Regulation of baseline vascular resistance in the canine diaphragm by nitric oxide

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1 The role played by nitric oxide (NO) in the regulation of blood flow to the canine isolated hemidiaphragm was evaluated by determining (a) the effects of the L-arginine analogues N^G-nitro-L-arginine methyl ester (L-NAME), N^G-nitro-L-arginine (L-NOARG), and argininosuccinic acid (ArgSA) on baseline vascular resistance and of the latter two agents on endothelium-dependent (acetylcholine, ACh) and endothelium independent (sodium nitroprusside, SNP) vasodilatation; (b) the effects of L- and D-arginine on baseline vascular resistance; and (c) the effects of L-glutamine, an inhibitor of intracellular recycling of L-citrulline to L-arginine, on baseline resistance and on the response to ACh and SNP.

2 L-NAME, L-NOARG and ArgSA (6×10^{-4} M final concentration) increased baseline diaphragmatic vascular resistance to a similar extent ($28.6 \pm 4.2\%$, $26.7 \pm 4.3\%$ and $32.8 \pm 4.6\%$ respectively). L-NOARG and ArgSA reversed the vasodilator effect of ACh but not of SNP.

3 L- and D-arginine had no effect on vascular resistance.

4 L-Glutamine (10^{-3} M) increased baseline vascular resistance by $10 \pm 1.9\%$ ($P < 0.05$) but did not alter responses to either ACh or SNP.

5 Basal NO release plays a role in the regulation of baseline diaphragmatic vascular resistance. L-Arginine analogues tested potently and specifically inhibited this process. Moreover, extracellular L-arginine appears to have no effect on baseline diaphragmatic vascular resistance.

Keywords: EDRF; nitroarginine; argininosuccinic acid; L-arginine; L-glutamine; blood flow; respiratory muscles

Introduction

Endothelial release of vasodilator substances, including nitric oxide (NO) has been shown to play a pivotal role in the regulation of blood flow in a number of vascular beds. The importance of the L-arginine-NO pathway in the regulation of resting vascular tone and as a mediator of endothelium-dependent vasodilatation has been evaluated by observing the effects of inhibiting nitric oxide synthase activity with L-arginine analogues. The effect of these false substrates, however, has varied among experimental preparations. While in the majority of studies, an increase in baseline vascular resistance and inhibition of endothelium-dependent dilatation have been observed, a few investigators have also described no inhibition of endothelium-dependent dilatation or non-selective inhibition of agonist-induced dilatation following the infusion of L-arginine analogues (Mugge *et al.*, 1991; Thomas *et al.*, 1989). Such discrepant results obtained in apparently similar experimental preparations indicate that conclusions drawn from these studies, must be restricted to the vascular bed under study. In addition, L-arginine analogues have been shown to differ in their potency (Vargas *et al.*, 1991), specificity (Thomas & Ramwell, 1988), reversibility by L-arginine (Thomas & Ramwell, 1992; Bogle *et al.*, 1992), method of endothelial cell entry (Bogle *et al.*, 1992) and mechanism of action (Gross *et al.*, 1990). Such pharmacological differences may, therefore, contribute to the conflicts which currently complicate interpretation of this literature.

The source of L-arginine on which the endothelial cell depends for substrate in the synthesis of NO also varies. In some isolated vessel preparations (Gold *et al.*, 1989; Kaley *et al.*, 1992) and in the pial circulation *in vivo* (Morikawa *et al.*, 1992) availability of extracellular L-arginine appears to limit basal NO formation. In other preparations (Fortes *et al.*, 1990; Gardiner *et al.*, 1990; Lahera *et al.*, 1990; Thomas *et al.*, 1989) administration of exogenous L-arginine has no

effect on vascular tone. These vessels presumably depend on intracellular synthesis of L-arginine and on the recycling of L-citrulline formed in the process of NO synthesis (Hecker *et al.*, 1990; Sessa *et al.*, 1990).

Failure to maintain substrate supply to the diaphragm in proportion to its metabolic requirements results in muscle failure (Macklem & Roussos, 1977; Supinski *et al.*, 1988). Study of the mechanisms by which diaphragm blood flow is regulated has, therefore, been of high priority among physicians seeking to develop rational treatment strategies for patients with respiratory failure (Aubier *et al.*, 1989). Since, for the reasons discussed above, the results obtained from previous studies cannot be extrapolated to the diaphragm, further progress in this field requires that the role of the nitric oxide pathway in this circulation be specifically determined.

