Short Communication

Different Influence of Intracellular Glutamine Depletion on Glutamate Uptake Mediated by Amino Acid Transport Systems; EAATs and X-$_c$, in Placental (BeWo) Cells

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Abstract

Glutamate transporters are responsible for active transport of glutamate over the cell membrane and play an important role in the glutamate-glutamine cycle. The purpose of the present study was to investigate the influence of intracellular glutamine depletion on glutamate uptake mediated by amino acid transport systems; EAATs and X-c in placental choriocarcinoma (BeWo) cells as a model of human trophoblasts. Intracellular glutamine was depleted by culturing the cells in regular medium without glutamine and by treating with an inhibitor of glutamine synthetase (methionine sulfoximine; MSX), for 16 hours. The uptake of glutamate was measured by the use of appropriate substrates and ionic conditions. When cultured in not only the absence of extracellular glutamine but also treatment with MSX, the differential uptake of glutamate depended on the glutamate transport systems. This study concluded that the depletion of intracellular glutamine decreased the activity of EAATs; as sodium-dependent manner and at the same time significantly enhanced the activity of X-c; as sodium-independent manner for glutamate transport in the BeWo cells. Thus, intracellular glutamine was obligatory for maintenance of optimal activity of system EAATs in the BeWo cells. The depletion of intracellular glutamine caused stress to the cells and the stress leaded to the upregulation of system X-c activity.

Keywords: amino acid transport system, glutamate, glutamine, placenta

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บทคัดย่อ

ความแตกต่างระหว่างอิทธิพลของสภาวะกลูทามีนภายในเซลล์รกลดลงต่อการนำเข้ากลูทาเมทที่ถูกเหนี่ยวนำโดยตัวขนส่งกรดอะมิโนสองระบบ

บุญฤทธิ์ ทองทรง

ตัวขนส่งกลูทาเมทช่วยในการขนส่งกลูทาเมทผ่านเซลล์แบบใช้พลังงาน และมีบทบาทในการเป็นส่วนประกอบที่สำคัญประการหนึ่งในวัฏจักรกลูทาเมท-กลูทามีน วัตถุประสงค์การศึกษาครั้งนี้ เพื่อศึกษาอิทธิพลของสภาวะกลูทามีนภายในเซลล์รกลดลงต่อการนำเข้ากลูทาเมทที่เหนี่ยวนำโดยตัวขนส่งกรดอะมิโนทั้งสองระบบ เซลล์รกถูกเพาะเลี้ยงด้วยอาหารเลี้ยงเซลล์ตามปกติแต่ไม่มีกรดอะมิโนกลูทามีนและทำการลดกลูทามีนภายในเซลล์โดยการเติมสารที่ทำหน้าที่ยับยั้งเอนไซม์กลูทามีนซินทีเทส ได้แก่ เมทไธโอนีนซัลโฟซิมีน เป็นระยะเวลานาน 16 ชั่วโมง ศึกษาการนำกรดอะมิโนกลูทาเมทผ่านเซลล์โดยใช้สารตั้งต้นและปรับสภาวะไอออนที่เหมาะสม จากการศึกษาพบว่าการเลี้ยงเซลล์ในสภาวะกลูทามีนภายในเซลล์ลดลง ทำให้การนำเข้ากรดอะมิโนกลูทาเมทระบบที่ใช้ตัวขนส่งชนิดต้องอาศัยโซเดียมไอออนลดลง แต่ในสภาวะเดียวกันกลับทำให้การนำเข้ากรดอะมิโนกลูทาเมทระบบที่ใช้ตัวขนส่งชนิดไม่ต้องอาศัยโซเดียมไอออนเพิ่มขึ้นอย่างมาก จากผลการศึกษาดังกล่าวแสดงให้เห็นว่าปริมาณกรดอะมิโนกลูทามีนภายในเซลล์ มีบทบาทหน้าที่ในการคงสภาวะที่เหมาะสมสำหรับระบบตัวขนส่งกรดอะมิโนกลูทาเมทชนิดที่ต้องอาศัยโซเดียมไอออน และการลดลงของระดับกลูทามีนภายในเซลล์รกนี้ อาจทำให้เกิดสภาวะเครียดซึ่งทำให้ระบบตัวขนส่งกรดอะมิโนกลูทาเมทชนิดที่ไม่ต้องอาศัยโซเดียมไอออนทำหน้าที่เพิ่มขึ้น

