SELECTIVE DISORGANISATION OF BIOCHEMICAL FUNCTION IN B CELLS OF ISLETS OF LANGERHANS INFECTED BY EMC-M VIRUS IN TISSUE CULTURE

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Received 4 August 1981

1. Introduction

The myocardial (M) variant of encephalomyocarditis (EMC) virus has been used for some years to produce diabetes in mice [1,2]. EMC virus normally rapidly destroys the cells in which it replicates and insulin deficiency in such infected animals may be the result of viral multiplication in the insulin producing B cells. While EMC virus may persist in such islets for up to 3 weeks, abnormal glucose tolerance curves can be detected in infected mice for up to a year, even in the absence of ultrastructural changes in the islets of Langerhans [3]. To explain this result, it has been suggested that EMC-M virus infection may trigger immunologically mediated islet cell damage [4] or alternatively that there is a persistent infection of the B cells. There is also the possibility that the islet cells may be functionally impaired rather than destroyed.

To date there has been little reported on the direct effects of EMC-virus on isolated islets of Langerhans, although an almost complete inhibition of proinsulin biosynthesis was reported in microdissected islets of Langerhans from mice infected with EMC virus, 60–70 h after infection [5]. Here, the effects of EMC-M virus infection on isolated islets of Langerhans maintained in tissue culture and their function were investigated in more detail. In general, the secretory function of infected islets was surprisingly well maintained, though from 2 days onwards there was a marked inhibition of insulin biosynthesis. Also, the rate of cyclic AMP accumulation in infected islets in response to 5 and 20 mM glucose showed a considerable increase when compared to the rate observed for control islets over the 4 day culture period. The relevance of these findings to the virally induced altered function of the infected islets is briefly discussed.

2. Methods

Islets of Langerhans were isolated from adult male QS mice, 10–12 weeks old, by a collagenase digestion technique [6]. Collagenase at 3 mg/ml was used throughout. The mice were fed with 'Allied Feed Rat' and 'Mouse Cubes' ad libitum. The isolated islets were washed several times in sterile Hank's balanced salt solution (HBSS) supplemented with penicillin (100 U/ml) and streptomycin (0.1 mg/ml). The islet cells were then infected with a virus to cell ratio of 1 plaque-forming unit (PFU) virus for each cell, incubated for 1 h to facilitate adsorption of the EMC virus and placed in a Falcon tissue culture flask (25 cm^2 growth area) and maintained in the tissue culture medium RPM1 1640 with the following additions: 10% foetal calf serum, penicillin (100 U/ml) and streptomycin (0.1 mg/ml). This artificial culture medium preserves the islet function well during culture [7]. Control islets were treated in the same way as infected islets except that HBSS was substituted for virus. Control and infected islets were harvested daily from the culture flasks up to day 4, washed 3 times in HBSS to remove culture medium and pre-incubated in buffered bicarbonate medium containing 2 mM glucose at 37°C for 30 min before each experiment. Groups of 5 islets were sonicated in 50 μl 0.1 M HCl, with a Branson Sonifier Cell Disrupter B 15. Samples were taken for measurement of insulin by radioimmunoassay. Total insulin content/islet was expressed as ng insulin/islet (± SEM).

Insulin release rate was determined by incubating groups of 5 islets of approximately equal size in a buffered bicarbonate medium with 5 or 20 mM glucose for 1 h at 37°C. At the end of this time the incubation vials were centrifuged at 1500 × *g* for 1 min,

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/$02.50

and the supernatant taken for assay of insulin by radioimmunoassay. Results for secretion are expressed as μunits insulin . islet^{-1} . min^{-1} (± SEM).

The rate of insulin biosynthesis was determined by incubating groups of 30 islets in a buffered bicarbonate medium containing 5 or 20 mM glucose and 0.1 mCi L-[4,5-^{3}H]leucine for 1 h at 37°C [8]. After the incubation period, the islets were washed and disrupted by sonication, and samples taken to measure the rate of incorporation of the labelled insulin from unspecific labelled proteins with the aid of anti-insulin affinity columns prepared as in [9]. Samples were also taken to determine the insulin content of the sonicate by radioimmunoassay. Leucine incorporation is expressed as dpm . ng insulin^{-1} . h^{-1} (± SEM). As is commonly the case when using systems of this kind, no attempt was made to differentiate between incorporation into insulin and into proinsulin.

