POSTNATAL CHANGES IN THE TEMPERATURE DEPENDENCE OF SYNAPTOSOMAL AMINO ACID UPTAKE

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1. Introduction

Synaptosomal particles isolated from rat brain are capable of accumulating amino acids by means of active, carrier-mediated transport systems [1–3]. The rates at which some amino acids are accumulated have been found to be dependent on the postnatal age of the donor animals [4]. The uptakes of threonine, leucine and glycine are exemplary in this respect, since each was found to undergo a different type of transition during development. In this present report we describe temperature-dependent parameters of the accumulations of these three amino acids by synaptosomal fractions obtained from rats at different stages of postnatal development.

2. Materials and methods

Cerebral cortices were obtained from brains of rats of different age groups. Synaptosomal fractions were isolated from homogenates of the cortices by centrifugation on discontinuous ficoll gradients [5] and were suspended in an incubation medium with the composition of 10 mM Tris–HCl (pH 7.4), 15 mM $MgCl_2$ and 300 mM sucrose. Incubations consisted of 0.25 mg synaptosomal protein, 0.1μCi of labeled amino acid and various amounts of unlabeled amino acid (final concentrations ranged from 0.5 to 21.6 μM) in a final vol of 1 ml. Incubations were carried out at 37° C for 2 min, at 30° C for 4 min, 25° C for 5 min, at 17° C for 10 min and at 10° C for 20 min. Following incubation the synaptosomal particles were harvested on 0.8μ Millipore filters and the filters were assayed for ^{14}C content by liquid scintillation counting [2].

3. Results

Double reciprocal plots were constructed for synaptosomal amino acid uptake at the various incubation temperatures. Values for the apparent K_m and V_{max} terms were derived from these plots, and the logs of these values were plotted against the reciprocal values of the absolute temperatures at which the incubations were performed. These latter plots were in all cases essentially linear throughout the temperature range studied, indicating the probable involvement of a rate-limiting step in each plot [6]. Values for the apparent energy parameters, ΔH_m and E_a, were derived from the slopes of these plots.

The ΔH_m values for threonine and leucine accumulation were both significantly higher in the fractions from newborn than in fractions from adult rats (fig.1). Over most of the temperature range studied the K_m term for threonine uptake was highest, and that for leucine uptake was lowest in fractions from newborn rats. In the case of glycine accumulation, there were minor differences in the K_m term but the values for ΔH_m were essentially the same at the three ages studied.

The slopes of the plots of log V_{max} vs 1/T were essentially the same, regardless of the amino acid or the age of the rat from wich the fractions were prepared. The values for E_a calculated from these latter plots ranged between 9800–12 000 cal (table 1).

4. Discussion

The results of this study show that the apparent K_m for the uptakes of threonine, leucine and glycine chan-

