Short Communication

The Depletion of Intracellular Glutamine by Methionine Sulfoximine on Amino Acid Uptake in Placental Cells (BeWo)

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Abstract

The intracellular concentration of glutamine is very high where it plays a central role as a ready source of nitrogen, carbon and energy in various metabolic processes. The purpose of the present study was to investigate the influence of intracellular glutamine status on amino acid uptake activity in placental choriocarcinoma (BeWo) cells, analogous to normal placental trophoblasts. Intracellular glutamine was depleted by culturing the cells in regular medium without glutamine and by treating with 2 mM methionine sulfoximine (MSX), an inhibitor of glutamine synthetase, for 16 hours. The uptake of various amino acids was measured by the use of appropriate substrates and ionic conditions. When cultured in the absence of glutamine and MSX treatment, the uptake of serine, threonine and histidine was not influenced. Under similar conditions, the uptake of glutamate, alanine, glycine, α-(methylamino)isobutyric acid (MeAIB), taurine and carnitine was reduced to a varied extent. These data showed that intracellular glutamine was obligatory for maintenance of optimal activity of amino acid uptake in BeWo cells.

Keywords: amino acid uptake, glutamine, methionine sulfoximine, placenta

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บทคัดย่อ

การทำให้กลูทามีนภายในเซลล์ลดลงด้วยเมทไธโอนีนซัลโฟซิมีนต่อการนำเข้ากรดอะมิโนของเซลล์รก

บุญฤทธิ์ ทองทรง

โดยทั่วไปปริมาณของกลูทามีนภายในเซลล์มีระดับสูง บทบาทที่สำคัญประการหนึ่งของกลูทามีนภายในเซลล์คือ การเป็นแหล่งไนโตรเจน คาร์บอน และพลังงานในกระบวนการสันดาปต่างๆ วัตถุประสงค์การศึกษาครั้งนี้ เพื่อเข้าใจผลของสถานะระดับกลูทามีนภายในเซลล์รกต่อการนำเข้ากรดอะมิโน เซลล์รกถูกเพาะเลี้ยงด้วยอาหารเลี้ยงเซลล์ตามปกติแต่ไม่มีกรดอะมิโนกลูทามีน และถูกลดระดับกลูทามีนภายในเซลล์โดยการเติมเมทไธโอนีนซัลโฟซิมีน ซึ่งทำหน้าที่ยับยั้งเอนไซม์กลูทามีนซินทีเทสเป็นระยะเวลานาน 16 ชั่วโมง หลังจากนั้นศึกษาการนำกรดอะมิโนชนิดต่างๆผ่านเซลล์ โดยใช้สารตั้งต้นและปรับสภาวะไอออนที่เหมาะสม ผลการศึกษาพบว่าการเลี้ยงเซลล์ในภาวะที่ไม่มีกลูทามีนทั้งจากภายนอกเซลล์และภายในเซลล์ ไม่มีผลต่อการนำเข้ากรดอะมิโน เซรีน ธรีโอนีน และฮีสทิดีน แต่ภายใต้สภาวะเดียวกันนี้ พบว่าการนำเข้ากรดอะมิโน กลูทาเมต อะลานีน ไกลซีน อัลฟาเมทธิลอะมิโนไอโซบิวทีริคแอซิด เทารีน และสารคาร์นิทีน ลดลงในระดับที่แตกต่างกัน จากผลการศึกษาดังกล่าวแสดงให้เห็นว่าปริมาณกรดอะมิโนกลูทามีนต่อเซลล์มีหน้าที่คงสภาวะที่เหมาะสมสำหรับการนำกรดอะมิโนผ่านเข้าสู่เซลล์

คำสำคัญ: การนำเข้ากรดอะมิโน กลูทามีน เมทไธโอนีนซัลโฟซิมีน รก

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Introduction

Nutrients play a critical role in interaction of various intracellular and extracellular factors. Many studies have shown that amino acids have marked ability to control many cellular processes (Kadowaki and Kanazawa, 2003) that respond to the nutritional status of the cells. Glutamine, a nonessential amino acid, is by far the most abundant free amino acid in circulation and is an important molecule that not only serves as a building block for protein synthesis but also performs a variety of additional biological functions (Karinch et al., 2001; Oehler and Roth, 2003). The intracellular concentration of glutamine is very high where it plays a central role as a ready source of nitrogen, carbon and energy in various metabolic processes. Glutamine is taken up into mammalian cells from extracellular medium by several active and passive amino acid transport systems (Ganapathy et al., 2003). In addition, glutamine is also synthesized by the amidation of glutamate. This ATP-dependent reaction is catalyzed by glutamine synthetase, an enzyme inhibitable by the glutamine analog methionine sulfoximine (MSX). The purpose of the present study was to investigate the influence of intracellular glutamine depletion on the uptake activity of the amino acids. This study was carried out using the BeWo cells, a human placental choriocarcinoma cell line. These cells have proven to be effective in studying the placental transporters (Bode et al., 2006). They express abundant and several amino acid transport systems that are subject to extensive regulation in human placenta under various physiological and pathological conditions (Mahendran et al., 1993; Glazier et al., 1997; Sibley et al., 1997; Godfrey et al., 1998; Harrington et al., 1999 ; Oehler and Roth, 2003). The different amino acid transporters can work together as an integrated system in the syncytiotrophoblast (Sengers et al., 2010). The uptake activity of some amino acids in the placental brush border membrane has been shown to directly correlate with the birth weight of babies under various physiological and pathological conditions (Mahendran et al., 1993; Glazier et al., 1997; Sibley et al., 1997; Godfrey et al., 1998; Harrington et al., 1999). Therefore, information on the identity of this factor that regulates the amino acid uptake measurements in these cells may be relevant to the understanding of fetal growth and development.

