Coenzyme Q$_1$ redox metabolism during passage through the rat pulmonary circulation and the effect of hyperoxia

Said H. Audi, Marilyn P. Merker, Gary S. Krenz, Taniya Ahuja, David L. Roerig, and Robert D. Bongard

Departments of 1Biomedical Engineering, 2Mathematics, Statistics and Computer Science, Marquette University; Departments of 3Pulmonary and Critical Care Medicine, 4Anesthesiology, and 5Pharmacology/Toxicology, Medical College of Wisconsin; and 6Zablocki Veterans Affairs Medical Center, Milwaukee, Wisconsin

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Audi SH, Merker MP, Krenz GS, Ahuja T, Roerig DL, Bongard RD. Coenzyme Q$_1$ redox metabolism during passage through the rat pulmonary circulation and the effect of hyperoxia. J Appl Physiol 105: 1114–1126, 2008. First published August 14, 2008; doi:10.1152/japplphysiol.00177.2008.—The objective was to evaluate the pulmonary disposition of the ubiquinone homolog coenzyme Q$_1$ (CoQ$_1$) on passage through lungs of normoxic (exposed to room air) and hyperoxic (exposed to 85% O$_2$ for 48 h) rats. CoQ$_1$, or its hydroquinone (CoQ$_1$H$_2$), was infused into the arterial inflow of isolated, perfused lungs, and the venous efflux rates of CoQ$_1$H$_2$ and CoQ$_1$ were measured. CoQ$_1$H$_2$ appeared in the venous effluent when CoQ$_1$ was infused, and CoQ$_1$ appeared when CoQ$_1$H$_2$ was infused. In normoxic lungs, CoQ$_1$H$_2$ efflux rates when CoQ$_1$ was infused decreased by 58 and 33% in the presence of rotenone (mitochondrial complex I inhibitor) and dicumarol [NAD(P)H-quinone oxidoreductase 1 (NQO1) inhibitor], respectively. Inhibitor studies also revealed that lung CoQ$_1$H$_2$ oxidation was via mitochondrial complex III. In hyperoxic lungs, CoQ$_1$H$_2$ efflux rates when CoQ$_1$ was infused decreased by 23% compared with normoxic lungs. Based on inhibitor effects and a kinetic model, the effect of hyperoxia could be attributed predominantly to a 47% decrease in the capacity of complex I-mediated CoQ$_1$ reduction, with no change in the other redox processes. Complex I activity in lung homogenates was also lower for hyperoxic than for normoxic lungs. These studies reveal that lung complexes I and III and NQO1 play a dominant role in determining the vascular concentration and redox status of CoQ$_1$ during passage through the pulmonary circulation, and that exposure to hyperoxia decreases the overall capacity of the lung to reduce CoQ$_1$ to CoQ$_1$H$_2$ due to a depression in complex I activity.

mathematical modeling; NADH: ubiquinone oxidoreductase; NAD(P)H: quinone oxidoreductase 1; ubiquinol-cytochrome-c oxidoreductase

THE LUNG IS WELL SUITED for influencing the chemical composition of the blood as it passes from the venous to the systemic arterial circulation. The nonrespiratory or metabolic functions of the lung are affected by various lung cell surface and intracellular enzymes and transporters that act on blood-borne compounds and are furthered by the large pulmonary endothelial surface area and the fact that the lung receives the entire venous return (28). Among the classes of compounds affected are blood-borne redox active pharmacological, physiological, and toxic compounds (e.g., quinones), whose redox status and concentrations in the blood may be influenced by lung cell surface or intracellular oxidoreductases (2–5, 9, 10, 16, 17, 27, 28, 47, 49–51). Thus the lung has the potential to influence the bioavailability and bioactivity of such compounds in lung tissue, blood vessels, and downstream organs.

Indicator dilution methods have been important research tools for studying metabolic functions of various intact organs, including the lung (2, 5, 12, 28, 55, 63). These methods involve the bolus injection or finite pulse infusion of two or more indicators into the arterial inlet to an organ, followed by measurement of concentrations of these indicators in the venous effluent as a function of time. The injected indicators usually include an intravascular indicator (also known as vascular reference indicator) plus a test indicator that is a substrate or ligand for the organ’s metabolic function(s) of interest. The interactions of the test indicator with these metabolic function(s) on passage though the organ result in characteristic differences between the vascular and test indicator venous effluent concentration vs. time curves. The information in these curves is deciphered using mathematical models representing the dominant physical and chemical processes hypothesized to be involved in the disposition of the test indicator within the organ (2, 5, 12, 28, 55, 63).

We have used indicator dilution methods and mathematical modeling to probe the activities of redox enzymes in the isolated, perfused lung and to evaluate the impact of these enzymes on the redox status and disposition of blood-borne redox active compounds on passage through the lung (2–5, 9, 27). For instance, we have observed that the redox active quinone compound duroquinone (DQ) is reduced to durohydroquinone (DQH$_2$) on passage through the pulmonary circulation of the isolated, perfused rat lung, wherein DQH$_2$ appears in the venous effluent. Based on inhibitor studies, NAD(P)H-quinone oxidoreductase 1 (NQO1) was implicated as the dominant reductase involved, and the capacity of the lung to reduce DQ to DQH$_2$ was shown to be a measure of lung NQO1 activity (2). NQO1, which is predominantly a cytoplasmic enzyme, is of interest because it is a phase II antioxidant enzyme involved in the detoxification of reactive electrophilic metabolites (e.g., quinones, lipid peroxides, organic peroxides) via two-electron reduction (2, 20, 50). As such, NQO1 competes with one-electron quinone reductases (e.g., P-450 reductases, cytochrome b$_5$) for reactive electrophilic metabolites and other oxidoreductases, thereby limiting redox cycling of these compounds and generation of prooxidant reactive oxygen species (ROS) (14, 20, 25, 31, 40, 56).

As a phase II antioxidant enzyme, NQO1 is induced via the antioxidant response element in response to oxidativ
trophic stress (3, 14, 20, 25, 31, 40, 47, 56). Accordingly, when rats were exposed to hyperoxia (85% O2 for 21 days) as a model of oxidative stress, lung tissue NQO1 activity and protein levels increased, as did the capacity to reduce DQ to DQH2 in the pulmonary circulation (3). Since this hyperoxic exposure is well known to confer adaptation to the otherwise lethal effects of 100% O2 in rats, the impact on NQO1 is consistent with an adaptive response to oxidative stress (3, 26).

In the present study, we directed our attention to the question of whether homologs of the endogenous quinone, ubiquinone, are reduced on passage through the rat pulmonary circulation and, if so, to evaluate the role of NQO1 and other redox enzymes. Ubiquinone is the quinone electron carrier in the mitochondrial electron transport chain located at the inner mitochondrial membrane and also participates in other redox functions, including as an anti- and prooxidant substance (23). To address this question, we selected the quinone compound 2,3-dimethoxy-5-methyl-6-[3-methyl-2-butenyl]-1,4-benzoquinone (coenzyme Q1; CoQ1) because it is an amphipathic homolog of ubiquinone (30, 35, 48, 53). The relatively high octanol-water partition coefficient (log10 octanol-water partition coefficients >3) and significant water solubility (1.3 mM) of CoQ1 are advantageous for evaluating its redox metabolism during passage through the pulmonary circulation using indicator dilution methods (30, 35, 53). Moreover, CoQ1 has been used as an electron acceptor to study not only NQO1, but also other oxidoreductases, including NADH-ubiquinone oxidoreductase (mitochondrial electron transport complex I), succinate-ubiquinone reductase (mitochondrial electron transport complex II), and transplasma membrane electron transport, in isolated enzymes, subcellular fractions, and intact cells in culture (30, 34, 46, 48, 57, 62).