Cyclic AMP was measured by radioimmunoassay with an antibody to the 2′-*O*-acetyl derivative [10]. Ten islets were incubated in 15 μl buffered bicarbonate medium with 5 or 20 mM glucose for 10 min at 37°C. After incubation, 35 μl ice-cold 0.1 M HCl containing 2 mM 3-isobutyl-1-methyl-xanthine was added and the mixture subjected to sonication. Samples were taken for measurement of cyclic AMP and insulin. Results are expressed as fmol cyclic AMP . ng insulin^{-1}. 10 min^{-1} (± SEM).

3. Results and discussion

The mean islet insulin content for cultured control islets on day 2 was 18.9 ng ± 2.9 ng insulin/islet and 16.4 ng ± 2.9 ng insulin/islet for infected islets. The means in each case were derived from ⩾9 obs. This difference is not statistically significant. Thereafter on days 3 and 4, the insulin content did not change. These results imply that significant lysis of B cells did not occur. Under these circumstances, interferon production may provide protection for some of the islet cells.

Insulin secretion rates from control and infected islets in response to 20 mM glucose were not significantly different for the first 3 days after infection. On day 4, however, the secretory response from infected islets was slightly reduced as compared with the values obtained from control islets (fig.1). Infected islets might therefore show a diminished secretory response to high glucose levels some time after infection, as in [11], using pancreatic fragments from EMC-infected mice. The secretory response to glucose may be severely depressed as long as 7,14 and 21 days after infection [11].

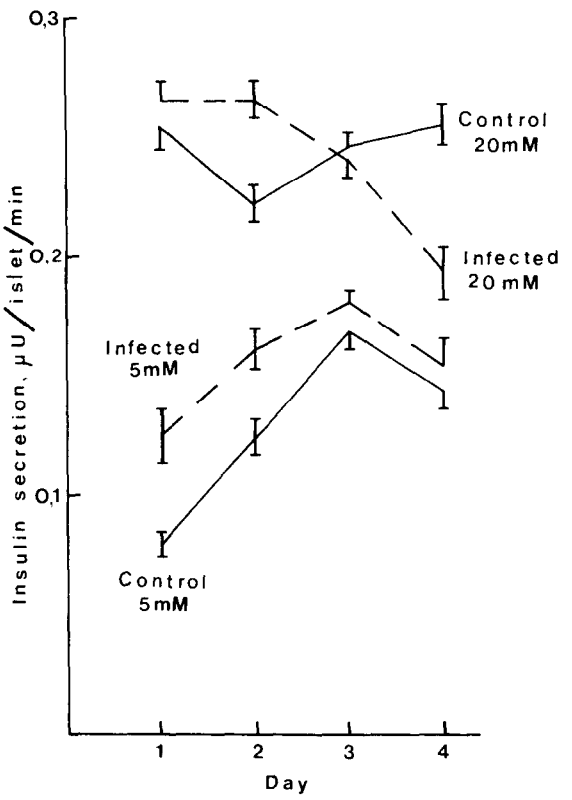

Fig.1. Insulin release in control and infected islets of Langerhans maintained in tissue culture. Islets were infected with EMC virus in vitro and the secretory response to 5 and 20 mM glucose was determined and compared with that of control islets over a 4 day culture (see text). Broken lines indicate the response of infected islets. Each point represents the mean (with SEM) of 5 obs.

