TRANSPORT OF NATURALLY OCCURRING AMINO ACIDS AND ALPHA-AMINO ISOBUTYRIC ACID BY NORMAL AND DIAPAUSING MOUSE BLASTOCYSTS

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SUMMARY

Uptake of ^{14}C-labelled α-amino isobutyric acid and a mixture of naturally occurring amino acids by normal mouse preimplantation blastocysts was found to be twice that of delayed implantation blastocysts. With both groups of embryos concentration of the amino acids against a gradient occurred. The results may explain, in part, the lower amino acid incorporation rates found with diapausing vs. normal blastocysts and open the question of "metabolic dormancy" of the diapausing embryo.

INTRODUCTION

When diapausing mouse blastocysts are incubated *in vitro* with radioactive amino acids, the amount of label found in proteins of these embryos is significantly less than in normal blastocysts treated similarly (1,2,3). Among factors which contribute to this difference and the efficiency of labeling are: absolute rates of protein synthesis and degradation, rate of amino acid transport, and the specific activity of the amino acid pool used for protein synthesis. Since a close correlation appears to exist between amino acid transport rates and rates of incorporation of the precursors into protein of embryos (2,3), it appears necessary, at this point, to determine whether amino acid transport is, indeed, diminished in

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diapausing embryos. This study, therefore, concerns itself with uptake of naturally occurring amino acids and α-amino isobutyric acid (AIB) by both normal and diapausing blastocysts. The latter compound was used to obviate possible differences associated with metabolism and/or utilization of the naturally occurring amino acids by the two classes of embryos and, thus, evaluate transport rates specifically.

MATERIALS AND METHODS

The method of Edwards and Gates (4) was used to synchronize estrus and superovulate sexually mature, random-bred Swiss-Webster mice. The presence of a vaginal plug the morning following mating ascertained mating and denotes Day 1 post coitus (p.c.). To obtain delayed blastocysts, bilateral ovariectomy was performed on pregnant mice ca. 80 hours p.c. (5).

Normal mothers and those in implantation delay were sacrificed on the same day between 0700 and 1600 hours at the times p.c. indicated in Tables I and II. Blastocysts were flushed from excised uteri with modified Brinster's medium, MBM-2, which incorporated the following changes: 68.94 mM NaCl,29.93 mM sodium lactate, 0.49 mM sodium pyruvate and 11.11 mM glucose (6). Blastocysts were washed once in MBM-2, then transferred in groups of 5 to 50 embryos to Maximov slides containing 0.20 to 0.50 ml of MBM-2 plus 4.4 μCi/ml of a ^{14}C-amino acid mixture (54 mCi/Milliatom: CFB 104; Amersham-Searle) or 5.0 μCi/ml of 1-^{14}C-AIB (60 mCi/mMole; Amersham-Searle).

After labeling, each experimental group was washed twice in MBM-2 before solubilization of groups of 2 to 10 embryos in microtiter wells containing 0.10 ml MBM-2 and 0.10 ml 4% (w/v) sodium dodecyl sulfate. Each solution was quantitatively transferred to a 3 MM filter paper disc which was air dried. The dried discs were either counted, as described below, for total uptake or processed according to Mans and Novelli (7) for measurement of incorporation. The scintillation fluid used for uptake measurements was like that used for incorporation determinations (7) except that the solvent system was toluene: cellosolve (1:1, v/v) to accommodate water in the samples. Cellosolve reduced the counting efficiency by 15% in contrast to a maximum efficiency of 90%. Discs used as controls were processed identically to experimental samples. To assess acid soluble uptake, the average value for incorporation was substracted from each individual total uptake determination.

Data is presented as the mean value for cpm/embryo after subtraction of appropriate control values. The 95% confidence limits (c.l.) are also reported. Means of groups where the embryo type was the only variable were compared by a group comparison test (8).

