Effects of exogenous insulin on placental transfer of maternal glucose to the rat fetus

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Summary. There is controversy concerning the possible modulation of glucose transfer to the fetus by insulin acting on the maternal side of the placenta. To study this question, 20.5 day pregnant rats were infused simultaneously with (U-¹⁴C)-D-glucose (via the jugular vein) and with different doses of insulin (via the left uterine artery) so that placentas from the left uterine horn were exposed to a higher insulin concentration than those from the right uterine horn. Placentas and fetuses from each uterine side were processed separately. No differences were detected in total blood radioactivity, plasma-¹⁴C-glucose, -¹⁴C-lactate, -glucose, or -radioimmunoassayable insulin in fetuses from the left versus the right uterine horn. Total placenta radioactivity and ¹⁴C-glycogen were also similar

in the left and right uterine sides at all insulin doses studied. Infusion of insulin (66 mU/min) to the pregnant rat caused hyperinsulinaemia and hypoglycaemia, decreased blood total radioactivity and plasma ¹⁴C-glucose, and increased plasma ¹⁴C-lactate in the mother. The level of fetal plasma ¹⁴C-glucose paralleled that of the mother. It is concluded that in the rat, placental glucose uptake, its transfer to the fetus, and fetal glucose utilization are not directly affected by maternal circulating insulin. Metabolic changes occurring in fetuses of hyperinsulinaemic mothers are secondary to the decreased availability of glucose.

Key words: Placenta, glucose transfer, insulin, rat fetus.

Maternal glucose is a basic fuel for fetal oxidative metabolism [1, 2]. Fetal circulating glucose parallels alterations in maternal glycaemia [3, 4] because glucose transfer across the placenta takes place by facilitated diffusion [5, 6] which depends on maternal glucose concentration [7]. Infusion of insulin into the sheep fetus reportedly increases fetal glucose utilization and its uptake from the mother [8] and similar results have been found in the rat [9]. Since the placenta is known to contain insulin receptors [10–13] and maternal hyperinsulinaemia occurs during late pregnancy [14, 15], the question has been raised whether maternal insulin acting on the placenta could stimulate glucose transfer to the fetus. The effects of insulin on the placenta have been investigated in human [16–20] and in ruminant preparations [21–24] yielding both positive [16, 17, 19, 22, 23] and negative [18, 20, 21, 24] results. No studies have been performed on insulin effects on the rat placenta which contains a much lower number of insulin receptors than the placentas of other species [10]. Changes in insulin levels in maternal circulation have been reported to affect fetal/maternal glucose relationships in the rat [25–27]. We therefore studied whether rat placenta glucose transfer is sensitive to increments in maternal

insulin levels. For this purpose we infused exogenous insulin via the left uterine artery, according to our recently described technique [28], simultaneously with an infusion of (U-¹⁴C)-D-glucose via the jugular vein. The effect of insulin was determined by comparing the ¹⁴C-variables in fetuses from the left and right uterine horns.

Materials and methods

Animals and surgical procedure

Fed pregnant Wistar rats at day 20.5 of gestation (determined by the appearance of spermatozooids in vaginal smears), weighing 270–290 g, were anaesthetized intravenously with sodium pentobarbital (33 mg/kg body weight). After laparotomy, a cannula (PE-10, Intramedic, Clay Adams, NY, USA) was introduced into the left external iliac artery to the beginning of the superior vesical artery which was clamped distally to the left uterine artery exit level (Fig. 1, cannula 1). The infusion medium, introduced through the cannula by means of a peristaltic pump, therefore combined with blood circulating through the left uterine artery. Other details of the surgical procedure were as previously described [28]. The rats were also infused through a cannula (PE-50, Intramedic, Clay Adams, NY, USA) placed in the jugular vein (Fig. 1, cannula 2).

