

Perinatal Hypoadrenalism in the Rat does not Alter Glucose Tolerance and Insulin Secretory Response to Glucose

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ABSTRACT

Fetal and neonatal hypoadrenalism was induced by treating rat mothers with metyrapone from day 12 of pregnancy to day 7 postnatally. Hypoadrenalism in the neonates was indicated by a slight reduction in body weight, adrenal hyperplasia and a tendency towards reduced adrenal corticosterone concentration. An intraperitoneal glucose tolerance test on the 7-day old neonates did not show any disturbances of glucose disposal or insulin secretory response to glucose. The data suggest that adrenal corticosteroids are not essential for the perinatal development of the B-cell secretory response to glucose.

INTRODUCTION

The functional development of the pancreatic B-cell takes place in an orderly fashion during fetal and neonatal life. In the rat fetus B-cells can be identified morphologically on day 12 of gestation (27, 35) but they do not attain functional competence until later in development. Glucose-stimulated insulin secretion is absent on gestational day 15 (28), can be demonstrated on day 18 (3, 19), develops further during the neonatal period (1) but does not reach its full capacity until adult life (11). The factors governing these developmental changes are not fully understood, but glucose itself has been demonstrated to stimulate the development of a glucose-sensitive insulin secretory response (2, 8). However, it is also of interest to note that serum concentrations of growth hormone (4, 29, 30), thyroid hormones (7) and corticosterone (16) all increase dramatically during the perinatal period and could potentially modulate B-cell develop-

ment. Growth hormone increases the insulin output of fetal pancreatic islets in vitro but fails to increase the glucose sensitivity of the secretory process or islet cell replication (32). Fetal decapitation in utero, which minimizes pituitary influence on pancreatic development, does not retard the development of insulin secretion (17, 20). Thyroid hormones are similarly without effect on B-cell maturation in vitro (32) and perinatal hypothyroidism does not affect the development of the insulin secretory response to glucose in vivo (31). So far the possible role of corticosterone in this process has not been investigated.

In the present study insulin secretory development is investigated in the offspring of rats treated with metyrapone during pregnancy and lactation. This substance crosses the placenta and diminishes both maternal and fetal corticosterone synthesis (10) by inhibition of adrenal hydroxylases (5, 22), thereby inducing a state of hypoadrenalism.

MATERIALS AND METHODS

Female Sprague-Dawley rats from a local colony (Biomedicum, Uppsala, Sweden) were caged overnight with males and vaginal smears were taken on the following morning. Day 0 of pregnancy was considered as the day on which a sperm-containing smear was found. In order to induce hypoadrenalism in the offspring, the rat mothers were treated with metyrapone from day 12 of pregnancy to the end of the experiment on day 7 postnatally. Two ml of an aqueous solution of metyrapone (Metopiron^R; kindly provided by Ciba-Geigy, Göteborg, Sweden) was administered with a gastric tube as a once daily dose of 50-70 mg/kg body weight. Control animals were not treated with gastric intubation. All animals had free access to drinking water and pelleted laboratory animal chow (R2 pellets; A-lab, Södertälje, Sweden) throughout the experiment. Delivery took place spontaneously on day 22 of pregnancy in both experimental and control group.

On day 7 postnatally a glucose tolerance test was performed on the offspring. The pups were weighed and some removed for sampling of basal serum concentrations of glucose and insulin or for dissection of pancreas and adrenals. The remaining pups received an i.p. injection of 2 g/kg body weight of glucose in a 30% (w/v)

aqueous solution. The injection site was sealed with vaseline and the animals returned to their mothers. At 30, 60 and 120 min after the injection animals from each litter were killed by decapitation and blood collected from the severed neck vessels. At the end of the experiment the mothers were weighed, killed by cervical dislocation and blood collected from the cut neck vessels.

Blood samples were allowed to clot for one hour at +4° before centrifugation and separation of serum. Serum samples were stored at -20° until analyzed. Serum glucose was determined with a glucose oxidase technique using a Beckman Glucose Analyzer 2 (Beckman, Fullerton, USA) and insulin was assayed radioimmunologically (12). Corticosterone was assayed with a commercially available radioimmunoassay (Radioimmunoassay Ltd, Cardiff, UK).

Pancreatic glands of neonates, dissected out immediately after decapitation, were weighed and sonicated in 500 μ l distilled water. After sonication 200 μ l of each sample was added to 2 ml acid ethanol (70% ethanol with 1.5 ml 1 M HCl/100 ml). The samples were thoroughly mixed and incubated overnight at +4°, centrifuged and the supernatants stored at -20° until assayed for insulin.

