O-04

Effects of Epidermal Growth Factor and Insulin-like Growth Factor-I on Placental Amino Acids Transport Activities in Rats

Kenichiro Ono

Laboratory of Veterinary Clinical Pathobiology, Graduate School of Agricultural and Life Sciences, The University of Tokyo

Epidermal growth factor (EGF) and insulin-like growth factor-I (IGF-I) have been shown to stimulate proliferation and differentiation of various somatic cells, including placental trophoblasts and also to enhance fetal growth and development when maternally administered. Since an increase of the expression of placental EGF and IGF-I receptors in rat, mouse, and human with the gestation advanced, both EGF and IGF-I were considered to play pivotal roles on fetal growth by regulating some function of placental cells.

Amino acids are crucial importance for both maternal and fetal requirements of energy source and essential constituent of fetal mass during pregnancy. Impaired fetal and placental uptake of amino acids has been observed in several models of growth retardation in the rat. Amino acid is concentrated in the fetal side through active transport by amino acid transporters and is one of the important metabolic fuels for the fetal growth.

Therefore, at first plasma amino acid concentrations in mothers and fetuses were measured as an index of uphill transport across the placenta associated with EGF and IGF-I. The EGF administration at the concentration of 0, 0.1, or 0.2 μ g/g to pregnant rats from day 18 to 21 of gestation apparently increased fetal/maternal ratio of serum proline concentration and also fetal growth in EGF dose-dependent manner. When IGF-I in doses of 0, 1, 2, and 4 μg/g were administrated, the ratio of leucine, isoleucine, tryptophan, phenylalanine, tyrosine and also fetal growth significantly increased with a dose-dependent manner. These results suggested that EGF and IGF-I enhanced fetal growth by, as one of its possible mechanisms, promoting placental activity to transfer some amino acid

supplies from the mother to the fetus in late pregnancy.

Thus, transport activities for fundamental amino acids, especially proline, leucine, and alanine into placental microvillous membrane vesicles (PMVs), which were prepared from pregnant rats administered with EGF or IGF-I at different doses, were kinetically analysed. These uptakes showed saturable hyperbolic curves that obeyed Michaelis-Menten kinetics in any doses of EGF and IGF-I. The values of Michaelis constant (Km) and maximum velocity (Vmax) were calculated. In EGF treatment, Vmax values for proline uptake into PMV were remarkably increased compared with those in control PMV, whereas no significant differences in Vmax and Km values for sodium-dependent leucine, and sodium-dependent (A-1 and A-2) and -independent alanine uptake were observed. On the other hand, Km and Vmax values for proline uptake into PMVs in IGF-I treatment at 1 μg were remarkably higher than those in control rats. A statistically significant increase of Km value for sodium-independent alanine uptake was also detected in IGF-I treatment at 2 μg, while no significant differences in those values were observed in sodium-independent leucine and sodium-dependent alanine uptake. These results suggested that EGF and also IGF-I enhance placental activities of proline uptake and supplies these amino acids from mother to fetuses in late pregnancy.

Transport of amino acid across plasma membrane is mediated carriers or transporters. Among them, system L amino acid transport activity is the most important and consists of two subunits, one of which is the heavy chain of the cell surface antigen 4F2 and the other is L-type amino acid transporter1 (LAT1) or L-type amino acid transporter2 (LAT2). The heterodimeric formation of LAT1 or LAT2 to 4F2hc is necessary for the transport function. Therefore, mRNA expression of system L amino acid transporter was investigated in late-term rat placentas after administration of IGF-I. The significant increase in mRNA expression of rLAT2 and slightly increase in that of r4F2hc were observed in placenta with IGF-I treatment in the dose of 2 μg. These results indicated that IGF-I regulated the expression of the system L subunits, especially LAT2, which formed LAT2/4F2hc heterodimer, resulted in the enhancement of transport activity for the neutral amino acids via the placenta.

In conclusion, it is suggested that EGF and IGF-I regulate the expression of amino acid transporters, which enhance amino acids uptake in placenta, to support fetal growth.