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Responses to bradykinin are mediated by NO-independent mechanisms in the rat hindlimb vascular bed

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Abstract

The vasodilator response to bradykinin (BK) appears to be mediated by a number of different endothelium-derived relaxing factors (EDRFs). The EDRFs mediating the response depend on the species and vascular bed studied. The mechanism by which BK dilates the hindlimb vascular bed was investigated in the anesthetized rat. BK produced dose-dependent increases hindlimb blood flow. The NOS antagonist L-NAME had little effect on the magnitude of the increase in flow when baseline hemodynamic parameters were corrected by an NO donor infusion. However, the duration of the response was slightly shortened by L-NAME. Charybdotoxin (Chtx) and apamin nearly abolished the L-NAME resistant component of the hindlimb vasodilator response to BK, but did not affect the hindlimb vasodilator response to the sodium nitroprusside (SNP). The cyclooxygenase inhibitor meclofenamate and the K^+-ATP channel blocker U37883A, in the presence of L-NAME, did not alter the vasodilator response to BK. These results suggest that a significant portion of the hindlimb vasodilator response to BK is mediated by the activation of K_{Ca} channels, and independent of NO synthesis, cyclooxygenase products, and activation of K^+-ATP channels. The present data suggest that the mechanisms mediating the vasodilator response to BK in the hindlimb vascular bed of the rat are complex, consisting of a Chtx and apamin sensitive, L-NAME resistant phase and a minor phase mediated by NO. In contrast, NO accounts for about half of the hindlimb vasodilator response to acetylcholine (ACh), with the other half of the response mediated by a Chtx and apamin sensitive mechanism. Additionally, the present results demonstrate that the NO donor infusion technique is able to compensate for the loss of basal NO production following inhibition of NOS, and to restore hemodynamic parameters to pre-L-NAME levels, making it a useful technique for the investigation of the role of NO in mediating vascular responses.

Keywords: Bradykinin; Nitric oxide; Potassium channels

1. Introduction

Bradykinin (BK), a potent vasodilator in vivo, activates vascular endothelial receptors initiating a cascade of events leading to the formation of a relaxing factor that diffuses to underlying vascular smooth muscle [1,2]. The existence of an endothelial-derived relaxing factor (EDRF) was first demonstrated by Furchgott and Zawadzki in 1980 in an aortic smooth muscle ring preparation [1]. Traditionally, it has been thought that EDRF is nitric oxide (NO) or an NO derivative [3–8]. NO, formed by nitric oxide synthase (NOS) in the vascular endothelium diffuses to smooth muscle and interacts with the heme moiety of soluble guanylate cyclase, increasing cGMP formation, leading to smooth muscle relaxation [9–12].

Evidence in the literature suggests, however, that NO may only be one of several molecules mediating the vasodilator response to BK. In human microcoronary arteries and

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small subcutaneous arteries, a significant component of the vasodilator response to BK was found to persist following inhibition of NOS [13,14]. Additionally, in porcine pial arteries, porcine pulmonary resistance arteries, rat coronary arteries, and bovine coronary arteries the vasodilator response to BK was found to possess a significant component that is independent of NO synthesis [15–18]. These studies suggest that the mechanism mediating vasodilator responses to BK is far more complex than originally thought.

The identity of the EDRF mediating the NO-independent response to BK remains uncertain. It has been shown that this factor causes vascular relaxation by hyperpolarizing smooth muscle, inactivating voltage-gated calcium channels and inhibiting calcium entry. However, the exact mechanism by which this endothelium-derived hyperpolarizing factor (EDHF) induces relaxation is uncertain. Potassium channels [13,19,20], gap junctions [14], arachidonic acid products [21], and *S*-nitrosothiols [19] have all been proposed as possible mediators.

Many studies use derivatives of the amino acid L-arginine to inhibit production of NO. In these studies, comparison of vasodilator responses before and after inhibition of NOS is complicated, since baseline arterial pressure and vascular resistance are increased following NOS inhibition [8,22–25]. In order to normalize data in some studies responses were compared as a percent change from baseline [22,24–28]. Other studies compared responses at different levels of vascular tone [29,30].

Since it has been hypothesized that second messengers other than NO may be involved in the response and because changes in vascular tone may complicate the analysis of vascular responses, the current study was undertaken to investigate the role of NO and K_{Ca} channels in mediating vasodilator responses to BK in vivo. In these studies, changes in vascular tone produced by inhibition of NOS were corrected using an infusion of an NO donor to replace basal NO and to restore pressure and flow to values similar to baseline values, making possible direct comparison of agonist-induced responses before and after NOS inhibition.

2. Methods

2.1. General surgical procedures

All experiments follow the American Physiological Society guiding principles in research [31]. All experiments were performed in male Sprague–Dawley rats weighing 300–450 g anesthetized with thiobarbital sodium (Inactin®) (120 mg kg^{-1} i.p.). The trachea was cannulated to maintain airway patency and the animals breathed room air spontaneously.

For experiments in which hindlimb vasodilator responses were evaluated, an external jugular vein and the left iliac vein were catheterized for the intravenous (i.v.) administration of drugs. The left common carotid artery was catheterized for the measurement of arterial pressure. A flow probe (Transonic Systems Inc.) was placed around the right iliac artery just below the aortic bifurcation and hindlimb blood flow was measured with a Transonic Systems T-106 small animal flowmeter. A catheter was inserted into the left iliac artery and was advanced to the aortic bifurcation for the intraarterial (i.a.) administration of agonists into the right hindlimb circulation. This procedure has been described previously [32,33]. Agonists were injected directly into the right hindlimb circulation in small volumes (10–30 ul) so that changes in right iliac blood flow could be measured with minimal changes in systemic arterial pressure. This simplified the analysis of responses since changes in blood flow, without changes in pressure, are inversely related to changes in resistance. Additionally, in some experiments, platinum electrodes were inserted subdermally in a lead II configuration for the measurement of the electrocardiogram (ECG). Arterial pressure, right iliac flow, and ECG data were recorded on a PC using a MP100 acquisition system (Biopac Systems Inc.).

For experiments in which ultrasonography was performed, the animal was anesthetized as described above. The left common carotid artery was cannulated for the measurement of systemic arterial pressure. The left external jugular vein was cannulated for the administration of drugs. Echocardiography was performed with a Toshiba Aplio 80 ultrasound machine (Toshiba America Medical Systems, Tustin, CA) with a linear array transducer (Toshiba PLT-1202S at 14 MHz). Two-dimensional echo guided M-mode images were obtained (2 cm depth, focus 0.75 cm) from a left parasternal window. M-mode left ventricular measurements were made according to American Society of Echocardiography guidelines [34]. Fractional shortening was calculated as [(LVEDD − LVESD)/LVEDD], where LVEDD is left ventricular end-diastolic dimension and LVESD is left ventricular end-systolic dimension. Color Doppler guided pulsed wave Doppler flow measurements (3 cm depth, focus 1.5 cm) at the level of the pulmonic valve were performed from a high left parasternal short-axis view. Values for peak pulmonary arterial flow and fractional shortening represent the average measurements from three consecutive cardiac cycles.

