Norepinephrine Metabolism in Humans

Kinetic Analysis and Model

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Abstract

The present study was undertaken to quantify more precisely and to begin to address the problem of heterogeneity of the kinetics of distribution and metabolism of norepinephrine (NE) in humans, by using compartmental analysis. Steady-state NE specific activity in arterialized plasma during [^{3}H]NE infusion and postinfusion plasma disappearance of [^{3}H]NE were measured in eight healthy subjects in the supine and upright positions. Two exponentials were clearly identified in the plasma [^{3}H]NE disappearance curves of each subject studied in the supine (r = 0.94–1.00, all $P < 0.01$) and upright (r = 0.90–0.98, all $P < 0.01$) positions. A two-compartment model was the minimal model necessary to simultaneously describe the kinetics of NE in the supine and upright positions. The NE input rate into the extravascular compartment 2, estimated with the minimal model, increased with upright posture (1.87±0.08 vs. 3.25±0.2 μg/min per m^2, $P < 0.001$). Upright posture was associated with a fall in the volume of distribution of NE in compartment 1 (7.5±0.6 vs. 4.7±0.3 liters, $P < 0.001$), and as a result of that, there was a fall in the metabolic clearance rate of NE from compartment 1 (1.80±0.11 vs. 1.21±0.08 liters/min per m^2, $P < 0.001$). We conclude that a two-compartment model is the minimal model that can accurately describe the kinetics of distribution and metabolism of NE in humans.

Introduction

Norepinephrine (NE)[1] is the major neurotransmitter of the sympathetic nervous system (SNS). The SNS plays an important role in homeostatic circulatory adjustments (1), and its involvement in the pathophysiology of disease is receiving considerable attention (2–4). Measurement of NE levels in plasma have been used to provide an index of SNS activity (5). However, because NE is rapidly removed from the circulation, changes of plasma NE could result from changes in the rates of release or removal, or both. The relative contributions of release and removal mechanisms to the level of NE in plasma have been assessed by infusion of tritiated-NE ([^{3}H]NE) and estimation of plasma NE kinetics by isotope dilution, providing useful information about regulation of SNS activity in vivo (6, 7).

The isotope dilution method that has been used assumes that plasma NE kinetics can be described by a one-compartment or so-called noncompartmental model. Although this approach is widely used and believed not to require specific assumptions regarding model structure, recent work (8–10) has helped to define the inherent structural assumptions and limitations of noncompartmental kinetic analysis. In particular, one major assumption of this approach is that all de novo sources of the substance under investigation enter into, and all irreversible losses leave from, the accessible compartment.

However, NE is released by sympathetic nerves into an inaccessible compartment, the neuroeffector junction, and only a small fraction of the released NE "spills over" into plasma (11). The majority of released NE is taken back up into postganglionic sympathetic neurons, another inaccessible site. Thus, for the NE system, the assumption about entry and irreversible loss from the accessible compartment is not justified. Furthermore, the disappearance of [^{3}H]NE from plasma in humans is at least biexponential (12), indicating that at least two compartments are needed to adequately describe NE kinetics.

Compartmental analysis is now commonly used in the study of metabolic and endocrine processes (13). Although this approach has been attempted for the study of NE kinetics in humans (14, 15), the early studies were limited by the lack of a sensitive and specific assay for plasma NE. Presently, methods to accurately measure plasma NE concentrations are readily available. In addition, a complete analytic theory (16, 17) and sophisticated computer modeling software (18, 19) for compartmental analysis are available.

Thus, the present study was undertaken to quantify more precisely and to begin to address the problem of heterogeneity of the kinetics of distribution and metabolism of NE in humans, by using compartmental analysis. To determine

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1. *Abbreviations used in this paper:* [^{3}H]NE, tritiated norepinephrine; L_{ij}, fractional transfer rate constants; MCR_1, circulating norepinephrine metabolic clearance rate; MRT_i, norepinephrine compartmental mean residence times; NE, norepinephrine; NE_2, extravascular NE input rate; NEAP, noncompartmental NE appearance rate in plasma; NECL, noncompartmental NE clearance rate from plasma; NE_R, percent NE_2 removed from compartment 2; NE_S, percent NE spillover into compartment 1; NE_{SF}, NE spillover fraction; Q_i, NE compartmental mass sizes; R_{ij}, NE mass flow rates; SNS, sympathetic nervous system; V_1, volume of distribution of NE in compartment 1.

whether this approach can be applied to a physiologic change in SNS activity, we used compartmental analysis to estimate how steady-state NE kinetics change from the supine position compared with the upright posture.

Methods

Subjects and protocols

Informed consent was obtained from eight healthy young subjects, four male and four female, ages 19–30 (mean±SEM, 23±3 yr). All subjects were within 15% ideal body weight according to Metropolitan Life Insurance Tables (1959). The protocols were approved by the University of Michigan Human Use and Radiation Control Committees. All subjects were admitted to the University of Michigan Clinical Research Center 48 h before the studies and all were studied after an overnight fast, both in the supine position and during upright posture, between the hours of 7:00 and 11:00 a.m. During the 12-h period before the studies they were prohibited from using nicotine, caffeine, marijuana, and other known stimulants of catecholamine release.

On the morning of the study, an intravenous catheter was placed in an antecubital vein of one arm for infusion of [^{3}H]NE. In the other arm, a scalp vein needle was inserted retrograde into a dorsal vein of the hand, which was placed in a warming box at 60°C to obtain arterialized venous samples. This approach has been used for studying the kinetics of a variety of substrates and hormones (20–22). The catheters were kept patent with 0.45% NaCl. The first 1 ml of blood sampled was discarded at each sampling time. Subjects were administered a 15 μCi/m^2 bolus of L-7-[^{3}H]NE (sp act, 18.8 Ci/mmol; New England Nuclear, Boston, MA), followed by a 0.35 μCi/min per m^2 infusion using a syringe pump (Harvard Apparatus Co., S. Natick, MA). Infusates contained 1 mg/ml ascorbic acid to prevent oxidation of NE. Arterialized blood samples (10 ml) were collected at 30, 40, and 50 min during [^{3}H]NE infusion for measurement of steady-state [^{3}H]NE and plasma NE concentrations. The [^{3}H]NE infusion was then stopped and blood samples (10 ml) were obtained at 1, 2, 4, 6, 8, 10, 15, and 20 min for measurement of [^{3}H]NE concentration. Plasma NE concentration was measured 20 min after stopping the infusion. The zero time value (t = 0) for analysis of the disappearance curves was taken as the mean of the 30, 40, and 50 min samples.

