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Involvement of monocarboxylate transporter 1 (SLC16A1) in the uptake of L-lactate in human astrocytes☆

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ABSTRACT

Purpose: Astrocytes, the most abundant glial cells in the central nervous system (CNS), help neurons survive. Monocarboxylate transporters (MCTs) are reported to transport L-lactate, which is important for CNS physiology and cognitive function. However, it remains unclear which MCT isoform is functionally expressed by human astrocytes. The aim of this study was to establish the contribution of each MCT isoform to L-lactate transport in human astrocytes.

Methods: The function of L-lactate transport was studied using NHA cells as a human astrocyte model and radiolabeled L-lactate. The expression of MCT in human astrocytes was detected by immunohistochemistry staining.

Results: The cellular uptake of L-lactate was found to be pH- and concentration-dependent with a *K*m value for L-lactate uptake of 0.64 mM. This *K*m was similar to what has been previously established for MCT1-mediated L-lactate uptake. α-Cyano-4- hydroxycinnamate (CHC) and 5-oxoproline, which are both MCT1 inhibitors, were found to significantly inhibit the uptake of L-lactate, suggesting MCT1 is primarily responsible for L-lactate transport. Moreover, MCT1 protein was expressed in human astrocytes.

Conclusion: pH-dependent L-lactate transport is mediated by MCT1 in human astrocytes.

1. Introduction

Monocarboxylate transporters (MCTs) are proton-linked transporters that transport monocarboxylates such as lactate, pyruvate, and ketone bodies. Of the 14 known MCTs, three isoforms, MCT1, MCT2, and MCT4 are expressed in the rodent brain. More specifically, MCT2 is expressed in neurons while MCT1 and MCT4 are expressed in glial cells [1]. Suzuki et al. [2] reported that these transporters play important roles in long-term memory formation. It has been reported that expression of MCTs in astrocytes showed decline in Alzheimer's disease model mice [3]. It is important to clarify whether MCT contributes to L-lactate uptake into NHA cells for finding the strategy to improve cognitive function and to inhibit the progression of Alzheimer's disease.

Astrocytes are the most abundant glial cells in the central nervous system (CNS) and have the potential to play a number of important roles in CNS physiology. For example, it has been reported that astrocytes help neurons to survive, with MCTs being involved in this mechanism [4]. MCT4 has been suggested to be functionally expressed for nicotinate transport in rat astrocytes [5]; however, it is not clear which MCT isoform is functionally expressed in human astrocytes. Past studies have also suggested that lactate next to glucose, is a fuel taken up and oxidized by human brain cells [6].

The present study therefore aimed to establish which MCT isoform is functional for L-lactate transport in human astrocytes and determine the contribution of each MCT isoform to L-lactate transport.

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2. Methods

2.1. Chemicals

NHA (CC-2565), astrocyte growth medium (AGM) BulleKit (CC-3186), and subculture reagents were purchased from Lonza, Japan. [^{14}C] L-lactate was purchased from American Radiolabeled Chemicals. All other compounds were of reagent grade.

2.2. Cell culture

NHA were cultured in AGM BulleKit in accordance with the protocol of Lonza Japan. NHA are fetal human cells. AGM BulleKit is formulated for optimal growth of specific types of normal human cells. Cells were seeded in 100-mm Petri dishes at a density of 5×10^3 cells/cm^2 and incubated at 37 °C in a humidified atmosphere of 95% air-5% CO_2. Media were replenished within 24 h of thawing cells, and every 48 h that followed. NHA were subcultured by partial digestion with ReagentPack™ subculturing reagents (Lonza; CC-5034) when cultures reached 80% confluence, five days after plating.

2.3. Transport studies using human astrocytes

For transport studies, NHA were plated onto 24-well plates and grown to confluence. Uptake of [^{14}C] L-lactate was initiated after washing the cells, by adding 0.5 mL of uptake buffer containing 140 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM D-glucose, 5 mM Good's buffer, 0.67 μM [^{14}C] L-lactate and unlabeled compounds. Homopiperazine-1,4-bis(2-ethanesulfonic acid) (HOMOPIPES) was used as the pH 5.0 buffer, 2-morpholinoethanesulfonic acid (MES) as the pH 5.5–6.5 buffer, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) as the pH 7.0–7.5 buffer. After incubation for a specific period, the cells were quickly washed with ice-cold buffer, solubilized in 0.5 mL of 0.5 N NaOH, and prepared for liquid scintillation analysis. The measurement of L-lactate uptake was determined as previously described [7].

2.4. Immunofluorescent analysis

NHA were washed twice with Phosphate buffered saline (PBS) and fixed with 4% formaldehyde for 15 min at room temperature, and then treated with 0.2% Triton X-100 for 5 min at 4 °C.

