The role of nitric oxide in the spatial heterogeneity of basal microvascular blood flow in the rat diaphragm

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Summary
The effects of N^o-nitro-L-arginine (L-NOARG) and N^G-monomethyl-L-arginine (L-NMMA) on the spatial distribution of diaphragmatic microvascular blood flow were assessed in anesthetized, mechanically ventilated rats. Microvascular blood flow was measured after different periods at either a fixed site (Q_stat) or 25 different sites (Q_scan) using computer-aided laser-Doppler flowmetry (LDF) scanning. The value of Q_stat was unaffected after 15–20 min superfusion with any one of the following agents: L-NOARG (0.1 mM), L-NMMA (0.1 mM), L-arg (10 mM). The cumulative frequency histogram of the Q_scan value in the control group displayed a non-Gaussian distribution that was not significantly affected after 15 min superfusion with the vehicle of L-NOARG. Superfusion with either L-NMMA or L-NOARG at 0.1 mM for 15 min displaced the histogram of cumulative frequency to the left, with the median value of blood flow decreasing by 10 to 20%. However, skewness and kurtosis of the distribution of basal Q_scan were unaffected after superfusion of either of the L-arg analogues. Pretreatment with L-arg (10 mM), followed by co-administration of L-arg (10 mM) with L-NOARG (0.1 mM) only partially prevented L-NOARG from exerting this inhibitory effect on the distribution of basal Q_scan and normalized changes in basal Q_scan after superfusion of either of the L-arg analogues. In conclusion, a basal NO activity is present in the diaphragmatic microvascular bed of rats. LDF scanning rather may yield more vivid information about the extent of overall tissue perfusion than conventional LDF whenever basal NO activity is involved. Moreover, the parallel flow profiles after NO synthase blockade suggest that the spatial inhomogeneity of basal diaphragmatic microvascular blood flow is not dependent on basal NO formation.

Introduction
Diaphragmatic blood flow is a crucial factor in determining the function of the diaphragm because decreased contractive force of the diaphragm in low blood flow states can lead to respiratory failure [1, 2]. It has been shown that diaphragmatic vascular tone in the isolated canine diaphragm under basal conditions is partially regulated by NO [3, 4]. In diaphragmatic microcirculation, which ultimately determines perfusion adequacy, N^o-nitro-L-arginine (L-NOARG) has been shown to cause a significant reduction in A2 arteriolar diameters in a dose-dependent manner [5]. On the contrary, our previous studies using laser-Doppler flowmetry (LDF) showed that baseline NO activity in the diaphragmatic...
microvascular bed of resting rats is quite low, and that neither \( \text{N}^\omega \)-nitro-L-arginine methyl ester (L-NAME) nor L-NOARG can affect basal microcirculatory blood flow [6]. Since there is a wide spatial heterogeneity of diaphragmatic microvascular blood flow [8], the issue is raised that vasomotor responses recorded over a single site in the diaphragm may not be representative of the entire area of the vascular bed being studied.

Observations in several preparations including coronary vascular beds and striated muscles have suggested that variation in the importance of the NO pathway among arterioles of different diameters is a common phenomenon [8–11]. Similarly, longitudinal gradients of NO-mediated dilatation of arterioles have been identified in isolated canine diaphragms, as shown by an ability of L-NOARG to induce vasoconstriction, which is linearly related to the basal diameters of arterioles [12]. To clarify the relationship between basal NO activity and spatial inhomogeneity of diaphragmatic microcirculation, study of flow information from multiple sites is necessary. Collection of this information can be accomplished with LDF, and multi-spots scanning can be done with either an integrating probe [13], or a laser-Doppler perfusion imager [14]. However, the former technique requires a luxurious space to accommodate the big probe head (diameter = 6 mm), while the latter method demands meticulous examination of off-line microscopic images to exclude flow values originating from major visible vessels. To avoid these problems, the present study used computer-aided LDF scanning to measure blood flow over multiple locations in the diaphragm. Information concerning the distribution of tissue microvascular blood flow was obtained by analysis of the central location and spread of frequency histograms.

The aim of this study was to assess the role of NO in the spatial heterogeneity of diaphragmatic microcirculation under basal conditions. Rat diaphragms were prepared to allow recording of information about the spatial heterogeneity of microcirculation by LDF scanning. Both L-NOARG and \( \text{N}^\text{G} \)-monomethyl-L-arginine (L-NMMA) were used as specific pharmacological tools to block NO synthase, and the ability of L-arginine (L-arg) to prevent these L-arg analogues from exerting their inhibitory effects on basal NO formation was also assessed.

### Methods

#### Animal preparation

Male Sprague–Dawley rats (weight 300–350 g) were housed at the Laboratory Animal Center of the College of Medicine at National Cheng Kung University. All animals were maintained on Purina rat chow and tap water ad libitum. The animals were fasted overnight the day before the experiment.

The animals were initially anesthetized with intraperitoneal sodium pentobarbital (30 mg/kg body wt) followed by intravenous 50% w/v urethane (1.2–1.5 g/kg body wt) and placed in a supine position on a rodent operating table. After tracheostomy, muscle relaxant (gallamine triethiodide; 60 mg/kg body wt) was administered, and the rats were artificially ventilated at tidal volumes between 6–7 ml/kg body wt and a rate of 70–80 breaths/min. Supplemental \( \text{O}_2 \) was given via the inspiratory port. Mean systemic blood pressure (MAP) was measured with a polyethylene catheter (PE-50) inserted via the right carotid artery and connected to a pressure transducer. A cardiograph triggered by the pulse pressure signal was used to monitor the heart rate. Normal saline (3–5 ml/h/100 g body wt) was infused into the left external jugular vein throughout the experiment via a peristaltic pump. Rectal temperature was continuously monitored with a thermistor and maintained at 36–38°C by a heating lamp and a temperature-regulated bed.

#### Diaphragm preparation

The techniques used for diaphragm preparation have been described in detail elsewhere [7]. Briefly, thoracotomy was performed in the right fifth and sixth intercostal spaces, and a 1 cm long segment of the right sixth rib was removed. The diaphragm was separated from the lungs and the mediastinal tissues. An ovoid-shaped stainless steel plate coated with white glossy acrylic was slipped behind the diaphragm to hold the left hemidiaphragm flat.

Midline and transverse abdominal incisions were made and the ligament between the liver and the central tendon was severed. With the animal in the Trendelenburg position, the upper abdominal wall was folded back and retracted. Abdominal