des-Arg⁹-Bradykinin Produces Tone-Dependent Kinin B1 Receptor–Mediated Responses in the Pulmonary Vascular Bed

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Abstract Responses to des-Arg⁹-bradykinin, a selective kinin B1 receptor agonist, were characterized in the pulmonary vascular bed of the intact-chest cat. Injections of des-Arg⁹-bradykinin into the perfused lobar artery under low–resting tone conditions caused dose-related increases in lobar arterial pressure; whereas in the same experiment under elevated tone conditions, injections of the B1 agonist caused dose-related decreases in lobar arterial pressure. Vasconstrictor responses to des-Arg⁹-bradykinin under low-tone conditions and vasodilator responses under elevated-tone conditions were antagonized by des-Arg⁹-[Leu⁸]-bradykinin, a kinin B1 receptor antagonist, whereas responses under low- and high-tone conditions were not altered by Hoe 140, a kinin B2 receptor antagonist. Vasconstrictor responses to des-Arg⁹-bradykinin under low-tone conditions were attenuated by phentolamine, prazosin, and reserpine but not by sodium meclofenamate, suggesting that release of catecholamines and activation of α-adrenergic receptors are involved. Pulmonary vasodilator responses under elevated-tone conditions were inhibited by N⁶-nitro-L-arginine methyl ester, suggesting that des-Arg⁹-bradykinin stimulates the release of nitric oxide, whereas meclofenamate and U-37883A, a nonsulfonamide ATP-sensitive K⁺ channel antagonist, did not alter vasodilator responses to the B1 receptor agonist. These results suggest that vasodilator responses to des-Arg⁹-bradykinin under low-tone conditions are mediated by the activation of kinin B1 receptors, the release of catecholamines within the lung, and the activation of α-adrenergic receptors, whereas vasodilator responses under elevated tone conditions are mediated by activation of B1 receptors and the release of nitric oxide from the endothelium. These data provide pharmacologic evidence for the existence of functionally active kinin B1 receptors that mediate tone-dependent vasconstrictor and vasodilator responses in the pulmonary vascular bed of the cat. (Circ Res. 1994;75:1064-1072.)

Key Words • des-Arg⁹-bradykinin • Hoe 140 • pulmonary vascular bed • kinin B1 receptor • nitric oxide

Decreases in blood pressure in the dog in response to injections of crude kinin extracts were first described in 1909.¹ It has been established that substrate kininogens are cleaved by tissue and plasma kallikreins to form the nonapeptide bradykinin (BK).²,³ BK is rapidly cleaved by kininase II (EC 3.4.15.1), a dipeptidyl carboxypeptidase shown to be identical to angiotensin I–converting enzyme, as well as by other peptidases on passage through the lung.⁴ However, BK can also be cleaved by kininase I (K1, EC 3.5.17.3),⁵ K1 accounts for 90% of kinin destruction in plasma and is a family of two carboxypeptidases, carboxypeptidase N (KI-CPN) and carboxypeptidase M (KI-CPM), which act to cleave the carboxy-terminal arginine of BK, resulting in the formation of des-Arg⁹-bradykinin (DABK).³,⁵,⁷ K1-CPN is synthesized by the liver and released into the circulation, whereas K1-CPM is a membrane-bound enzyme that is located in the human kidney, lung, fibroblast, pulmonary artery endothelial cells, and placenta.⁶ Inasmuch as K1 is specific for degrading arginine bonds, K1 is not capable of further BK degradation, so that increased levels of DABK occur in plasma.⁵,⁹ The release of arginine by K1 could provide substrate for the formation of endothelium-derived nitric oxide by nitric oxide synthase.⁸,¹⁰-¹²

Received May 31, 1994; accepted August 22, 1994.
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There is considerable pharmacologic evidence in support of the concept of BK receptor heterogeneity.²,³,¹⁰,¹⁴ The classic BK receptor subtypes have been characterized as B1 and B2, and the action of BK on the B2 receptor is thought to mediate the majority of physiologic responses to the kinins.²,³ The B1 receptor is selectively stimulated by DABK on the basis of high-affinity contractile activity of the peptide in the rabbit aorta, mesenteric vein, and basilar artery; however, this receptor is not believed to play an important role under physiologic conditions.²,³,¹⁴ The development of competitive, potent, selective kinin receptor antagonists has provided support for the identity of B1 receptors.²,³ DABK does not activate B2 receptors but stimulates kinin B1 receptors, and a change in the balance between levels of BK and its metabolite, DABK, could change the emphasis and effects of the kinin system.²,³,¹⁵

