The Influence of Antenatal Corticosteroids on Hypoglycemia in Newborn Rats with Intrauterine Growth Retardation

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Summary

This study examines the effect of maternally injected glucocorticoid on the pattern of hypoglycemia exhibited by rat pups with intrauterine growth retardation (IUGR). The majority of surgical procedures designed to produce small-for-gestational age (SGA) newborns for biochemical studies were carried out on days 18 and 19 of gestation because of favorable yields of pups with IUGR at those operative days. At birth, normal controls showed a mean $\pm$ SE plasma glucose value of 63 ± 2 mg/dl; mean glucose for the group with IUGR was significantly lower at 43 ± 2 mg/dl. There was a further decrease in the plasma glucose concentration of pups with IUGR at 2-4 hr of age, whereas values in the control littermates did not fall during this interval. Through the first 2 hr of neonatal life, 46% of the pups with IUGR exhibited plasma glucose values less than 40 mg/dl, whereas only 18% of the control littermates manifested hypoglycemia. During the 2-4-hr interval, the incidence of hypoglycemia in animals with IUGR increased to 91%; however, the incidence in controls remained at 18% from 2-4 hr and fell to 4% at 4-6 hr of age. At birth, the pups with IUGR had a lower mean liver weight compared to their control littermates, but glycogen concentration of liver was similar to the control mean $\pm$ SE of 25.7 ± 1.8 (IUGR = 22.2 ± 1.3 mg/g wet weight). Total hepatic glycogen stores, however, were markedly lower in dysmature rat pups (IUGR = 2.96 ± 0.17 mg; control = 7.23 ± 0.43 mg). Concentrations of plasma glucose at birth of individual control and IUGR animals were found to correlate significantly ($r = 0.64, p < 0.001$) with total liver glycogen content.

The decline in plasma glucose values in pups with IUGR was not present in animals whose dams received glucocorticoid injection 24 and 48 hr before delivery. At 4-6 hr of age, for instance, the mean plasma glucose concentration in the corticoid-treated IUGR group (70.1 ± 6.9 mg/dl) approximated that of the control group. Instead of the 91% incidence of hypoglycemia noted in the nontreated dysmature pups, an incidence of 55% was found at 2-4 hr of age in offspring of mothers given glucocorticoid. At 4-6 hr, the treated group showed an incidence of 18% compared to a 67% figure in the nontreated IUGR animals. The concentration of liver glycogen in these animals also differed in that the treated IUGR pups showed significantly higher values (26.9 ± 1.7 mg/g wet weight, mean $\pm$ SE) than nontreated progeny. It is concluded that antenatally administered corticosteroids influence the development of neonatal hypoglycemia in the dysmature rat pup and that the major effect is not at birth, but during the 2-4-hr period of neonatal life.

Speculation

These data indicate that hypoglycemia may be prevented by antenatally administered steroids in an animal model of the SGA infant and raise the possibility of prenatal intervention in susceptible fetuses. The protective mechanism that manifests itself in

early neonatal life may be attributable to better utilization of gluconeogenic substrates and induction of key enzymes in developing liver.

Poor fetal growth may result from maternal factors such as toxemia of pregnancy, fetal factors such as chromosomal aberrations, and first trimester viral infections and other unknown causalities. It is estimated that the incidence of IUGR or SGA infants resulting from these combined factors is between 1.5% and 3% of all live births (4). These infants may manifest several clinical problems, including hypoglycemia, and may show abnormal growth patterns during infancy and impaired neurologic development (5, 7-9, 12, 15, 25, 32, 37). Investigations concerning the clinical and metabolic problems encountered in infants with IUGR have been limited by the number of appropriate patients available in a given center and the techniques which can be utilized to study the underlying pathophysiology and pathobiochemistry. Thus, interest in an animal model for the dysmature infant developed several years ago and led to a method reported by Wigglesworth in 1964 to produce growth retardation in fetal rats (39). The method involves unilateral ligation of uterine vessels and produces a uteroplacental insufficiency state. The progeny from the ligated uterine horn are characteristically small, defined by convention as 2 SD or 15% less than the weight of littermates (20). The yield of dysmature fetuses per pregnancy, however, varies widely and documentation of optimum operative approaches and results has not appeared in the literature.