The objective of the present study was to utilize indicator dilution methods to determine whether CoQ1 is reduced on passage through the isolated, perfused rat lung and, if so, to identify and quantify the dominant redox processes that contribute to lung CoQ1 redox metabolism. The potential contribution of multiple redox enzymes to the redox metabolism of CoQ1 on passage through the pulmonary circulation was addressed using inhibitors and kinetic modeling, the latter of which allowed us to quantify the contributions of these enzymes. A further objective was to evaluate the impact of oxidative stress (in vivo exposure to 85% O2 for 48 h) on lung CoQ1 redox metabolism.

**Glossary**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
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<tr>
<td>CoQ1</td>
<td>Coenzyme Q1</td>
</tr>
<tr>
<td>CoQ1H2</td>
<td>CoQ1 hydroquinone</td>
</tr>
<tr>
<td>[CoQ1](x, t) and [CoQ1H2](x, t)</td>
<td>Vascular concentrations of free CoQ1 and CoQ1H2, respectively, at distance x from the capillary inlet and time t (µM)</td>
</tr>
<tr>
<td>[CoQ1]</td>
<td>Total (free + BSA bound) vascular concentration of CoQ1 (µM)</td>
</tr>
<tr>
<td>[CoQ1H2]</td>
<td>Total (free + BSA bound) vascular concentration of CoQ1H2 (µM)</td>
</tr>
<tr>
<td>CV</td>
<td>Asymptotic coefficient of variation (%)</td>
</tr>
<tr>
<td>DQ</td>
<td>Duroquinone</td>
</tr>
<tr>
<td>DQH2</td>
<td>Durohydroquinone</td>
</tr>
<tr>
<td>h(t)</td>
<td>Capillary transit time distribution</td>
</tr>
<tr>
<td>FAPGG</td>
<td>N-[3-(2-Furyl)acryloyl]-Phe-Gly-Gly</td>
</tr>
<tr>
<td>FITC-dex</td>
<td>Fluorescein isothiocyanate dextran</td>
</tr>
<tr>
<td>[FITC-dex](x, t)</td>
<td>Vascular concentration of FITC-dex at distance x from the capillary inlet and time t (µM)</td>
</tr>
<tr>
<td>Km1a</td>
<td>Apparent Michaelis-Menten constant for complex I-mediated CoQ1 reduction (µM)</td>
</tr>
<tr>
<td>Km2a</td>
<td>Apparent Michaelis-Menten constant for NQO1-mediated CoQ1 reduction (µM)</td>
</tr>
<tr>
<td>Km3a</td>
<td>Apparent Michaelis-Menten constant for rotenone-dicumarol-insensitive reductase(s)-mediated CoQ1 reduction (µM)</td>
</tr>
<tr>
<td>Km4a</td>
<td>Apparent Michaelis-Menten constant for CoQ1H2 oxidation via complex III (µM)</td>
</tr>
<tr>
<td>NQO1</td>
<td>NAD(P)H-quinone oxidoreductase 1</td>
</tr>
<tr>
<td>PAEC</td>
<td>Bovine pulmonary arterial endothelial cells</td>
</tr>
<tr>
<td>PS</td>
<td>Permeability-surface area product (ml/min), which is a measure of plasmatic clearance of FAPGG and an index of perfused capillary surface area</td>
</tr>
<tr>
<td>Ve</td>
<td>Volume of the vascular region of the single capillary element model (ml)</td>
</tr>
<tr>
<td>Vc</td>
<td>Volume of the tissue or extravascular region of the single capillary element model (ml)</td>
</tr>
<tr>
<td>Vmax1</td>
<td>Maximum rate for CoQ1 reduction via complex I (µmol/min)</td>
</tr>
<tr>
<td>Vmax2</td>
<td>Maximum rate for CoQ1 reduction via NQO1 (µmol/min)</td>
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<tr>
<td>Vmax3</td>
<td>Maximum rate for CoQ1 reduction via rotenone-dicumarol insensitive reductase(s) (µmol/min)</td>
</tr>
<tr>
<td>Vmax4</td>
<td>Maximum rate for CoQ1H2 oxidation via complex III (µmol/min)</td>
</tr>
<tr>
<td>VFI/α1</td>
<td>Virtual volume of distribution for CoQ1 (ml)</td>
</tr>
<tr>
<td>VF2/α2</td>
<td>Virtual volume of distribution for CoQ1H2 (ml)</td>
</tr>
<tr>
<td>W</td>
<td>Convective transport velocity (cm/min)</td>
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**Greek Letters**

<table>
<thead>
<tr>
<th>Greek Symbol</th>
<th>Description</th>
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<tr>
<td>α1 and α2</td>
<td>Constants that account for the rapidly equilibrating interactions of CoQ1 and CoQ1H2 with the 5% BSA (i.e., Pc) perfusate</td>
</tr>
<tr>
<td>α3 and α4</td>
<td>Constants that account for the rapidly equilibrating interactions of CoQ1 and CoQ1H2 with lung tissue sites (Pc) of association, respectively</td>
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</table>

**METHODS**

**Materials.** CoQ1 and other chemicals, unless noted, were purchased from Sigma (St. Louis, MO). CoQ1H2 was prepared by reduction of CoQ1 with potassium borohydride (KBH4), as previously described.
the venous effluent to reach steady state during CoQ1 or CoQ1H2 providing insights into the tissue permeation of CoQ1H2. What flow was equilibrated with 15% O2-6% CO2-balance N2, resulting in perfusate (Masterflex roller pump) with the control perfusate maintained at 37°C and BSA (2). The single-pass perfusion system was primed (Masterflex roller pump) with the control perfusate maintained at 37°C and equilibrated with 15% O2-6% CO2-balance N2, resulting in perfusate PO2, P CO2, and pH of ~105 Torr, 40 Torr, and 7.4, respectively. Initially, control perfusate was pumped (Masterflex roller pump) through the lung until the lung was evenly blanched, and venous effluent was clear of blood by visual inspection. The lung was ventilated (40 breaths/min) with end-inspiratory and end-expiratory pressures of ~6 and 3 mmHg, respectively, with the above gas mixture. The pulmonary arterial pressure was referenced to atmospheric pressure at the level of the left atrium and monitored continuously during the course of the experiments. The venous effluent pressure was atmospheric pressure. At the end of each experiment, the lung was weighed and then dried (60°C) to a constant weight for the determination of lung dry weight.

Experimental protocols. To evaluate the disposition of CoQ1 and CoQ1H2 during passage through the lung, pulse infusion and bolus injection indicator dilution experiments were carried out. Pulse infusion experiments were carried out to provide data about the capacity of the lung to reduce CoQ1 and to oxidize CoQ1H2 and the contributions of various oxidoreductases. The bolus injection experiments were carried out to provide data in which transient information about CoQ1H2 disposition on passage through the lung is emphasized, thus providing insights into the tissue permeation of CoQ1H2. What follows is a description of the main pulse infusion and bolus injection experimental protocols.

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To determine the capacity of the lung to reduce CoQ1, each lung received four successive 30-s CoQ1H2 pulse infusions at concentrations of 50, 100, 200, and 400 M at a flow of 30 ml/min. Two venous effluent samples (~0.5 ml) were collected between 25 and 30 s after the initiation of each pulse infusion. This protocol was carried out in six normoxic and seven hyperoxic lungs.

To determine the redox processes that contribute to CoQ1 reduction on passage through the pulmonary circulation, each lung was perfused for 5 min with perfusate containing the complex I inhibitor rotenone (20 M) or the NQO1 inhibitor dicumarol (400 M) (3), or a combination of the two (rotenone plus dicumarol). This was followed by four successive CoQ1 pulse infusions, as above, where the inhibitors were also present throughout the infusion protocol. Two venous effluent samples (~0.5 ml) were collected between 25 and 30 s after the initiation of each pulse infusion. The number of normoxic lungs perfused with perfusate containing rotenone, or dicumarol, or both were 4, 5, and 5, respectively, for a total of 13 lungs. For the same sequence of inhibitors, the number of hyperoxic lungs studied were 4, 5, and 4, respectively, for a total of 13 lungs.

