Effect of Environmental Temperature on Glucose-induced Insulin Response in the Newborn Rat

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SUMMARY

Blood glucose and plasma insulin and glucagon concentrations were determined in full-term rats delivered by cesarean section and exposed to 37° C. or 24° C. environmental temperature during the first hours of extrauterine life.

When newborn rats were maintained at thermal neutrality (37° C.), a transient period of hypoglycemia of two hours occurred, associated with a rapid fall in plasma insulin and a rise in plasma glucagon concentrations. During cold exposure (24° C.), the blood glucose level remained stable over the four hours studied; the decrease of plasma insulin was sluggish while the rise of plasma glucagon was unchanged.

In newborn rats maintained at 37° C., an intraperitoneal glucose load one hour after delivery produced a marked rise in blood glucose and plasma insulin concentrations one hour later. The distribution of experimental points suggested a sigmoidal dose-response curve. By contrast in newborn rats kept at room temperature (24° C.) the same glucose load did not induce any increase in plasma insulin in spite of hyperglycemia. However, phentolamine resulted in pronounced plasma insulin rise in hypothermic newborns in response to glucose administration. From these observations it is concluded that the in-vivo unresponsiveness of the beta cells to glucose at birth, reported by others, is mainly due to the experimental conditions.

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Most investigators have claimed that the fetal pancreas of the rat responds poorly or not at all to glucose stimulation both in vivo[8,18] and in vitro.[1,7,17,18,21,26] However, glucose infused to pregnant rats for one hour produces a threefold increase of the basal plasma insulin concentration in 21.5-day-old fetuses in utero.[13,20] This was recently confirmed in 21-day-old rat fetuses in utero.[7]

Thus, the apparent unresponsiveness of the newborn rat pancreas to glucose in vivo[2,7,18] cannot be explained only by an immature glucose-induced insulin-secretory mechanism. It may be more likely related to experimental conditions and to the complex metabolic and hormonal changes that occur during the first hours after birth.[12]

It has been observed that hypothermia impairs insulin release to glucose in adult animals.[5,9] The newborn rats have a not fully developed thermoregulatory mechanism[27] and are much more sensitive to changes in the external temperature. In the present paper we report on the influence of environmental temperature on blood glucose, plasma insulin, and glucagon concentrations during the early neonatal period in the rat. The insulin response to a glucose load administered one hour after delivery was also studied at thermal neutrality (37° C.) or at room temperature (24° C.).

MATERIAL AND METHODS

Animals. Albino rats of the Sherman strain were used. They were fed a laboratory chow (UAR.B03) ad libitum. The duration of pregnancy was estimated

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from the assumed time of ovulation, which usually occurred around 0100 hour the night of cohabitation with a male. Since a precise timing of birth was desired, the fetuses were delivered by cesarean section at 21.5 days of gestation within two minutes after cervical dislocation of the mothers.

Environmental temperature. The newborn rats, weighing about 5 gm., were either transferred to a Humidicrib (Jouan S.A., Paris) in which the temperature was maintained at 37° C. and relative humidity at 70 per cent or kept at room temperature (23-25° C.). They were unfed for the whole of the experiments. Core temperature was estimated in some newborns placed at 24° C. with an iron-constantan thermocouple introduced deeply in the rectum and recorded on a millivoltmeter (Sefram, Paris).

Glucose load procedure. After one hour at 37° C. or 24° C., the neonates were injected intraperitoneally with varying volumes of a 30 per cent (w/v) glucose solution (5 to 30 mg. glucose per animal) and were placed again for another hour at their previous experimental temperature. Control newborns were treated in the same way but injected with 0.9 per cent (w/v) NaCl solution.

Sampling. Blood samples were collected on newborns by puncture at the level of the armpit vessels, in heparinized tubes cooled in ice and containing an antiprotease (Iniprol, Choay Laboratory, France). Plasma was separated immediately at +4° C. and stored at −20° C. until assay.