Metabolic studies utilizing the animal model of IUGR have demonstrated that hypoglycemia occurs in dysmature rat pups at birth and during early neonatal life. The fall in plasma glucose below normal values is accompanied by decreased liver glycogen stores in these neonates, as compared to control littermates (6). Observations relative to glycogen production by the livers from IUGR pups have suggested that enzymatic competence exists to carry out the biosynthetic pathway. Inadequate glucose transport from the maternal pool, therefore, may be the main cause for reduced hepatic glycogen levels (26). Prevention of early neonatal hypoglycemia in the animal with IUGR (11) and in the dysmature human infant (35) by prenatal glucose administration in large amounts further supports this hypothesis. Other methods of influencing neonatal glucose homeostasis, such as administering adrenocorticoids antenally, have been evaluated in normal rat fetuses, but not in those subject to intrauterine growth retardation.

These findings have led to the present study which examines the effect of maternally injected glucocorticoid on the pattern of neonatal hypoglycemia exhibited by rat pups with IUGR. The purposes of this investigation included (1) further characterization of the surgical procedure used in producing animals with IUGR; (2) determination of the incidence and severity of hypo-

glycemia in the fetally malnourished progeny at birth and under the stress condition of delayed feeding during the first 6 hr of life; (3) assessment of liver glycogen concentrations in these animals, as compared to plasma glucose levels; and (4) evaluation of the effects of antenatally administered corticoid on plasma glucose and liver glycogen values in animals at risk for hypoglycemia.

MATERIALS AND METHODS

ANIMALS AND SURGICAL PROCEDURES

Pregnant Sprague-Dawley rats with accurately timed gestations (within 12 hr) were utilized in this study. Under ether anesthesia, a low, midline laparotomy was performed on the 17th to the 19th day after mating. The uterus was then exposed and the uterine vessels were ligated as described by Wigglesworth (39) near the lower end of one horn using a silk suture. The majority of the surgical procedures designed to produce animals for biochemical studies were carried out on days 18 and 19 because of more favorable yields of dysmature pups found at these operative times. The vascular supply to the opposite uterine horn was left intact and these animals served as controls. At term (22 days of gestation), delivery by cesarian section was carried out under similar operative conditions. Rat pups were weighed immediately after delivery on a Mettler PT1200 electronic balance (42). A reduction in weight of more than 15% compared to the mean of control littermates was considered evidence of fetal growth retardation (20).

EXPERIMENTAL GROUPS OF NEONATES WITH IUGR

The progeny of 126 pregnant rats comprised the following experimental groups.

Group 1: Newborns at birth. Control and fetally malnourished pups from 25 litters were killed as soon as possible (within 1–5 min) after birth. Blood sampling from severed neck vessels for glucose determination was done before death. The entire liver was then rapidly excised and frozen at 30° for glycogen determination.

Group 2: Nutritionally Deprived Newborns. The progeny from 41 litters yielding controls and dysmature pups were maintained without nutritional support for 2–6 hr at an ambient temperature of 32°. Sampling of blood and liver was carried out at either 2, 4, or 6 hr of age. The weight of these animals was noted at birth and at the time of death. No attempt was made to follow plasma glucose or liver glycogen values in these animals after 6 hr of life.

Group 3: Glucocorticoid-treated Animals. The progeny from 60 pregnant rats comprised this group. Each dam received an intramuscular injection of 9- α -fluoroprednisolone acetate (1 mg/kg) 24 and 48 hr before delivery (43). This steroid is a hydrocortisone analog with potent glucocorticoid activity and was chosen because of its proven competence in previous studies with fetal animals (19, 38). The rat pups from these pregnancies were divided as follows. Progeny from 28 litters were killed at birth with sampling of blood and removal of the liver as noted previously. The pups from 32 litters were maintained under conditions of starvation similar to group 2. Blood sampling was performed and the pups killed at 2–6 hr of age.