For experiments in which cardiac output was measured, the animal was anesthetized as described above, and the left iliac artery was cannulated for the measurement of arterial pressure. The left external jugular vein and the left iliac vein were cannulated for the administration of isotonic saline and drugs, respectively. A thermistor catheter was advanced from the left common carotid artery to the aortic arch, and cardiac output was determined by thermal dilution technique [35] with a Cardiotherm 500 cardiac output computer.

For experiments in which carotid blood flow was measured, the animal was anesthetized as described above, and the left iliac artery was cannulated for the measurement of systemic arterial pressure. The left iliac vein was cannulated for the administration of drugs. An electromagnetic flow probe (Carolina Medical, King, NC) was placed around the

right common carotid artery. Arterial pressure and carotid flow were recorded on a Grass model 7D polygraph.

2.2. Effect of L-NAME on the hindlimb vascular responses

Injections of BK and acetylcholine (ACh) were carried out in the control period, and L-NAME (25 mg kg^{-1} i.v.) was then administered. After ~5 min, systemic arterial pressure began to increase, and both cardiac output and hindlimb blood flow began to decrease. Injections of BK and ACh were repeated. An i.v. infusion of the NO-donor SNP (10–30 ug min^{-1} i.v.) was then initiated in order to correct for the loss of basal NO production, and restore systemic arterial pressure, cardiac output, and hindlimb blood flow to pre-L-NAME baseline levels. Using this technique, the loss of basal NO production following inhibition of NOS is compensated for, while agonist-induced stimulation of NOS is still inhibited. Responses to BK and ACh were again determined after baseline hemodynamic parameters were restored to near control levels and were stable.

In separate sets of experiments, the effect of L-NAME and an SNP infusion on carotid vascular parameters and on cardiac function, as assessed by Doppler ultrasound, were investigated. Baseline measurements were recorded, and then L-NAME (25–100 mg kg^{-1} i.v.) was administered. Measurements of cardiac function and carotid vascular parameters were again recorded. An infusion of SNP was initiated to compensate for the loss of basal NO production, and carotid vascular parameters and cardiac parameters were again recorded once the infusion had stabilized.

2.3. Effect of charybdotoxin and apamin on hindlimb vascular responses

In another set of experiments, the effect of the K_{Ca} channel blockers charybdotoxin (Chtx) and apamin was investigated. The hindlimb was prepared as described above. Bolus systemic administration of Chtx and apamin resulted in electrocardiographic abnormalities and irregular blood flow to the hindlimb vascular bed. Therefore, an additional catheter was inserted into the abdominal aorta just above the ilioaortic bifurcation for the local infusion Chtx and apamin into the right hindlimb. A local infusion of Chtx and apamin did not result in the electrocardiographic and blood flow irregularities observed with systemic administration of these compounds. Injections of BK, ACh, and SNP were carried out in the presence of L-NAME (25 mg kg^{-1} i.v.) in the control period. Chtx and apamin were then infused locally to the hindlimb via the aortic catheter. The infusion rate of Chtx and apamin was adjusted for the basal blood flow rate in each animal and calculated to achieve concentrations in the hindlimb equivalent to those shown to be effective in isolated artery preparations (Chtx 3×10^{-8} M; apamin 2.5×10^{-7} M) [29,32]. After the infusion of Chtx and apamin had been running for 10 min, responses to BK, ACh, and SNP were again recorded. In a separate set of animals, Chtx and apamin were infused before the administration of L-NAME so that their effect on the responses to BK and L-NAME could be determined.

2.4. Effect of meclofenamate and U37883A on NO-independent hindlimb vascular responses

L-NAME (25 mg kg^{-1} i.v.) was administered, and an i.v. infusion of the NO-donor SNP (10–30 g min^{-1} i.v.) was initiated in order to correct for the loss of basal NO production, as described previously. Responses to BK were determined after baseline hemodynamic parameters were restored to near control levels and were stable. The non-selective cyclooxygenase inihibitor meclofenamate was then administered (5 mg kg^{-1} i.v.) slowly over 5 min. Responses to BK were determined after baseline hemodynamic parameters were stable. The K^+-ATP channel blocker U37883A was then administered (5 mg kg^{-1} i.v.) slowly over 5 min. Responses to BK were determined after baseline hemodynamic parameters were stable.

Hemodynamic data are expressed as mean ± S.E. and were analyzed using one-tailed paired and unpaired *t*-tests, and ANOVA with a Scheffe post test. The duration of the blood flow response was defined as the period of time from the injection of the agonist to the time at which the blood flow returned to the pre-injection baseline. The area under the blood flow versus time tracing during was determined by integration using Biopac AcqKnowledge software. A *P*-value of <0.05 was used as the criterion for statistical significance.

3. Results

3.1. Use of an SNP infusion to normalize baseline hemodynamic parameters

NOS inhibitors increase baseline arterial pressure and vascular resistance, and because of a change in initial value, the analysis of vascular responses are complicated. In order to restore baseline hemodynamic parameters to control levels after administration of a NOS inhibitor, an infusion of the NO-donor SNP was used. The effect of the L-NAME and an infusion of SNP on systemic and hindlimb vascular parameters is shown in Fig. 1 and Table 1. Administration of L-NAME (25 mg kg^{-1} i.v.) produced a significant increase in mean systemic arterial pressure, total peripheral resistance, carotid vascular resistance, and hindlimb vascular resistance, while producing a significant fall in cardiac output, left ventricular fractional shortening, pulmonary arterial blood velocity, carotid blood flow, and hindlimb blood flow (Fig. 1 and Table 1). A continuous i.v. infusion of SNP was used to return mean systemic arterial pressure, total peripheral resistance, cardiac output, left ventricular fractional shorten-