Although B1 receptors are not thought to be physiologically important in mediating cardiovascular responses to kinins, such as increased vascular permeability, smooth muscle contraction, vasodilation, and hypotension, they have recently been shown to play a role in mediating decreases in systemic arterial pressure in the dog under physiologic conditions.¹⁶-¹⁹ Kinin B1 receptors are also thought to coexist with B2 receptors and are upregulated in response to pathological stimuli.³,¹⁹-²⁴ Kinin B1 receptors have also been reported to promote cell division and secretion of collagen by fibroblasts.²⁵ However, responses to DABK in the cat have not been documented, and little, if anything, is known about the presence of kinin B1 receptors in the pulmonary vascular bed. Therefore, the present study was undertaken to investigate responses to
DABK and the mechanism by which the kinin B1 receptor agonist induces changes in vascular resistance in the pulmonary vascular bed of the cat under conditions of controlled pulmonary blood flow.

Materials and Methods

Ninety-eight adult cats unselected as to sex, weighing 2.4 to 5.4 kg, were sedated with ketamine hydrochloride (10 to 15 mg/kg IM) and were anesthetized with sodium pentobarbital (30 mg/kg IV). The animals were strapped in the supine position to a fluoroscopic table, and supplemental doses of anesthetic were administered as needed to maintain a uniform level of anesthesia. The trachea was intubated with a cuffed pediatric endotracheal tube, and the animals spontaneously breathed room air enriched with 95% O2/5% CO2. Systemic arterial (aortic) pressure was measured from a catheter inserted into the aorta from a femoral artery, and intravenous injections were made into a catheter positioned in the inferior vena cava from a femoral vein.

For perfusion of the left lower lung lobe, a specially designed 28-cm 6F triple-lumen balloon perfusion catheter (Arrow International, Inc) was passed under fluoroscopic guidance (Picker C-Arm) from the left external jugular vein into the artery to the left lower lobe. After the cats had been heparinized (1000 U/kg IV), the lobar artery was isolated by distension of the balloon cuff on the perfusion catheter. The lobe was then perfused by way of the catheter lumen beyond the balloon cuff with blood from a femoral artery with a perfusion pump (model 1210, Harvard Instrument Co). Lobar arterial pressure was measured from a second port 5 mm from the cuff on the perfusion catheter. The perfusion rate was adjusted so that lobar arterial perfusion pressure approximated mean pressure in the main pulmonary artery and was not changed thereafter during the experiment. The perfusion rate ranged from 25 to 49 mL/min in these experiments. The agonists were injected in small volumes directly into the perfusion circuit distal to the pump in a random sequence. Left atrial pressure was measured with a 6F double-lumen catheter (Arrow International, Inc) or a 6F radiopaque polyethylene catheter (Cook, Inc) passed transeptally into the vein draining the left lower lobe. The catheter tip was positioned so that the left atrial pressure port on the distal lumen was 1 to 2 cm into the lobar vein, and the second catheter port was near the venoatrial junction. When necessary, blood could be withdrawn or infused through this second catheter lumen to maintain constant left atrial pressure. Left atrial pressure ranged from 2 to 5 mm Hg and was not changed by DABK and BK.

In the first series of experiments, responses to DABK were investigated when lobar vascular resistance was at low–resting tone conditions (lobar arterial pressure ranged from 10 to 16 mm Hg). The range of doses of DABK was established in pilot experiments and was 0.1 to 1 µg. The relative potency of vasconstrictor responses to DABK was assessed by comparing responses with serotonin, U46619, angiotensin II, and BK. In the next series of experiments, responses to DABK were obtained in 10-minute intervals to determine whether responses were reproducible and stable.

