Oxygen dependency of monoamine oxidase activity in the intact lung

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Audi, Said H., Christopher A. Dawson, Susan B. Ahlf, and David L. Roerig. Oxygen dependency of monoamine oxidase activity in the intact lung. Am J Physiol Lung Cell Mol Physiol 281: L969–L981, 2001.—Hydrogen peroxide generated by monoamine oxidase (MAO)-mediated deamination of biogenic amines has been implicated in cell signaling and oxidative injury. Because the pulmonary endothelium is a site of metabolism of monoamines present in the venous return, this brings into question a role for MAO in hyperoxic lung injury. The objective of this study was to evaluate the O2 dependency of the MAO reaction in the lung. To this end, we measured the pulmonary venous effluent concentrations of the MAO substrate [14C]phenylethylamine and its metabolite [14C]phenylacetic acid after the bolus injection of either phenylethylamine or phenylacetic acid into the pulmonary arterial cells; pargyline; semicarbazide; multiple indicator dilution; mathematical modeling; endothelial cells; pargyline; semicarbazide

MONOAMINE OXIDASES (MAOs) purified from various tissues have been found to have rather high Michaelis constants for O2 ($K_{mO2}$), generally >200 μM (18, 19, 24–26, 29, 33, 37, 44, 45) and well above the physiological intracellular O2 concentrations in those tissues. This along with the observation that oxidative deamination of biogenic amines via MAOs can be a significant source of hydrogen peroxide (H2O2) involved in cell signaling (34, 41, 46) and oxidative injury (12, 14, 16, 51) suggests that the MAO O2 sensitivity may be physiologically important. For example, the protective effect of MAO inhibition against central nervous system O2 toxicity (51) and renal ischemia-reperfusion injury (16) has been attributed to the inhibition of the H2O2 produced during MAO-mediated monoamine metabolism. However, there is a large discrepancy between the $K_{mO2}$ for MAO from the several studies carried out with purified MAO (18, 19, 24–26, 29, 33, 37, 44, 45) and the apparent $K_{mO2}$ (12–34 μM) from the few studies carried out in cultured cells (26, 27). This suggests that in addition to possible species and/or tissue (31, 52) differences, the sensitivity for O2 may depend on the cellular MAO environment (9, 40, 52).

The pulmonary endothelium is an important site of metabolism of certain monoamines present in the systemic venous return (15) and is subject to injury during high O2 exposure (13). This brings into question the influence of PO2 on pulmonary endothelial monoamine metabolism and a possible contribution of MAO-generated H2O2 to pulmonary hyperoxic injury. The discrepancy between results from purified enzymes and cell culture indicates that the O2 dependency of MAO activity for cells residing in the intact organ cannot necessarily be predicted from studies on such reduced systems. Thus the objective of this study was to evaluate the O2 dependency of the pulmonary endothelial MAO activity in intact lungs.

To this end, we developed a bolus injection multiple indicator dilution (MID) method for measuring MAO kinetics in the intact organ where the factors affecting substrate disposition can be more complex than in either cell culture or purified enzyme systems (2–6, 10, 22, 38). Thus a key aspect of the approach is the ability to separate the kinetics of substrate-tissue interactions (e.g., membrane transport, enzymatic metabolism, nonspecific plasma and tissue protein interactions) from each other and from the kinematics of organ perfusion (e.g., perfusion heterogeneity, transit time distribution) (4, 6, 10, 38). We measured the effect of PO2 on the pulmonary venous effluent concentration of the MAO substrate β-[ethyl-1-14C]phenylethylamine hydrochloride ([14C]PEA) and its [1-14C]phenylacetic acid ([14C]PAA) metabolite after the bolus injection of...
either $[^{14}C]$PEA or $[^{14}C]$PAA into the pulmonary artery of perfused rabbit lungs. The apparent $K_m$ estimated
with a kinetic model of the pulmonary disposition of
$[^{14}C]$PEA was $\sim 18$ $\mu$M and therefore much lower than
that measured with purified enzymes (18, 19, 24–26,
29, 33, 37, 44, 45) and closer to that measured in
cultured cells (26, 27).

LUNG MONOAMINE OXIDASE ACTIVITY

Glossary

$B$ Site of sequestration of PEA within cells

$C_P(t)$ $[^{3}H]$2O effluent concentration versus time curve

$C_{in}(t) = (q/F)h_n(t)$ Capillary input function

$C_{B}(t)$ FITC-dextran (Dex) effluent concentration versus time curve

$C_T(t)$ Indicator concentration versus time outflow curve for perfusion tubing system without the lung

$h_c(t)$ Capillary transit time distribution

$h_n(t)$ Noncapillary (arteries, veins, connecting tubing, and injection system) transit time distribution

$K_1$ and $K_2$ PEA and PAA endothelial surface equilibrium dissociation constants, respectively

$k_{p_b}$ Cell sequestration rate constant for PEA

$[H_2O][O_2]_{k_a}$ MAO association rate constant

$K_m$ Michaelis constant for O2

$k_{met} = ([H_2O][O_2]k_a)/\alpha_3$ Measure of the rate of PEA deamination by pargyline-sensitive MAO, where $\alpha_3 = 1 + ([Pr_e]/K_{eq3})$

$k_{met2}$ Measure of the rate of PEA metabolism via semicarbazide-sensitive form of MAO (SSMAO)

$K_{eq2}$ and $K_{eq1}$ PEA and PAA plasma protein equilibrium dissociation constants, respectively

$K_{eq3}$ and $K_{eq4}$ PEA and PAA equilibrium dissociation constants for nonspecific intracellular associations, respectively

$k_{PAA} = PS_2/\alpha_4 Q_e$ Measure of PAA egress from the cells where $\alpha_4 = 1 + ([Pr_e]/K_{eq4})$

$k_{PEA} = PS_1/\alpha_3 Q_e$ Measure of PEA egress from the cells where $\alpha_3 = 1 + ([Pr_e]/K_{eq3})$

$k_{seq} = k_{p_b}[B]Q_e/\alpha_3$ Measure of the PEA sequestration rate within the lung tissue where $\alpha_3 = 1 + ([Pr_e]/K_{eq3})$

$\text{MAO}$ Monoamine oxidase

$\text{Pa and Pv}$ Arterial and venous pressures, respectively

$\text{PAA}$ Phenylacetic acid

$\text{PAA}_{e}(x,t)$ where $\alpha_3 = 1 + ([Pr_e]/K_{eq3})$

$\text{PEA}$ Phenylethylamine

$\text{PEAB}$ PEA bound to site of sequestration within cells

$\text{PEAP}_{e}$ and $\text{PAA}_{e}$ PEA and PAA associated with nonspecific intracellular sites, respectively

$\text{PEAP}_{e}$ and $\text{PAA}_{e}$ PEA and PAA bound to plasma protein, respectively

$[\text{PAA}_{e}](x,t)$ Vascular concentration of PAA at distance $x$ from capillary inlet and time $t$

$[\text{PAA}_{e}](x,t)$ Endothelial cell concentration of PAA at distance $x$ from capillary inlet and time $t$