Chemical analyses. Blood glucose concentrations were determined on 20-μl. blood samples by the glucose oxidase method.[19] Plasma insulin concentrations were measured by radioimmunoassay. The pork insulin antiserum (SCAIP, no. 41-3) used in these experiments had previously been shown to react poorly with pork proinsulin.[25] ^{131}I-labeled human insulin was supplied by the Centre National de Transfusion Sanguine (France). Standard curves were obtained with crystalline rat insulin (24 U./mg.; batch R 169; Novo-Denmark). The sensitivity of the technique allowed insulin to be measured in 10-μl. duplicate plasma samples. Plasma immunoreactive glucagon was determined with an antiserum specific for pancreatic glucagon K 47, kindly supplied by Dr. L. G. Heding (Novo Research Institute, Denmark) in an assay system described previously.[11]

The results were given as means ± S.E.M., and statistical analysis was done by unpaired Student's *t* test.

RESULTS

Glucose, insulin, and glucagon changes at birth. In newborn rats kept at thermal neutrality (37° C.), the blood glucose concentrations (table 1 and figure 1) fell rapidly one hour after delivery (27 ± 2 mg./100 ml.); then it rose slowly for up to four hours, to a level above the value measured at birth. The plasma insulin concentration decreased sharply during the first hour of extrauterine life; during the next hour it decreased slowly and remained stable for the following two hours. By contrast, plasma glucagon concentration increased threefold to a peak at 30 minutes, then it decreased but remained at a value significantly greater than that observed at birth.

Effect of cold exposure. The measured rectal temperature of newborn rats placed at birth at room temperature decreased rapidly to reach this environmental temperature within the first half hour (figure 2). At 24°

TABLE 1

Effect of 37° C. or 24° C. environmental temperature on blood glucose and plasma insulin and glucagon concentrations in newborn rats during the first four hours of extrauterine life

		Hours after birth				
		0	0.5	1	2	4
Blood glucose (mg./100 ml.)	37° C.	54 ± 2 (45)	38 ± 4* (28)	27 ± 2‡ (11)	45 ± 3* (10)	72 ± 5† (43)
	24° C.	—	54 ± 2 (8)	60 ± 4 (13)	53 ± 4 (10)	59 ± 3 (10)
Plasma insulin (μU./ml.)	37° C.	206 ± 12 (22)	73 ± 8‡ (9)	42 ± 3‡ (13)	27 ± 3‡ (10)	32 ± 2‡ (14)
	24° C.	—	105 ± 8‡ (23)	93 ± 6‡ (23)	70 ± 6‡ (25)	40 ± 4‡ (16)
Plasma glucagon (pg./ml.)	37° C.	330 ± 30 (15)	1,038 ± 110‡ (16)	950 ± 70‡ (16)	620 ± 40† (14)	560 ± 50† (14)
	24° C.	—	707 ± 65† (10)	967 ± 35‡ (12)	712 ± 68† (14)	572 ± 29† (10)

The values are means ± S.E.M. Numbers in parentheses indicate number of observations. Significant differences when compared with 0 time value * $p < 0.05$; † $p < 0.02$; ‡ $p < 0.01$. The plasma glucagon levels in newborn rats kept at 37° C. are taken from Girard et al.[12]

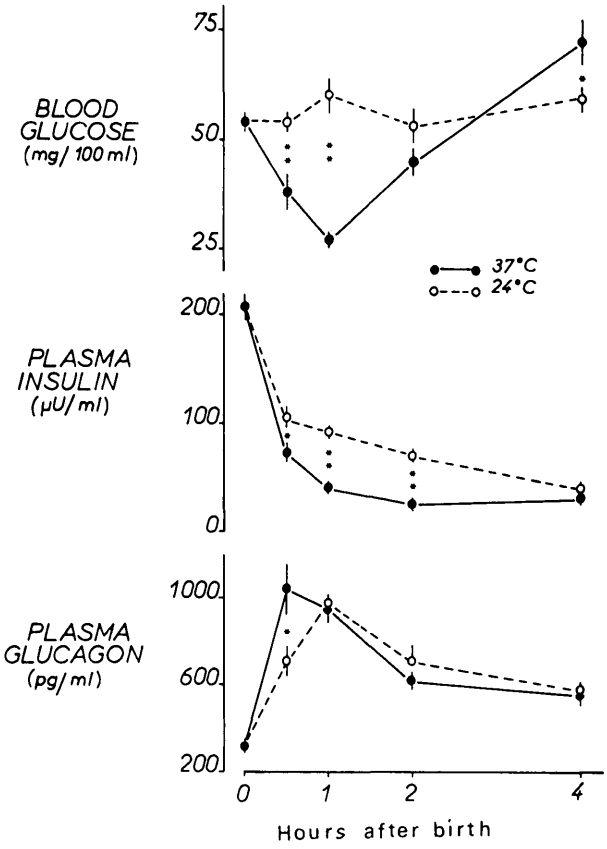

FIG. 1. Effect of environmental temperature on blood glucose and plasma insulin and glucagon concentrations in newborn rats during the first four hours of extrauterine life. Values are means ± S.E.M. Significance of differences between the 37° C. and 24° C. groups for each time: *p < 0.05; †p < 0.001.