RESULTS

With both normal and diapausing embryos, incorporation

Table I

Net radioactivity (total uptake), acid soluble radioactivity (total uptake minus acid insoluble) and radioactive protein (acid insoluble) accumulated by delayed and day 5 normal blastocysts after 3.0 hours and 6.0 hours of labeling *in vitro*.

Type of embryo	Day p.c.	Number of experiments	Number of determinations	Labeling period (hours)	CPM/blastocyst + 95% c.l. acid soluble	protein	Total
normal	5	6	32[a]	3.0	795 ± 198	1100 ± 107	1915 ± 205
delayed	7-9	2	14	3.0	348 ± 295	555 ± 146	903 ± 309
normal	5	4	19	6.0	1433 ± 383	2041 ± 251	3473 ± 401
delayed	7-9	4	21	6.0	686 ± 364	1285 ± 239	1971 ± 383

a. Twenty six determinations were made for radioactive protein. Subtraction of the average radioactivity in protein from the total uptake determinations in each experiment accounts for the slight discrepancy when acid soluble and insoluble radioactivity are summed.

Table II

Total uptake of 1-^{14}C-α-amino isobutyric acid by blastocysts after 1.7 or 3.0 hours. In each case there were 2 independent experiments and a total of 12 samples.

Type of embryo	Day p.c.	Labeling period (hours)	Total CPM/blastocyst taken up ± 95% c.l.
normal	5	1.7	43 ± 13
delayed	8	1.7	18 ± 5
normal	5	3.0	74 ± 18
delayed	8	3.0	32 ± 7

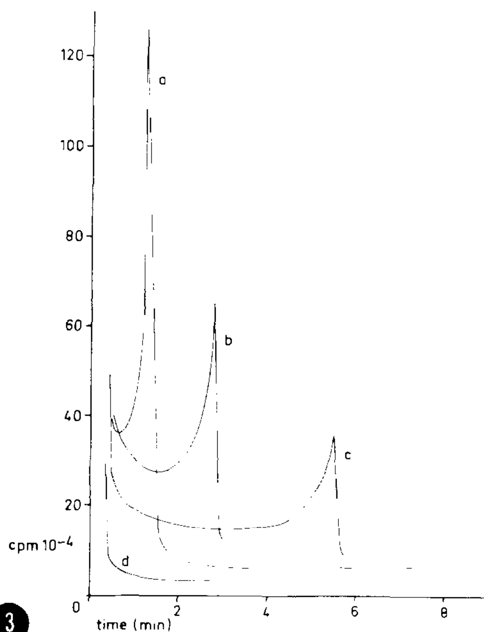
values parallel the rate of appearance of radioactive precursors into acid soluble pool, Table I. However, normal day 5 embryos accumulate radioactive protein and take up exogenous radioactive amino acids at twice the rate of diapausing blastocysts, Tables I and II. Although it might be argued that the enhanced rate of utilization of the precursors for protein synthesis caused the increased amino acid uptake by normal blastocysts, this does not seem to be the case. AIB, which shares transport systems of the neutral naturally occurring amino acids (9), is also transported more rapidly by normal vs. diapausing blastocysts although it is not known to be metabolized, or incorporated into protein (10). An alteration, therefore, in the biochemistry of blastocysts as a consequence of diapause is diminished amino acid transport capability. Moreover, diminished incorporation rates observed with diapausing vs. normal blastocysts are explicable, at least in part, in terms of differences in precursor transport rates.

Less than 10% of the total ^{14}C-AIB taken up was found associated with protein; this is believed to be the result of non-specific adsorption rather than incorporation per se. One cannot, however, discount the possibility that transfer of the radioactive carboxyl group of AIB by an unidentified enzyme(s) in the embryos occurred and resulted in formation of a protein precursor.