Fig. 1. Scheme of our experimental procedure showing location of the infusion cannulas. Media containing either insulin or saline was introduced via cannula 1, and media containing (U- 14 C)-D-glucose was introduced via cannula 2

Experiments with (U- 14 C)-D-glucose

Rats were infused simultaneously with a solution of 20 UCi·ml $^{-1}$ of (U- 14 C)-D-glucose (specific activity 199.6 Ci·mol $^{-1}$, Amersham International, Amersham, Bucks, UK) via the jugular vein (Fig. 1, cannula 2) at a rate of 20 μ l·min $^{-1}$ and with a solution of pork monocomponent insulin (Actrapid, Novo Industri, Copenhagen, Denmark) (20 μ l·0.66 mU $^{-1}$ ·min $^{-1}$ or 20 μ l·66 mU $^{-1}$ ·min $^{-1}$). In a control group of rats 0.9% NaCl (20 μ l·min $^{-1}$) was infused via the left uterine artery (Fig. 1, cannula 1). Eight to eleven rats were studied in each experimental group. After 15 min of infusion, 3–4 ml of blood were collected from the aorta into heparinized syringes and the fetuses and placentas from both uterine sides were excised. Livers from the mothers and from some fetuses, as well as the placentas, and one whole fetus from each uterine horn were placed immediately in liquid N $_2$. Aliquots of blood (25 μ l) were decoloured with hydrogen peroxide for counting total radioactivity. Plasma from other blood aliquots was used for measurement of insulin [29] with a radioimmunoassay kit for rat insulin (generously provided by Novo), and for deproteinization with Ba(OH) $_2$ -ZnSO $_4$ [30].

When separation of 14 C-glucose and 14 C-lactate was required, 100–200 μ l plasma aliquots were deproteinized with 10% HClO $_4$, and KHCO $_3$ -neutralized supernatants were used for both glucose measurement [31] and subjected to ascending chromatography on Whatman 3MM paper in n-butanol-water-methanol-formic acid (320:320:81:1, by volume) [32]. Spots were identified using purified standards run in parallel and spots were directly used for counting their radioactivity. Frozen livers, placentas, and whole fetuses were pounded in a porcelain mortar under liquid N $_2$ and weighed aliquots (approximately 0.5 mg) were used for digestion in 2 ml of 30% KOH in a boiling water bath for 10 min. Glycogen was precipitated three times with ethanol in the cold [33]. Glycogen precipitates were hydrolyzed with 5 N H $_2$ SO $_4$ at 100°C for 2 h and after neutralization with NaOH, aliquots were used for determination of glucose with glucose-oxidase [31]. This procedure has previously been validated by us [14].

14 C-radioactivity measurements were performed in a scintillation mixture containing 750 ml of xylene, 250 ml of triton-X-100, 3 g of PPO and 100 mg of POP-OP, and results were corrected by considering 1×10^6 dpm as the total infused radioactivity per rat.

Statistical analysis

Statistical comparisons between the groups were performed with the Student's paired and unpaired t-test.

Results

Plasma insulin, glucose, and liver glycogen

Rats at 20.5 days of gestation were infused simultaneously for 15 min with saline or insulin via the left uterine artery (Fig. 1, cannula 1) and (U- 14 C)-D-glucose via the jugular vein (Fig. 1, cannula 2). Insulin increased plasma radioimmunoassayable-insulin and decreased plasma glucose levels in the infused mother, although this effect was significant only when insulin was administered at the rate of 66 mU per min. Maternal insulin infusion via the left uterine artery did not modify either plasma insulin or glucose levels in fetuses from both left and right uterine horns (Table 1). In basal conditions, during maternal saline infusion, plasma fetal insulin levels were slightly higher than in their mothers ($p < 0.05$) whereas after maternal infusion with 66 mU per min of insulin plasma fetal insulin levels became significantly and similarly reduced in both the left and right uterine horns (Table 1). Fetal plasma glucose levels were lower than maternal levels after saline infusion ($p < 0.001$), but this difference disappeared after maternal insulin infusion (Table 1). Liver glycogen concentration did not differ in fetuses from the left and right uterine horns in any of the experimental groups studied (Table 1) and values were always much higher in fetuses

