Effect of glucose on beta cell proliferation and population size in organ culture of foetal and neonatal rat pancreases

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SUMMARY

The effect of glucose on growth of the beta cell population was characterized in rat pancreatic organ culture. The effect was monitored by measuring $[^3H]$thymidine labelling indices of beta cells during the culture period and by quantitation of beta cell population size at the end of the culture period. Foetal and neonatal pancreases and different glucose levels were compared.

Glucose was found to be effective in stimulating the beta cell proliferation and beta cell population increase at 300 mg/100 ml in 18-day foetal pancreatic explants, but not in 3-day neonatal explants, when compared to the control level of 100 mg/100 ml. A higher level of glucose (500 mg/100 ml) was ineffective and may even inhibit beta cell population growth. The higher than control levels of glucose (300 mg/100 ml and 500 mg/100 ml) were able to stimulate insulin secretion in neonatal tissue, but not in foetal tissue, although foetal tissue may develop such response later in culture. These results suggest that glucose stimulates beta cell proliferation and insulin secretion through different mechanisms. They further show that the potentiality for beta cell proliferation under glucose stimulation decreases with age of the explants and that the capacity for beta cell to proliferate as a function of glucose stimulation is limited.

INTRODUCTION

Increased beta cell proliferation or increased total beta cell volume has been reported in a number of in vivo and in vitro studies. In experimental animals, partial pancreatectomy (Martin & Lacy, 1963), treatment with glucocorticoids (Kern & Logothetopoulos, 1970; Like & Chick, 1974), alloxan, streptozotocin (Logothetopoulos, Brodsky & Kern, 1970), insulin antibody (Logothetopoulos & Bell, 1966), growth hormone (Martin, Akerblom & Garay, 1968), and glucose (Brodsky, Kern & Logothetopoulos, 1972) all have been shown to stimulate islet cell proliferation or increase in islet cell volume. Increased beta cell proliferation and beta cell volume is also found in the genetic diabetic mouse during development (Like & Chick, 1970) and in foetuses of diabetic mothers (Kim, Runge, Wells & Lazarow, 1960; Wellmann & Volk, 1977). It is believed that hyperglycemia is the immediate cause for beta cell hyperplasia in these conditions. Such glucose-caused beta cell hyperplasia, however, is usually transient; beta

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cell degeneration ensues with prolonged stimulation by high levels of glucose. The direct effect of glucose on beta cell proliferation and beta cell growth is further demonstrated in *in vitro* studies. Lambert *et al.* (1967) observed an increased beta cell volume in organ-cultured foetal rat pancreases with high glucose medium. Chick (1973), in monolayer cultures, demonstrated increased beta cell proliferation by high glucose. Similar results were also reported by Anderson (1975) and Swenne, Bone, Howell & Hellerstrom (1980) in cultures of isolated islets.

The mechanisms by which glucose regulates beta cell proliferation and the relationship between regulation of insulin synthesis and/or release and that of beta cell proliferation is still unknown despite efforts to characterize the effects of hexose sugars and insulin release regulators on beta cell proliferation (King & Chick, 1976; King, Kitchen & Chick, 1978). In view of the important role which glucose plays in regulating beta cell function and proliferation, the present study was undertaken to further characterize the effect of glucose on beta cell proliferation activity and long-term growth of beta cell population.

An organ culture system was used to study the effect of glucose on the growth of beta cell population. The proliferative activities of insulin-immunoreactive beta cells throughout the culture period and the total quantity of insulin-immunoreactive beta cells at the end of the culture period were the parameters used to assess the growth of the beta cell population. The following specific questions were asked: (1) Does elevated glucose level in culture medium stimulate beta cell population growth? (2) Is there a dose relationship of glucose to beta cell population growth? (3) Is there a relationship between growth response and insulin secretory response of beta cells under glucose stimulation? (4) Foetal and neonatal islet tissue differ in functional differentiation (Kaung, Hegre & Lazarow, 1975) and cell proliferation behaviour (Kaung, unpublished) in organ culture. What is the effect of age of pancreatic explants on beta cell growth response to glucose stimulation?

**MATERIALS AND METHODS**

**Animals**

Sprague-Dawley strain rats of 18-days foetal (17.5 days after mating) and 3 days neonatal (4 days after birth) age were used.