Within minutes after completion of the study in the supine position, subjects assumed upright posture. The [^{3}H]NE study was repeated beginning 10 min after standing. Arterialized blood samples were again collected after 30, 40, and 50 min of [^{3}H]NE infusion (corresponding to 40, 50, and 60 min of upright posture), and the infusion was stopped. The t = 0 value for analysis of the disappearance curves during upright posture was taken as the mean of the three samples obtained during [^{3}H]NE infusion. As during the supine study, samples for measurement of [^{3}H]NE concentration were obtained for 20 min after discontinuation of the [^{3}H]NE infusion. Plasma NE concentration was measured 20 min after stopping the infusion.

Three additional young healthy subjects underwent the supine [^{3}H]NE infusion protocol for a second time without standing, beginning 10 min after the end of the [^{3}H]NE disappearance of the first supine [^{3}H]NE infusion. The [^{3}H]NE and NE concentration sampling protocol was otherwise identical to the above for the supine and upright posture studies.

Analytical methods

Blood samples were collected into chilled plastic tubes containing EGTA and reduced glutathione, and immediately placed on ice. They were promptly centrifuged at 4°C and the plasma was stored at −20°C until assay. Plasma NE was quantified by a single isotope radioenzymatic assay using unextracted plasma (23). All samples from a single subject were analyzed in the same run of the assay. The intra-assay coefficient of variation for the method in this laboratory is 5%. [^{3}H]NE concentrations were determined by liquid scintillation counting of radiolabeled catecholamines after alumina extraction as previously described (24). Alumina extraction and measurement of [^{3}H]NE were performed within 24 h of each study. Recovery of [^{3}H]NE after alumina extraction, calculated in each study by adding an aliquot of [^{3}H]NE infusate to a plasma sample obtained before the start of the infusion, was 59±5% (mean±SD).

We tested the purity of the [^{3}H]NE isolated by alumina extraction in the samples taken during the second [^{3}H]NE infusion in the three subjects who underwent the double supine control study. We injected 200 μl of alumina eluate onto a high-performance liquid chromatography (HPLC) Zorbac ODS column (Dupont Co., Wilmington, DE) coupled to electrochemical detection (Bioanalytical Systems, West Lafayette, IN) with a mobile phase consisting of monochloroacetic acid, 14.2 g; NaOH, 4.67 g; Na_2EDTA, 0.75 g; sodium octyl sulfate, 0.4 g; and 2% acetonitril per liter. > 95% of the counts recovered eluted in the NE peak. In contrast, < 5% eluted in the void volume, where the alumina-extractable metabolite 3,4,dihydroxyphenylglycol elutes in our system.

Model development

Identification of exponential models. One-, two-, and three-exponential models, g(t) (Eq. 1), were fitted to the data of the two experiments for each subject.

$$g(t) = \sum_{i=1}^{n} A_i e^{-a_i(t)}, \quad A_i \geq 0, a_i \geq 0 \tag{1}$$

The selection of the best exponential model was made on the basis of partial F-test analyses (25), and the accuracy of the fit for the exponential curves was evaluated by r values obtained for the fitted curves (26). Two exponentials gave a much better fit than one (see Results). More than two exponentials gave no significant improvement in the fit over that obtained with two exponentials.

Compartmental analysis. Our results show that in both the supine and upright positions, the plasma NE concentration was constant over the time of the measurements, so we may assume the NE system was in a steady state. The [^{3}H]NE infusion rate (equal to ~ 5 ng/min) used in these studies was 25 times less than a NE infusion rate previously shown to have no measurable effects on heart rate, blood pressure, or other metabolic parameters (27), and it was 16 times less than a NE infusion rate previously shown to cause an increment of only 3.0 pg/ml in plasma NE (28). Furthermore, in the present study, the [^{3}H]NE infusion contributed 1.48% to total plasma NE. Thus we may assume that the [^{3}H]NE (tracer) application level was small enough so as not to perturb endogenous NE (tracee). Under such circumstances, the distribution of [^{3}H]NE follows the kinetics of a linear dynamic system with constant coefficients (16, 17).

The NE system in vivo is characterized by double exponential functions both in the supine position and during upright posture (see Results), demonstrating that a two-compartment model is the minimal model that can accurately describe the NE system in humans. The known physiology for the fate of NE after its release from sympathetic nerve terminals was then used to develop and interpret the potential compartmental structures, as illustrated in Fig. 1.

In these models, compartment 1 includes circulating NE and so is accessible for sampling. Compartment 2 is less sharply defined anatomically, but is a distributed pool into which NE is released from sympathetic nerve varicosities and which exchanges with the circulating pool. Compartment 2 is a lumped compartment whose heterogeneity includes the heart, kidney, and gut, i.e., tissues that locally release, take-up, metabolize, and "spill" NE into the circulation. As suggested by the shaded area for Model A in Fig. 1, some removal of circulating NE by these tissues could theoretically occur directly from compartment 1. However, since NE only secondarily spills over into the circulation after its release into compartment 2, it is likely that a large fraction of endogenously released NE is removed from compartment 2. The results of our mathematical modeling strongly support this hypothesis.

The NE mass in compartment 2 (Q_2) represents an extravascular source of released NE that is available for neuronal reuptake into

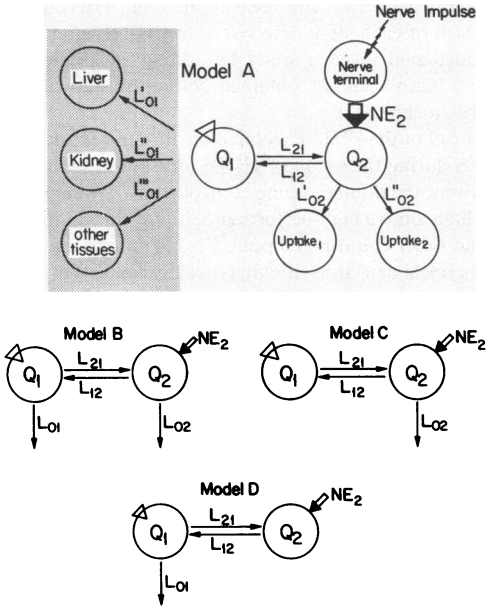

Figure 1. Potential two-compartment models. Model A is a translation of the known physiology for the fate of NE after its release from sympathetic nerve terminals into the compartmental mode. Lower panels show three parametric model reductions of Model A with different structures that are candidates to describe the observed NE system. Triangles cut into sampled compartments, while arrows point to sites of endogenous NE release. Note that NE stored in postganglionic sympathetic nerve terminals, shown as a third compartment in Model A, is not included in the three two-compartment models. Irreversible removal of NE from compartment 1, shown as the shaded area of Model A, is reduced to L_{01} in Model B. Similarly uptake$_1$ and uptake$_2$ are reduced to L_{02} in Model B. For further discussion of these different models see text.

sympathetic nerve terminals (*Uptake*$_1$), $Q_2L'_{02}$, and for local metabolism (*Uptake*$_2$), $Q_2L''_{02}$. The NE mass in compartment 2 most likely represents NE anywhere in the vicinity, and/or at a distance from, the neuroeffector junctions of sympathetic nerve synapses. However, Q_2 does not represent NE within sympathetic nerve endings, because our results show that the value we obtained for Q_2 represents only a small fraction of total tissue NE stores (e.g., the heart alone contains about 1 μg/g of NE [24]).