We have, therefore, evaluated the role of NO in the regulation of blood flow in the canine diaphragm by determining (a) the effects of three L-arginine analogues, N^G-nitro-L-arginine methyl ester (L-NAME), N^G-nitro-L-arginine (L-NOARG) and argininosuccinic acid (ArgSA) on baseline vascular resistance and of the latter two agents on endothelium-dependent (acetylcholine, ACh) and -independent sodium nitroprusside, SNP) vasodilatation; (b) the effects of L- and D-arginine on baseline vascular tone resistance; and (c) the effects of L-glutamine, an inhibitor of L-arginine synthesis from L-citrulline (Sessa *et al.*, 1990) on baseline resistance and on ACh and SNP-induced vasodilatation.

Methods

Animal preparation

Studies were performed on mongrel dogs (weight = 27.8 ± 1.27 kg s.d.). The animals were anaesthetized with sodium thiopentone (10 mg kg^{-1}) followed by α -chloralose

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(60–80 mg kg⁻¹). Supplemental doses of α -chloralose were given as needed to eliminate the jaw tone but maintain knee reflexes. All animals were treated with indomethacin 5 mg kg⁻¹ to block prostaglandin synthesis. The animals were supine, intubated with cuffed endotracheal tubes and mechanically ventilated (tidal volume 15 ml kg⁻¹, frequency adjusted to maintain the arterial PCO_2 from 38 to 42 mmHg). Supplemental oxygen was supplied through the inspiratory line and arterial PO_2 maintained above 100 mmHg. Positive end-expiratory pressure (5 cmH₂O) was applied at the expiratory line. A catheter was placed in the aorta through the right carotid artery to monitor arterial pressure (P_{art}) and another catheter was placed in the right femoral vein to administer supplemental anaesthetic. Core body temperature was kept constant at approximately 37°C by a heating pad placed under the animal.

Isolated left hemidiaphragm

In all animals, we used the *in situ*, vascularly isolated left hemidiaphragm preparation previously described in detail (Hussain *et al.*, 1989). Briefly, through an abdominal incision, silk sutures were placed 1 cm apart in the left hemidiaphragm to be used for future reference in adjusting the muscle's resting length. The intercostal vessels and the left internal mammary artery were ligated and the lower left ribs were removed. The diaphragm was divided into two halves and the ribs and cartilages of the lower costal margin were freed and suspended vertically from metal bars. Resting diaphragm length was adjusted to that *in situ* by use of the reference sutures. The stomach, liver, spleen, and left kidney were retracted to expose the left phrenic artery. This artery was ligated proximally and a polyethylene catheter (PE 160, 1.14 mm i.d., 1.57 mm o.d., 3 cm long) was introduced into the distal portion. A side port in the catheter was used for measurement of phrenic perfusion pressure (P_{phr}). The catheter was connected to an electromagnetic flow probe (Carolina Medical Electronics, 1.91 mm i.d.) in order to measure phrenic arterial flow (Q_{phr}). The pressure-flow relationship of the catheter-probe system was linear over the range of flows in the study with conductance = 2.6 ml min⁻¹ mmHg⁻¹. The other side of the probe was connected to a Y shaped connector, one arm of which was connected to a catheter in the left femoral artery, thereby allowing the diaphragm to be perfused with blood diverted from this source. The other arm of the Y was connected via a constant perfusion pump to a catheter in the right femoral artery. The diaphragm could, thereby, be pump perfused using a constant flow pump (Cole-Parmer Masterflex No. 7523-00, head No. 7016-20, No. 16 tubing) with blood from the right femoral artery by clamping the left femoral arterial catheter. Drugs were administered to the phrenic circulation through a side port in the Y connector using a Harvard infusion pump. All animals were heparinized after catheterizing the phrenic artery.

The temperature of the diaphragm was monitored continuously with a temperature probe fixed to the surface of the isolated hemidiaphragm (Mon-a-therm, Model 6000, Zimmer of Canada Ltd., Mississauga, Ont, Canada) and maintained between 37.5 and 38.5°C with a heat lamp if required. At the end of the experiment the animals were exsanguinated and the diaphragm was dissected free and weighed.

