INFLUENCE OF FASTING ON BASAL AND PITUITARY-STIMULATED INSULIN SECRETION FROM PERIFUSED PANCREATIC ISLETS OF LEAN AND OBESE MICE

Anne BELOFF-CHAIN and S. BOGDANOVIC
Department of Biochemistry, Imperial College of Science and Technology, London SW7

and

M. A. CAWTHORNE
Beecham Pharmaceutical Research Division, Walton Oaks, Tadworth, Surrey, England

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1. Introduction

An impairment of insulin secretion in vitro from pancreatic islets of mice [1] and rats [2] following prolonged fasting has been reported. It has previously been demonstrated in this laboratory [3] that pancreatic islets from fed lean mice showed a greater response to stimulation of insulin secretion by a pituitary factor from the obese mice than did the pancreatic islets of fed obese mice. However islets from obese mice maintained on a restricted diet from the time of weaning showed a normal response to the pituitary stimulation [4].

The present work was carried out to establish whether fasting influenced the basal rate of insulin secretion from islets of lean and obese mice, and the response of the islets to stimulation of insulin secretion by the perifused pituitary gland of fed and fasted obese mice. Experiments were carried out with microdissected pancreatic islets of approximately the same size. The pooled results showed that basal insulin secretion from a given number of islets per channel varied considerably. Most of the variation could be accounted for by an analysis based on the assumption that islets were sometimes damaged during preparation or by subsequent handling, and that such islets failed to secrete insulin. Hence a 'true' value for the number of surviving islets per channel could be calculated for each group of animals studied. The results showed that islets from starved or fed lean mice did not differ significantly in their basal insulin secretion, whereas islets from fed obese mice had a higher basal-rate of insulin secretion which was reduced to below the level of lean mice islets by 24–48 h fasting.

The results showed also that fasting slightly increased the response of lean mouse islets to stimulation by the factor from pituitary glands from fasted obese mice but not from fed obese mice. The increased stimulation by pituitaries from fed obese mice as compared with fasted obese mice was only significant with islets from fed lean mice. The islets from fed obese mice showed a decreased response to stimulation by the pituitary glands from fed ob/ob mice as compared with islets from fed lean mice. An increased response was obtained by fasting obese mice for 48 h but not after 24 h fasting.

2. Materials, methods and calculation of results

The animals, insulin assay, preparation of tissues and perifusion technique were described previously [3]. All the lean mice were homozygotes (+/+) bred in the Department [5].

To calculate percentage stimulation by the pituitary gland in any group of experiments, the basal insulin secretion was determined for a period of 30 min before introducing the pituitary gland into the system. The peak value was measured following pituitary stimulation (maximum stimulation was obtained in

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Table 1
Influence of the pituitary gland from obese mice on insulin secretion from isolated pancreatic islets

Conditions	Basal (μU ml^{-1} insulin secreted in 5 min from perifused islets)	Peak	% Stimulation
Fed ob/ob pituitary fed +/+ islets	76 ± 12 (6)	213 ± 31 (6)	184 ± 16 (6)
Fed ob/ob pituitary 24-h-fasted +/+ islets	32 ± 4 (6)	102 ± 9 (6)	225 ± 28 (6)
24-h-Fasted ob/ob pituitary fed +/+ islets	86 ± 10 (6)	184 ± 30 (6)	133 ± 9 (6)
24-h-Fasted ob/ob pituitary 24-h-fasted +/+ islets	80 ± 7 (9)	209 ± 26 (9)	173 ± 19 (9)
Fed ob/ob pituitary fed ob/ob islets	90 ± 7 (8)	197 ± 18 (8)	122 ± 16 (8)
Fed ob/ob pituitary 24-h-fasted ob/ob islets	83 ± 6 (9)	177 ± 9 (9)	119 ± 17 (9)
Fed ob/ob pituitary 48-h-fasted ob/ob islets	64 ± 4 (4)	231 ± 25 (4)	256 ± 17 (4)

Results are mean values ± SE. Number of experiments in parentheses. % Stimulation calculated from individual experiments

Table 2
Significance tests and conclusions from table 1

Compared	p-Values	Conclusions
Fed ob/ob pituitary on fed or fasted +/+ islets	0.2	Not significant
Fasted ob/ob pituitary on fed or fasted +/+ islets	0.05–0.1	Borderline significance Fasted islets slightly more sensitive
Fed or fasted ob/ob pituitary on fed +/+ islets	0.02	Significant +/+ Islets more sensitive to fed pituitary
Fed ob/ob pituitary on fed or 18 - 24-h-fasted ob/ob islets	0.1–0.3	Not significant
Fed ob/ob pituitary on fed or 48-h-fasted ob/ob islets	< 0.001	Significant 48-h Fasting increases sensitivity of islets
Fed ob/ob pituitary on fed +/+ or fed ob/ob islets	0.01–0.02	Significant +/+ Islets more sensitive

approx. 5 min in all groups of experiments). These values are expressed as μU insulin secreted/5 min/ number of islets introduced into each perifusion channel; this was usually 6 islets. The time taken to collect one fraction of 2 ml was 5 min.

Percentage stimulation was calculated as:

$$\frac{\text{peak value} - \text{basal value}}{\text{basal value}} \times 100$$

thus the exact number of viable islets was not important since % stimulation refers to basal insulin secretion in the same experiment. These results are given in table 1 and Student's t-test, for the difference between sample means, was used to establish the significance of the comparisons (table 2).