In fact, as suggested by Model A in Fig. 1, the intraneuronal storage pool of NE represents a third compartment. However, the turnover rate of the tissue NE pool is measured in hours (29). The present study was designed to focus on the kinetics of NE released from nerve terminals and spilling over into the circulation, processes with turnover rates measured in minutes. Thus, the present study design excludes the intraneuronal storage compartment. The fractional transport rate coefficients (L_{ij}) of the models represent the translocations of NE mass within the two-compartment systems. The values of these parameters may or may not have physiological counterparts.

For sampling solely from compartment 1, there is insufficient information to uniquely determine all of the parameters of Model A (Fig. 1). Thus, we set $L_{01} = L'_{01} + L''_{01} + L'''_{01}$ and $L_{02} = L'_{02} + L''_{02}$, thereby reducing Model A to Model B (Fig. 1). As illustrated in the Appendix, Model B is also unidentifiable. But it is known from published data that only a small fraction of the NE released by sympathetic nerves spills over into the circulation (11). Values for this fraction have been estimated to be approximately between 0.10 and 0.30 (27, 30). Incorporation of this information into the structure of Model B (Fig. 1) allows calculation of L_{12} as a function of the NE spillover fraction (NE_{SF}): the ratio of the NE mass transport rate from compartment 2 to compartment 1 to NE released into compartment 2. With L_{12} determined, Model B becomes identifiable (see Appendix).

Our results indicate that within the range of NE_{SF} consistent with the best fit to our data and the published data (27, 30), for Model B (Fig. 1), the mass transport rate of NE via the pathway $L_{01}(R_{01} = Q_1L_{01})$ is quantitatively identical to values for urinary excretion of NE reported in the literature (30–33). Thus, as an alternative approach to solving Model B uniquely, we assumed that L_{01} represents the fractional rate of urinary NE excretion, and its values were calculated from the published data (30–33). With L_{01} determined, Model B also becomes identifiable (see Appendix) without any assumptions about the NE_{SF}.

Moreover, because urinary NE excretion represents a very small percentage of the rate of NE release into compartment 2 (see Results) and the values for L_{01} are very close to zero, we performed additional numerical parameter identification analyses setting L_{01} equal to zero. In this case, Model B reduces to Model C (Fig. 1), which is identifiable without further assumptions (see Appendix). To the extent that Model C, which requires only the assumption that L_{01} equals zero, can accurately describe the observed NE kinetics, it becomes the minimal model that can be used for the analysis of the kinetics of distribution and metabolism of NE in humans. The minimal modeling approach is consistent with the principle of parsimony and it has been shown to be useful in the study of metabolic systems analysis (34). Model D is as simple as Model C, but it cannot account for irreversible removal of NE from the inaccessible compartment 2, which is known to occur. Therefore, Model D was excluded from further analysis.

We used Berman's minimal change postulate (35) to determine the effect of upright posture on NE kinetics. This approach involves seeking the minimum set of parameters whose change is necessary and sufficient to simultaneously fit the data of the supine and upright steady states. The concepts of necessary and sufficient conditions are defined and discussed in the context of SAAM and CONSAM methodology by Foster and Boston (36).

Goldstein and co-workers (28) have pointed out the potential importance of mixing in interpreting data from the first 2 min of the [^{3}H]NE disappearance from plasma after discontinuation of [^{3}H]NE infusion. To define the influence of the early sample time points of the [^{3}H]NE disappearance curve on the parameter estimates of the minimal model and its fit to the experimental data, we studied four additional subjects in the supine position. Extra samples were obtained at 12, 14, 16, and 18 min after stopping the [^{3}H]NE infusion so that the data could also be analyzed without either the 0 or 1 min time points. Although there was an improvement in the goodness-of-fit of the [^{3}H]NE disappearance curve when the 0 time point was excluded, no differences in the L_{ij} or NE_2 were observed. Thus the parameters of the minimal model were not influenced significantly by any early mixing effects on [^{3}H]NE levels.

Computational methods

Mathematical modeling was performed on a VAX 11/730 computer (Digital Equipment Corp., Maynard, MA) using the SAAM27 (18) and CONSAM (19) simulators. All [^{3}H]NE and NE data were fit simultaneously by solving the differential equations of both the [^{3}H]NE tracer and NE tracee systems by the method of weighted nonlinear least squares (18). The criteria of goodness-of-fit for the compartmental parameter estimates are those of Berman, Weiss, and Shahn (37).

The unknown compartmental parameters estimated included the fractional transport rate constants, $L_{ij}(min^{-1})$, the volume of distribution of NE in compartment 1 (V_1 [liters]), and the steady-state input rate of NE into compartment 2 (NE_2) (μg/min per m^2). These parameters were automatically obtained by SAAM along with statistical measures of their estimability. Other kinetic parameters calculated by SAAM were the NE mass transport rates (R_{ij}) (μg/min per m^2), the NE compartment sizes Q_1 and Q_2 (μg/m^2), the NE metabolic clearance rate from compartment 1 (MCR_1) (liters/min per m^2), and the NE compartmental mean residence times (MRT_1 and MRT_2) in minutes. The percent of NE_2 removed from compartment 2 via the pathway L_{02}

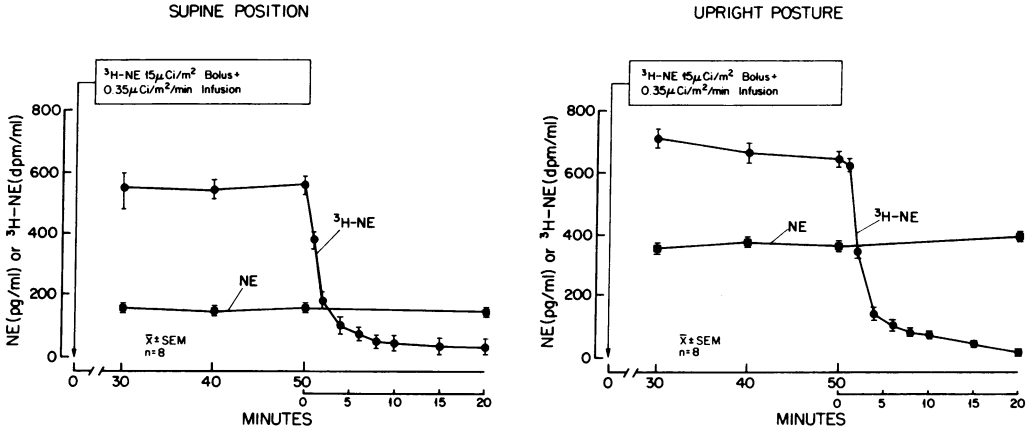

Figure 2. Plasma levels of NE and [^{3}H]NE during [^{3}H]NE infusion in the supine position and during 60 min of upright posture (corresponding to 50 min of [^{3}H]NE infusion) in young healthy subjects. Stable levels of [^{3}H]NE were obtained during [^{3}H]NE infusion, and there was rapid disappearance of [^{3}H]NE once the infusion was stopped. Plasma levels of NE were stable in the supine position and increased to a higher stable level during upright posture.