$[\text{PEA}_{e}](x,t)$ Vascular concentration of PEA at distance $x$ from capillary inlet and time $t$

$[\text{PEA}_{e}](x,t)$ Endothelial cell concentration of PEA at distance $x$ from capillary inlet and time $t$

$\text{Pr}_e$ Plasma and intracellular proteins, respectively

$\text{PS}_1$ and $\text{PS}_2$ Endothelial influx permeability-surface area products for PEA and PAA, respectively

$q$ Mass of the injected indicator

$\dot{Q}$ Total organ flow

$Q_c$ Capillary volume

$Q_e$ Extravascular volume accessible to PEA and PAA

$Q_v$ Pulmonary vascular volume

$Q_w$ Extravascular water volume

$Q_1$ and $Q_2$ Measures of the magnitude of the rapidly equilibrating nonspecific interactions of PEA and PAA, respectively, with luminal endothelial surface

$[R](x,t)$ Vascular concentration of the vascular reference indicator at distance $x$ from the capillary inlet ($x = 0$) and time $t$

$\alpha_3$ Time
Experimental Methods

Isolated Rabbit Lung Preparation

As previously described (3, 6), each New Zealand White rabbit [2.68 ± 0.16 (SD) kg; n = 24; New Franken Research Rabbits, New Franken, WI] was given chlorpromazine hydrochloride (25 mg/kg im) followed by pentobarbital sodium (20–25 mg/kg) via an ear vein, heparinized (1200 IU/kg), and exsanguinated via a carotid artery catheter. After cannulation of the pulmonary artery and vein and the trachea, the lungs were removed from the chest and attached to the perfusion system primed with a physiological salt solution containing (in mM) 4.7 KCl, 2.51 CaCl_2, 1.19 MgSO_4, 2.5 KH_2PO_4, 118 NaCl, 25 NaHCO_3, 5.5 glucose, and 4.5% bovine serum albumin (BSA) (3, 7). The perfusion system included a perfusate reservoir and a MasterFlex roller pump that pumped the perfusate from the reservoir into the pulmonary artery and vein and the trachea, the lungs were removed from the chest and attached to the perfusion system primed with a physiological salt solution containing (in mM) 4.7 KCl, 2.51 CaCl_2, 1.19 MgSO_4, 2.5 KH_2PO_4, 118 NaCl, 25 NaHCO_3, 5.5 glucose, and 4.5% bovine serum albumin (BSA) (3, 7). The perfusion system included a perfusate reservoir and a MasterFlex roller pump that pumped the perfusate from the reservoir into the pulmonary artery. In the recirculation mode, the perfusate drained from the left atrium back into the reservoir. Pulmonary arterial (Pa) and venous (Pv) pressures referenced to 

\[ W \] Average linear flow velocity within \( Q_c \)

\[ x \] Distance from the capillary inlet (\( x = 0 \))

\[ Z_1 \] and \[ Z_2 \] Nonspecific PEA and PAA surface binding sites, respectively

For the high and low PO_2 values, the system had not completely equilibrated with the ventilating gas at the time the bolus injections were made when the respective PO_2 values were 20 and 12 Torr for 0% O_2, with a PCO_2 of 36.8 ± 6.0 (SD) Torr and pH of 7.36 ± 0.07 (SD) at 35°C after the change to the respective gas mixtures. For subsequent evaluation of the O_2 dependency of MAO activity, the PO_2 was taken to be the average of the inflow and outflow PO_2 values measured immediately before the injection and after the sample collection. Although for the high and low PO_2 values, the system had not completely equilibrated with the ventilating gas at the time the measurements were made, the values did not change significantly over the duration of the data collection period.

To produce a bolus injection, a solenoid-operated injection loop (3) was situated in the inflow tubing so that a 1.0-ml bolus could be introduced into the inflow stream without changing the flow or pressure. Just before the injection, the ventilator was stopped at end expiration and the venous outflow was directed into the sample tubes of a modified (3) Gilson Escargot fraction collector. One hundred 2-ml samples were collected at a sampling interval ranging from 0.3 to 2.4 s depending on the flow as described in Experimental Protocols.

After each experiment, the lungs were removed from the perfusion system, and additional bolus injections were made at the various flows studied, with the arterial and venous cannulas connected directly together. The data from these injections were used to obtain the concentration versus time curves [\( C_{RT}(t) \)] and moments thereof (3, 6, 7) for the passage of the bolus through the tubing from injection to fraction collector in the absence of the lungs at each of the flows studied.

Bolus Composition

The 1.0-ml bolus contained 2.5 mg of fluorescein isothiocyanate-labeled 40,000 molecular weight dextran (FITC-Dex) and 0.1 μCi of either [14C]PEA or [14C]PAA. In 13 of the 24 lungs studied, the injected bolus also included 0.3 μCi of 3H_2O. The specific activities for [14C]PEA and [14C]PAA were 50 and 52 mCi/mmol, respectively. The total volume containing the indicators was ~0.1 ml, with the balance composed of perfusate removed from the reservoir just before injection so that the injectate PO_2 was approximately equal to that of the arterial inflow.

Sample Composition

The concentration of FITC-Dex in the outflow samples was measured spectrophotometrically (494 nm) with a Bausch and Lomb (Rochester, NY) Spectronic 100 spectrophotometer. 3H and 14C were measured by liquid scintillation counting with a Packard Instrument (Downers Grove, IL) model 4530 liquid scintillation spectrometer. For samples collected after [14C]PEA injection, 1.0-ml aliquots were stored at -70°C until lyophilized before the [14C]PEA and [14C]PAA concentrations were measured with ion-exchange chromatography (see below). Measured quantities of the injectate solution were added to the sample tubes collected before the emergence of the indicators. These samples, which were treated as the effluent samples, served as standards for the calculation of indicator concentrations.

The identities of the compounds in the venous effluent samples collected after [14C]PEA injection were established with thin-layer chromatography (TLC) on silica gel 60 TLC plates developed in an ethyl acetate-isopropyl-25% NH_3OH (50:35:10 vol/vol/vol) mobile phase. With the use of authentic standards, the solute-to-solvent migration ratio values for PEA, PAA, and the intermediary metabolite phenylacetaldehyde were 0.46, 0.26, and 0.92, respectively. TLC analysis of selected peak venous outflow samples collected over the range of conditions studied demonstrated the presence of only [14C]PEA and [14C]PAA. After the identities of the [14C]-labeled compounds in the venous effluent were established, [14C]PEA and [14C]PAA in each sample collected were separated with ion-exchange chromatography (43).

Bio-Rex 70 cation-exchange resin (200–400 mesh) was washed and equilibrated to pH 6.0 with 0.05 M sodium phosphate buffer. The resin was packed to a bed height of 1 cm in a plastic Poly-Prep column (Bio-Rad). The lyophilized 1.0-ml samples were redissolved in 2.0 ml of the pH 6.0 buffer before being passed through the columns. This was followed by two 1.0-ml water washes, and the total effluent from each column, which contained mostly [14C]PAA, was collected in a scintillation vial. The [14C]PEA was eluted from each column with two 2.0-ml aliquots of 0.25 M HCl, and the effluent was collected in a separate scintillation vial. The 14C counts in all samples were determined after the addition of 8 ml of Liquiscint (National Diagnostics, Atlanta, GA) on a Packard model 4530 liquid scintillation spectrometer. Recovery of 14C was >95%. The crossovers of PAA into PEA and of PEA into PAA were <0.5 and <5%, respectively, as measured with standards treated as the samples. These percentages include the inherent crossover of the ion-exchange separation procedure and any metabolism that might have occurred in postcollection samples (1). The venous effluent data measured after the bolus injection of [14C]PEA under the various experimental conditions described in Experimental Protocols were cor-
rected for the crossover of PAA into PEA and of PEA into PAA.