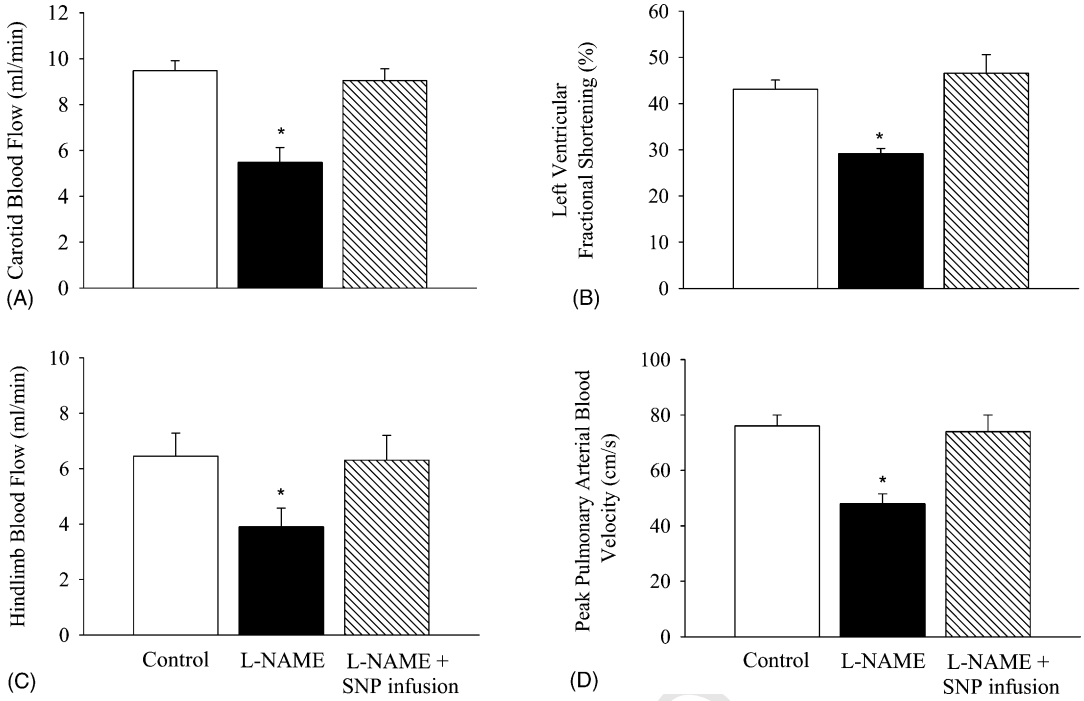

Fig. 1. The effect of L-NAME (25 mg kg^{-1} i.v.) and an SNP infusion on (A) carotid blood flow; (B) left ventricular fractional shortening; (C) hindlimb blood flow; and (D) peak pulmonary arterial blood velocity; (*) denotes $P < 0.05$ when compared to control; n = 5–7 for each group.

ing, pulmonary arterial blood velocity, carotid and iliac blood flow, as well as carotid and iliac vascular resistance (Fig. 1 and Table 1) to control, or pre-L-NAME values. This allowed for the direct comparison of agonist responses at a similar level of baseline resistance before and after administration of the NOS inhibitor. The return of mean arterial pressure to pre-L-NAME levels occurred within 5 min of the initiation of the SNP infusion. Return of hindlimb and carotid blood flow to pre-L-NAME levels lagged the pressure response by several minutes. The other parameters measured—cardiac output, left ventricular fractional shortening, and pulmonary arterial blood velocity—were not measured continuously. Thus, the timecourse of the return to pre-L-NAME values following initiation of an SNP infusion cannot be precisely stated.

3.2. Effect of L-NAME and an SNP infusion on hindlimb responses to BK

Intraarterial injections of BK (10–300 ng) into the hindlimb circulation produced dose-dependent increases in hindlimb blood flow (Fig. 2) and dose-dependent decreases in hindlimb vascular resistance with minimal changes in systemic arterial pressure. When hindlimb vasodilator responses to i.a. injections of BK and ACh were compared before and after administration of L-NAME (25 mg kg^{-1} i.v.), in the presence of an SNP infusion to normalize vascular tone, the magnitude of the flow responses to ACh and BK were not altered (Fig. 2). The duration of the change in blood flow in response to BK was slightly attenuated at the 10 and 30 ng doses, but not at the higher doses (Fig. 3, left).

Table 1
Effects of various treatments on baseline hindlimb vascular parameters

Treatment	Mean arterial pressure (mmHg)	Cardiac output (ml min^{-1})	Total peipheral resistance (mmHg ml^{-1} min)	Hindlimb blood flow (ml min^{-1})	Hindlimb resistance (mmHg ml^{-1} min)
None	120 ± 7	121 ± 4	0.95 ± 0.04	8.1 ± 0.8	15.8 ± 0.7
L-NAME	165 ± 5*	75 ± 5*	2.19 ± 0.15*	3.7 ± 0.3*	47.9 ± 5.2*
Chtx + apamin	128 ± 8	–	–	6.4 ± 0.7	20.9 ± 3.1
L-NAME + Chtx + apamin	178 ± 6*	–	–	3.3 ± 0.5*	57.7 ± 5.8*
L-NAME + SNP infusion	119 ± 8	126 ± 6	0.86 ± 0.05	6.3 ± 1.0	20.0 ± 4.0

L-NAME (25 mg kg^{-1} i.v.) produced significant decrease in cardiac output and hindlimb blood flow and produced significant increases in mean arterial pressure, total peripheral resistance, and hindlimb resistance. L-NAME + an infusion of Chtx (0.9 μg min^{-1} i.a.) + apamin (3.5 μg min^{-1} i.a.) did not further alter mean arterial pressure, hindlimb blood flow or hindlimb resistance. L-NAME and an SNP infusion returned mean arterial pressure, cardiac output, hindlimb blood flow, total peripheral resistance, and hindlimb resistance back to pre-L-NAME control levels. An infusion of Chtx and apamin, alone or in the presence of L-NAME, did significantly not alter mean arterial pressure, hindlimb blood flow, or hindlimb resistance. Reported values were measured after baseline vascular parameters had stabilized following administration of the appropriate antagonist(s); n = 5–10.

* $P < 0.05$ when compared to no treatment.

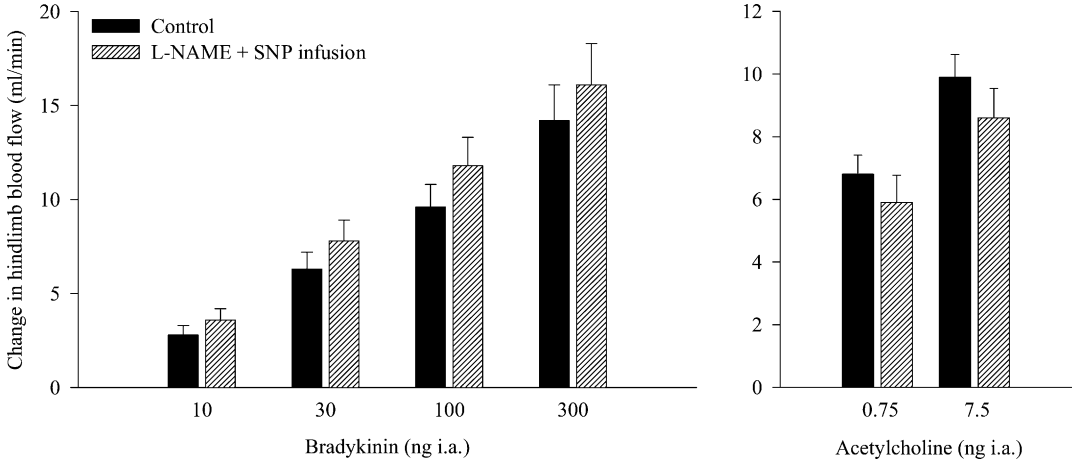

Fig. 2. The effect of L-NAME (25 mg kg^{-1} i.v.) and an SNP infusion on the magnitude of the hindlimb vasodilator responses to BK (left) and ACh (right); n = 8 for each group.