In the second series of experiments, the inhibitory effects of the kinin B1 receptor agonist des-Arg9-[Leu]-BK, BK, and angiotensin II were investigated. In the next series of experiments, the effects of Hoe 140, a potent BK B2 receptor agonist, were investigated on responses to DABK, BK, and angiotensin II. In the next series of experiments, the effects of Hoe 140, a potent BK B2 receptor agonist, were investigated on responses to DABK, BK, and serotonin. deArg9-[Leu]-BK had no significant sustained effect on lobar arterial pressure.

In the next series of experiments, the effects of sodium meclofenamate on responses to DABK, BK, and arachidonic acid were compared before and after the administration of sodium meclofenamate (2.5 mg/kg IV). The cyclooxygenase inhibitor produced small (1 to 4 mm Hg) increases in lobar arterial pressure. In the fifth series of experiments, vasoconstrictor responses to DABK, norepinephrine, and U46619 were compared after the administration of the α-receptor blocking agent phentolamine (1 mg/kg IV) and prazosin (0.2 mg/kg IV), and the effects of reserpine (1 to 1.5 mg/kg IV) on the response to DABK were investigated. The extent of reduction of the pressor response to tyramine was used as an index for the extent of catecholamine depletion. Phentolamine, prazosin, and reserpine produced small (1 to 3 mm Hg) decreases in lobar arterial pressure. To determine whether DABK was releasing catecholamines within the pulmonary vascular bed or from the adrenal medulla and other peripheral sites, responses were compared when the standard perfusion circuit was used and when a 1-minute delay coil was added to the perfusion circuit.

In other experiments, responses to DABK were investigated when lobar vascular resistance was elevated by infusion of the stable prostaglandin/endothelium analogue U46619. In these experiments, under elevated-tone conditions, lobar arterial pressure was increased to the same level during the control and treatment periods. The range of doses of DABK was established in pilot experiments and was 0.1 to 3 µg. The potency of vasodilator responses to DABK was assessed by comparing responses with BK. In a second series of experiments, the effects of kinin B1 receptor antagonist des-Arg9-[Leu]-BK, BK, and (S)-5-methoxytryptamine (S)-5-methoxytryptamine were compared after the administration of DABK (1 to 3 µg/kg IV) and nitric oxide. In the third series of experiments, the effects of Hoe 140 were investigated on responses to DABK, BK, and nitric oxide.

In the fourth series of experiments, the effects of captopril (4 mg/kg IV) on responses to DABK and BK were compared to determine whether both peptides were inactivated by kininase II (angiotensin-converting enzyme). Captopril was administered as a slow intravenous bolus.

In the next series of experiments, the effects of sodium meclofenamate were studied under elevated-tone conditions, and responses to DABK, BK, and arachidonic acid were compared before and after the administration of sodium meclofenamate (2.5 mg/kg IV). In this series of experiments, the effects of U-37883A on DABK, BK, and lemakalim were investigated. In another series of experiments, vasodilator responses to DABK were compared when lobar vascular arterial pressure was elevated with U46619 alone (15 to 200 ng/min) and when tone was increased by the administration of N'-nitro-l-arginine methyl ester (L-NNAME, 100 mg/kg IV), followed by an intralobar infusion of BK (8 to 10 µg/min) in these experiments, control responses to the vasodilator agents were obtained when lobar arterial pressure was raised to an average value of 35±2 mm Hg with U46619 infused directly into the perfused lobar artery. The U46619 infusion was terminated, and lobar arterial pressure was permitted to return to near control value. L-NNAME (100 mg/kg IV) was then administered as a slow intravenous bolus. After 20 to 30 minutes, the infusion of U46619 was resumed at a rate (20 to 200 ng/min) sufficient to raise lobar arterial pressure to 35±3 mm Hg, and responses to vasodilator agents were again determined. In all experiments under elevated-tone conditions, vasodilator responses were compared when baseline lobar arterial pressure was at similar levels during the control period (32 to 40 mm Hg) and during the treatment (32 to 39 mm Hg).

