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Insulin and Glucagon Secretion by Islets Isolated from Fetal and Neonatal Rats

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Summary. Islets were isolated by mild collagenase digestion and microdissection from rat fetuses 2 days before term and pups 1 or 2 days after birth and their insulin and glucagon secretion studied in vitro. Fetal B cells were stimulated by 16.7 mmol/l glucose, 20 mmol/l leucine or 20 mmol/l arginine. Fetal A cells were not affected by glucose or leucine, but were significantly stimulated by arginine. Somatostatin abolished the effect of arginine on both IRI and IRG output. Neonatal islets proportionally released more insulin and glucagon than their fetal counterparts, but reacted to the tested agents in a similar fashion. During the perinatal period, pancreatic insulin storage increased at a higher rate than that of glucagon. It is concluded that fetal B cells are equipped with sensors to a variety of agents and able to modulate their secretory rate according to the concentration of these agents. A cells are reactive to arginine 2 days before term but do not become glucose reactive until several days after birth.

Key words: Insulin, glucagon, rat, fetus, neonate, isolated islets, glucose, arginine, leucine, somatostatin.

Whereas insulin (IRI) secretion has been extensively studied during the perinatal period in the rat, much less information is available concerning the regulation of glucagon (IRG) secretion by the fetal and neonatal rat pancreas. Glucose had little or no stimulatory effect on the IRI secretion of fetal pancreatic pieces [1, 2], cultured explants of fetal pancreas [3] or isolated fetal islets [4, 5]. The glucose stimulated insulin release mechanism was reported to mature after birth [1, 2] or after one week culture in a high glucose medium [5]. A few authors, however, observed that glucose significantly enhanced the rate of IRI secretion of fetal pancreatic pieces or cultured explants [6, 7] and the rate of IRI biosynthesis by fetal rat islets [8]. Leucine and arginine had no stimulatory effect on the IRI secretion of fetal pancreas removed on the 18th day of gestation and incubated either immediately or following a 4 day culture [9]. The addition of 10 mmol/l caffeine to the incubation medium unmasked the secretagogue effect of these 2 amino acids [9]. After birth, arginine enhanced both IRI and IRG secretion [10].

Experiments performed in various species lent support to the contention that fetal A cells were also immature. In particular, in the newborn rat, glucose did not modulate IRG secretion either in vivo [11] or in vitro [10, 12], whereas somatostatin significantly lowered plasma IRG concentration [11]. To our knowledge, data concerning the effect of glucose and somatostatin on fetal rat A cells are presently unavailable.

It could perhaps be concluded that during the perinatal period, the endocrine pancreas undergoes critical maturation steps possibly related to the switch from intra- to extrauterine life. In order to better define these maturation steps, we have compared the IRI and IRG secretion of rat islets isolated 2 days before term and 1 day after birth.

Materials and Methods

Female albino rats weighing 230 to 260 g were caged overnight with a male and the exact duration of pregnancy was known with a 12 h error. On the 20th day of gestation, the pregnant rats were anaesthetized with nembutal (60 mg/kg, sc), the pups were rapidly removed, their pancreases dissected and transferred into 2 ml of Hanks solution to which 1 mg of collagenase (Clostridium histolyticum, lot 1406317, Boehringer, West Germany) was added. Other pups were killed by decapitation 24 to 48 h after spontane-

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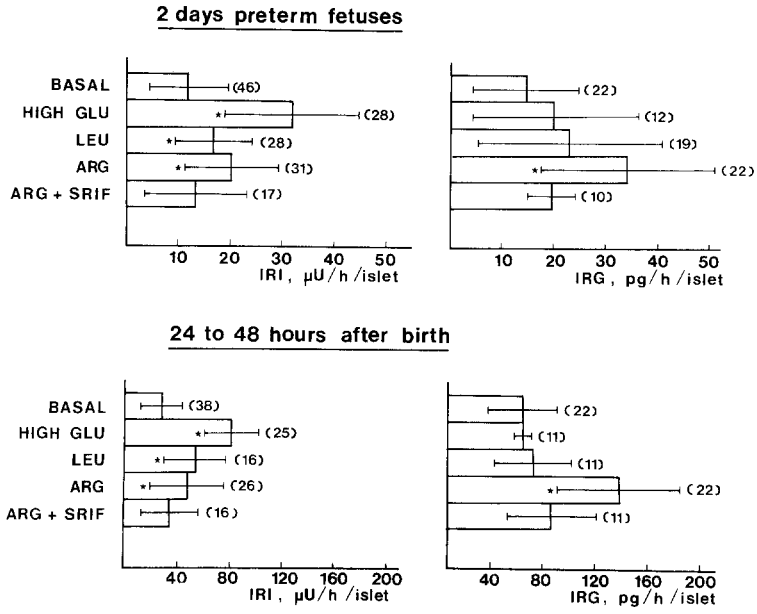