To evaluate the capacity of the lung to oxidize CoQ1H2, each lung was perfused for 5 min with perfusate containing rotenone (20 M) and dicumarol (400 M) to minimize CoQ1 reduction, followed by four successive CoQ1H2 pulse infusions at concentrations of 50, 100, 200, and 400 M at a flow of 30 ml/min. The inhibitors were present throughout this infusion protocol. Four normoxic and four hyperoxic lungs were studied using this protocol. In four of these lungs (two normoxic and two hyperoxic), an additional 200 M CoQ1H2 pulse infusion was carried out in the presence of cyanide (KCN) (2 mM, complex IV inhibitor) in addition to dicumarol and rotenone. This pulse infusion was carried out to evaluate the contribution of mitochondrial electron transport complex III to CoQ1H2 oxidation on passage through the lung. Two venous effluent samples (~0.5 ml) were collected between 25 and 30 s after the initiation of each pulse infusion.

To evaluate the accessibility of CoQ1H2 to lung tissue on passage through the pulmonary circulation, bolus injection experiments were carried out in one normoxic and one hyperoxic lung. Each lung was first perfused with cyanide (2 mM) containing perfusate for 5 min, after which the respirator was stopped at end expiration, and a 0.1 M bolus of perfusate containing cyanide and either 400 M CoQ1H2 or 35 M FITC-dex was injected into the pulmonary arterial inflow tubing. At the same time that the bolus was injected, the venous effluent was diverted into a fraction collector for continuous collection of the lung effluent at a rate and duration depending on the perfusate flow (2). The FITC-dex and CoQ1H2 bolus injections were repeated with the flow set to 10 and 30 ml/min.

To determine the tubing transit time of the perfusion system, at the end of the above bolus injection protocol the lung was removed from the perfusion system, and the arterial and venous cannulas were connected, and two FITC-dex (35 M) bolus injections were carried out at 10 and 30 ml/min.

To determine the perfused lung surface area, a 150 M 20-s pulse infusion of the angiotensin-converting enzyme substrate N-[3-(2-furyl) acryloyl]-Phe-Gly-Gly (FAPGG) was introduced into the lung with the flow set at 30 ml/min (3). Two venous effluent samples (~1.0 ml each) were collected between 15 and 20 s after the start of the infusion (3). This FAPGG pulse infusion was carried out at the beginning of each of the above experimental protocols in normoxic and hyperoxic lungs.

**Determination of CoQ1, CoQ1H2, and FITC-dex in venous effluent samples.** Venous effluent samples were first centrifuged (1 min at 5,600 g). For each sample, 100 M of supernatant were then added to each of two centrifuge tubes: one containing 10 M potassium ferricyanide (1.21 M in deionized H2O) to oxidize any CoQ1H2 to CoQ1, and the other containing 10 M EDTA (1 M in deionized H2O) to minimize autoxidation of CoQ1H2. Ice-cold absolute ethanol (0.4-0.8 ml) was added, and the tubes were mixed on a vortex mixer followed by centrifugation at 9,300 g for 5 min at 4°C. A perfusate sample that had passed through the lungs but contained no CoQ1 or CoQ1H2 was treated in the same manner to be used as the blank for absorbance measurements. The absorbances were measured at 275 nm using a Beckman DU 7400 spectrophotometer. Sample concentrations of CoQ1 and CoQ1H2 (in M) were calculated from the absorbance values of the fully oxidized (following the addition of ferricyanide) (Abs1) and the EDTA-treated (Abs2) supernatant in the tubes using extinction coefficients at 275 nm of 14.30 mM-1 cm-1 for CoQ1 and 2.29 mM-1 cm-1 for CoQ1H2 (41, 57) as follows

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where [CoQ1] and [CoQ1H2] are the concentrations of CoQ1 and CoQ1H2 sample concentrations, respectively.

For a given venous sample, [CoQ1] and [CoQ1H2] are given by Eqs. 1 and 2:

\[
[\text{CoQ1}] = \frac{\text{Abs1} - 0.00229}{0.0143} \quad \text{and} \quad [\text{CoQ1H2}] = \frac{\text{Abs1} - 0.00229}{0.0143} - [\text{CoQ1}]
\]

FITC-dex concentrations were determined from the absorbance at 495 nm using a molar extinction coefficient of 93.5 mM⁻¹ cm⁻¹ (2, 3).

**RESULTS**

Figure 1 shows the venous effluent concentrations of CoQ1 and CoQ1H2, represented as fractions of the infused concentration of CoQ1 (Fig. 1A) or CoQ1H2 (Fig. 1B) in a normoxic lung. Also shown are the venous effluent FITC-dex concentrations as fractions of the infused FITC-dex concentration. Since FITC-dex is an intravascular indicator that is not otherwise metabolized or taken up by the lung, the FITC-dex fractional concentration vs. time curves represent what the CoQ1 (Fig. 1A) and CoQ1H2 (Fig. 1B) curves would have looked like, if the only effect of passage through the lung were convection (2, 3). The fact that the CoQ1 (Fig. 1A) and CoQ1H2 (Fig. 1B) curves deviate from that for FITC-dex suggests that processes in addition to convection, e.g., CoQ1 reduction and CoQ1H2 oxidation, are taking place on passage through the lung.

Regardless of whether CoQ1 or CoQ1H2 was infused, the fractional concentrations of CoQ1, CoQ1H2, and FITC-dex all approached steady state by ~25 s (Fig. 1). At steady state, ~93 and 96% of the infused CoQ1 (Fig. 1A) and CoQ1H2 (Fig. 1B), respectively, were recovered in the venous effluent as CoQ1 + CoQ1H2, with the loss attributable primarily to binding of both forms to the experimental tubing (data not shown). At steady state, ~40% of the infused CoQ1 appeared as CoQ1H2, in the venous effluent (Fig. 1A), and ~13% of the infused CoQ1H2 appeared as CoQ1 (Fig. 1B). In subsequent pulse infusion studies, the steady-state data were used to calculate steady-state CoQ1 or CoQ1H2 efflux rates, calculated as the product of the perfusate flow and the steady-state CoQ1 or CoQ1H2 concentrations, respectively, in venous samples collected between 25 and 30 s.

To evaluate the processes contributing to CoQ1 reduction in the lung, the steady-state CoQ1H2 efflux rates were measured over a range of infused CoQ1 concentrations in normoxic lungs in the absence (control) and presence of the complex I inhibitor rotenone, the NQO1 inhibitor dicumarol, and the combination...
of the two (Fig. 2). The steady-state CoQ\(_1\)H\(_2\) efflux rates increased over the concentration range studied and approached saturation at the highest CoQ\(_1\) concentration. Figure 2 also shows that the steady-state CoQ\(_1\)H\(_2\) efflux rates decreased by \(58\) and \(33\)% in the presence of rotenone and dicumarol, respectively, and by \(85\)% in the presence of both inhibitors, suggesting a dominant role for complex I and NQO1 in CoQ\(_1\) reduction on passage through the lung. The data also reveal the contribution of a rotenone-dicumarol-insensitive reductase(s) to CoQ\(_1\) reduction on passage through the lung.

The rotenone concentration (20 \(\mu\)M) used to inhibit complex I-mediated CoQ\(_1\) reduction is a saturating concentration based on the results of a rotenone dose-response experiment (data not shown), which showed no additional inhibition of the steady-state rate of CoQ\(_1\)H\(_2\) efflux during CoQ\(_1\) (200 \(\mu\)M) infusion in a normoxic lung for rotenone concentrations \(>20\) \(\mu\)M. The concentrations of dicumarol (400 \(\mu\)M) and cyanide (2 mM) used were also saturating based on the results of previous studies (2, 3).