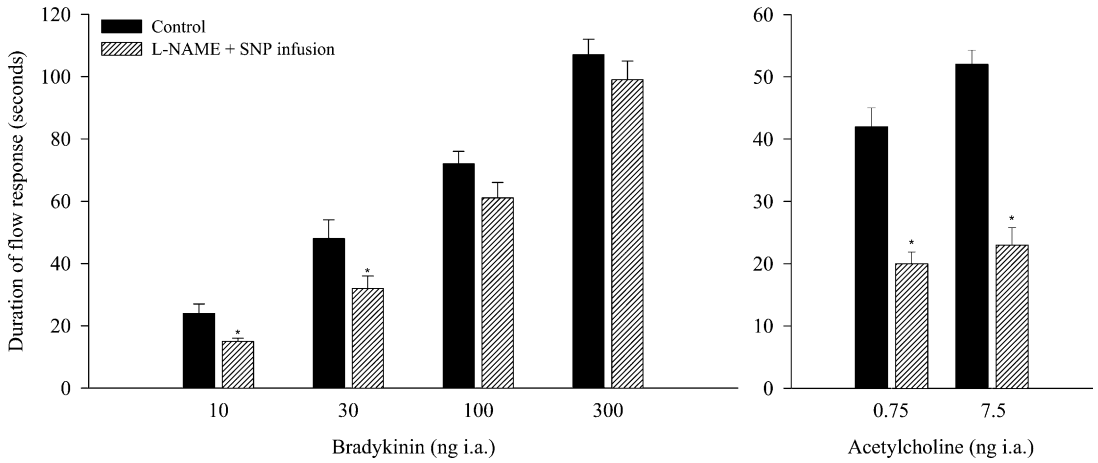

Fig. 3. The effect of L-NAME (25 mg kg^{-1} i.v.) and an SNP infusion on the duration of the hindlimb vasodilator responses to BK (left) and ACh (right). The duration of the response to ACh was shortened by about half, whereas the duration of the response to BK was only slightly reduced at the two lowest doses studied; (*) denotes $P < 0.05$; n = 8 for each group.

These results are similar to those seen when L-NAME alone was administered (data not shown). The duration of the response to ACh was significantly decreased at all doses studied by L-NAME and an SNP infusion (Fig. 3, right). The reduction in the duration of the response to BK or ACh was not greater when the dose of L-NAME was increased to 100 mg kg^{-1} i.v. (data not shown). Representative tracings of the blood flow responses to BK and ACh before and after L-NAME and an SNP infusion are illustrated in Figs. 4 and 5.

3.3. Effect of charybdotoxin, and apamin on hindlimb vascular responses

Administration of L-NAME (25 mg kg^{-1} i.v.) produced an increase in systemic arterial pressure and hindlimb resistance, and a decrease in hindlimb blood flow (Table 1). Intraarterial injections of BK in the presence of L-NAME (25 mg kg^{-1}

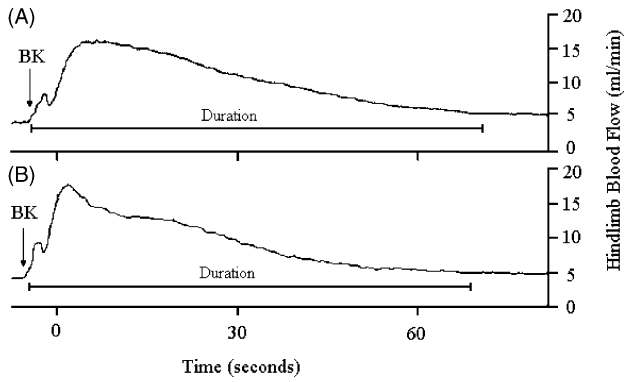

Fig. 4. Representative tracings of the hindlimb blood flow response to BK (100 ng i.a.) in the control situation (A); and following the administration of L-NAME (25 mg kg^{-1} i.v.) and the initiation of an SNP infusion (B). The time of injection of BK is indicated by the arrow in each tracing. The response was not significantly altered in the presence of L-NAME and an SNP infusion.

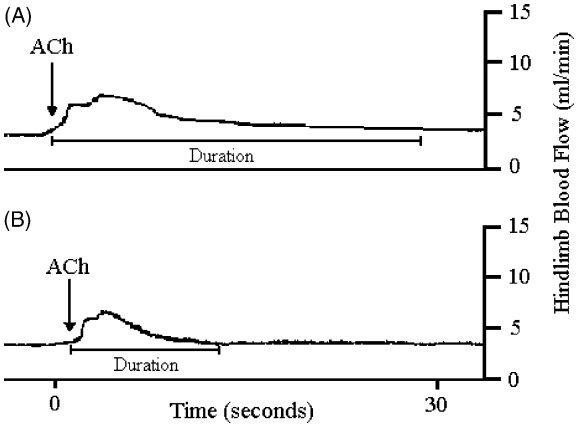

Fig. 5. Representative tracings of the hindlimb blood flow response to ACh (0.75 ng i.a.) in the control situation (A); and following the administration of L-NAME (25 mg kg^{-1} i.v.) and the initiation of an SNP infusion (B). The time of injection of ACh is indicated by the arrow in each tracing. The duration of the response was significantly shortened in the presence of L-NAME and an SNP infusion (B) when compared to the control situation (A).

i.v.) produced dose-dependent increases in hindlimb blood flow (Fig. 5A). In the presence of L-NAME, an infusion of Chtx + apamin did not further alter baseline hindlimb vascular parameters (Table 1). An infusion of Chtx + apamin did, however, attenuate the magnitude and duration of the vasodilator responses to BK and ACh (Figs. 6 and 7, left and right), while not altering the vasodilator response to SNP (Figs. 6 and 7, center). A representative tracing of the blood flow response to BK, in the presence of L-NAME, before and after an infusion of Chtx and apamin is shown in Fig. 8. The blood flow response to BK was greatly diminished in the presence of L-NAME and an infusion of Chtx and apamin (Fig. 8B) when compared to the response in the presence of L-NAME alone (Fig. 8A). When an infusion of Chtx and apamin was initiated without prior administration of L-NAME, baseline vascular parameters were unaltered (Table 1). However, the vasodilator response to BK was decreased, while the response to SNP was unaffected (Table 2.)

3.4. Effect of meclofenamate and U37883A on NO-independent hindlimb vascular responses

The increase in systemic arterial pressure and the decrease in hindlimb blood flow produced by L-NAME were reversed using an SNP infusion. Intraarterial injections of BK in the presence of L-NAME and an infusion of SNP produced dose-dependent increases in hindlimb blood flow. Administration of meclofenamate (5 mg kg^{-1} i.v.), in a dose that attenuated the systemic vasodepressor response to the cyclooxygenase substrate arachidonic acid, did not alter hindlimb vascular parameters (data not shown). Following administration of meclofenamate, the vasodilator response to BK was not significantly altered (Fig. 9). Subsequent administration of U37883A (5 mg kg^{-1} i.v.), in a dose that attenuated the vasodilator to the K^+-ATP channel opener levcromakalim, did not alter hindlimb vascular tone (data not shown). U3788A also did not significantly attenuate the vasodilator response to BK (Fig. 9).

