The Effect of Flow Rate on Substrate Metabolism in the Isolated Perfused Lung Model

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Most lung perfusion models use very low flow rates to minimize edema. We tested the effect of flow rates on pulmonary function and cellular oxidative metabolism. Isolated rat lungs were perfused with a blood-based solution containing ^{13}C-labeled substrates. Pulmonary artery flow rates were set at either 5, 10 or 20 ml/min. ^{13}C enrichment of glutamate C4 (reflecting substrate oxidation) was determined by ^{13}C and ^{1}H spectroscopy. Oxygenation was worse at 20 mL/min. Glutamate ^{13}C-enrichment was reduced at 5 mL/min, implying inadequate substrate delivery for normal metabolism. Data derived from perfusion models incorporating low flow rates should be interpreted with caution.

Background:

Most isolated lung perfusion models incorporate pulmonary artery flow rates approximating 10% of normal cardiac output to achieve stability and minimize lung edema[1,2]. Although it is assumed that these flow rates are sufficient to support cellular metabolism, direct quantification of substrate oxidation in the perfused lung tissue has not been reported. This study was designed to investigate oxidative metabolism in isolated perfused rat lungs under conditions of varying flow rates and to examine the relationship between substrate oxidation and pulmonary function.

Methods:

Four groups (n=6 each) of Sprague-Dawley rats were anesthetized and heparinized. A tracheostomy was performed and animals were ventilated (RR=50, FIO_2=0.21, V_T=10 ml/kg, PEEP=2 cm H_2O). After sternotomy, the pulmonary artery (PA) was cannulated and the lungs were flushed with 4°C Euro-Collins solution (60 ml/kg, 25 cm H_20 pressure). The left atrium was incised to allow unrestricted flow of effluent. The heart and lungs were then excised *en bloc* and perfused ex-vivo[3] for 45 minutes with a red blood cell-based Krebs-Heinseleit buffer (Hct 20%) containing a combination of ^{13}C-labeled substrates (5.5 mM U-^{12}C glucose, 1.2 mM 3-^{13}C lactate, 0.12 mM 3-^{13}C pyruvate, 0.17 mM 1,3-^{13}C acetoacetate, and 0.35 mM U-^{13}C mixed free fatty acids). Temperature (37°C), PO_2 (35-45 mm Hg), PCO_2 (35-45 mmHg), and pH (7.35-7.45) of the perfusate were carefully controlled. The perfusate was delivered to the PA catheter via a peristaltic pump and was not recirculated. PA flow rates were controlled for each group: 5 ml/min (Group1), 10 ml/min (Group2), or 20 ml/min (Group3). Group 4 was perfused at 10 ml/min with the concentrations of all substrates doubled to mimic the total substrate delivery of Group 3. During this interval, all lungs were ventilated with an FIO_2 of 0.40. Other ventilator parameters were unchanged from their initial values. Inflow and outflow PO_2, pulmonary artery pressure, and airway pressure were measured throughout the perfusion interval. Pulmonary compliance was calculated as (V_T / Δ airway pressure). At the end of perfusion, non-parenchymal tissue was trimmed. Lungs were then freeze-clamped, extracted in perchloric acid, neutralized in KOH, lyophilized, and reconstituted in D_2O. Substrate oxidation was quantified by two techniques. First, ^{13}C NMR spectra of extracts were obtained in a 9.4T Bruker spectrometer. Total glutamate ^{13}C enrichment was determined by integrating multiplet peaks at each of the five glutamate carbon resonances referenced to an internal standard of known concentration (3-^{13}C lactate.) When sufficient signal was identified, the relative contribution of each substrate to oxidation within the citric acid cycle was also calculated by ^{13}C isotopomer analysis[4]. A complementary technique to assess glutamate enrichment was also applied. ^{1}H NMR spectra of the same extracts were acquired on a 7.0T Varian Inova spectrometer. Spectra from each extract were obtained both with and without ^{13}C decoupling centered at glutamate C4 (34.2 ppm). Fractional ^{13}C enrichment of glutamate was determined by the increase in the integral of the H4 (protons bound to C4 of glutamate) signal with ^{13}C-^{1}H decoupling[5].

Results:

Pulmonary function remained stable in each group for the entire perfusion period. Functional data collected after 45 minutes of perfusion are displayed in **Table 1**. Pulmonary artery pressures rose and oxygenation deteriorated at flows of 20 ml/min ($p<0.05$, group 3 vs. all other groups). Quantification of ^{13}C enrichment in lung tissue measur-ed at the end of the perfusion interval by both techniques is shown in **Table 2**. Glutamate enrichment was significantly reduced at flow rate of 5 mL/min compared to all other groups. The relative contributions of each substrate to acetyl-CoA oxidized within the citric acid cycle are provided in **Table 3**.

Table 1. *Pulmonary Function at End Experiment*

Group	PO_2:FIO_2 Ratio	Compliance (Δml/Δmm Hg)	Mean PA Pressure (mm Hg)
1	490±18	0.47±0.13	10±1
2	525±13	0.33±0.02	10±3
3	429±38*	0.39±0.07	16±2*
4	525±13	0.36±0.04	10±1

Mean±SEM; *$P < 0.05$ by ANOVA

Table 2. *Fractional Enrichment of Glutamate C4*

Group	Total Glutamate ^{13}C Enrichment (arbitrary units)	^{13}C enrichment of Glutamate C4 (%) By ^{1}H NMR
1	71±15*	8.44±1.62*
2	163±22	24.19±5.76
3	155±18	21.26±2.04
4	152±19	23.87±2.41

Mean±SEM; *$P < 0.05$ by ANOVA

Table 3. *Relative Contributions of Substrate Classes to AcetylCoA*

Group	Fatty Acids	Aceto-acetate	Lacate or Pyruvate	Unlabeled Substrates
2	23±2%	16±3%	24±2%	36±6%
3	26±3%	11±2%	28±2%	36±4%
4	22±2%	17±1%	26±2%	35±5%

Mean±SEM; P = NS by ANOVA

Conclusions

Gas exchange and pulmonary mechanics appeared well maintained at all flow rates, although pulmonary functional parameters deteriorated at flows of 20 ml/min. This finding suggests that higher flow rates may be detrimental to pulmonary function. Substrate oxidation, as evidenced by the appearance of enriched glutamate in the ^{13}C spectrum, was significantly decreased at flow rates less than 10 ml/min. This finding was consistently observed in both methods of quantifying glutamate enrichment. Decreased glutamate enrichment may imply inadequate de-livery of substrates for normal oxidative metabolism at lower flows, a condition that may result in lung tissue with inadequate energy reserves for vital cellular processes.

We conclude that substrate oxidation in isolated perfused lung models is compromised at very low flow rates. The rationale for using low flow rates in isolated perfused lung models is to avoid the functional deterioration that occurs under higher flow conditions. However, because of reduced oxidative metabolism, data derived from ex-vivo perfusion models incorporating very low flow rates should be interpreted cautiously. This may be most important in studies that evaluate energy-dependent functions.

References:

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