**Experimental Protocols**

The initial experiments were carried out under the various experimental conditions and protocols required to provide the information necessary for the development of the kinetic model and to evaluate the influence of \( P_{O_2} \) on the kinetics of the pulmonary disposition of PEA.

**Flow.** One experimental approach for separating the various processes affecting the pulmonary disposition of a given indicator is to vary the flow (2, 3, 6), which, in turn, varies the time the injected indicators are in contact with the pulmonary endothelium. To determine a useful range of flows in this context, a bolus containing FITC-Dex and either \([^{14}C]\)PEA \((n = 7 \text{ lungs})\) or \([^{14}C]\)PAA \((n = 1 \text{ lung})\) was injected with the flow set at 400, 200, 100, or 50 ml/min at outflow sampling intervals of 0.3, 0.6, 1.2, or 2.4 s, respectively. The \([^{14}C]\)PEA and \([^{14}C]\)PAA bolus injections were carried out in different lungs, and the flow sequence for these and subsequent experiments was randomized. The lungs were ventilated with the high \( O_2 \) gas mixture to maximize \([^{14}C]\)PEA metabolism. The effect of flow on the vascular volume was minimized by setting the mean pulmonary vascular pressure \( (|P_a - P_v|/2) \) at all flows to approximately equal that at 400 ml/min by adjusting the height of the venous outflow (2, 3, 6).

Once it was determined, as indicated in Estimation of Model Parameters, that the effluent concentration versus time data at the two extremes of this flow range provided sufficient information to separately identify the kinetic parameters descriptive of the pulmonary disposition of PEA, two flows, 400 and 50 ml/min, were used in subsequent experiments.

**MAO inhibition.** To evaluate the role of MAO in the pulmonary metabolism of PEA and to provide a positive control for evaluating the ability of the kinetic analysis (see KINETIC MODEL) to distinguish between changes in PEA uptake and metabolism, experiments with the MAO inhibitors pargyline and semicarbazide (21, 43) were carried out by perfusing the lungs with perfusate containing 20 mM pargyline and 1.0 mM semicarbazide for 5 min before the \([^{14}C]\)PEA \((n = 4 \text{ lungs})\) or \([^{14}C]\)PAA \((n = 1 \text{ lung})\) bolus injections at 400 and 50 ml/min and high \( P_{O_2} \). Pargyline (20 mM) and semicarbazide (1.0 mM) were also included in the injected boluses. The pargyline and semicarbazide concentrations were chosen because they had been previously shown to completely inhibit \([^{14}C]\)PEA metabolism by perfused rabbit lungs (21, 43).

Further evaluation of the separate effects of pargyline and semicarbazide on \([^{14}C]\)PEA metabolism was carried out in lungs perfused at 50 ml/min and ventilated with high \( O_2 \) to provide the maximum window for detecting the effects of MAO inhibition. \([^{14}C]\)PEA was injected before and after the lung was perfused with perfusate containing either pargyline (20 mM) or semicarbazide (1.0 mM). Separate lungs were used for pargyline and semicarbazide, and the concentration of the MAO inhibitor in the injectate was the same as that in the perfusate during the injection-sampling period.

**Varying \( P_{O_2} \).** The \( O_2 \) dependency of \([^{14}C]\)PEA and \([^{14}C]\)PAA pulmonary disposition was measured by injecting boluses containing either \([^{14}C]\)PEA or \([^{14}C]\)PAA at one or more of the \( O_2 \) levels at 400 and 50 ml/min. During the transition to the low \( P_{O_2} \), there was a transient increase in perfusion pressure, reflecting hypoxic vasoconstriction. This constriction had dissipated by the time the low \( P_{O_2} \) had been reached as previously described (39).

Additional Experiments

At the 4.5% BSA concentration of the perfusate, 12% of \([^{14}C]\)PEA and 89% of \([^{14}C]\)PAA were albumin bound as measured with centrifugal ultrafiltration as previously described (3, 5, 6). The octanol-water partition coefficients for \([^{14}C]\)PEA and \([^{14}C]\)PAA at pH 7.4 were 0.123 and 0.047, respectively, measured as previously described (3, 5, 6).

**Drugs and Isotopes**

\([^{14}C]\)PEA and \([^{14}C]\)PAA were obtained from American Radiolabeled Chemicals (St. Louis, MO). \( ^3H_2O \) was purchased from Amersham Pharmacia Biotech (Piscataway, NJ). FITC-Dex, pargyline, and semicarbazide hydrochloride were obtained from Sigma (St. Louis, MO). BSA was the Bovuminar standard powder obtained from Intergen (Purchase, NY). The gases were obtained from Praxair (Waukesha, WI). All other chemicals used were of reagent grade.

**EXPERIMENTAL RESULTS**

Figure 1 exemplifies the venous \([^{14}C]\)PEA and \([^{14}C]\)PAA concentration curves after pulmonary arterial injection of either \([^{14}C]\)PEA or \([^{14}C]\)PAA over the range of perfusate flows indicated. Figure 1, top, demonstrates that \([^{14}C]\)PEA is extensively extracted by the lungs. For instance, at 400 ml/min, which results in a capillary mean transit time of <1 s (5), >70% of the injected \([^{14}C]\)PEA was extracted after a single pass through the pulmonary circulation. The ability of the lungs to metabolize \([^{14}C]\)PEA is demonstrated by the appearance of \([^{14}C]\)PAA in the venous effluent after the injection of \([^{14}C]\)PEA. At 400 ml/min, the amount of \([^{14}C]\)PAA in the venous effluent was relatively low. Decreasing the flow increased both \([^{14}C]\)PEA uptake and the appearance of \([^{14}C]\)PAA. At 50 ml/min, >77% of the injected \([^{14}C]\)PAA was recovered as \([^{14}C]\)PAA in the venous effluent compared with ~21% at 400 ml/min as indicated in Table 1 and Fig. 1.

The \([^{14}C]\)PAA curves obtained after \([^{14}C]\)PAA injection provide information about the pulmonary disposition of \([^{14}C]\)PAA independent of \([^{14}C]\)PEA metabolism, which is required for subsequent kinetic analysis. It is clear from the data in Fig. 1, bottom, that the rate of \([^{14}C]\)PAA uptake is much slower than that of \([^{14}C]\)PEA.