4. Discussion

The results of the present study demonstrate that the NOS inhibitor L-NAME, in a dose that attenuates the hindlimb vasodilator response to ACh, does not have a major effect on either the magnitude or duration of the hindlimb vasodilator response to BK. Administration of L-NAME increased arterial pressure, systemic, carotid, and hindlimb vascular resistance, and decreased cardiac output, left ventricular fractional

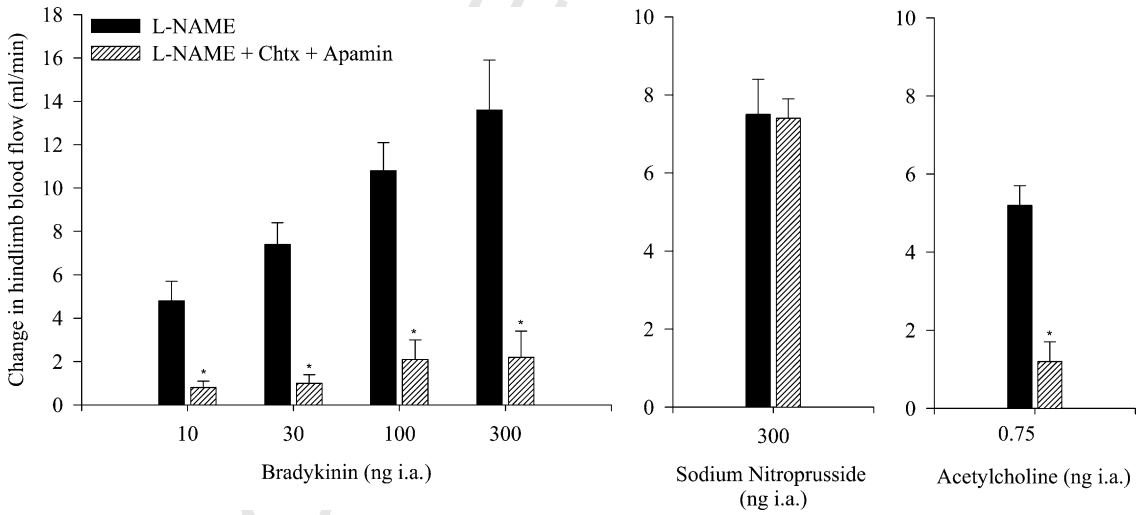

Fig. 6. The effect of an infusion of the K_{Ca} channel blockers Chtx and apamin, in the presence of L-NAME (25 mg kg^{-1} i.v.), on the magnitude of the hindlimb vasodilator responses to BK (left); SNP (center); and ACh (right). The magnitude of the responses to BK and ACh was diminished, while the response to SNP was unaffected; (*) denotes $P < 0.05$; $n = 5$ for each group.

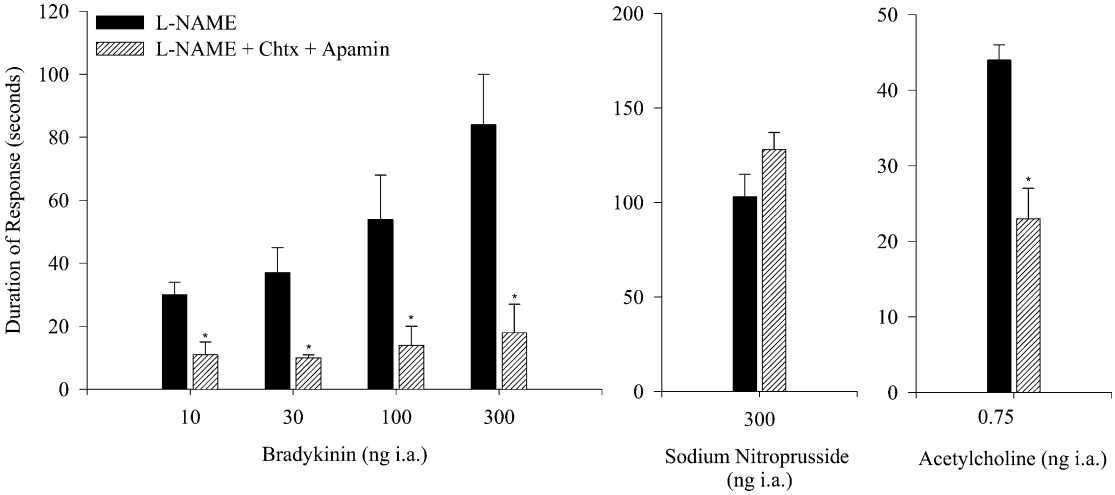

Fig. 7. The effect of an infusion of the K_{Ca} channel blockers Chtx and apamin, in the presence of L-NAME (25 mg kg^{-1} i.v.), on the duration of the hindlimb vasodilator responses to BK (left); SNP (center); and ACh (right). The duration of the responses to BK and ACh was diminished, while the response to SNP was unaffected; (*) denotes $P < 0.05$; $n = 5$ for each group.

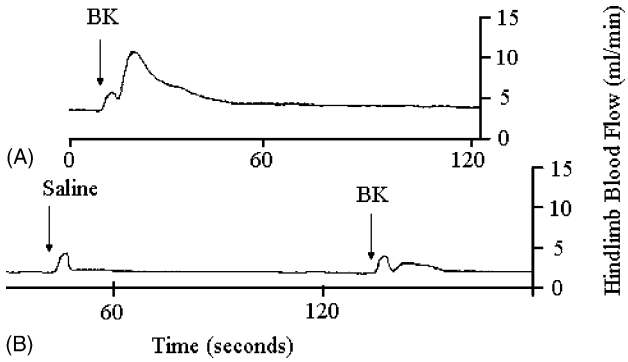

Fig. 8. Representative tracings of the hindlimb blood flow response to BK (100 ng i.a.): (A) in the presence of L-NAME (25 mg kg^{-1} i.v.); and (B) in the presence of L-NAME and an infusion of Chtx and apamin (Chtx 0.4–0.9 μg min^{-1} i.a.; apamin 1.5–3.5 μg min^{-1} i.a.).

shortening, pulmonary arterial blood velocity, and carotid and hindlimb blood flow. The increases in pressure and vascular resistance and the decreases in left ventricular fractional shortening, pulmonary arterial blood velocity, and regional blood flow were reversed by an infusion of the NO donor SNP, essentially restoring normal cardiovascular function while maintaining the inhibition of agonist-induced NO release. The K_{Ca} channel antagonists Chtx and apamin attenuated the L-NAME resistant component of the hindlimb vasodilator response to BK, while not affecting responses to SNP or L-NAME. Additionally, the cycoloxygenase inhibitor meclofenamate and the K^+-ATP channel blocker U37883A did not have an effect on the NO-independent portion of the BK response. These results are in agreement with previous studies and are in agreement with the concept that NO plays an important role in the regulation of baseline tone in the systemic vascular bed.