The rate of insulin biosynthesis showed a different pattern (fig.2). The initial rate of biosynthesis from infected islets in response to 20 mM glucose was not very different from that observed from control islets in response to 20 mM glucose on day 2. On days 3 and 4, however, there was a progressive decline in the rate of insulin biosynthesis from infected islets in response to 20 mM glucose. The suppression of proinsulin synthesis may well represent the frequently documented 'shut off' phenomenon seen with respect to other host proteins in cells infected with viruses [12,13]. It is also of interest that the decline in the

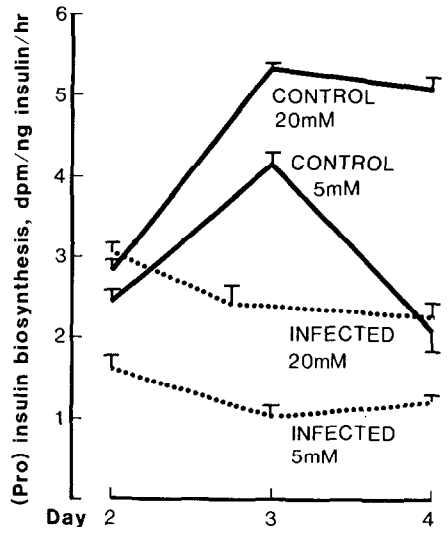

Fig.2. (Pro)insulin biosynthesis in response to 5 and 20 mM glucose in control and EMC-infected islets. Mouse islets were infected with EMC virus in tissue culture and (pro)insulin biosynthesis studied in them at various times following infection. The response to 5 and 20 mM glucose was compared with that of uninfected islets. Biosynthesis was studied by removing the islets from the culture medium and measuring the rate of incorporation of [^{3}H]leucine into (pro)insulin over 1 h (see text). Broken lines indicate infected islets. Each point is the mean (with SEM) of 5 obs.

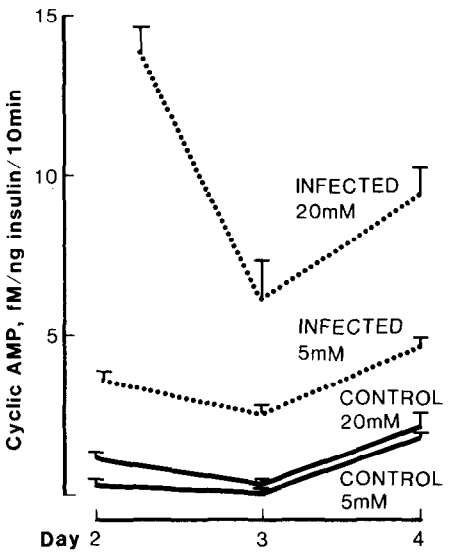

Fig.3. Cyclic AMP accumulation in control and EMC-infected islets of Langerhans in response to glucose. Mouse islets were infected with EMC virus in tissue culture. After removal from the culture medium at various times, cyclic AMP accumulation over 10 min was determined in them in response to 2 concentrations of glucose (see text). Broken lines indicate the response of infected islets. The results represent means (with SEM) of 5 obs. (fM = fmol).

rate of insulin biosynthesis appears to precede any change in the insulin release pattern.

The cyclic AMP content of isolated mouse islets of Langerhans is known to increase with increasing concentrations of glucose in short-term experiments in vitro [14] as well as in long-term experiments in which isolated islets are maintained in tissue culture [15]. Here, cultured mouse control islets also showed an increase in cyclic AMP content when glucose was increased from 5–20 mM glucose over the 4 day culture period. However, the mean cyclic AMP content for infected islets in response to 5 and 20 mM glucose was very much higher than that observed for control islets (fig.3). Such high levels of cyclic AMP seen in infected islets were unexpected. The very high levels may reflect a serious metabolic abnormality involving either the synthesis or breakdown of cyclic AMP soon after infection.

Though raised cyclic AMP levels have often been associated with increased islet secretory activity, this does not seem to be the case with EMC-infected islets. Various biochemical parameters after EMC infection of islets are quite differently affected during this 4 day period. Such changes cannot merely be a consequence of uniform cell destruction. There appears to be some selectivity in the metabolic response to the virus. This is well exemplified by the near-normal secretory response to glucose and the considerable increase in cyclic AMP in infected islets.

It remains to be seen whether such changes are related to the long-term secretory and biosynthetic changes observed up to 60 days following EMC infection [16].

Acknowledgements

Aspects of this work were supported by grants to K. W. T. from the National Health and Medical Research Council of Australia, the British Diabetic Association and the Medical Research Council. We also wish to thank Professor John Turtle for help with facilities.

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