Treatment with both pargyline and semicarbazide decreased the \([^{14}C]\)PAA concentration in the venous effluent after \([^{14}C]\)PEA injection to undetectable levels at both high and low flows (Fig. 2 and Table 1). Figure 3C shows that treatment with only pargyline decreased the fractional recovery of \([^{14}C]\)PAA in the collected venous effluent samples after \([^{14}C]\)PEA bolus injection at 50 ml/min by ~80%. On the other hand, any effect of treatment with semicarbazide alone was undetectable (Fig. 3B).

The \( O_2 \) dependency of \([^{14}C]\)PEA metabolism in the intact lung is exemplified in Fig. 4. No \( O_2 \) effect is detectable in the range of \( P_{O_2} \) values from 518 to 106 Torr. However, at a \( P_{O_2} \) of ~16 Torr, \([^{14}C]\)PAA concentrations in the venous effluent after \([^{14}C]\)PEA injection
were lower (most notably at 50 ml/min) than those at a high PO2 (Fig. 4).

Neither the MAO inhibitors nor PO2 had a significant effect on the [14C]PAA outflow curves after [14C]PAA bolus injection (see Estimation of Model Parameters). Thus only the PAA outflow curves measured after [14C]PAA bolus injection in lungs ventilated with the high PO2 gas mixture are shown (Fig. 1).

The fractions of injected [14C]PEA recovered in the collected venous effluent samples as [14C]PEA or [14C]PAA are given in Table 1 for each of the experimental conditions studied. The fractions of injected [14C]PAA and FITC-Dex recovered were 98.5 ± 2.7 (SD) and 95.2 ± 1.6%, respectively.

The Pa and Pv values at 400 and 50 ml/min under the various experimental conditions studied are given in Table 2. The pulmonary vascular volume (Qv) and extravascular water volume (QW) calculated from the FITC-Dex, CR(t), 3H2O, CF(t), and tubing CT(t) outflow curves (7) under the various experimental conditions studied were 8.6 ± 1.3 (SD) and 6.8 ± 1.4 ml, respectively, and were not significantly affected by experimental condition or flow.

KINETIC MODEL

Reactions

The metabolism of PEA to PAA is a two-step reaction (21, 43) where the first step involves the oxidative deamination of PEA to the intermediary metabolite phenylacetaldehyde via MAO and the second step involves the oxidation of phenylacetaldehyde to PAA via aldehyde dehydrogenase. The kinetic model developed in the present study for the pulmonary disposition of PEA and PAA assumes the MAO reaction to be the limiting step (28). This assumption is consistent with the TLC results in the present study where only [14C]PEA and [14C]PAA could be detected in venous effluent samples collected after [14C]PEA injection. With this assumption, the metabolism of

Table 1. Fractional recovery of 14C in venous effluent samples collected after [14C]PEA bolus injection at flow rates of 400 and 50 ml/min

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>n</th>
<th>400 ml/min</th>
<th>50 ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO2 = 518 Torr</td>
<td></td>
<td>49.2 ± 1.3</td>
<td>21.3 ± 2.0</td>
</tr>
<tr>
<td>PO2 = 106 Torr</td>
<td>6</td>
<td>55.3 ± 1.0*</td>
<td>23.7 ± 1.4</td>
</tr>
<tr>
<td>PO2 = 16 Torr</td>
<td></td>
<td>57.5 ± 2.9*</td>
<td>19.6 ± 1.4</td>
</tr>
<tr>
<td>Pargyline + semicarbazide (PO2 = 518 Torr)</td>
<td>4</td>
<td>74.5 ± 3.4*</td>
<td>0.13 ± 0.03*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of lungs. PEA, phenylethylamine; PAA, phenylacetic acid. *Significantly different from PO2 = 518 Torr at each flow rate, P < 0.05 (by 1-way analysis of variance followed by Dunnett’s method).

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PEA to PAA can be summarized by the following reaction

\[
\text{PEA} + \text{O}_2 + \text{NAD}^+ + 2\text{H}_2\text{O} \rightarrow \text{PAA} + \text{H}_2\text{O}_2 + \text{NADH} + \text{H}^+ + \text{NH}_3
\]

where \([\text{H}_2\text{O}]\text{[O}_2]\) is the MAO association rate constant. In addition to reaction \(a\), the kinetic model assumes that PEA participates in the following reaction within the tissue

\[
\text{PEA} + B \rightarrow \text{PEAB}
\]

where \(B\) is the site of accumulation or sequestration of PEA within the cells (21, 43, 47) with the cell sequestration rate constant \(k_B\). The PEA and PAA are also allowed to participate in nonspecific, rapidly equilibrating interactions with the perfusate BSA, on the luminal cell surface, and within the cells.

**Single-Capillary Element**

A single-capillary element of this model is composed of a capillary volume \(Q_c\) and an extravascular volume \(Q_e\) accessible to PEA or PAA. The spatial and tempo-

**Table 2. Pa and Pv at 40 and 50 ml/min**

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>50 ml/min</th>
<th>400 ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\text{Pa}_5)</td>
<td>(\text{Pv}_5)</td>
</tr>
<tr>
<td>(\text{PO}_2=518) Torr</td>
<td>11.3 ± 0.7</td>
<td>11.3 ± 0.7</td>
</tr>
<tr>
<td>(\text{PO}_2=106) Torr</td>
<td>12.2 ± 1.5</td>
<td>12.2 ± 1.5</td>
</tr>
<tr>
<td>(\text{PO}_2=16) Torr</td>
<td>14.4 ± 0.9</td>
<td>14.4 ± 0.9</td>
</tr>
<tr>
<td>(\text{Pargyline+semicarbazide} (\text{PO}_2=518) Torr</td>
<td>11.5 ± 0.7</td>
<td>11.5 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n\), no. of lungs. Pa, arterial pressure; Pv, venous pressure.
nal variations in the concentrations of the vascular indicator, PEA and PAA within \(Q_c\) and \(Q_e\), are described by the following species balance equations based on the above reactions and the assumption that the O2 concentration was constant during the passage of a bolus

\[
\frac{\partial [\text{R}]}{\partial t} + W \left( \frac{\partial [\text{R}]}{\partial x} \right) = 0 \quad (1)
\]

\[
\frac{\partial [\text{PEA}_c]}{\partial t} + W \left( \frac{\alpha_1 Q_e}{\alpha_1 Q_e + Q_1} \right) \left( \frac{\partial [\text{PEA}_c]}{\partial x} \right) = \frac{k_{\text{PEA}} \text{PEA}_c - PS_1 \text{[PEA]}_e}{\alpha_1 Q_e + Q_1} \quad (2)
\]

\[
\frac{\partial \text{PEA}_e}{\partial t} = PS_1 \text{[PEA]}_e - k_{\text{met}} \text{PEA}_e \quad (3)
\]

\[
\frac{\partial [\text{PAA}_e]}{\partial t} + W \left( \frac{\alpha_2 Q_e}{\alpha_2 Q_e + Q_2} \right) \left( \frac{\partial [\text{PAA}_e]}{\partial x} \right) = \frac{k_{\text{PAA}} \text{PAA}_e - PS_2 \text{[PAA]}_e}{\alpha_2 Q_e + Q_2} \quad (4)
\]

\[
\frac{\partial \text{PAA}_c}{\partial t} = PS_2 \text{[PAA]}_e - k_{\text{PAA}} \text{PAA}_c + k_{\text{met}} \text{PAA}_e \quad (5)
\]