To evaluate the capacity of the lung to oxidize CoQ\(_1\)H\(_2\), the steady-state CoQ\(_1\) efflux rates were measured during the infusion of CoQ\(_1\)H\(_2\) over a range of concentrations. For these studies, CoQ\(_1\) reduction was minimized by including dicumarol and rotenone in the perfusate. The steady-state CoQ\(_1\) efflux rates increased over the range of CoQ\(_1\)H\(_2\) concentrations studied, with no detectable difference between normoxic and hyperoxic lungs (Fig. 3). In the presence of cyanide, the steady-state CoQ\(_1\) efflux rate dropped to nearly zero (data not shown), suggesting that CoQ\(_1\)H\(_2\) oxidation on passage through normoxic and hyperoxic lungs occurs predominantly via the hydroquinone oxidase-cytochrome \(c\) reductase activity of complex III.

Exposure to hyperoxia had a significant effect on the capacity of the lung to reduce CoQ\(_1\). The steady-state CoQ\(_1\)H\(_2\) efflux rates over the range of CoQ\(_1\) concentrations studied were \(23\)% lower in hyperoxic than in normoxic lungs (Fig. 4A). This decrease can be almost completely eliminated with rotenone (Fig. 4B) or rotenone plus dicumarol (Fig. 4C), but not with dicumarol alone (Fig. 4D), suggesting a decreased contribution of the complex I-mediated component of CoQ\(_1\) reduction in hyperoxic lungs. On the other hand, the contributions of the dicumarol (NQO1)-sensitive component (Fig. 4, B and C) and the rotenone-dicumarol-insensitive component (Fig. 4C) appeared to be unaffected by hyperoxia. Figure 4 also indicates that complex I and NQO1 are the dominant CoQ\(_1\) reductases on passage through hyperoxic as well as normoxic lungs.

Bolus injection experiments were used to evaluate the accessibility of CoQ\(_1\)H\(_2\) to lung tissue from the vasculature.
Normoxic lung and one hyperoxic lung. Cyanide was present in the perfusate lung mean transit time (vascular mean transit time). Solid lines are model fits time from each sample time and then normalizing the values to the FITC-dex both flows. The time scale was obtained by subtracting tubing mean transit they were virtually superimposable for the normoxic and hyperoxic lung at flows of 10 and 30 ml/min. Only one FITC-dex curve (10 ml/min, normoxic lung) is shown, since injections of FITC-dex (35 M) or CoQ1H2 for the normoxic and hyperoxic lung were similar, implying that CoQ1H2 accesses equivalent tissue volumes from the vascular region of normoxic and hyperoxic lungs. We were unable to carry out such bolus injection studies for CoQ1 because of the high dicumarol concentration (400 M) required to inhibit NQO1 in the presence of an albumin-containing medium. This dicumarol concentration would interfere with spectrophotometric measurements of CoQ1 at the low CoQ1 concentrations (<5 M) that are present in effluent samples in the early rising portion and the tail of the CoQ1 concentration vs. time outflow curve following CoQ1 bolus injection. Additionally, we were unable to inhibit the unidentified rotenone-dicumarol-insensitive reductase(s) contributing to CoQ1 reduction.

Because the data in Fig. 4 suggested that the hyperoxic exposure decreased the complex I-mediated CoQ1 reduction, complex I activity was measured in the P2 fractions obtained from lung homogenates. Table 1 shows that complex I activity normalized to protein was ~48% lower in P2 fractions derived from hyperoxic than normoxic lungs. There was no significant difference between normoxic and hyperoxic lungs with respect to mitochondrial electron-transport complex IV activity or whole lung O2 consumption rate (Table 1). The latter is an approximation of the total steady-state consumption rate of reducing equivalent by the entire lung (2).

Table 2 shows that lung perfusion pressure, wet weight, wet-to-dry weight ratio, and perfused capillary surface area were not significantly different between normoxic and hyperoxic lungs, consistent with previous studies (2, 3, 15, 26).

Data analysis. The data in Figs. 1–5 are the net result of CoQ1 and CoQ1H2 interactions with competing nonlinear tissue redox processes, CoQ1 and CoQ1H2 interactions with BSA in the vascular space, and capillary perfusion kinematics [i.e., capillary transit time distribution, h(t)]. The latter affects the efficiency of the lung in reducing CoQ1 and oxidizing CoQ1H2 on passage through the pulmonary circulation. For instance, the longer the capillary mean transit time, the more time available for CoQ1 and CoQ1H2 interactions with lung tissue redox.

Table 1. Mitochondrial complex I and IV activities measured in P2 fractions of lung homogenates and whole lung oxygen consumption rate

<table>
<thead>
<tr>
<th></th>
<th>Complex I Activity, mmol min⁻¹ mg protein⁻¹</th>
<th>Complex IV Activity, mmol min⁻¹ mg protein⁻¹</th>
<th>Lung O2 Consumption, μmol min⁻¹ g dry lung wt⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxic</td>
<td>11.89±1.05</td>
<td>121.29±22.34</td>
<td>3.44±0.11</td>
</tr>
<tr>
<td>Hyperoxic</td>
<td>6.25±1.01*</td>
<td>135.02±24.99</td>
<td>2.80±0.48</td>
</tr>
</tbody>
</table>

Values are means ± SE. For complexes I and IV activities, n = 9 and 7 for normoxic and hyperoxic lungs, respectively. For lung O2 consumption rate, n = 4 and 4 for normoxic and hyperoxic lungs, respectively. *Significantly different from the corresponding normoxic value, P < 0.05.
Within the vascular volume of the kinetic model, CoQ₁ and CoQ₁H₂ participate in nonspecific and rapidly equilibrating interactions with the perfusate albumin, Pₚ (processes 1 and 2). Within the tissue volume, CoQ₁ is reduced via complex I (process 3), NQO₁ (process 4), and otherwise unidentified rotenone-dicumarol-insensitive reductase(s) (process 5), and CoQ₁H₂ is oxidized via complex III (process 6). Also, within the tissue volume, CoQ₁ and CoQ₁H₂ undergo nonspecific, rapidly equilibrating interactions with lung tissue sites (Pₚ) of association (processes 7 and 8). CoQ₁ reduction and CoQ₁H₂ oxidation are assumed to follow Michaelis-Menten kinetics, with Vmax and Km representing the maximum reduction or oxidation rate and Michaelis-Menten constant, respectively. All nonspecific CoQ₁ and CoQ₁H₂ interactions are assumed to follow the law of mass action.

The governing differential equations of the single capillary element model (see Appendix) are the basis of the whole organ model, which accounts for the distribution of pulmonary capillary transit times, tₜ, as described in the Appendix. The tₜ was previously determined for the normoxic rat lung (2) and is assumed to be unaffected by exposure to 85% O₂ for 48 h. This is based on the results in Table 1, which show that exposure to hyperoxia had no significant effect on lung vascular flow distribution.

The kinetic model parameters are Vmax₁, Vmax₂, and Vmax₃, which are the maximum rates (µmol/min) for CoQ₁ reduction via complex I, NQO₁, and rotenone-dicumarol-insensitive reductase(s), respectively; Km₁ₐ, Km₂ₐ, and Km₃ₐ (µM), the apparent Michaelis-Menten constants (µM) for complex I, NQO₁, and rotenone-dicumarol-insensitive reductase(s) mediated CoQ₁ reduction, respectively (3); Vmax₄

Table 2. Rat body weights, lung perfusion pressures, lung weights, lung wet-to-dry ratio, and angiotensin-converting enzyme activity

<table>
<thead>
<tr>
<th>Body Weight, g</th>
<th>Wet Weight, g</th>
<th>Wet-to-Dry Ratio</th>
<th>PS, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxic 316±5</td>
<td>9.8±0.2</td>
<td>1.28±0.03</td>
<td>5.6±0.1</td>
</tr>
<tr>
<td>Hyperoxic 311±3</td>
<td>9.4±0.3</td>
<td>1.34±0.04</td>
<td>5.8±0.1</td>
</tr>
</tbody>
</table>

Values are mean ± SE. For normoxic lungs, n = 43, 34, 43, 30, and 28 for body weight, Pₚ, lung wet weight, wet-to-dry ratio, and PS, respectively, where 43 is the total number of normoxic lungs studied. For hyperoxic lungs, n = 40, 34, 39, 31, and 30, respectively, where 40 is the total number of hyperoxic lung studied. Pa, lung perfusion pressure with perfusate flow set at 30 ml/min; PS (permeability surface area product), measure of plasmatic clearance of N-[3-(2-furyl) acryloyl]-Phe-Gly-Gly and an index of perfused capillary surface area.