(NE_R), the percent spill over of NE_2 into compartment 1 via the pathway L_{12} (NE_S), for Model C, and the fraction of NE_2 leaving via the pathway L_{01} as a function of the NE_{SF}, for Model B, were also calculated by SAAM.

Calculation of the rate of NE appearance into plasma (NEAP) and the rate of NE clearance from plasma (NECL) by the isotope dilution technique, were performed as previously described (6, 7).

Statistics

Values are presented as mean±SEM unless otherwise stated. Statistical analyses were performed using the CLINFO system (Bolt, Beranek, and Newman Inc., Cambridge, MA). The Kolmogorov-Smirnov test was used to check normality. All parameters were normally distributed. Student's pair-wise *t* tests were used to compare population means between the supine and upright positions, and multivariate analysis of variance (MANOVA) was used to test for differences between the model parameters for the different values of NE_{SF} and L_{01} for urinary NE excretion.

Results

Levels of [^{3}H]NE and NE in plasma during the baseline [^{3}H]NE infusion study are illustrated in Fig. 2. Both in the supine position and during upright posture [^{3}H]NE levels achieved a plateau during [^{3}H]NE infusion and then disappeared rapidly after stopping the infusion. Plasma NE levels were stable throughout the sampling periods. [^{3}H]NE levels at the start of the decay ($t = 0$) fell to 8.2±2% of their initial value in the supine position and to 11.5±2% of their initial value during upright posture, by $t = 20$, indicating that the [^{3}H]NE disappearance curves were followed for an adequate period of time after stopping the [^{3}H]NE infusion. In addition, the low levels of [^{3}H]NE at the end of the decay periods indicate that any residual [^{3}H]NE due to the infusion during the supine study would have little effect if any on NE kinetics during upright posture.

Identification of exponential models. The disappearance of [^{3}H]NE is not fit well by a single exponential. When a second exponential is added, an excellent fit of the observed decay of [^{3}H]NE is obtained. For each subject studied the range of *r* values for the fit with the monoexponential model was 0.68 to 0.75, all P = NS. The *r* values for the fit with the biexponential model (range, 0.94–1.00; all $P < 0.01$) were greater than the *r* values obtained with the monoexponential model for each subject.

The close fit of the plasma [^{3}H]NE decay data by the biexponential model for a representative subject both in the supine position and upright posture is illustrated in Fig. 3. For each subject studied the *r* values of the biexponential model fit to the upright posture studies were 0.90–0.98, all $P < 0.01$. Partial *F*-test analyses of the exponential model fits for each subject in the supine position and during upright posture revealed the biexponential model fit to be superior in explaining the postinfusion [^{3}H]NE data (all P values < 0.01).

Compartmental analysis. As shown in Fig. 4, for one representative subject, a $NE_{SF} \leq 10\%$ and $\geq 20\%$ does not fit the [^{3}H]NE disappearance curve well. For this subject, the curve fit with the smallest weighted least squares residual error (W_R) was obtained at NE_{SF} equal to 15%. For all subjects, the best fit W_R value obtained from SAAM was used to define the best fit curve to the observed data. The ratio of the fit constrained to a particular NE_{SF} to the best fit W_R was then calculated for each value of NE_{SF} tested. For the data shown in Fig. 4, $W_R(NE_{SF} = 15\%)/W_R = 1.0$ (range of the ratios, 11.3 [$NE_{SF} = 5\%$] to 4.3 [$NE_{SF} = 35\%$]). The tail-end of the curve is the phase of the curve that is due to the [^{3}H]NE that enters the circulation from its extravascular sites of distribution after stopping the [^{3}H]NE

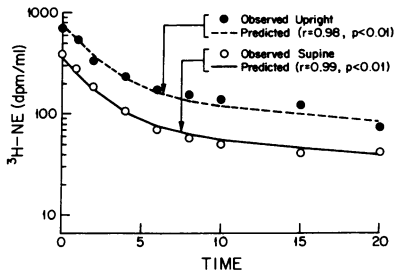

Figure 3. The disappearance of [^{3}H]NE after stopping [^{3}H]NE infusion in one subject in the supine position and during upright posture. The excellent fits of the observed data by the biexponential model or two-compartment model are apparent.

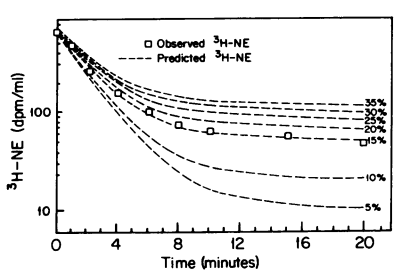

Figure 4. Observed [^{3}H]NE disappearance vs. predicted [^{3}H]NE disappearance as a function of NE spillover fraction in percent, in one subject (supine position) using Model B (Fig. 1). Note that the best fit to the observed data occurred at a NE spillover of 15%.

Table I. Norepinephrine Tracee Data as a Function of the NE_{SF} Using Model B

NE_{SF}*	R_{01}	R_{21}	R_{12}	R_{02}	NE_2 (01)‡
%	μg/min per m^2	μg/min per m^2	μg/min per m^2	μg/min per m^2	%
5	0.0008±0.0005	0.2550±0.0293	0.2563±0.0292	5.15±0.60	0.03±0.03
10§	0.0123±0.0074	0.2538±0.0258	0.2650±0.0218	2.66±0.22	0.5±0.3
15§	0.0272±0.0102	0.2604±0.0230	0.2879±0.0184	1.92±0.12	1.4±0.6
20§	0.0367±0.0587	0.3384±0.0704	0.2940±0.0142	1.47±0.07	2.3±1.0
25	0.0541±0.0197	0.2473±0.0224	0.3016±0.0183	1.21±0.07	4.0±1.5
30	0.0796±0.0197	0.2224±0.0244	0.3201±0.0216	1.01±0.07	6.8±1.7
35	0.1104±0.0280	0.2175±0.0275	0.2861±0.0249	0.86±0.06	10.2±2.3
MANOVA *P* values	0.041	0.29	0.67	<0.001	<0.001

All values are mean±SEM, n = 8 subjects. * NE_{SF} = norepinephrine spillover fraction × 100 (see Methods). ‡ Percent of NE input into compartment 2 leaving via the pathway L_{01}. § The best fit to the experimental data for each subject occurred between NE_{SF} = 10 and 20%.

infusion. Note that at low NE_{SF}s very little [^{3}H]NE enters the circulation, and that as the NE_{SF} increases the amount of [^{3}H]NE entering the circulation increases. Similar results were observed for each subject. For all subjects, the range of W_R ratios was 9.1±1.2 (NE_{SF} = 5%) to 3.5±4.3 (NE_{SF} = 35%). The NE_{SF} consistent with the best fit to the observed data using Model B was 18±3%.