where \([\text{R}](x,t), [\text{PEA}_c](x,t),\) and \([\text{PAA}_c](x,t)\) are the vascular concentrations of the vascular reference indicator, PEA, and PAA, respectively, at distance \(x\) from the capillary inlet (\(x = 0\)) and time \(t\). \(\text{PEA}_c(x,t) = (\alpha_3 Q_e)[\text{PEA}_c](x,t)\) and \(\text{PAA}_c(x,t) = (\alpha_4 Q_e)[\text{PAA}_c](x,t)\), where \([\text{PEA}_c](x,t)\) and \([\text{PAA}_c](x,t)\) are the endothelial cell concentrations of PEA and PAA, respectively, at distance \(x\) from the capillary inlet and time \(t\); \(\alpha_1 = 1 + ((\text{Pr}_c)/K_{kq1})\) and \(\alpha_2 = 1 + ((\text{Pr}_c)/K_{kq2})\), where \(K_{kq1}\) and \(K_{kq2}\) are the plasma protein equilibrium dissociation constants for PEA and PAA, respectively, with the perfusate albumin \((\text{Pr}_c); \alpha_3 = 1 + ((\text{Pr}_c)/K_{kq3})\) and \(\alpha_4 = 1 + ((\text{Pr}_c)/K_{kq4})\), where \(\text{Pr}_c\) represents nonspecific intracellular sites of association for PEA and PAA with the equilibrium dissociation constants \(K_{kq3}\) and \(K_{kq4}\), respectively; \(k_{\text{met}} = ([\text{H}_2\text{O}][\text{O}_2]_{\alpha 3} / \alpha 3\) and \(k_{\text{seq}} = (k_0 [\text{B}]Q_c / \alpha 3)\); \(W\) is the average linear flow velocity within \(Q_c\); \(\text{PS}_1\) and \(k_{\text{PEA}} = \text{PS}_1/\alpha 3\) are the rates of mass transport of PEA in and out of the endothelial cells, respectively; \(\text{PS}_2\) and \(k_{\text{PAA}} = \text{PS}_2/\alpha 4\) are the rates of mass transport of PAA in and out of the endothelial cells, respectively; \(Q_1 = Q_c K_1[Z_1] + Q_a = Q_c Q_e K_2[Z_2],\) where \(Z_1\) and \(Z_2\) represent nonspecific sites of association for PEA and PAA on the endothelial surface, respectively, with the equilibrium dissociation constants \(K_1\) and \(K_2\), respectively.

The identifiable model parameters are \(k_{\text{met}} (s^{-1})\), which is the measure of the rate of PEA deamination by MAO; \(k_{\text{seq}} (s^{-1})\), which is the measure of the PEA sequestration rate within the lung tissue; \(\text{PS}_1 (ml/s)\) and \(\text{PS}_2 (ml/s)\), which are the endothelial permeability-surface area products for PEA and PAA, respectively; \(k_{\text{PEA}} (s^{-1})\) and \(k_{\text{PAA}} (s^{-1})\), which are measures of the respective rate of PEA and PAA egress from the cells; and the virtual volumes \(Q_1 (ml)\) and \(Q_2 (ml)\), which are measures of the magnitude of the rapidly equilibrating cell surface interactions of PEA and PAA, respectively. For PAA injections, Eqs. 2–5 reduce to Eqs. 4 and 5 with \(\text{PEA}_c\) set to zero, and the number of identifiable parameters reduces to three, namely \(PS_2 (ml/s), k_{\text{PAA}} (s^{-1})\), and \(Q_2 (ml)\). The values of \(\alpha_1\) and \(\alpha_2\) were set at 1.14 and 9.1, respectively, based on the measured PEA-BSA and PAA-BSA binding.

**Whole Organ**

To construct an organ model from the single-capillary element model, the distribution of pulmonary capillary transit times \(h_c(t)\) needs to be taken into account (4–6, 10, 38). Previously, Audi et al. (5) estimated that for normal rabbit lungs in this perfusion system, the mean transit time \(t_c\) of the \(h_c(t)\) was \(\sim 44\%\) of the total vascular mean transit time, the relative dispersion \(h_c(t)\) \(RD_c = \sigma_c t_c\) was \(\sim 0.9\); and the skewness coefficient (4) of the \(RD_c(t)\) \(m_2/c^2\) was \(\sim 2\), where \(c^2\) and \(\sigma_c\) are the third central moment and standard deviation of \(h_c(t)\), respectively. For the analysis described in *Estimation of Model Parameters*, we used these values to approximate \(h_c(t)\) using a shifted random walk function as previously described (4–6). The capillary transit time distribution was accounted for by first discretizing \(h_c(t)\) into a finite number of capillary transit times as previously described (4, 6). The organ output for a given indicator was then obtained by summing the corresponding solutions of Eqs. 1–5 for all these capillary transit times, each weighted according to \(h_c(t)\) (4).

Estimation of the kinetic model parameters described in *Estimation of Model Parameters* involved numerically (finite difference method (4)) solving Eqs. 1–5 for the appropriate boundary conditions at each iteration of a Levenberg-Marquardt optimization routine (35). The time step was chosen by successively halving an initial time step until the coefficients of variation between the solutions of Eqs. 1–5 at successive time steps was <2%.

**Estimation of Model Parameters**

Preliminary investigation of the kinetic model behavior revealed that data from a single bolus injection are not sufficient to robustly estimate all of the identifiable model parameters. Previously, Audi et al. (2, 3, 6) demonstrated the utility of manipulating flow to reduce correlations between model parameters. In the present study, the range of flows studied was chosen as follows. Initial injections at 400 ml/min, which is in the range of rabbit cardiac output \(\sim 340\) ml/min for a 2.7-kg rabbit (5), revealed that the outflow concentration curves after \([^{14}\text{C}]\) PEA injections are dominated by the virtual volumes \(Q_1 (ml)\) and \(Q_2 (ml)\), respectively little information on metabolism (Fig. 1 L975).
[14C]PAA rather than [14C]PEA. This occurred by 50 ml/min as seen in Fig. 1D. Therefore, the outflow curves measured at 400 and 50 ml/min after [14C]PEA or [14C]PAA injection were used for parameter estimation. The utility of these two flows for reducing correlations between model parameters, particularly [14C]PAA uptake and metabolism, is revealed by the sensitivity functions described in DISCUSSION.