PROCESSING OF SAMPLES AND ANALYTIC PROCEDURES

Blood, collected in heparin lithium fluoride tubes, was rapidly centrifuged in a Beckman 152 microfuge (41) and the plasma utilized for duplicate determinations of true glucose. The latter was performed using the specific glucose oxidase method (22) with a Beckman ERA 2001 analyzer (41). Liver samples, frozen for 1–3 weeks before extraction, were weighed and extracted with alcoholic KOH (30% KOH in ethanol) as described by Roehrig and Allred (33). This procedure entailed sonication and heating in a boiling water bath for 1 hr. After cooling the extract, 1.2 volumes cold 95% ethanol were added in order to precipi-

tate glycogen. Precipitation was carried out overnight at 4°, and the particulate matter collected by centrifugation. Pellets of glycogen were then washed three times with 70% ethanol and hydrolyzed enzymatically by incubation for 10 min at 55° with amyloglucosidase (150 units). After centrifugation, the glucose concentration of the sample was measured as described above. The extraction, precipitation, and hydrolysis steps were carried out in the same test tube. Complete hydrolysis of glycogen was shown to result from amyloglucosidase treatment and excellent reproducibility was achieved with this approach.

RESULTS

EVALUATION OF OPERATIVE PROCEDURE

For animals subjected to surgery at 17, 18, or 19 days of gestation, the mean body weight at birth of 184 dysmature pups was 3.46 ± 0.05 g, whereas that of 344 normal control littermates was 5.26 ± 0.03 g ($P < 0.001$). Minimal variation in body weights was noted in fetuses delivered from the ligated uterine horn on each operative day.

The yield of fetally malnourished pups varied depending on day of surgery. Operative procedures carried out at 17 days of gestation produced 15 fetuses with IUGR out of a total number of 66, i.e., 23% on the ligated side, whereas 103 out of 374 (28%) were obtained at 18 days. In contrast, a 39% yield (66 dysmature pups produced from a total ligated side number of 167 fetuses) was noted in those animals who underwent uterine artery ligation at a gestational age of 19 days. By the chi square analysis of operative day success rates, significance was noted at the $P < 0.005$ level, indicating a trend toward better yield at later operative times.

PLASMA GLUCOSE CONCENTRATIONS

IUGR and control plasma glucose values obtained during the first 6 hr of neonatal life are presented in Figure 1. At birth, normal controls showed a mean $\pm$ SE plasma glucose value of 63 ± 2 mg/dl. The incidence of hypoglycemia (plasma glucose < 40 mg/dl) in these controls was 13%. Plasma glucose values for the group with IUGR at birth were significantly decreased ($P < 0.001$) with a mean $\pm$ SE concentration of 43 ± 2 mg/dl. There was a decline in the plasma glucose level of the dysmature pups during the 2–4-hr of age period. The mean $\pm$ SE value fell from 39.5 ± 2 mg/dl at 0–2 hr of age to 20.5 ± 3 mg/dl, but then returned by 6 hr of age to a level approximating that at birth. The marked decline in plasma glucose values was not found in the control littermates and mean plasma glucose values were

Fig. 1. Mean plasma glucose values of control and intrauterine growth retarded (IUGR) rat pups (nontreated and corticoid-treated groups). Brackets indicate SE. Asterisks (*) indicate $P < 0.001$ compared to the nontreated IUGR group, and plus signs (+) denoted $P < 0.005$ compared to the nontreated IUGR group.

maintained in the normoglycemic range throughout early neonatal life (56.5 ± 1 at 0–2 hr, 66.5 ± 3 at 2–4 hr, and $74.2 \pm \text{mg/dl}$ at 4–6 hr).

These observations on mean glucose levels were reflected in the incidences of hypoglycemia in the two groups. At birth and through the first 2 hr of neonatal life, 46% of the pups with IUGR exhibited hypoglycemia, whereas only 18% of the control littermates manifested low plasma glucose levels. During the 2–4 hr interval, the hypoglycemia rate in animals with IUGR was 91%; the control animals did not show an increase in the percentage of hypoglycemia during that time period. By 4–6 hr of age the hypoglycemic tendency in those with IUGR dropped to 67%, and only 4% of the control plasma glucose values were less than 40 mg/dl.

LIVER GLYCOGEN CONTENT AND LIVER WEIGHT

Table 1 presents the interrelationships between glucose, liver glycogen, and liver weight noted at birth and during early neonatal life in all the experimental groups.

