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Clinical Pharmacokinetics with WinSAAM

Oscar A. Linares, MD, and Raymond C. Boston, PhD

Abstract In this article we study clinical pharmacokinetics using the NIH-WinSAAM software.

Keywords: Compartmental analysis; pharmacokinetics; WinSAAM; modeling

1.1 Introduction

To determine whether differences in neuronal reuptake contribute to age-related changes of sympathetic nervous system activity, we compared norepinephrine (NE) release and metabolism during [3H]NE infusion and decay in six young (age 126 yr) and seven older (age 61-73 yr) healthy nonobese subjects. Subjects were studied on a control day and on a separate day after desipramine (DMI; 125 mg orally), a neuronal reuptake blocker. Compartmental analysis of plasma NE specific activity was used to determine several NE kinetic parameters. Plasma NE levels and NE spillover rates were higher in the elderly. Although plasma NE was unaffected by DMI in both age groups, both the metabolic clearance rate of NE from plasma and the rate of NE spillover into plasma fell in young and older groups during DMI. Furthermore, DMI dramatically lowered the mass of NE in the extravascular compartment and the rate of NE entry into the extravascular compartment. Thus neuronal uptake blockade has major effects on NE release as well as NE metabolism in humans. However, age-related differences in NE kinetics cannot be explained by differences in neuronal uptake.

ALTERATIONS of sympathetic nervous system function have been implicated in impaired adaptive circulatory mechanisms in aging (25). Plasma levels of norepinephrine (NE), the major neurotransmitter of the sympathetic nervous system in humans, are higher in the elderly not only at rest (42) but also during the stresses of upright posture, exercise, glucose ingestion, and a mental stress test (25, 41). The increase in plasma NE in the elderly is due to both diminished NE clearance from the circulation and increased appearance of NE in the plasma (39). The relative contributions of release and removal mechanisms to the level of NE in plasma have been previously assessed primarily by use of tritiated NE ([3H]NE) to estimate plasma NE kinetics. We have recently employed compartmental analysis to provide further information about NE kinetics in humans, particularly about NE kinetics in a large extravascular compartment (26, 27, 35).

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NE is cleared from the neuroeffector junction by neuronal (uptake 1) and nonneuronal (uptake 2) mechanisms. Disturbances in these clearance mechanisms may have pathogenetic significance in cardiovascular disease states. For example, in some hypertensive patients, defective uptake 1 has been reported (12, 21). The present study was designed to determine whether impaired neuronal reuptake contributes to the age-related changes of NE kinetics. An oral dose of 125 mg of a tricyclic antidepressant, desipramine (DMI), has been used previously to block neuronal reuptake in humans (22). In the present study, DMI was given to both young and elderly subjects before tracer NE infusion to separate the effects of uptakes 1 and 2 on NE metabolism. This study demonstrates major effects of DMI on NE release and metabolism and qualitatively similar effects of DMI on young and old humans.

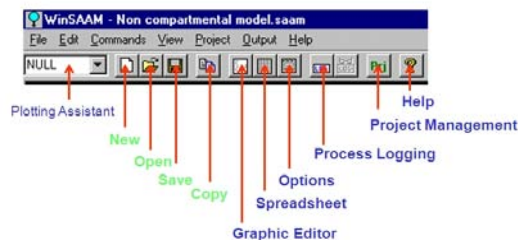

Figure 1.1: Menu items and toolbar buttons.

1.2 Methods

Protocol. Six young subjects (5 men and 1 woman) 19- 26 yr of age and seven older subjects (3 men and 4 women) 61-73 yr of age were studied. All were healthy and within 30Life Insurance tables). There was no difference in body weight between the young and old groups (112 t 4 vs. 109 t 4any medication, and all were screened with a medical history, physical examination, and routine clinical blood tests to ensure that they were without any clinical condition known to affect catecholamine metabolism. The subjects were prohibited from the use of known modulators of catecholamine release and metabolism, including nicotine, caffeine, and marijuana, for 12 h before the studies. The protocols were approved by the University of Michigan Human Use and Radiation Control Committees. All subjects were studied in the supine position after an overnight fast on 2 consecutive days at the University of Michigan Hospitals.