Sodium meclofenamate (Warner-Lambert–Parke-Davis), acetycholine chloride, BK, sodium arachidonate, DABK, des-Arg9-[Leu]-BK, serotonin creatinine sulfate, norepinephrine hydrochloride, and tyramine hydrochloride (Sigma Chemical Co), and captopril (Bristol-Myers Squibb) were dissolved in saline. Nitric oxide solutions were prepared by first bubbling 20 mL of reagent grade methanol (EM Industries, Inc) for 20 minutes with nitrogen in a gastight vial. The doses of nitric oxide administered are expressed as volume of nitric oxide solution injected. A gastight syringe was used to inject the nitric oxide solution into the perfusion circuit. The methanol
vehicle for the nitric oxide solution had no consistent effect on lobar arterial pressure when injected into the perfused lobar artery in volumes of 30 or 100 μL. The thromboxane A2 receptor agonist U46619 (9,11-dideoxy-11α,9α-epoxymethanoprostaglandin F2α, Upjohn), was dissolved in 100% ethanol at a concentration of 10 mg/mL, and further dilutions were made in normal saline. All drugs were kept frozen or on ice in brown glass bottles, and working solutions were prepared on a frequent basis. Hoe 140 (Hoechst-Roussel Pharmaceuticals), L-NAME (Sigma), prazosin hydrochloride (Pfizer), and phen tolamine mesylate (CIBA-GEIGY) were dissolved in normal saline. Reserpine (Serpasil, CIBA-GEIGY) was dissolved in 20% ethanol and saline. U-37883A (Upjohn) was dissolved in saline solution and sonicated.

Blood gases and pH were measured with a Corning model 178 analyzer and were in the normal range: pH, PCO2, and PO2 averaged 7.39±0.3, 32.3±0.4 mm Hg, and 108±2 mm Hg, respectively, during the control period. All hemodynamic data are expressed in absolute units and presented as mean±SEM. Responses represent peak changes, unless otherwise noted. The data were analyzed by a one-way ANOVA and Scheffe’s F test or Student’s t test.28 A value of P<.05 was used as the criterion for statistical significance.

Results

Responses to DABK Under Low–Resting Tone Conditions

Under baseline conditions, when tone in the pulmonary vascular bed was at resting levels (12 to 16 mm Hg), injections of DABK in doses of 0.1 to 1 μg into the perfused lobar artery caused dose-related increases in lobar arterial pressure, whereas injections of BK in doses of 0.3 and 1 μg in these same experiments had no significant effect on lobar arterial pressure (Fig 1). Pressor responses to DABK under low-tone conditions were not diminished when the kinin B1 receptor agonist was injected in a dose of 1 mg at 10-minute intervals, suggesting that tachyphylaxis to the pressor response does not develop when the peptide is injected at 10-minute intervals (data not shown). In terms of relative vasoconstrictor activity in the pulmonary vascular bed, DABK was more potent than serotonin, less potent than the thromboxane A2 mimic (U46619), and only slightly less potent than angiotensin II when doses were compared on a nanomolar basis (Fig 1). Increases in lobar arterial pressure in response to DABK under low-tone conditions were inhibited by the kinin B1 receptor antagonist des-Arg9-[Leu1]-BK, administered in a dose of 10 μg, followed by an infusion of 4 to 8 μg/min into the perfused lobar artery (Fig 2). des-Arg9-[Leu1]-BK was without significant effect on pulmonary vasoconstrictor responses to norepinephrine, serotonin, U46619, or angiotensin II (Fig 2). Increases in lobar arterial pressure in response to DABK were not altered by the kinin B2 receptor antagonist Hoe
140 in a dose of 100 μg/kg IV, and Hoe 140 did not alter the pressor response to serotonin (Fig 3A). The role of cyclooxygenase product release in mediating or modulating pressor responses to the B1 receptor agonist was investigated; after the administration of sodium meclofenamate in a dose of 2.5 mg/kg IV, increases in lobar arterial pressure in response to DABK were increased significantly, whereas the pressor response to the prostaglandin precursor, arachidonic acid, was decreased significantly (Fig 3B).

