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Aminoisobutyric Acid Transport in Primary Cultures of Normal Adult Rat Hepatocytes[1] (38947)

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Suspensions of freshly isolated rat liver cells have been employed to differentiate between hepatic parenchymal and sinusoidal cell functions and to investigate hepatic physiology, biochemistry, and metabolic regulation (1–3). However, rat liver cells prepared in this manner manifest a number of altered metabolic and morphologic properties (4, 5). To circumvent these problems, methods for establishing primary nonproliferating monolayer cultures of isolated hepatocytes have been developed (6, 7) which permit the separation of irreversibly damaged cells from undamaged or minimally damaged cells and allow for repair of minor cellular injuries incurred during isolation. Such cultures demonstrate a number of metabolic functions characteristic of liver *in vivo* (7, 8).

We now report that primary monolayer cultures of rat hepatocytes, in contrast to suspensions of freshly isolated cells (3, 5), exhibit properties of active transport for the nonmetabolizable amino acid analog, α-aminoisobutyric acid (AIB).

Materials and Methods. Male Sprague–Dawley rats weighing 190–250 g were used. Rats were fed Purina pellets *ad libitum* and were maintained in light- and temperature-controlled quarters.

For isolation of hepatic parenchymal cells (HPC), the liver of anesthetized rats was perfused *in situ* (1) with a solution of 0.05% collagenase (Type II, Worthington Biochemicals) and 0.1% hyaluronidase (Calbiochem) in Hanks' balanced salt solution buffered to a pH of 7.35 with 25 m*M* HEPES (HBSS). The perfused liver was excised, sliced, and processed according to the method of Howard *et al.* (2). Dispersed cells were sedimented by centrifugation at 50*g* for 2 min and washed twice in HBSS supplemented with 0.05% fibrinogen. After the final wash HPC viability was assessed by trypan blue exclusion and the concentration of cells adjusted to 3.50×10^5 viable HPC/ml in L 15 culture medium (Microbiological Associates) with 10% fetal calf serum and 50 μg/ml Gentamicin (Schering Corp.).

One-half milliliter aliquots of HPC suspensions were dispensed onto fibrinogen-coated (0.05% in HBSS), 16-mm tissue culture, multidish dispo-trays (Linbro Chemical Co., Inc.) and incubated for 22 hr at 37° in air. Prior to use cultures were washed with HBSS and were evaluated for the viable attached-cell count with trypan blue. Plating efficiency of 57–87% was attained for cells isolated in different experiments. However, well-to-well variability in a single experiment was within 5%.

Uptake of AIB by cultured cells was assessed with ^{14}C-labeled AIB (New England Nuclear), specific activity of 2–10 mCi/m*M*, essentially as described by Foster and Pardee (9). At appropriate intervals, the incubation medium was aspirated and the cultures were rinsed rapidly with two 3-ml aliquots of ice-cold HBSS. After the cells were disrupted by addition of 0.5 ml of 0.1 *N* NaOH; aliquots were mixed with 1 ml of Scintisol Complete (Isolab Inc.) and radioactivity was counted in a Nuclear Chicago β-scintillation counter. Experiments with radiolabeled inulin added to the culture medium demonstrated that the washing procedure employed was effective

[1] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences —National Research Council. The facilities are fully accredited by the American Association of Accreditation of Laboratory Animal Care.

in removing all but 0.04% of the added inulin. In contrast, the intracellular content of AIB was unaffected by additional washes with ice-cold HBSS, indicating minimal removal of AIB from monolayers during the washing procedure.

Uptake of AIB by HPC in suspension was determined as previously described (3). Cells were collected by suction onto glass fiber filters and washed with ice-cold HBSS. The filters were transferred to scintillation vials, dried, and radioactivity determined as above.

Results. Fresh suspensions of HPC appeared to be freely permeable to AIB. As shown in Table I, rapid equilibration of AIB occurred in HPC incubated for 0.5 min at 37° or 4°, and the intracellular concentration of AIB was unaffected by incubation for up to 2 hr. The rapid removal of intracellular AIB by successive rinses with ice-cold HBSS is a clear indication of the free permeability of fresh suspensions of HPC to this amino acid analog.

In contrast to fresh suspensions, cultured HPC were not freely permeable to AIB. The rate of AIB uptake by cell monolayers was essentially linear during the first 2 hr when incubated at 37°. Transport of AIB appeared to be energy-dependent: (i) it was inhibited by 2 m*M* KCN and 10 m*M* iodoacetate and (ii) failed to occur when cells were incubated at 4° (Fig. 1). Addition of 100 n*M* glucagon stimulated uptake of AIB by approximately 23%.

The apparent K_m for AIB uptake by monolayer cultures of HPC was approximately 19 m*M* (Fig. 2). When glucagon was present, this value was unaffected but V increased from 49 to 59 p*M*/10³ HPC/10 min.

TABLE I. EFFECT OF TEMPERATURE ON UPTAKE OF AIB BY SUSPENSIONS OF FRESHLY ISOLATED HPC.[a]

Time (min)	cpm/filter ± SE Temperature 37°	4°
0.5	3380 ± 394	3437 ± 275
30	3760 ± 361	3930 ± 335
120	3644 ± 197	3440 ± 378

[a] 10⁶ HPC were incubated in 1 ml of HBSS with 2 m*M* AIB and 0.25 μCi[1-¹⁴C]AIB, then harvested under suction on glass fiber filters and rapidly rinsed with 8 ml of ice-cold HBSS. $N = 6$.

