SHORT COMMUNICATION

HUMAN PLACENTAL TRANSFER OF GLUCAGON

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SUMMARY

1. The permeability of human chorion laeve in vitro to $^{125}$I-labelled glucagon was investigated. The problem caused by degradation of $^{125}$I-labelled glucagon in the preparation and a means to prevent this are described.

2. The human chorion laeve was found to be impermeable to iodinated glucagon.

Key words: glucagon, human placental transfer.

Recent studies measuring fetal cerebral oxygen and glucose uptake in sheep indicate that the requirements of the fetal brain are comparable to those of the adult (Makowski, Schneider, Tsoulos, Colwill, Battaglia & Meschia, 1972). Since oxygen and glucose contents of arterial blood perfusing the fetal head are considerably less than in the adult animal, there would appear to be a low margin of safety with respect to oxygen and glucose supply to the fetal brain. During periods of stress in utero the requirements of the fetal brain for glucose might be met by the role of glucagon as the regulator of nutrient mobilization. The role of maternal glucagon, if any, would depend upon its ability to cross the placenta. The permeability of human chorion to $^{125}$I-labelled glucagon is described below.

METHODS

The method of Battaglia, Hellegers, Meschia & Barron (1962) for investigation of chorion in vitro as a membrane system provides a quantitative description of the permeability of the placental membrane. The tissue referred to as chorion laeve and amnion represents a piece of intact membranous portion of the placenta taken between 2 and 6 cm from the edge of the 'cake' and obtained within 5 min of delivery. The method provides for the mounting of tissue as a vertical membrane which separates two fluid chambers, each containing 10 ml of the...
bicarbonate buffered solution of Gey & Gey (1936), supplemented with glucose (2.78 mmol/l), trasylol (200 000 units/100 ml) and bovine serum albumin (100 mg/100 ml).

Two preparations from each of six placentas were studied. In the experiments involving placentas numbers 1 and 2 125I-labelled glucagon (150 pg/ml) was added to the solution in contact with the maternal surface. In the experiments involving placentas numbers 3 and 4, the glucagon concentration was increased to 1500 pg/ml. In the experiments involving placentas numbers 5 and 6 labelled glucagon (1500 pg/ml) was added to the solution in contact with the fetal surface. The incubations in vitro were carried out in a constant temperature water bath at 37°C. In each experiment 0.2 ml samples were taken from the solution in contact with the fetal and maternal sides of the membrane at 60 min intervals throughout the 180 min of the incubation. The samples were subjected to chromatoelectrophoresis by a modification of the technique of Berson, Yalow, Bauman, Rothschild & Newerly (1956). The chromatoelectrophoresis was carried out at room temperature in barbital buffer, pH 8.6 (0.1 mol/l), on Whatman 3 MM paper strips (4 cm x 48 cm). Bromophenol blue (5 μl; 10 g/l) was added to each

![Paper radiochromatoelectrophoretograms of 125I-labelled glucagon as a function of time of contact with the maternal and fetal sides of human chorion. A, Unchanged hormone; B, hormone fragments; C, free iodide. Arrowheads denote the origin.](image-url)
sample to act as a marker, and 25 µl of the resultant mixture was applied to each paper strip as a 1 cm band, 3 cm from the cathode. A constant 500 V was applied to the system and run until the bromophenol blue markers had travelled 10 cm to the centre of the strips. The strips were then dried at 60°C, cut into 20 cm x 1 cm portions and counted in a Wallac Auto-gamma counter for 1 min, with background count 25–30 c.p.m.

The 125I-labelled glucagon (from lot GLF599A, Lilly Research Laboratories, Indianapolis, Ind., U.S.A.) used in this investigation was prepared by a modification of the procedure of Greenwood, Hunter & Glover (1963) and was partially purified by salting out with saturated sodium chloride in the presence of albumin. Further purification was carried out by the precipitation of impurities with 96% ethanol, leaving the pure (97–99%) hormone in the supernatant (Heding, 1971). The specific radioactivities of 125I-labelled glucagon prepared in this way ranged from 311 to 484 mCi/mg.