It has been reported that DABK can release catecholamines, and to determine whether catecholamine release and activation of α-receptors are involved in mediating the pressor response to DABK in the pulmonary vascular bed, the effects of phentolamine, prazosin, and reserpine were investigated. These results are summarized in Fig 4. After the administration of phentolamine in a dose of 1 mg/kg IV, increases in lobar arterial pressure in response to DABK were reduced significantly (Fig 4A). Phentolamine decreased the pressor response to norepinephrine in the pulmonary vascular bed but was without effect on the increase in lobar arterial pressure in response to an injection of U46619 (Fig 4A). Prazosin, a selective α1-adrenergic receptor antagonist, in a dose of 0.2 mg/kg also reduced pressor responses to DABK and norepinephrine but did not alter the pressor response to U46619 (Fig 4C).

In experiments in which the influence of reserpine on the response to DABK was investigated, responses to DABK, tyramine, and U46619 were determined beginning 45 to 60 minutes after the injection of reserpine (1 to 1.5 mg/kg) over a 10-minute period into the femoral vein. After treatment with reserpine, increases in lobar arterial pressure in response to DABK and tyramine were reduced significantly, whereas the pressor response to U46619 was not altered (Fig 4B).

To determine whether DABK releases catecholamines within the lung or from peripheral organs, the
effect of a 1-minute-delay coil on the time course of the pressor response to the peptide was investigated, and these results are summarized in Fig 5. The time course of the increase in lobar arterial pressure in response to DABK was similar when the peptide was injected in a dose of 1 μg into the perfused lobar artery, when the standard perfusion circuit was used, or when the 1-minute-delay coil was positioned in the perfusion circuit (Fig 5A and 5B).

Responses to DABK Under Elevated-Tone Conditions

Under elevated-tone conditions, when lobar arterial pressure was increased to a high steady level (32 to 41 mm Hg) with U46619, intralobar injections of DABK in doses of 0.1 to 3 μg caused dose-related decreases in lobar arterial pressure (Fig 6). Decreases in lobar arterial pressure in response to DABK under elevated-tone conditions were not diminished when the kinin B1 receptor agonist was injected in a dose of 1 μg at 10-minute intervals (data not shown). In terms of relative vasodilator activity in the pulmonary vascular bed of the cat, BK was approximately threefold more potent than DABK in decreasing lobar arterial pressure (Fig 6). The effects of the kinin B1 receptor antagonist des-Arg9,[Leu]^t^-BK on decreases in lobar arterial pressure in response to DABK were investigated, and these data are summarized in Fig 7A. After the administration of the kinin B1 receptor antagonist in a dose of 10 μg, followed by an infusion at 4 to 8 μg/min into the perfused lobar artery, decreases in lobar arterial pressure in response to DABK were reduced significantly (Fig 7A). Although des-Arg9,[Leu]^t^-BK decreased vasodilator responses to DABK, the kinin B1 receptor antagonist was without significant effect on the pulmonary vasodilator response to BK or to nitric oxide (Fig 7A). The kinin B2 receptor antagonist Hoe 140 was without significant effect on the decrease in lobar arterial pressure in response to DABK when administered in a dose of 100 μg/kg IV (Fig 7B). Hoe 140 (100 μg/kg IV) significantly decreased the vasodilator response to BK, but not to nitric oxide, in the pulmonary vascular bed of the cat (Fig 7B).

BK is inactivated by angiotensin-converting enzyme (kininase II), whereas DABK is not reported to be a substrate for the enzyme. In the present experiments, the effects of the angiotensin-converting enzyme inhibitor captopril on pulmonary vasodilator responses to DABK and BK were compared, and these results are summarized in Fig 8. Decreases in lobar arterial pressure and the time required for lobar arterial pressure to return to one-half baseline (preresponse) value (T1/2) of the vasodilator response to DABK were not changed after the administration of captopril (4 mg/kg IV) (Fig 8). However, captopril significantly enhanced the decrease in lobar arterial pressure and the T1/2 of the decrease in lobar arterial pressure in response to BK but did not alter the vasodilator response to nitric oxide (Fig 8).

