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# STIMULATION OF INSULIN SECRETION FROM MOUSE PANCREATIC ISLETS, MAINTAINED IN TISSUE CULTURE, BY THE PITUITARY NEUROINTERMEDIATE LOBE OF THE GENETICALLY OBESE MOUSE (OB/OB)

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## SUMMARY

The rapid stimulation of insulin release by a perifusate from the pituitary neurointermediate lobe of the ob/ob mouse has been demonstrated by perifusing collagenase prepared mouse islets maintained for 48 hours in tissue culture. The maximal stimulation occurred in about 2 minutes and insulin secretion remained slightly above basal levels for 10 minutes. Freshly prepared collagenase islets showed no response to the pituitary factor and after 24 or 72 hours in culture there was a significant but reduced response compared to the 48 hour cultured islets.

## INTRODUCTION

Stimulation of insulin release from microdissected pancreatic islets by the intact pituitary gland and by the pituitary neurointermediate lobe from genetically obese mice (ob/ob) has been reported from this laboratory.[1][2] The problems encountered working with microdissected islets have been the variability of response of different islet preparations and the limited number of islets that can be prepared by this time-consuming technique. The present work was undertaken to ascertain whether a more consistent response to the perifused pituitary neurointermediate lobe of the obese mouse could be obtained by maintaining collagenase prepared islets in tissue culture.

## MATERIALS AND METHODS

The animals, insulin assay and perifusion technique was as previously described [1][3]. The perifusion system was further modified by using two-way stop-cocks to switch from the buffer to the pituitary perifusate. The method used for the isolation of pancreatic islets was based on that described by Lacy and Kostianovsky [4] with some modifications. Two homozygous lean mice (+/+/) were killed by cervical dislocation and the pancreas removed under sterile conditions and placed into 5 ml of Hank's solution in a sterile dish. The tissue was distended by injection of Hank's solution, then minced into fine pieces with scissors and transferred into a 10 ml tube containing 4 mg collagenase (2 mg collagenase Worthington Biochem. Corp. Type IV

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Batch 485019 activity 180 units$^{-1}$ mg, 2 mg collagenase Sigma Chemical Corporation, Type V, Batch 28C 6920, activity 125 units$^{-1}$ mg). The total volume in the tube was 5 ml. The tube was stoppered and shaken by hand for approximately 8 min. until individual islets could be seen under a stereodissecting microscope. The collagenase was removed by washing three times with Hank's solution. After the final wash the islets (approximately 100) were suspended in Hank's solution and harvested either for maintenance in culture as described by Andersson and Hellerstrom (5, 6), or perifused directly. The islets were cultured in plastic petri-dishes containing RPMI 1640 media (Flow Laboratories) plus 10% foetal calf serum and 5.5 mM glucose. Under these conditions the islets do not attach themselves to the base of the culture dish but remain free in the medium. The islets were maintained in culture in an incubator (95% $0_2$/5% $C0_2$, 37°C, 100% humidity) for periods indicated in the results. In the case of the 3-day cultured islets the medium was changed after 24 hours. Groups of approximately 10 islets were transferred to each channel of the perifusion block.

## CALCULATION OF RESULTS

Results in Table 1 are expressed as basal rate of insulin secretion calculated over a 10 minute period and maximum rate (peak) in μU insulin islet$^{-1}$ min.$^{-1}$. The percentage stimulation of insulin secretion was calculated as previously described (7).

## RESULTS AND DISCUSSION

The results given in Fig. 1 show that the response of islets maintained in culture for 48 hours to the pituitary neurointermediate lobe perifusate is extremely rapid. The maximal stimulation occurs at about 2 minutes and remains slightly above basal levels for 10 min; a very similar time course to that observed previously with microdissected islets, although the % stimulation was greater (3).

Work is in progress in this laboratory on the isolation and identification of the neurointermediate lobe peptide from ob/ob mice which stimulates insulin release,

Table 1 Stimulation of insulin release from pancreatic islets maintained in culture by the neurointermediate lobe of genetically obese mice (ob/ob).
(Preparation of islets, conditions of tissue culture and calculation of results described in text. Results expressed as mean values ± S.E.M. number of experiments in parenthesis).

| Hours Maintained in culture | | INSULIN RELEASE (μU Islet$^{-1}$ Min$^{-1}$) BASAL | PEAK | % Stimulation |
|---|---|---|---|---|
| 0 | (7) | 1.31 ± 0.13 | 1.40 ± 0.13 | 7.7 ± 5.7 |
| 24 | (5) | 1.72 ± 0.21 | 5.12 ± 0.89 | 194 ± 35.0 |
| 48 | (12) | 1.40 ± 0.22 | 14.14 ± 2.4 | 946 ± 131.0 |
| 72 | (5) | 0.84 ± 0.06 | 2.24 ± 0.26 | 163 ± 18.4 |

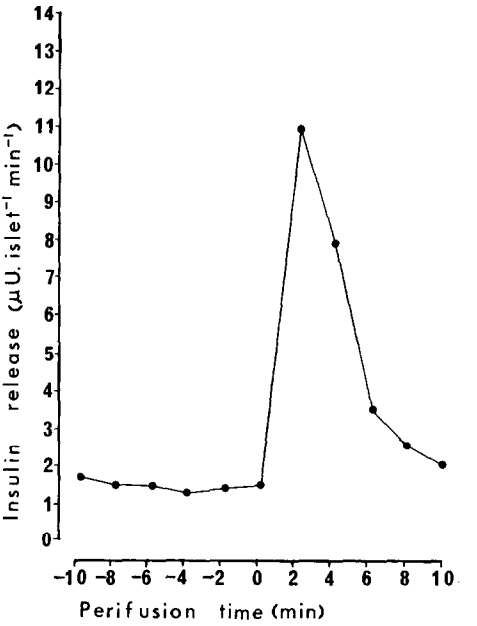


Fig. 1 Stimulation of insulin release from pancreatic islets maintained for 48 hours in culture by perifused pituitary neurointermediate lobes of the obese mouse (ob/ob)
Experimental conditions described in the text. Results expressed as mean of 7 experiments with S.E.M. represented by vertical lines. Switch from Krebs Ringer Bicarbonate buffer to neurointermediate lobe perifusate at 0 time.

and it is concluded from these experiments that the use of pancreatic islets maintained for 48 hours in culture will provide a useful biological assay system for these studies. The results given in Table 1 show that freshly prepared collagenase treated islets showed no response. Islets maintained in culture for 24 or 72 hours gave a mean percentage stimulation of 194 and 163 respectively, whereas the percentage stimulation of islets maintained for 48 hours in culture gave a mean of 946. These results suggest that islets maintained for 48 hours in culture show a maximal sensitivity to the pituitary insulin releasing factor from ob/ob mice.

This finding is of interest in relation to a report [8] that the inhibiting effect of somatostatin on glucose stimulation of insulin release was more marked in islets maintained for 48 hours in culture than in freshly prepared islets using collagenase digestion. It was suggested by these authors that collagenase treatment may damage the cell surface and that there is a regeneration of the required surface components after 48 hours in culture. Similarly the experiments reported above suggest that collagenase damages the receptor to the pituitary releasing factor and that maximal regeneration of the receptor occurs in 48 hours. (It may be relevant that Kahn [9]

has reported that membrane proteins have an average half life of 30-60 hours). These findings suggest that freshly prepared collagenase treated islets utilised by many investigators may not be a satisfactory preparation for studies on insulin release.

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