Impaired Insulin Secretion after Intravenous Glucose in Neonatal Rhesus Monkeys that Had Been Chronically Hyperinsulinemic *In Utero* (43364)

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Abstract. **Chronic hyperinsulinemia in the fetal rhesus monkey results in fetal macrosomia without change in fetal plasma glucose concentration. After 18 days of hyperinsulinemia, fetuses were delivered by cesarean section, at which time experimental animals had significantly ($P < 0.05$) elevated umbilical artery plasma insulin concentrations of 2039 ± 854 p*M* compared with 129 ± 72 p*M*. Plasma immunoreactive C peptide (IRCP) was significantly reduced to 39 ± 17 p*M* compared with 286 ± 134 p*M*. Eight hours after the insulin-delivering pumps were removed, plasma glucose, insulin, and IRCP were the same in both the experimental and control groups. At this time, 0.5 g glucose/kg was given intravenously and insulin and IRCP secretion was measured over a 1-hr period. The secretion, as assessed by integrating the incremental response of both insulin and IRCP, was significantly ($P < 0.05$) lower by 80% in the experimental animals compared with the controls. Our data show that experimentally produced *in utero* euglycemic hyperinsulinemia in the fetal rhesus monkey produces a defect in the glucose-mediated insulin secretory mechanism that is detectable in the neonatal period even when hyperinsulinemia is no longer present. This study provides more support for the concept that fuel/hormone-mediated fetal teratogenesis may explain some of the fetopathy of the infant of the diabetic mother.** [P.S.E.B.M. 1992, Vol 199]

We reported previously that the newborn rhesus monkey that was made hyperinsulinemic *in utero* exhibited impaired insulin secretion during the immediate neonatal period (1). This defect was characterized by the inability of intravenous glucagon to stimulate insulin release. Those studies also identified a potential hepatic glucagon receptor defect in those animals. Whether the impaired insulin secretion was due to a pancreatic β cell plasma membrane glucagon receptor defect or at some point distal to the receptor could not be determined in those studies. Although glucagon is able to independently stimulate insulin release, its use *in vivo* also results in an increase in plasma glucose concentration (2, 3).

It was not possible to definitively separate the effects of those two insulin secretagogues on insulin secretion. Studies of the offspring of rats made diabetic during pregnancy have demonstrated that impaired insulin secretion is present after a glucose challenge (4, 5). Those studies raise the possibility that the defect is due to fuel-mediated teratogenesis caused by fetal hyperglycemia (6). In order to determine whether the insulin secretion defect was due to a glucagon receptor defect, neonatal rhesus monkeys that had been hyperinsulinemic *in utero* were given an intravenous glucose tolerance test at 8 hr of age. We reasoned that if the glucagon receptor defect was the cause of the impaired insulin secretion, glucose would cause a normal insulin release. If glucose was also not able to elicit a normal insulin release, it would indicate that a more generalized defect exists. Therefore, we tested the hypothesis that experimentally produced chronic *in utero* euglycemic hyperinsulinemia in the fetal rhesus monkey produces

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Received September 3, 1991. [P.S.E.B.M. 1992, Vol 199]
Accepted October 18, 1991.

0037-9727/92/1993-0327$3.00/0

a defect in the glucose-mediated insulin secretory mechanism that is detectable during the neonatal period.

Materials and Methods

The fourteen rhesus monkeys (*Macaca mulatta*) used in this study were born and cared for in the American Association for the Accreditation of Laboratory Animal Care (AAALAC)-approved New England Regional Primate Research Center (NERPRC) in Southborough, Massachusetts. The studies were reviewed and approved by the Institutional Animal Care and Use Committees of Rhode Island Hospital, Brown University, and the NERPRC. The insertion of the Alzet minipumps into the rhesus fetuses and the delivery of these fetuses by cesarean section was carried out aseptically in an approved surgical suite at the NERPRC.

At approximately 130 days gestation, Alzet minipumps delivering 4.75 units of insulin in 1.6% glycerol per day, or glycerol solution alone, were implanted into six experimental and eight control fetuses. This was followed by delivery by cesarean section at approximately 150 days gestation, as described previously (7).

Free-flowing umbilical artery and vein blood samples were taken before the umbilical cord was severed. The Alzet minipumps were removed and the neonates were transferred to an incubator where spontaneous respiration occurred while the neonates were being vigorously towel dried. At 30 min of age, a femoral vein of the neonates was catheterized. While the neonates were kept in a fasted state for an 8-hr period, blood samples were taken at hourly intervals. The red blood cells taken during sampling were returned to the neonate as described previously.

