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ANIMAL VARIATION IN ALANINE UPTAKE BY RABBIT ILEAL MUCOSA

J.Y.F. PATERSON, M.W. SMITH and F.V. SEPÚLVEDA

A.R.C. Institute of Animal Physiology, Babraham, Cambridge CB2 4AT (U.K.)

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Summary

Alanine uptake by rabbit ileal mucosa has been measured in the presence and absence of Na^+ to establish the characteristics of biological variation. Common K_m values, calculated for two systems of alanine entry, were used for estimation of two J_{max} values for individual rabbits. The distribution of J_{max_1} and J_{max_2} within the population was normal and there was minimal interaction between these two parameters, judged by a cross-correlation analysis. Ways of processing data to account for this type of animal variation are discussed.

Evidence has been presented recently to suggest that alanine uptake by rabbit ileum takes place through two carrier-mediated processes. System 1 has a requirement for Na^+, a high affinity for alanine and a small J_{max}. System 2 has a low affinity for alanine, a high J_{max} and no Na^+ requirement [1—4]. A characteristic of this previous work was the finding that J_{max}, but not K_m, varied between different groups of rabbits. A similar variation had been reported in earlier work, where it was assumed that only one entry system for alanine existed in the rabbit ileum [5, 6]. The number of animals used in these experiments (up to eight to obtain mean estimates of K_m and J_{max}) appeared to be too small to represent fully the scatter in J_{max} values encountered within the whole population of rabbits. The fact that this scatter was wider for J_{max} than K_m was interesting, since it implied a possible regulation of alanine entry systems based on a control of the number of carrier molecules present rather than on any change in carrier structure. This situation would be similar to that reported previously for multi-transport systems for amino acids found in other tissues (see Ref. 7 for review). The object of the present work was to investigate the source of J_{max} variation in more detail

in an attempt to find ways of minimizing its effect on future analysis of data.

Uptake was measured in the same piece of ileum in the presence and absence of Na^+ at alanine concentrations of 1, 4, 8, 16, 32 and 64 mM. The same experiment was performed on 101 rabbits used over a period of 3 months. The model equations:

$$J_{mc\ (Na = 140)} = \frac{J_{max_1}\ [Ala]}{K_{m_1} + [Ala]} + \frac{J_{max_2}\ [Ala]}{K_{m_2} + [Ala]}$$

$$J_{mc\ (Na = 0)} = \frac{J_{max_2}\ [Ala]}{K_{m_2} + [Ala]}$$

were used to estimate parameters for the two systems simultaneously. The methods for calculating uptake and estimating kinetic constants have been described previously [1—4].

Analysis of curves obtained from individual rabbits using the equations given above led to the generation of a wide range of K_m as well as J_{max} values. The distribution of these populations was lognormal. Before proceeding further, it is worthwhile considering the errors inherent in estimating these constants in a situation where biological variation is absent. Fig. 1 shows a contour diagram of the residual sum of squares surface produced using error-free theoretical data describing a hyperbolic curve having a K_m of 50 mM and a J_{max} of 100 nmol · cm^{-2} · min^{-1}. Substrate concentrations of 1, 2, 4, 8, 16, 32 and 64 mM were chosen to generate a set of seven data points (equivalent to J_{mc} uptake values). Different values of K_m (40, 42, 44, ...60 mM) and J_{max} (90, 92, 94, ...110 nmol · cm^{-2} · min^{-1}) were inserted into the program for calculation of the residual sum of squares. Inverse interpolation

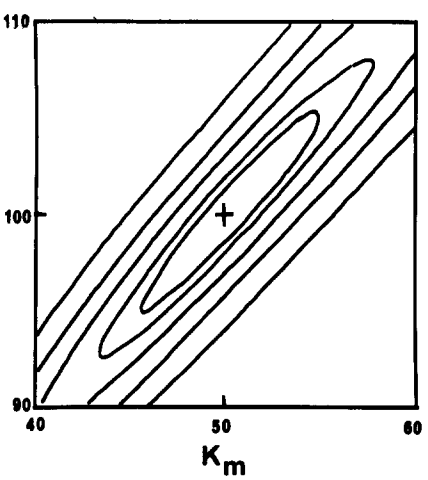

Fig. 1. Contour diagram of the residual sum of squares surface in the region of the solution locus for a theoretical uptake system having a K_m of 50 mM and a J_{max} of 100 nmol · cm^{-2} · min^{-1}. The height of the surface is 0 at the centre and the contours have the values 1, 2, 5, 10 and 20.