Although the increase in arterial pressure and vascular resistance following administration of a NOS inhibitor supports a role for NO in the maintenance of tone, it complicates the analysis of the role of NO in mediating vasodilator responses in the hindlimb vascular bed. An increase in baseline tone can alter the response to a vasodilator agent as predicted by the law of initial value [36]. In order to account for the change in baseline tone following NOS inhibition previous studies have described responses as a percent change from baseline or have infused vasoconstrictor agents to increase baseline

Table 2
Effect of an infusion of Chtx and apamin on the hindlimb vasodilator responses to BK and SNP

Drug	Dose (ng i.a.)	*n*	Treatment	Change in hindlimb blood flow (ml min^{-1})	Area of response (ml)
BK	30	4	Control	8.3 ± 2.7	225 ± 70
		4	Chtx + apamin	2.5 ± 0.5*	100 ± 23*
	300	4	Control	13.1 ± 3.5	586 ± 150
		4	Chtx + apamin	4.8 ± 1.2*	230 ± 80*
SNP	300	3	Control	3.5 ± 0.8	115 ± 27
		3	Chtx + apamin	3.0 ± 0.5	128 ± 29

An infusion of Chtx (0.9 μg min^{-1} i.a.) + apamin (3.5 μg min^{-1} i.a.) decreased the magnitude and the area of the hindlimb vasodilator response to BK. The magnitude and area of the response to SNP were not altered by the infusion of Chtx and apamin; *n* is the number of animals.

* $P < 0.05$ when compared to no treatment.

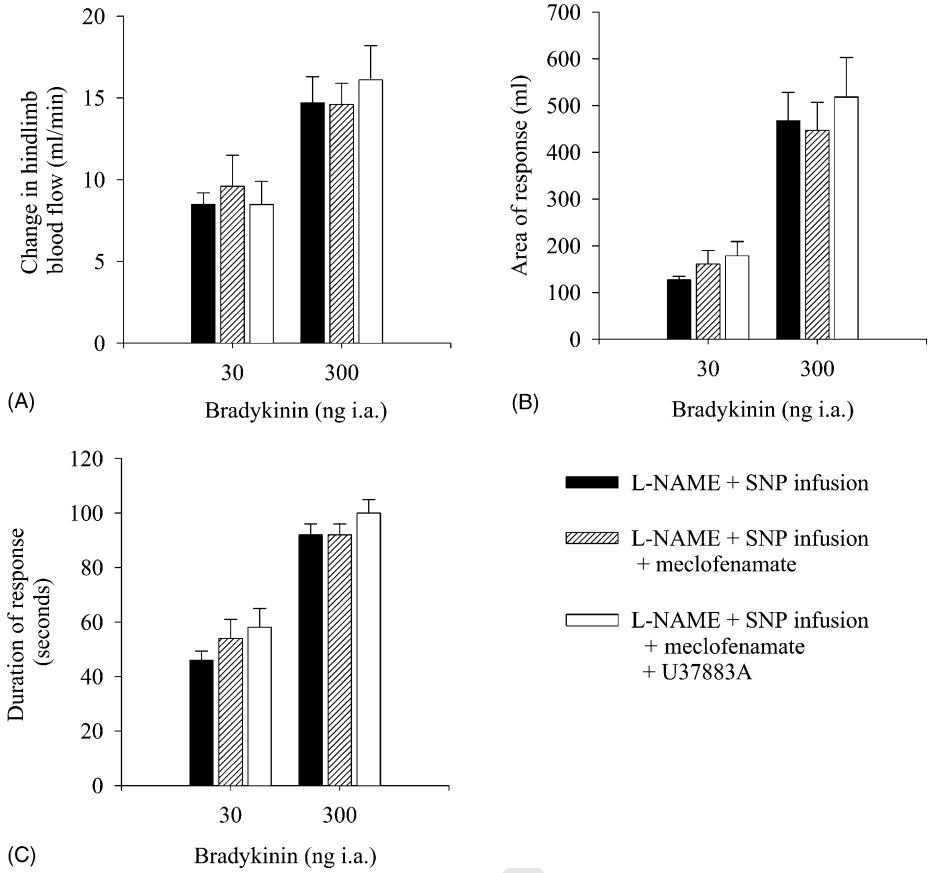

Fig. 9. The effect of the cyclooxygenase inhibitor meclofenamate and the K^+-ATP channel blocker U37883A, in the presence of L-NAME (25 mg kg^{-1} i.v.) and an SNP infusion, on the magnitude (A); area (B); and duration (C) of the hindlimb vasodilator response to BK.

vascular tone [22,24–27]. Our laboratory has previously used an infusion of the NO donor SNP to compensate for the loss of basal NO production following inhibition of NOS. Using this technique it is possible to restore arterial pressure, cardiac output, hindlimb blood flow, and vascular resistance to pre-L-NAME levels [32,37]. The present study again uses this technique to normalize vascular tone and demonstrates that, in addition to restoring arterial pressure, cardiac output, and hindlimb blood flow to pre-L-NAME levels, the SNP infusion technique is also able to restore left ventricular fractional shortening, pulmonary arterial blood velocity, carotid blood flow, and carotid vascular resistance to pre-L-NAME levels. Left ventricular fractional shortening is a measure of left ventricular contractility, or the inotropic state of the left ventricle, and can be influenced by changes in left ventricular preload or afterload. While administration of L-NAME produced a decrease in left ventricular fractional shortening, it cannot be determined from the present data if L-NAME is having a direct effect on the contractility of the left ventricle, or if the reduction in left ventricular fractional shortening is due to an increase in afterload. Regardless of the mechanism by which L-NAME reduces left ventricular fractional shortening, the SNP infusion technique is able to restore left ventricular fractional shortening to pre-L-NAME levels. Thus, the use of L-NAME and the SNP infusion technique allows for a direct comparison of the vascular responses to BK at similar baseline hemodynamic values before and after NOS inhibition, eliminating the need for mathematical normalization of the data.