Table 1. Plasma insulin, glucose, and liver glycogen concentrations in 20.5-day-pregnant rats and their fetuses after infusion of either saline or insulin, via the left uterine artery and (U-¹⁴C)-D-glucose via the jugular vein

Infusion via the left uterine artery	Plasma insulin (mU/l)			Plasma glucose (mmol/l)			Liver glycogen (mg/g)		
	Mother	Fetuses		Mother	Fetuses		Mother	Fetuses	
		Left horn	Right horn		Left horn	Right horn		Left horn	Right horn
Saline	146 ± 10	195 ± 24	218 ± 19 ^a	6.00 ± 0.29	3.74 ± 0.29 ^c	3.52 ± 0.40 ^c	8.8 ± 3.1	51.5 ± 3.1 ^c	46.8 ± 3.0 ^c
Insulin (0.66 mU · min ⁻¹)	180 ± 29	206 ± 13	198 ± 14	4.67 ± 0.89	3.42 ± 0.38	3.05 ± 0.61	9.7 ± 4.6	55.8 ± 7 ^c	46.4 ± 7.7 ^b
<i>p</i>	NS	NS	NS	NS	NS	NS	NS	NS	NS
Insulin (66 mU · min ⁻¹)	446 ± 13	247 ± 30 ^c	248 ± 28 ^c	3.00 ± 0.46	3.31 ± 0.42	3.35 ± 0.33	3.4 ± 1.6	4.11 ± 3.6	36.7 ± 3.5 ^c
<i>p</i>	<0.001	NS	NS	<0.001	NS	NS	NS	<0.05	<0.05

Infusions were performed for 15 min under pentobarbital anaesthesia. Values correspond to mean ± SEM of 8–11 rats/group. Statistical comparisons for each insulin-treated group versus the saline control group are indicated by the *p* values whereas those for fetuses versus mothers are indicated by “a” (*p* < 0.05), “b” (*p* < 0.01) or “c” (*p* < 0.001). Comparisons between fetuses from left and right horns were not significant (*p* > 0.05) in any of the groups studied

Table 2. Total radioactivity in blood, whole fetuses and placentas, and placental ¹⁴C-glycogen in 20.5-day-pregnant rats and their fetuses after the infusion of either saline or insulin via the left uterine artery and (U-¹⁴C)-D-glucose via the jugular vein

	Blood total radioactivity (dpm/ml)			Total radioactivity (dpm/ml)				Placental ¹⁴ C-glycogen (dpm/g)	
	Mother	Fetuses		Whole fetus (dpm/g)		Placental (dpm/g)		Left horn	Right horn
		Left horn	Right horn	Left horn	Right horn	Left horn	Right horn		
Saline	6058 ± 248	4365 ± 270 ^c	4612 ± 314 ^b	5294 ± 290	5417 ± 300	5847 ± 190	5842 ± 359	90.8 ± 55.2	78.9 ± 31.5
Insulin (0.66 mU · min ⁻¹)	6506 ± 442	4650 ± 229 ^b	4827 ± 252 ^b	5527 ± 293	5350 ± 307	5836 ± 225	6160 ± 770	98.7 ± 27.6	106.6 ± 47.3
<i>p</i>	NS		NS	NS	NS	NS	NS	NS	NS
Insulin (66 mU · min ⁻¹)	3601 ± 254	3424 ± 229	3360 ± 208	3452 ± 113	3793 ± 334	3680 ± 162	3976 ± 224	71.0 ± 35.0	90.7 ± 48.6
<i>p</i>	<0.001	<0.05	<0.05	<0.001	<0.01	<0.001	<0.01	NS	NS

Infusions were performed for 15 min under pentobarbital anaesthesia. Values correspond to mean ± SEM of 8–11 rats/group. Statistical comparisons for each insulin treated group versus the saline control group are indicated by *p* values whereas those for fetuses versus mothers are indicated by “b” (*p* < 0.01) or “c” (*p* < 0.001). Comparisons between fetuses from left and right horns were not significant (*p* < 0.05) in any of the groups studied

than in their respective mothers. Maternal insulin infusion decreased liver glycogen concentration in fetuses from mothers receiving the highest insulin concentration (66 mU · min⁻¹) (*p* < 0.05).

