KINETIC STUDIES OF LEUCINE FLOW IN THE NONABSORPTIVE PHASE OF DIGESTION IN RATS

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UDC 612.332.08:(616.015.3:577.112.382.4

KEY WORDS: leucine flow; compartment model; nonabsorptive phase of digestion

The dynamics of amino acids during the phases of digestion have been investigated previously [1, 2], but a realistic estimate of the flow rates of amino acids has been obtained by continuous infusion [3, 9], which does not take into account flow changes based on phases and rhythms. In the investigation described below an attempt was made to model the leucine flow in a cyclic system of suitable compartments on the basis both of previously determined kinetics constants and of experimental data on the kinetics of radioactivity during pulsed injection of ^{14}C-leucine. The most convenient physiological state from this point of view is the nonabsorptive phase of digestion, which reflects the state of relations between the organs rather than the flow of nutrients into the body from the intestine.

EXPERIMENTAL METHOD

A scheme of four successive compartments was chosen as the test model: portal (I), hepatic (II), arterial (III), and muscular (IV) (Fig. 1). Each compartment is divided into two pools: free pools of amino acids – X_1, X_3, X_5, and X_7, and bound pools of amino acids (protein pools) – X_2, X_4, X_6, and X_8. The flow rate of L-leucine can be described by a set of first-order velocity constants k_{ij} and the leucine content in the pool x_i, where i and j are the corresponding indices of the pools. It is also postulated that the rate of oxidation of L-leucine, which according to some data comprises about 9-10% of the total flow [8], is unimportant during quantitative description. The ground for the choice of these assumptions can be more clearly defined during the final analysis of the model.

The system of ordinary linear differential equations describing the dynamics of the change in specific radioactivity of L-leucine in the test pools was postulated for this scheme. Solution of this system, which was done by the 4th-order Runge–Kutta method, with automatic control of stability at each step, was obtained by the use of numerical values of the velocity constants which can be divided into two groups: values known from the literature – the constants K_{13}, K_{34}, K_{43}, K_{46}, K_{57}, K_{78}, K_{87}, K_{21} and unknown approximate values – constants K_{15}, K_{51}, K_{26}, K_{62}, K_{35}, K_{75} (Table 1). One of the tasks of the numerical investigation is to determine the precise values of the unknown constants.

In the first stage of the investigation a qualitative study was made of this dynamic system in order to determine interval values of the constants, i.e., to establish those values of unknown constants at which the given system has a stable solution. It was found that for all reasonable values of the constants, the eigenvalues of the matrix of the λ_1 system have a negative real part, and consequently, the solution of the dynamic system is asymptotically stable.

To determine the numerical values of the constants more accurately the following procedure was adopted. In experiments on rats values were obtained for the content of total and, correspondingly, specific radioactivity of L-leucine in all the pools studied. For this purpose, ^{14}C-L-leucine (Czechoslovakia) was injected intraperitoneally in a dose of 50 mCi per animal into Wistar rats weighing 150-170 g and in the nonabsorptive phase of digestion, 6 h after eating food. These parameters were recorded for 24 h at 24 times points. Weighing samples of the organs and blood samples from the portal and aortic systems were homogenized in 5% sulfosalicylic acid, the precipitates were separated by centrifugation and dissolved in 0.25N NaOH, and then transferred into a scinillation cocktail for counting. The supernatant was passed through Dowex 50×8, eluted, and its radioactivity determined in

Institute of Nutrition, Academy of Medical Sciences of the USSR. Computer Center, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR I. P. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 1, pp. 11-13, January, 1990. Original article submitted June 19, 1989.

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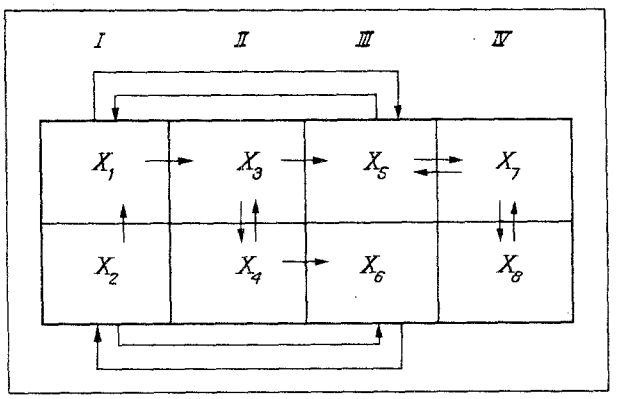

Fig. 1. Transfer of L-leucine in four successive compartments. Compartment: I) portal; II) hepatic; III) arterial; IV) muscular. Pools here and in Fig. 2: X_1, X_3, X_5, X_7 – free, X_2, X_4, X_6, X_8 – bound.