The role of nitric oxide release in mediating the pulmonary vasodilator response to DABK was investigated, and these data are summarized in Fig 9. After the administration of L-NAME in a dose of 100 mg/kg IV, decreases in lobar arterial pressure in response to DABK were reduced significantly (Fig 9A). Pulmonary vasodilator responses to BK were also decreased significantly, and the vasodilator response to nitric oxide was increased significantly after treatment with L-NAME.
(Fig 9A). To determine whether the release of vasodilator products in the cyclooxygenase pathway is involved in mediating the pulmonary vasodilator response to the kinin B1 receptor agonist, the effects of sodium meclofenamate on decreases in lobar arterial pressure in response to DABK were investigated. After the administration of sodium meclofenamate at a dose of 2.5 mg/kg IV, decreases in lobar arterial pressure in response to DABK and to BK were not altered (Fig 9B).

The role of ATP-sensitive K⁺ (K<sub>ATP</sub>) channel activation in mediating the pulmonary vasodilator response to DABK was investigated, and these data are summarized in Fig 10. After the administration of U-37883A, a novel K<sub>ATP</sub> channel blocking agent, at a dose of 5 mg/kg IV, vasodilator responses to DABK and BK were not altered, whereas the pulmonary vasodilator response to lemakalim, a K<sub>ATP</sub> channel agonist, was reduced significantly (Fig 10).

**Discussion**

Results of the present study show that DABK, a kinin B1 receptor agonist, increases lobar arterial pressure when injected into the perfused lobar artery under normal–resting tone conditions. The present data also show that injections of DABK decrease lobar arterial pressure when tone in the pulmonary vascular bed has been raised to a high steady level. The increases in lobar arterial pressure under low-tone conditions and the decreases under elevated-tone conditions are antagonized by des-Arg<sup>9</sup>[Leu<sup>8</sup>] BK, a kinin B1 receptor antagonist, whereas Hoe 140, a kinin B2 receptor antagonist, did not alter responses to DABK under low- or high-tone conditions.27,28 The inhibitory effects of des-Arg<sup>9</sup>[Leu<sup>8</sup>] BK on responses to DABK were selective in that vasoconstrictor and vasodilator responses to agonists that act on different membrane receptors in the pulmonary vascular bed were not altered by the kinin...
B1 receptor antagonist, des-Arg^9,[Leu]^1-BK did not alter vasodilator responses to BK, and DABK did not induce tachyphylaxis when responses were elicited at 10-minute intervals. These data suggest that the inhibitory effects of des-Arg^9,[Leu]^1-BK on responses to DABK were selective and that tachyphylaxis to DABK was not a factor in the present studies. The present data demonstrate that DABK induces tone-dependent pulmonary vasoconstrictor and vasodilator responses that can be elicited in the same experiment and suggest that the tone-dependent responses to DABK are mediated by the activation of kinin B1 receptors in the pulmonary vascular bed of the cat. To our knowledge, this is the first pharmacologic demonstration of the presence of kinin B1 receptors that mediate tone-dependent responses in the pulmonary vascular bed. These data are consistent with the results of a recent study in which DABK was shown for the first time to decrease systemic arterial pressure in the anesthetized dog under normal physiologic conditions. Hypotensive responses to DABK have been extensively documented in pathologic states, such as after the administration of Escherichia coli endotoxin. Moreover, it has also been demonstrated recently that the isolated canine renal artery contains functional kinin B1 receptor, mediating vasorelaxation. The results of the present investigation extend the work of previous studies demonstrating the presence of kinin B1 in the systemic vascular bed of the dog and in isolated renal arteries by providing evidence for the existence of functional kinin B1 receptors, which mediate vasoconstriction and vasodilation in the pulmonary vascular bed of the cat.

In terms of relative pulmonary vasoconstrictor activity, DABK was only slightly less potent than angiotensin II in its ability to increase lobar arterial pressure, and in terms of relative vasodilator activity, DABK was only slightly less potent than BK in the pulmonary vascular bed. In addition to providing functional evidence in support of the presence of kinin B1 receptors, which elicit tone-dependent responses in the pulmonary vascular bed, the present data show that the kinin B1 receptor–mediated vasoconstrictor response is inhibited by phenolamine and prazosin but not by sodium meclofenamate. The inhibitory effects of phenolamine and prazosin were selective in that the pressor response to norepinephrine was decreased but that the response to U46619 was not altered. Treatment with reserpine inhibited the vasoconstrictor response to DABK and to tyramine, an indirect-acting sympathomimetic amine. These data provide support for the hypothesis that the pulmonary vasoconstrictor response to DABK is dependent on the release of catecholamines. The site of release of catecholamine by DABK was localized in experiments in which a 1-minute-delay coil was imposed.

