Analysis of L-NAME-dependent and -resistant responses to acetylcholine in the rat

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Dabisch PA, Liles JT, Baber SR, Golwala NH, Murthy SN, Kadowitz PJ. Analysis of L-NAME-dependent and -resistant responses to acetylcholine in the rat. Am J Physiol Heart Circ Physiol 294: H688–H698, 2008. First published November 21, 2007; doi:10.1152/ajpheart.00394.2007.—The mechanism by which acetylcholine (ACh) decreases systemic arterial pressure and hindlimb vascular resistance was investigated in the anesthetized rat. ACh injections caused dose-dependent decreases in systemic arterial pressure and hindlimb vascular resistance. Nω-nitro-L-arginine methyl ester (L-NAME) had little effect on the magnitude of depressor and vasodilator responses but decreased response duration when baseline parameters were corrected by a nitric oxide (NO) donor infusion. The decrease in the duration of the ACh depressor response was prevented by the administration of excess L-arginine. The L-NAME-resistant component of the depressor response to ACh was attenuated by ebselen, a glutathione peroxidase mimic. The calcium-activated potassium (KCa) antagonists charybdotoxin (ChTX) and apamin decreased the magnitude but not the duration of the hindlimb vasodilator response to ACh. The combination of L-NAME, ChTX, and apamin reduced the magnitude and duration of the vasodilator response to ACh but not to sodium nitroprusside. Vasodepressor and hindlimb vasodilator responses to ACh were not modified by cytochrome P-450 and cyclooxygenase pathway inhibitors. These results suggest that the hindlimb vasodilator response to ACh has an initial L-NAME-resistant component mediated by the activation of KCa channels and a sustained L-NAME-dependent component. The results with ebselen suggest that the L-NAME-resistant component of the depressor response involves a peroxide-sensitive mechanism. The present study suggests that vasodilator responses to ACh are not mediated by cytochrome P-450 products, since miconazole and 1-aminobenzotriazole alone or in combination did not affect either component of the response. The present data suggest that the hindlimb vasodilator response to ACh in the rat is mediated by two mechanisms with an initial ChTX- and apamin-sensitive, L-NAME-resistant phase not mediated by cytochrome P-450 products and a secondary sustained phase mediated by NO.

endothelium-dependent response; decrease in arterial pressure; hindlimb vasodilator response; calcium-activated potassium channels; cytochrome P-450

ACETYLCHOLINE ACTIVATES vascular endothelial muscarinic receptors, leading to the formation of a factor or factors that relax the vascular smooth muscle (19, 20). Although evidence in the literature indicates that the endothelium-derived relaxing factor is nitric oxide (NO), in large arteries, the results of many studies indicate that more than one factor is involved in mediating cholinergic vasodilator responses (2, 4, 5, 7, 19, 20, 31, 41). Moreover, the factors and mechanisms that mediate endothelial-dependent vasodilation vary with species, vascular bed, and the vessel size studied (11, 19, 25, 26, 31, 32, 41).

When NO and prostaglandin-dependent mechanisms are inhibited, ACh-induced vasodilation is associated with arterial smooth muscle hyperpolarization (10, 15, 16, 24, 29, 30, 35). The process of smooth muscle hyperpolarization is believed to involve the formation of an endothelial-derived hyperpolarizing factor (EDHF) (4, 9, 11, 16, 21, 29–31, 35, 41). Although our knowledge of the identity and mechanisms by which EDHF mediates vasodilator responses to ACh is incomplete, there is agreement about the role of calcium-activated potassium (KCa) channels in mediating smooth muscle hyperpolarization (11, 16, 17, 21, 28–30, 35). It was initially thought that large-conductance KCa channels on smooth muscle cells were important in EDHF-mediated responses (6, 9, 18, 28, 32, 40). However, recent evidence indicates a role for small- and intermediate-conductance KCa channels on the endothelium (8, 10, 11, 17, 29, 33, 36). It has been shown that there is a role for small- and intermediate-conductance KCa channels in mediating the vasodilator response to ACh in the regional vascular bed of the rat in vivo (33). It has been reported that epoxyeicosatrienoic acids (EETs) are endothelium-derived, cytochrome P-450 metabolites of arachidonic acid, which act as EDHFs (4, 6, 9, 18, 21, 23, 27, 40). However, knowledge about the role of the cytochrome P-450 system in mediating the vasodilator response to ACh in the hindlimb vascular bed of the rat is incomplete (33). It has also been suggested that H2O2 can serve as an endothelial-derived factor in regulating vascular tone (38). However, the effects of ebselen, a glutathione peroxide mimic, on the ACh-induced vasodilation is associated with arterial smooth muscle hyperpolarization (10, 15, 16, 24, 29, 30, 35).