At 8 hr of age, each animal was given an intravenous glucose tolerance test with 0.5 g glucose/kg body wt. Blood samples were taken immediately prior to the 30-sec glucose bolus into a cephalic vein and 5, 15, 30, and 60 min through the femoral vein catheter after glucose. Plasma was immediately separated and stored frozen at −70°C until analyzed for glucose, insulin, and immunoreactive C peptide (IRCP).

Plasma IRCP was measured by radioimmunoassay in 100 μl each of assay buffer (0.05 *M* sodium phosphate (pH 7.4), 25 m*M* disodium EDTA, and 1% bovine serum albumin), standard (human C peptide serially diluted from 2.133 to 0.033 pmol/ml) or sample (20 μl of plasma plus 80 μl of buffer), guinea pig antihuman C peptide serum (1/200 dilution), and I-125 human Tyr-C peptide (50 pg in 100 μl of buffer). After incubation for 24 hr at 4°C, 100 μl of goat antiguinea pig IgG (received pretitered and diluted 1/20 with precipitation buffer, which is assay buffer containing 3% polyethylene gel) and 100 μl of guinea pig serum (diluted 1/70 with precipitation buffer) were added and incubation continued for an additional 2 hr at 4°C. The precipitable radioactivity was obtained after centrifugation at 3000*g* for 30 min at 4°C. Human C peptide was obtained from Eli Lilly and Co., Indianapolis, IN. Guinea pig anti-human C peptide serum and goat antiguinea pig IgG were from Linco Research, Inc., Eureka, MO. The Novo laboratories, Inc., of Danbury, CT, were the source of I-125 human Tyr-C peptide.

Data analysis was performed on a Macintosh SE computer using the StatView SE Program by Brain-Power, Inc. Differences between groups were tested by the unpaired Student's *t* test, with differences being considered significant that had *P* values of < 0.05. All summary data are presented as mean ± SD.

Results

Table I summarizes the data collected at delivery for the control and experimental animals. There were no significant differences in the gestational age and umbilical artery or vein plasma glucose concentrations. Although euglycemic, the experimental fetuses were significantly ($P < 0.05$) hyperinsulinemic compared with controls with insulin concentrations approximately 16 times basal, 1946 ± 840 p*M* vs 122 ± 66 p*M*. The significant ($P < 0.05$) increase in fetal weight of 514 ± 15 g for the experimental animals compared with 437 ± 62 g for controls was observed as expected. Also expected based on previous studies was the significantly ($P < 0.05$) lower plasma IRCP in the experimental animals compared with the controls. The experimental animals' IRCP concentration of 39 ± 17 p*M* reflects a suppression of endogenous insulin secretion in these animals compared with the controls with concentrations of 286 ± 124 p*M*.

Figure 1 summarizes the plasma glucose (Fig. 1A), insulin (Fig. 1B), and IRCP (Fig. 1C) of the two groups of animals 1 hr prior to the glucose tolerance test that was given after 8 hr of fasting, and the changes in their concentrations after 0.5 g glucose/kg. After 7 hr of fasting, plasma glucose, insulin, and IRCP in the two groups were not different. By this point, plasma insulin concentrations in the experimental animals had

Table I. Neonatal Delivery Data after 18 ± 1 Days of 4.75 Units of Insulin/Day in 1.6% Glycerol or 1.6% Glycerol Alone

	Control	Experimental
n	8	6
Gestational age (days)	150 ± 2	150 ± 2
UA glucose (m*M*)	2.58 ± 0.40	2.17 ± 0.90
UV glucose (m*M*)	2.86 ± 0.34	2.41 ± 1.12
UA insulin (p*M*)	122 ± 66	1946 ± 840[a]
UA insulin (p*M*)	129 ± 72	2039 ± 854[a]
IRCP (p*M*)	286 ± 134	39 ± 17[a]
Weight (g)	437 ± 62	514 ± 15[a]

[a] $P < 0.05$.