A control NE kinetics study was performed on the 1st day and a second NE kinetics study was performed on the following day 3 h after oral ingestion of 125 mg of DMI (Norpramin, desipramine hydrochloride, Merrell Dow Pharmaceuticals, Cincinnati, OH). The study protocol (Fig. 1) was identical on both days. A fixed sequential protocol design was chosen to avoid the possibility of a carryover effect of DMI to the following day if sequence randomization had been used. We have not observed a sequence effect in sequential NE kinetic studies done on the same day (26) or in

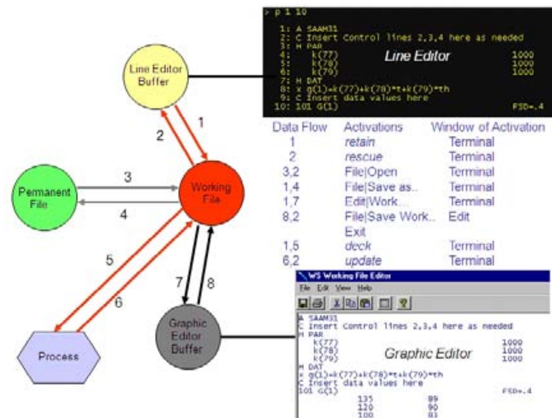

Figure 1.2: Menu items and toolbar buttons.

baseline studies done 4 wk apart in a double-blind placebo controlled crossover design (32, 34).

An intravenous catheter was placed in an antecubital vein of one arm for infusion of [3H]NE. In the contralatera1 arm, a scalp vein needle was inserted retrogradely into a dorsal vein of the hand, which was placed in a warming box at 60C to obtain arterialized blood samples. This approach has been used for studying the kinetics of a variety of substrates and hormones (17, 28) and has previously been validated for catecholamines (5, 38). The catheters were kept patent with 0.45saline. The first 1 ml of blood sampled was discarded at each sampling time. Subjects received infusions of L- [ring-2,5,6-3H]NE (sp act 40.4 Ci/mmol; Du Pont-New England Nuclear, Boston, MA) at an infusion rate of -0.35 &i 1 min. mm2 for 60 min using a syringe pump (Harvard Apparatus, S. Natick, MA). Infusates contained 1 mg/ml ascorbic acid to prevent oxidation of NE. Arterialized blood samples (10 ml) were collected at 40, 50, and 60 min during [3H]NE infusion for measurement of steady-state [3H]NE and plasma NE concentrations. A sample was also obtained for measurement of plasma DMI concentrations on the study day. Blood pressure and heart rate measurements were obtained after blood withdrawal at 40, 50, and 60 min during the [3H]NE infusion (automated sphygmomanometer BP203NA, Air Shields, Hatboro, PA).

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WinSAAM: Software for Pharmacokinetic Modeling

Oscar A. Linares, Darko Stefanovski and Ray C. Boston

Abstract Compartmental modeling is the backbone of pharmacokinetics and its has contributed substantially to our understanding of normal and abnormal system states in living and nonliving systems.

2.1 Introduction

Pharmacokinetics is the study of the absorption, distribution, metabolism, and excretion (ADME) of drugs in the body and compartmental modeling is the backbone of pharmacokinetics. Compartmental modeling has a long history of application in the study of metabolism and pharmacokinetics in humans [2, 3, 4]. Efficient and effective computational pharmacokinetics [5] calls for an array of integrated computer modeling tools for knowledge discovery, optimum dose regimen design, and pharmacokinetic system identification. The SAAM (Simulation, Analysis, And Modeling) software is one such system which has evolved over 50 years and is underpinned by the pioneering contributions of Mones Berman at the National Institutes of Health (NIH), Bethesda, MD [6, 7, 8]. WinSAAM [9, 10] is the direct descendent of SAAM, albeit with considerable enhancement of capacity and performance. The evolutionary nature of the software has led to a consistent methodology so that investigators can dynamically explore systems and processes using pharmacokinetic models in real-time. Although WinSAAM is software for compartmental modeling, the backbone of pharmacokinetics, there remains some confusion with regard to WinSAAM's scope of application in pharmacokinetic work [12].