Fig. 6. A schematic representation of the hypothesized vascular and lung tissue interactions of CoQ₁ and CoQ₁H₂ in a single capillary element consisting of a vascular region and its surrounding tissue region. Within the vascular region, CoQ₁ and CoQ₁H₂ participate in nonspecific and rapidly equilibrating interactions with the perfusate BSA, Pₚ (processes 1 and 2). Within the tissue region, CoQ₁ is reduced via complex I (process 3), NQO₁ (process 4), and otherwise unidentified rotenone-dicumarol-insensitive reductase(s) (process 5), and CoQ₁H₂ is oxidized via complex III (process 6). Also, within the tissue volume, CoQ₁ and CoQ₁H₂ undergo nonspecific, rapidly equilibrating interactions with lung tissue sites (Pₚ) of association (processes 7 and 8). CytoC (oxid.) and cytoC (red.) are the oxidized and reduced forms of cytochrome c, respectively. DH and D⁺ represent the reduced and oxidized forms of electron donors for the unknown rotenone-dicumarol-insensitive CoQ₁ reductase(s), respectively.

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(μmol/min) and $K_{m3a}$ (μM), the maximum rate and apparent Michaelis-Menten constant for CoQ1H$_2$ oxidation via complex III, respectively; $V_{F1/\alpha 1}$ (ml) and $V_{F2/\alpha 2}$ (ml), the respective virtual tissue volumes of distribution accessible to CoQ1 and CoQ1H$_2$ from the vascular region (see Glossary). The constants $\alpha_1 = 14.6$ and $\alpha_2 = 16.5$ account for the rapidly equilibrating interactions of CoQ1 and CoQ1H$_2$ with the 5% BSA (i.e., $P_2$) perfusate calculated from the fractions of CoQ1 and CoQ1H$_2$ bound to BSA obtained by ultrafiltration.

The governing differential equations (see Appendix) show that the steady-state concentrations of CoQ1 and CoQ1H$_2$ during CoQ1 or CoQ1H$_2$ infusion are independent of the values of $V_{F1/\alpha 1}$ and $V_{F2/\alpha 2}$. Hence, the relevant kinetic model parameters under steady-state conditions during pulse infusion of CoQ1 or CoQ1H$_2$ are $V_{max1}$, $V_{max2}$, $V_{max3}$, $V_{max4}$, $K_{m1a}$, $K_{m2a}$, $K_{m3a}$, and $K_{m4a}$.

Estimation of kinetic model parameters descriptive of the various oxidoreductases. As described below, model parameters descriptive of the various oxidoreductases were estimated by fitting the model solution simultaneously to averaged pulse infusion data from multiple lungs (Figs. 2–4). This approach was chosen since data from multiple experimental protocols are needed to estimate these model parameters, and since it is not practical to carry out multiple protocols in the same lung. We have used this approach previously, and it is similar to the simultaneous nonlinear regression approach used by others (2, 50, 61).

For the data in Figs. 3 and 4C, complex I and NQO1 were inhibited by rotenone and dicumarol, respectively. Thus parameters descriptive of CoQ1 reduction via rotenone-dicumarol-insensitive reductase(s) ($V_{max3}$ and $K_{m3a}$) and CoQ1H$_2$ oxidation via complex III ($V_{max4}$ and $K_{m4a}$) were estimated by fitting the steady-state solution of the organ model (Appendix) simultaneously to the mean values of the normoxic data in Figs. 3 and 4C. Inhibition of complex I and NQO1-mediated components of CoQ1 reduction were simulated by setting the values of $V_{max1}$ and $V_{max2}$ to zero. The estimated values of $V_{max3}$, $K_{m3a}$, $V_{max4}$, and $K_{m4a}$ are shown in Table 3. Also shown in Table 3 are the asymptotic coefficients of variation (CVs) of these estimates and correlation coefficients between these parameters, which were determined as previously described (5, 44). Since Figs. 3 and 4C show no significant differences between normoxic and hyperoxic data in the presence of dicumarol plus rotenone, the values of $V_{max3}$, $K_{m3a}$, $V_{max4}$, and $K_{m4a}$ for hyperoxic lungs were set to those estimated for normoxic lungs (Table 3).

Knowing $V_{max3}$, $K_{m3a}$, $V_{max4}$, and $K_{m4a}$, the model parameters descriptive of complex I-mediated CoQ1 reduction ($V_{max1}$ and $K_{m1a}$) and NQO1-mediated CoQ1 reduction ($V_{max2}$ and $K_{m2a}$) in normoxic and hyperoxic lungs were then estimated. This was achieved by fitting the steady-state solution of the organ model (CoQ1H$_2$ efflux rates when CoQ1 was infused) simultaneously to the mean values of the normoxic and hyperoxic data in Fig. 4, A, B, and D, in the absence (control) and presence of rotenone or dicumarol. For this parameter estimation step, $V_{max1}$ was assumed to be the only parameter affected by exposure to hyperoxia. The other parameters, namely $K_{m1a}$, $V_{max2}$, and $K_{m2a}$, were assumed to have the same values for normoxic and hyperoxic lungs. The estimated values of $V_{max1}$, $K_{m1a}$, $V_{max2}$, and $K_{m2a}$ for normoxic and hyperoxic lungs, CVs of these estimates, and correlation coefficients between these parameters are shown in Table 4.

The asymptotic CV is a measure of the relative precision of the estimate of a given parameter. The CVs for the estimated values of $V_{max1}$ for normoxic and hyperoxic lungs (Table 4) are relatively small, consistent with a significant difference (likelihood ratio test; $P < 0.005$) between the estimated values of $V_{max1}$ for normoxic and hyperoxic lungs. On the other hand, the CVs for the estimated values of $K_{m2a}$ and $K_{m3a}$ (Tables 3 and 4) are relatively high. One possible reason is that the actual values of these parameters are low relative to the range of CoQ1 concentrations achieved in this study.

Tables 3 and 4 show that the absolute values of the correlation coefficients between the model parameters descriptive of the various oxidoreductases were < 0.85. This suggests that the pulse infusion data (Figs. 2–4) have enough discriminating information about these parameters.

Estimation of the virtual tissue volume ($V_{F2/\alpha 2}$) accessible to CoQ1H$_2$ on passage through normoxic and hyperoxic lungs. The virtual tissue volume $V_{F2/\alpha 2}$ accessible to CoQ1H$_2$ from the vascular region was calculated as the product of perfusate flow and the difference between the mean transit times of the venous effluent curves of FITC-dex and CoQ1H$_2$ (Fig. 5), estimated as previously described (3). The estimated values of $V_{F2/\alpha 2}$ for the one normoxic lung studied were 1.68 and 1.78 ml at 10 and 30 ml/min, respectively. For the one hyperoxic lung studied, the estimated values of $V_{F2/\alpha 2}$ are 1.70 and 1.85 ml at 10 and 30 ml/min, respectively. For normoxic and hyperoxic lungs, the value of the tissue volume accessible to CoQ1 from the vascular region, $V_{F1/\alpha 1}$, was set to that estimated for $V_{F2/\alpha 2}$ from the data in Fig. 5. Table 5 provides a summary of the values of all of the kinetic model parameters for normoxic and hyperoxic lungs.