METHODS

All experiments followed the American Physiological Society guiding principles in research and were approved by the Institutional Animal Care and Use Committee. The experiments were performed in male Sprague-Dawley rats weighing 280–450 g and anesthetized with thiobarbital sodium (100–140 mg/kg ip; Inactin; Sigma Chemical) with supplemental doses given as needed to maintain a uniform level of anesthesia. The trachea was cannulated to maintain airway patency, and the animals breathed room air spontaneously. An external jugular vein and femoral vein were catheterized with polyethylene (PE)-50 tubing for an intravenous injection of vasoactive agonists and for the

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intravenous infusion of sodium nitroprusside (SNP) in experiments in which baseline tone was normalized. The femoral artery was catheterized with PE-50 tubing for the measurement of systemic pressure, and the pressure was measured with a Statham pressure transducer. For the measurement of cardiac output, a 3-Fr thermistor microprobe P23X (Columbus Instruments) was positioned in the aortic arch from the left carotid artery, and cardiac output was determined by the thermal dilution technique with a Cardiotherm 500 cardiac output computer with a small animal interface (Columbus Instruments) and a cold 0.9% NaCl indicator injected into the jugular-vein catheter with its tip positioned in the superior vena cava near the right atrium.

In experiments in which L-NAME was administered and baseline values were corrected with an infusion of a NO donor, SNP was infused at rates of 10 – 40 \( \mu \text{g/min} \) iv to restore hemodynamic values to pre-L-NAME baseline levels. In experiments in which L-arginine was used to restore systemic arterial pressure to pre-L-NAME levels, the NO synthase (NOS) substrate was injected in a dose of 90 mg/kg iv, and the injection was followed by an infusion of 3.6 – 7.2 mg/min iv, which was adjusted to restore baseline systemic arterial pressure to a pre-L-NAME value. In experiments in which the role of endogenous peroxide was investigated, the effects of the glutathione peroxide mimic ebselen on decreases in systemic arterial pressure in response to ACh were investigated in animals treated with L-NAME (50 – 100 mg/kg iv).

In experiments in which hindlimb blood flow was measured, a flow-probe was placed around the right iliac artery just below the aortic bifurcation, and iliac blood flow was measured with a Transonic Systems T-106 small-animal flow meter. A catheter was advanced from the left femoral artery to the aortic bifurcation for intra-arterial administration of agonists into the hindlimb circulation. In the experiments in which charybdotoxin (ChTX) and apamin were infused, a second infusion catheter was advanced from the left femoral artery to the aortic bifurcation. ChTX and apamin were infused into this catheter, and the infusion rate was adjusted in each animal based on iliac blood flow to achieve concentrations equal to 3 \( \times 10^{-8} \) M for ChTX and 2.5 \( \times 10^{-7} \) M for apamin in the hindlimb circulation. The \( K_{\text{a}} \) inhibitors were infused for 10 – 15-min periods.

In experiments in which the effects of 1-aminobentriazole (1-ABT) and miconazole were studied, the inhibitors were injected intravenously. L-NAME, atropine sulfate, 1-ABT, miconazole, ChTX, apamin, ACh iodide, SNP, and sodium meclofenamate (Sigma) were dissolved in 0.9% saline solution. Ebselen (Cayman Chemical) was dissolved in DMSO (Sigma Chemical) and was injected intraperitoneally.