This paper demonstrates WinSAAM use for pharmacokinetic modeling and parameter estimation (WinSAAM is free software [http://www.winsaam.com]). The paper thus serves as a user and applications manual for WinSAAM in general, and pharmacokinetic data analysis with WinSAAM, in particular. The mathematical formulation and solution of compartmental and pharmacokinetic models are discussed in detail in texts by Norwich [14], Lassen & Perl [15], Godfrey [16], Brown [1], Jacquez [3], Cobelli, Foster, and Toffolo [13], Gibaldi and Perrier [17], Bates and Watts [18], and Seber and Wild [19], and the papers by Jacquez and Simon [20, 21]. General strategies for modeling with compartments have been presented by Jacquez [22] and Wastney et. al. [23]. For ease of exposition, derivations of mathematical solutions are not included. The reader is referred to the references for details. Certain applications may require the use of nonstandard models for which the reader will be required to consult other sources to derive his or her own solutions. This paper emphasizes the principles underlying the WinSAAM solutions to pharmacokinetic models and their interpretation.

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2.2 Background

Brief History

In 1956, Berman and Shoenfeld [24] and Mones Berman in his PhD thesis [25] developed a mathematical approach to quantify radioisotopic experiments in terms of linear constant coefficient differential equations reflected by compartments. Shortly thereafter, Berman developed the Simulation, Analysis, And Modeling (SAAM) software for compartmental analysis [6, 7]. In the late 70's and early 80's, Boston optimized the SAAM software and developed its user–interface [12]. The latter gave birth to computer-assisted computational compartmental modeling and allowed its wide application in biology and medicine. Since its development, the SAAM software series has been used for experimental kinetic data analysis in over 1000 peer reviewed scientific publications in the literature.

Using WinSAAM

WINSAAM INTERFACE

Figure 2.1 shows WinSAAM's menu items and toolbar buttons interface. To start a new problem, select `FILE|NEW` from the menu. To work on an existing problem, select `FILE|OPEN`. This opens a WinSAAM text input file (a ASCII text file with extension *.saam) and makes it the *working* file.

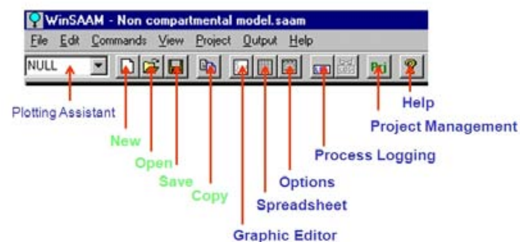

Figure 2.1: Menu items and toolbar buttons.

MODELING CONSTRUCTS

WinSAAM has its own dictionary of modeling constructs (also called operational units) which allow the user to sketch a proposed compartmental/pharmacokinetic model, and from that sketch, enter the model directly into the software (Figure 2.1).

2.3 PHARMACOKINETIC MODELING

INTRODUCTION

Pharmacokinetic modeling is a convenient tool to analyze drug data. When the distribution of drug in one state is distinguishable from the distribution of drug in another state, i.e., the drug undergoes homogeneous distribution, and/or redistribution and partitioning into one or more regions in the body, in this case, the time-course of the concentration of drug in the body can be described by a set of simultaneous ordinary differential equations. We develop both the basic theory and nomenclature for this paper in parallel with the nomenclature of the SAAM dictionary, i.e., the language used to describe a model to WinSAAM.

KINETIC PROCESSES

The time-scale for the processes involved in the transfer of drug into, within, and out of the body is finite, and it is mostly due to the time it takes for drug to transfer across body membranes. For example, the rate-limiting step to absorption of drug from the gastrointestinal tract is the transfer of drug across the epithelial tissue of the gastrointestinal tract, whereas the elimination of drug from the body may be slowed due to glomerular filtration and transport across Bowman's capsule membranes. Other potential rate-limiting processes include distribution of drug to body organs and tissues, tubular reabsorption in the kidney, enterohepatic cycling, hepatoportal circulation and biochemical reactions.

Drug transport across rate-limiting barriers such as the cell membrane is fundamental to pharmacokinetics. A cell membrane normally refers to the plasma membrane that surrounds the cytoplasm of a cell and forms the cell boundary. The cell membrane consists of a lipid bilayer with embedded proteins. Depending on the membrane's location and role in the body, lipids can make up anywhere from 20 to 80 percent of the membrane, with the remainder being proteins. Lipids generally give membranes their flexibility. Cholesterol is a type of lipid that, by contrast, helps stiffen the membranes of mammalian cells.

