Expression of glucose transporter and glucose uptake in human oocytes and preimplantation embryos

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The expression of glucose transporters 1, 2, 3 and 4 was evaluated in human oocytes and polyploid preimplantation embryos. Only glucose transporter 1 (GLUT-1) isoform was detected in oocytes and in 2-12-cell stage embryos. Glucose uptake was markedly increased in embryos as compared to oocytes (19.7 ± 3.4 pmol/min/embryo and 2.3 ± 0.3 pmol/min/oocyte), and GLUT-1 was inhibited by cytochalasin B. These results suggest that, although GLUT-1 is expressed in human oocytes and throughout preimplantation development, its function in mediating the rise in glucose uptake is triggered following fertilization.

Keywords: glucose transporter/glucose uptake/GLUT-1/oocytes/preimplantation embryos

Introduction

The relationship between the developmental competence of the preimplantation embryo and its metabolism has been the subject of extensive studies (Barnett and Bavister, 1996). The response of embryos to different energy substrates may provide an insight into their metabolic requirements. Thus, recent studies in mouse oocytes and preimplantation embryos have demonstrated the continuous expression of the glucose transporter GLUT-1 isoform, while GLUT-2 and GLUT-3 were detectable only at the blastocyst stage (Hogan et al., 1991; Aghayan et al., 1993; Chi et al., 1993; Pantaleon et al., 1997). In addition, during preimplantation development, the utilization of glucose and the activity of hexokinase increase in an exponential manner (Takahazu et al., 1994).

Human early preimplantation embryos develop better in glucose-free medium (Conaghan et al., 1993; Bavister, 1995; Quinn et al., 1995). The inability of glucose to support invivo development of early stage embryos stimulated our interest in evaluating the expression of glucose transporters and glucose uptake in human oocytes and preimplantation embryos.

Materials and methods

Gametes and preimplantation embryos

Oocytes and embryos were obtained following routine in vitro fertilization (IVF) treatments performed in Bikur Cholim Hospital IVF Unit. Unfertilized oocytes and 2-12-cell stage polyploid preimplantation embryos were collected 48–72 h after oocyte retrieval and insemination. For ethical reasons, experiments with normal embryos were not performed.

Gametes and embryos were maintained in glucose- and phosphate-free medium (P1 medium, Irvine Scientific, Santa Ana, CA, USA). The medium was supplemented with 10% (v/v) synthetic serum substitute (Irvine Scientific). Control oocytes were subjected to two cycles of freeze-thaw at −70°C. For immunoblot analysis, oocytes and embryos were collected and cryopreserved under liquid nitrogen using the ultra-rapid freezing method (Feichtinger et al., 1991).

Semen samples were obtained at random from men who had been referred to our Fertility Laboratory for sperm analysis. The samples were processed by the discontinuous Percoll gradient as previously described (Van Der Zwalmen et al., 1991). After washing in phosphate-buffered saline (PBS) pH 7.4, the samples were centrifuged for 5 min at 400 g. Approximately 10⁷ motile sperm cells were used for immunoblot experiments.

The study protocol was reviewed and approved by the Bikur Cholim Hospital Review Board for Clinical Research.

Anti-GLUT antibodies

Polyclonal anti-GLUT antibodies were produced in rabbits against synthetic peptides (18 amino acids). Antibodies to GLUT-1 and GLUT-3 were directed against the C-terminal domain. There was no cross reactivity between the antibodies. Anti GLUT-4 antibodies were raised against the amino terminal of the peptide. Anti GLUT-2 antibodies were a generous gift from Dr B. Thorens (Thorens et al., 1988). All antibodies were used at a dilution of 1:10⁵.