Labelled circulating metabolites

Plasma ¹⁴C-glucose levels did not differ in fetuses from the left and right uterine horns in any of the groups studied (Fig. 2) and values were always lower in fetuses than in their respective mothers. In maternal plasma, ¹⁴C-glucose values decreased after the infusion via the left uterine artery with 66 mU · min⁻¹, but not with 0.66 mU · min⁻¹ of insulin, and this effect was paralleled in values of fetuses from both left and right horns which remained significantly below maternal levels (Fig. 2). In contrast to ¹⁴C-glucose, maternal plasma ¹⁴C-lactate increased with insulin infusion (Fig. 3). Plasma ¹⁴C-lactate levels were always higher in fetuses than in their mothers and values never differed in fetuses from the left versus the right uterine side, nor did maternal insulin infu-

sion affect this variable in their respective fetuses (Fig. 3).

Total radioactivity in blood, fetuses and placentas and placental ¹⁴C-glycogen

Blood total radioactivity did not differ in fetuses from the left and right horns and fetal were lower than maternal values when mothers received either saline or the low concentration of insulin via the left uterine artery (Table 2). Both fetal and maternal total radioactivity levels in blood, however, decreased significantly when mothers received 66 mU · min⁻¹ insulin instead of saline (Table 2). There were no differences in the total radioactivity of placentas and fetuses from the left versus the right uterine horns (Table 2), and whereas these values were similarly unaffected by the low dose of insulin and by saline infused through the left uterine artery, the higher insulin dose used (66 mU · min⁻¹) caused a significant reduction (*p* < 0.01). This decrease was similar in placentas and fetuses from both uterine sides (Table 2)

Fig. 2. Plasma ^{14}C -glucose in 20.5-day pregnant rats and their fetuses after 15 min infusion of either saline or insulin via the left uterine artery, and (U- ^{14}C)-D-glucose via the jugular vein. Values correspond to means $\pm$ SEM of 6 rats per group. Statistical comparisons of mean values for fetuses from the left (L) and right (R) uterine horns were not significant in any of the groups studied ($p > 0.05$). Statistical comparisons between mean values of all fetuses versus their respective mother are shown by $\blacktriangle$ ($\blacktriangle\blacktriangle\blacktriangle = p < 0.001$), and values of insulin versus saline infusion for both mothers and fetuses are indicated by * ($= p < 0.05$; $*** = p < 0.001$)

Fig. 3. Plasma ^{14}C -lactate in 20.5 day pregnant rats and their fetuses after 15 min infusion of either saline or insulin via the left uterine artery, and (U- ^{14}C)-D-glucose via the jugular vein. Values correspond to means $\pm$ SEM of 6 rats per group. Statistical comparisons of mean values for fetuses from the left (L) and right (R) uterine horns were not significant in any of the groups studied ($p > 0.05$). Statistical comparisons between mean values of all the fetuses versus their respective mothers are indicated by $\blacktriangledown$ ($\blacktriangledown\blacktriangledown\blacktriangledown = p < 0.001$) and those of insulin infusion versus saline for both mothers and fetuses by * ($= p < 0.05$)

and also paralleled the effect on total blood radioactivity produced by $66 \text{ mU} \cdot \text{min}^{-1}$ in the mother. A small proportion of placental radioactivity corresponded to ^{14}C -glycogen and maternal insulin infusion did not affect this variable in placentas from either left or right uterine horns (Table 2).