The first step in the parameter estimation procedure was to utilize the [14C]PAA data after [14C]PAA injection to estimate the PAA model parameters, namely PS2, kPAA, and Q2, independently of PEA uptake and metabolism to PAA. This was accomplished by simultaneously fitting the solutions of Eqs. 1–5 with the initial conditions [R](x,0) = [PAAe](x,0) = [PEAc](x,0) = 0 and PAA0(x,0) = 0 and the boundary conditions [R](0,t) = Cin(t); [PAAe](0,t) = (1/Q2) Cin(t); and PAA0(0,t) = 0 to the [14C]PAA concentration versus time data measured after [14C]PAA bolus injections at 400 and 50 ml/min. Cin(t) = (q/Q)hin(t) is the capillary input concentration curve (2–6), where hin(t) is the noncapillary (arteries, veins, connecting tubing, and the injection system) transit time distribution, and q and Q are the mass of the injected indicator and total flow through the organ, respectively. Cin(t) is related to the vascular reference indicator curve C0(t) and the hin(t) by the convolution relationship Cin(t) = Cin(t)*hin(t) as previously described (2–6). Table 3 shows the estimates of the PAA model parameters and measures of precision of these estimates, namely the 95% confidence intervals and the correlation matrix (3, 30). To determine whether the values of the PAA model parameters were affected by any of the experimental conditions, we compared the model fit obtained using the parameter values estimated from each individual experiment with the fit obtained using the mean set of PAA model parameters values estimated from all the PAA experiments given in Table 3. The F ratios (36) indicated that the fits to the individual data sets using the mean parameters were not significantly worse than those using individual parameters estimated from each data set. Thus it is concluded that any effects of the different experimental conditions were not detectable, and the kinetic model parameters descriptive of the pulmonary disposition of PAA were set to the mean values of 0.27 ml/ml of vascular volume, 0.063 s⁻¹, and 0.50 ml·s⁻¹·ml⁻¹ of vascular volume for PS2/Qc, kPAA, and Q2/Qc, respectively. The normalization to the Qc measured for each lung is to accommodate small differences in lung sizes.

Knowing the PAA kinetic parameters, the parameters descriptive of PEA disposition, namely kmet (s⁻¹), kseq (s⁻¹), PS1 (ml/s), kPEA (1/s), and Q1 (ml) were estimated by fitting the model to the [14C]PEA and [14C]PAA data obtained after [14C]PEA injection. This was accomplished by simultaneously fitting the solutions of Eqs. 1–5 with the initial conditions [R](x,0) = [PEA0](x,0) = [PEAc](x,0) = 0 and PEA0(x,0) = PAA0(x,0) = 0 and the boundary conditions [R](0,t) = Cin(t); [PEAc](0,t) = (1/Q1) Cin(t); [PAA0](0,t) = 0; and PEA0(0,t) = PAA0(0,t) = 0 to the [14C]PEA and [14C]PAA data after [14C]PEA bolus injections at 400 and 50 ml/min. With this parameter estimation approach, the effects of MAO inhibitors and PO2 on the pulmonary disposition of PEA were evaluated.

Table 4 shows the estimated values of the PEO model parameters and measures of precision of these estimates (3, 30) under the various experimental conditions studied. The resulting model fit is exemplified in Figs. 2 and 4. The estimated values of the PEO model parameters are also shown in Table 5, where the extensive parameters PS1 and Q1 were normalized to the Qc to account for small differences in lung sizes. With pargyline and semicarbazide treatment, kmet became undetectable with little effect on PS1. The effects on kseq and Q1 were small but significant. In the range from 518 to 106 Torr, PO2 had no significant effect on the PEA kinetic parameters (Table 5). However, at PO2 = 16 Torr, the estimated value of kmet was significantly smaller than that estimated at the higher PO2 levels. The effect of PO2 = 16 Torr on kseq was also significant. The apparent Km for MAO in the intact lung was estimated from kmet = [(kmet)max PO2]/(PO2 + Km) (Fig. 5), where (kmet)max is the maximum kmet. The value was 17.0 Torr (18.2 μM).

**DISCUSSION**

The results indicate that the apparent Km for MAO in the intact lung is significantly smaller than that estimated from studies carried out with purified MAO (18, 19, 24–26, 29, 33, 37, 44, 45) and closer to that estimated from studies carried out in cultured cardiac myocytes (27) and hepatocytes (26). The value is well below normal alveolar PO2, suggesting a minimal influence of PO2 on lung MAO activity. The results are consistent with differences in the activities and specificities of

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**Table 3. Estimated values of the kinetic model parameters descriptive of the pulmonary disposition of PAA and measures of precision of these estimates**

<table>
<thead>
<tr>
<th>Estimated Model Parameters</th>
<th>Measures of Precision of Model Parameter Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>95% Confidence interval</td>
</tr>
<tr>
<td>PS2, ml/s</td>
<td>2.43 ± 0.39</td>
</tr>
<tr>
<td>Q2, ml</td>
<td>4.55 ± 0.37</td>
</tr>
<tr>
<td>kPAA, s⁻¹</td>
<td>0.063 ± 0.018</td>
</tr>
</tbody>
</table>

Values are means ± SE for estimated model parameters and means ± SD for 95% confidence interval and correlation matrix; n = 3 lungs. See glossary for definition of parameters. The ith entry of the correlation matrix is the correlation coefficient between the ith and jth model parameters (30).
enzymes in general (8, 49) and of MAO in particular (9, 49) in their in situ cellular environments versus the purified enzymes. Although the estimated value of $K_{m}^{O_2}$ is much smaller than most of those estimated for purified MAO and probably not relevant in the hyperoxic range, it is still in a range high enough for sensing changes in $O_2$ in the hypoxic range. Furthermore, the results do not preclude an important role for MAO-generated $H_2O_2$ in the hypoxic range. The $O_2$ concentrations used to estimate $K_{m}^{O_2}$ (Fig. 5) were measured in the perfusate. These values may be higher than the $O_2$ concentrations near the outer mitochondrial membrane site of MAO under the assumption that the mitochondria are sinks for $O_2$. However, in the lungs, where the rate of metabolism is low compared with the rate of gas transport across the alveolar capillary barrier and where diffusion distances are small, the expectation is that the local $O_2$ is not much lower than the perfusate $O_2$ (27). Thus although the estimated $K_{m}^{O_2}$ in this sense represents an upper bound on the actual $K_{m}^{O_2}$, the difference is probably quite small and in the direction of increasing the discrepancy between intact lungs and purified enzymes. Previous studies (19, 27) on the $O_2$ dependency of the MAO reaction have demonstrated that the $K_{m}^{O_2}$ is also somewhat sensitive to the monoamine substrate concentration, consistent with the nature of the MAO reaction mechanism (19, 27). In the present study, the estimate is for tracer PEA concentration.