Following washing of the fixed cells with PBS containing 0.5% normal donkey serum (Jackson ImmunoResearch), they were incubated with rabbit anti-MCT1 antibody (sc-50324) (Santa Cruz Biotechnology) (diluted 1:50) and goat anti-glial fibrillary acidic protein (GFAP) antibody (sc-6170) (Santa Cruz Biotechnology) (diluted 1:50) in PBS containing 0.2% BSA for 2 h at room temperature. Cells were then washed with PBS and thereafter incubated for 2 h at room temperature with Texas Red-conjugated donkey anti-rabbit (sc-2784) and fluorescein isothiocyanate-conjugated donkey anti-goat (sc-2024) secondary antibodies (Santa Cruz Biotechnology) (diluted 1:100). Cells were again washed with PBS and then analyzed using the FLUOVIEW FV10i (OLYMPUS).

2.5. Data analysis

The results were expressed as mean ± S.D. or S.E. Statistical comparisons of mean values were performed by one-way analysis of variance and Dunnett's test. Non-linear regressions were fitted using SigmaPlot 12.5 (HULINKS) and confirmed by linear regression.

The following equation was utilized to establish the cellular uptake rate of L-lactate:

$$V = V\max[S]/(K\mathrm{m} + [S]) + K\mathrm{d}[S]$$

V represents the uptake rate of substrate; Vmax, the maximum uptake rate; [S], concentration of the substrate in the medium; Km, the Michaelis–Menten constant; and Kd, the coefficient of passive diffusion.

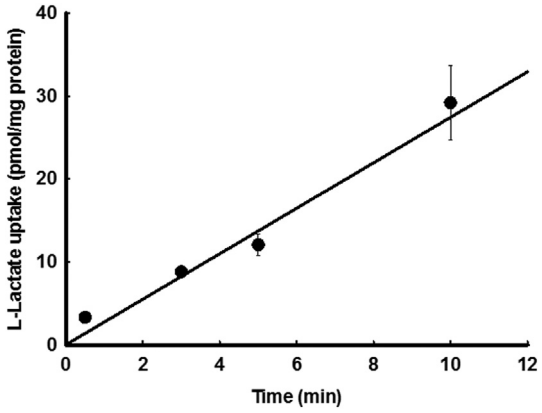

Fig. 1. Time-dependent cellular accumulation of L-lactate. The uptake of [^{14}C] L-lactate (0.67 μM) by NHA was measured in MES buffer (pH 5.5) at 37 °C. Data are means ± S.E. of three independent experiments.

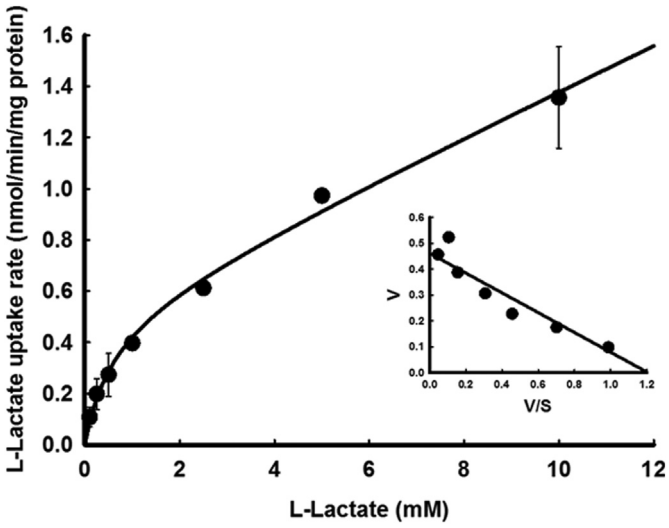

Fig. 2. Kinetics of L-lactate transport. Uptake of L-lactate (0.05–10 mM) by NHA was measured in MES buffer (pH 5.5) for 10 min at 37 °C. The inset shows the Eadie–Hofstee plot of the data after subtraction of non-saturable [^{14}C] L-lactate uptake. V represents the uptake rate of substrate; [S], concentration of the substrate in the medium. Data are means ± S.E. of four independent experiments.

3. Results

3.1. Time-dependent cellular accumulation of L-lactate

The time course for the accumulation of L-lactate by NHA is shown in Fig. 1. L-lactate accumulation was linear up to 10 min after incubation with [^{14}C] L-lactate. The initial uptake rate was therefore determined within 10 min after the onset of L-lactate uptake.

3.2. Kinetics of L-lactate uptake

The relationship between the initial uptake rate and L-lactate concentration indicates that the uptake rate was saturated at high concentrations of L-lactate (Fig. 2). The Fig. 2 inset shows an Eadie–Hofstee plot of the data following subtraction of the non-saturable transport components. Kinetic parameters were calculated by non-linear regression of the Michaelis–Menten kinetics, and Km, Vmax, and Kd were estimated to be 0.64 ± 0.27 mM, 0.54 ± 0.11 nmol/min/mg protein, and 0.09 ± 0.01 μL/min/mg protein, respectively. These results