The cell membrane regulates what enters and leaves the cell, maintains the correct intracellular pH level, and provides a means of separating charges so that the cell can, for example, generate the energy-carrying molecule adenosine triphosphate. Proteins transmit chemical messages into the cell, and they also monitor and maintain the cell's chemical climate. On the outside of cell membranes, attached to some of the proteins and lipids, are chains of sugar molecules that help each cell type do its job.

The cell membrane is both a physical and chemical barrier which defines the boundary between the individual and its environment. Its origin is intimately connected with the origin of life as we know it. Cell membranes are sheet-like structures, typically 7.5 nm thick. They consist mainly of lipid and protein, and may also contain sugars. Membrane lipids are relatively small molecules that have both hydrophilic and hydrophobic properties. Specific proteins mediate specific functions in membranes, e.g., transport and energy generation. Membranes are asymmetric fluid structures.

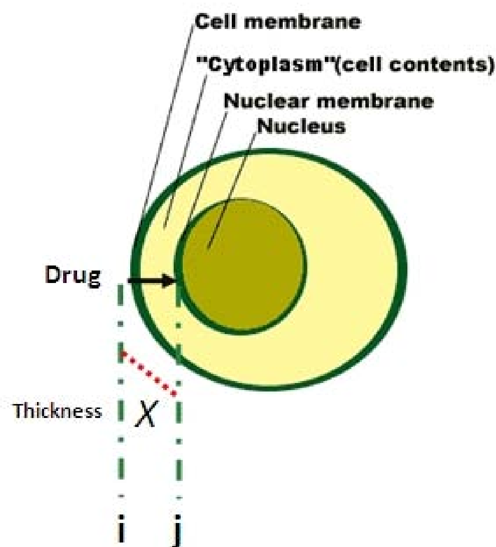

Figure 2.2: Membrane.

EXPONENTIAL MODELS

Selecting the "best" model among a set of multiexponential models of increasing dimension is a common problem in pharmacokinetics because the solution of the linear differential equations of pharmacokinetic models all turn out to be polyexponential in form. Therefore, the integrated differential equations can be generalized by Equation 2.1,

$$C(t_k) = \sum_{i=1}^{n} A_i e^{-L_i t_k} + e_k, \tag{2.1}$$

where $C(t_k)$ is drug concentration over time, A_i and L_i are unknown constant parameters corresponding to eigenvector and eigenvalue pairs, respectively, and e_k is the measurement error at each discrete time point t_k. The data are usually fitted using weighted-least squares [27, 28, 29].

The number of compartments in a compartmental model are determined by the number of exponential terms in Equation 2.1. Models with three or more exponential terms (compartments) may be configured in different ways, leading to different possible solutions, not all of which may be identifiable [13]. For this reason, pharmacokinetic models involving

three or more exponential terms are not common and are motivated more on a statistical rather than a biological basis [31].

The exponential model provides a convenient model for statistical analysis, but will be difficult to interpret in terms of simple compartmental models when *n* is moderately large. Multiexponential models of the form given by Equation 2.1 arising as solutions to compartmental systems may also be subject to certain constraints.

Following first-order kinetics, the amount $F_1(t)$ of drug in the central compartment satisfies the following differential equation,

$$\frac{dF_1(t)}{dt} = -k_e F_1(t). \tag{2.2}$$

Since $F_1(0) = D$, the solution to Equation 2.2 is

$$F_1(t) = De^{-k_e t}. \tag{2.3}$$

Assuming the drug is distributed uniformly throughout the apparent volume of distribution V_1, Equation 2.3 can be divided by V_1 to obtain

$$C_1(t) = Ae^{-\alpha t}, \tag{2.4}$$

where $C_1(t)$ denotes the concentration of drug in the central compartment equal to $A = D/V_1$ and $\alpha = k_e$. Linearizing Equation 2.4 gives

$$\ln C_1(t) = \ln A - \alpha t. \tag{2.5}$$

Thus a semi-logarithmic plot of concentration $C_1(t)$ over time t plots a straight line.