**Organ culture**

Pancreatic pieces of 1 mm³ size from 18-day foetal and 3-day neonatal rats were organ cultured in 0.5 ml medium containing 50% medium 199 (Grand Island Biological Co.) and 50% chicken serum (Grand Island Biological Co.) with different glucose concentrations. Cultures were incubated at 37°C in an atmosphere of 95% O₂ and 5% CO₂. Medium was changed every 2 days. At the
end of every 2-day culture period, [methyl-\(^{3}\)H]thymidine (specific activity 6 Ci/mM; Schwarz Mann) was added to the culture medium at 10 μCi/ml for 1 h. The explants were then rinsed in fresh medium and fixed in Bouin’s fluid.

**Immunohistochemistry and radioautography**

The fixed pancreatic explants were dehydrated, embedded in paraffin and serially sectioned at 4 μm. Mounted tissue sections were immunohistochemically stained with guinea-pig antiporcine insulin serum by the peroxidase–antiperoxidase method of Sternberger (1974). These were subsequently processed for radioautography by coating with Kodak NTB liquid emulsion according to Kopriwa & Leblond (1962). After exposure of 28 days, the radioautographs were developed and counterstained with haematoxylin.

**Quantitation of cell proliferative activity**

The labelling index or percent labelled cells was used to represent the proliferative activities of pancreatic cells and insulin-immunoreactive beta cells. This was calculated by counting labelled cells in every 500 cells for each cell type. A nucleus containing a minimum of five silver grains above background of the same-sized area was considered labelled.

**Quantitation of beta cell population size**

The quantity and percent quantity of the insulin-immunoreactive beta cells were estimated by scanning methods (Carpenter & Lazarow, 1962). The tissue sections which were serially cut at 4 μm thickness and mounted at 18-section intervals were linearly scanned at 75 μm vertical intervals using a linear scanner developed by Lazarow & Carpenter (1962). The linear distances (expressed as mm of scan) of the whole pancreatic explants and of the insulin-immunoreactive beta cells were used to represent the quantity or the population size of these two components and were used to calculate the percent quantity of beta cells in the whole pancreatic explants.

**Quantitation of insulin in the medium**

At the time of media change during the course of the culture, the insulin in the culture media was quantitated by radioimmunoassay (Morgan & Lazarow, 1963).

**Experimental design**

Eighteen-day foetal and 3 day neonatal pancreatic pieces were grown in organ culture for 6 days in media containing three different levels of glucose: 100 mg/100 ml, 300 mg/100 ml and 500 mg/100 ml. At every 2-day interval some explants were labelled with [\(^{3}\)H]thymidine, fixed, processed, immunohistochemically stained and radioautographed. The labelling indices of insulin-immunoreactive beta cells and of the total explants at each 2-day interval and the population size of beta cells at the sixth day of culture were quantitated. Insulin content in the media was also assayed at 2-day intervals.
RESULTS

The foetal and neonatal pancreases showed different responses to high levels of glucose, both with respect to insulin secretion and beta cell population growth.

Effect of glucose on insulin secretion

The 300 mg/100 ml and 500 mg/100 ml glucose levels, when compared to 100 mg/100 ml, were ineffective in stimulating insulin secretion from the foetal pancreas during the 6 days of organ culture (Fig. 1, upper panel, left). On day 2 of culture, the insulin secreted by foetal explants grown in media containing 100 mg/100 ml, 300 mg/100 ml and 500 mg/100 ml glucose were 936 ± 92 μi.u./ml, 932 ± 60 μi.u./ml and 744 ± 68 μi.u./ml, respectively. The respective insulin values for 4-day cultures were 1596 ± 152 μi.u./ml, 1700 ± 116 μi.u./ml and 1264 ± 108 μi.u./ml. At day 6 of culture, the insulin secreted by foetal explants were 2132 ± 192 μi.u./ml, 1824 ± 116 μi.u./ml, and 1632 ± 132 μi.u./ml under 100 mg/100 ml, 300 mg/100 ml, and 500 mg/100 ml glucose stimulation respectively.