คำสำคัญ: ระบบขนส่งกรดอะมิโน กลูทาเมท กลูทามีน รก

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Introduction

Most of the nutrients for fetal growth and development are supplied from maternal blood circulation while some of them are synthesized in the placenta. Both glutamate and aspartate, unlike others, are concentrated higher within the placental trophoblast than in the maternal and fetal circulations. These amino acids may provide an energy source to the placenta (Battaglia, 2000) and are thought to be important for fetal growth and development. The ability to transport glutamate across cellular membranes by glutamate transporters was evidenced in many tissues including placenta (Moe, 1995; Matthews et al., 1998; Noorlander et al., 2004). By molecular identification in mammals, at least five isoforms (Excitatory Amino Acid; EAA, transporters) of high affinity sodium-dependent glutamate transporters representing system EAATs were identified in both apical and basal membranes of syncytiotrophoblasts (Cariappa et al., 2003; Noorlander et al., 2004). These transporters can transport not only L-glutamate but also L- and D-aspartate (Kanai and Hediger, 1992). Additionally, sodium-independent glutamate transporter representing system X-c (Christensen, 1990) was identified from placenta as a transport system which exchanges glutamate and cystine (Wagner et al., 2001). The work of Noorlander et al. (2004) demonstrated that expression patterns of placental glutamate transporters were indicated in active transport of glutamate in blood circulation between fetus and mother. Placental transport of maternal glutamine establishes the fetoplacental glutamine-glutamate cycle. In addition, placental uptake of glutamate from fetal blood circulation is important to maintain low concentration of extracellular glutamate and prevent toxic levels to reach the brain (Robinson and Dowd, 1997).

The intracellular concentration of glutamine is very high. There are two routes to obtain glutamine; taken up into mammalian cells from extracellular medium by several active and passive amino acid transport systems (Ganapathy et al., 2003) and synthesized by the amidation of glutamate. The ATP-dependent reaction is catalyzed by glutamine synthetase, an enzyme inhibited by the glutamine analog methionine sulfoximine (MSX). Knowledge concerning the intracellular glutamine depletion of glutamate uptake in trophoblast cells is necessary to understand the glutamate-glutamine regulation. In the placenta, the glutamate-glutamine cycle plays a crucial role in contributing glutamine to the fetus and excreting glutamate and ammonia from fetal circulation (Battaglia, 2000) as well as regulating the concentrations of some amino acids. It is known that glutaminase produces glutamate from glutamine in many organs. This transformation between glutamine and glutamate has an essential role in some regulations such as intracellular pH (Neu, 2001). Furthermore, glutamine synthetase is responsible

enzyme for glutamine synthesis in the placenta to keep up with fetal demand (Battaglia, 2000). Little is known about the effect of depletion of intracellular glutamine on uptake activity of amino acid transport systems in placenta. Therefore, the purpose of the present study was to investigate the influence of intracellular glutamine depletion on glutamate uptake mediated by amino acid transport systems; EAATs and X-c in the placental choriocarcinoma (BeWo) cells as a model of the human trophoblasts. These cells express abundant and several amino acid transport systems that are subject to extensive regulation in human placenta under various physiological and pathological conditions (Sibley et al., 1997; Oehler and Roth, 2003; Thongsong et al., 2005). Therefore, *in vitro* study into the condition of intracellular glutamine depletion for mediating uptake of glutamate transport systems in BeWo cells may lead to a better understanding of the glutamate transporters in the maternal-placental-fetal unit.

Materials and Methods

Cell culture and reagents: BeWo choriocarcinoma cell line, cell culture media, fetal bovine serum, unlabeled and radiolabeled amino acid of L-[^{3}H] glutamic acid (40 Ci/mmol) were provided by Professor Dr. Vadivel Ganapathy and Professor Dr. Puttur D Prasad.

Cell culture and treatment: BeWo cells were cultured in 12-well culture plates for uptake assays 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 conditions and the rationale of intracellular glutamine depletion for the study were described in my previous report (Thongsong, 2012[a]). Confluent cultures were treated in a glutamine-free culture medium to lead to the absence of extracellular glutamine. Under this condition, cells were treated without MSX to control availability of intracellular glutamine and with MSX for 16 hours to induce the depletion of intracellular glutamine because of the inhibition of endogenous synthesis of glutamine. After treatment of the cells with or without MSX, the cells were used for measurement of glutamate uptake.

Glutamate uptake measurement: Uptake measurement was 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 glutamic acid. The composition of the uptake buffer was as previously described (Thongsong, 2012[b]). For sodium-dependent glutamate uptake, the uptake of 5 µM glutamate was measured in the presence of sodium chloride. For sodium-independent glutamate uptake, the uptake of 5 µM glutamate was measured in the presence of N-methyl-D-glucamine chloride. The incubation was continued for a desired length of time, following which the uptake was terminated by aspirating the uptake medium. 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 glutamate uptake in BeWo cells

Since glutamate and glutamine are involved in many biosynthesis and metabolism processes, control of the concentration of these amino acids among the maternal-placenta-fetal unit is essential. Some studies described that maternal glutamine rather than glutamate was transferred across the placenta to the fetal circulation (Liechty et al., 1991; Vaughn et al., 1995). In the consistent study of Battaglia (2000), a large amount of glutamine was released from the placenta into fetal circulation and reversely,