In regard to experimental design, responses to ACh were investigated in the systemic and hindlimb vascular bed. The effects of ChTX and apamin were only investigated in the hindlimb vascular bed, since systemic (intravenous) administration of these agents resulted in toxicity. The doses of inhibitors used were determined from studies in the literature, previous studies in our laboratory, and pilot experiments. The doses of 1-ABT and the EET-depletion protocol were derived from a review by Roman (34).

Systemic arterial pressure and heart rate determined from the pressure pulses with a tachograph were recorded on a Grass model 7 polygraph and were digitized and stored on a personal computer using a Biopac MP100 data acquisition system. The duration of the response, defined as the time from the agonist injection to the time the measured parameter returned to the preinjection baseline value, and the area of the curve during this interval were determined by the Biopac data system. The data are expressed as means ± SE and were analyzed using paired and unpaired \( t \)-tests ANOVA with the Fisher post hoc test. A \( P \) value of <0.05 was used as the criterion for statistical significance.

### RESULTS

**Effect of L-NAME.**

The effect of L-NAME on vasodepressor responses to ACh was investigated when baseline tone was restored by an infusion of SNP. The administration of L-NAME (25 – 100 mg/kg iv) significantly increased systemic and hindlimb vascular resistance and decreased cardiac output (Fig. 1). A continuous infusion of SNP restored systemic and hindlimb vascular resistance and cardiac output to control

![Fig. 1](http://ajpheart.physiology.org/)

**Fig. 1.** The effect of \( \text{N}^{\omega} \)-nitro-L-arginine methyl ester (L-NAME; 25 – 100 mg/kg iv) and an intravenous sodium nitroprusside (SNP) infusion on mean arterial pressure, cardiac output, total peripheral resistance, hindlimb blood flow, and hindlimb vascular resistance. *\( P < 0.05 \) compared with control; \( n \), number of experiments.
pre-L-NAME values, so that responses to ACh could be compared at similar levels of baseline tone (Fig. 1). The injection of ACh in doses of 0.1–3 μg/kg iv caused dose-related decreases in systemic arterial pressure, and vasodepressor responses were reduced 15–25% by treatment with L-NAME (25–100 mg/kg iv) when baseline values were restored with the SNP infusion (Fig. 2A). The duration of the vasodepressor response to ACh and the area of the curve was decreased by ~50% when baseline values were restored with the SNP infusion (Fig. 2, B and C), and a typical response to an iv injection of ACh (0.3 μg/kg) is shown in Fig. 2D.

**Effect of L-arginine and ebselen in L-NAME-treated animals.**

The effect of L-arginine administration on the response to ACh was investigated in animals treated with L-NAME. The injection of L-arginine (90 mg/kg iv) and an intravenous infusion of 3.6–72 mg/min attenuated the increase in systemic arterial pressure in response to L-NAME (100 mg/kg iv) (control, 118 ± 4 mmHg; and L-NAME and L-arginine, 121 ± 5; *P > 0.05) and prevented the inhibitory effect of L-NAME on the vasodepressor response to intravenous injections of ACh. The decreases in systemic arterial pressure in response to ACh, the response duration, and area of the curve were not significantly different from control when L-NAME and L-arginine were administered together (Fig. 3). The effect of ebselen on responses to ACh was investigated in L-NAME-treated animals, and these data are summarized in Fig. 3. The injection of ebselen (10–20 mg/kg ip) in animals treated with L-NAME (50–100 mg/kg iv) caused a significant reduction in the depressor response to intravenous injections of ACh (Fig. 3D).