TABLE 1. Numerical Parameters of Leucine Flow

Index of flow $i \rightarrow j$	k_{ij}, velocity constant, h^{-1}	Source of data	x_i, leucine content in i-th pool, μmoles	Flow rate, μmoles/h
1—5	527,0	Calculation	0,36	189,7
5—1	86,3	»	2,2	189,9
2—6	527,0	»	15,0	7900,0
6—2	86,3	»	58,0	5000,0
1—3	1,0	[9]	0,36	0,36
3—4	0,035	[4]	50,0	1,75
4—3	0,032	[3]	1060,0	33,9
4—6	0,017	[6]	1060,0	17,7
3—5	0,032	Calculation	50,0	1,6
5—7	1,13	[3]	2,2	2,5
7—5	0,004	Calculation	52,0	0,21
7—8	0,007	[4]	52,0	0,36
8—7	0,004	[5]	3700,0	14,8
2—1	0,10	[6]	15,0	1,5

a mixture of amino acids. The leucine content was determined indirectly by calculating the ratio of leucine to total aromatic amino acids, using data in the literature [7], and by direct measurement of absorption of the aromatic amino acids at 280 nm, allowing for the coefficient of extinction for an equimolar mixture. The content of L-leucine in the protein pools also was calculated on the basis of data in the literature, using the ratio of the leucine content to total protein.

EXPERIMENTAL RESULTS

The values will be given in the form $X_i^e(t_j)$, where i = 1, ..., 8 denotes the number of pools and j = 1, ..., 24 denotes the number of measurements in each pool. In this case the problem of multidimensional minimization is solved:

$$\sum_{i=1}^{8}\sum_{j=1}^{24}[X_i^T(t_j, A)-X_i^e(t_j)]^2 \rightarrow min\ A,$$

where A is the matrix of a dynamic system with fixed values of the known constants, and $X_i^T(t_j, A)$ is the numerical solution of the dynamic system by the 4th-order Runge-Kutta method with automatic control.

The Hook–Jeeves method was chosen to solve this problem because this direct minimization method does not require calculations of derivatives and can be quite easily realized on modern computers. The program for this method was written in FORTRAN-IV language on an EC-1061 computer. The following values of the constants were obtained (in h^{-1}): K_{15} = 527.0; K_{51} = 86.3; K_{26} = 527.0; K_{62} = 86.3; K_{35} = 0.032; K_{75} = 0.004.

The time course of the change in specific radioactivity of L-leucine is shown in Fig. 2 as a continuous curve, while the theoretical values obtained in a numerical experiment with established values of the constants, is shown as a dotted curve. On the whole reliable agreement was obtained between the theoretical and empirical data. The system of leucine flows in the nonabsorptive phase of digestion can therefore be described by a "rough" system of linear differential equations provided that the initial assumptions are valid. Overestimation of the theoretical values in the X pool is approximately twofold, evidence of the presence of true reutilization of leucine in the liver, which thus accounts for about 50% of the total flow of leucine supplied for protein synthesis in the liver. There are also anoma-

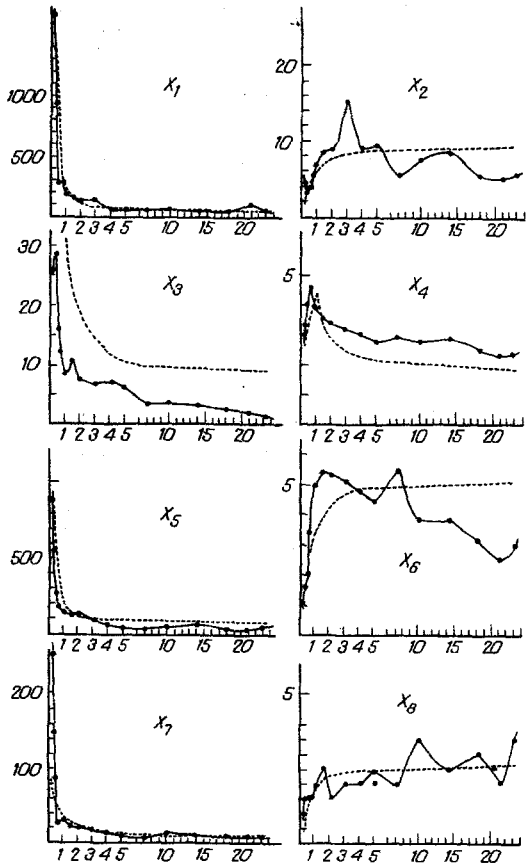

Fig. 2. Time course of ^{14}C-specific radioactivity of ^{14}C-L-leucine in free and bound pools based on experimental data and numerical model. Ordinate, specific radioactivity of leucine (in cpm/μmole); abscissa, time after injection (in h).

lies in the character of specific radioactivity for proteins of the portal system in contrast to total blood proteins.