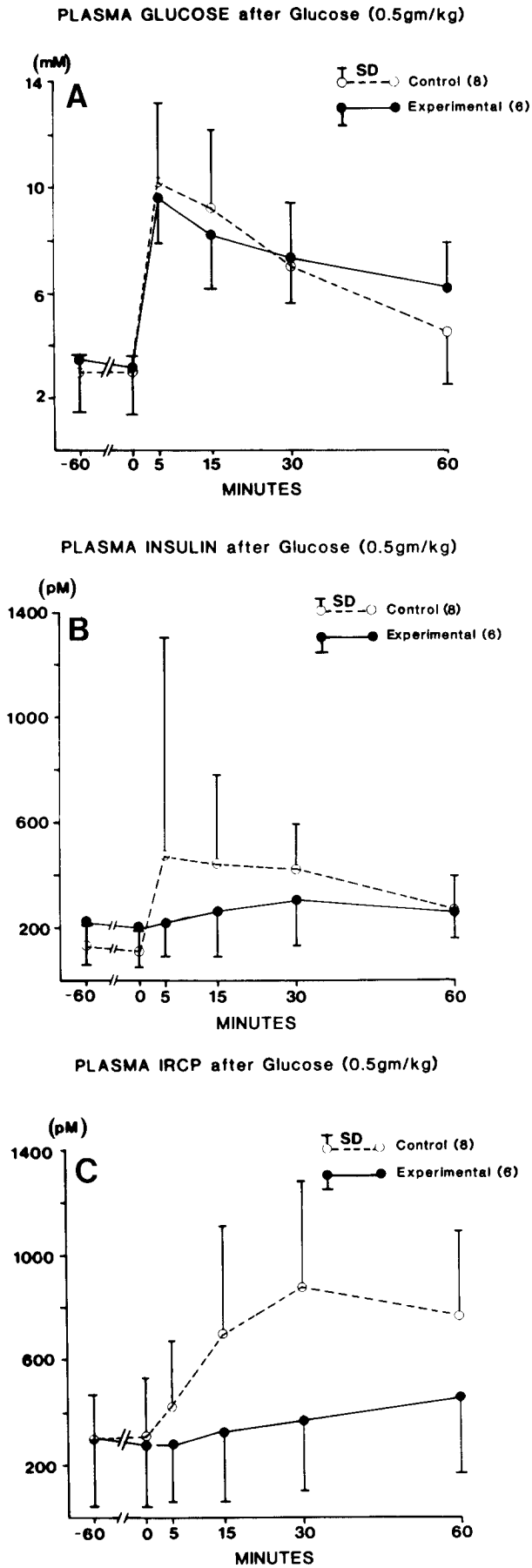

Figure 1. Plasma concentration of (A) glucose, (B) insulin, and (C) IRCP in response to 0.5 g of glucose/kg given intravenously to neonatal rhesus monkeys at 8 hr of age.

dropped into the normal range (220 ± 160 p*M* for the experimental animals and 140 ± 90 p*M* for the control animals). IRCP levels in the experimental animals increased to 300 ± 170 p*M*, bringing them to the normal level of 300 ± 270 p*M*. The fasting plasma glucose concentration was also not different in the two groups at this time, with 3.5 ± 2.0 m*M* in the experimental animals and 3.0 ± 0.7 m*M* in the controls.

Significant differences exist between the two groups of animals in their response to the intravenous glucose challenge, as summarized in Table II. The rate of glucose disappearance (k) as measured by plotting the log of the glucose concentration versus time was significantly ($P < 0.05$) lower in the experimental group, whereas the time interval for glucose concentration to fall by 50% ($t_{½}$) was increased. The integrated incremental insulin response to the glucose load was also significantly ($P < 0.05$) reduced in the experimental animals by over 80%. A similar significant ($P < 0.05$) parallel reduction in the integrated incremental IRCP response was also present in the experimental animals. The insulinogenic index calculated by dividing the Δ Insulin by the Δ Glucose was, therefore, significantly lower in the animals that had been exposed to chronic euglycemic hyperinsulinemia *in utero*.

Discussion

The insulin secretory response to intravenous glucose in neonatal rhesus monkeys that had been exposed to nearly 3 weeks of chronic euglycemic hyperinsulinemia was significantly reduced compared with age-matched controls. This 80% reduction in insulin and IRCP secretion indicates that the impaired insulin secretion to intravenous glucagon that had been observed previously in this model (1) was not due to a defect in the glucagon receptor-mediated stimulatory mechanism. This data are consistent with the hypothesis that insulin at chronically high concentrations is able to exert a teratogenic effect on the developing fetal pancreas that alters its functional sensitivity to insulin secretagogues, such as glucagon and glucose, during the neonatal period.

