Insulin Biosynthesis in Isolated Pancreatic Islets of Fetal and Newborn Rats

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SUMMARY

The effect of glucose and glucose plus glucagon on the incorporation of H^3-L-leucine into proinsulin and insulin was examined in isolated islets of twenty-one-day old fetal and five- and ten-day old newborn rats. Maximal stimulation of (pro-) insulin biosynthesis was achieved with 100 mg. per cent of glucose in isolated islets of twenty-one-day old fetal rats. No additional effect was observed with 300 mg. per cent of glucose. On the other hand, in islets of five- and ten-day old newborn rats the incorporation of H^3-L-leucine into proinsulin and insulin was gradually augmented by glucose up to concentrations of 300 mg. per cent. Addition of glucagon to the various glucose concentrations only enhanced the synthesis of insulin in ten-day old newborn islets, whereas it had no effect on the islets of the younger age groups. The results show a different pattern of insulin biosyntheses in fetal and newborn islets, which may be related to the varied plasma glucose concentrations of the perinatal period. DIABETES *24*:373-77, April, 1975.

The single-chain protein, proinsulin, is a precursor in the biosynthesis of insulin.[1] It accounts for less than 5 per cent of the total immunoreactive insulin extracted from the adult[2] or fetal pancreas.[3,4] For the adult β-cell, glucose appears to be the main stimulus for insulin release and biosynthesis.[5-7] The mechanism by which glucose enhances the latter has been characterized more recently.[8] On the other hand, the effect of glucose on both insulin biosynthesis and release is further potentiated if glucagon is added to the incubation medium.[9,10] However, for the fetal and newborn endocrine pancreas it has been shown that glucose is a poor stimulus of insulin release[11,12] while the addition of glucagon significantly enhances the glucose-induced insulin secretion from the β cells of fetal and newborn rats.[13] Furthermore, during development of the fetal islets of Langerhans, glucagon has been detected slightly earlier and in higher concentration than insulin.[14] Thus it is assumed that glucagon might also influence the biosynthesis of insulin in prenatal life. Therefore, the effect of glucose and glucagon was studied on the incorporation of radioactive leucine into proinsulin and insulin in isolated islets of twenty-one-day old fetal and five- and ten-day old newborn rats.

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MATERIAL AND METHODS

Wistar albino rats weighing about 200 gm. were caged together for thirty-six hours. The first day was designated as day 1 of fecundation. Twenty-one days later fetuses were obtained by cesarean section under light ether anesthesia while other pregnant rats were allowed to deliver vaginally. Each pregnant rat had usually eight to twelve offspring which were all taken for a single experiment. The fetuses or newborns were decapitated, the pancreatic glands were quickly removed, and all glands of one litter were pooled. The islets were then isolated by the collagenase method[15] and collected with a Pasteur pipette using a stereomicroscope, where the light was placed in such a position that it was shining through the Petri plate from below. The islets appear to be a light brown color compared to the gray color of the exocrine tissue. Thus, the islets are easily harvested. Twenty islets were incubated in 1 ml. of a Krebs-Ringer bicarbonate buffer, pH 7.4. The buffer was supplemented with L-leucine-4,5-H-3 (50 μCi, 20 Ci, per millimole, Radiochemical Center, Amersham), 2 mg. per milliliter of the protease inhibitor, Trasylol (Bayer, Leverkusen, Germany), seventeen amino acids (20 μg. per milliliter of each amino acid, leucine excluded), and 10 μg. per milliliter of glucagon. The insulin concentration in this preparation was 0.9 mg. per

gram of glucagon. The glucose concentration varied from no glucose to 300 mg. per cent. Under constant shaking, the islets were incubated in an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide (v/v) for three hours at 37° C.

The incubation was stopped with ice cold trichloracetic acid with a final concentration of 10 per cent, followed by an ultrasonic disintegration of 15 seconds. The precipitate was washed with 5 per cent trichloracetic acid and dissolved in 0.5 ml. of 1 molar acetic acid. The islet proteins were then separated on a Sephadex G 50 fine column, 1.2 x 55 cm., which had been equilibrated with 1 M acetic acid and calibrated with albumin, porcine proinsulin and insulin, glucagon and leucine. One molar acetic acid was used as the effluent. The flow rate was 10 ml. per hour and the fraction volume 1 ml. The UV absorption was continuously recorded with an absorptiometer (Uvicord II, LKB-Produkter AB, Stockholm, Sweden) and 0.1 ml. of each fraction was assayed for radioactivity in a liquid scintillation counter, using Bray's scintillation liquid.[16] The remaining volume of each fraction was dried in vacuo over $CaCl_2$ and NaOH, and the immunoassay buffer for the insulin determinations was then added.[17]