At birth, the pups with IUGR had a lower mean liver weight as compared to their control littermates (0.15 ± 0.06 versus $0.28 \text{ g} \pm 0.04$, respectively, $P < 0.001$). A weight difference was also found between normoglycemic and hypoglycemic dysmature pups (0.17 ± 0.01 and $0.12 \pm 0.01 \text{ g}$, respectively, $P < 0.001$).

Comparison of glycogen concentration per g of wet liver weight revealed slightly lower values in the IUGR group (22.2 ± 1.3) but no statistically significant difference in the overall mean (control = 25.7 ± 1.8). Total liver glycogen stores at birth, however, were markedly lower ($P < 0.001$) in dysmature rat pups ($2.96 \pm 0.17 \text{ mg}$) compared to control littermates ($7.23 \pm 0.43 \text{ mg}$).

Figure 2 demonstrates the relationship between mean plasma glucose levels at birth for all animals and total liver glycogen content. As indicated, a significant correlation ($r = 0.64$, $p < 0.001$) was observed.

In nutritionally deprived animals of 2–4 hr of age, the liver glycogen concentration of the pups with IUGR ($13.6 \pm 1.1 \text{ mg/g}$ wet weight) was also lower ($p < 0.005$) than control littermates (28.3 ± 3.9). This relationship was likewise noted in comparing total hepatic glycogen content. Differences in liver weights between the IUGR and control littermates were again noted at 2–4 hr of age; however, within the group with IUGR, no difference was noted between the normoglycemic and the hypoglycemic animals.

EFFECT OF ANTENATAL GLUCOCORTICOID ON PLASMA GLUCOSE, LIVER GLYCOGEN CONTENT, AND LIVER WEIGHT IN PUPS WITH IUGR

Plasma glucose patterns of the pups with IUGR not exposed to exogenous corticoids are compared in Figure 1 to those treated antenatally and also to control pups. The decline in plasma glucose values of dysmature pups during the 2–4-hr period was not present in animals whose dam received glucocorticoid injection 24 and 48 hr before delivery. Further analysis of the 2–4-hr and 4–6-hr time periods revealed significant differences in plasma glucose values between untreated and treated groups with IUGR. At 4–6 hr, the difference was particularly impressive in that the treated group with IUGR maintained a plasma glucose value (70.1 ± 6.92) which approximated the control group value and was almost 2-fold greater than the mean value of $37.5 \pm 7.4 \text{ mg/dl}$ in the nontreated IUGR group ($p < 0.005$). No differences in plasma glucose values were noted in normal control littermates during the same study time periods whether subjected to antenatal corticoids or not.

The average incidence of hypoglycemia in the two experimental groups of dysmature pups did not differ in the first 2 hr of life (46% in nontreated animals, 48% in those exposed to exogenous corticoid). In the subsequent 4 hr, however, the incidence

rates differed. Instead of 91%, an incidence of 55% was found at 2–4 hr of age. At 6 hr, the corticoid-treated group showed an incidence of 18% as compared to a 67% figure in nontreated, small-for-gestational age animals.

Although mean glycogen concentration at birth in corticoid-treated IUGR pups was relatively low, a substantial increase was noted in these animals by 2–4 hr of age (Table 1). Thus, mean liver glycogen concentration (26.9 ± 1.7) in the treated animals at 2–4 hr was significantly greater than the value observed in untreated dysmature pups. During the 2–4-hr age period, total liver glycogen in the corticoid-treated pups with IUGR was also increased significantly compared to the nontreated group, as shown in Table 1. The treated group's mean in fact approximated that of controls at 2–4 hr of age. Liver weights were likewise elevated in the treated group.

DISCUSSION

Hypoglycemia in human fetuses has been documented in four conditions: fetal growth retardation, pre-eclampsia, accidental hemorrhage, and maternal hypoglycemia (31). Studies in humans obviously are limited, but the animal model of intrauterine

