Biochemical changes induced by Coxsackie B4 virus in short-term culture of mouse pancreatic islets

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Isolated mouse pancreatic islets were infected in vitro with two strains of Coxsackie B4 virus - a tissue culture-adapted strain and a mouse pancreas-adapted strain. Within 48 h of infection changes had occurred in the biochemical activities of islets infected with the mouse pancreas-adapted strain of virus. Basal insulin release was increased two-fold in these islets, while glucose-induced insulin secretion remained unchanged. Insulin biosynthesis was greatly reduced at a stimulatory concentration of glucose (20 mM), thus leading to a reduced insulin content in these islets. These effects are of importance because they demonstrate that certain strains of Coxsackie B4 virus, like encephalomyocarditis virus, may selectively alter β-cell function in vitro.

A number of viruses are implicated in the direct induction of diabetes in animals (1-4). Among these viruses the most completely studied is encephalomyocarditis (EMC) virus. This virus causes biochemical changes in mouse islets infected in vitro that are not attributable to cell destruction (5). These changes include depressed insulin synthesis and alterations in cyclic AMP metabolism. To date there have been no similar studies carried out with Coxsackie B4 virus, even though this virus has been associated with the development of diabetes in man (6-11). We report here the direct biochemical effects of Coxsackie B4 virus on mouse islets in vitro.

Materials and Methods

Islet isolation

Mouse pancreatic islets were obtained from 10 - 12-week old male DBA/2 mice using a collagenase digestion technique (12), and standard aseptic procedures throughout. The islets were washed three times with RPMI 1640 tissue culture medium containing 5 mM glucose, 10% foetal calf serum and antibiotics (penicillin 100 U/ml and streptomycin sulphate 0.1 mg/ml).
Inoculation of islets with virus and tissue culture

The tissue culture-adapted Coxsackie B4 was the prototype strain which had been passaged a number of times in primary Rhesus monkey kidney cell cultures and more recently in Vero cells (a continuous cell line derived from the kidney of African green monkey, *Cercopithecus aethiops*). The mouse pancreas-adapted strain was prepared serially by passing the prototype strain through 3-week-old Swiss mice at 5 day intervals, using pancreatic tissue as the inoculum. Pancreatic suspensions from the eleventh passage were used as the inoculum in these experiments.

In each case the titre of the stock viral suspension was determined by titrating infectivity on monolayers of Vero cells and establishing the TCID$_{50}$ (TCID$_{50}$ is the dose of virus that gives rise to cytopathic changes in 50% of the inoculated cultures).

Groups of 10 islets were incubated for 1 h at 37°C with 100 TCID$_{50}$ of mouse pancreas-adapted virus or 100 TCID$_{50}$ of tissue culture-adapted virus in 0.2 ml of tissue culture medium. A further 0.3 ml of tissue culture medium was then added to the islets, and they were cultured for 48 h at 37°C in a humidified atmosphere with 95% O$_2$: 5% CO$_2$. Control islets were treated identically except that an equivalent volume of tissue culture medium was used in place of viral suspension.

Biochemical assays

At the end of the culture period incubations of islets were carried out as follows: The islets were washed twice with bicarbonate-buffered medium (13) containing 2 mM glucose and pre-incubated in this buffer for 30 min at 37°C in a shaking water bath. After a brief centrifugation the buffer was removed and replaced with 150 µl of fresh buffer containing either 2 mM or 20 mM glucose and 50 µCi/ml of L-[4,5-3H]leucine. Islets were then incubated for 2 h at 37°C, following which they were centrifuged and the supernatant was removed and frozen at -20°C for subsequent radioimmunoassay of insulin (14). The islets were resuspended and washed with phosphate-buffered saline (PBS) containing 10 mM leucine and then suspended in PBS prior to sonication. Aliquots of the sonicate were used to determine rates of (pro)insulin biosynthesis using a Protein-A Sepharose technique (15). Total protein synthesis was determined in TCA-precipitated fractions of the sonicate (16). Islet insulin content was measured by direct application of the immunoassay procedure to suitably diluted sonicates.

Results and Discussion

The results presented show clearly that Coxsackie B4 viruses can directly infect mouse pancreatic islets in tissue culture and may selectively alter biochemical function. The tissue culture-adapted strain of Coxsackie B4 virus affected only the insulin content of the islets (Table 1) whereas the mouse pancreas-adapted strain altered several aspects of islet function (Tables 2-4).

It is of interest that the secretory response to 20 mM glucose was almost unchanged by either strain of Coxsackie B4 (Table 2). By contrast, the biosynthesis of insulin was severely reduced in islets
Table 1. The insulin content of islets 48 h after infection with tissue culture-adapted and mouse-adapted strains of Coxsackie B4 virus (CB4) (ng insulin/islet)

Results are given as means ± S.E.M. with numbers of observations in parentheses.

<table>
<thead>
<tr>
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<th>2 mM glucose</th>
<th>20 mM glucose</th>
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<tbody>
<tr>
<td>Control</td>
<td>7.68 ± 0.91 (15)</td>
<td>9.07 ± 0.97 (15)</td>
</tr>
<tr>
<td>Tissue culture-adapted CB4</td>
<td>5.95 ± 0.99 (8)</td>
<td>6.69 ± 0.57 (8)*</td>
</tr>
<tr>
<td>Mouse pancreas-adapted CB4</td>
<td>6.35 ± 0.61 (15)</td>
<td>5.24 ± 0.36 (14)**</td>
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*P < 0.05 and **P < 0.001, for differences between mean values in control and infected islets.