Second, now suppose that the drug of interest is administered orally. The amount of drug $F_0(t)$ present in the gastrointestinal tract satisfies the following differential equation:

$$\frac{dF_0(t)}{dt} = -k_a F_0 t, \tag{2.6}$$

where k_a denotes the fractional transfer rate coefficient for gastrointestinal absorption. Thus, for oral dosing, $F_1(t)$ is given by

$$\frac{dF_1(t)}{dt} = k_a F_0 t - k_e F_1(t). \tag{2.7}$$

Equations 2.6 and 2.7 can be solved simultaneously using Laplace transforms to obtain

$$F_1(t) = \frac{Dk_a}{(k_a - k_e)} \left(e^{-k_e t} - e^{-k_a t}\right), \tag{2.8}$$

iff $k_a \neq k_e$. Dividing Equation 2.8 by the apparent volume of distribution V_1 gives

$$C_1(t) = A\left(e^{-\alpha t} - e^{-\beta t}\right) \tag{2.9}$$

where $A = Dk_a\left[(k_a - k_e)\, V_1\right]^{-1}$, $\alpha = k_e$ and $\beta = k_a$. A semi-logarithmic plot of $C_1(t)$ versus t has a terminal linear component with slope equal to α.

Lastly, if the drug is administered by constant infusion, drug is delivered into the central compartment at an essentially constant rate equal to k_0, with

$$\frac{dF_1(t)}{dt} = k_0 - k_e F_1(t). \tag{2.10}$$

Integration of Equation 2.10 and division by the apparent volume of distribution (V_1) gives

$$C_1(t) = A\left(1 - e^{-k_e t}\right), \tag{2.11}$$

where $A = \frac{k_0}{[k_e \cdot V_1]}$.

Two-Compartment Model: If the one compartment model fails to provide an adequate fit to the data, two compartment models may be entertained. For example, consider the two-compartment model in Figure 2.2 Panel B. Lets apply a single intravenous dose of drug. Then, the amounts of drug present in the central and peripheral compartments, $F_1(t)$ and $F_2(t)$, satisfy

$$\begin{aligned} \frac{dF_1(t)}{dt} &= -\left(k_e + k_{21}\right) F_1(t) + k_{12} F_2(t) + D \\ \frac{dF_2(t)}{dt} &= k_{21} F_1(t) - k_{12} F_2(t) \end{aligned} \tag{2.12}$$

where k_e is the fractional elimination rate constant from the central compartment and k_{21} and k_{12} are intercompartmental fractional rates of drug transfer between the central and peripheral compartments, respectively. The solution to Equation 2.12 is given by

$$\begin{aligned} F_1(t) &= \frac{D}{(\beta - \alpha)}\left[\left(k_{12} - \alpha\right) e^{-\alpha t} - \left(k_{21} - \beta\right) e^{-\beta t}\right] \\ F_2(t) &= \frac{Dk_{12}}{(\beta - \alpha)}\left(e^{-\alpha t} - e^{-\beta t}\right) \end{aligned} \tag{2.13}$$

where α and β satisfy the equation

$$\alpha + \beta = k_{21} + k_{12} + k_e \tag{2.14}$$

and

$$\alpha\beta = k_{12} k_{01}. \tag{2.15}$$

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Effect of Desipramine on Norepinephrine Metabolism in Humans: Interaction with Aging

Jannifer S. Stromberg, Oscar A. Linares, Mark A. Supiano, Marla J. Smith, Andrew H. Foster, and Jeffrey B. Halter

Abstract To determine whether differences in neuronal reuptake contribute to age-related changes of sympathetic nervous system activity, we compared norepinephrine (NE) release and metabolism during [3H]NE infusion and decay in six young (age 126 yr) and seven older (age 61-73 yr) healthy nonobese subjects. Subjects were studied on a control day and on a separate day after desipramine (DMI; 125 mg orally), a neuronal reuptake blocker. Compartmental analysis of plasma NE specific activity was used to determine several NE kinetic parameters. Plasma NE levels and NE spillover rates were higher in the elderly.