Previous studies have demonstrated that the vasodilator response to BK consists of both NO-dependent and NO-independent components, and that the relative contributions of these components depend on the diameter of the vascular segment being studied, the species examined, and the vascular bed being studied. In small isolated porcine retinal arteries and conducting pulmonary arteries, the vasodilator response to BK was found to dependent entirely upon NO production [15,38]. In contrast, in porcine pial arteries, porcine pulmonary resistance arteries, human subcutaneous arteries, human microcoronary arteries, rat coronary arteries, and bovine coronary arteries the vasodilator response to BK was found to possess a significant component that was independent of NO synthesis [13–18]. The results of the present study suggest that NO plays a minor role in mediating the hindlimb vasodilator response to BK. The magnitude of the hindlimb vasodilator response to BK was not reduced by L-NAME in a dose of 25 mg kg^{-1} i.v. when baseline flow and vascular resistance were restored by an SNP infusion. The duration of the hindlimb vasodilator response to BK was not significantly reduced at the higher doses, although the re-

duction did reach statistical significance at the two lowest doses studied. The reduction of the duration of the vasodilator response to BK following administration of a NOS inhibitor has been reported previously in the rat coronary circulation [16]. The results of the present study suggest that NO does not play a major role in determining the magnitude of the hindlimb vasodilator response to BK, but that it may play a minor role in modulating the duration of the response. In contrast, NO appears to account for about half of the hindlimb vasodilator response to ACh, with the other half of the response mediated by a Chtx and apamin sensitive mechanism.

It has been reported that NOS inhibitors, such as L-NAME, do not completely inhibit NO production in the rabbit carotid artery and it is possible that L-NAME, even in high doses, may not completely inhibit NO production [39]. However, several studies in the literature have demonstrated complete blockade of NO-dependent responses with similar NOS inhibitors [3,40]. In addition, in the present study, the doses of L-NAME used significantly reduced the hindlimb vasodilator response to ACh, while the response to BK was not greatly affected. Furthermore, doses of L-NAME as high as 100 mg kg^{-1} i.v. did not produce any further decrease in the magnitude or duration of the hindlimb vasodilator response to BK or ACh beyond that observed with the 25 mg kg^{-1} i.v. dose, suggesting that the blockade of NOS by L-NAME was maximal. It has also been reported that NO is an inhibitor of endogenous NOS activity [41]. Therefore it is possible that the infusion of the NO donor SNP after L-NAME administration provides additional blockade of endogenous NO synthesis.

It has been reported that a significant component of the vasodilator response to BK and other endogenous vasodilators may be mediated by the release of an endothelium dependent hyperpolarizing factor (EDHF) and hyperpolarization of vascular smooth muscle via activation of a K_{Ca} channel [14,29,32]. Hyperpolarization of smooth muscle leads to inactivation of voltage dependent calcium channels and vasorelaxation. K_{Ca} channels can be blocked by the combination of the toxins Chtx and apamin [29]. Since it is hypothesized that BK releases an EDHF, the effects of K_{Ca} channel antagonists Chtx and apamin on the L-NAME resistant component of the response to BK were investigated. An infusion of Chtx and apamin, both alone and in the presence of the NOS inhibitor L-NAME, significantly attenuated the vasodilator response to BK in the hindlimb vascular bed, suggesting that activation of K_{Ca} channels play a role in mediating this response. Responses to the vasodilator agent SNP were not altered, demonstrating that the blockade produced by Chtx and apamin is selective for BK. Meclofenamate, a non-selective cyclooxygenase inhibitor, did not have an effect on the response to BK in the presence of L-NAME, suggesting that prostaglandins do not play a significant role in mediating the response. This finding is in agreement with previous studies [14,19]. While it appears that prostaglandins do not play a role in mediating the response to BK, other arachidonic acid derived mediators, such as the epoxyeicosatrienoic acids, cannot be ruled out. Additionally, U37883A, in the presence of L-NAME and meclofenamate, did not have an effect on the response to BK, suggesting that the response is independent of K^+-ATP channel activation. Similar findings have been reported previously for EDHF-dependent agonists, including BK and ACh [19,32,42].

In summary, the present study utilized an SNP infusion to restore arterial pressure, cardiac function, regional blood flow, and vascular resistance to baseline values after administration of a NOS inhibitor, and demonstrates that the vasodilator response to BK in the hindlimb vascular bed of the rat is mediated by both NO- and K_{Ca} channel-dependent mechanisms. However, the NO-dependent component of the response appears to be minor relative to the component of the response that is dependent upon the activation of K_{Ca} channels. Additionally, cyclooxygenase products and K^+-ATP channel activation do not appear to play a role in mediating the NO-independent component on the response. Finally, the present results demonstrate that the SNP infusion technique is able to compensate for the loss of basal NO production following inhibition of NOS, and to restore hemodynamic parameters to pre-L-NAME levels, making it a useful technique for the investigation of the role of NO in mediating vascular responses.

References

[1] Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373–6.
[2] Furchgott RF, Carvalho MH, Khan MT, Matsunaga K. Evidence for endothelium-dependent vasodilation of resistance vessels by acetylcholine. Blood Vessels 1987;24:145–9.
[3] Bellan JA, Longnecker LL, Kadowitz PJ, McNamara DB. Selective and complete blockade of acetylcholine-induced relaxation in rabbit aortic rings by *N* omega-nitro-L-arginine but not by glybenclamide. Eur J Pharm 1993;234:273–6.
[4] Broten TP, Miyashiro JK, Moncada S, Feigl EO. Role of endothelium-derived relaxing factor in parasympathetic coronary vasodilation. Am J Physiol 1992;262:1579–84.
[5] Ignarro LJ, Harbison RG, Wood KS, Kadowitz PJ. Dissimilarities between methylene blue and cyanide on relaxation and cyclic GMP formation in endothelium-intact intrapulmonary artery caused by nitrogen oxide-containing vasodilators and acetylcholine. J Pharmacol Exp Ther 1986;236:30–6.
[6] Ignarro LJ, Byrns RE, Buga GM, Wood KS. Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide radical. Circ Res 1987;61:866–79.
[7] Ignarro LJ, Buga GM, Byrns RE, Wood KS, Chaudhuri G. Endothelium-derived relaxing factor and nitric oxide possess identical pharmacologic properties as relaxants of bovine arterial and venous smooth muscle. J Pharmacol Exp Ther 1988;246:218–26.
[8] McMahon TJ, Hood JS, Bellan JA, Kadowitz PJ. N^{ω}-Nitro-L-arginine methyl ester selectively inhibits pulmonary vasodilator responses to acetylcholine and bradykinin. J Appl Physiol 1991;71:2026–31.
[9] Edwards JC, Ignarro LJ, Hyman AL, Kadowitz PJ. Relaxation of intrapulmonary artery and vein by nitrogen oxide-containing vasodilators and cyclic GMP. J Pharmacol Exp Ther 1984;228:33–42.