**Effect of inhibitors.**

The effects of meclofenamate, 1-ABT, and atropine on decreases in systemic arterial pressure in response to ACh were investigated, and these data are summarized in Fig. 4. The injection of sodium meclofenamate (5

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**Fig. 2.** The effect of L-NAME and an infusion of SNP on decreases in systemic arterial pressure (A), duration of the vasodepressor response (B), and area of the curve (C) in response to intravenous injections of ACh in doses of 0.1–3 g/kg. Responses to ACh were compared before (control) and after injection of L-NAME (25–100 mg/kg iv) when baseline values were restored with an infusion of SNP. D: records from experiments showing the effect of L-NAME on the response to an intravenous injection of ACh (0.3 μg/kg iv) when baseline parameters were restored with an intravenous infusion of SNP. *P < 0.05, response different than control; n, number of experiments.
IV) or 1-ABT (50 mg/kg ip or iv) had no significant effect on the magnitude or duration of the vasodepressor response to ACh (Fig. 4, A and C). The muscarinic receptor antagonist atropine (1 mg/kg iv) significantly decreased the magnitude and duration of the vasodepressor response to ACh (Fig. 4B). The administration of sodium meclofenamate or 1-ABT had no significant effect on the area of the curve of the vasodepressor response to ACh, whereas atropine significantly decreased the area of the curve (data not shown). Meclofenamate, 1-ABT, or atropine had no significant effect on systemic arterial pressure when the values were compared 20–30 min after the administration of the antagonists (data not shown).

Analysis of responses to ACh in the hindlimb vascular bed. Intra-arterial injections of ACh into the hindlimb vascular bed caused dose-related increases in hindlimb blood flow with minimal changes in systemic arterial pressure. The increases in hindlimb blood flow in response to ACh were not significantly changed by the administration of L-NAME (25–100 mg/kg iv) when baseline hindlimb blood flow was restored by a SNP infusion (control, 6.6 ± 0.6 ml/min; and L-NAME + SNP infusion, 6.5 ± 0.5 ml/min; *P > 0.05; Fig. 5A). However, the duration of the vasodilator response to ACh and the area of the curve were significantly reduced by the L-NAME treatment when baseline values were restored with the SNP infusion (Fig. 5, B and C).

Effect of ChTX and apamin. The effect of KCa channel antagonists ChTX and apamin on the hindlimb vasodilator response to ACh is shown in Fig. 6. The intra-arterial infusion of ChTX (0.4–0.9 μg/min) and apamin (1.5–3.5 μg/min) caused a significant decrease in the magnitude of the vasodilator response to ACh but had no significant effect on the duration of the vasodilator response to ACh (Fig. 6, A and B). The administration of ChTX and apamin had no significant
effect on the hindlimb vasodilator response to an intra-arterial injection of SNP (Fig. 6, A and B) or on hindlimb blood flow. The administration of L-NAME (25 mg/kg iv), ChTX, and apamin (intra-arterial) caused a significant reduction in the magnitude and duration of the hindlimb vasodilator response to ACh but had no significant effect on the vasodilator response to SNP (Fig. 7, A and B).

Effect of cytochrome P-450 inhibitors. The effect of 1-ABT on the response to ACh was investigated in the hindlimb vascular bed, and these data are summarized in Fig. 8. The administration of 1-NAME (25 mg/kg iv), ChTX, and apamin (intra-arterial) caused a significant reduction in the magnitude and duration of the hindlimb vasodilator response to ACh but had no significant effect on the vasodilator response to SNP (Fig. 7, A and B).

ACh in animals treated with L-NAME (25 mg/kg iv) when baseline blood flow was restored with a SNP infusion (Fig. 8B). The administration of 1-ABT + miconazole had no significant effect on the vasodilator response to intra-arterial injections of SNP in the hindlimb vascular bed of the rat (Fig. 8C). The effect of sodium meclofenamate on the hindlimb vasodilator response to ACh was investigated and, following an injection of the cyclooxygenase inhibitor in a dose of 5 mg/kg iv, vasodilator responses to ACh were not significantly changed (Fig. 9, A and B).

Fig. 5. The effect of L-NAME (25–100 mg/kg iv) and SNP infusion on the magnitude (A), duration (B), and area of the curve (C) of the increase in hindlimb blood flow in response to ACh in the rat. *P < 0.05, significantly different than control; n, number of experiments.

