperpolarization *via* apamin- plus charybdotoxin-sensitive K^+ channels to smooth muscle cells through gap junctions, but not by diffusible substance(s). It is suggested that, in anesthetized rats, the EDHF-induced depressor response is attenuated in the presence of endogenous and exogenous NO via an increment in cGMP.

1. Introduction

Endothelium-dependent relaxations induced by vasodilator substances such as acetylcholine or bradykinin are mediated by prostacyclin (PGI_2), nitric oxide (NO), and endothelium-derived hyperpolarizing factor (EDHF). It has been proposed that the vasodilator action of EDHF, which hyperpolarizes cell membranes and induces smooth muscle relaxation, is mediated via initial activation of small and intermediate conductance Ca^{2+}-activated potassium channels ($K_{Ca}2.3$, $K_{Ca}3.1$) present on the endothelium (Félétou and Vanhoutte, 2009). Variable responses to EDHF have been reported among species and vascular beds, and possible candidates for EDHF include epoxyeicosatrienoic acid (a metabolite of cytochrome P450 epoxygenase; Fisslthaler et al., 1999), endothelium-derived potassium ions (K^+; Edwards et al., 1998), and hydrogen peroxide (H_2O_2; Matoba et al., 2000). It has also been suggested that the EDHF phenomenon is primarily electrical in origin and driven by endothelial hyperpolarization that spreads radially into the vessel wall via myoendothelial gap junctions (Griffith, 2004). EDHF primarily mediates vasodilation in resistance arteries, whereas NO primarily mediates vasodilation in conduit arteries (Shimokawa et al., 1996). The nature of EDHF has often been investigated using isolated arteries and arterioles; however, systematic analyses of EDHF in anesthetized rats are rarely reported because *in vivo* studies of EDHF measure localized blood flow, such as in the rat mesenteric, hindlimb, and sciatic nerve circulation (Parkington et al., 2002; Thomsen et al., 2000).

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Desai et al. (2006) reported that EDHF-induced depressor responses in anesthetized rats were inhibited by combination treatment with apamin plus charybdotoxin, suggesting that EDHF-mediated systemic blood pressure responses are also mediated by $K_{Ca}2.3$ and $K_{Ca}3.1$. However, identification of EDHF in anesthetized rats remains contentious.

Nitric oxide (NO) is a well-known vasodilator substance, but it may also modulate EDHF-type responses. Some investigators have demonstrated that EDHF-type responses are potentiated by inhibition of NO synthase (NOS) and reduced by exogenous NO, 8-br-cGMP, or the overproduction of NO induced by proinflammatory mediators in isolated perfused arterial segments (Kessler et al., 1999; Bauersachs et al., 1996). In addition, Nishikawa et al. (2000) proposed that EDHF served as a back-up vasodilator when NO production is impaired, because a small dose of an NO donor almost completely inhibited EDHF-type responses in the canine coronary microcirculation. Together, the findings of these studies indicate that NO inhibits EDHF-induced dilation in isolated arteries, but the interaction between NO and EDHF in the maintenance of systemic blood pressure has not been clarified.

Thus, the aim of the present study was to investigate the mechanisms underlying EDHF-type depressor responses induced by different agonists in anesthetized rats and to investigate the effect of endogenous and exogenous NO on EDHF-induced responses.

2. Materials and methods

2.1. Animals and experimental design

Male Sprague-Dawley rats (300–400 g; Japan SLC, Shizuoka, Japan) were used in the present study. Rats were housed in a light-controlled room under a 12-h light–dark cycle and were allowed free access to food and water. Experimental protocols and animal care methods were approved by the Animal Experimental Committee at Hyogo University of Health Sciences (Hyogo, Japan).

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the femoral artery and vein were cannulated with a polyethylene catheter. The arterial catheter was connected to a pressure transducer (Deltran II; Utah Medical Products, Midvale, UT, USA) and systemic blood pressure was monitored continuously on a PowerLab (ML870; ADInstruments, Bella Vista NSW, Australia). All drugs were administered through the venous catheter. Acetylcholine and bradykinin were used as agonists in the present study; both cause endothelium-dependent vasodilation. All rats were subsequently given bolus injections of acetylcholine (0.1, 0.3, 1 and 3 μg/kg, 1 ml/kg, at 5-min intervals) and bradykinin (1, 3, 10 and 30 μg/kg, 1 ml/kg, at 5-min intervals). Responses to each dose of acetylcholine and bradykinin were recorded as the maximum fall in mean arterial blood pressure immediately after drug administration. Infusion of drug was performed through contralateral venous catheter. Five different experiments were performed, as detailed below.

