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Differential Effect of Cupric Ions on the *In Vitro* Utilization of Glucose by Blastocysts and Pancreatic Islets

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ABSTRACT

To evaluate a possible interference of cupric ions on the trophoblast cell metabolism, glucose utilization was studied in mouse blastocysts delayed in utero and later activated by oestradiol for 18 h. Isolated pancreatic islets from guinea pigs were used for comparative purposes. The pancreatic islets were not affected when the copper concentration was 0.1 mmol/l whereas for the blastocysts a marked effect was found already at a concentration of 0.01 mmol/l. This indicates that copper concentrations even lower than that of the uterine secretion in presence of a Cu-IUD might affect different enzymes linked with the glucose metabolism of the trophoblast cells.

INTRODUCTION

Blastocysts of the rat, mouse and rabbit when exposed in vitro to cuprous or cupric ions are detrimentally effected (5,7,8,13,20), although the degree of the damage is modified by the amount of albumin present in the incubation medium (13) making comparisons between concentrations in utero and in vitro difficult. The blastocyst sensitivity to copper develops gradually, probably appearing at the period of transition from the morula to the blastocyst stage (7,23). Furthermore, the influence of the copper ions seems to be reversible since the rat blastocysts transferred from a copper containing to a non-treated uterine horn developed normally (6).

Although the gradual appearance of the copper effect and its reversibility are compatible with many types of adverse mechanisms, a blocking of some vital process such as the uptake or metabolism of nutrients seems likely, since it is known that the achievment of the blastocyst stage is accompanied by a rapid increase in metabolic rate of the zygote (4). Glucose is one nutrient efficiently utilized by cells. The blastocyst at initiation of implantation increases its capacity of glucose utilization (21). To evaluate a possible interference by copper on the cell metabolism, glucose utilization was studied in isolated mouse blastocysts. Isolated islets of guinea pigs were similarly chosen for comparative studies since their rate of glucose utilization is well

known from previous studies (25) and since they have a similar size and shape as the blastocyst.

MATERIALS AND METHODS

The blastocysts were obtained from NMRI mice, which had been ovariectomized and injected s.c. with 0.02 ml Depo-Provera (containing 1 mg medroxiprogesteron acetate; Upjohn Co., Kalamazoo, USA) about a week previously in order to obtain an experimental delay of implantation (3,15). The mice in delay were injected s.c. with 0.1 µg oestradiol 18h before the experiment. Animals were killed by an overdose of Mebumal (ACO, Stockholm, Sweden), the uterus flushed with Hanks solution containing 2 mg albumin/ml, and the blastocysts collected with a small pipette.

The islets were isolated aseptically from pancreatic glands of normal guinea pigs using the collagenase digestion technique according to the method of Howell and Taylor (14). The islets were then maintained in tissue culture for three days (1) prior to the experimental procedure.

Glucose utilization was measured using a technique previously applied to isolated islets of Langerhans (2,25), in which the formation of ^{3}H-water from 5-^{3}H-glucose is determined. This reflects glycolysis and the hexose monophosphate shunt (HMPS), but not the use of glucose for glycogen synthesis. Batches of 20-30 blastocysts or 15 isolated pancreatic islets were incubated in small vials (16), containing 20 µl of Krebs-Ringer bicarbonate buffer, supplemented with 2 mg/ml bovine plasma albumin, fraction V (Armour Pharmaceutical Co., Eastbourne, U.K.), 16.7 mmol/l glucose and the labelled substrate 5-^{3}H-glucose (The Radiochemical Centre, Amersham, U.K.) of specific activity 6 Ci/mmol. Copper chloride was dissolved in redistilled water and added to the buffer in a final concentration of cupric ions of 100 or 10 µmol/l. In some vials cupric ions were omitted. The vials were placed inside scintillation flasks containing 0.5 ml redistilled water and sealed with a rubber membrane. Incubation was performed for 60 minutes at +37°C in a shaker and in a gas phase of 95% O_2:5% CO_2. The metabolism of the blastocysts or the islets was then stopped by an injection of 10 µl 0.2 mol/l HCl through the rubber membrane. A further incubation at room temperature was carried out to allow the tritiated water formed by the living material to equilibrate with the water in the scintillation flasks. Controls without biological material or pancreatic islets were included in each experiment to allow correction for the blank. The radioactivity in the water was then measured by liquid scintillation spectrometry. The recovery of ^{3}H-water during the equilibration period was tested by measurements of the diffusion of a known amount of ^{3}H-water, and was in this system found to be approximately 70%.

The rate of glucose utilization was calculated according to a formula given

by Ashcroft et al (2) and expressed as either pmol of glucose utilized per blastocyst per hour or as pmol per µg islet dry weight per hour.

RESULTS

The implanting blastocysts, obtained 18 hrs after the injection of oestrogen, in contrast to pancreatic islets, showed a markedly decreased utilization of glucose when cupric ions were added to the incubation medium (Table 1).