The values for the NE mass transport rates for Model B as a function of the NE_{SF} are shown in Table I. Values are provided for NE_{SF} from 5 to 35%. However, we emphasize that only values in the 10–20% range are compatible with the best fit to the experimental data. Although the fraction of NE_2 leaving via the pathway L_{01} increased as the NE_{SF} increased, its value did not exceed 10%. Furthermore, within the range of the NE_{SF} that best fit our data, it did not exceed 2.3%, indicating that the major irreversible removal of endogenously released NE is from compartment 2.

Within the range of values for NE_{SF} that best fit our data (18±3%), the NE mass transport rate representing irreversible loss of NE from compartment 1 (R_{01}) was within the range of values for urinary excretion of NE reported in the literature (30–33). Therefore, L_{01} was fixed at values estimated from the published data for urinary NE excretion, and the sensitivity of the parameter values for Model B were examined within that range.

In Table II the parameters of Model B are tabulated for L_{01} fixed at values estimated from the published data for urinary NE excretion. It is apparent that the values for L_{21}, L_{12}, and L_{02} are not influenced significantly over the range of L_{01} values based on urinary NE excretion. Similarly, values for NE_2 and V_1, both supine and upright are not affected. To determine if the model parameters are sensitive to a change in L_{01} of the magnitude that may occur with upright posture, it was possible to also estimate L_{01} for upright posture from the data of the study by Cuche et al. (31). Note that similar results for the model parameters are also obtained with this value of L_{01}, indicating that the assumption that L_{01} does not change with upright posture is valid for values of L_{01} comparable with those expected for urinary excretion of NE. One study subject was excluded from the analysis in Table II because of a high degree of uncertainty in some of the estimated parameters of the model at all fixed values of L_{01}.

Model B vs. Model C. Because our findings indicated that irreversible loss of NE via L_{01} is very small (Table I), we rea-

Table II. Norepinephrine Kinetic Parameters For Model B Using Published Values for Urinary NE Excretion

					Supine position		Upright posture	
Studies	L_{01}*	L_{21}	L_{12}	L_{02}	NE_2	V_1	NE_2‡	V_1‡
	min^{-1}	min^{-1}	min^{-1}	min^{-1}	μg/min per m^2	liters	μg/min per m^2	liters
Hoeldtke (30)	0.0707	0.5044±0.03	0.0066±0.001	0.0350±0.001	1.80±0.2	6.9±0.7	2.89±0.3	4.2±0.2
Cuche (31)								
Supine§	0.0451	0.5264±0.03	0.0065±0.001	0.0346±0.005	1.70±0.1	7.0±0.7	2.78±0.3	4.3±0.3
Upright‖	0.0649	0.4878±0.07	0.0070±0.001	0.0340±0.001	1.86±0.2	7.2±0.6	3.00±0.4	4.7±0.3
Alexander (32)	0.0296	0.5425±0.03	0.0064±0.001	0.0347±0.001	1.72±0.1	7.0±0.7	2.82±0.3	4.3±0.3
Carey (33)	0.0211	0.5386±0.03	0.0063±0.001	0.0352±0.001	1.74±0.1	7.0±0.8	2.86±0.3	4.2±0.3
MANOVA *P* values	—	0.79	0.99	1.00	1.00	1.00	0.99	1.00

All values are mean±SEM, n = 7 subjects. * L_{01} represents fixed parameter values calculated from urinary NE excretion data in the reports cited. ‡ Supine vs. upright posture P values < 0.001 for all comparisons. § All parameter values supine or upright estimated using the value for L_{01} obtained from urinary NE excretion in the supine position. ‖ All parameter values supine or upright estimated using the value for L_{01} obtained from urinary NE excretion in the upright posture.

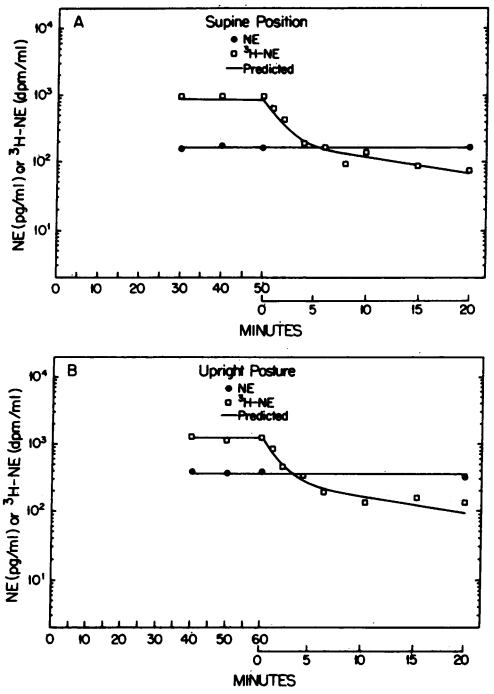

Figure 5. Simultaneous fit of plasma NE and [^{3}H]NE concentrations in the supine position and during upright posture using Model C, in one subject. Note the good fit to all the experimental data collected.

soned that setting L_{01} equal to zero should have only a small effect on the estimates of the parameter values for Model C. This hypothesis was tested by comparing results of the parameter identification analyses using Model B with those using Model C (which assumes $L_{01} = 0$). There was no statistically significant difference between the estimated parameters with Models B and C over the range of fixed values for L_{01} calculated from urinary excretion data, indicating that for small values of L_{01} of the magnitude represented by urinary NE excretion, Model C does not differ significantly from Model B.

Model C. Fig. 5 illustrates a simultaneous fit to all the experimental data collected for one subject in the supine position and during upright posture with Model C. Similar good simultaneous fits were obtained for each of the other studies. Simultaneous modeling using the minimal-change criterion allowed us to determine whether a subset of parameters could be identified whose changes were necessary and sufficient to explain the changes seen in NE kinetics following the perturbation of upright posture. The results of this analysis for Model C are shown in Table III. There was no single L_{ij} whose change was necessary and sufficient to explain the changes seen in NE kinetics with upright posture. Only changes in V_1 and NE_2 were necessary and sufficient. As illustrated in Table III, upright posture was associated with an average 2.8-liter drop in V_1 and a 1.38 μg/min per m^2 increase in NE_2 (both $P < 0.001$). Table IV summarizes the population tracee parameters obtained by SAAM in the supine position and during upright posture for Model C.