Discussion

Infusion of the late pregnant rat via the left uterine artery as described in the present study has been validated recently as a sensitive technique to detect placental D-glucose, L-alanine, and glycerol transfer mechanisms [28, 34]. Infusion of insulin through the left uterine artery should produce a higher concentration of insulin in blood irrigating the left versus the right uterine horn. The simultaneous infusion of ^{14}C -glucose through the jugular vein of the rat mother permits determination of possible direct effects of insulin on placental function by comparison of samples from the left versus the right uterine sides. The present results show that infusion for 15 min of sufficient amounts of insulin to double maternal radioimmunoassayable-insulin concentration does not alter the radioactivity values in uteroplacental struc-

tures of the left and right uterine horns. Experiments in which infusion of insulin was prolonged up to 1 hour led to similar negative results (data not shown), indicating that this hormone does not affect glucose transfer in the rat placenta. Our conclusion differs from that of Paxon et al. [22] and Crandall et al. [23] in sheep, but is in agreement with Hay et al. [24] who, using the glucose clamp methodology, showed that variations in maternal insulin concentration in pregnant sheep did not modify placental glucose uptake or transfer to the fetus. While sheep placenta specifically binds insulin [35], this hormone does not modulate glucose uptake or utilization in in vitro preparations [21]. In the human placenta, which is rich in insulin receptors [10, 36], it has been reported that glucose metabolism is either sensitive [16, 19] or insensitive [18, 37] to added insulin in vitro. Similarly contrasting results have also been observed in the regulation by insulin of amino acid uptake by human placenta [17, 20]. Placental insulin binding capacity is lower in the rat than in other mammals [10] and, to our knowledge, there is no published evidence that placental metabolism is directly influenced by maternal insulin. The present study revealed no effect on placental glucose uptake (as indicated by placental radioactivity) or on glucose incorporation into placental glycogen,

in agreement with reports that several enzyme activities, influenced by insulin in other rat tissues, are not insulin-sensitive in the placenta [27, 38].

These findings, together with present results showing that increased maternal insulin levels do not modify placental permeability to glucose, indicate that this hormonal change does not modify placental function and metabolism in the rat. This conclusion coincides with reports that, in severely diabetic pregnant rats, fetal blood glucose levels passively follow maternal values [39] and enzyme activities in placentas do not differ from those in controls [38]. A "permissive" role for insulin in certain placental functions cannot be totally discarded as no extreme conditions of insulin deficiency and replacement were tested in the present study.

As expected, administration of high insulin doses ($66 \text{ mU} \cdot \text{min}^{-1}$) to the mother decreased both glucose concentration and ^{14}C -glucose levels and enhanced ^{14}C -lactate values in maternal plasma. These changes were paralleled by the fetus only in ^{14}C -glucose, indicating that it was affected as a secondary consequence of maternal alterations due to insulin. As indicated by the mother/fetus plasma ^{14}C -glucose ratios, the plasma ^{14}C -glucose mother-to-fetus gradient was of similar magnitude in both the saline- and insulin-infused rats.

The fetal plasma glucose concentration deserves special comment. Fetuses from mothers infused with saline showed plasma glucose levels predictably lower than in their respective mothers. With the insulin infusions, the decrease in maternal glycaemia was not paralleled in the fetus, and maternal and fetal glucose concentrations became similar. Comparable results have been observed in the fasted rat and after maternal insulin administration [40]. Since liver glycogen content decreased in fetuses from mothers infused with insulin ($66 \text{ mU} \cdot \text{min}^{-1}$), enhanced glycogenolysis probably compensates for the reduced availability of incoming maternal glucose. Fetal glucose metabolism does not seem, however, to be affected by maternal insulin infusion, as indicated by the unchanged ^{14}C -lactate level in fetal plasma. Therefore we may conclude that in the rat, placental glucose uptake, its transfer to the fetus, and fetal glucose utilization are not directly affected by maternal circulating insulin. Moreover, metabolic changes occurring in fetuses of hyperinsulinaemic mothers are secondary to the decreased availability of glucose and presumably of other substrates crossing the placenta.

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