Table 4. Effect of $P_0_2$ and MAO inhibition on the estimated values of the kinetic model parameters descriptive of the pulmonary disposition of PEA and measures of precision of these estimates

<table>
<thead>
<tr>
<th>Estimated Model Parameters</th>
<th>$P_0_2 = 518$ Torr ($n = 11$)</th>
<th>$P_0_2 = 108$ Torr ($n = 6$)</th>
<th>$P_0_2 = 16$ Torr ($n = 5$)</th>
<th>Pargyline + semicarbazide ($P_0_2 = 518$ Torr; $n = 4$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{S_1}$, ml/s</td>
<td>$16.78 \pm 0.84$</td>
<td>$14.89 \pm 0.47^a$</td>
<td>$14.39 \pm 1.95$</td>
<td>$11.15 \pm 0.16$</td>
</tr>
<tr>
<td>$Q_{l_1}$, ml</td>
<td>$6.94 \pm 0.27$</td>
<td>$6.91 \pm 0.89$</td>
<td>$7.36 \pm 1.49$</td>
<td>$7.84 \pm 0.56$</td>
</tr>
<tr>
<td>$k_{PEA}$, s$^{-1}$</td>
<td>$0.193 \pm 0.025$</td>
<td>$0.167 \pm 0.009$</td>
<td>$0.125 \pm 0.021$</td>
<td>$0.122 \pm 0.015$</td>
</tr>
<tr>
<td>$k_{met}$, s$^{-1}$</td>
<td>$8.13 \pm 0.991$</td>
<td>$0.81 \pm 0.0655$</td>
<td>$0.406 \pm 0.069^b$</td>
<td>$0.127 \pm 0.017^a$</td>
</tr>
<tr>
<td>$Q_{1,m}$, l/s</td>
<td>$0.325 \pm 0.032$</td>
<td>$0.329 \pm 0.009$</td>
<td>$0.127 \pm 0.017^a$</td>
<td>$0.163 \pm 0.042^a$</td>
</tr>
</tbody>
</table>

Values are means ± SE for estimated model parameters and means ± SD for 95% confidence interval and correlation matrix; $n$, no. of lungs. See glossary for definition of parameters. MAO, monoamine oxidase. *Significantly different from $P_0_2 = 518$ Torr, $P < 0.05$ (by 1-way analysis of variance followed by Dunnett’s method).

Table 5. Effect of $P_0_2$ and MAO inhibition on the values of the kinetic model parameters descriptive of the pulmonary disposition of PEA

<table>
<thead>
<tr>
<th>Model Parameters</th>
<th>$n$</th>
<th>$P_{S_1}$, $Q_{l_1}$, s$^{-1}$</th>
<th>$Q_{l_1}$, Ql</th>
<th>$k_{PEA}$, s$^{-1}$</th>
<th>$k_{met}$, s$^{-1}$</th>
<th>$k_{mae}$, s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_0_2 = 518$ Torr</td>
<td>11</td>
<td>$1.81 \pm 0.08$</td>
<td>$0.747 \pm 0.028$</td>
<td>$0.193 \pm 0.025$</td>
<td>$0.813 \pm 0.091$</td>
<td>$0.325 \pm 0.032$</td>
</tr>
<tr>
<td>$P_0_2 = 108$ Torr</td>
<td>6</td>
<td>$1.69 \pm 0.07$</td>
<td>$0.767 \pm 0.063$</td>
<td>$0.167 \pm 0.009$</td>
<td>$0.767 \pm 0.055$</td>
<td>$0.329 \pm 0.009$</td>
</tr>
<tr>
<td>$P_0_2 = 16$ Torr</td>
<td>5</td>
<td>$1.61 \pm 0.15$</td>
<td>$0.809 \pm 0.128$</td>
<td>$0.125 \pm 0.021$</td>
<td>$0.406 \pm 0.069^b$</td>
<td>$0.127 \pm 0.017^a$</td>
</tr>
<tr>
<td>Pargyline + semicarbazide ($P_0_2 = 518$ Torr)</td>
<td>4</td>
<td>$1.34 \pm 0.13$</td>
<td>$0.945 \pm 0.016^a$</td>
<td>$0.122 \pm 0.015$</td>
<td>$0^a$</td>
<td>$0.163 \pm 0.042^a$</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, no. of lungs. See glossary for definition of parameters. *Significantly different from $P_0_2 = 518$ Torr, $P < 0.05$ (by 1-way analysis of variance followed by Dunnett’s method).
LUNG MONOAMINE OXIDASE ACTIVITY

Fig. 5. Rate of PEA deamination by pargyline-sensitive monoamine oxidase (k_{max}) at the 3 O_2 levels studied. Solid line superimposed on data is the fit of k_{met} = (k_{met}max P/O_2/K_{O_2}^m), where (k_{met}max) is the maximum k_{met} and K_{O_2}^m is the Michaelis constant for O_2. Values are means ± SE. The apparent K_{O_2}^m is 17.0 Torr (18.2 μM).

One aspect of studies on intact organs and cells that distinguishes them from studies on purified enzymes is that the rate of entry into the cells needs to be accounted for. In the above model, PEA uptake is represented by a linear transport mechanism (9) having a permeability-surface area product (PS_1). Previous investigations (21, 48) have concluded that this mechanism is passive diffusion. Given the relatively low lipid solubility of PEA, its extensive pulmonary uptake via passive diffusion is somewhat surprising (48). The data in Figs. 1–4 and the relative rates of uptake and metabolism indicate that the intracellular concentration of PEA during bolus passage became much greater than the vascular concentration. This would be consistent with a large PEA tissue-to-perfusate partition coefficient, which in the model would be a large α(Q_c-to-Q_c) ratio. Alternatively, if an active uptake mechanism were involved, the model representation would be a larger permeability-surface area product for PEA uptake (PS_1) than for egress, the latter being lumped with other processes in the group parameter k_{PEA}. These two effects are not separable with the data obtained in the present study. However, with additional experimental protocols, the kinetic model and the MID method developed in the present study may be useful for further evaluation of the underlying PEA uptake mechanism.

Another difference between studies with intact organs or cells and studies with purified enzymes is that in intact organs and cells, competing processes such as substrate interactions with perfusate constituents (e.g., plasma proteins) and with any number of cellular and/or tissue constituents can also affect substrate availability by altering its partitioning within the cells and between the medium (perfusate) and the cells. These factors are represented in the model by Q_1 and Q_2 for nonspecific cell surface and/or tissue interactions, α_1 and α_2 for perfusate albumin interactions, and α_3, α_4, and k_{met} for intracellular interactions.

The major difference between intact organs and isolated cells is that in the intact organ, access to the cells is generally via the vascular system, which further complicates the evaluation of intracellular functions. For example, changing the flow by itself has a substantial effect on the extracted fraction of PEA (Fig. 1), and at a given flow, the overall extraction fraction is determined by contributions from the capillary pathways with different flows and/or transit times (4, 5, 10) and hence different extraction fractions. This is taken into account in the model by allowing for longitudinal spatial variations in the concentration of the injected indicators within a given capillary element (Eqs. 1–5), by representing the organ by parallel capillary elements with different transit times (4), and by weighing the contributions of these capillary elements according to h_c(t) as previously discussed in more detail (4).

Previous studies (21, 43) have concluded that the endothelium is the main site for the MAO responsible for the oxidative deamination of PEA in the lung. Three forms of MAO have been identified in the rabbit lung (20, 21, 42, 43), including MAO-A, MAO-B, and a semicarbazide-sensitive form (SSMAO), with distinct subcellular localizations and substrate and/or inhibitor affinities. MAO-A and MAO-B are both located on the outer mitochondrial membrane (20, 21, 32, 42, 43), but MAO-A has a higher affinity for serotonin and norepinephrine and is selectively inhibited by clorgyline (21, 43), whereas MAO-B has a higher affinity for PEA and is more sensitive to inhibition by pargyline (21, 43). PEA is also a substrate for SSMAO, which is thought to be located on the plasma membrane (11, 17, 21, 43, 50). Roth and Gillis (43) and Gillis and Roth (21) found that treatment with pargyline followed by the addition of semicarbazide reduced PEA metabolism by 70 and ~100%, respectively. This is consistent with the results in the present study where treatment with pargyline alone decreased the fractional recovery of [14C]PAA in the collected venous effluent samples after a [14C]PEA bolus injection at 50 ml/min by ~80% (Fig. 3C) and that treatment with both pargyline and semicarbazide was needed for full inhibition (Fig. 2). However, the lack of effect of semicarbazide alone on PEA metabolism (Fig. 3B) has some interesting implications as indicated below.