In three separate experiments, radioactive I^{125}-porcine proinsulin, I^{125}-porcine insulin, and I^{125}-human-C-peptide were added to the incubation buffer and treated in the same way as the incubations with the islets. The TCA precipitate was chromatographed on the Sephadex G 50 fine column, and the radioactivity was then determined in each fraction. In addition, according to Steiner and Oyer,[1] the fractions of the proinsulin peak P (see figure 1) were pooled, dried in vacuo, and incubated in Tris-HCL, pH 8.5, with 100 μg. trypsin (p-tosyl-L-phenylalanine-chlormethylketone-trypsin) (Serva, Heidelberg, Germany), for ten minutes at 37° C. After rechromatography on the Sephadex G 50 fine column, most of the radioactive material was eluted in the region of insulin, giving strong reactions with antibodies to insulin.

Since the islets were taken from the same islet pool and equally distributed to the different test tubes, Student's paired *t* test was used for statistical analysis.

RESULTS

Figure 1 shows the elution profile obtained from the islet proteins on the Sephadex G 50 fine column. The first peak of radioactivity contains as yet unidentified islet proteins,[8,18] while the second peak (P) contains proinsulin and proinsulin intermediates,[19] and

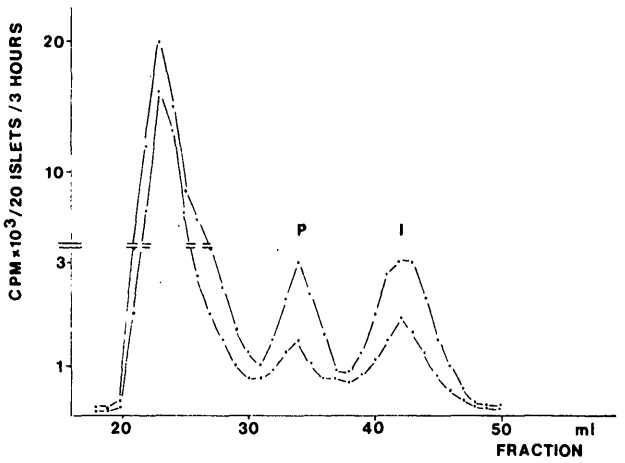

FIG. 1. The elution profile on the Sephadex G 50 fine column for islet proteins of isolated islets from twenty-one-day old fetal, lower line, and ten-day old newborn rats, upper line. P represents the proinsulin and I the insulin peak.

the third peak (I) insulin and the C-peptide.[19] This was established by the immunoreactivity of these fractions to insulin antibodies. Furthermore, when I^{125}-porcine proinsulin, I^{125}-porcine insulin or I^{125}-human C-peptide were chromatographed on the Sephadex G 50 fine column, the radioactivity appeared in the fractions of the corresponding peaks. The two lines in figure 1 represent separate experiments with twenty-one-day old fetal and ten-day old newborn rat islets. The incorporation of the radioactivity into the proinsulin and insulin peaks was higher in the twenty islets of the ten-day old newborn rats than in the twenty islets of the twenty-one-day old fetal rats, while the radioactivity which appeared in the first peak was almost the same in both age groups.

In figures 2 through 4 the total radioactivity of all the proinsulin or insulin peaks are presented as columns. Each column represents the summation of counts over seven to ten fractions from individual chromatographs.

Figure 2 shows the results from twenty-one-day old fetal islets. The first column, with no glucose in the incubation medium, is the sum of those seven to ten fractions where the proinsulin or insulin peak appeared when glucose was added to the incubation medium. There was neither a proinsulin nor an insulin peak detectable when the islets were incubated without glucose in the Krebs-Ringer bicarbonate buffer. The addition of 50 mg. per cent of glucose significantly enhanced the incorporation of radioactive leucine into proinsulin and insulin peak. When the glucose concentration was raised to 100 mg. per cent, the biosynthesis of proinsulin and insulin was further

