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IGF regulation of neutral amino acid transport in the BeWo choriocarcinoma cell line (b30 clone): Evidence for MAP kinase-dependent and MAP kinase-independent mechanisms

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Abstract

Objective: IGF-1 and IGF-1 receptors are major determinants of fetal growth and are expressed primarily on the maternal-facing surface of the syncytiotrophoblast cell membrane in the human placenta. IGF-1 regulates fetal growth, in part, by regulating amino acid transport across the placenta. The objective of these studies was to study the role of IGF-1 and its signaling pathway in regulating neutral amino acid transport in a human trophoblast cell culture model.
Design: The regulation of neutral amino acid transport by IGF-1 was studied in cultured BeWo^{b30} choriocarcinoma cells using the non-metabolizing amino acid analog, [^{3}H]-α-aminoisobutyric acid (AIB). Transport in the absence of Na was used to distinguish system L from total AIB transport. Similarly, Na-dependent transport in the presence of excess methyl-AIB (MeAIB) permitted discrimination of systems A (MeAIB-sensitive) and ASC (MeAIB-insensitive). Specific inhibitors of intracellular signaling pathways were then used to determine the signaling pathway utilized by IGFs to regulate each amino acid transport system. Specificity of inhibition was assessed using specific markers of p70 S6 kinase activity and MAP kinase activation.
Results: Maximal stimulating concentrations of IGF-I (100 ng/ml) stimulated AIB transport by 30–40% exclusively through system A. Wortmannin (100 nM), an inhibitor of PI-3-kinase activity, inhibited all IGF-I-stimulated transport. Rapamycin (100 ng/ml), an inhibitor of p70 S6 kinase, and bisindolylmaleimide, an inhibitor of protein kinase C (PKC), had no effect. PD-098059 (50 μM), an inhibitor of MAP kinase activation, inhibited 20–30% of basal AIB transport but did not inhibit IGF-I-stimulated transport under the conditions studied. IGF-1 did not increase steady state mRNA levels of the system A transporters, SNAT1 and SNAT2, suggesting IGF-1 stimulates transport via post-transcriptional mechanisms.
Conclusions: These data demonstrate that IGF-I stimulates neutral amino acid transport system A by a PI3-kinase dependent, post-transcriptional pathway in the BeWo^{b30} cell line. Additionally, system A activity appear to be sensitive to MAP kinase-dependent pathways not regulated by IGFs.

Keywords: IGF; Placenta; Transport; Trophoblast; Choriocarcinoma

1. Introduction

Fetal growth is dependent on the placental transport of nutrients derived from the maternal circulation. Specific transport systems have been identified in the placenta that regulate the transport of a variety of nutrients including vitamins, calcium, fatty acids, and glucose, the primary energy source during fetal life [1–6]. Additionally, specific transport systems have been identified involved in the transport of amino acids [7–9]. Neutral amino acids are transported primarily

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via Na^+-dependent systems A and ASC, and Na^+-independent system L. The hormonal regulation of neutral amino acid transport in a variety of tissues has been studied, including the placenta. Karl demonstrated that both insulin and IGF-I regulate neutral amino acid transport in the normal trophoblast, each apparently via their respective receptors [10]. He further demonstrated that IGF-I specifically stimulates Na^+-dependent system A transport, and not systems ASC or L in the undifferentiated cytotrophoblast. Using metabolic inhibitors, his work further suggested that IGF-stimulated transport in the normal cytotrophoblast is PKC-dependent and PKA-independent. We have shown that the type I IGF receptor is expressed on the maternal-facing surface of the syncytiotrophoblast, similar to the insulin receptor, suggesting that the differentiated syncytiotrophoblast is regulated by IGFs derived from the maternal circulation [11]. Discriminating insulin and IGF signaling pathways in the trophoblast is complicated by the fact that a significant percentage of insulin and IGF receptors exist in the form of Ins/IGF hybrids [12–15]. As many as 70–80% of the placental type I IGF receptor has been shown to exist in hybrid form [16,17]. These receptors are thought to act primarily as high affinity IGF-1 receptors based on binding affinity, although signaling specificity may be influenced by the intracellular domain of the insulin receptor β-subunit. The physiologic role of hybrid receptors has not been defined but they are likely to have a significant effect on the specificity of insulin and IGF signaling at the cellular level.