Materials and Methods

Cell culture and chemicals: The BeWo choriocarcinoma cell line, cell culture media, methionine sulfoximine (MSX), fetal bovine serum, unlabeled amino acids and radiolabeled amino acids such as L-[^{3}H]glutamine, L-[^{3}H]glutamic acid, L-[^{3}H]serine, L-[^{3}H]threonine, L-[^{3}H]histidine,

[^{3}H]taurine, L-[^{3}H]Alanine, [^{3}H]glycine, L-[^{3}H]carnitine and [^{14}C]α-(methylamino) isobutyric acid (MeAIB) were provided by Professor Dr. Vadivel Ganapathy and Professor Dr. Puttur Prasad.

Cell culture and treatment: The BeWo cells were cultured in 12-well culture plates in 150 cm^2 flasks in DMEM/F-12 (50:50) medium containing 2.5 mM glutamine and supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin. The culture condition was in a 37^{0}C incubator supplied with 5% CO_2 and 95% relative humidity. Confluent cultures were treated with MSX in a glutamine-free culture medium. These culture conditions effectively lead to the depletion of intracellular glutamine because of the lack of glutamine in the medium and also because of the inhibition of endogenous synthesis of glutamine by MSX. After treatment of the cells with or without MSX for 16 hours, the cells were used for the amino acid uptake measurements. Cells cultured in glutamine-free medium but not treated with MSX were used as control.

Amino acid uptake measurements: Uptake measurements were carried out at 37^{0}C. The medium was aspirated and the cell monolayer was washed once with the uptake buffer. Uptake was then initiated by the addition of 500 µl of uptake buffer containing 0.5 µCi of radiolabeled amino acids. The incubation was continued and then measured time course for 1, 3, 5, 10, 20 and 40 min, following which the uptake was terminated by aspirating the uptake medium. All subsequent measurements were done within this linear phase of uptake. The transport of various amino acids was measured for 5 minutes. After the termination of the uptake, the cells were washed two times with 1.5 ml of ice-cold uptake buffer. The cells were then solubilized with 0.5 ml of 1% SDS/0.2 N NaOH and transferred to scintillation vials for the determination of the radioactivity associated with the cells. Experiments were made in triplicate. The results are given as means ± SEM.

Results and Discussion

Influence of intracellular glutamine depletion on amino acid uptake in BeWo cells: As for in vitro model, the effect of extracellular and intracellular glutamine levels on the uptake of amino acids in BeWo cells was investigated. Initially, determination of the uptake activity was measured in cells grown in medium with and without glutamine in the absence and presence of MSX. The rationale for these experiments was as follows; MSX is an inhibitor of glutamine synthetase and therefore treatment of cells would prevent endogenous generation of glutamine inside the cells. When cells are grown in the absence of glutamine in the culture medium but in the presence of MSX, this might lead to depletion of glutamine inside the cells. Such depletion may not occur when cells are grown in the presence of glutamine. Confluent cells were treated with or without MSX (2 mM) for 16 hours in the presence or absence of glutamine (2.5 mM). Uptake of various amino acids was then measured for 5 minutes. The

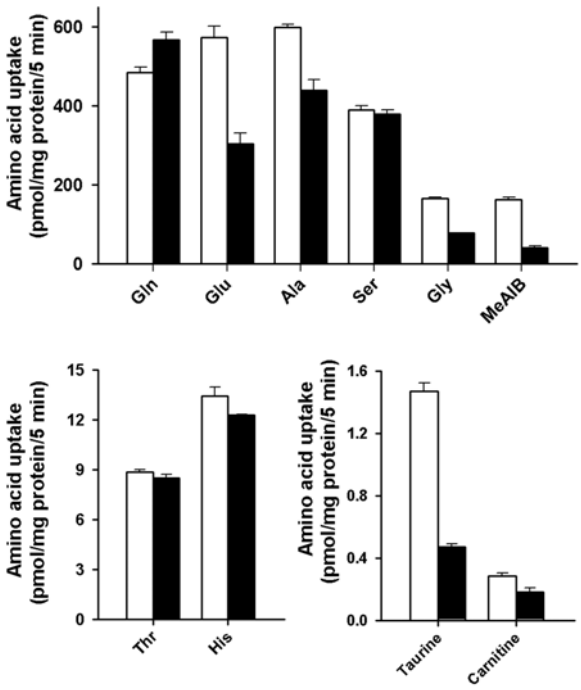

Figure 1 Specificity of MSX effect. BeWo cells were treated in the absence of extracellular glutamine without (open bars) or with (filled bars) MSX (2 mM) for 16 hours. Transport of various amino acids was then measured for 5 min. The concentrations of these amino acids during uptake measurement were as follows: 5 µM for glutamine, glutamate, alanine, serine, glycine, MeAIB, and histidine; 700 nM for threonine; 50 nM for taurine; and 15 nM for carnitine.