Experimental protocols

Baseline vascular resistance Studies were performed on separate groups of animals, each group (6 animals in each group) receiving a different inhibitor of NO release. In each experiment, the left diaphragm was autoperfused from the left femoral artery for 30 min. Following this stabilization period, pump perfusion was begun, as described above, at the flow rate identical to that recorded during the period of autoperfusion. The Harvard pump was then used to infuse

saline into the phrenic circulation at a rate equal to 1/100th of the phrenic arterial blood flow. After 20 min of infusion, control measurements of P_{art} and P_{phr} were made. The saline syringe was then removed from the Harvard pump and replaced with one containing one of the following solutions: N^G-nitro-L-arginine (L-NOARG) (LNA), 6×10^{-2} M; N^G-nitro-L-arginine methyl ester (L-NAME), 6×10^{-2} M; argininosuccinic acid (ArgSA), 6×10^{-2} M; L-glutamine, 10^{-1} M. The test agent was infused for 20 min, at which time measurements of P_{art} and P_{phr} were repeated. All agents, except L-NOARG, were dissolved in normal saline with correction of pH to 7.4. L-NOARG solution of 6×10^{-2} M was prepared by dissolving L-NOARG in 0.5 ml of 1 N HCl and 19.5 ml of normal saline. We then added enough NaOH to correct the pH to 7.4. Infusion of the L-NOARG vehicle elicited no changes in diaphragmatic vascular resistance.

Response to ACh and SNP In 3 animals, the dose-response curves for ACh and SNP were determined. Increasing concentrations of these agents were infused into the phrenic circulation, each at 1/100th of the total phrenic arterial flow rate. The final concentrations found to produce 80% of maximal vasodilatation averaged 10^{-5} M for ACh and $2 \mu\text{g ml}^{-1}$ for SNP. These concentrations were then used in the following studies of the effects of the test agents on the responses to these vasodilators.

Studies were performed on separate groups of animals (6 per group), each receiving a different inhibitor of NO release. Following an initial 30 min stabilization period of autoperfusion, the diaphragm was pump-perfused at the same rate as that recorded during autoperfusion. A dual syringe pump was loaded with two syringes containing saline. Saline from both syringes was then infused into the phrenic circulation at a rate equal to 1/100th of the phrenic arterial flow per syringe. After 20 min, baseline measurements of P_{art} and P_{phr} were performed. One of the saline syringes was then removed from the pump and replaced with one containing either ACh (10^{-3} M) or SNP ($200 \mu\text{g ml}^{-1}$), and infusion was continued at the same rate. After 20 min of simultaneous infusion of saline and either ACh or SNP, by which time a steady state vascular resistance had been achieved in all animals, measurements of P_{art} and P_{phr} were repeated. The second saline syringe was then removed and replaced with a syringe containing one of the following solutions: L-NOARG, 6×10^{-2} M; ArgSA, 6×10^{-2} M; L-glutamine, 10^{-1} M. After 20 min of simultaneous infusion of either ACh or SNP and the test agent, measurements of P_{art} and P_{phr} were repeated.

L-Arginine and D-arginine In 2 separate groups of animals (6 per group) the left hemi-diaphragm was autoperfused for 30 min and then pump perfused at the natural flow rate as described above. The Harvard pump was subsequently used to infuse saline into the phrenic circulation at a rate equal to 1/100th of the phrenic arterial blood flow. After 20 min of infusion, control measurements of P_{art} and P_{phr} were made. The saline syringe was then removed from the Harvard pump and replaced with one containing either L-arginine or D-arginine (pH = 7.4) and infusion was continued at the same rate. The initial concentration in the syringe was 0.6×10^{-1} M to yield a final concentration of 0.6×10^{-3} M in the phrenic arterial blood. This syringe was replaced sequentially with syringes containing solutions which yielded final concentrations of 3×10^{-3} M and 6×10^{-3} M. The test agent was infused for 10 min at each concentration, at which time repeat measurements of P_{art} and P_{phr} were performed.

Data analysis

Results are expressed as mean $\pm$ s.e.mean. Two mean comparisons were performed with two tailed paired *t* tests. Comparisons of multiple means were performed using analysis of variance (ANOVA) corrected for multiple measures when appropriate and analysed *post-hoc* using the Neuman-Keuls

procedure. Differences were deemed significant when $P < 0.05$.