Table II. Kinetic Parameters and Integrated Incremental Response to 0.5 g/kg iv of Glucose at 8 Hours of Age

	Control	Experimental
n	8	6
$t_{½}$ (min)	46.5 ± 10.0	84.8 ± 32[a]
k (%/min)	1.57 ± 0.43	0.93 ± 0.38[a]
ΔG (m*M*/hr)	18.9 ± 6.2	19.3 ± 3.0
ΔI (p*M*/hr)	1206 ± 826	237 ± 352[a]
ΔIRCP (p*M*/hr)	1610 ± 450	320 ± 520[a]
ΔI/ΔG	81 ± 62	12 ± 20[a]

[a] $P < 0.05$.

It was difficult to obtain fetal blood samples during the period of fetal insulin delivery to confirm that the fetus was never hypoglycemic during our study period. To do so would have required that the mother be subjected to the stress of long-term restraint, which might have altered glucose homeostasis or resulted in premature termination of pregnancy. We have assumed that the fetal glucose levels obtained at delivery reflect levels present throughout the hyperinsulinemic period. This assumption is justified because the primate placenta is very permeable to glucose, making it possible for the fetal-maternal glucose gradient to be smaller than in other mammals. If *in utero* hypoglycemia was a feature of this model, then an increase in the plasma concentration of the hypoglycemic counterregulatory hormones cortisol, glucagon, epinephrine, or norepinephrine should be observed. That this is not the case for these animals has been reported previously (1). Although there is no evidence for hypoglycemia in the insulin-treated fetuses, 3 weeks of slightly reduced plasma glucose may have put the islets into a resting state resulting in reduced insulin production and storage. The diminished insulin stores could then limit an acute secretory response to glucose. This hypothesis cannot be evaluated easily with our model because of the technical barriers, but it could be tested with chronically catheterized fetal preparation or *in vitro* with cultured islets.

In vivo studies in which chronic fetal hyperglycemia has been experimentally produced have been performed in rats. These studies have documented that chronic maternal and fetal hyperglycemia during the latter part of pregnancy results in impaired insulin secretion in the offspring of these mothers during the neonatal period (8). Glucose tolerance tests performed at 1 month of age in offspring delivered from mothers that were made chronically hyperglycemic by constant glucose infusion revealed impaired insulin secretion. This insulin secretion impairment persists throughout life and is transmitted to subsequent generations (9). Similar results have been reported when maternal diabetes is produced during late gestation (4, 5, 10). These rat studies and now our own monkey studies demonstrate that the *in utero* metabolic conditions in which the fetal pancreas is developing are important determinants of future pancreatic function.

The precise nature of the pancreatic defect in the hyperinsulinemic rhesus fetus is not well characterized, but our studies point to the involvement of steps in the control of insulin synthesis and secretion that are shared by a number of insulin secretagogues. Although we have not evaluated the insulin response to arginine or tolbutamide during the neonatal period, we have carried out those studies in infant monkeys. In 4- and 5-month-old rhesus monkeys that had been exposed to 3 weeks of euglycemic hyperinsulinemia *in utero*, insulin secretion to arginine and tolbutamide was significantly reduced by at least 50% (11). MacDonald proposed a schema in which he describes signals that are secretagogue specific as proximal and those that are more removed from those specific signals and used by many secretagogues as distal (12). Our studies both during the neonatal period and later on in life point to the possibility that a common distal signaling mechanism may be involved.

The offspring of women with diabetes during pregnancy are at greater risk for developing glucose intolerance and diabetes later in life (13–15). Animal studies have shown the presence of both short-term and long-term insulin secretion defects in the offspring of hyperglycemic pregnant rats. Our own studies provide evidence that insulin secretory defects may be caused by isolated fetal euglycemic hyperinsulinemia in the nonhuman primate. Since the human infant of the diabetic mother is chronically hyperinsulinemic *in utero* when her mother's diabetes is not well controlled, and possibly even when it is, insulin-mediated teratogenesis could be responsible for the abnormal functional development of the fetal pancreas of the infant of the diabetic mother.

The work described was funded in part by grants from the NIH (HD-11343 and RR-00168) and the Rhode Island Hospital Research Fund. The authors are especially grateful to Donna Berger for her expert assistance in the preparation of the manuscript.

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