Keywords: Norepinephrine kinetics; catecholamines; sympathetic nervous system; compartmental analysis

Although plasma NE was unaffected by DMI in both age groups, both the metabolic clearance rate of NE from plasma and the rate of NE spillover into plasma fell in young and older groups during DMI. Furthermore, DMI dramatically lowered the mass of NE in the extravascular compartment and the rate of NE entry into the extravascular compartment. Thus neuronal uptake blockade has major effects on NE release as well as NE metabolism in humans. However, age-related differences in NE kinetics cannot be explained by differences in neuronal uptake.

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baseline studies done 4 wk apart in a double-blind placebo controlled crossover design (32, 34).

An intravenous catheter was placed in an antecubital vein of one arm for infusion of [3H]NE. In the contralatera1 arm, a scalp vein needle was inserted retrogradely into a dorsal vein of the hand, which was placed in a warming box at 60C to obtain arterialized blood samples. This approach has been used for studying the kinetics of a variety of substrates and hormones (17, 28) and has previously been validated for catecholamines (5, 38). The catheters were kept patent with 0.45saline. The first 1 ml of blood sampled was discarded at each sampling time. Subjects received infusions of L- [ring-2,5,6-3H]NE (sp act 40.4 Ci/mmol; Du Pont-New England Nuclear, Boston, MA) at an infusion rate of -0.35 &i 1 min. mm2 for 60 min using a syringe pump (Harvard Apparatus, S. Natick, MA). Infusates contained 1 mg/ml ascorbic acid to prevent oxidation of NE. Arterialized blood samples (10 ml) were collected at 40, 50, and 60 min during [3H]NE infusion for measurement of steady-state [3H]NE and plasma NE concentrations. A sample was also obtained for measurement of plasma DMI concentrations on the study day. Blood pressure and heart rate measurements were obtained after blood withdrawal at 40, 50, and 60 min during the [3H]NE infusion (automated sphygmomanometer BP203NA, Air Shields, Hatboro, PA).

The [3H]NE infusion was stopped at 60 min, and blood samples (10 ml) were obtained at 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 min for measurement of [3H]NE concentration. Blood pressure and heart rate were again recorded after final sample withdrawal. Plasma NE concentrations and plasma DMI levels were also measured at 10 and 20 min after the infusion was stopped. Figure 1 shows plasma [3H]NE levels of one subject on both a control and a DMI-pretreated day. Analytic methods. Blood samples for measurement of plasma NE and plasma [3H]NE were collected in chilled plastic tubes containing ethylene glycol-bis(P-aminoethyl ether)-N,N,N,N-tetraacetic acid and reduced glutathione and immediately placed on ice. They were promptly centrifuged at 4C, and the plasma was stored at -70C until assay. Plasma NE was quantified by a single-isotope radioenzymatic assay using unextracted plasma (15). All samples from a single subject were analyzed in the same run of the assay. The intra-assay coefficient of variation for plasma NE in our laboratory is 5scintillation counting of the radiolabeled catecholamine after alumina extraction as previously described (13). Alumina extraction and measurement of [3H]NE were performed within 24 h of each study. Recovery of [3H]- NE after alumina extraction, calculated in each study by adding an aliquot of [3H]NE infusate of known concentration to a plasma sample obtained before infusion, was 70 t 2high pressure liquid chromatography as previously described (10, 33).

Computational methods

Two-compartment mathematical modeling was performed on a VAX 11/730 computer (Digital Equipment, Maynard, MA) using the SAAM version 29 (3) and CONSAM version 29X (2) simulators, as previously described (26). All [3H]NE and NE data were fit simultaneously by solving the differential equations of both the [3H]NE tracer and tracee systems by the method of weighted nonlinear least squares (3). The criteria of goodness of fit for the compartmental parameter estimates are those of Berman et al. (4).

The minimal two-compartment model used for these analyses is illustrated in Fig. 2. The unknown compartmental model parameters estimated by simultaneous fits of the basal and DMI-pretreated NE data included the fractional transport rate constants (Lij; min-l), the volume of distribution of NE in compartment 1 (liters), and the steady-state input rate of NE into compartment 2 (NE,; nmol . min- . m-). These parameters were derived by SAAM along with statistical measures of their estimability. Other kinetic parameters calculated by SAAM were the NE mass transport rates (nmol l min- . mB2), the NE compartmental mass sizes (Q1 and Q2; nmol/m2),

Mean arterial pressure (MAP) was calculated by using the following formula: MAP = diastolic blood pressure + 1/3(systolic blood pressure - diastolic blood pressure). MAP was calculated for each of the five sets of blood pressure readings recorded for each subject and was then averaged to calculate a mean MAP for each subject on each day of the study. The heart rate measurements for each subject were similarly averaged on each day of the study.