That both normal and diapausing blastocysts accumulate AIB against a gradient can be shown by means of the following calculation. When one considers that: (a) the volumes of delayed and day 5 normal blastocysts are ca. 0.91 (11) and 0.45 nl (12), respectively; (b) one μCi equals 2.2 c 10^6 dpm; and (c) the ^{14}C-AIB concentration is 5.0 μCi/ml, then the maximum radioactivity in diapausing and normal blastocysts would be 10 and 5.0 dpm per blastocyst, respectively, if equilibration with the medium occurred. However, the average amount of ^{14}C-AIB in normal and delayed blastocysts after three hours in 99 dpm (74 ± 18 cpm/0.75 cpm per dpm) and 43 dpm (32 ± 7 cpm/0.75 cpm per dpm), respectively, Table II. Thus concentration against a gradient must have occurred. Concentration of the naturally occurring amino acids can be shown similarly.

DISCUSSION

The biological significance of the observed diminished amino acid transport activity in diapausing vs. normal blastocysts is that these results: (a) delineate a change in one of the steps involved in utilization of exogenous precursors for protein biosynthesis during delayed implantation, and (b) demonstrate the effect of the diminished transport activity on measurements of incorporation rates. The latter is of particular importance since incorporation rates have been equated to absolute synthetic rates and have been used as indicators of



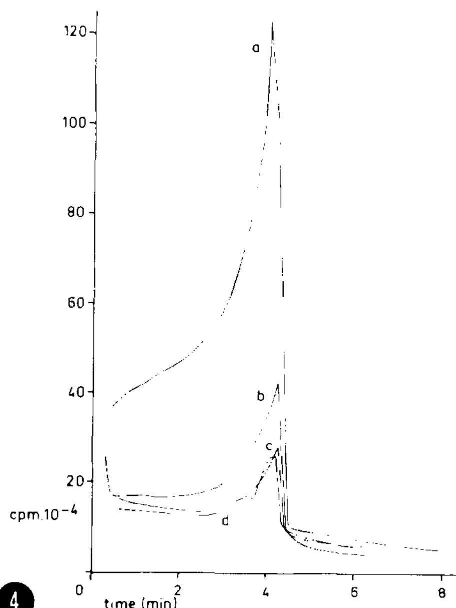


Fig. 3. Effect of lipoxygenase concentrations on the time course of luminescence. Curve a) 5.2×10^{-8}M; b) 2.6×10^{-8}M; c) 1.3×10^{-8}M; d) Noise level. No luminol.

Fig. 4. Effect of superoxide dismutase (SOD) on the chemiluminescence. Curve a) no SOD; b) 1.2×10^{-8}M SOD; c) 6×10^{-8}M SOD; d) 1.2×10^{-7}M SOD. No luminol.

linoleic acid starts at an oxygen concentration below 5 μM. Thus, gradual increase and the sharp decline of luminescence just precede the onset of the anaerobic reaction.

Fig. 2 shows the effect of varying linoleic acid concentrations. At substrate concentrations below the oxygen concentration (240 μM) there is no significant light emission in contrast to the effect of a substrate concentration well above 240 μM. Increasing the enzyme concentration at a sufficiently high substrate concentration (> 240 μM) brings about a proportional decrease in time required to attain the luminescence maximum (Fig. 3).

In Fig. 4 the effect of superoxide dismutase on the light emission from the lipoxygenase/linoleic acid system is shown. Small amounts (70 units/ml) already cause an almost complete quenching of the luminescence which is indicative of the involvement of $O_2^{\cdot}$ in the light-emitting process.

The addition of catalase had no effect while carbonate (up to 0.25 M)

12. Cole, R. J., (1967) J. Embrol. Exp. Morph. 17, 481-490.
13. McLaren, A., (1973) Regulation of Mammalian Reproduction pp. 321-334, Thomas, Springfield, Ill.
14. Psychoyos, A. (1973) Handbook of Physiology, Section 7, vol. 2, part 2, 187-215.
15. Weitlauf, H. M. (1974) J. Reprod. Fert. 39, 213-224.
16. Borland, R. M. and Tasca, R. J. (1974) Dev. Biol. 36, 169-182.
17. Epstein, C. J. (1975) Biol. of Reprod. 12, 82-105.