Western blot analysis

Oocytes and 2-12-cell stage embryos were thawed, washed extensively in PBS, and resuspended in Laemmi sample buffer (Laemmi, 1970). Groups of 20–30 oocytes or 10–15 embryos were subjected to one-dimensional 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis, using a mini-gel system (Bio-Rad laboratories, Richmond, CA, USA). The proteins were electrotransferred to nitrocellulose membranes at 4°C for 1 h. The membranes were washed for 10 min in double-distilled water, 20 min in Tris-buffered saline (TBS: 20 mM Tris, 150 mM NaCl, pH 7.4) with 0.1% Tween-20 followed by an overnight incubation in 5% non-fat milk. Membranes were incubated with anti-GLUT antibodies (1:500 dilution) for 2 h at room temperature and then for 20 min with horseradish peroxidase conjugated to goat anti-rabbit IgG (Sigma Chemical Co., St Louis, MO, USA). The filters were finally washed for 30 min in TBS containing 0.05% Tween-20 and incubated for 1 min with enhanced chemiluminescence reagents (ECL Western blotting detecting system, Amersham, Chalfont, Bucks, UK). Exposure time to Kodak X-OMATAR film was 5–30 s. Rat brain, liver and muscle membranes were used as a positive control, and human sperm as a negative control.
The purpose of our study was to evaluate the expression of GLUT-1, -2, -3 and -4 in human oocytes and early stage preimplantation embryos. The developmental expression of glucose transporters has been studied in mice (Hogan et al., 1991; Aghayan et al., 1992; Morita et al., 1992; Chi et al., 1993; Pantaleon et al., 1997), but to the best of our knowledge has not been reported in humans hitherto.

Although the antibodies to GLUT-1–4 all exhibited strong reactions in control tissues on Western blot analysis, only the GLUT-1 isoform was detected in oocytes and was expressed at least throughout the first two to four cleavage divisions of the preimplantation embryo.

Due to stringent ethical safeguards on research with normal human embryos, our study was performed with polyploid embryos which have restricted developmental capacity. This limited our ability to evaluate the developmental expression of GLUT isoforms during the more advanced stages of human preimplantation development. Thus, whether GLUT-2, -3 and -4 transporters are expressed in human blastocysts remains unanswered. Because GLUT-1 is already expressed in unfertilized oocytes, our findings from polyploid embryos may be extrapolatable to the normal diploid preimplantation human embryo.

Previous studies with murine oocytes and preimplantation embryos similarly demonstrated the presence of GLUT-1 as early as the unfertilized oocyte, whereas GLUT-2 and -3 were expressed at the blastocyst stage and GLUT-4 was undetectable (Hogan et al., 1991; Aghayan et al., 1992; Morita et al., 1992; Pantaleon et al., 1997). As indicated in our study GLUT-4, which is the major insulin-regulatable isoform, is not expressed in the early human preimplantation embryo. This suggests that in the early stages of development, glucose uptake is insulin insensitive. In fact, in mouse embryos, insulin receptors have not been detected until the 64-cell stage (Schultz et al., 1993).

Fertilization is accompanied by polar body extrusion. Thus, the total surface area of the zygote and of the early cleavage stage embryo is smaller than that of the unfertilized oocyte. Therefore, the 8-fold increase in glucose uptake observed in embryos as compared to oocytes may indicate their increasing metabolic needs. Moreover, this may also indicate the operation of a switch in substrate preference from pyruvate to glucose. Comparable rates of increased glucose uptake in mouse oocytes and 2–8-cell embryos have been reported (Morita et al., 1992). In addition, a recent study has demonstrated that hexokinase activity in human embryos became elevated during development into blastocysts (Martin et al., 1993). On the basis of these findings it may be suggested that although in vivo the early development of the human zygote is not dependent on glucose, following fertilization an increased uptake of glucose via the GLUT-1 transporter occurs. Furthermore, preimplantation development may also be associated with increased activity of the glycolytic pathway (Martin et al., 1993).

Although 2-deoxyglucose is known to be toxic in some conditions, this is unlikely to be relevant to this study. ATP depletion occurs after incubation with >1 mM 2-deoxyglucose for several hours, and is correlated with a significant decrease in the active transport of the hexose (Sasson et al., 1997). In our experiments the substrate concentration was 0.1 mM and its uptake was linear over the period studied.

In summary, these findings suggest that the glucose trans-
porter GLUT-1, rather than GLUT-2, -3 or -4, is expressed in oocytes and early preimplantation human embryos. Subsequent experiments aim to evaluate transcripts of GLUT transporters using polymerase chain reaction techniques.

References

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