Fig. 4. The effect of meclofenamate (5 mg/kg iv; A), atropine (1 mg/kg iv; B), and 1-aminobenztriazole (1-ABT; 50 mg/kg ip or iv; C) on decreases in systemic arterial pressure and on duration of the response to ACh in the rat. *P < 0.05, significantly different than control; n, number of experiments.
Effect of repeated injections of ACh. The effect of repeated injections of ACh was investigated to determine whether responses decreased over time in animals treated with L-NAME or 1-ABT. Decreases in systemic arterial pressure in response to ACh or response duration and increases in hindlimb blood flow or response duration were not changed by repeated injections of ACh and by time over a 5-h period in animals treated with L-NAME (25–100 mg/kg iv; Fig. 10) or 1-ABT (50 mg/kg ip or iv; Fig. 11).

DISCUSSION

The results of the present study indicate that responses to ACh are complex and that more than one mechanism is involved in mediating responses to ACh in the rat. The present results show that the NOS inhibitor L-NAME decreases the duration of the systemic vasodepressor and hindlimb vasodilator response to ACh but does not have a major effect on response magnitude when baseline values are restored with a NO donor infusion. The results showing that L-NAME had a minor effect on response magnitude and that the decrease in the duration of the systemic depressor response to ACh could be prevented by the administration of excess L-arginine (the NOS substrate) provide support for the hypothesis that the sustained component of the ACh response is dependent on NO formation (1). The results showing that the initial peak transient response to ACh is L-NAME resistant are consistent with previous studies in the literature (1, 5, 33). It has been reported that when NO and prostaglandin synthesis are inhibited, ACh-induced vasodilation is associated with smooth muscle hyperpolarization (28, 29, 33, 35). It has been hypothesized that the initial component of the vasodilator response to ACh in the rat involves the release of an EDHF and that the response is mediated by the opening of intermediate and small-conductance KCa channels (33). The intermediate- and small-conductance KCa channels can be blocked by ChTX and apamin (3, 10, 17, 33, 36). The EDHF that is blocked by ChTX and apamin has been well studied in isolated blood vessels from the rat, and it is possible to administer ChTX and apamin into the regional vascular bed of the rat without a major effect on systemic arterial pressure (10, 14, 17, 33). It has been reported that L-NAME and indomethacin reduced the ACh-induced increase in hindlimb vascular conductance by 40% in the rat. However, the infusion of ChTX and apamin in L-NAME-treated animals reduced the ACh response by 90% (33). It has also been reported that apamin and ChTX inhibited systemic vasodilator responses to ACh in L-NAME-treated rats (14).