The following conclusions can be drawn from analysis of values of the flow rates of leucine, calculated on the basis of exact values of the constants and values of the leucine content in the different pools: a) in the nonabsorptive phase of digestion the L-leucine flow is shifted toward breakdown of liver and muscle proteins. The rate of release of leucine from liver and muscle proteins is about equal (16.2 and 14.8 μmoles/h). It is suggested that in the absorptive phase the opposite shift takes place: the flow of synthesis increases, while at the same time the breakdown flow undergoes minimization. The mean hourly leucine consumption of the rats, which according to calculations is about 40 μmoles/h, can serve as the limit for the increase; b) the rate of circulation of leucine, reaching 190 μmoles/h, may perhaps be the natural limit of the total rate of renewal of ^{14}C-leucine in the body, including the rate of its reutilization.

In addition to the above, it must be pointed out that construction of the model of amino-acid transfer in consecutive compartments is sufficientdly convenient both methodologically and for testing certain assumptions. In particular, this applied to the relations between oxidative and structural pathways of leucine metabolism. Moreover, there may also be the possibility of estimating true rates of breakdown of biological structures and of processes of interorgan specialization in amino-acid metabolism and in physiological mapping of areas of protein synthesis and breakdown.

The effect of circidian rhythms, evaluated during parallel incorporation of ^{3}H-leucine 10 min before each fixed point, and normalization of ^{14}C- radioactivity to the value of ^{3}H-radioactivity did not lead to any appreciable smoothing of the empirical curves. Thus, the phasic character of digestion is unquestionably of the greater importance when amino-acid flow rates are estimated.

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CHARACTER OF BLOOD PRESSURE CHANGES UNDER THE COMBINED ACTION OF PRESSOR AND DEPRESSOR AGENTS

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UDC 612.146.2:[615.357:577.175.523+615.217.32].08

KEY WORDS: systemic arterial pressure; pressor agents; depressor agents; paired combinations

During contact of an organism with the external environment, as a rule it is subjected to the action of several stimuli, which is realized through neurohumoral influences, including those on the heart and vessels. The resultant response of the hemodynamics under these conditions is the combined response of the hemodynamics under these conditions is the combined resonse of neurohumoral stimuli which differ in direction and in the mechanism of their action, and it is this which determined interest in the problem of the combined effect of these stimuli on the circulatory system [1, 3, 4]. Changes in arterial blood pressure (BP) in response to electrical stimulation of depressor and carotid nerves, leading to shifts in the opposite direction, have been demonstrated in the literature [4, 7]. A mainly depressor effect was exhibited, but its magnitude was less than that in response to separate application of these stimuli. Predominance of dilator responses of blood vessels of skeletal muscles and the small intestine was observed during simultaneous stimulation of the constrictor and dilator zones of the hypothalamus in cats [2, 6] and of the carotid and median nerves in dogs [1]. A similar effect also was obtained to the combined action of histimine and noradrenalin (NA) on vessels of the microcirculatory system in rats [5]. It is not yet clear what changes take place in BP as an integral parameter of the systemic hemodynamics in response to the combined action of two vasoactive substances with opposite effects.

The aim of this investigation was to study the character and magnitude of changes in the systemic BP in response to a combination of pairs of pressor and depressor substances, giving rise to opposite and equal effects on it: NA and angiotensinamide (ATA), and acetylcholine (ACH) and histamine (HA).

EXPERIMENTAL METHOD

Experiments were carried out on 27 male and female cats weighing 3-5 kg and anesthetized with urethane (1 g/kg), with the use of heparin (1500 U/kg), with an open chest and with artificial ventilation of the lungs. Changes in BP in response to combined intravenous injections of two vasoactive substances, opposite in their action but equal in the magnitude of their effect, were studied in the experiments. As pressor agents we used NA hydrotartrate (in a dose of 1-32 μg/kg) and ATA (0.25-10 μg/kg), and as the depressor agents — ACh chloride (0.0001-10 μg/kg) and HA dihydrochloride (0.25-8 μg/kg). Doses of the substances causing changes in BP by 25 ± 3, 50 ± 5, and 75 ± 10% relative to its initial level when injected intravenously and separately, determined in preliminary experiments, were used. These vasoactive substances were diluted in physiological saline so that their test doses were contained in a volume of solution equal to 0.5 ml. The preparations were injected into the animal's femoral vein. The systemic BP was measured in the left subclavian artery by means of a pressure indicator with mechanotron transducer (made by the experimental workshops of the Research Institute of Experimental Medicine, Academy of Medical Sciences of the USSR). The pressure

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Department of Normal Physiology, I. P. Pavlov First Leningrad Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 1, pp. 13-15, January, 1990. Original article submitted September 13, 1988.

0007-4888/90/0001-0015$12.50