Supine position. As shown in Table IV, the estimated mass of NE in compartment 2 (Q_2) was ~ 100-fold larger than Q_1 ($P < 0.001$). As would be expected in a steady-state for Model C, R_{12} and R_{21} were equal and so were R_{02} and NE_2 (P values = NS). The mean residence time of NE in compartment 1 (MRT_1) was significantly less than MRT_2. The estimated percent of NE_2 removed from compartment 2 (NE_R) was 85.4±1% with only 14.6±0.6% spilling over into compartment 1 (NE_S). Note that by using Model C, NE_R and NE_S can be estimated directly from the data without having to assume values for NE_{SF} as with Model B. Also note that the estimate of percentage spillover agrees closely with the NE_{SF} giving the best fit of the data using Model B.

Upright posture. Both the mean [^{3}H]NE level (706±47 vs. 562±39 dpm/ml, $P < 0.001$) and plasma NE level (386±30 vs. 140±13 pg/ml, $P < 0.001$) were higher during upright posture. As can be seen in Table IV, with upright posture there was a modest absolute increase in Q_1, but a large absolute increase in Q_2. However, the relative increases in NE mass during upright posture in compartments 1 and 2 were comparable. Twice as much NE mass exchanged between compartments 1 and 2 during upright posture than in the supine position (R_{21} and R_{12}) and there was a twofold increase in NE_2 and R_{02}. Unexpectedly, there was a fall in MCR_1 which was a direct result of the fall in V_1. There were no differences in the MRTs for NE,

*Table III. Estimated Parameter Values For Model C**

				Supine		Upright	
Subjects	L_{21}	L_{12}	L_{02}	NE_2	V_1	NE_2	V_1
	min^{-1}	*min^{-1}*	*min^{-1}*	*μg/min per m^2*	*liters*	*μg/min per m^2*	*liters*
1	0.5889±0.05	0.0080±0.002	0.0520±0.01	1.71±0.6	6.6±0.7	4.55±1	3.7±0.2
2	0.4199±0.05	0.0046±0.003	0.0263±0.01	1.70±0.4	9.0±0.9	2.80±0.6	6.3±0.7
3	0.5040±0.04	0.0031±0.001	0.0198±0.001	2.12±0.9	8.0±0.7	3.31±1	5.4±0.6
4	0.4894±0.04	0.0024±0.001	0.0143±0.009	1.87±1	8.1±0.7	2.95±2	5.5±0.6
5	0.4929±0.05	0.0073±0.003	0.0348±0.006	2.07±0.3	9.3±1	3.32±0.5	4.2±0.5
6	0.5833±0.09	0.0119±0.005	0.0612±0.007	1.64±0.2	4.8±0.6	2.43±0.3	3.5±0.4
7	0.4833±0.03	0.0013±0.001	0.0097±0.001	2.16±0.4	4.9±0.4	2.77±2	4.1±0.4
8	0.5659±0.04	0.0099±0.004	0.0572±0.01	1.71±0.5	9.1±1	3.84±2	4.9±0.6
Mean±SEM	0.5172±0.02	0.0061±0.001	0.0344±0.007	1.87±0.08	7.5±0.6	3.25±0.2‡	4.7±0.3‡

Values are parameter±SD of estimate and mean±SEM of the population. * L_{ij} remained invariant with upright posture. A change in only NE_2 and V_1 were necessary and sufficient to explain the kinetics during upright posture. ‡ Supine position vs. upright posture, P values < 0.001.

Table IV. Population Parameter Values Using Model C

Parameters	Supine position (n = 8)	Upright posture (n = 8)	Pairwise t test P values
Q_1 ($\mu g/m^2$)	0.62±0.05	1.01±0.08	0.0002
Q_2 ($\mu g/m^2$)	82±23	125±29	0.0006
R_{21} ($\mu g/min$ per m^2)	0.316±0.02	0.519±0.04	0.0003
R_{12} ($\mu g/min$ per m^2)	0.316±0.02	0.519±0.04	0.0003
MCR_1 (*liters/min per* m^2)*	1.80±0.11	1.21±0.08	0.0002
MRT_1 (*min*)‡	1.83±0.14	1.83±0.14	NS
MRT_2 (*min*)§	36.9±10	36.9±10	NS
NE_S (%)‖	14.6±0.6	14.6±0.6	NS
NE_R (%)¶	85.4±1	85.4±1	NS

All values mean±SEM 8 subjects.
* Metabolic clearance rate of NE from compartment 1 = $L_{21} \times (L_{02}/L_{12} + L_{02}) \times V_1$.
‡ Mean residence time for NE in compartment 1 = $(1/L_{21})$.
§ Mean residence time for NE in compartment 2 = $(1/L_{02} + L_{12})$.
‖ NE spillover (%) = $L_{12}/(L_{12} + L_{02}) \times 100$.
¶ NE removal (%) = $L_{02}/(L_{12} + L_{02}) \times 100$.

nor in the NE_R or NE_S, with upright posture. These kinetic parameters are functions of the L_{ij}, which did not change with upright posture (Table III).

Isotope dilution technique. NEAP increased with upright posture, 0.24±0.02 vs. 0.46±0.04, $P < 0.001$, and NECL fell, 1.61±0.07 vs. 1.22±0.06, $P < 0.01$. R_{12}, the parameter of the two-compartment model most analogous to NEAP, tended to be greater than NEAP in the supine position (0.32±0.02 vs. 0.24±0.02, $P = 0.01$) but not significantly so during upright posture (0.54±0.04 vs. 0.46±0.04, $P = 0.12$). NEAP was much less than NE_2 both in the supine position (0.24±0.02 vs. 1.87±0.08, $P < 0.0001$) and during upright posture (0.46±0.04 vs. 3.25±0.24, $P < 0.0001$). NECL was not statistically different than MCR_1 both in the supine position (1.61±0.07 vs. 1.80±0.10, $P = 0.21$, respectively) and during upright posture (1.22±0.06 vs. 1.21±0.08, $P = 0.98$, respectively).

Control supine [^3H]NE infusion studies. Mean steady-state plasma [^{3}H]NE levels were similar between both supine [^{3}H]NE infusion studies 1 and 2 (509±22 vs. 478±22 dpm/ml, respectively, $P = 0.19$), demonstrating the lack of significant residual [^{3}H]NE from the first study, during the second. As during the supine and upright posture studies, in the three subjects studied, a single set of L_{ij} accurately fit the double supine [^{3}H]NE infusion and disappearance data and the NE tracee data. The NE compartmental system minimal model tracee data for these subjects is shown in Table V. There was no single parameter whose percentage change was > 45%. Although during the second study both the plasma NE concentration and NE_2, Q_1, Q_2, and R_{12} increased in two of the three subjects, the changes in these were not as marked as with upright posture. NE volume of distribution and MCR_1 remained essentially the same.