In the kinetic model represented by Eqs. 2–5, there is no explicit accommodation for two types of MAO. To evaluate the possible implications of this simplification, we modified the kinetic model represented by Eqs. 1–5 to allow for PEA metabolism via both luminal surface (SSMAO) and intracellular MAO (MAO-B). This was accomplished by substituting Eqs. 2 and 5 with Eqs. 6 and 7

\[
\frac{\partial[PEA_c]}{\partial t} + W\left(\frac{\alpha_2 Q_c + Q_1}{\alpha_2 Q_c + Q_1}\right)\frac{\partial[PEA_c]}{\partial x} = k_{PEA}\text{PEA} - (PS_1 + k_{met2})[\text{PEA}_c] \quad (6)
\]

\[
\frac{\partial[PAAC_c]}{\partial t} + W\left(\frac{\alpha_2 Q_c + Q_2}{\alpha_2 Q_c + Q_2}\right)\frac{\partial[PAAC_c]}{\partial x} = k_{PAAPAA}\text{PAAC} - PS_2[\text{PAAC}_c] + k_{met2}[\text{PEA}_c] \quad (7)
\]

where k_{met2} (ml/s) is a measure of the rate of PEA metabolism by SSMAO. The value of k_{met2} was estimated by fitting the solution of Eqs. 3 and 5–7 to the
MAO inhibitors significantly decreased the total amount of $^{14}$C recovered in the venous effluent samples collected after a $^{14}$CPEDAE bolus injection at 50 ml/min (47). This decrease in total recovery reveals competition between the two intracellular processes, namely $^{14}$CPEDAE metabolism and sequestration. The normal rate of $^{14}$CPEDAE metabolism ($k_{\text{met}}$) is faster than that of $^{14}$CPEDAE sequestration ($k_{\text{seq}}$) as shown in Table 5. Thus, in the absence of MAO inhibitors, most of the $^{14}$CPEDAE extracted by the pulmonary endothelium was metabolized to $^{14}$CPAA and ultimately returned to the perfusate (Fig. 1C). After treatment with MAO inhibitors, metabolism was no longer competing with sequestration for $^{14}$CPEDAE, and hence a larger fraction of the PEA taken up was sequestrated and a smaller fraction of the injected $^{14}$C was recovered in the venous effluent. This competition between intracellular PEA metabolism and sequestration was not detectable at 400 ml/min (Table 1) because at this flow, less time was available for either $^{14}$CPEDAE metabolism or sequestration than at the lower flow. Thus a larger fraction of the extracted PEA returned to the perfusate. This observation further demonstrates the utility of varying the flow for revealing competing parallel processes.

To help put this flow dependency (2, 3, 6) in perspective, the normalized sensitivity function $\theta S(t)$ (6) obtained for the PEA model parameters estimated from the $^{14}$CPEA and $^{14}$CPAA data after $^{14}$CPEA injections at 400 and 50 ml/min are shown in Fig. 6. For the $i$th model parameter $\theta_i$, $S_i(t) = \partial C/t/\partial \theta_i$, where $C(t)$ is the calculated PEA or PAA indicator effluent concentration. The sensitivity function $S_i(t)$ was approximated by the change in $C(t)$ resulting from a 1% change in $\theta_i$ divided by the change in $\theta_i$ (6). Multiplying $S_i(t)$ by the value of the parameter estimate, $\theta_i$, provides an indication of the relative contribution of the parameter to the model fit to the data at a given time (6). Comparison of these normalized sensitivity functions reveals the extent and the time epoch to which the optimized model parameters make their contributions to the model fit. The shapes of the sensitivity function relative to each other reveal how independent the contributions of the individual parameters are to the model fit. For example, at 400 ml/min, the dominant role of PEA uptake in the model fit is revealed by the sensitivity function of $PS_1$ (Fig. 6A). The contribution of $k_{\text{met}}$ at 400 ml/min is small relative to that for $PS_1$ (Fig. 6A). At 50 ml/min, on the other hand, $k_{\text{met}}$ plays a dominant role, whereas the contribution of $PS_1$ is relatively small (Fig. 6D). Thus fitting the model to the data at both flows reduces the correlation between

Table 6. Comparison of values of PEA model parameters estimated with data measured at 4 flow rates vs. those at 2 flow rates

<table>
<thead>
<tr>
<th>Model Parameters</th>
<th>n</th>
<th>PSO/Qc, s$^{-1}$</th>
<th>Qc/Qc</th>
<th>kPEA, s$^{-1}$</th>
<th>kmet, g$^{-1}$×10$^{-1}$</th>
<th>kesq, g$^{-1}$×10$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>400 and 50 ml/min</td>
<td>7</td>
<td>1.77 ± 0.11</td>
<td>0.716 ± 0.028</td>
<td>0.211 ± 0.037</td>
<td>0.885 ± 0.136</td>
<td>0.331 ± 0.051</td>
</tr>
<tr>
<td>400, 200, 100, and 50 ml/min</td>
<td>7</td>
<td>1.77 ± 0.11</td>
<td>0.713 ± 0.025</td>
<td>0.221 ± 0.033</td>
<td>0.997 ± 0.131</td>
<td>0.398 ± 0.057</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of lungs. See glossary for definition of parameters. PO$_2$ = 518 Torr.
model parameters by increasing the extent of their contributions to the model fit to the data and by extending and segregating the time epochs over which they make their contributions.

For the above analysis, the data at only the 400 and 50 ml/min flows were utilized for parameter estimation. However, in several experiments, data were collected at four flows within the same flow range (Fig. 1). As one measure of the robustness of the above parameter estimation approach, we determined whether dividing up the flow range into the four smaller increments would have a significant impact on the estimated values of the model parameters. To this end, the PEA model parameters estimated by simultaneously fitting the solution of Eqs. 1–5 to the [14C]PEA and [14C]PAA data measured after [14C]PEA injections at two and four flows were compared. The values of the kinetic model parameters from two or four flows were not significantly different (Table 6).

The MID method has been used to measure intracellular reactions in organs such as the heart (10) and liver (38). Although widely used in the lungs for measuring tissue composition (4, 7), transcapillary transport (2, 15, 22), and reactions that take place at the luminal endothelial surface (6, 15), its use for studying intracellular metabolism in the lungs has been limited (6, 7). The kinetic model represented by Eqs. 1–5 is similar to the one used by Pang et al. (38) to evaluate the intracellular metabolism of acetaminophen in the intact liver. The kinetic model and experimental protocol developed in the present study establish a basis for utilizing the MID method for evaluating intracellular functions such as MAO activity in the intact lung. The present results with this approach suggest a minimal influence of O2 concentration on the MAO reaction in the normal rabbit lung.

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