results in figure 1 show that while the uptake of glutamate, alanine, glycine, MeAIB, taurine and carnitine is reduced to varying extent in MSX-treated cells, the uptake of serine, threonine and histidine is not affected under similar conditions. Surprisingly, the uptake of glutamine is slightly (16%) higher in MSX-treated cells compared to the glutamine uptake in control cells (567±35 pmol/mg protein/5 min vs 485±24 pmol/mg protein/5 min). These results indicate that the effect of intracellular depletion of glutamine on amino acid uptake activity can affect the variety of amino acid properties. In addition, the present studies show that intracellular glutamine status may influence not only the uptake activity of one amino acid but also that of another uptake nutrient such as the carnitine. Uptake of various nutrients that are different properties from glutamine such as taurine and carnitine are also decreased significantly in MSX-treated cells.

This report describes the effect of intracellular glutamine depletion on the uptake of amino acids in BeWo cells. The effect of glutamine depletion on amino acid transport is not limited to specific amino acid. Thus, extracellular and intracellular glutamines appear to have a differential effect on the regulation of some amino acid activities.

These findings may have important physiological implications. They should be investigated consequently the alternate possibility whether MSX treatment may interfere with the expression of some amino acid transporter genes at the level of transcription and with protein expression

at the level of translation. Further investigation is needed to be done with the specific amino acid transport system, system A, since it is one of the transporters that mediate glutamine entry into normal placental syncytiotrophoblast at the brush border membrane (Novak and Beveridge, 1997). Thus, intracellular glutamine status may be a significant player as a regulator of transcellular transfer of amino acids from mother to fetus across the placenta. This amino acid will be considered to be conditionally essential.

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References

Bode, C.J., Jin, H., Rytting, E., Silverstein, P.S., Young, A.M. and Audus, K.L. 2006. *In vitro* models for studying trophoblast transcellular transport. Methods Mol Med. NIH Public access. 122: 225-239.

Ganapathy, V., Inoue, K., Prasad, P.D. and Ganapathy, M.E. 2003. Cellular uptake of amino acids: systems and regulation, in: Metabolic and Therapeutic Aspects of Amino Acids in Clinical Nutrition, L.A. Cynober (ed), CRC Press, New York, NY. 63-78.

Glazier, J.D., Cetin, I., Perugino, G., Ronzoni, S., Grey, A.M., Mahendran, D., Marconi, A.M., Pardi, G. and Sibley, C.P. 1997. Association between the activity of the system A amino acid transporter in the microvillous plasma membrane of the human placenta and severity of fetal compromise in intrauterine growth restriction. Pediatr Res. 42: 514-519.

Godfrey, K.M., Matthews, N., Glazier, J., Jackson, A., Wilman, C. and Sibley, C.P. 1998. Neutral amino acid uptake by the microvillous plasma membrane of the human placenta is inversely related to fetal size at birth in normal pregnancy. J Clin Endocrinol Metab. 83 : 3320-3326.

Harrington, B., Glazier, J., D'Souza, S. and Sibley, C. 1999. System A amino acid transporter activity in human placental microvillous membrane vesicles in relation to various anthropometric measurements in appropriate and small for gestational age babies. Pediatr Res. 45 : 810-814.

Kadowaki, M. and Kanazawa, T. 2003. Amino acids as regulators of proteolysis. J Nutr. 133: 2052S-2056S.

Karinch, A.M., Pan, M., Lin, C.M., Strange, R. and Souba, W.W. 2001. Glutamine metabolism in sepsis and infection. J Nutr. 131: 2535S-2538S; discussion 2550S-2551S.

Mahendran, D., Donnai, P., Glazier, J.D., D'Souza, S.W., Boyd, R.D. and Sibley, C.P. 1993. Amino acid (system A) transporter activity in microvillous membrane vesicles from the placentas of appropriate and small for gestational age babies. Pediatr Res. 34 : 661-665.

Novak, D.A. and Beveridge, M.J. 1997. Glutamine transport in human and rat placenta. Placenta. 18: 379-386.

Oehler, R. and Roth, E. 2003. Regulative capacity of glutamine. Curr Opin Clin Nutr Metab Care. 6: 277-282.

Sibley, C., Glazier, J. and D'Souza, S. 1997. Placental transporter activity and expression in relation to fetal growth. Exp Physiol. 82: 389-402.

Sengers, B.G., Please, C.P. and Lewis, R.M. 2010. Computational modeling of amino acid transfer interaction in the placenta. Exp Physiol. 95: 829-840.

Smith, C.H., Moe, A.J. and Ganapathy, V. 1992. Nutrient transport pathways across the epithelium of the placenta. Annu Rev Nutr. 12: 183-206.