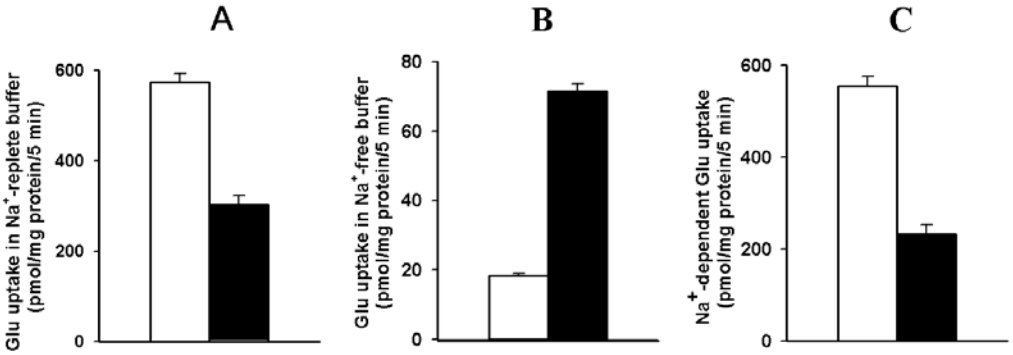

Figure 1 Influence of intracellular glutamine depletion with MSX treatment on glutamate uptake mediated by amino acid transport system EAATs and X-c. BeWo cells were treated in the absence of extracellular glutamine without (open bars) or with (filled bars) MSX (2 mM) for 16 hours. Uptake of 5 µM glutamate was then measured at 37^{0}C in the presence of sodium chloride or N-methyl-D-glucamine chloride for 5 min. Figure 1A represents the activity of the combined activity of EAATs and X-c; the uptake of glutamate measured in the sodium ion replete buffer. Figure 1B represents the activity of system X-c; the sodium-independent glutamate uptake measured in the presence of N-methyl-D-glucamine chloride. Figure 1C represents the activity of EAATs; the difference between the uptake in the presence of sodium chloride and the uptake in the presence of N-methyl-D-glucamine chloride.

glutamate was taken up by the placenta from fetal blood. To examine activity of glutamate transport systems in this study, confluent cells were treated in the absence of extracellular glutamine with or without MSX for 16 hours and the uptake of glutamate was then measured in the presence of sodium chloride or N-methyl-D-glucamine chloride. The uptake of glutamate measured in the sodium ion replete buffer represents the combined activity of EAATs and X-c (Fig 1A). The sodium-independent glutamate uptake measured in the presence of N-methyl-D-glucamine chloride represents the activity of system X-c (Fig 1B). The difference between the uptake in the presence of sodium chloride and the uptake in the presence of N-methyl-D-glucamine chloride represents the activity of EAATs (Fig 1C). These data involve both sodium-dependent and sodium-independent manner of glutamate uptake in the control cells. In addition, they demonstrate and conclude that the depletion of intracellular glutamine by MSX treatment decreased the sodium-dependent glutamate uptake (Fig. 1C) as the activity of system EAATs by ~60%, whereas the sodium-independent glutamate uptake as the activity of system X-c increased by ~350% (Fig 1B) under identical conditions. To explain the decreasing system EAATs activity, Novak et al. (2001) demonstrated that EAAT2 played a vital role under some conditions, especially in conditions of amino acid depletion. This transporter was detected in the syncytiotrophoblast and could be involved in transporting into or out of this cell (Noorlander et al., 2004). Thus, intracellular glutamine is obligatory for maintenance of optimal activity of system EAATs in the BeWo cells and the depletion of glutamine in the cells induces stress leading to the increase in sodium-independent glutamate uptake via the glutamate/cystine exchanger. According to my previous reports, the influence of intracellular glutamine depletion in placental cells on the regulation of amino acid transport was not limited to specific amino acid (Thongsong, 2012[a]) and depended on amino acid transport systems (Thongsong, 2012[b], 2013).

To speculate what the mechanism of MSX action on system X-c is, my previous experiments had shown that the kinetic pattern and uptake activity of system A inhibition were associated with MSX treatment without changes in steady-state levels of mRNA specific for system A (Thongsong, 2012[b], 2013). In contrast, the steady state levels of xCT mRNA were elevated by MSX treatment (personal communication with Miyauchi), showing that the increase in X-c activity seen with MSX treatment is due to enhanced *de novo* synthesis of the transporter protein. Therefore, this *in vitro* study on mediating uptake by glutamate transport systems under condition of intracellular glutamine depletion in BeWo cells may provide more insight about the role of the glutamate transporters in transyncytial and maternal-placental-fetal nutrient transport.

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