**Fig 9.** A, Bar graphs showing the effect of N^ω^-nitro-L-arginine methyl ester (L-NAME, 100 mg/kg IV) on decreases in lobar arterial pressure in response to des-Arg^9-bradykinin, bradykinin, and nitric oxide solution under elevated tone conditions. B, Bar graphs showing the effect of sodium meclofenamate (2.5 mg/kg IV) on decreases in lobar arterial pressure in response to des-Arg^9-bradykinin and bradykinin under elevated-tone conditions. n indicates the number of experiments. *Significantly different from the control value.

**Fig 10.** Bar graphs showing the influence of U-37883A, a new nonsulfonylurea ATP-sensitive K^+^ channel blocking agent, on vasodilator responses to des-Arg^9-bradykinin, bradykinin, and lemakalim. The ATP-sensitive K^+^ channel blocking agent was administered at a dose of 5 mg/kg IV. n indicates the number of experiments. *Significantly different from the control value.
in the perfusion circuit. The observation that the time course of the vasoconstrictor response to DABK was similar when the standard perfusion circuit and the 1-minute-delay coil were used suggests that the major site of catecholamine release resides within the pulmonary vascular bed and that release from peripheral sites and recirculation of catecholamines play little, if any, role in mediating the pressor response to DABK in the pulmonary vascular bed of the cat.

The results of experiments with phenolamine, prazosin, reserpine, and meclofenamate suggest that the pulmonary kinin B1 receptor-mediated vasoconstrictor response is dependent on intrapulmonary catecholamine release and activation of α-adrenergic receptors and does not involve the release of vasoconstrictor metabolites in the cyclooxygenase pathway. The observation that the vasoconstrictor response to DABK was enhanced by sodium meclofenamate may suggest that products in the cyclooxygenase pathway modulate the pressor response to the B1 receptor agonist under low-tone conditions. However, the nature of this interaction requires further study. Although DABK has significant vasoconstrictor activity in the pulmonary vascular bed of the cat, this activity is not shared by BK when the peptide is administered in a similar range of doses. The explanation for the differences in response to BK and DABK under low–resting tone conditions is uncertain.29,30 However, it is possible that the removal of arginine at the carboxy terminal may confer a catecholamine-releasing activity to the peptide.30

Vasodilator responses to DABK were not altered by the cyclooxygenase inhibitor sodium meclofenamate or by U-37883A, a K_ATP channel antagonist, but were reduced by L-NNAME in a dose that decreased vasodilator responses to BK but not to nitric oxide. It has been reported that L-NNAME blocks muscarinic receptors;31 however, studies in pulmonary and peripheral vascular beds of the cat show that the inhibitory effects of L-NNAME and nitro-L-arginine, an inhibitor that does not block muscarinic receptors, on responses to BK and acetylcholine are similar.32–35 These studies indicate that the predominant effect of L-NNAME is on nitric oxide synthase and that muscarinic receptor blockade plays little, if any, role in the pulmonary and systemic vascular bed in the cat.32–35 In terms of selectivity, studies in the pulmonary and systemic vascular beds of the cat indicate that L-NNAME does not impair vasodilator responses to agents that act by a variety of mechanistically distinct mechanisms.32–35 The results of studies with L-NNAME suggest that the pulmonary vasodilator response to DABK is mediated, at least in part, by the release of nitric oxide and that release of vasodilator prostaglandins or membrane hyperpolarization by way of K_ATP channel activation is not involved or plays a minimal role in mediating the response to the peptide. The results with U-37883A showing that vasodilator responses to lemakalim, but not to DABK or BK, are blocked suggest that this nonsulfonylurea K_ATP blocking agent may provide another useful probe for studying K_ATP-mediated vascular responses in the pulmonary vascular bed.36 The results with U-37883A are similar to results with glibenclamide and provide support for the hypothesis that BK does not dilate the pulmonary vascular bed by activating a K_ATP channel and hyperpolarizing resistance vessel elements.35 The observation that responses to BK are reduced only ∼50% by L-NNAME may suggest that nitric oxide synthase is not completely inhibited by the dose of L-NNAME used or that additional mechanisms may be involved in mediating the vasodilator response to BK in the pulmonary vascular bed. These mechanisms may involve membrane hyperpolarization by activating K+ channels other than the K_ATP channel. The results of the present studies suggest, however, that kinin B1 and B2 receptors mediating the release of nitric oxide are present on endothelial cells in the pulmonary vascular bed of the cat.34,35,37