[10] Gruetter CA, Gruetter DY, Lyon JE, Kadowitz PJ, Ignarro LJ. Relationship between cyclic guanosine 3′:5′-monophosphate formation and relaxation of coronary arterial smooth muscle by glyceryl trinitrate, nitroprusside, nitrite, and nitric oxide: effects of methylene blue and methemoglobin. J Pharmacol Exp Ther 1981;219:181–6.
[11] Napoli SA, Gruetter CA, Ignarro LJ, Kadowitz PJ. Relaxation of bovine coronary arterial smooth muscle by cyclic GMP, cyclic AMP, and analogs. J Pharmacol Exp Ther 1980;212:469–73.
[12] Rapoport RM, Draznin MB, Murad F. Endothelium-dependent relaxation in rat aorta may be mediated through cyclic GMP-dependent protein phosphorylation. Nature 1983;306:174–6.
[13] Batenburg WW, Garrelds IM, van Kats JP, Saxena PR, Danser AH. Mediators of bradykinin-induced vasorelaxation in human coronary microarteries. Hypertension 2004;43:488–92.
[14] Luksha L, Nisell H, Kublickiene K. The mechanism of EDHF-mediated responses in subcutaneous small arteries from healthy pregnant women. Am J Physiol 2004;286:1102–9.
[15] Aschner JL, Smith TK, Kovacs N, Pinheiro JM, Fuloria M. Mechanisms of bradykinin-mediated dilation in newborn piglet pulmonary conducting and resistance vessels. Am J Physiol 2002;283:373–82.
[16] Baydoun AR, Woodward B. Effects of bradykinin in the rat isolated perfused heart: role of kinin receptors and endothelium-derived relaxing factor. Br J Pharmacol 1991;103:1829–33.
[17] Hecker M, Bara AT, Bauersachs J, Busse R. Characterization of endothelium-derived hyperpolarizing factor as a cytochrome P450-derived arachidonic acid metabolite in mammals. J Physiol 1994;481:407–14.
[18] Lacza Z, Puskar M, Kis B, Perciaccante JV, Miller AW, Busija DW. Hydrogen peroxide acts as an EDHF in the piglet pial vasculature in response to bradykinin. Am J Physiol 2002;283:406–11.
[19] Batenburg WW, Popp R, Fleming I, de Vries R, Garrelds IM, Saxena PR, et al. Bradykinin-induced relaxation of coronary microarteries: *S*-nitrosothiols as EDHF? Br J Pharmacol 2004;142:125–35.
[20] Inokuchi K, Hirooka Y, Shimokawa H, Sakai K, Kishi T, Ito K, et al. Role of endothelium-derived hyperpolarizing factor in human forearm circulation. Hypertension 2004;42:919–24.
[21] Rosolowsky M, Campbell WB. Role of PGI2 and EETs in the relaxation of bovine coronary arteries to arachidonic acid. Am J Physiol 1993;264:327–35.
[22] Bellan JA, Minkes RK, McNamara DB, Kadowitz PJ. N^{ω}-Nitro-L-arginine selectively inhibits vasodilator responses to acetylcholine and bradykinin in cats. Am J Physiol 1991;260:1025–9.
[23] Brock RW, Tschakovsky ME, Shoemaker JK, Halliwill JR, Joyner MJ, Hughson RL. Effects of acetylcholine and nitric oxide on forearm blood flow at rest and after a single muscle contraction. J Appl Physiol 1998;85:2249–54.
[24] Rees DD, Palmer RMJ, Moncada S. Role of endothelium-derived nitric oxide in the regulation of blood pressure. Proc Natl Acad Sci USA 1989;86:3375–8.
[25] Rees DD, Palmer RMJ, Schulz R, Hodson HF, Moncada S. Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. Br J Pharmacol 1990;101:746–52.
[26] Aisaka K, Gross SS, Griffith OW, Levi R. L-Arginine availability determines the duration of acetylcholine-induced systemic vasodilatation in vivo. Biochem Biophys Res Commun 1989;163:710–7.
[27] Champion HC, Bivalacqua TJ, Zadina JE, Kastin AJ, Hyman AL, Kadowitz PJ. Role of nitric oxide in mediating vasodilator responses to opioid peptides in the rat. Clin Exp Pharmacol Physiol 2002;29:229–32.
[28] Loke KE, Sobey CG, Dusting CJ, Woodman AL. Requirement for endothelium-derived nitric oxide in vasodilatation produced by stimulation of cholinergic nerves in rat hindquarters. Br J Pharmacol 1994;112:630–4.
[29] Parkington HC, Chow JM, Evans RG, Coleman HA, Coleman M. Role for endothelium-derived hyperpolarizing factor in vascular tone in rat mesenteric and hindlimb circulations in vivo. J Physiol 2002;542:929–37.
[30] Whittle BJR, Lopez-Belmonte J, Rees DD. Modulation of the vasodepressor actions of acetylcholine, bradykinin, substance P, and endothelin in the rat by a specific inhibitor of nitric oxide formation. Br J Pharmacol 1989;98:646–52.
[31] American Physiological Society. Guiding principles for research involving animals and human beings. Am J Physiol Regulat Integrative Comp Physiol 2002;283:281–3.
[32] Dabisch PA, Liles JT, Taylor JT, Sears BW, Saenz R, Kadowitz PJ. Role of potassium channels in the nitric oxide-independent vasodilator response to acetylcholine. Pharmacol Res 2004;49:207–15.
[33] Feitelson JBA, Kulenovic E, Beck DJ, Harris PD, Passmore JC, Malkani AL, et al. Endogenous norepinephrine regulates blood flow to the intact rat tibia. J Ortho Res 2002;20:391–6.
[34] Sahn 34, DeMaria DJ, Kisslo A, Weyman A J. Recommendations regarding Quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation 1978;58:1072–83.
[35] Kissling G, Ross C, Brandle M. Validity of thermal dilution technique for measurement of cardiac output in rats. Am J Physiol 1993;265.
[36] Jancso T, Rabloczky G, Csukas M. Examination of the law of initial value in connection with the blood pressure response of anaesthetized and non-anaesthetized cats. Acta Physiol Acad Sci Hung 1972;42:333–43.
[37] Dabisch PA, Liles JT, Kadowitz PJ. Effect of inhibition of nitric oxide synthase on the vasopressor response to ephedrine. Can J Physiol Pharmacol 2003;81:966–71.
[38] Jeppesen P, Aalkjaer C, Bek T. Bradykinin relaxation in small porcine retinal arterioles. Invest Opthamol Vis Sci 2002;43:1891–6.
[39] Cohen RA, Plane F, Najibi S, Huk I, Malinski T, Garland CJ. Nitric oxide is the mediator of both endothelium-dependent relaxation and hyperpolarization of the rabbit carotid artery. Proc Natl Acad Sci USA 1997;94:4193–8.
[40] Nagao T, Illiano S, Vanhoutte PM. Heterogenous distribution of endothelium-dependent relaxations resistant to N^{ω}-nitro-L-arginine in rats. Am J Physiol 1992;263:1090–4.
[41] Griscavage JM, Hobbs AJ, Ignarro LJ. Negative modulation of nitric oxide synthase by nitric oxide and nitroso compounds. Adv Pharmacol 1995;34:215–34.
[42] Chen G, Yamamoto Y, Miwa K, Suzuki H. Hyperpolarization of arterial smooth muscle induced by endothelial humoral substances. Am J Physiol 1991;260:1888–92.