Model predictions. One approach for evaluating the above kinetic model is to test its ability to predict CoQ1 and/or CoQ1H$_2$ indicator dilution data not used for estimating the kinetic model parameters (2, 13). To this end, we carried out the following experiments and tested the ability of the model to predict their results.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimated Values</th>
<th>CV, %</th>
<th>Correlation Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{max1}$, μmol/min</td>
<td>0.86</td>
<td>20.1</td>
<td>1 0.76 −0.06 0.16</td>
</tr>
<tr>
<td>$K_{m3a}$, μM</td>
<td>65.6</td>
<td>77.4</td>
<td>1 −0.08 0.56</td>
</tr>
<tr>
<td>$V_{max4}$, μmol/min</td>
<td>4.6</td>
<td>3.2</td>
<td>1 0.60</td>
</tr>
<tr>
<td>$K_{m4a}$, μM</td>
<td>75.9</td>
<td>14.5</td>
<td>1</td>
</tr>
</tbody>
</table>

$V_{max3}$ and $K_{m3a}$ are the respective maximum rate and apparent Michaelis-Menten constant for coenzyme Q$_1$ (CoQ$_1$) reduction via rotenone-dicumarol-insensitive reductase(s); $V_{max}$ and $K_{m}$ are the respective maximum rate and apparent Michaelis-Menten constant for CoQ$_1$ hydroquinone (CoQ$_1$H$_2$) oxidation via complex III. Parameter values were estimated from the mean values of the normoxic data in Figs. 3 and 4C. The values of $V_{max1}$, $K_{m1a}$, $V_{max2}$, and $K_{m2a}$ for hyperoxic lungs are assumed to be the same as those estimated for normoxic lungs. CV, asymptotic coefficient of variation.

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Table 4. Estimated values of the kinetic parameters
descriptive of rotenone and dicumarol-sensitive CoQ1
reduction on passage through normoxic and hyperoxic lungs,
a measure of the relative precision of the estimates of these parameters, and correlation coefficients between
these parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimated Values</th>
<th>CV, %</th>
<th>Correlation Matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{max1}$ (normoxic), $\mu$mol/min</td>
<td>2.17</td>
<td>8.6</td>
<td>0.61</td>
</tr>
<tr>
<td>$V_{max2}$ (hyperoxic), $\mu$mol/min</td>
<td>1.15*</td>
<td>12.0</td>
<td>1</td>
</tr>
<tr>
<td>$K_{m1a}$, $\mu$M</td>
<td>33.7</td>
<td>42.3</td>
<td>1</td>
</tr>
<tr>
<td>$V_{max2}$, $\mu$mol/min</td>
<td>1.17</td>
<td>11.5</td>
<td>1</td>
</tr>
<tr>
<td>$K_{m2a}$, $\mu$M</td>
<td>13.4</td>
<td>93.6</td>
<td>1</td>
</tr>
</tbody>
</table>

$V_{max1}$ and $K_{m1a}$ are the respective maximum rate and apparent Michaelis-Menten constant for CoQ1 reduction via complex I; $V_{max2}$ and $K_{m2a}$ are the respective maximum rate and apparent Michaelis-Menten constants for NAD(P)H:quinone oxidoreductase 1 (NQO1)-mediated CoQ1 reduction. Parameters were estimated from the mean values of the normoxic and hyperoxic data shown in Figure 4. A, B, and D, with $V_{max1}$ as the only parameter affected by exposure to hyperoxia. *Significantly (likelihood ratio test; $P < 0.005$) different from the corresponding normoxic value.

The first experiment was to evaluate the effect of inhibiting CoQ1 oxidation using cyanide (2 mM) on the steady-state rate of CoQ1 oxidation during CoQ1 infusion in normoxic ($n = 4$) and hyperoxic ($n = 4$) lungs. Figure 7 shows that, in the presence of cyanide, which closes complex III for CoQ1 oxidation, the steady-state rates of CoQ1 efflux were ~30–100% higher than in the absence of cyanide (control) over the range of CoQ1 concentrations studied in normoxic (Fig. 7A) and hyperoxic (Fig. 7B) lungs.

The second experiment was to evaluate the effect of inhibiting complex I-mediated CoQ1 reduction on the venous effluent concentration vs. time outflow curves for CoQ1 and CoQ1H2 following CoQ1 arterial bolus injection in one normoxic lung. Again, the FITC-dex venous effluent curve in Fig. 8 represents what the CoQ1 venous effluent curve would have been had CoQ1 not interacted with the lung as it passed through the pulmonary vessels. Figure 8 shows that ~71% of the injected CoQ1 was recovered as CoQ1H2 in the venous effluent in the absence of the complex I inhibitor rotenone (Fig. 8A) compared with ~49% in the presence of rotenone (Fig. 8B). Furthermore, despite the differences in the venous effluent curves of CoQ1 and CoQ1H2 in the absence (Fig. 8A) and presence (Fig. 8B) of rotenone, the venous effluent curves of (CoQ1 + CoQ1H2) are virtually superimposable, suggesting that CoQ1 and CoQ1H2 have similar tissue volumes of distribution accessible from the vascular region.

The solid lines superimposed over this data in Figs. 7 and 8 are the predicted organ model solutions. For these predictions, the values of the model parameters for normoxic and hyperoxic lungs were set to those in Table 5, estimated from the data in Figs. 2–5. The ability to predict the dominant features of the data in Figs. 7 and 8 is further evidence that the model in Fig. 6 provides a reasonable explanation of the dominant vascular and tissue processes that determine the pulmonary disposition of CoQ1 and CoQ1H2 on passage through the lungs of normoxic and hyperoxic rats.

**DISCUSSION**

The results demonstrate the capacity of the rat lung to reduce CoQ1 to CoQ1H2 and to oxidize CoQ1H2 to CoQ1 on passage through the rat pulmonary circulation. The dominant CoQ1 reductases are mitochondrial electron transport complex I and NQO1, as revealed by the inhibitory effects of rotenone and dicumarol, respectively (11, 23, 30, 32, 35, 45, 46). The data also shows that additional rotenone and dicumarol-insensitive reductase(s) contribute to CoQ1 reduction on passage through the pulmonary circulation. This was revealed by the observation that dicumarol plus rotenone did not completely eliminate CoQ1H2 efflux during CoQ1 infusion over the range of CoQ1 concentrations studied (Fig. 4C). Potential rotenone-dicumarol-insensitive CoQ1 reductases include mitochondrial electron transport complex II, transplasma membrane electron transport, and glycerol-3-phosphate-CoQ reductase (34, 52, 57, 62). There is also the possibility of CoQ1 reduction at a rotenone-insensitive site on complex I (30, 46).

The oxidation of CoQ1H2 on passage through the pulmonary circulation was inhibited by cyanide, suggesting that the predominant pulmonary CoQ1H2 oxidase is mitochondrial electron transport complex III. Cyanide promotes a reduced state for complex III, thereby eliminating complex III as a pathway for oxidation of quinones, including CoQ1H2 in pulmonary endothelial cells (23, 48). The ability of CoQ1H2 to serve as an electron donor for complex III has also been previously observed in purified yeast complex III and hepatocytes (23, 42).

Table 5. Summary of the values of kinetic model parameters
for normoxic and hyperoxic lungs

<table>
<thead>
<tr>
<th>Model Processes and Parameters</th>
<th>Normoxic Lungs</th>
<th>Hyperoxic Lungs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{max1}$, $\mu$mol/min</td>
<td>2.17</td>
<td>1.15*</td>
</tr>
<tr>
<td>$K_{m1a}$, $\mu$M</td>
<td>33.7</td>
<td>33.7</td>
</tr>
<tr>
<td>NQO1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{max2}$, $\mu$mol/min</td>
<td>1.17</td>
<td>1.17</td>
</tr>
<tr>
<td>$K_{m2a}$, $\mu$M</td>
<td>13.4</td>
<td>13.4</td>
</tr>
<tr>
<td>Rotenone-dicumarol-insensitive reductase(s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{max3}$, $\mu$mol/min</td>
<td>0.86</td>
<td>0.86</td>
</tr>
<tr>
<td>$K_{m3a}$, $\mu$M</td>
<td>65.6</td>
<td>65.6</td>
</tr>
<tr>
<td>Complex III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{max4}$, $\mu$mol/min</td>
<td>4.6</td>
<td>4.6</td>
</tr>
<tr>
<td>$K_{m4a}$, $\mu$M</td>
<td>75.9</td>
<td>75.9</td>
</tr>
<tr>
<td>Apparent tissue volumes accessible to CoQ1 and CoQ1H2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_f/\alpha_1$, ml</td>
<td>1.73</td>
<td>1.78</td>
</tr>
<tr>
<td>$V_f/\alpha_2$, ml</td>
<td>1.73</td>
<td>1.78</td>
</tr>
</tbody>
</table>

