LINEAR KINETIC MODEL TO ESTIMATE PROTEIN SYNTHESIS RATE AFTER [^{14}C]TYROSINE INFUSION IN DOGS

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1. Introduction

Garlick et al. [1] have described a procedure for estimating protein synthesis rates in experimental animals. Radiolabelled amino acid is infused for a short time, usually 6 h, and serial plasma samples are taken during this period to determine the rate at which a steady state of labelling in the plasma is obtained. After the infusion the animal is sacrificed, the selected tissue excised and the specific radioactivities of the intracellular free amino acid pool and the protein bound pool determined. Equations were derived to transform these data into estimates of k_s, the rate constant for transfer of amino acid from the free intracellular pool to the protein bound pool and the procedure has been used in a number of subsequent publications by these workers [2–4].

For optimal reliability of k_s estimates, serial tissue biopsies should also be taken during the infusion. While this is difficult with current technology it should become more feasible in the near future. In the meantime, however, the terminal sampling of the tissue compartments provides data which should be utilized to the fullest extent. The equations of Garlick et al. produce k_s values which give no indication of their reliability. An alternative treatment is proposed here based on a 3 compartmental model which gives k_s together with an estimate of its uncertainty. It is used with data on [^{14}C]tyrosine incorporation in myocardial tissue of dogs.

2. Methods

2.1. *Experimental*

Mongrel dogs of both sexes were used. Body weights remained stable on a diet of goat meat and dry vegetable fibre given each afternoon. The animals were fasted for 16 h prior to an experiment while water was allowed ad lib. A polyethylene cannula (i.d. 0.8 mm) was inserted into a fore limb vein, taped to the limb and connected to an infusion pump (Braun Apparatebau, Melsingen, FRG). L-[U-^{14}C]-Tyrosine (Radiochemical Centre, Amersham, 483 mCi/mmol) was diluted with heparinized saline (50 units/ml) to an activity of 1.67 μCi/ml. Unlabelled tyrosine was added to the infusate to produce the average tyrosine concentration in dog plasma (33 μmol/l). This was infused at 5 ml/h for 6 h; total infused tyrosine represented a negligible fraction of the total body pool. Animals were conscious and unrestrained during the infusion. Blood samples (5 ml) were withdrawn from a cannula inserted in a superficial brach of the femoral vein. At 6 h the dog was killed rapidly by an intravenous injection of pentobarbitone sodium (330 mg/ml). The heart was rapidly excised, rinsed in ice-cold Krebs solution for several seconds to remove entrapped blood and placed on ice. Subsequent procedures were performed in a cold room (4°C). A 5 g sample was homogenized in ice-cold 10% (w/v) trichloroacetic acid and centrifuged. The supernatant was assayed for its free tyrosine specific radioactivity [5]. The precipitate was washed three times in 10% trichloro-

acetic acid, then single washes of acetone, ethanol and diethyl ether. The protein was then hydrolyzed in 6 M HCl for 20 h at 110°C in sealed tubes and then tyrosine specific radioactivity was determined [5]. Radioactive samples were counted on a Nuclear Chicago Isocap-300 with a scintillant of 35% Triton X-100 and 0.6% diphenyloxazole in toluene. Corrections were made for quenching and the counting error was better than 2% (relative standard deviation).

2.2. *The model and computations*

A three compartment model was chosen to represent the process (fig.1). Assumptions were:

(i) First order transfer between compartments.
(ii) k_{12} equalled k_{21}.
(iii) Unlabelled total body tyrosine was in a steady-rate during the experiment.
(iv) Return of labelled tyrosine from compartment 3 to 2 was insignificant during the experiment.
(v) The build-up of activity in the plasma could be described by a simple exponential equation.

Differential equations for the model were:

$$\frac{dA_1}{dt} = k\,(A_{1\,max} - A_1) \qquad (1)$$

$$\frac{dA_2}{dt} = k_{12}\,(A_1 - A_2) - k_{23}A_2 \qquad (2)$$

$$\frac{dA_3}{dt} = k_{23}A_2 \qquad (3)$$

where A_i is the specific activity of tyrosine in compartment i, k is the rate constant for build up in compartment 1 and A_{1max} is the maximum level in compartment 1. These equations were solved numerically on a DEC System-10 computer using NONLIN, an iterative non-linear least squares regression computer program [6]. The estimated uncertainties of data from compartments 1, 2 and 3 were 20%, 5% and 5%, respectively, and all data points were weighted with their reciprocal variance. Initial estimates for the four parameters were obtained as accurately as possible to ensure rapid and reliable convergence.

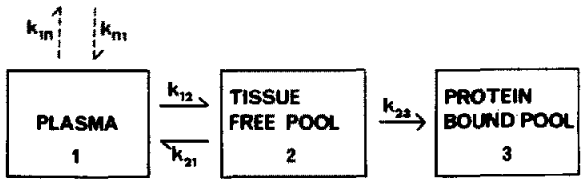

Fig.1. Compartmental representation of tyrosine incorporation into myocardial protein. The dotted rate processes indicate transfers to and from other body compartments. Estimation of k_{23}, the rate of protein synthesis, is the prime objective.