It was noticed that the basal insulin secretion was very variable. If this variation was solely or primarily due to random inactivation by injury of islets during the course of preparation, plotting each observation from one group of experiments on a horizontal scale showing insulin concentration from 0–120 μU ml^{-1} (i.e., the full range observed) should produce a number of clusters of values, each corresponding to a particular number of viable islets, this number increasing as the basal value (obtained in the experiment) increases. The distance between adjacent peaks would represent the secretion (5 min) of one islet, or an integral multiple of this, a constraint being imposed by the total number of islets placed in the perifusion channel. An example of such a diagram is given in fig.1 using fed +/+ islets. The basal insulin secretion can in this way be expressed as μU insulin/islet/min for any given group of animals.

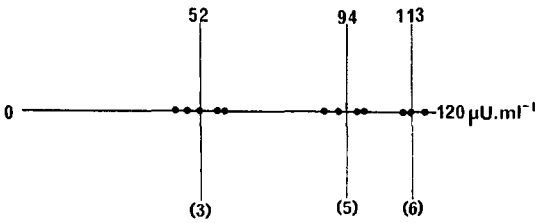

Fig.1. Basal insulin secretion, in μU insulin ml^{-1}.5 min^{-1}, from fed +/+ islets. Horizontal line represents 0–120 μU insulin ml^{-1} and spans the full range of basal secretion obtained over 5 min. The average for a cluster of values is shown by a vertical line and is written above it, rounded-off to the nearest whole number. (•) Represents an individual value. Figures in parentheses below the line show the number of islets thought to be viable. Smallest difference = 19 μU ml^{-1} . islet^{-1} . 5 min^{-1}. Fraction volume = 2 ml, time = 5 min therefore 19 × 2/5 = 7.6 μU insulin secreted/ min/islet.

3. Results and discussion

The results given in tables 1 and the significance tests (table 2) suggest the following conclusions.

The response of islets from the lean mice to stimulation of insulin secretion by the pituitary glands from fed obese mice is unaffected by 24 h fasting. However when the pituitary glands are taken from 24-h-fasted obese mice the +/+ islets show a slightly greater response on fasting. This may be due to a maximum stimulation being obtained with the pituitary glands from fed ob/ob mice because the putative secretagogue is produced in sufficient quantity to saturate the β-cell surface receptors. After only 24 h fasting the maximum β-cell response appears similar to that in fed animals. If it is assumed that fasting lowers the effective concentration of the pituitary factor so that the β-cell receptors are no longer saturated, the response by the β-cells would be affected by their differing surface receptor concentrations, so that increasing receptor concentrations would tend to produce increased sensitivity to a constant concentration of the pituitary factor. Fed +/+ islets do respond less strongly than fasted ones to perifused 24-h-fasted ob/ob pituitary, so this suggests that 24 h fasting increases the receptor concentration on the β-cells. Additionally islets from fed ob/ob mice respond less to fed ob/ob pituitary perifusate than do islets from fed lean mice. Pituitaries from fed obese mice are known to produce much more of the factor than pituitaries from homozygous lean mice [5]. However the sensitivity of the obese mouse islets is fully restored by 48 h fasting, although not after 24 h.

This suggests that the pituitary factor can, in common with other hormones, modulate the magnitude of the response of its own receptor [6,7]. The results reported here indicate that fasting decreases the secretion of the pituitary factor, and therefore the receptors on the β-cell would be expected to increase. This reciprocal relationship between

Table 3
Basal insulin secretion from islets of obese and lean mice

	Islets	
(a)	Fed +/+	7.5 ± 0.18 (12)
(b)	24-h-Fasted +/+	7.4 ± 0.25 (15)
(c)	Fed ob/ob	10.3 ± 0.36 (8)
(d)	24 - 48-h-fasted ob/ob	6.4 ± 0.12 (13)

(a) (b) Do not differ significantly
(c) Differs significantly from each of (a) (b) (d)
$p < 0.001$ in each case
(d) Differs significantly from both (a) and (c)
$p < 0.001$ in each case, and from (b), $p = 0.01-0.05$.

Results expressed as μU insulin/islet/min ± SE calculated as explained in text
No. observations in parentheses

hormone and receptor concentrations has been extensively studied in relation to insulin [8].

Another possible explanation for the increased sensitivity of the islet cells from obese mice after prolonged fasting could be that the high circulating insulin levels in the fed obese mice directly inhibit the response of the islets to stimulation of insulin secretion by the pituitary factor [9]. This would also explain why after prolonged fasting, which reduces the circulating insulin levels to near normal values [10], the response of the islets will be restored.

The results given in table 3 show that the islets from fed ob/ob mice have a higher basal rate of insulin secretion than islets from lean mice, and that this is reduced to below the level of that in lean mice after 24 h or 48 h fasting. This may indicate that ob/ob islets in vivo and in fed animals are exposed to prolonged more intense stimulation of insulin release than is the case in fasted ob/ob mice or in lean mice. The decline in basal secretion upon fasting may be a consequence of the previously mentioned decreased output of the pituitary factor or decreased plasma glucose concentration which are considerably elevated in fed ob/ob mice, or both. The ob/ob islets seems to be more sensitive to the effects of short-term diet restriction than do the lean islets.

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