The values of $V_{max1}$, $K_{m1a}$, $V_{max2}$, and $K_{m2a}$ were estimated from the mean values of the normoxic and hyperoxic data shown in Figure 4. A, B, and D, with $V_{max1}$ as the only parameter affected by exposure to hyperoxia. The values of $V_{max3}$, $K_{m3a}$, $V_{max4}$, and $K_{m4a}$ were estimated from the mean values of the normoxic data in Figure 3 and Figure 4C. For hyperoxic lungs, the values of $V_{max4}$, $K_{m4a}$, $V_{max5}$, and $K_{max5}$ were set to those estimated for normoxic lungs. The values of $V_f/\alpha_1$ (virtual volume of distribution for CoQ1H2) for normoxic and hyperoxic lungs were estimated from the data in Figure 5. The values of $V_f/\alpha_2$ (virtual volume of distribution for CoQ1H2) for normoxic and hyperoxic lungs were estimated from the data in Figure 5. The values of $V_f/\alpha_1$ (virtual volume of distribution for CoQ1H2) for normoxic and hyperoxic lungs were estimated from the data in Figure 5. The values of $V_f/\alpha_2$ (virtual volume of distribution for CoQ1H2) for normoxic and hyperoxic lungs were estimated from the data in Figure 5. The values of $V_f/\alpha_1$ (virtual volume of distribution for CoQ1H2) for normoxic and hyperoxic lungs were estimated from the data in Figure 5.
The utility of the proposed kinetic model is in its ability to account for and evaluate potential changes in one or more of the dominant processes that determine the disposition of CoQ1 and CoQ1H2 on passage through the pulmonary circulation. This is important, especially if rotenone-sensitive CoQ1 reduction is to be used as an index of complex I activity in other models of lung injury or oxidative stress, where the activities of multiple redox processes might be altered (60).

The kinetic model analysis revealed that the ∼23% decrease in CoQ1 reduction capacity in hyperoxic lungs could be accounted for by ∼47% decrease in the maximum rate or capacity ($V_{\text{max}}$) of complex I-mediated CoQ1 reduction, with no effect on any of the other tissue or vascular processes hypothesized in the kinetic model. This is in contrast to longer exposure to 85% O2 (21 days), which, in addition to increasing the capacity of NQO1-mediated DQ reduction, decreased vascular volume and perfused capillary surface area (PS) and increased the heterogeneity of the vascular transit time distribution (3).

The observations of a lack of an effect of rat exposure to 85% O2 for 48 h on NQO1-mediated CoQ1 reduction and mitochondrial complex III-mediated DQH2 oxidation are consistent with the results of our laboratory’s previous study of DQ and DQH2 redox metabolism in the rat lung (3). Our previous study revealed that rat exposure to 85% O2 for 48 h had no significant effect on NQO1-mediated DQ reduction or complex III-mediated DQH2 oxidation during passage through the lung (3). Moreover, exposure to 85% O2 for 48 h had no significant effect on NQO1 activity or protein measured in cytosolic fractions of lung homogenates (3).

The decrease in complex I activity in the present study represents a relatively early in situ metabolic consequence of hyperoxia in that it precedes effects on lung histology, morphometry, and hemodynamic and perfusion kinematics observed with rat exposure to 85% O2 for 21 h (3, 26). Of the few studies evaluating the metabolic consequences of hyperoxia in the 18- to 48-h period, a decrease in serotonin clearance and an increase in lactate production have been observed in lungs from rats exposed to 100% O2 for 18 and 36 h, respectively (15, 36, 43). Additionally, Klein et al. demonstrated a decrease in the metabolism of prostaglandin E2 in intact lungs of rats exposed to 97% O2 for 36 h (43).

The 48% decrease in mitochondrial complex I activity measured in P2 fractions from hyperoxic compared with normoxic lung homogenates was not associated with a detectable change in whole lung O2 consumption rate (Table 1), which is an approximation of the total steady-state consumption rate of reducing equivalents by the entire lung (2). One possible explanation is that complex I activity is normally in excess compared with the rate of electron flow through the respiratory...
chain. This is consistent with the results of a study by Barrientos and Moraes (11), in which the effects of complex I impairment on cell respiration were evaluated in a rotenone-treated human osteosarcoma-derived cell line. They showed that complex I activity was inhibited by as much as ~35%, with little effect (~5%) on cell respiration. Another possible explanation is activation of compensatory mechanisms (e.g., mitochondrial electron transport complex II, glycerol phosphate dehydrogenase) to sustain respiration in the complex I-impaired hyperoxic lung.

There is ample evidence that increased production of ROS is a major factor in hyperoxic lung injury (19, 22, 37, 38). Thus one strategy that cells may follow to protect against hyperoxic lung injury is to mitigate the activities of ROS sources (18, 22, 59). Mitochondrial electron transport complex I is a major source of ROS (18, 59). Moreover, studies have shown that the rate of ROS formation at complex I in endothelial cells increased with an increase in O₂ tension and that complex I inactivation using rotenone decreased ROS generation in sheep pulmonary microvascular endothelial cells exposed to hyperoxia (100% O₂ for 30 min) (18, 54). Additional studies would be needed to evaluate the effect of the depression in complex I activity observed in hyperoxic lungs on ROS production at complex I and to determine whether this depression is an injury resulting from the increase in mitochondrial ROS production (24) or an early manifestation of an adaptive response to the hyperoxic environment (26).

Previous studies have addressed the quinone specificity of mitochondrial complex I and NQO1 (1, 29, 30, 33, 45, 48). Complex I, which catalyzes the transfer of electrons from NADH to ubiquinone, appears to be specific in terms of the structural and steric requirements for quinones to act as good electron acceptors (29, 30, 45). For instance, complex I has relatively high catalytic efficiencies for ubiquinone homologs with short isoprenoid side chains (e.g., CoQ₁), and ubiquinone analogs having straight saturated chains (e.g., decyl-ubiqui- none) (29, 30, 45). On the other hand, the benzoquinone DQ is considered a poor electron acceptor from complex I due to steric factors (30, 45). This is consistent with our laboratory’s results from a previous study in which we showed that DQ reduction on passage through the rat pulmonary circulation is a major factor in hyperoxic lung injury (19, 22, 37, 38). Moreover, studies have shown that the rate of ROS formation at complex I in endothelial cells increased with an increase in O₂ tension and that complex I inactivation using rotenone decreased ROS generation in sheep pulmonary microvascular endothelial cells exposed to hyperoxia (100% O₂ for 30 min) (18, 54). Additional studies would be needed to evaluate the effect of the depression in complex I activity observed in hyperoxic lungs on ROS production at complex I and to determine whether this depression is an injury resulting from the increase in mitochondrial ROS production (24) or an early manifestation of an adaptive response to the hyperoxic environment (26).

Quantitative structure-function studies have suggested that quinones with van der Waals volumes of <200 Å are better electron acceptors from NQO1 than quinones with van der Waals volumes of >200 Å (1, 33). Based on the van der Waals volumes of DQ (162.9 Å) and CoQ₁ (243.96 Å), one would expect DQ to be a better NQO1 substrate than CoQ₁. This might explain the lower capacity of NQO1-mediated CoQ₁ reduction (1.17 μmol/min) compared with NQO1-mediated DQ reduction (1.95 μmol/min) during passage through the rat lung (3).