Discussion

The present study demonstrates that a minimal-change two-compartment model is the minimal model that provides a good description of the kinetics of distribution and metabolism of NE in humans both in the supine position and during upright posture. We also identified the specific two-compartment model with the minimum number of parameters and assumptions, but which still is consistent with all the data used to construct it. This minimal model (Model C, Fig. 1) was able to provide a quantitative estimate for the physiological increase of NE release into compartment 2 (extravascular) that accompanied assumption of upright posture. Because the minimal model makes full use of the kinetic data, it can provide more information about the kinetics of distribution and metabolism of NE in vivo than simple measurement of NE levels in plasma or estimates of plasma NE kinetics using the isotope dilution technique.

Compartmental analysis is a tool which allows estimation of parameters of interest in inaccessible regions of the body. Thus the use of this technique is attractive for the study of the NE system in vivo, because NE is released from, and into, inaccessible anatomic sites. Using compartmental analysis we identified an extravascular source of NE (compartment 2) which was many times larger than that of the sampled compartment 1. This finding is consistent with what is known anatomically about the noradrenergic system; i.e., circulating

Table V. Double-Supine [^3H]NE Infusion Studies Minimal Model NE Tracee Data

Subjects	Plasma NE*	NE_2	R_{12}	Q_1	Q_2	V_1	MCR_1
	pg/ml	*$\mu g/min$ per m^2*	*$\mu g/min$ per m^2*	*$\mu g/m^2$*	*$\mu g/m^2$*	*liters*	*liters/min per m^2*
Supine infusion No. 1							
1	104	1.00±0.04	0.114±0.004	0.23±0.01	27±3	3.9±0.2	1.93±0.05
2	247	1.70±0.09	0.172±0.001	0.61±0.04	43±7	6.1±0.3	1.71±0.04
3	136	1.59±0.22	0.175±0.005	0.76±0.04	130±17	10.3±0.5	2.39±0.03
Mean±SEM	162±43	1.43±0.22	0.154±0.017	0.53±0.16	67±32	6.8±1.9	2.01±0.20
Supine infusion No. 2							
1	101	1.00±0.04	0.105±0.003	0.21±0.01	25±6	3.6±0.2	1.80±0.05
2	274	2.41±0.14	0.228±0.007	0.81±0.05	56±9	6.1±0.3	1.71±0.04
3	226	1.91±0.21	0.210±0.006	0.91±0.05	155±26	8.5±0.4	1.98±0.04
Mean±SEM	200±51	1.77±0.41	0.181±0.040	0.64±0.22	79±39	6.1±1.4	1.83±0.08

Values are parameter estimate±SD of estimate unless otherwise indicated. * Individual values are mean of four samples taken at 30, 40, and 50 min, and 20 min after stopping the [^{3}H]NE infusion.

NE represents only a small fraction of the NE that is released at extravascular sites (5, 11). Moreover, during physiologic activation of SNS discharge with upright posture, there was a large increase in the absolute value of NE mass in compartment 2 compared with the sampled compartment 1. However, the relative increases of NE in both compartments 1 and 2 were comparable, indicating that during upright posture an increase of NE in compartment 2 was paralleled by an equal increase in both magnitude and direction of NE in compartment 1. This finding is consistent with and supports previous results that suggest that circulating NE levels parallel SNS release of NE with upright posture (5).

The estimate of NE mass in compartment 2 obtained in the present study is an underestimate of the aggregate endogenous NE in the storage sites of peripheral sympathetic nerves, because the heart alone contains about 1 μg/g of NE (24). Therefore, the most likely explanation for the NE mass in compartment 2 is that it represents NE anywhere in the vicinity of, and/or at a distance from, the neuroeffector junctions of sympathetic nerve synapses plus reversibly bound NE in tissues, but not intraneuronal NE. The finding that this value increased with upright posture, and that it was over 100 times larger than the NE mass in compartment 1, fits that hypothesis.

There is evidence that NE in plasma and extracellular fluid equilibrates rapidly so that these two compartments can be lumped into one (14, 38). Because the NE molecule is small, its diffusion out of capillary beds into the surrounding tissue fluids is very rapid, so that by the time the [^{3}H]NE concentration in plasma has become reasonably stable, the NE has already penetrated into a larger volume than plasma. Our results indicate that the average V_1 is somewhat larger than plasma volume, but much less than total extracellular fluid volume. Thus, we postulate that there is rapid equilibration of NE with part, but not all, of the extracellular fluid space. Compartment 1 of the minimal model therefore must represent a lumped compartment which contains the plasma and part of the extracellular fluid space.

Calculations based on the isotope dilution method suggested that the increase in plasma NE levels with upright posture were due both to an increase in NEAP into plasma and a decrease in NECL from plasma. Compartmental analysis confirmed these findings and demonstrated that the fall in NECL (or MCR_1) could be explained by a fall in V_1, rather than a change in $uptake_1$ or $uptake_2$, as would have been indicated by a change in the L_{ij}. The most likely explanation for the fall in NE clearance with upright posture is that compensating peripheral vasoconstriction resulted in decreased perfusion of tissues where NE is removed from the circulation.

It is unlikely that the fall in NE clearance during upright posture resulted from changes in blood distribution, which are known to occur with upright posture (39), because we found that a single set of L_{ij} could explain the changes seen in the kinetics of distribution and metabolism of NE in both the supine and upright positions. The L_{ij} represent a distributed total body average of various organ and tissue specific fractional rate constants. If upright posture resulted in a change in perfusion from organs that remove NE more efficiently to those which remove NE less efficiently, the observed fall in NE clearance should have been accompanied by a change in the overall estimated fractional rate constants.

Bufano, Vanoa, and Starcich (15) studied a two-compartment model of *dl*-7-[^{3}H]NE distribution in plasma for assessing noradrenergic activity in man. Their proposed model (similar to Model D in Fig. 1) included loss of [^{3}H]NE only from compartment 1. We rejected this model on physiologic grounds, because it does not include NE removal from a compartment remote to the accessible compartment. In addition, the unavailability of a sensitive and specific assay for plasma NE prevented them from following plasma NE kinetics. Our study has clearly extended the modeling methodology to overcome the problems encountered in the study of Bufano and co-workers (15).