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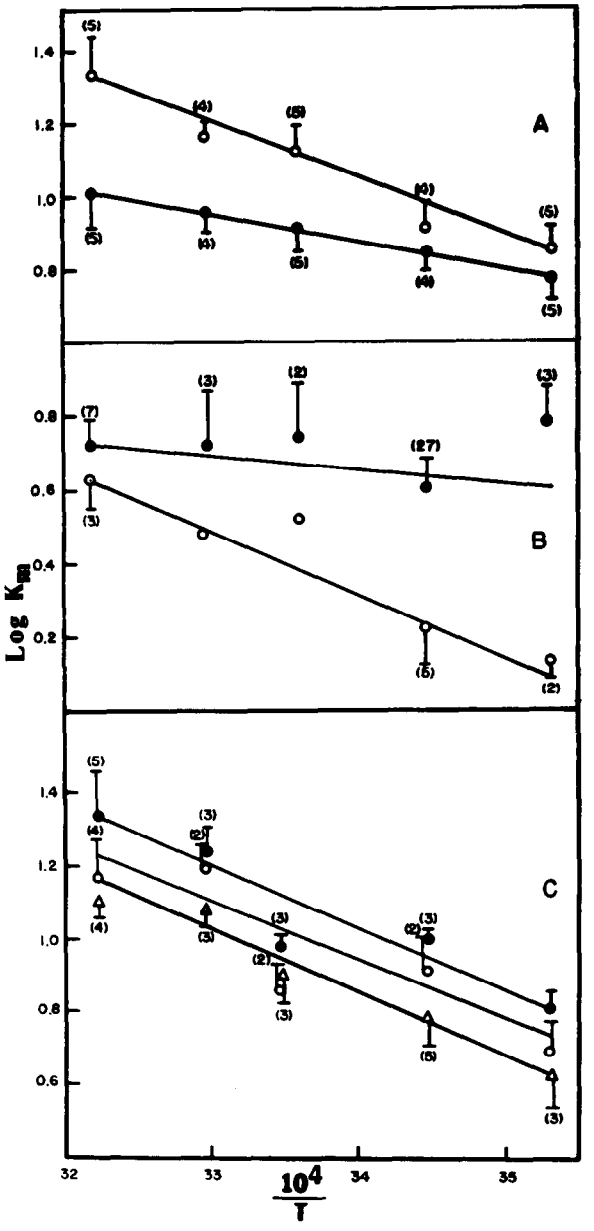

Fig. 1. Effect of temperature on the K_m for amino acid accumulation by synaptosomal fractions. Fractions were isolated from cerebral cortices of adult rats (●–●–●), rats 1–3 days of postnatal age (○–○–○), and rats 11–16 days of postnatal age (△–△–△). Experiment with threonine, A; with leucine, B; and with glycine, C. Values for ΔH_m, computed from the slopes of the lines, were as follows: 2500 cal for threonine uptake by fractions from adult rats, 5600 cal for threonine uptake by fractions from 1–3 day-old rats, 1090 cal for leucine uptake by fractions from adult rats, 5145 cal for leucine uptake by fractions from 1–3 day-old rats, 5200 cal for glycine uptake by fractions from adult rats, 4860 cal for glycine uptake by fractions from 1–3 day-old rats and 5600 cal for glycine uptake by fractions from 11–16 day-old rats. The number of K_m determinations is given in parentheses and the bars represent S.D.

ged during development, and that, in the case of threonine and leucine, the slopes of the Van't Hoff isochore also appeared to be age-dependent. We interpret these findings to be indicative of intrinsic changes during development in the uptake mechanisms per se. If the K_m term represents an equilibrium constant between the amino acid and a Michaelis-type complex which is formed between amino acid and a transport carrier in the initial stages of the transport mechanism, then the ΔH_m term is the change in heat content accompanying the formation of this complex. Under these conditions the reduction, from newborn to adult animals, in the ΔH_m value observed with threonine and leucine accumulation would represent an increase in the entropy of combination of amino acid with its transport carrier. In the case of threonine, this change is from about 3.5 cal/°K × mol in the newborn to about 15 cal/°K × mol in the adult, and, for leucine, 8.5 cal/°K × mol in the newborn to about 21 cal/°K × mol in the adult. Thus, we would suspect that, in the case of these two amino acids, a less ordered structure in an amino acid-carrier complex evolves during development.

If the initial step in the amino acid entry is the formation of a Michaelis complex between amino acid and a carrier, the value for the apparent E_a derived from

Table 1
E_a values for amino acid uptakes by synaptosomal fractions

Amino Acid	Age of Donor Animal	E_a (cal)
Threonine	Adult	9800
Threonine	1–3 days	9800
Leucine	Adult	9500
Leucine	1–3 days	9500
Glycine	Adult	12 000
Glycine	1–3 days	10 700
Glycine	11–16 days	10 700

Arrhenius plots of the V_{max} term would be a measure of the amount of energy acquired by the complex. The consistency of the value for this activation energy, both amongst the different amino acids and in fractions prepared from animals of the different pastnatal ages (table 1), suggests the operation of a common activation mechanism which does not undergo modification during postnatal development.

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