Our laboratory’s previous study of CoQ₁ reduction in bovine pulmonary arterial endothelial cells (PAEC) provides insight into the results of the present study, and vice versa (48). Like the lung, the cells mediated reduction of CoQ₁ to CoQ₁H₂, the latter of which appeared in the extracellular medium. The reduction rate (~0.7 nmol·min⁻¹·cm⁻² of endothelial cell surface area) was sufficient to account for most of CoQ₁ reduction on passage through the rat pulmonary circulation (~0.9 nmol·min⁻¹·cm⁻², assuming a rat lung endothelial surface area of ~4,500 cm²) (3). Also, as in the lung, exposure of the cells to hyperoxia (albeit 95% O₂ for 48 h) decreased CoQ₁ reduction capacity via a depression in complex I activity, although the decrease in CoQ₁ reduction capacity and complex I activity in cells (52 and 80%, respectively) was larger than those in the lung (23 and 47%, respectively).

Whereas CoQ₁ reduction could be accounted for predominantly by complex I activity in cultured PAEC, CoQ₁ reduction in the lung is shared by complex I and NQO1. Whether the difference between lungs and cultured PAEC is attributable to cell culture conditions, contribution of other lung cell types to CoQ₁ reduction on passage through the pulmonary circulation, species difference in NQO1 electron acceptor preference (33), or the fact that cultured PAEC were derived from a large vessel, wherein the reduction in the intact lung presumably takes place largely in the capillaries, or other factors, is not known.

For blood-borne electron acceptors that are permeable to the lung tissue from the vasculature and are complex I substrates, the concept is that the lung would play a role in determining their redox status and concentrations in plasma and hence in their bioactivity and bioavailability upon entry into the systemic circulation. This might include a range of pharmacological, physiological, and toxic redox active compounds (e.g., quinones) (2, 7, 21, 45, 50). The further implication is that, when complex I activity is depressed, such as in the hyperoxic exposure we described, and perhaps other models of oxidative stress, the impact of the lung on such substances would be altered. Previously, our laboratory demonstrated the utility of various other redox active compounds as probes in indicator dilution methods for evaluating the activities of pulmonary endothelial transplasma membrane electron transport systems, NQO1, and monoamine oxidases in the intact lung (2–5, 9, 16, 27). The present study demonstrates the addition of CoQ₁ and CoQ₁H₂ to this series of nondestructive probes for evaluating lung redox functions, including mitochondrial complexes I and III, in experimental models of lung injury and disease.

APPENDIX

Based on the model depicted in Fig. 6, the species balance equations descriptive of spatial and temporal variations in the concentrations of CoQ₁, CoQ₁H₂ in vascular volume (Vᵥ), and tissue or extravascular volume (Vₑ) of a single capillary element, and in the concentration of FITC-dex in the vascular volume are:

\[
\frac{\partial [\text{FITC-dex}]}{\partial t} + W \frac{\partial [\text{FITC-dex}]}{\partial x} = 0 \tag{A1}
\]

\[
\frac{\partial [\text{CoQ₁}]}{\partial t} + \left( \frac{V_v}{V_v + \frac{V_{fr}}{\alpha_1}} \right) \frac{\partial [\text{CoQ₁}]}{\partial x} = \left( \frac{V_{max1}}{V_v + \frac{V_{fr}}{\alpha_1}} \right) \left( \frac{K_{m1a} + [\text{CoQ₁}]}{K_{m1a} + [\text{CoQ₁}]} \right) \tag{A2}
\]

\[
- \left( \frac{\text{CoQ₁H₂}}{V_v} \right) \left( \frac{V_{max2}}{K_{m2a} + [\text{CoQ₁}]} + \frac{V_{max3}}{K_{m3a} + [\text{CoQ₁}]} \right)
\]
where \( W \) is convective transport velocity = \( L/\kappa; \ x = 0 \) and \( x = L \) are the capillary inlet and outlet, respectively; \( \kappa \) is the capillary mean transit time; \([\text{FITC-dex}]_t(x, t), [\text{CoQ}_1]_t(x, t), \) and \([\text{CoQ}_1H_2]_t(x, t)\) are vascular concentrations of FITC-dex and free \( \text{CoQ}_1 \) and \( \text{CoQ}_1H_2 \) forms, respectively, at distance \( x \) from the capillary inlet and time \( t; [\text{CoQ}_1] = \alpha_1 [\text{CoQ}_1H_2] \) and \([\text{CoQ}_1H_2] = \alpha_2 [\text{CoQ}_1] \) are the total (free + BSA bound) vascular concentrations of \( \text{CoQ}_1 \) and \( \text{CoQ}_1H_2 \), respectively; \( \alpha_1 = 1 + (\text{CoQ}_1H_2 \) bound fraction/\( \text{CoQ}_1 \) free fraction) = 14.6 and \( \alpha_2 = 1 + (\text{CoQ}_1 \) bound fraction/\( \text{CoQ}_1H_2 \) free fraction) = 16.5 are constants that account for the rapidly equilibrating interactions of \( \text{CoQ}_1 \) and \( \text{CoQ}_1H_2 \) with the 5\% BSA (i.e., P,) perfusate obtained by ultrafiltration; \( V_{c1}/\alpha_1 \) \( V_c \) and \( V_{c2}/\alpha_2 \) \( V_c \) are the respective virtual volumes of distribution for \( \text{CoQ}_1 \) and \( \text{CoQ}_1H_2 \), where \( \alpha_3 \) and \( \alpha_4 \) are constants that account for the rapidly equilibrating interactions of \( \text{CoQ}_1 \) and \( \text{CoQ}_1H_2 \) with lung tissue sites \( (P_c) \) of association, respectively.

For the steady state during pulse infusion of \( \text{CoQ}_1 \), \( \text{CoQ}_1H_2 \), or FITC-dex, Eqs. A1–A3 reduce to:

\[
\frac{\partial [\text{FITC-dex}]}{\partial x} = 0 \quad (A4)
\]

\[
W V_c \frac{\partial [\text{CoQ}_1]}{\partial x} = \left[ \frac{[\text{CoQ}_1H_2]V_{\text{max}}}{K_{\text{m}1a} + [\text{CoQ}_1H_2]} \right] - \left[ \frac{[\text{CoQ}_1]}{K_{\text{m}1a} + [\text{CoQ}_1]} \right]
\]

\[
+ \left( \frac{V_{\text{max}}}{K_{\text{m}2a} + [\text{CoQ}_1]} \right)
\]

\[
- \left( \frac{V_{\text{max}}}{K_{\text{m}3a} + [\text{CoQ}_1]} \right)
\]

\[
W V_c \frac{\partial [\text{CoQ}_1H_2]}{\partial x} = - \left[ \frac{[\text{CoQ}_1H_2]V_{\text{max}}}{K_{\text{m}4a} + [\text{CoQ}_1H_2]} \right]
\]

\[
+ \left( \frac{V_{\text{max}}}{K_{\text{m}5a} + [\text{CoQ}_1H_2]} \right)
\]

\[
+ \left( \frac{V_{\text{max}}}{K_{\text{m}6a} + [\text{CoQ}_1H_2]} \right)
\]

\[
(A5)
\]

\[
(A6)
\]

Equations A1–A6 are for a single capillary element. To account for the effect of capillary perfusion kinematics on the plasma concentrations and redox status of \( \text{CoQ}_1 \) and \( \text{CoQ}_1H_2 \) on passage through the pulmonary circulation, an organ model was constructed that accounts for the distribution of pulmonary capillary transit times, \( h_i(t) \), which was previously determined for the normoxic rat lung (2), and was represented in the model using a random walk function (2,3).

To model \( \text{CoQ}_1 \) or \( \text{CoQ}_1H_2 \) pulse infusions or bolus injections, given \( h_i(t) \), Eqs. A1–A3 are solved numerically with appropriate initial \( (t = 0) \) and boundary \((x = 0) \) conditions (2, 5, 7). To provide the whole organ output \([\text{CoQ}_1]\) and \([\text{CoQ}_1H_2]\), the outputs for all transit times are summed, each weighted according to \( h_i(t) \), as previously described (2, 5, 7, 28).

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**REFERENCES**