In the present study, L-NAME had no significant effect on the initial peak increase in hindlimb blood flow in response to ACh but reduced the duration of the vasodilator response when baseline blood flow was restored with a SNP infusion after the administration of the NOS inhibitor. The infusion of ChTX and apamin into the hindlimb vascular bed reduced the initial or
peak increase in blood flow in response to ACh without altering baseline blood flow but had no significant effect on the duration of the vasodilator response. Moreover, when L-NAME, ChTX, and apamin were administered together, the initial peak increase and the duration of the hindlimb vasodilator response to ACh were significantly reduced without an effect on the response to SNP. These data provide support for the hypothesis that the initial peak increase in hindlimb blood flow in response to ACh is dependent on the release of an EDHF, which mediates the initial phase of the vasodilator response that, in a large part, determines response magnitude. These data also provide support for the hypothesis that the sustained component of the ACh response, which is reduced by L-NAME, is mediated by the release of NO (1, 5, 33). The results of the present study in the hindlimb vascular bed are consistent with previous results and with the hypothesis that vasodilator responses to ACh in the rat hindlimb are mediated by at least two mechanisms involving ChTX-apamin- and L-NAME-sensitive components (33). The effects of L-NAME on vasodepressor and vasodilator responses to bradykinin have been investigated. The NOS inhibitor L-NAME in doses of 50–100 mg/kg iv had very little, if any, effect on decreases in systemic arterial pressure in response to intravenous injections of bradykinin in the rat when baseline values were restored with an intravenous SNP infusion (12). The hindlimb vasodilator response to bradykinin was significantly attenuated by the infusion of ChTX and apamin into the hindlimb vascular bed (12). These data suggest that responses to bradykinin are mediated for the most part by a NO-independent mechanism in the rat hindlimb vascular bed (12). The results of the present study suggest that responses to bradykinin and ACh in the rat may be mediated by different mechanisms in respect to the role of NO. The results of the present study suggest that responses to ACh are mediated by NO-dependent and -independent mechanisms and indicate that responses to bradykinin and ACh in the rat may be mediated by different mechanisms in respect to the role of NO. The results of the present study with sodium meclofenamate indicate that cyclooxygenase products, including PGI2, have no significant role in mediating systemic vasodepressor or hindlimb vasodilator responses to muscarinic receptor stimulation in the anesthetized rat. The observation that the L-NAME-sensitive component of the depressor response to ACh is not altered by a cyclooxygenase inhibitor and is reversed by excess L-arginine, which is hypothesized to overcome the NOS inhibition, is consistent with the hypothesis that the sustained component of the response is mediated by NO and that cyclooxygenase products are not involved (1, 5, 33). However, the identity of the mediator of the initial ChTX-apamin-sensitive component has not yet been determined (33).

Fig. 7. The effect of an intra-arterial infusion of ChTX and apamin on the magnitude (A) and duration (B) of the hindlimb vasodilator response to intra-arterial injections of ACh and SNP in L-NAME-treated (25 mg/kg iv) animals. *P < 0.05 compared with control; n, number of experiments.
The EDHF that is blocked by ChTX-apamin is well characterized and has been attributed to KCa channels and the involvement of myoendothelial gap junctions (8, 10, 11, 17, 35). The EETs are endothelium-derived cytochrome P-450 epoxygenase metabolites of arachidonic acid (34). It has been reported that the EETs hyperpolarize vascular smooth muscle and have vasodilator activity, and it has been hypothesized that the EETs act as a EDHF (4, 6, 9, 18, 23, 27, 40). It has also been reported that the EETs play a significant role in mediating vasodilator responses to ACh in some vascular beds (4, 15, 25, 32, 37). However, a role for the EETs in mediating vasodilator responses varies with species and the vascular bed studied and has not been studied in the hindlimb vascular bed in the rat (10, 11, 18, 33, 36).

In the present study, the role of the cytochrome P-450-epoxygenase pathway products in mediating responses to ACh was investigated using pathway inhibitors (34). Miconazole and 1-ABT have been reported to inhibit the P-450 epoxygenase pathway and were used alone and in combination in the present study (3, 27, 34, 35). The administration of 1-ABT in
A dose of 50 mg/kg ip, which has been reported to reduce the formation of the EETs in the rat kidney, liver, and lung (33), was without significant effect on the magnitude or duration of the systemic vasodepressor or hindlimb vasodilator response to ACh in the rat. The antimycotic agent miconazole, which has also been reported to reduce EET formation, had no significant effect on the vasodepressor or hindlimb vasodilator responses to ACh (27, 34). The administration of 1-ABT and miconazole in combination in the same experiment had no significant effect on the vasodepressor or hindlimb vasodilator responses to ACh. It has been reported that it may take considerable time to reduce tissue EET pools after the administration of a cytochrome P-450 inhibitor (34). To allow time for tissue EET depletion to occur and to enhance the depletion process, responses to ACh were examined at periods up to 5 h after the administration of the P-450 inhibitor, and in the first hour after inhibitor administration, 10–14 sequential ACh injections were made to promote the depletion of tissue pools. The results of these experiments show that the magnitude and duration of the systemic vasodepressor and hindlimb vasodilator responses to ACh are not changed over a 5-h period when responses are studied using a ACh-repeated injection protocol in an effort to deplete tissue EET pools after the administration of a cytochrome P-450 inhibitor.