The BeWo choriocarcinoma cell line (b30 clone) is a trophoblast cell line of human origin. When exposed to forskolin intracellular levels of cAMP are increased resulting in morphological and biochemical differentiation, analogous to the normal trophoblast [18]. They have subsequently been shown to express neutral amino acid transport systems analogous to the normal trophoblast [19]. The relative contributions of systems A, ASC and L are similar in the undifferentiated normal trophoblast and $BeWo^{b30}$ cell. Furthermore, system ASC expression declines to almost undetectable levels during cell differentiation. This suggests that the $BeWo^{b30}$ cell line shares some important functional characteristics with the normal trophoblast and suggests it may be a useful model to study trophoblast transport function. We have also shown that the $BeWo^{b30}$ cell line expresses the type I IGF receptor [20]. Receptor expression increases during $BeWo^{b30}$ cell differentiation consistent with important roles for IGFs in the regulation of normal trophoblast metabolism. Additionally, this cell line does not express insulin receptors detectable by cell binding assays, and thus are a useful model to study IGF receptor-mediated regulation in the absence of endogenous insulin receptors and IGF/insulin receptor hybrids. We have therefore begun to develop the $BeWo^{b30}$ cell as a model system to study IGF regulated amino acid transport in the human trophoblast.

2. Methods and materials

2.1. Sources of material

The $BeWo^{b30}$ cell line was provided by Dr. A. Schwartz (St. Louis, MO). Human IGF-I was obtained from Bachem (Torrance, Calif.). [^{3}H]-AIB was obtained from Amersham, Inc. (Arlington Heights, IL) AIB and MeAIB were obtained from Sigma (St. Louis, MO). Wortmannin, PD-098059, rapamycin, and bisindolylmaleimide were obtained from Calbiochem (San Diego, CA). Earle's Balanced Salt solution (EBSS), Dulbecco's Modified Essential Medium (DMEM), fetal bovine serum (FBS), and antibiotics (penicillin/streptomycin) were obtained from Grand Island Biological Co. (GIBCO, Grand Island, NY).

2.2. Transport studies

AIB uptake by $BeWo^{b30}$ cells was performed in 24 well cluster trays as previously described [21]. Cells were grown in DMEM, 10% FBS in T-25 flasks until confluent. The cells were then split into 2×24 well cluster plates and grown until approximately 90% confluent. They were incubated overnight in serum-free media and washed 3 times in EBSS. The cells were then incubated in 1 ml EBSS with or without IGF-I (100 ng/ml) for 2 h. This also served to deplete amino acid pools within the cells. Uptake measurements were initiated by replacing Earle's solution with *N*-2-hydroxyethylpiperazine-*N*′-2-ethansulfonic acid (HEPES)-buffered KREB's solution containing 50 μM [^{3}H]-AIB (1μCi/well). The cells were incubated for 10 mins for each assay. Preliminary time course experiments demonstrated that uptake was linear up to this time point. Cells were disrupted by the addition of 0.25 ml of 1 M NaOH SDS to each well followed by shaking for 30 min at 37 °C. The pH of each well was then neutralized with 26 μl concentrated HCl. The sample was removed from each well and added to vials containing 10 ml Optifluor (Packard Instruments, Downer's Grove, IL).

To discriminate between Na^+-dependent and Na^+-independent transporters, AIB uptake was measured in the presence of excess (50 mM) MeAIB or the absence of Na^+. The Na^+-dependent fraction was determined by subtraction of the Na^+-independent uptake.

2.3. Inhibition of MAP-kinase and p70 S6-kinase activation

Cells were grown under conditions identical to those described for amino acid transport studies and incubated