Fig. 1. Insulin and glucagon release (mean ± SD) of fetal and neonatal islets during a 1 h incubation in the presence of 2.8 mmol/l glucose (BASAL), 16.7 mmol/l glucose (HIGH GLU), 2.8 mmol glucose + 20 mmol/l leucine (LEU), 2.8 mmol/l glucose + 20 mmol/l arginine (ARG) and 2.8 mmol/l glucose + 20 mmol/l arginine + 5 µg/dl somatostatin (ARG + SRIF). Number of observations in parentheses. Note difference in scale for fetal and neonatal islets. * = $p < 0.001$ vs basal

ous delivery and their pancreases removed. After vigorous shaking for 10 min at 37°, collagenase digestion was stopped by ice cold Hanks solution and the islet suspension was washed 3 times. Using a dissecting microscope, the small fetal islets were best seen on a black background with lateral light. Since collagenase digestion was kept minimal, most islets were still attached to exocrine or connective tissue. They were carefully freed of extraneous tissue by microdissection, handpicked with a wire loop and transferred to Erlenmeyer flasks containing 2 ml of ice cold incubation medium kept under a 95% 0_2 + 5% CO_2 atmosphere. Each flask received 10 islets and was incubated for 60 min at 37° under gentle shaking. At the end of the incubation, the medium was aspirated through a Millipore filter holder equipped with a nylon filter of 5 µm pore, selected because it retained free cells but did not adsorb insulin. The cell-free medium was immediately frozen at −25°. Ten additional islets were directly transferred to a Potter homogenizer containing 2 ml of acid ethanol and the homogenate allowed to stand 24 h at 4°. The incubation medium consisted of Krebs-Ringer bicarbonate buffer supplemented with 10 mg/ml bovine serum albumin (crystallized, Sigma, St Louis) and 2.8 mmol/l glucose. When indicated, this basic medium was enriched by the addition of 13.9 mmol/l glucose, 20 mmol/l L-leucine or 20 mmol/l L-arginine, with or without 50 ng/ml cyclic somatostatin (Beckman, Geneva, lot C0513).

After appropriate dilution, IRI and IRG concentrations were measured by standard immunoassay procedures [13, 14] using a rat insulin standard (Novo Industri AS, Copenhagen, lot R 171) and a pork glucagon standard (Novo Industri AS, lot G 501575). The antiglucagon serum (AGS 10) was prepared by P. P. Foà and [^{125} I] monoiodoglucagon was purchased from New England Nuclear, Boston, Mass.

Differences between groups were evaluated by Student's *t*-test.

Results

The insulin and glucagon secretions of islets isolated from fetuses after 20 days of gestation and from pups aged 24 to 48 h are illustrated in Fig. 1. Two days before term, IRI output was increased 3 fold by 16.7 mmol/l glucose and to a lesser extent by 20 mmol/l leucine or arginine. The stimulatory effect of arginine was almost completely abolished by 50 ng/ml somatostatin. Glucagon secretion was not significantly altered by high glucose or leucine. Arginine enhanced the IRG output of fetal islets and this effect was abolished by somatostatin. Islets isolated from newborn rats reacted in a similar fashion, although they released about 2.5 times more insulin and 4 times more glucagon than the fetal islets.

The islets and total pancreas IRI and IRG contents of both age groups are summarized in Table 1. During the perinatal period, the pancreatic storage of insulin appeared to increase at a higher rate than that of glucagon.

After 1 h incubation in the presence of 2.8 mmol/l glucose, both fetal and neonatal islets released an amount of glucagon equivalent to approximately 2% of their content. The percentage of insulin released in 1 h was also about 2% in neonatal islets, but almost 4% in fetal islets.

Table 1. IRI and IRG extractable from isolated islets and total pancreas of 2 days preterm fetuses and pups aged 24 to 48 h

	2 days preterm	24 to 48 h after birth
Islet hormone content		
IRI, µU/islet	319 ± 142 (26)	1143 ± 345 (19)
IRG, pg/islet	669 ± 210 (25)	2446 ±1088 (22)
Pancreas hormone content		
IRI, mU/pancreas	52 ± 12 (26)	325 ± 80 (19)
IRG, ng/pancreas	147 ± 65 (22)	576 ± 210 (18)
Pancreas hormone concentration		
IRI, mU/mg	2.32 ± 1.65 (26)	11.0 ± 6.8 (19)
IRG, ng/mg	6.85 ± 2.82 (22)	16.0 ± 4.8 (18)

Mean ± SD. Number of observations in parentheses

Discussion

Fetal and neonatal islet cells qualitatively reacted to glucose, leucine, arginine and somatostatin as would adult cells, the only exception being that A cell secretion was not inhibited by a high glucose concentration. This peculiarity might be secondary to alteration in the cells during the process of islet isolation. Such an explanation seems unlikely since the functional integrity of these cells is evidenced by their reactivity to arginine and somatostatin. Before stating that fetal and neonatal A cells are completely glucose blind, one should, in addition, verify that glucagon secretion is not stimulated in a very low glucose medium and that the arginine stimulated IRG release is not inhibited by a high glucose concentration. In its present form however this finding is in agreement with those of Girard et al. and of Edwards et al. [11, 12]. Thus, B cell sensitivity to fuels and A cell sensitivity to arginine appear before the 20th day of gestation, whereas A cell reactivity to glucose appears later, at least several days after birth.

Neonatal islets contain and release more insulin and glucagon than fetal islets and the IRI/IRG molar ratio of released hormones is slightly lower for the islets of newborn rats. The slight decrease of the released hormones molar ratio observed in vitro markedly contrasts with the dramatic changes of IRI and IRG plasma concentrations which occur at birth [11, 15]. These observations strongly suggest that, in vivo, changes in IRI and IRG secretion rates are not determined by intrinsic changes of the islets themselves but rather by modifications of their neuroendocrine and/or metabolic environment. At birth, cord cutting would remove the B cell stimulating influence of the placenta [16] while, as suggested by Sperling et al. [17], postnatal stimulation of the adrenergic system would enhance glucagon and further depress insulin secretion.

Our data do not support the hypothesis that critical maturation steps take place in the endocrine pancreas during the perinatal period. We are tempted to conclude that late fetal islet cells should not be looked upon as „not yet complete adult cells". They are already equipped with receptors to a variety of substances and are able to modulate their secretion according to changes in their environment, of which the most dramatic occur at birth.

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