2.1.1. Effects of blocking agents on agonist-induced depressor responses in the presence of indomethacin plus N^G-nitro-L-arginine

The effects of K^+ channel inhibitors and putative EDHF inhibitors were examined to identify EDHF. Various EDHF-blocking drugs have been reported by Fujioka et al. (2002). In the present study, we used carbenoxolone, a water-soluble gap junction inhibitor, rather than 18α-glycyrrhetinic acid (another gap junction inhibitor), which is not soluble in water.

Rats were pretreated with indomethacin (10 mg/kg, a cyclooxygenase inhibitor), and N^G-nitro-L-arginine (L-NA; 10 mg/kg, a NOS inhibitor), 30 min before construction of dose–response curves to acetylcholine or bradykinin. Rats were then pretreated for 10 min with blocking agents, 4-aminopyridine (a voltage-dependent K^+ channel inhibitor), glibenclamide (an ATP-sensitive K^+ channel inhibitor), apamin (a $K_{Ca}2.3$ inhibitor), charybdotoxin (a $K_{Ca}3.1$ inhibitor), iberiotoxin (a large conductance Ca^{2+}-activated K^+ channel [$K_{Ca}1.1$] inhibitor), catalase (an H_2O_2-degrading enzyme), quinacrine (a phospholipase A_2 inhibitor), ouabain (an Na^+/K^+-ATPase inhibitor) plus $BaCl_2$ (an inhibitor of inward rectifier K^+ channels) or carbenoxolone, before a second dose–response curve to acetylcholine or bradykinin was obtained.

The doses of the blocking agents indomethacin, L-NA, 4-aminopyridine, glibenclamide, apamin, charybdotoxin, iberiotoxin, and carbenoxolone used in the present study were based on previous reports (Ayajiki et al., 2005; Berg and Koteng, 1997; Edgley et al., 2008; Desai et al., 2006, Gigout et al., 2006; Smits et al., 1997). The doses of catalase (67500–87500 U/kg), quinacrine (0.8–1.0 mg/kg), ouabain (0.39–0.51 mg/kg), and $BaCl_2$ (0.40–0.51 mg/kg) used in the present *in vivo* studies were extrapolated from concentrations of each drug used in *in vitro* studies (1250 U/mL, 30 μM, 10 μM, and 30 μM, respectively; Matoba et al., 2000, Fujioka et al., 2002) taking blood volume (54–70 mL/kg) into account because there are no previous reports of *in vivo* doses. Moreover, for some drugs we used a higher dose than the extrapolated dose to confirm effectiveness within a range that did not elicit death.

2.1.2. Effects of carbenoxolone on sodium nitroprusside- and salbutamol-induced depressor responses in the presence of indomethacin plus L-NA

Non-specific effects of carbenoxolone were evaluated using sodium nitroprusside and salbutamol, which cause endothelium-independent vasodilation. We hypothesized that carbenoxolone treatment would have no effect on sodium nitroprusside- and salbutamol-induced depressor responses.

Rats were pretreated with a combination of indomethacin plus L-NA, followed 30 min later by construction of a dose–response curve to sodium nitroprusside (an exogenous source of NO; 1, 3, 10, 30 μg/kg; injected at 5-min intervals) or the selective β_2-adrenoceptor agonist salbutamol (0.1, 0.3, 1, 3 μg/kg; injected at 5-min intervals). Rats were then treated with carbenoxolone (150 mg/kg) followed, 10 min, by a second dose–response curve to sodium nitroprusside or salbutamol.

2.1.3. Effects of L-NA on agonist-induced depressor responses in the presence of indomethacin

The effects of endogenous NO on EDHF-induced depressor responses were evaluated using L-NA, a NOS inhibitor. We hypothesized that L-NA treatment would potentiate EDHF-induced depressor responses.

Rats were pretreated with indomethacin, followed by construction of dose–response curves to acetylcholine or bradykinin. Then, rats were treated with L-NA or phenylephrine (8–15 μg/kg per min, i.v. infusion to adjust basal mean arterial blood pressure) followed, 30 min later, by a second dose–response curve to acetylcholine or bradykinin.

2.1.4. Effects of sodium nitroprusside or 8-br-cGMP on agonist-induced depressor responses in the presence of indomethacin plus L-NA

The effects of exogenous NO or cGMP on EDHF-induced depressor responses were evaluated using the sodium nitroprusside (NO donor) and 8-br-cGMP (a cell-permeable cGMP analog). We hypothesized that treatment with either sodium nitroprusside or 8-br-cGMP would inhibit EDHF-induced depressor responses.