In contrast to the situation in foetal pancreatic cultures, the 300 mg/100 ml and 500 mg/100 ml glucose levels compared to 100 mg/100 ml were effective in stimulating insulin release from the neonatal pancreatic explants (Fig. 1, upper panel, right). The stimulatory effect of glucose was more extensive during the initial 2 days of culture (2800 ± 315 μi.u./ml insulin at 300 mg/100 ml glucose, 2928 ± 180 μi.u./ml insulin at 500 mg/100 ml glucose, compared to 2164 ± 184 μi.u./ml insulin at 100 mg/100 ml glucose P<0.05). The explants became less responsive to high glucose under prolonged stimulation. At day 4 of culture, the explants secreted 2492 ± 252 μi.u./ml insulin at 500 mg/100 ml glucose stimulation, 2376 ± 172 μi.u./ml insulin at 300 mg/100 ml glucose compared to 1768 ± 188 μi.u./ml insulin at 100 mg/100 ml glucose (P<0.1). At day 6 of culture, the insulin secreted at 500 mg/100 ml glucose was 1512 ± 148 μi.u./ml, at 300 mg/100 ml glucose was 1652 ± 120 μi.u./ml. These values are slightly higher than 1108 ± 208 μi.u./ml of insulin at 100 mg/100 ml glucose (P<0.1). Furthermore, 300 mg/100 ml glucose stimulated these neonatal explants maximally and 500 mg/100 ml glucose did not stimulate more insulin secretion than 300 mg/100 ml glucose.

In conclusion, glucose at 300 mg/100 ml or above stimulated insulin release over 100 mg/100 ml glucose in neonatal pancreatic tissue, but not in foetal pancreatic tissue.

Effect of glucose on beta cell population growth

[3H]thymidine was found to be incorporated into the nuclei of differentiated beta cells in both foetal and neonatal pancreatic explants (Fig. 2). The results of labelling indices of beta cells for foetal and neonatal explants under different levels of glucose stimulation is shown in Fig. 1, lower panel.
Glucose on beta cell population growth in culture

307

Days in culture

% of labelled beta cells

Fig. 1. The labelling indices of insulin-immunoreactive beta cells and the insulin secreted in media during 6 days of culture period for 18-day foetal and 3-day neonatal pancreases. ** Significantly different than values of 100 mg/100 ml controls (P < 0.05). * (P < 0.1).

Fig. 2. Incorporation of [3H]thymidine in the nuclei of insulin-immunoreactive beta cells of 18-day foetal pancreatic explant cultured for 2 days in 300 mg/100 ml glucose medium. x650.

Beta cell proliferative activities of neonatal pancreatic explants were not significantly altered by either 300 mg/100 ml or 500 mg/100 ml glucose. As illustrated in Fig. 1 (lower panel, right), at day 2 of culture, the labelling indices
of beta cells were 5·5 ± 0·7, 4·7 ± 1·0, and 6·5 ± 1·4 for explants grown in media containing glucose at 100 mg/100 ml, 300 mg/100 ml, and 500 mg/100 ml, respectively. At day 4 of culture, the corresponding labelling indices were 3·1 ± 0·6, 2·5 ± 0·4, and 1·9 ± 0·5, and at day 6 of culture, these respective values were 1·5 ± 0·2, 1·9 ± 0·3 and 1·7 ± 0·4. Throughout 6 days of culture, no difference was observed between labelling indices of beta cells grown in high glucose media and those grown in 100 mg/100 ml glucose medium.

In foetal pancreas explants, only 300 mg/100 ml glucose was effective in producing an increased rate of beta cell proliferation at early stages of explantation (Fig. 1 lower panel, left). At day 2 of culture, the labelling index of beta cells at 300 mg/100 ml glucose was 8·2 ± 0·9 which is significantly higher (P<0·05) than that of beta cells at 100 mg/100 ml glucose (4·6 ± 1·0). At day 4 of culture, the beta cell labelling index was 10·4 ± 0·8 at 300 mg/100 ml significantly higher (P<0·1) than the value (8·2 ± 0·7) at 100 mg/100 ml glucose. This stimulatory effect of 300 mg/100 ml glucose on foetal tissue dropped to control levels (7·5 ± 0·7 for 300 mg/100 ml glucose, 6·1 ± 0·6 for 100 mg/100 ml glucose) at day 6 of explantation.

Glucose at 500 mg/100 ml did not increase beta cell labelling indices over 100 mg/100 ml glucose level during the 6-day culture period. The beta cell labelling indices for 500 mg/100 ml glucose were 4·4 ± 1·4, 5·3 ± 1·3 and 6·0 ± 1·5 on day 2, day 4, and day 6 of culture, respectively, not significantly higher than respective values of 4·6 ± 1·0, 8·2 ± 0·7 and 6·1 ± 0·6 for 100 mg/100 ml glucose.