was used to estimate specific values of residual sum of squares (1, 2, 5, 10 and 20). The nonlinear regression methods used provide a variance-covariance matrix for the two parameters. Of particular interest is the covariance found between J_{max} and K_m, for which the correlation coefficient is 0.948. The residual sum of squares assuming a K_m of 50 mM and a J_{max} of 100 nmol · cm^{-2} · min^{-1} is zero (error-free data). Shifting either the K_m or J_{max} value by ± 5 units increases the residual sum of squares to between 10 and 20. However, if it is decided to move both parameters in the same direction (SW—NE) one can tolerate a shift of K_m from 45 to 55 mM, with very little increase in the residual sum of squares (between 1 and 2). Moving along this diagonal axis will, in fact, always give a residual sum of squares (i.e., goodness of fit) close to that found at the solution locus.

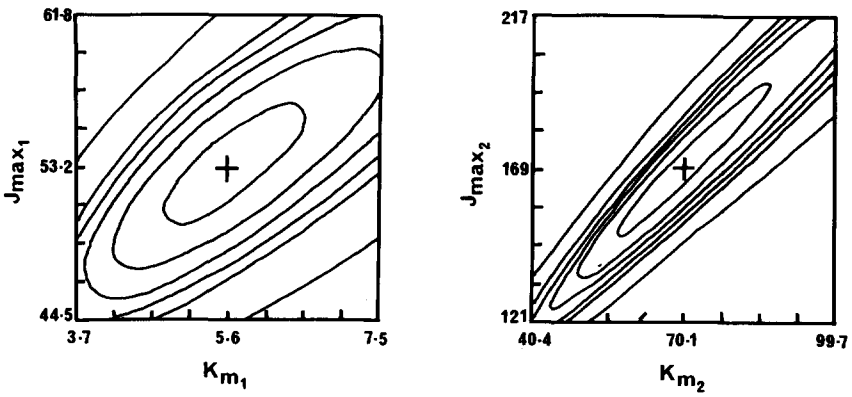

Fig. 2. Contour diagrams of the residual sum of squares surface in the region of the solution loci for the K_m (mM) and J_{max} (nmol · cm^{-2} · min^{-1}) of the two systems. The height of the surface is 6.0 at the centre and the contours have the values 10, 20, 30, 40, 50 and 100. Each axis is divided into eight standard errors with mean values lying at the centre of each graph.

Fig. 2 shows similar contour diagrams for observations on alanine uptake carried out on 101 rabbits. The shape of these contour lines is, in general, very similar to that obtained using error-free data. There is, however, some asymmetry in moving SW from the solution locus, the valley at this point sloping more steeply than in the NE direction. This again is likely to arise from the way data are analysed rather than from any normal variation in the population of rabbits. The S.D. of alanine uptake is known to be directly proportional to the rate of uptake [3]. Experimental error encountered in estimating alanine uptake at high substrate concentrations will tend to generate high rather than low values for K_m and J_{max} (the program analysing an erroneously high value for uptake can adjust the K_m to an infinitely high value but when dealing with an erroneously low uptake the computed K_m will still have a value significantly different from zero). The contour diagrams shown in Fig. 2 are therefore entirely consistent with the assumption that two systems exist for alanine uptake in this tissue, each having its own K_m. This would agree with earlier findings showing a constant K_m and variable J_{max} in the ileum [1—6] and with work on other cell types subjected to hormonal stimulation where K_m remains constant and J_{max} varies [7]. It was

therefore decided, in the present work, to calculate a common K_m value for systems 1 and 2. These values were then used to calculate respective J_{max} values for results obtained on individual rabbits. This procedure resulted in an increase in the residual mean square from 0.033 (allowing all parameters to vary) to 0.059 (using values for K_{m_1} and K_{m_2} derived from analysis of total data).