Table 1. Liver weight and glycogen content in newborn rats¹

	Control	IUGR	IUGR + corticoid
Liver weight (g)			
At birth	0.28 ± 0.04^2 (n = 25)	0.15 ± 0.06 (n = 53)	0.15 ± 0.04 (n = 24)
At 2–4 hr	0.19 ± 0.02^3 (n = 12)	0.11 ± 0.01 (n = 40)	0.14 ± 0.01^3 (n = 26)
Glycogen (mg/g wet weight)			
At birth	25.7 ± 1.8 (n = 25)	22.2 ± 1.3 (n = 53)	16.9 ± 0.79^4 (n = 18)
At 2–4 hr	28.3 ± 3.9^3 (n = 12)	13.6 ± 1.1 (n = 40)	26.9 ± 1.7^3 (n = 25)
Glycogen (mg total)			
At birth	7.23 ± 0.43^2 (n = 25)	2.96 ± 0.17 (n = 53)	2.55 ± 0.30 (n = 18)
At 2–4 hr	5.26 ± 0.68^3 (n = 12)	1.45 ± 0.17 (n = 40)	3.56 ± 0.32^2 (n = 25)

¹ IUGR: intrauterine growth retardation. All values represent mean $\pm$ SE; number of animals in parentheses.

² $P < 0.001$, as compared to the nontreated IUGR group.

³ $P < 0.005$, as compared to the nontreated IUGR group.

⁴ $P < 0.02$, as compared to the nontreated IUGR group.

Fig. 2. Correlation between mean plasma glucose concentrations and the total liver glycogen content of control and IUGR pups at birth.

growth retardation has enabled investigators to gather data on early neonatal hypoglycemia and other aspects of carbohydrate metabolism in the small-for-gestational age animal. Work by Oh and colleagues (28) led to the hypothesis that impaired placental transfer of substrate (glucose) to the fetus because of decreased blood flow was the mechanism responsible for low blood glucose values noted in rat pups with IUGR. Exploration into the cause of neonatal hypoglycemia in the dysmature rat pup was carried out by Nitzan and Groffman (26, 27) who used *in vitro* techniques. They demonstrated impaired glucose production from labeled alanine in liver slices from these animals and suggested that a delay in postnatal development of hepatic gluconeogenesis might contribute to the hypoglycemia associated with fetal malnutrition. An examination of hepatic enzymes by Chanez and coworkers (6), however, failed to reveal any significant abnormalities in the various enzymes involved in gluconeogenesis. On the other hand, data suggesting a decrease in phosphoenolpyruvate carboxykinase (PEPCK) activity, a key step in gluconeogenesis, was gathered by Philippidis and Ballard (30). A relative deficiency of this enzyme in fetal liver was observed, and it was postulated that a defect in the development of PEPCK activity existed in the dysmature newborn animal.

It was the purpose of our investigation to concentrate on the first 6 hr of neonatal life in the dysmature rat and to determine the incidence and severity of hypoglycemia and whether prenatally administered glucocorticoid could alter the pattern of hypoglycemia in intrauterine growth retardation. Placental transfer of cortisol analogs similar to the agent used in this study has been demonstrated by Ballard *et al.* (2) and Barrett *et al.* (3).

Data from this study indicate that the 2–4 hr postnatal period is a critical time for the development of hypoglycemia in that 91% of growth-retarded newborns became hypoglycemic during that time. The hypoglycemic tendency appeared immediately after birth when 39% of the animals with IUGR were noted to have plasma glucose values less than 40 mg/dl. When compared to control littermates at birth, the dysmature pups had similar mean values for glycogen concentration per g liver, but lower total liver weight and total liver glycogen content. A correlation was identified between mean plasma glucose levels at birth and total liver glycogen content. During the 2–4-hr neonatal period, significant differences continued to be present between control and fetally malnourished pups, including values for plasma glucose, glycogen, and total liver weight.

Protection from the trend toward low plasma glucose values in the dysmature rat pup was demonstrated in the group of experimental animals who were exposed 48 hr before birth to maternally administered glucocorticoid. The lowered incidence of hypoglycemia in the corticoid-treated group of pups with IUGR during the 2–4-hr postnatal period was accompanied by higher mean plasma glucose concentrations compared to those of nontreated IUGR animals. The difference in mean plasma glucose in these two groups extended to the 4–6-hr neonatal period. However, no significant effect of antenatal corticoids was noted in the glucose pattern of controls during the same time period. At birth, hepatic glycogen concentration in corticoid-exposed controls was also equivalent to values in nontreated controls. The corticoid-treated, dysmature group exhibited a marked increase in total liver weight and glycogen concentration per wet liver weight; this was reflected in the markedly greater hepatic glycogen stores in the treated animals. Thus, it would appear that antenatally administered glucocorticoid influences the development of neonatal hypoglycemia in the fetally malnourished rat and that the major effect is not at birth, but during the 2–4-hr period of neonatal life.