The results of the present study do not provide evidence in support of a role for the cytochrome P-450 epoxygenase pathway in mediating the initial or sustained component of the
systemic vasodepressor or hindlimb vasodilator response to ACh in the rat. These results are in agreement with studies in other vascular beds in the literature (10, 11, 18, 36). The reason for the difference in results is uncertain but may involve differences in species or in the regional vascular bed studied.

It has been reported that responses to NO-dependent vasodilator agents exhibit tachyphylaxis after the administration of a NOS inhibitor (13). It has been suggested that the tachyphylaxis is due to a depletion of a stored NO pool (13). However, in the present study, responses to ACh do not exhibit tachyphylaxis in the systemic and hindlimb vascular beds of the rat. These data do not provide evidence in support of the hypothesis that NO released by ACh is derived from a stored pool in the endothelium (13). It is possible that l-NAME may not completely inhibit NO production in the present study. However, several studies in the literature show complete blockade of NO responses with similar doses of NOS inhibitors (25, 32, 33, 35). In addition, in the present study, l-NAME in doses as high as 300 or 400 mg/kg iv (data not shown) did not produce any further decrease in the magnitude or duration of the vasodepressor response to ACh beyond that observed with the dose of 100 mg/kg iv. It has been reported that NO inhibits NOS activity (22). It is, therefore, possible that the use of a NO donor infusion after l-NAME administration to restore baseline values may provide additional support for the premise that NOS is inhibited in the present study. It has been suggested that endogenous peroxide can serve as a key endothelial-derived factor in regulating vascular smooth muscle tone (38). The results of the present study show that ebselen a glutathione peroxidase mimic, significantly attenuated the vasodepressor response to ACh in l-NAME-treated animals. These data may be interpreted to suggest that the l-NAME-resistant vasodepressor response to ACh is mediated by endogenous peroxide. However, more data with other forms of catalase suitable for in vivo experiments are needed before an endogenous peroxide mechanism can be established.

In conclusion, the results of the present study using a NO donor infusion to restore baseline values after the administration of a NOS inhibitor show that the response to ACh has an initial transient and sustained phase. L-NAME had little, if any, effect on the initial peak increase in blood flow or decrease in systemic arterial pressure in response to ACh but shortened the duration of the response, an effect on the vasodepressor response that could be prevented by excess L-arginine. The initial phase of the vasodilator response could be attenuated by the local administration of ChTX and apamin, and both phases of the ACh hindlimb vasodilator response could be attenuated by ChTX-apamin and l-NAME. 1-ABT and miconazole, cytochrome P-450 inhibitors, alone and in combination and when used in a protocol designed to deplete tissue EET pools had no measurable effect on the vasodilator or vasodepressor response to ACh. The cyclooxygenase inhibitor sodium meclofenamate had no significant effect on responses to ACh and provides no evidence in support of a role for cyclooxygenase products. The present data provide support for the hypothesis that the hindlimb vasodilator response to ACh is dependent on the release of a EDHF, which opens KCa channels and mediates the initial phase that determines response magnitude, and on the release of NO, which mediates the sustained phase of the response. The results of the present study do not provide evidence in support of a role for cytochrome P-450 products in mediating the initial or sustained component of the response to ACh in the systemic and hindlimb vascular bed in the rat. The present data

Fig. 11. Effect of repeated injections of ACh (10–14 injections in 45 min) on the magnitude and duration of decreases in systemic arterial pressure in response to intravenous injections of ACh (A and B) and increases in hindlimb blood flow and duration in blood flow in response to injections of ACh at 1, 3, and 5 h after ACh (C and D) in 1-ABT-treated animals (50 mg/kg iv or ip). n, Number of experiments.
suggest that the L-NAME-resistant vasodepressor response to ACh may involve a peroxide-sensitive mechanism.

GRANTS

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