Although the present data suggest that DABK increases lobar arterial pressure by releasing catecholamines in the lung and activating α-adrenergic receptors under low-tone conditions but decreases lobar arterial pressure by releasing nitric oxide under elevated-tone conditions, the exact mechanism by which the increase in tone changes the direction and mechanism of the response is uncertain. Since both pressor and depressor responses are reduced by des-Arg9,[Leu8]-BK, they are mediated by activation of kinin B1 receptors. It is possible that changes in tone could induce changes in the kinin B1 receptor signaling mechanism, so that under low-tone conditions, the release of catecholamines is dominant, whereas under elevated-tone conditions, the release of nitric oxide from the endothelium predominates. It has been established that BK is rapidly inactivated in the pulmonary vascular bed by angiotensin I–converting enzyme (kininase II).2,3,23,35 Moreover, in the present study the decreases in lobar arterial pressure in response to BK were enhanced by captopril, whereas the kininase II inhibitor was without significant effect on the pulmonary vasodilator response to DABK. These data are consistent with results of studies showing that the actions of kininase II on DABK are slower and of lower affinity than on BK.6,9

The biological relevance of the results of the present study is uncertain. However, it is possible that when angiotensin-converting enzyme is inhibited by agents such as captopril or enalapril, BK levels will increase in the plasma. This would provide more substrate for K1 and further increase DABK levels in the blood. Therefore, it is possible that DABK levels would be sufficient to exert a biological effect on the pulmonary circulation and that this response could be blocked by kinin B1 receptor antagonists, such as des-Arg9,[Leu8]-BK.38 Although results of the present studies suggest the presence of functional kinin B1 receptors in the pulmonary vascular bed of the cat, injections of DABK in doses up to 10 µg had no significant effect on perfusion pressure in the isolated rat lung and have only a small effect on perfusion pressure in the hindlimb circulation of the cat (J.A. Santiago, B.D. Nossaman, and P.J. Kadowitz, unpublished data). Moreover, responses to BK are different in the pulmonary vascular bed of the cat, where vasodilation was observed, and in the rat, where vasoconstriction was observed.38 These data suggest that the distribution of functional kinin B1 receptors is dependent on species and on regional vascular bed studied.

In summary, the present results show that under resting-tone conditions, DABK increases pulmonary vascular resistance, whereas under elevated-tone conditions, DABK decreases pulmonary vascular resistance. The vasoconstrictor response under low-tone conditions and the vasodilator response under elevated-tone conditions are blocked in a selective manner by des-Arg9,[Leu8]-BK, a kinin B1 receptor antagonist. The pressor response under low-tone conditions is attenuated by phenolamine, prazosin, and reserpine, suggesting that release of catechol-
amines and an α-receptor mechanism is involved. The depressor response under elevated-tone conditions is reduced by L-NAME, suggesting that it is mediated in part by the release of nitric oxide. These studies provide support for the hypothesis that functional kinin B1 receptors mediating tone-dependent responses are present in the pulmonary vascular bed of the cat.

Acknowledgments

This study was supported by National Institutes of Health grant HL-15580 and a grant from the American Heart Association. The authors thank Janice Ignarro for editorial assistance.

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_Circ Res._ 1994;75:1064-1072
doi: 10.1161/01.RES.75.6.1064

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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