Adrenal glands of neonates, dissected out immediately after decapitation, were weighed and sonicated in 500 μ l distilled water. Aliquots were removed for determination of their DNA content (14, 21). The remaining samples were diluted with equal volumes of absolute ethanol and stored at -20° until assayed for corticosterone.

Results are given as means \pm S.E.M. and statistical analysis was performed using Student's two-tailed t-test for independent observations.

RESULTS

Metyrapone-treated pregnant rats had an apparently normal pregnancy and delivery occurred at the same gestational age as in the control rats. Litter size was not affected, nor was there any difference in maternal body weight on day 7 postnatally (Table 1). The basal serum glucose and insulin concentrations of metyrapone-treated rat mothers were not different from those of control mothers. However, the serum corticosterone concentration was

Table 1. Effects of metyrapone treatment during pregnancy and lactation in the rat.

	Controls		Metyrapone-treated	
Maternal				
body weight (g)	303 ± 6	(8)	316 ± 8	(11)
serum glucose (mM)	6.5 ± 0.3	(8)	6.5 ± 0.3	(8)
serum insulin (µg/l)	0.41 ± 0.04	(8)	0.43 ± 0.08	(7)
serum corticosterone (nM)	5.4 ± 2.4	(8)	1.3 ± 0.2	(8)*
Neonatal				
litter size (n)	11.0 ± 1.0	(8)	10.2 ± 0.2	(11)
body weight (g)	14.9 ± 0.2	(88)	14.4 ± 0.2	(104)*
serum insulin (µg/l)	0.40 ± 0.04	(27)	0.29 ± 0.01	(24)***
adrenal wet weight (mg)	0.81 ± 0.08	(25)	1.32 ± 0.12	(24)***
adrenal wet weight/DNA (mg/ng)	39 ± 3	(25)	52 ± 10	(22)***
corticosterone/adrenal wet weight (pmol/mg)	57 ± 10	(25)	37 ± 6	(23)
insulin/pancreatic wet weight (ng/mg)	111 ± 7	(27)	68 ± 11	(25)**

Table 1. Pregnant and lactating rats were administered metyrapone, 50-70 mg/kg body weight, by gastric intubation from day 12 of gestation to day 7 postnatally. On day 7 postnatally both mother and pups were killed and the adrenal and pancreatic glands and serum were collected for assay of hormones. Values are given as means ± S.E.M. for the number of rats (n) indicated. Significance of difference between metyrapone-treated rats and their offspring and control animals: * p<0.05; ** p<0.01; *** p<0.001.

lowered to 24% of the value of the control mothers.

The litters of metyrapone-treated mothers suckled well and upon examination their gastrointestinal tracts were filled with milk, indicating that metyrapone-treatment of the mothers probably did not interfere with their ability to feed the offspring. Nevertheless, at 7 days of age their neonates had a slightly reduced body weight (Table 1).

Adrenal wet weight was increased in the neonates of metyrapone-treated mothers (Table 1). The ratio between adrenal wet weight and DNA content was also increased, indicating a cellular hypertrophy. There was also a tendency towards a reduced adrenal concentration of corticosterone in the offspring of metyrapone-treated mothers, but this did not reach statistical significance.

When an i.p. glucose tolerance test was performed on the 7-day old neonates the glucose tolerance was similar in the offspring of metyrapone-treated mothers and controls (Fig 1). The basal serum insulin concentration was lower in the offspring of metyrapone-