Results

Effect on baseline vascular resistance

Baseline $\dot{Q}_{phr}$ before L-NOARG, L-NAME, ArgSa and L-glutamine infusion averaged 20.8, 26.4, 29.2 and 28.5 ml⁻¹ 100 g min⁻¹, respectively. Figure 1 illustrates the mean values of P_{phr} measured at baseline and after 20 min infusion of these agents. L-NAME (6×10^{-4} M), L-NOARG (6×10^{-4} M) and ArgSA (6×10^{-4} M) significantly ($P < 0.01$) increased phrenic vascular resistance ($28.6 \pm 4.2\%$, $26.7 \pm 4.3\%$ and $32.8 \pm 4.6\%$ respectively compared to baseline), whereas L-glutamine (10^{-3} M) increased vascular resistance by a statistically significant ($P < 0.05$) but much smaller amount ($10 \pm 1.9\%$, Figure 1). The increase in phrenic vascular resistance in response to all of these agents reached a steady state level within 10 min of the infusion with no change thereafter. In addition, the pressor response to L-NOARG, L-NAME and L-glutamine was well maintained and persisted for over 60 min after the cessation of infusion. By comparison, phrenic vascular resistance recovered completely after 20 min of the cessation of ArgSA infusion. Figure 2 illustrates an example of the changes in phrenic perfusion pressure in response to 6×10^{-4} M L-NAME infusion. The increase in phrenic vascular resistance plateaued within 10 min of L-NAME infusion. Infusion of normal saline into the phrenic artery over 20 min period elicited no significant alterations in phrenic vascular resistance.

Effect on response to ACh and SNP

Figure 3 illustrates the effects of L-NOARG (a), ArgSA (b), and L-glutamine (c), on the vasodilator response to ACh and SNP. Infusion of L-NOARG and ArgSA completely reversed the vasodilatation induced by ACh while having no significant effect on the SNP response. L-Glutamine had no effect on the magnitude of vasodilatation produced by either ACh or SNP.

Figure 1 Change in phrenic arterial perfusion pressure following infusion of N^G-nitro-L-arginine methyl ester (L-NAME, 6×10^{-4} M), N^G-nitro-L-arginine (L-NOARG, 6×10^{-4} M), argininosuccinic acid (ArgSA, 6×10^{-4} M) and L-glutamine (L-Glut, 10^{-3} M). * $P < 0.05$; ** $P < 0.01$ for the change compared to baseline perfusion pressure.

Effects of L-arginine and D-arginine

Figure 4 presents the values of P_{phr} during infusion of L-arginine and D-arginine. Neither isomer had any effect on diaphragmatic vascular resistance at any of the concentrations tested.

Discussion

The main findings of this study are: (1) infusion of the L-arginine analogues L-NOARG, L-NAME and ArgSA increased the baseline vascular resistance of the phrenic circula-

Figure 2 A representative tracing of the changes in phrenic perfusion pressure in response to 6×10^{-4} M N^G-nitro-L-arginine methyl ester (L-NAME).

Figure 3 Effects of N^G-nitro-L-arginine (L-NOARG, 6×10^{-4} M), argininosuccinic acid (ArgSA, 6×10^{-4} M) and L-glutamine (L-Glut, 10^{-3} M) on the changes in phrenic arterial perfusion pressure during acetylcholine (ACh, 10^{-5} M) and sodium nitroprusside (SNP, 2μ l ml⁻¹) infusion. * $P < 0.05$ compared with response to vasodilator alone.

Figure 4 Effects of (a) L-arginine and (b) D-arginine on baseline phrenic arterial perfusion pressure.

tion *in vivo*; (2) infusion of L-NOARG or ArgSA attenuated the vasodilator effect of ACh infusion while having no effect on vasodilatation in response to SNP; (4) L-arginine and D-arginine at physiological pH have no effect on baseline diaphragmatic vascular resistance; (5) L-glutamine (10^{-3} M) increased baseline vascular resistance but had no effect on either ACh or SNP induced vasodilatation.