The stimulatory effect of moderately high glucose (300 mg/100 ml) on beta cell population growth in foetal tissue and the lack of effect of high glucose on beta cell growth in neonatal tissue were again reflected in the percent quantities of beta cells at the end of 6-day culture periods (Table 1). The foetal explants contained significantly more (P<0·05) beta cells (5·5 ± 0·2 %) after growth for 6 days in medium containing 300 mg/100 ml glucose than explants grown in

### Table 1. Beta cell quantity and percent of beta cells in pancreas cultured for 6 days in different glucose levels

<table>
<thead>
<tr>
<th>Age of pancreas at time of explantation</th>
<th>Glucose in media</th>
<th>Total quantity of explant* (mm scan)</th>
<th>Quantity of beta cells* (mm scan)</th>
<th>Percent quantity of beta cells* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-day foetal</td>
<td>100 mg/100 ml</td>
<td>31·0 ± 3·5 (4)</td>
<td>1·1 ± 0·3 (4)</td>
<td>3·8 ± 0·9 (4)</td>
</tr>
<tr>
<td></td>
<td>300 mg/100 ml</td>
<td>49·0 ± 8·0 (4)</td>
<td>2·7 ± 0·4 (4)</td>
<td>5·5 ± 0·2 (4)**</td>
</tr>
<tr>
<td></td>
<td>500 mg/100 ml</td>
<td>53·4 ± 3·1 (4)</td>
<td>1·5 ± 0·3 (4)</td>
<td>3·0 ± 0·6 (4)</td>
</tr>
<tr>
<td>3-day neonatal</td>
<td>100 mg/100 ml</td>
<td>40·3 ± 5·9 (4)</td>
<td>3·8 ± 0·4 (4)</td>
<td>9·8 ± 1·5 (4)</td>
</tr>
<tr>
<td></td>
<td>300 mg/100 ml</td>
<td>33·9 ± 0·6 (4)</td>
<td>3·9 ± 0·5 (4)</td>
<td>11·3 ± 2·1 (4)</td>
</tr>
<tr>
<td></td>
<td>500 mg/100 ml</td>
<td>33·1 ± 3·7 (4)</td>
<td>1·7 ± 0·3 (4)</td>
<td>4·9 ± 0·4 (4)**</td>
</tr>
</tbody>
</table>

* Mean ± s.e.m. (n = 4).

** Significantly different from 100 mg/100 ml control levels (P<0·05).
Glucose on beta cell population growth in culture

Table 2. Labelling indices of whole pancreas explants on day 2 and day 4 of culture of 18-day foetal pancreas grown in different glucose levels

<table>
<thead>
<tr>
<th>Glucose in media</th>
<th>Day 2</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg/100 ml</td>
<td>14.6 ± 1.7</td>
<td>10.2 ± 1.2</td>
</tr>
<tr>
<td>300 mg/100 ml</td>
<td>13.8 ± 0.8</td>
<td>12.8 ± 2.5</td>
</tr>
<tr>
<td>500 mg/100 ml</td>
<td>15.7 ± 1.5</td>
<td>13.3 ± 1.2</td>
</tr>
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</table>

*Mean ± s.e.m. (n = 4).

100 mg/100 ml glucose medium (3.8 ± 0.9% total beta cell quantity). Explants grown in 500 mg/100 ml glucose medium did not contain more beta cells (3.0 ± 0.6%) than explants grown in 100 mg/100 ml glucose medium. On the other hand, in the neonatal pancreas, 300 mg/100 ml was not effective in increasing beta cell quantity (11.3 ± 2.1% compared to 9.8 ± 1.5% for 100 mg/100 ml glucose level). Furthermore, the 500 mg/100 ml glucose seemed to have detrimental effects for the long-term survival of beta cells in these neonatal pancreatic explants (at 500 mg/100 ml glucose, the beta cell quantity was 4.1 ± 0.4%, significantly lower, P < 0.05, than 9.8 ± 1.5% of beta cells in explants grown in 100 mg/100 ml glucose medium).