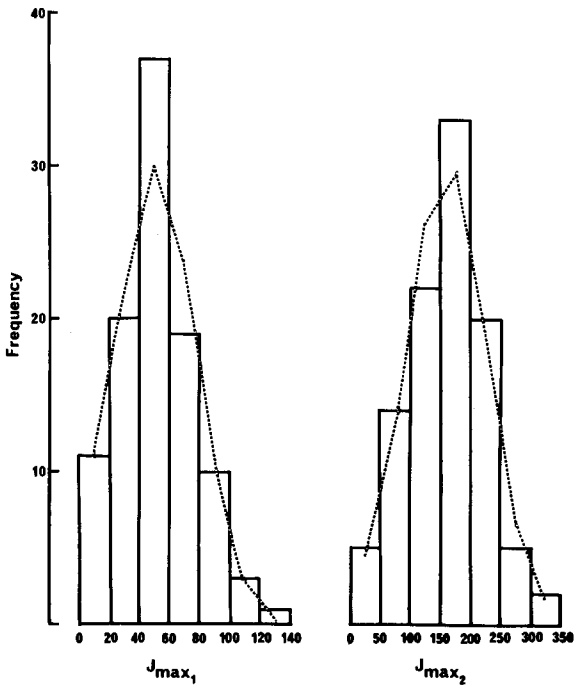

Fig. 3. Frequency histograms of J_{max_1} and J_{max_2} (n = 101) with the frequency polygons expected for normal distributions (broken line). For J_{max_1} the mean is 51.5 with S.D. 26.1. nmol · cm $^{-2}$ · min^{-1}, and for J_{max_2} the mean and S.D. are 160 and 65.2 nmol · cm^{-2} · min^{-1}, respectively.

The variation in J_{max} values within the population is shown in Fig. 3. Values for J_{max_1} and J_{max_2} are shown to be distributed normally around the mean with a large scatter between individual results (J_{max_1} 51.5 ± 26.1 and J_{max_2} 160 ± 65.2 nmol · cm^{-2} · min^{-1}, means ± S.D.). A two-way classification of these data was performed to test for any association between the values of J_{max_1} and J_{max_2}. Results have been grouped into low, medium and high values for this analysis (A, B and C, respectively; see legend to Table I for further explanation). The frequencies expected for a normal distribution of J_{max_1} and J_{max_2} under the headings, A, B and C are 16, 69 and 16, respectively. This corresponds closely to the sums found (16, 66 and 19 for J_{max_1}; 17, 70 and 14 for J_{max_2}). There is an association between the two J_{max} values, but this is confined to the AA portion of the table (6% of the total giving low values for J_{max_1} and J_{max_2}). Apart from this there is good agreement between expected and observed results suggesting that the vast majority of rabbits have J_{max} values which vary independently.

TABLE I

TWO-WAY CLASSIFICATION OF J_{max} VALUES OBTAINED FROM ANALYSIS OF ALANINE UPTAKE BY RABBIT ILEUM ASSUMING COMMON VALUES FOR K_{m_1} AND K_{m_2}

A, Results falling 1 S.D. or more below the mean; B, results falling 1 S.D. above or below the mean; C, results falling 1 S.D. or more above the mean. Numbers in brackets give the values expected for random variation between J_{max_1} and J_{max_2}

J_{max_2}	J_{max_1}		
	A	B	C
A	6 (2.7)	11 (11.1)	0 (3.2)
B	7 (11.1)	46 (45.7)	17 (13.2)
C	3 (2.2)	9 (9.2)	2 (2.6)

Part of the wide scatter in results seen previously [1—6] could result from this independent variation in J_{max} values. The routine use of large numbers of rabbits to minimize this effect is not feasible. An alternative approach is to include measurements relating to the present work (e.g., six estimates of alanine uptake in the presence or absence of Na^+) in all future experiments, the remaining six estimates being carried out under some new experimental condition. Information gained from the control part of each experiment could then be used, in relation to the data presented here, to judge whether the experiments represented an adequate sample of the whole population. The number of rabbits used in any one set of experimental conditions would then depend on the satisfaction of this criterion.

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