TABLE II. EFFECT OF RINSING WITH ICE-COLD HBSS ON REMOVAL OF AIB FROM FRESH SUSPENSIONS OF HPC.[a]

HBSS rinse volume (ml)	Cellular AIB content cpm ± SE (x 10²)	% Remaining
0	3370 ± 78	100.0
2	703 ± 22	20.8
4	282 ± 20	8.3
8	124 ± 7	3.7
16	77 ± 4	2.3
32	46 ± 5	1.4

[a] Cells were incubated for 15 min at 37° as described in Table I, then harvested under suction on glass fiber filters and flushed with 2 ml increments of ice-cold HBSS as indicated. $N = 6$.

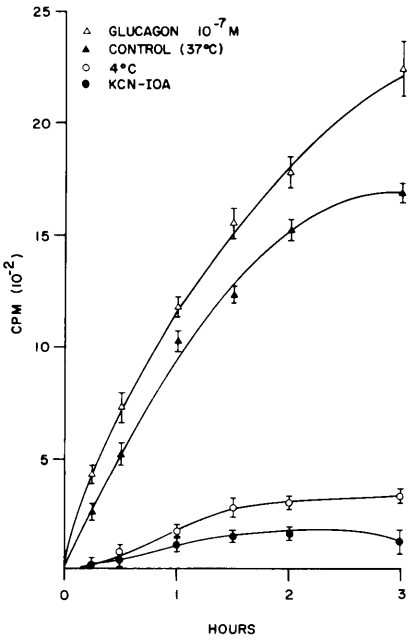

FIG. 1. Uptake of AIB by monolayer cultures of HPC. The incubation medium consisted of 0.5 ml of HBSS with 2.0 m*M* AIB and 0.4 μCi [1-¹⁴C]AIB. Concentrations of 100 n*M* glucagon, 2 m*M* KCN, and 10 m*M* iodoacetate were employed where indicated. Each point represents data calculated from the uptake of AIB by six monolayer cultures ± SE.

Discussion. The various enzymatic and mechanical procedures developed to dissociate liver cells into a single cell suspension inevitably result in biochemical and

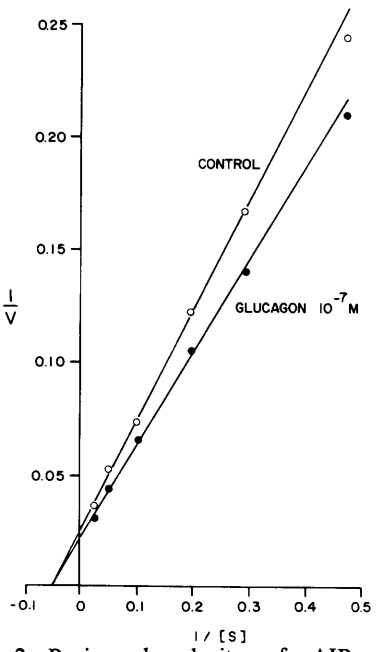

FIG. 2. Reciprocal velocity of AIB uptake (pmoles/10^3 HPC/10 min) plotted against reciprocal of AIB concentration (mM). Incubations were for 10 min. Each data point represents the mean from six monolayer cultures. Glucagon was added to a final concentration of 100 nM.

morphological aberrations (10, 11). Repair of cellular injuries incurred during isolation with restoration of many physiologic functions, however, can be achieved by subjecting dissociated HPC preparations to tissue culture conditions. Hence, nonproliferating, short-term, suspension or monolayer cultures of isolated HPC exhibit functional properties such as protein synthesis, respiration, gluconeogenesis, enzyme activation, and metabolite concentrations which are comparable to those of *in vivo* or isolated perfused liver (7, 8, 12).

Our observations have extended this list by demonstrating that the characteristics of active transport for the nonmetabolizable amino acid analog, AIB, absent in freshly isolated HPC, are restored following short-term culture.

The reported value of 19 mM for the apparent K_m of AIB transport by cultured hepatocytes was unexpectedly higher than the 8.7 mM value estimated for rat liver slices by Tews and Harper (13). Whether our higher value represents differences in experimental procedures, an aberration of the amino acid transport system of cultured HPC, or is a consequence of cellular heterogeneity of tissue slices versus our homogeneous HPC preparation is presently unknown.

Of more significance is the observation that the stimulation of AIB transport caused by glucagon in perfused (14) and sliced liver (13) can be duplicated in cultured hepatocytes. Hence, the mechanisms and control of amino acid transport and metabolism can be evaluated directly in homogeneous populations of normal adult parenchymal liver cells, and the use of isolated perfusion or liver slice techniques which fail to discriminate between hepatic parenchymal and sinusoidal cell function can be circumvented.

Summary. In contrast to suspensions of freshly isolated hepatic parenchymal cells (HPC), short-term monolayer cultures of HPC displayed properties of active transport for the amino acid analog aminoisobutyric acid (AIB). The uptake of AIB was inhibited by KCN and iodoacetate, failed to occur at 4°, and was stimulated by glucagon. The apparent K_m for AIB uptake by cultured HPC was approximately 19 mM. Glucagon did not alter the apparent K_m but did increase V.

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