To see whether the stimulatory effect of 300 mg/100 ml glucose on foetal beta cell proliferation reflected a promotion of cell proliferation of whole foetal explants by this glucose level, the labelling indices of whole foetal pancreatic explants were quantitated at different glucose levels through the culture period (Table 2). At day 2, the labelling indices of whole foetal explants were 14.6 ± 1.7, 13.8 ± 0.8, 15.7 ± 1.5, at 100 mg/100 ml glucose, 300 mg/100 ml glucose and 500 mg/100 ml glucose levels. At day 4, the respective labelling indices were 10.2 ± 1.2, 12.8 ± 2.5 and 13.3 ± 1.2. Thus, the labelling indices of whole explants were not affected by higher glucose levels. This indicated that the stimulatory effect of 300 mg/100 ml glucose on beta cell proliferation in the foetal tissue was not a promotion of general pancreatic cell growth of the explants by this glucose level, but a beta cell specific event.

In summary, only moderately high glucose, 300 mg/100 ml, stimulated beta cell growth in the foetal pancreas, and this stimulatory effect was transient.

DISCUSSION

The present investigation characterizes the effect of glucose on growth of beta cell population in an organ culture system. The effect is monitored by quantitation of [3H]thymidine-labelling indices of beta cells during the culture period and
by quantitation of beta cell population size at the end of the culture period. The
effect is compared in foetal and neonatal pancreases and for different glucose
levels.

The results showed that foetal and neonatal tissue responded differently to
glucose stimulation on beta cell population growth. High glucose (500 mg/100 ml
and 300 mg/100 ml) did not stimulate any increased beta cell population growth
in neonatal tissue over that observed with 100 mg/100 ml glucose. In foetal
pancreases, only a moderately high level of glucose (300 mg/100 ml) was able to
stimulate an increased beta cell population growth, and this response diminished
to a non-significant level with prolonged stimulation (day 6).

The stimulatory effect of 300 mg/100 ml glucose on foetal beta cell growth was
demonstrated by an initial increase of beta cell proliferation rate and an increase
in total beta cell population size at the end of 6 day culture. This effect was beta
cell specific. The increased quantity of beta cell population at the end of the
culture period probably resulted from the increased rate of beta cell proliferation
at the earlier stage of culture period, since the proliferative activities of the whole
explants were not stimulated by 300 mg/100 ml of glucose.

Foetal and neonatal pancreases also showed different responses to glucose in
insulin secretion. Consistent with our earlier report (Kaung, et al. 1975), neo-
natal tissue responded to high glucose stimulation, whereas foetal pancreas was
unresponsive to high glucose stimulation during the first 6 days of culture. Foetal
tissues, however, are capable of developing insulin secretory responsiveness to
glucose with time when cultured longer than 6 days (Kaung et al. 1975). The non-
responsiveness to glucose during the first 6 days of culture indicates that these
foetal tissues were still functionally immature during that period.

Thus, beta cells respond to high glucose differently with respect to insulin
secretion and cell proliferation. This differential pattern of response to high
glucose suggests that glucose stimulates insulin secretion and beta cell prolifera-
tion by different mechanisms. The same conclusion was also reached by King
et al. (1978) and Swenne et al. (1980). Furthermore, the present observations on
beta cell differential response patterns of insulin secretion and proliferation to
high glucose demonstrate that functionally immature beta cells exhibit a higher
potential for proliferation under glucose stimulation.

The observation that foetal beta cell proliferation rate eventually declined to
control levels after initial increase by 300 mg/100 ml glucose, together with the
complete lack of response of beta cell proliferation to high glucose in neonatal
pancreas and the lack of response to 500 mg/100 ml glucose in foetal tissue
supports the interpretation, from in vivo studies (Logothetopoulos & Bell, 1966;
Logothetopoulos et al. 1970; Kern & Logothetopoulos, 1970; Brodsky et al.
1972), that the potential for glucose-stimulated proliferation in beta cells is
limited.

Glucose-stimulated beta cell proliferation and beta cell volume increase have
been shown in both foetal (Lambert et al. 1967; Swenne et al. 1980) and neonatal
Glucose on beta cell population growth in culture

(Chick, 1973; King & Chick, 1976) cultures. Under the culture conditions of the present study, only foetal beta cells were capable of glucose-stimulated beta cell population growth. The different responses of neonatal tissue in present and reported (Chick, 1973; King & Chick, 1976) studies are difficult to interpret since the culture conditions are entirely different in these cases.

In conclusion, beta cells are capable of increased proliferation under glucose stimulation. However, the growth potential as a function of glucose stimulation for beta cell population is limited. Excessively high levels of glucose, prolonged duration of glucose stimulation, and older age (3 days neonatal) limit the growth response of beta cell population to glucose. Furthermore, beta cells respond to glucose stimulation to proliferate and to secrete insulin through different mechanisms.

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