C. the blood glucose values did not differ significantly from the level observed at birth, ranging from 54 to 60 mg./100 ml. during the four hours studied (table 1). Plasma insulin concentration decreased more slowly than in the 37° C. group, the difference being statistically significant at 0.5, 1, and 2 hours (figure 1); however, at four hours it reached values close to this group's (P >0.05). Plasma glucagon at 24° C. rose more slowly during the first hour; later on the differences with the 37°-C. group were not significant.

Effect of glucose. At thermal neutrality, the sustained hyperglycemia measured one hour after the glucose load was associated with a rise in plasma insulin concentrations (figure 3). The variable blood glucose concentrations obtained by injection of increasing amounts of glucose were connected with proportional insulin responses. The distribution of experimental points suggested a sigmoidal glucose-insulin dose-response curve. The plateau of maximal plasma insulin response

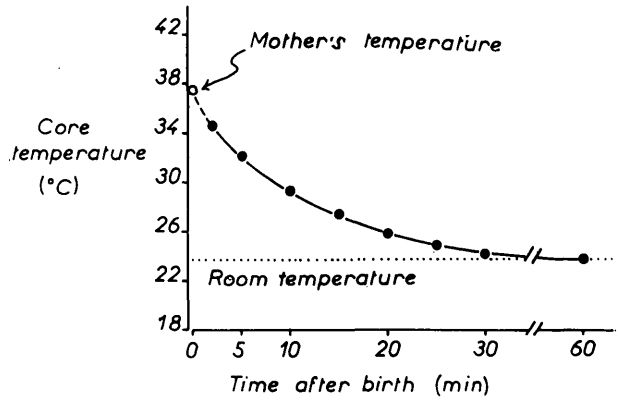

FIG. 2. Changes in rectal temperature in newborn rats (n = 5) placed at birth at room temperature (23.8° C.). Values are means ± S.E.M.

was reached at about 200 mg. glucose/100 ml., and the half-maximal response was seen at about 130 mg. glucose/100 ml. By contrast, in the newborn rats kept at room temperature (24° C.), the plasma insulin did not rise at all in spite of similar sustained hyperglycemia (figure 3).

Effect of phentolamine. In order to test a possible mechanism for the unresponsiveness of the β cells to glucose in hypothermic (24° C.) newborn rats, an alpha-adrenergic blockade was produced by a subcutaneous injection of phentolamine (Regitine, 20 mg./kg. body weight) 10 minutes prior to glucose or NaCl administration. As shown in table 2, phentolamine restored the insulin response to glucose (P < 0.001). A slight but significant increase of both blood glucose and plasma insulin concentrations was observed in control animals injected with phentolamine plus NaCl.

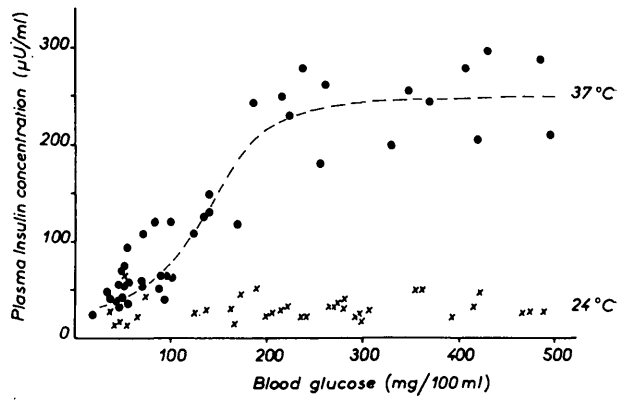

FIG. 3. Effect of environmental temperature on plasma insulin response to a one-hour intraperitoneal glucose load in one-hour-old newborn rats.