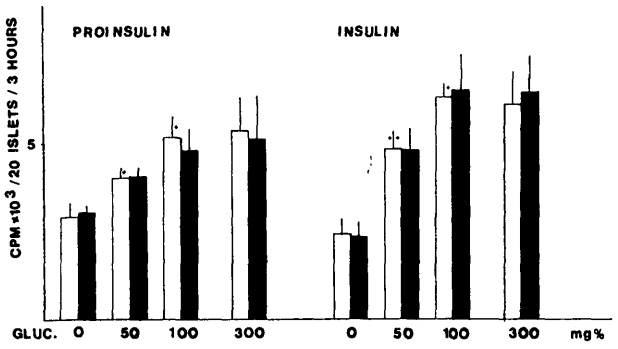

FIG. 2. The effect of glucose □ and glucose plus glucagon ■ on the biosynthesis of proinsulin and insulin in twenty-one-day old fetal rat islets. M ± S.E.M.; n = 5; +p<0.05; ++p<0.01; all other values are not significant.

stimulated while the addition of 300 mg. per cent did not increase this effect any further. At that age, the combination of 10 μg. per milliliter of glucagon and the various glucose concentrations did not increase the biosynthesis of proinsulin or insulin when compared to the preparations with no glucagon in the incubation medium. A direct comparison is justified, since in each experiment all the islets used for the incubations with glucose and the combinations of glucose and glucagon were collected from the same islet pool.

In islets of five-day old newborn rats, glucose at all concentrations stimulated the incorporation of radioactive leucine into the proinsulin and insulin peak (figure 3). There was a small but significant increase in the biosynthesis of proinsulin and insulin at the low concentrations of 50 mg. per cent and 100 mg. per cent. However, the effect of glucose on the biosynthesis of proinsulin and insulin was further increased when the glucose concentration was raised from 100 mg. per cent to 300 mg. per cent. In islets of five-day old newborn rats, the combination of glucose and 10 μg. per milliliter of glucagon did not

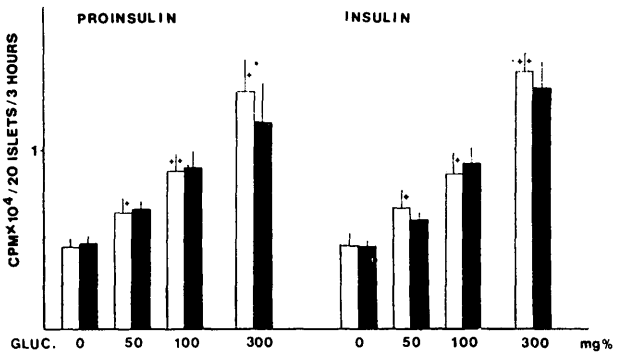

FIG. 3. The effect of glucose □ and glucose plus glucagon ■ on the biosynthesis of proinsulin and insulin in five-day old newborn rat islets. M ± S.E.M.; n = 5; +p<0.05; ++p<0.01; all other values are not significant.

stimulate the biosynthesis of proinsulin and insulin any further compared to the experiments with glucose alone.

In islets of ten-day old newborn rats, glucose provoked the same effect as in islets of five-day old newborn rats: it enhanced the biosynthesis of proinsulin and insulin from 50 mg. per cent to 300 mg. per cent (figure 4). The combination of glucagon and the low glucose concentrations had no additional stimulatory effect on the incorporation of radioactive leucine into the proinsulin fractions. However, 10 μg. per milliliter of glucagon at the high glucose concentration of 300 mg. per cent enhanced the biosynthesis of proinsulin and insulin. This effect was statistically significant for insulin (for proinsulin: $p < 0.1$, $n = 5$).

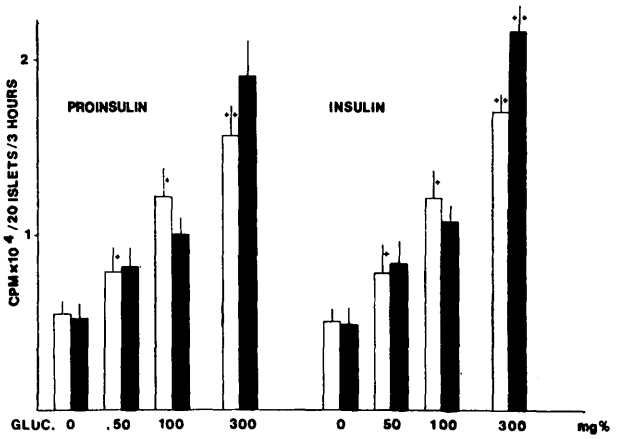

FIG. 4. The effect of glucose □ and glucose plus glucagon ■ on the biosynthesis of proinsulin and insulin in ten-day old newborn rat islets. M ± S.E.M.; n = 5; +p<0.05; ++p<0.01; all other values are not significant.