Basal NO release

Basal release of EDRF has been detected by bioassay from isolated vessels (Rubanyi *et al.*, 1985; Kelm & Schrader, 1990). *In vivo* studies have demonstrated increased baseline arterial pressure following systemic infusion of L-arginine analogues in rats (Gardiner *et al.*, 1989), guinea-pigs (Aisaka *et al.*, 1989), cats (Bellan *et al.*, 1991) and dogs (Klabunde *et al.*, 1990). Regionally, treatment with analogues of L-arginine have been found to increase vascular resistance in rabbit isolated hearts (Lamontagne *et al.*, 1991), rabbit hindlimb (Mugge *et al.*, 1991), dog hindlimb (White *et al.*, 1993) and human forearm (Vallance *et al.*, 1989). In rat cremaster muscle, arteriolar vasoconstriction has been demonstrated following light-dye injury to the endothelium (Koller & Kaley, 1990) and following topical application of L-arginine analogues (Kaley *et al.*, 1992). Similarly, Persson *et al.* (1990) have shown N^G -monomethyl-L-arginine (L-NMMA) to reduce microvascular diameters in rabbit tenuissimus muscle. Consistent with this evidence, we have found the baseline resistance of the diaphragmatic vasculature to increase significantly following the administration of L-arginine analogues. This effect could not have been due to suppression of vasodilator prostanoid synthesis (Koller *et al.*, 1990) since all animals had been pretreated with indomethacin. These results, therefore, suggest a role for modulation of basal endothelial release of NO in the control of resting smooth muscle tone in this vascular bed as well.

Endothelium-dependent and -independent vasodilatation

The results of studies of the role of NO in agonist-induced vasodilator responses have been less consistent than those concerning its role in regulation of baseline vascular tone. Some studies have shown that the *in vivo* responses to endothelium-dependent agents are selectively inhibited by treatment with L-arginine analogues, while responses to endothelium-independent agents are not significantly decreased (Aisaka *et al.*, 1989; Gold *et al.*, 1989; Gardiner *et al.*, 1989; Bellan *et al.*, 1991; 1993; Kaley *et al.*, 1992; White *et al.*, 1993). Other studies, however, have shown either no decrease in responses to endothelium-dependent vasodilators (Mugge *et al.*, 1991; Ross *et al.*, 1991) or a non-selective inhibition of both endothelium-dependent and -independent vasodilatation (Thomas *et al.*, 1989; Klabunde *et al.*, 1990). The degree to which L-arginine analogues are able to inhibit the vasodilatation induced by ACh has also been variable, prompting some authors to propose additional mechanisms of action for this vasodilator in their preparations (Gold *et al.*, 1989; Bellan *et al.*, 1991; Ross *et al.*, 1991). The dose and duration of treatment have also been reported to alter the relative effect of L-arginine analogues on baseline vascular tone in comparison to their effects on the response to ACh (Bellan *et al.*, 1991). Furthermore, the relative potencies of two of these agents L-NOARG and L-NMMA, in the *in vivo* canine femoral circulation has been shown to differ from that predicted from *in vitro* studies (Kirkeboen *et al.*, 1992). Differences in species, vessel type, agent used, dose and duration of treatment may all, therefore, contribute to these apparent discrepancies.

Three L-arginine analogues were used in the current study. We found that exposure of the diaphragmatic vasculature to equimolar concentrations of these three analogues increased baseline vascular resistance to a similar extent. In addition, L-NOARG and ArgSA, in the concentration used, attenuated the vasodilatation induced by ACh but did not increase phrenic vascular resistance beyond the baseline values. These results indicate that L-NOARG and ArgSA did not completely reverse ACh-induced vasodilatation. Moreover, these findings suggest that in the indomethacin pretreated canine diaphragmatic circulation, the vasodilatation induced by ACh infusion is mediated partly by augmentation of NO release and that these L-arginine analogues are specific inhibitors of this effect. We speculate also that part of the ACh-induced phrenic vasodilatation is mediated by NO-independent mechanisms such as endothelium-derived relaxing factor (EDHF, Komori & Vanhoutte, 1990). In rat isolated vessels, ACh induces endothelium-dependent hyperpolarization which partially mediate the vasodilator effect of ACh (Van de Voorde *et al.*, 1992).