RESULTS

The results from the six experiments are similar. During the chromatoelectrophoresis the undamaged hormone remains at the origin, while the damaged hormone, i.e. small peptides, and free iodide migrate to differing degrees. Under the conditions described above the hormone fragments show at 8–15 cm and the free iodide shows from 15 to 20 cm along the strip. The results from one preparation from placenta number 1 are represented in Fig. 1. Zone C on the chromatoelectrophoresis strip containing labelled hormone corresponds to the area of activity on a similar strip containing only Na125I, run at the same time in the same system.

On the maternal side there is a decrease of the pure hormone on the origin with time. There is an increase in hormone fragments, probably due to degradation caused by placental enzymes. On the fetal side no pure undamaged glucagon appears during the 180 min of the incubation, and hormone fragments and free iodide increase with time. Additional experiments to determine the sensitivity of the method showed that 1.25 pg of 125I-labelled glucagon/ml is the lowest concentration of labelled hormone which, at the origin, can be significantly (P<0.01) detected in comparison with the background count.

DISCUSSION

The bulk of transfer of nutrients from mother to fetus probably takes place across the chorion frondosum (Seeds, 1968). While the absence of a perfusing circulation must diminish the amount of transfer those portions of chorion laeve that are distant from the chorion frondosum, the most significant exchange across the chorion laeve is likely to occur in those areas adjacent to the chorion frondosum. Hence the tissues studied were taken from this region. Chorion laeve was not studied as an isolated tissue layer because stripping off the amnion may be difficult and chorion could thus be damaged. Earlier work (Battaglia & Hellegers, 1964; Moore, Hellegers & Battaglia, 1966) had shown that both tissue layers, chorion and amnion, are permeable to stachyhexose (molecular weight 666) and impermeable to an inulin preparation of molecular weight 990. Although compounds of small molecular size diffuse more rapidly across the thinner amnion, the molecular weight range which demarcates the permeability of these tissues to oligosaccharides is similar in both. Chez, Mintz, Horger & Hutchinson (1970) purported to demonstrate bi-directional placental transfer of 131I-labelled...
glucagon in the pregnant rhesus monkey. However, the demonstration that chorion laeve in vitro is impermeable to glucagon is in agreement with the findings in vivo of Johnston, Bloom, Greene & Beard (1972).

In preliminary experiments when $^{125}$I-labelled glucagon alone was added to the medium used excessive degradation of the $^{125}$I-labelled glucagon occurred. Eisentraut, Whissen & Unger (1968) and Nars, Stahl, Dambacher, Baumann & Girard (1972) demonstrated that incubation of labelled glucagon with plasma results in marked damage to the labelled molecule. Trasylol, a proteinase inhibitor, considerably inhibits plasma-induced degradation. Some impure enzyme-rich albumin preparations are also able to degrade tracer and glucagon standards. When trasylol was added to the incubation medium on both sides of the placental membrane the degradation decreased to less than 1% before contact with the membrane.

Glucose is generally considered to be the major energy substrate in the mammalian fetus, but recent experiments (Beatty, Young, Dwyer & Bocek, 1972) suggest the possibility that the fetus utilizes non-carbohydrates as a major energy source. The work of Tsoulos, Schneider, Colwill, Meschia, Makowski & Battaglia (1972) suggests that the amount of glucose acquired by the fetal lamb via the umbilical circulation can satisfy, at most, one-half of the total fetal aerobic needs. Thus the fetal brain seems much more dependent on glucose than do other fetal organs, and it may be particularly vulnerable to periods of hypoglycaemia. The actions of glucagon and insulin oppose each other in a net sense with respect to hepatic glucose balance. It has been suggested that a relative insufficiency of glucagon, of phosphorylase and of gluconeogenic enzymes during fetal life would favour the net accumulation of hepatic glycogen and the synthesis of protein (Foa, 1972). As glucagon, like insulin, is extremely important in the moment to moment control of the homeostasis of glucose and certain amino acids, and as maternal glucagon does not cross the placenta, the need to investigate the development of glucagon production in the fetus becomes apparent.

REFERENCES


Human placental transfer of glucagon