TABLE 2
Effect of phentolamine on one-hour glucose-induced plasma insulin increase in newborn rats maintained at 24° C.

		Blood glucose (mg./100 ml.)	Plasma insulin (μU./ml.)
NaCl	(7)	53 ± 3	47 ± 2
Glucose	(7)	435 ± 23†	45 ± 1
NaCl + phentolamine	(7)	68 ± 2*	63 ± 4*
Glucose + phentolamine	(6)	439 ± 20†	165 ± 26†

The values are means ± S.E.M. Numbers in parentheses indicate number of observations.
*Difference significant for $p < 0.01$.
† $p < 0.001$ from newborn rat injected with NaCl alone.

DISCUSSION

The maintenance of a constant body temperature requires the complete development of thermoregulatory systems. Since in the newborn rat these regulatory processes are partially present,[27] the blood glucose and hormonal changes observed after cold exposure (24° C.) may be the direct consequences of hypothermia or be related to the attempted adaptation of the newborn to cold. A large and abrupt drop in the external temperature from 37° C. to 24° C. leads to a rapid tissue cooling, as demonstrated in figure 2. The stable blood glucose concentrations observed in the present work as well as by others[14] may be due to this tissue cooling and to the reduction of glucose utilization at 24° C.

The neonatal fall in plasma insulin and the rise in plasma glucagon previously observed in cesarean-delivered rats maintained at 37° C.[11,12] are not modified by cold exposure. These hormonal changes occur without any variation of blood glucose concentration in cold-exposed neonates. This strongly suggests that hypoglycemia, which is observed in 37°-C.-exposed newborns cannot account alone for the spontaneous variations of these hormones at birth. This is in agreement with work of others,[6] although the environmental temperature was not indicated in that paper. An alternative mechanism to explain hormonal changes may be an increased activity of the autonomous nervous system triggered by the removal of the fetus from its intrauterine environment. In this way, stimulation of the nerve supply of the pancreas of the adult dog inhibits insulin[24] and stimulates glucagon[22] secretions. Injection of an alpha-adrenergic blocking agent (phentolamine) at birth prevents the fall of plasma insulin and the rise of glucagon that normally occurred during the first hour;[15] the administration of norepinephrine to term rat fetuses lowers insulin and increases glucagon concentrations.[13]

The endocrine pancreas of the one-hour-old newborn rat kept at thermal neutrality can release insulin in vivo in response to glucose (figure 3). The relationship between blood glucose and plasma insulin is a sigmoidal curve, in which the plateau and half-maximal insulin responses are close to those observed in isolated adult rat islets incubated one hour with glucose.[16] Thus, in vivo an adult-like sensitivity to glucose can be obtained from the beta-cell of the early newborn rat regarding at least the late (second-phase) insulin response. A glucose-induced biphasic insulin release has been reported in newborn infants after an intravenous glucose tolerance test.[10]

The unresponsiveness of the beta-cells to glucose in newborn rats kept at 24° C. (figure 3) is in harmony with previous data[1,7] in which external temperature was not reported. This lack of insulin response in vivo cannot be related to an immature glucose-induced insulin-secretory mechanism since newborns maintained at 37° C. showed a 10-fold increase of plasma insulin in response to glucose administration. An impaired glucose-induced insulin release has been reported in hypothermic adult dogs[5] that could be reversed by an alpha-adrenergic blocking agent.[4] Our present results support these data and suggest that alpha-adrenergic-receptor stimulation by increased sympathetic activity during cold exposure may account, at least in part, for the unresponsiveness of the β cells to glucose in vivo. Another possible mechanism might be a direct effect of cooling upon the pancreatic β-cell function as previously reported in isolated perfused rat pancreas.[9] On the other hand, in vaginally delivered rats maintained at 37° C., the lack of insulin response of the pancreas to a gastric glucose load[7] could result from the stressful route of glucose administration.

The observed insulin response of the fetal[7,13,20] and neonatal rat pancreas to glucose in vivo is not necessarily conflicting with its unresponsiveness to the same stimulation in vitro.[1-3,7,17,18,21,26] The metabolic environment in the two systems is quite different. The high plasma amino acid level reported during the perinatal period[12] could potentiate glucose-induced insulin release in vivo as it does in vitro on fetal,[17] newborn,[3] and adult[23] rat pancreas.

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