A modification of the [^{3}H]NE isotope dilution technique combined with analysis of integrated urinary catecholamine metabolite excretion, with corrections for contributions from brain metabolites, has been developed to estimate the rate of NE secreted and metabolized by peripheral sympathetic neurons (30). Using this method, Hoeldtke et al. (30) estimated the mean NE secretion rate in a group of healthy young subjects in the supine position to be 1.82±0.2 μg/min per m^2, and estimated that at least 78% of the NE secreted by the SNS undergoes local removal (uptakes 1 and 2) and that no more than 22% spills over into the circulation. On the basis of measurements of the increment in circulating NE levels required to cause significant hemodynamic effects, in relation to increases of endogenous plasma NE accompanying SNS stimulation, Silverberg et al. (27) concluded that over 80% of NE released by human sympathetic neurons is removed locally.

Using Model C, our estimate of the steady-state input rate of NE into compartment 2 was 1.87±0.08 μg/min per m^2. We estimate that 85±1% of the NE input into the extravascular compartment 2 is removed from that site by at least uptakes 1 and 2, and that 15±0.6% "spills over" into compartment 1. These results are in agreement with those of Hoeldtke et al. (30) and the conclusion reached by Silverberg et al. (27). However, compartmental analysis is not limited by assumptions about the relationship between urinary excretion of various NE metabolites and apparent NE secretion (or spillover rate) with total NE secretion, and we have demonstrated that compartmental analysis can be readily applied to the study of steady-state activation of the SNS, as during upright posture.

Sensitivity analysis (40) was performed to examine the extent to which the assumptions introduced during the model development would influence the estimated parameter values of the models. This analysis revealed that the presence of a pathway out of compartment 1 (L_{01}) using the technique of determining L_{12} as a function of the NE_{SF} for Model B, affected L_{01}, R_{01}, and NE_2 by increasing the former and decreasing the latter. However, within the range of NE_{SF} compatible with the best fit to the observed data, the changes were small. When L_{01} was assumed to equal the fractional rate of urinary NE excretion, an assumption supported by our analysis, or L_{01} was assumed to equal zero, the presence of a loss path from compartment 1 (L_{01}) had little effect on the parameter estimates obtained using either Model B or C. Thus Model C emerged as the simplest model that provides a good description of the kinetics of distribution and metabolism of NE in human beings, is consistent with all the data used to construct it, and is useful for practical applications.

We emphasize that our minimal model represents a working hypothesis that provides the best objective integration of the current experimental data and which still approximates the true NE metabolic system. Validation may be viewed as an

effort to support such a model through alternate independent approaches, including the design of new experiments to test the model.

In conclusion, our proposed minimal two-compartment model is consistent with previously published results of important quantitative aspects of NE metabolism in humans obtained by completely different methods, which serves to support the model and the previous results. Nevertheless, it is quite possible that by the use of qualitatively different experimental technique, new types of data may be derived which are beyond the predictive domain of the model. Under these circumstances, a new more extensive model would have to be proposed that could provide more precise information and a more complete description of the NE system. But, from the point of view of assessing overall SNS activity, the minimal model appears to be useful for practical applications because it detected the increase in SNS activity during upright posture. The [^{3}H]NE isotope dilution technique for in vivo estimation of NE kinetics, which is based on a one-compartment model, did not predict the [^{3}H]NE disappearance well and does not provide information about NE kinetics in the inaccessible compartment. Compartmental analysis of plasma NE kinetics provides a new radioisotopic approach for the study of NE metabolism in normal physiology and in disease states in humans.

Appendix

The equations for the identification experiment for Model B (Fig. 1, text) are Eqs. 2 and 3:

$$\dot{q} = Lq + Bu, \; q(0) = 0 \tag{2}$$

$$y = Cq, \; y_1 = q_1/V_1 \tag{3}$$

where $q = [q_1 \quad q_2]^T$ is the state vector of tracer quantities; $u = [1 \quad 0]^T$ is the tracer input vector; y_1 is the scalar of observations (tracer concentration in compartment 1); L is the state compartmental matrix which determines the kinetics of the system:

$$L = \begin{pmatrix} -(L_{01} + L_{21}) & L_{12} \\ L_{21} & -(L_{02} + L_{12}) \end{pmatrix} \tag{4}$$

B and C are the input and output design matrices.

$$B = (1 \quad 0); \quad C = (1/V_1 \quad 0) \tag{5}$$

The reachability matrix for the identification experiment is

$$R = \begin{pmatrix} 1 & 1 \\ 1 & 1 \end{pmatrix} \tag{6}$$

Hence, the system is structurally identifiable. The transfer function is

$$H_{11}(s) = \frac{(s + L_{02} + L_{12})/V_1}{s^2 + (L_{02} + L_{12} + L_{01} + L_{21})s + L_{01}L_{02} + L_{01}L_{12} + L_{21}L_{02}} \tag{7}$$

Thus, the input-output experiment determines Eqs. 8:

$$\begin{aligned} \phi_1 &= (L_{02} + L_{12}) \\ \phi_2 &= L_{02} + L_{12} + L_{01} + L_{21} \\ \phi_3 &= L_{01}(L_{02} + L_{12}) + L_{21}L_{02} \\ \phi_4 &= 1/V_1 \end{aligned} \tag{8}$$

Clearly, the model is not identifiable. However, if L_{12} can be determined, the model becomes identifiable. Let $L_{12} = z_1$ (a value based on published data), then

$$\begin{aligned} L_{02} &= \phi_1 - z_1 \\ L_{21} &= \phi_2 - (L_{02} + z_1 + L_{01}) \\ L_{01} &= \phi_2 - (L_{02} + z_1 + L_{21}) \\ V_1 &= 1/\phi_4 \end{aligned} \tag{9}$$

With independent knowledge of L_{01}, the model also becomes identifiable. Let $L_{01} = z_2$ (a value also based on published data), then

$$\begin{aligned} L_{21} &= \phi_1 - \phi_3 - z_2 \\ L_{12} &= \phi_2 - (z_2\phi_3)/L_{02} \\ L_{02} &= \phi_2 - (z_2\phi_3)/L_{12} \\ V_1 &= 1/\phi_4 \end{aligned} \tag{10}$$

L_{01} equals zero makes the model identifiable and reduces it to Model C (Fig. 1, text). The transfer function becomes

$$H_{11}(s) = \frac{(s + L_{02} + L_{12})/V_1}{s_2 + (L_{02} + L_{12} + L_{21})s + L_{02}L_{12}} \tag{11}$$

The input-output experiment determines Eqs. 12:

$$\begin{aligned} \phi_1 &= L_{02} + L_{12} + L_{21} \\ \phi_2 &= L_{02}L_{12} \\ \phi_3 &= L_{02} + L_{12} \\ \phi_4 &= 1/V_1 \end{aligned} \tag{12}$$

ϕ_4 uniquely determines V_1, and ϕ_2 and ϕ_3 uniquely determine L_{02} and L_{12}. With L_{02} and L_{12} determined, L_{21} is fully determined by ϕ_1.

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