L-Arginine

Endothelium-dependent relaxing factor/NO is synthesized from the guanido nitrogen of L-arginine and molecular oxygen by the NADPH and calmodulin-dependent action of NO synthase (NOS) (Busse & Mulsch, 1990; Forstermann *et al.*, 1991). Citrulline is a coproduct of this reaction. FMN, FAD, haeme and tetrahydrobiopterin are co-factors (Nathan, 1992). Substrate levels adequate to support ongoing NO synthesis are maintained by uptake of extracellular L-arginine and through the synthesis of L-arginine by a process linked to the release of NO (Mitchell *et al.*, 1990b). Endothelial cells can generate L-arginine from at least two sources. These include the peptidyl L-arginine pool which is available through the action of peptidyl arginine deiminase (Thomas & Ramwell, 1988) and recycling of accumulated L-citrulline to L-arginine through the intermediate formation and subsequent cleavage of argininosuccinate (Hecker *et al.*, 1990). Intracellular levels of L-arginine, therefore, normally remain high (0.1–1 mM, approximately 30 times the apparent K_m for

purified NOS, (Pollock *et al.*, 1991) even during prolonged stimulation of NO release (Mitchell *et al.*, 1990a).

In isolated vessel preparations, the effect of administration of exogenous L-arginine on vascular smooth muscle tone has been variable. In preparations in which vasodilatation was elicited, intracellular L-arginine had been depleted either because of prolonged (> 2 h) preparation time (Shini & Vanhoutte, 1991; Sun *et al.*, 1992) or by continuous stimulation of NO synthesis in arginine-free media (Gold *et al.*, 1989). In fresh preparations, no effect of L-arginine is observed (Shini & Vanhoutte, 1991; Sun *et al.*, 1992).

In the majority of blood perfused tissue preparations (Thomas *et al.*, 1989; Fortes *et al.*, 1990; Gardiner *et al.*, 1990; Lahera *et al.*, 1990), infusion of L-arginine had no effect on vascular resistance. Nevertheless, in the pial circulation, exogenous L-arginine, but not D-arginine administration resulted in vasodilatation which was reversible by the topical administration of L-NAME (Morikawa *et al.*, 1992). In the human forearm, furthermore, Panza *et al.* (1993) found that infusion of L-arginine but not D-arginine, enhanced the vasodilator response to acetylcholine, although such treatment had no effect on baseline vascular resistance. The role of extracellular L-arginine availability as a rate limiting factor in endothelial NO synthesis under physiological conditions, therefore, varies depending upon the tissue and the species under study. Our current finding that neither of the arginine isomers significantly altered vascular resistance indicates that L-arginine availability does not normally limit basal NO release in the canine diaphragmatic circulation.

L-Glutamine

In cultured endothelial cells, Sessa *et al.* (1990) found that L-glutamine resulted in significant (46.5%) inhibition of EDRF release as detected by bioassay. The effect was observed over a concentration range of 0.1–2.0 mM, with maximum effect at concentrations of 0.2 mM and above. L-glutamine exerted this action through inhibition of intracellular conversion of L-citrulline to L-arginine. It did not interfere with the conversion of L-Arg-L-Phe or L-Argsucc to

L-arginine nor did it inhibit the uptake of L-arginine into the endothelial cells. The inhibitory action of L-glutamine on L-citrulline conversion is most likely effected by allosteric interaction with argininosuccinate synthetase or by competitive interference due to the structural similarity between these two amino acids.

In the current study we found that L-glutamine, at a final concentration in the perfusing blood of 1 mM, produced a small but significant increase in diaphragmatic vascular resistance. The exact mechanism behind the increase in phrenic vascular resistance after L-glutamine infusion is not clear. Based on the experiments of Sessa *et al.*, one may speculate that L-glutamine inhibits basal NO release by preventing the conversion of L-citrulline to L-arginine. However, L-arginine requirements for basal NO synthesis can also be provided through an influx of intracellular L-arginine. Our experiments also indicated that L-glutamine infusion did not attenuate the vasodilator response to stimulation of NO release by ACh. These results suggest that during ACh infusion, intracellular L-arginine concentration may not be sufficiently reduced by L-glutamine to influence NOS activity. Alternatively, other sources of L-arginine may become more important or else conversion of L-citrulline to L-arginine by an as yet unknown transamination reaction may be activated during stimulation of NO production.

In summary, modulation of basal NO release by the endothelial cell plays an important role in the regulation of baseline diaphragmatic vascular resistance. Extracellular L-arginine availability does not appear to be rate limiting in this process. Intracellular recycling of L-citrulline, however, is likely to be an important source of substrate for this reaction under basal conditions. The L-arginine analogues, L-NOARG and ArgSA are potent and specific inhibitors of basal NO release in the canine phrenic circulation.

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