Statistical analysis. Values are presented as means t SE unless otherwise stated. Statistical analyses were performed using the Statview 512+ program (Abacus Concepts). Analysis of variance for repeated measures was used to test for age and drug effects. Students twosample paired t tests were also used for testing differences in study parameters during the control day vs. the DMIpretreated day. Students two-sample unpaired t tests were used for testing differences in study parameters between the young and elderly.

3.2 Results

A summary of the effects of DMI on NE kinetic parameters in young and elderly subject groups is provided in Table 1. There was no significant difference in plasma DMI levels between the two study groups (45.5 t 5.5 and 46.8 t 5.9 rig/ml for young and old, respectively). This finding is in agreement with previous studies that show no difference between the young and elderly in plasma concentrations of DMI achieved after a single oral dose (1, 9). No age differences were observed in the NE fractional transport rate constants Lzl, LIZ, and Lo. After DMI, L21 fell significantly, LIZ increased significantly, and Lo2 did not change.

Plasma NE, blood pressure, and heart rate. The mean plasma NE levels were 44 and 32compared with the young patients on the control and DMI-pretreated days, respectively (both P c 0.01). The administration of DMI did not cause a significant change in plasma NE levels within either group (Fig. 3A). The MAP was significantly higher in the elderly on both the control and DMI-pretreatment days. However, MAP

did not significantly change during the protocol in either age group after DMI administration. The baseline heart rate was similar in both age groups, increased significantly in both age groups after pretreatment with DMI, but tended to increase more in the elderly (15 t 3 vs. 8 t 4increase, P = 0.06).

NE removal The plasma MCR, of NE fell significantly in both age groups after DMI administration (Fig. 3B). Although the MCRl tended to be lower in the elderly than in the young, this was not a statistically significant difference. As summarized in Table 1, there were no significant age effects on the fractional catabolic rate of NE in compartment 1 or 2 (FCR1 or FCR2, respectively). FCRl fell significantly after DMI administration, whereas FCR2 increased after DMI. DMI also caused a moderate increase of the volume of distribution of NE in compartment 1.

3.3 Discussion

Although DMI has a potent effect to block neuronal reuptake of NE, we observed no significant change in plasma NE levels after DMI administration in healthy young and old people. This finding agrees with previous studies (l4,21). The lack of effect of DMI on plasma NE levels might suggest that neuronal uptake blockade has little effect on sympathetic nervous system (SNS) activity or on NE removal in vivo. On the contrary, the technique of compartmental analysis used in this study demonstrated that DMI has profound and complex effects on NE metabolism and cannot therefore be used as a simple uptake 1 blocker.

One of the important effects of DMI observed in this study is the significant reduction in the rate of NE release into the extravascular compartment (NE2). Although NE, is not a direct measure of NE release from sympathetic nerve terminals, this finding is consistent with several previous reports concerning DMI effects on NE metabolism. The reduction in NE, after DMI may represent both peripheral and concurrent central inhibition of NE release. Presynaptic inhibition of NE release would be expected to increase in the setting of uptake 1 blockade because of higher intrasynaptic NE concentrations (24). Recent studies in experimental animals have documented an effect of DMI to decrease sympathetic nerve firing rates by direct nerve recording (7).

In addition to a local feedback mechanism, the decreased release of NE after DMI may also be mediated by a central action of DMI (12, 36, 40). It has been shown that SNS outflow is inhibited in the central nervous system by pathways involving aZ-adrenergic receptors (6). The effects of DMI on efferent renal and lumbar sympathetic nerve traffic in rabbits suggest that DMI inhibits SNS activity via central cuz-receptors (7). Another study has shown that DMI may decrease whole body NE turnover while enhancing 6-hydroxymelatonin output (19). This study suggests not only that sympathetic nerve firing rates are decreased as a result of DMI but also that the total NE production rate may be decreased.