DISCUSSION

During the last days of pregnancy the insulin concentration of the fetal rat pancreas increases by about 50 to 100 per cent per day.[20] When the insulin concentration was expressed per gram of the pancreatic tissue, the highest value was reached in the newborn period and was tenfold higher than for the adult rat.[21]

Our results show that in twenty-one-day old fetal rat islets, glucose at low concentrations augmented the incorporation of tritiated leucine into proinsulin and insulin, while the amount of insulin synthesized at 100 mg. per cent of glucose was not different from the amount produced at 300 mg. per cent. This contrasts with the adult rat islets of Langerhans where it has been repeatedly shown that the biosynthesis of proinsulin and insulin was mainly stimulated by high glucose concentrations.[6,22] In fact, one group reported

a threshold level of 5 mmol of glucose for the biosynthesis of insulin.[5] One possible explanation for this difference between fetal and adult islets could be the difference in the blood sugar which is considerably lower in the fetal than in the adult rat.[23] It is well known that newborn infants of diabetic mothers exhibit islet hyperplasia and a significant increase in the amount of insulin in their pancreas, when compared to normal newborn infants.[24,25] Furthermore, these newborns react with an augmented insulin secretion after a glucose load.[25] On the other hand, if pregnant rats were made overtly diabetic with alloxan, thereby reaching constant blood sugar levels above 250 mg. per 100 ml., the pancreata of their offspring contained less insulin than normal newborn rats.[27] In addition, a constant infusion of glucose for a few days to normal adult rats which increased the blood sugar to 300 mg. per 100 ml. resulted in an augmented insulin biosynthesis.[28] Thus, the blood sugar appears to be a critical factor in the regulation of insulin biosynthesis and release in the adult and in the perinatal period. Shortly after delivery, the blood glucose reaches the adult level, and the results obtained with five- and ten-day old newborn rats demonstrate that at that age the islets incorporated more tritiated leucine into the proinsulin and insulin peak when they were incubated with 300 mg. per cent of glucose than with 100 mg. per cent, comparable to adult islets.

A direct comparison between the amount of insulin synthesized by islets of twenty-one-day old fetal and five- and ten-day old newborn rats is not justified since fetal islets are smaller than newborn islets,[23] and our results are related to the twenty islets used in each experiment. The finding that glucose stimulates the biosynthesis of (pro-) insulin in fetal and newborn islets is in agreement with a previous report[29] where the incorporation of tritiated leucine into proinsulin and insulin was related to the protein content of the islets. A higher biosynthetic rate per protein unit was found in fetal than in newborn islets. From our qualitative data we cannot comment on this report, though our data indicate an apparent increased biosynthesis of proinsulin and insulin in newborn islets in relation to the total protein biosynthesis, shown in figure 1, where the first peak of radioactivity was similar in fetal and in newborn islets.

Somewhat unexpected was the finding that glucagon did not influence the biosynthesis of proinsulin and insulin in fetal and five-day old newborn rat islets, since it has been reported that glucagon appears slightly earlier and in higher concentrations than insulin in the fetal endocrine pancreas of the rat.[14] However, the plasma glucagon level in the rat fetus is low when compared to the adult animal,[30] and it appears that during the first postnatal week, the mechanisms for glucagon secretion are not fully developed[31] which suggests that the β cells are not exposed to a constant glucagon concentration until the seventh day after delivery. Clearly, no conclusive explanation can be offered for the difference in biosynthesis between fetal and adult islets, since little is known about the mechanism by which glucagon influences protein biosynthesis during fetal life.[32] Similar to adult islets, the combination of glucagon and 300 mg. per cent of glucose increased the incorporation of radioactive leucine into the insulin peak in ten-day old newborn rat islets. Thus it appears that in the perinatal period of the rat, the glucose- and glucagon-dependent mechanisms for the biosynthesis of insulin are not necessarily correlated.

Furthermore, during that period of life, glucose is a poor stimulus for insulin secretion while the combination of glucose plus glucagon stimulates the release of insulin from the pancreas of twenty-one-day old fetal and five-day old newborn rats,[11,13,33] suggesting that insulin biosynthesis and release are not closely dependent as shown by our results for the fetal and newborn rat and by others for the adult rat.[5,10]

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