A number of possibilities exist to explain the mechanism of glucocorticoid action on neonatal glucose homeostasis. Control of glucose metabolism by adjustments in enzyme activities is a well established phenomenon (13). Developmental patterns of liver enzymes involved in glucose metabolism have been studied by a number of investigators (10, 13, 16, 36, 40); results of this work have been reviewed in detail by Greengard (13). It has

been shown that injection of thyroxine and cortisol enhances the development of enzyme activities normally present in the late fetal period. Among these are glycogen synthetase, which appears in a more active form in fetuses given steroids (14), and tyrosine aminotransferase (17). In studying the effect of glucocorticoids on key hepatic enzymes of gluconeogenesis, Yeung and associates (40) reported a slight depression of glucose-6-phosphatase activity and a 50% depression of hexose diphosphatase in the fetal rat liver. In further investigations, it was shown that pyruvate kinase and aspartate aminotransferase activities in fetal rat liver were both depressed by treatment, whereas phosphofructokinase activity was elevated. These investigators concluded that glucocorticoids did not initiate the synthesis of gluconeogenic enzymes and that steroids might play a role in control mechanisms for regulation of enzyme activities once synthesis was initiated (14).

Other investigators have evaluated the activity of PEPCK, an enzyme which catalyzes the transformation of oxaloacetate into phosphoenolpyruvate and which plays an important role in gluconeogenesis. It has been shown that glucocorticoid administration causes an increase in the activity of this enzyme. Presumably, this could enhance the capacity of the gluconeogenic pathway (29).

Clinical studies in small-for-gestational age infants have examined glucose responses and plasma amino acid patterns (21) after intravenously injected alanine, and compared the changes with those of full term infants (23, 24). Marked hyperaminoacidemia was noted in SGA infants, particularly affecting alanine, proline, valine, and glycine. It has therefore been proposed by Mestyan *et al.* (23, 24) that factors responsible for the provision of substrates for glucose synthesis may be present in the small-for-gestational age infant, but that insufficient hepatic gluconeogenic enzymes contribute to impaired glucose homeostasis.

Animal correlates utilizing the Wigglesworth model of IUGR indicate a somewhat similar change in blood amino acid patterns during the first 3 days of life (1, 34). The ratio of nonessential to essential amino acids in the rat pup with IUGR is increased over that of the control animal. Between 3 and 5 days after birth, all amino acid changes seem to disappear except for high levels of alanine in dysmature progeny. It is perhaps this relative inability to produce glucose from alanine that may explain the persistent pattern of hypoglycemia in some of the newborns with IUGR. If this is the case, then one might postulate that glucocorticoids act favorably on glucose homeostasis by increasing amino acid transport and bringing about more effective utilization of gluconeogenic substrate. Further investigations are needed, however, regarding the precise mechanism of action of steroids in protecting the rat pup with IUGR from neonatal hypoglycemia.

CONCLUSION

An animal model (rat) of the IUGR state associated with uteroplacental insufficiency was utilized to evaluate the effect of maternally administered glucocorticoid on the hypoglycemic tendency of growth-retarded progeny. Through the first 2 hr of neonatal life, 46% of the pups with IUGR exhibited hypoglycemia, whereas only 18% of control pups manifested this state. The incidence of hypoglycemia in animals with IUGR increased to 91% during the 2–4-hr time interval, whereas control pups maintained their low incidence with the value eventually falling to 4% at 4–6 hr of age. Concentration of plasma glucose at birth of individual control and IUGR progeny were found to correlate significantly with total liver glycogen content. Total hepatic glycogen stores in the fetally malnourished animals were markedly lower than those of controls. With prenatal glucocorticoid administration, there was no decline in the plasma glucose values of pups with IUGR and at 4–6 hr of age, the dysmature pups attained mean plasma glucose concentrations approximating those of nontreated control animals. Liver glycogen content in the treated pups with IUGR also showed significantly higher values than the nontreated IUGR progeny.

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