3. Results

A typical data set for a normal dog is shown in fig.2. Usually 8–14 plasma samples were taken and because of the high specific activity of the infusate and finite distribution times it was difficult to reduce the scatter in these data to less than ±20%. Within this variability, an exponential curve (eq. 1) adequately described the data as previously observed [1]. Final levels in the tissue free and protein bound pools were usually 70–80% and 1–3% of those in plasma respectively. This would indicate that transfer from compartment 1 to 2 is rapid, although not infinitely so, and that transfer from compartment 2 to 3 is many times slower. Discrepancy between observed data for compartments 2 and 3 and those calculated from the model was usually better than 2%. Table 1 gives least squares estimates for the four parameters in four days. It can be seen that they have the relative magnitudes expected from the primary data and that k, k_{12} and k_{23} span two orders of magnitude. The uncertainties in k_{23} ranged from 30–60% of the

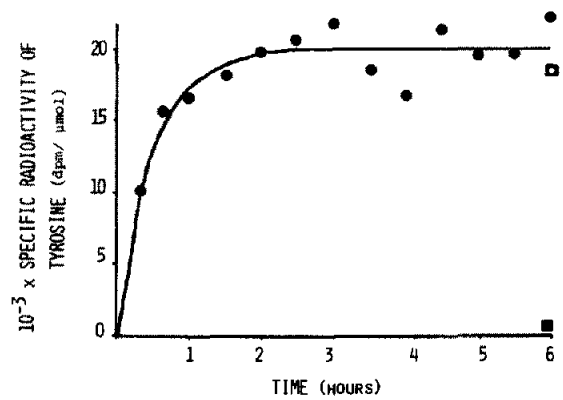

Fig.2. Tyrosine specific radioactivity of the tissue free pool (□) and protein bound pool (■) of the left ventricle and of the plasma (●) after infusion in a normal dog.

Table 1
Estimates of compartmental model parameters obtained from incorporation of tyrosine into protein of the left ventricle after infusion for 6 h

Dog	k	k_{12}	k_{23}	$A_{1max} \times 10^{-3}$
1	3.1 ± 1	0.32 ± 0.05	0.0041 ± 0.0015	20.0 ± 0.82
2	4.03 ± 1	0.26 ± 0.016	0.0053 ± 0.0023	15.1 ± 0.33
3	5.04 ± 0.9	0.42 ± 0.06	0.0036 ± 0.002	18.4 ± 0.5
4	2.06 ± 0.3	0.44 ± 0.06	0.0033 ± 0.0012	20.1 ± 0.53

Units are h^{-1} for rate constants and dpm/μmol for A_{1max}. Uncertainties are given as standard deviations.

estimates. We feel that this level of uncertainty (30%) in k_{23} is as good as can be obtained with the technique and that any conclusions regarding apparent changes in k_{23} must take this into account.

4. Discussion

The data in these experiments show that three compartments make up the least complex model compatible with the data. Expansion of this basic model does not seem possible with the present experimental technique, despite what may be known biochemically about protein synthesis because parameters become increasingly difficult to estimate and parameter uncertainties become unacceptably large.

The assumption of first order transfer is a common one in compartmental modelling [7]; even energy-dependent transport processes approximate such behaviour when well below their maximum. Since the animals had maintained constant body weight prior to the experiment, changes in unlabelled body tyrosine would be insignificant compared with the much larger changes in radioactive tyrosine during the experiment. Therefore monitoring specific radioactivity would provide data on the kinetics of [^{14}C]tyrosine rather than being compounded with coincident changes in unlabelled amino acid. Use of an exponential function to describe plasma tyrosine levels (eq. 1) has some theoretical basis as the same exponential rise to plateau is found in a one compartment model with zero order input and first order output. Negligible return of radioactivity from compartment 2 to 3 during the course of the experiment seems reasonable in view of the very small levels attained in compartment 3. With the high levels found in compartment 2, return to the plasma can not be ignored. It is likely that k_{12} does not exactly equal k_{21} but it does not seem possible to allow for this eventuality with the present technique. Reliability of convergence was poor and parameters were variably biased when k_{21} was made a separate variable. In common with the earlier method [1], the assumption is made that compartment 2 represents the true protein precursor pool. This is probably an oversimplification because there is evidence for the compartmentalisation of the tissue-free pool [8].

The progress curve for compartment 2 with time will not be a simple exponential except when k_{12} is infinitely large, otherwise it will have a sigmoidal-like form. With the method of Garlick et al. [1] an exponential form for compartment 2 is assumed provided that the infusion time is large compared with the time taken for the specific radioactivity of the plasma to reach a plateau. The error introduced by this approximation will vary for different experiments; for the parameters in this study it amounted to a 12% error in k_{23}. Consequently, it is perhaps simpler and more general to use the readily available NONLIN package than to modify the analytic approximation or to adjust it for each data set. The final estimates of k_{23} by the earlier method [1] were 20–34% smaller than those shown in table 1.

Compared with the earlier method [1] the procedure described herein for estimating protein synthetic rates is conceptually simpler with the mathematical details left to the computer. In addition, estimates of the reliability of the parameters are automatically calculated and the method should prove more robust over different experimental conditions. The

experimental procedure does not have to be adjusted to make the mathematics easy but can be varied to optimize the reliability of parameter estimation. It would be readily adaptable to serial sampling of compartments 2 and 3.

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