The basal utilization of glucose (at 16.7 mmol/l), calculated on a dry weight basis, was 113 pmol/µg/h for the pancreatic islets, and 315 pmol/µg/h for the blastocysts, considering that a mouse blastocyst in delayed implantation has an average dry weight of 35 ng (12).

TABLE 1

Effect of Cu^{2+} on the in vitro glucose utilization of mouse blastocysts and guinea pig pancreatic islets - determined as the formation of ^3H_2O from 5-^{3}H-glucose

Concentration of $CuCl_2$ (mmol/l)		Implanting blastocysts (pmol/blastocyst/h)		Pancreatic islets (pmol/µg/h)	
0		11.1 ± 1.1	(8)	113.1 ± 10.0	(9)
	$P < 0.01$				
0.01		6.5 ± 0.9	(7)	131.7 ± 8.9	(6)
	$P < 0.05$				
0.1		4.0 ± 0.6	(5)	124.6 ± 8.6	(11)

The results are given as mean values ± S.E.M., and with the number of experiments in each group given in parentheses.

DISCUSSION

The degree of development among the uterine zygotes during a normal implantation in the mouse differs substantially(11). Since the functional changes in the zygotes are rapid, this developmental variability renders observations on metabolic changes less valid. In an attempt to obtain a better synchrony of zygote development, the blastocysts were recovered from animals in an experimentally delayed implantation. At implantation, these blastocysts display similar properties as do normal blastocysts.

The present results show that the glucose utilization of the blastocysts is markedly affected by cupric ions at a concentration of only 10 µmol/l. This inhibitory level of copper corresponds to the concentration of about 10 µmol/l that Cross (8) has found to produce a decrease in membrane ion transport, and to the concentration of about 20 µmol/l which Holland and Pike (13) have found to influence the in vitro development of mouse embryos. Considering that the copper concentration in the uterine secretion from women carrying a Cu-IUD is

approximately 700 μmol/l (13), the reported inhibition of the blastocyst metabolism in vitro could well be active also in vivo in spite of a protecting effect by proteins in the secretion.

The fluxes of glucose after its phosphorylation in a cell are generally mediated via glycolysis, the hexose monophosphate shunt (HMPS), and glycogen synthesis. Anaerobic glycolysis is the main pathway, while the contribution of the HMPS varies considerably in different cell types, from approximately 0 to at least 30 per cent (24). The HMPS in B-cells of mouse pancreatic islets accounts for only a few per cent of the total glucose utilization, even at a high glucose concentration (2). In the cells of a blastocyst at an early stage of implantation and with a high synthesizing activity, this percentage may be much higher. The flux of glucose into glycogen is similarly low in the pancreatic islet cells, estimated at less than 10 per cent of the glucose utilization (2). Although we have not measured the rate of glycogen synthesis in the trophoblasts, morphological studies have indicated that a rapid increase in glycogen granules occurs in these cells 18 hours after activation of the implanting blastocyst (21). Thus, it appears that the HMPS and the glycogenic pathway are quantitatively more important in the blastocyst cells than in the pancreatic islet cells.

The mechanism of a copper-induced inhibition of the glucose utilization could involve some or all of these metabolic pathways. A plausible mode of interference of cupric ions with glucose metabolicm could be as an antagonist to other divalent ions, e.g. zinc and magnesium ions, in some of the glycolytic enzymatic reactions (22). A step that might be affected in this way is the enolase reaction, where a part of the tritiated water in the determination of glucose utilization is obtained.

An influence of copper on the HMPS is also possible. Earlier findings in erythrocytes implicated a copper-induced decrease of the HMPS activity, caused by inhibition of the enzymes glucose-6-phosphate dehydrogenase and glutathion reductase (9,10,17). However, it was later shown that copper, at a concentration less than 100 μmol/l, was actually stimulating the HMPS, and that the decreased amount of reduced glutathion previously mentioned, was due to an accelerated oxidation of this compound (18). Indeed, the overall mode of action of cupric ions on the HMPS seems to be complex and difficult to fully interpret.

Glycogen synthetase in human endometrial cells has been reported to be stimulated by copper in utero (19). If this effect is also present in trophoblast and islet cells, a copper induced increase of glycogen synthesis could contribute to a reduced rate of glucose utilization in our assay, by making less 5-^{3}H-glucose available to glycolysis.

It still remains to be explained why the glucose utilization of the blastocysts but not of the islets, was affected by cupric ions. It is possible that

differences in glucose handling, where the blastocyst may have higher fluxes into glycogen synthesis and the HMPS, are responsible for this opposite reaction to copper. Moreover, it is worthy of note that a pancreatic islet consists of a vasculated, compact mass of a few thousand highly specialized cells, while a blastocyst at this stage only contains a shell of approximately 50 undifferentiated cells around the blastocele. Thus, penetration of cupric ions into the tissue, as well as transport across the cell membranes, may be factors that differ between these two organs. However, it can be concluded that cupric ions markedly affect the glucose utilization of the blastocyst at a copper concentration that is lower than that of the uterine secretion from a woman with a copper-containing intrauterine device.

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