Despite the significant decrease in NE2, there was no significant change in plasma NE levels in either subject group during DMI. This result agrees with previous studies (14, 21, 29). Plasma NE levels depend on the dynamics of NE appearance and clearance from the intravascular compartment. The administration of DMI caused a decrease in MCRl in both the young and elderly subjects. This decrease in MCRl after DMI confirms previous work (14) and is expected, because DMI blocks one of the major mechanisms for NE removal from the sympathoeffector junction. The lack of difference in the reduction of MCR, after DMI in young and old suggests that there is not an age-related difference in neuronal reuptake mechanisms.

Despite the decrease in MCRl after DMI, plasma NE levels did not increase because the rate of NE spillover into plasma also decreased in both groups. The alterations in the NE release rate after DMI support the hypothesis suggested in previous studies (8, 14) that changes in catecholamine clearance, in this case induced by DMI, may serve a homeostatic role to dampen or amplify, respectively, increases or reductions in SNS activity. Because there were no age-related differences in these changes in NE kinetics after DMI, it appears that this compensatory adaptation is intact in healthy elderly subjects.

The relative contribution of neuronal reuptake to the total plasma clearance of NE is difficult to determine. Although a single oral dose of DMI has been thought to completely and selectively block uptake 1, allowing estimation of the contribution of neuronal uptake to overall removal, there is some controversy about whether neuronal uptake block is complete at this dose (22, 30). If the neuronal uptake block is incomplete at this dose, then the effects of DMI on MCRl may underestimate the component of total plasma clearance due to neuronal reuptake. Using plasma levels of dihydroxvphenvl-glycol to estimate neuronal reuptake of NE, DMI has been shown to decrease neuronal reuptake by 8590(11, 20). It has also been suggested that other pathways of NE removal, such as 0-methylation, may be activated by neuronal uptake block, to some degree compensating for the decrease in removal resulting from uptake block (14).

Metabolic clearance rate is determined by FCR1, the fraction of NE irreversibly lost from compartment 1 per unit time, and the volume of distribution of NE in compartment 1. The young and elderly subjects showed a 35 and 30but only a 20 and 24The degree of decrease in FCRl after DMI is greater than the degree of decrease in MCRl because there was a concurrent significant increase in the NE volume of distribution in both age groups after DMI (Table 1). Although the mechanism causing the volume of distribution to increase is not clear, the net result is that the MCR, values underestimate the effect of DMI to inhibit NE removal from plasma, which is better represented by FCR1.

Elderly people may be particularly sensitive to the adverse effects of the tricyclic antidepressants, including DMI (37). One common cardiovascular side effect reported with

DMI treatment is orthostatic hypotension (18). The mechanism of DMI-induced orthostatic hypotension has not been widely studied. Our study suggests that DMI induces a marked reduction of the rate of NE entry into the extravascular compartment in both young and elderly people, potentially caused by both local and central inhibitory phenomena. Previous studies with clonidine have shown that sensitivity to a Z-receptor-mediated suppression of SNS activity is not diminished in the elderly (16). Hypotension would be the expected result of diminished adaptation to suppression of NE release. We observed in both age groups that supine blood pressure was maintained during DMI administration despite decreased values of NE, and QZ.

This implies an adaptive circulatory response to DMI. One such adaptive response with DMI administration may be increased heart rate, which was seen in both the young and elderly after DMI. An increase in heart rate after a single oral dose of DMI has been observed previously in healthy subjects (31).

Further studies with larger sample sizes of both young and elderly subjects may further delineate differences in NE kinetic parameters that contribute to the development of DMI-induced side effects. In summary, plasma NE levels in young and elderly did not change after DMI administration because of a balance between a decrease in MCRl and a decrease in NE spillover. The fall of NE spillover after DMI was a result of marked suppression of the rate of NE entry into the extravascular compartment.

The age-related difference in MCRl does not appear to be due to a difference in neuronal reuptake because uptake 1 blockade with DMI did not reveal an age-related differential effect on MCR1. These findings suggest that differences in nonneuronal mechanisms may contribute to the age-related difference in MCR1. Moreover, the technique of compartmental analysis used in this study has demonstrated that DMI cannot be used as a simple uptake 1 blocker.

The drug has profound and complex effects on NE metabolism in the extravascular compartment. Further study is required to understand the complex effects of DMI on NE metabolism and the mechanisms of its pharmacological effects.

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