Table 2. Insulin secretion in islets infected with tissue culture-adapted and mouse pancreas-adapted strains of Coxsackie B4 virus (CB4) in vitro after 48 h (pg insulin/islet -1 min -1 )

Results are shown as means ± S.E.M. with numbers of observations in parentheses.

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<th>2 mM glucose</th>
<th>20 mM glucose</th>
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<tbody>
<tr>
<td>Control</td>
<td>2.36 ± 0.39 (14)</td>
<td>17.72 ± 1.74 (15)</td>
</tr>
<tr>
<td>Tissue culture-adapted CB4</td>
<td>3.52 ± 0.72 (8)</td>
<td>13.01 ± 2.25 (6)</td>
</tr>
<tr>
<td>Mouse pancreas-adapted CB4</td>
<td>6.61 ± 1.14 (15)*</td>
<td>15.42 ± 2.53 (15)</td>
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*P < 0.001 for difference between mean values in control and infected islets.

infected with the mouse pancreas-adapted strain of Coxsackie B4 (Table 3). The effects of the virus cannot therefore be due simply to a non-specific lysis of the β-cells. These results closely resemble those obtained with EMC virus (5) where a similar dichotomy of effects was noted.

In addition, there was a marked increase in insulin release into the incubation medium at low (2 mM) glucose concentration (Table 2). This effect may be due to a 'leakage' of insulin as a consequence of changes in the β-cell membrane. Again, a similar response was seen with EMC virus in vitro (5).

Cells infected by a virus are damaged not only by cell lysis following viral replication and by effects on host protein synthesis, but also by changes in the permeability characteristics of the plasma membrane following the attachment of the virus to the cells. It has been found in cells infected by paramyxoviruses that as a consequence
of these changes cellular constituents leak out (17-19). Since the insulin concentration in the 2 mM glucose incubation medium was greater in islets infected by mouse pancreas-adapted virus than in control islets this suggests that the adapted Coxsackie B4 may have had such an effect on the β-cells, leading to a 'leakage' of insulin.

Total protein synthesis was lowered only in islets infected with the mouse pancreas-adapted strain of virus (Table 4). A reduction in host protein synthesis commonly occurs in cells infected by picornaviruses in tissue culture (20). Picornaviruses are known to vary greatly in their biological and chemical characteristics (21). Antigenic drift (22-24) occurs frequently in these viruses and also virulent strains can be selected out by serial passage (25-27). Specifically, Yoon et al. (27) found that by passaging a Coxsackie B4 through β-cell cultures, strains of virus were produced which had a distinctly increased tropism for β-cells. Such a selection procedure probably occurred with the virus used in these experiments.

Table 3. (Pro)insulin secretion in islets 48 h after infection with Coxsackie B4 virus (CB4)

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<tr>
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<th>2 mM glucose</th>
<th>20 mM glucose</th>
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<tr>
<td>Control</td>
<td>12.55 ± 2.82</td>
<td>180.78 ± 15.53</td>
</tr>
<tr>
<td>Tissue culture-adapted CB4</td>
<td>11.35 ± 3.10</td>
<td>182.51 ± 18.34</td>
</tr>
<tr>
<td>Mouse pancreas-adapted CB4</td>
<td>18.63 ± 3.24</td>
<td>72.43 ± 6.82</td>
</tr>
</tbody>
</table>

*P < 0.001 for difference between mean values in control and infected islets.

Table 4. Total protein synthesis in islets 48 h after infection with tissue culture-adapted and mouse pancreas-adapted strains of Coxsackie B4 virus (CB4)

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<thead>
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<th>2 mM glucose</th>
<th>20 mM glucose</th>
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<tbody>
<tr>
<td>Control</td>
<td>323.03 ± 51.84</td>
<td>798.93 ± 57.43</td>
</tr>
<tr>
<td>Tissue culture-adapted CB4</td>
<td>306.50 ± 55.05</td>
<td>746.24 ± 77.43</td>
</tr>
<tr>
<td>Mouse pancreas-adapted CB4</td>
<td>236.82 ± 21.52</td>
<td>504.88 ± 38.80</td>
</tr>
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*P < 0.001 for difference between mean values in control and infected islets.
The absence of any major effects on the secretory activity of the islets, even though insulin biosynthesis was impaired, argues against a cytolytic destruction of the islets as an explanation of the results obtained. In addition, a similar selective effect has already been recorded for EMC virus (5). Moreover the structure of islets in these present experiments appeared undamaged when they were examined by light microscopy. Non-cytopathic viruses can infect cells without causing their destruction. In a recent study it was found that the pituitary cells of newborn mice infected with lymphocytic choriomeningitis virus (LCMV), although structurally undamaged, produced lower levels of growth hormone than uninfected controls (28).

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References


25. Pappeneheimer AM, Kunz LJ & Richardson S (1951) Passage of Coxsackie virus (Conn-5 strain) in adult mice with the production of pancreatic disease. J. Exp. Med. 94, 45-64.