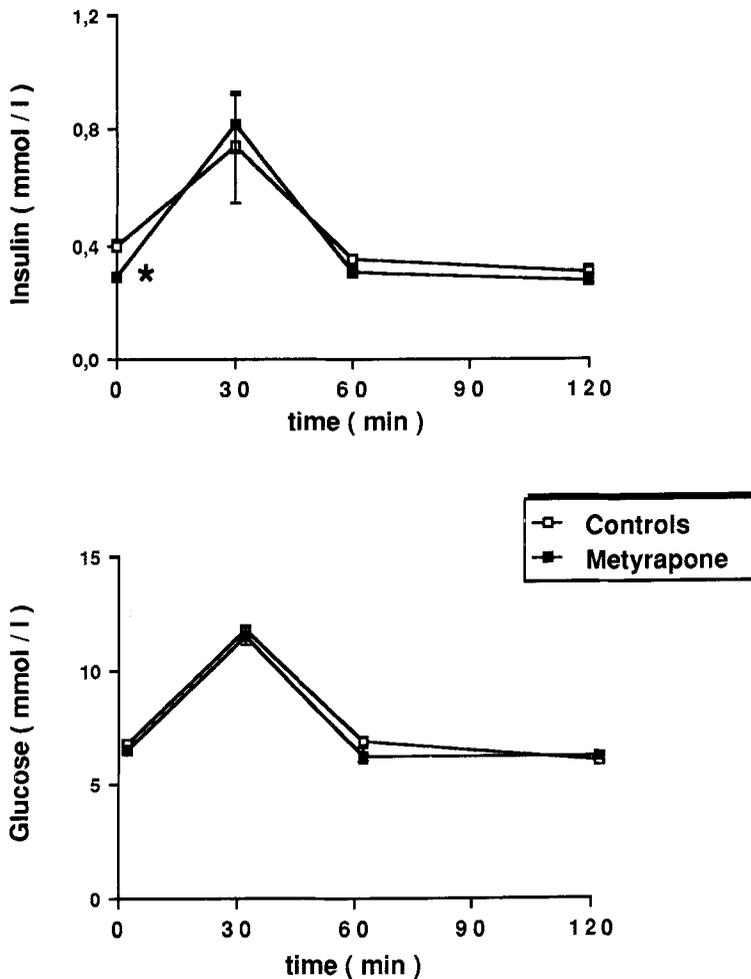


Fig 1. Effect of metyrapone treatment of rats during pregnancy and lactation on the glucose tolerance and insulin secretory response to glucose in the offspring. Rat mothers were treated with metyrapone (50-70 mg/kg body weight/day) from day 12 of gestation to day 7 postnatally when an intraperitoneal glucose tolerance test (2 g glucose/kg body weight) was performed on the offspring and serum concentrations of glucose (lower panel) and insulin (upper panel) measured. Values are given as means \pm S.E.M. for 7-11 animals. Significance of difference between the offspring of metyrapone-treated animals and the offspring of control animals: * $p < 0.05$.

treated mothers, but the insulin secretory response to glucose during the test was similar in both groups (Fig 1).

The pancreatic insulin concentration in the neonates of metyrapone-treated mothers was only two thirds of that of the

control group (Table 1).

DISCUSSION

Treatment with metyrapone during pregnancy and lactation induces a state of hypoadrenalism in both mother and offspring (9, 10, 26). In the present study this was confirmed by the decreased serum concentration of corticosterone in the mothers. Despite the hypoadrenalism the rat mothers thrived, did not lose weight and carried their offspring to term without losses. The neonates appeared healthy although signs of hypoadrenalism such as adrenal hyperplasia and a tendency towards decreased adrenal corticosterone concentration could be demonstrated. Serum concentrations of corticosterone in the offspring (data not shown) showed variations in both the experimental and control group. This was considered a stress response due to handling and changes in environmental temperature during the experiment and would not reflect a failure of metyrapone to inhibit fetal adrenal function. Indeed, in previous studies of metyrapone-induced fetal hypoadrenalism a decrease of serum corticosterone concentration was not observed despite adrenal hypertrophy and diminished adrenal corticosterone content (26).

The peak values of the glucose tolerance test in the neonates were higher than in adult rats of the same strain (6) and the insulin secretory response to the glucose peaks lower than the corresponding response in adults (33). These observations confirm the immaturity of the insulin secretory response to glucose in 7-day-old rats (11) which as a consequence dispose of glucose less efficiently than adults. The insulin secretory response to glucose in vivo is similar to the response in vitro in the offspring (11). The presently demonstrated lack of difference between the offspring of metyrapone-treated rats and the control animals would therefore suggest the adrenal corticosteroids are not essential for the perinatal development of a glucose-sensitive insulin secretion.

Chronic hypoadrenalism in the adult rat diminishes insulin secretion (25, 34) whereas administration of glucocorticoids to normal adult animals increases insulin output by stimulation of B-cell growth (13, 18, 24) and insulin secretion (23, 24). The

present finding of lowered serum and pancreatic insulin concentrations could be interpreted to indicate a hypoplasia of the islet organ in the neonates with hypoadrenalism. The total capacity for insulin secretion would thus be diminished although the glucose sensitivity of the B-cell has been reported to remain intact (15). Further experiments, including the morphometric analysis of the endocrine pancreas of the neonates, would be needed to clarify this hypothesis.

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