THE RELEASE OF INSULIN FROM PANCREATIC ISLETS OF LEAN AND OBESE MICE STIMULATED IN VITRO BY PITUITARY GLANDS FROM OBESE MICE AND BY HIGH GLUCOSE CONCENTRATIONS

Anne BELOFF-CHAIN and Janet HAWTHORN
Department of Biochemistry, Imperial College, London SW7 2AY, England

Received 24 February 1976

1. Introduction

It has been reported in previous publications from this laboratory [1] that a factor or factors secreted in vitro by the pituitary glands of genetically obese mice (ob/ob) and heterozygote lean mice (ob/+) [2] stimulate insulin secretion from isolated pancreatic islets of lean mice. The patterns of insulin secretion described in these publications showed that there was a rapid stimulation in the first ten minutes, reaching a maximum in about 5 min, followed by a prolonged less marked elevation over the basal control values for a further 40 min period. The present investigation was carried out to try and establish whether this pattern of insulin secretion was due to the depletion, during the first twenty minutes of perifusion, of a pituitary factor stimulating insulin secretion or to the rapid release of insulin, probably from a labile insulin pool [3]. In order to gain further information on the mechanism of stimulation of insulin secretion by the pituitary, the effect on islets from lean and obese mice was compared with the effect of high glucose concentrations on similar islet preparations.

Experiments reported here show (a) that the pituitary factor responsible for the stimulation of insulin secretion is secreted by the isolated glands for at least 30 min (b) it is suggested that the rapid release of insulin under the influence of pituitary stimulation is due to secretion from a labile insulin pool and (c) that islets from obese mice show a reduced response to the pituitary whereas they have an increased response to high glucose concentrations.

2. Materials and methods

The animals, insulin assay, preparation of tissues and perifusion techniques were as previously described [1]. The pituitaries were prepared from ob/ob mice and the islets from the pancreas of lean mice (ob/+ or +/+) or obese mice (ob/ob) as indicated in the legends. No differences in the response of islets from lean ob/+ or lean +/+ have been observed.

In one set of experiments (fig.1) two lots of islets from the same lean mouse were placed in the perifusion chamber and perifused for 20 min. Pituitary glands from obese mice were then placed in series with one lot of islets and insulin secretion from the stimulated and unstimulated islets was measured for a further 35 min. The stimulated islets were then connected in series with a new preparation of pituitary glands. The first pituitary preparation being connected to the control islets. Insulin secretion was measured for a further 30 min.

In the second series of experiments (fig.2) two groups of pancreatic islets were isolated from different animals with a 25 min period between their preparation. The pituitary glands were perifused in series first with one lot of islets and after 25 min the same pituitary preparation was connected to the second lot of islets.

In the third group of experiments (fig.3) pituitary glands from ob/ob mice were perifused in series with islets from obese and lean mice.

In the last group of experiments (fig.4) islets from obese and lean mice were perifused for 15 min with

North-Holland Publishing Company – Amsterdam

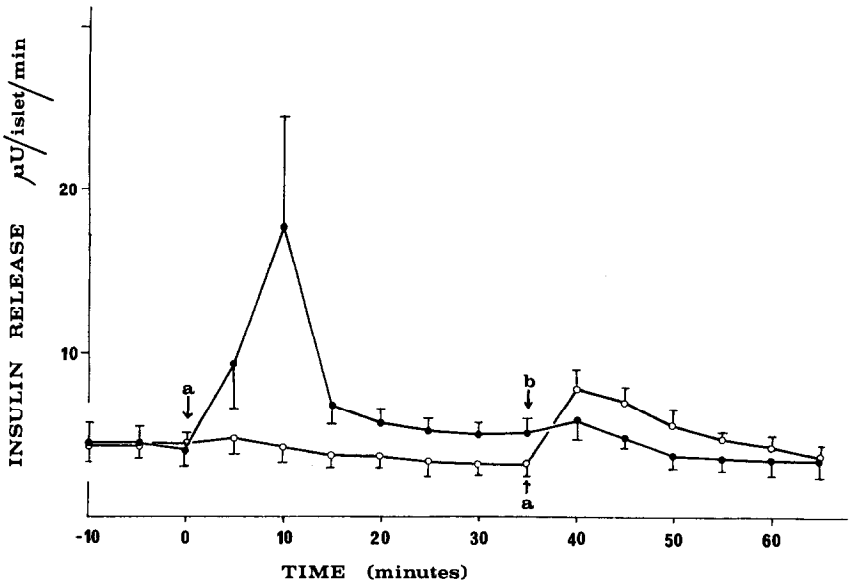

Fig.1 Influence of two preparations of pituitary glands on insulin secretion from isolated lean mouse pancreatic islets. The perifusion medium was Krebs–Ringer bicarbonate buffer containing 0.2% bovine plasma albumin and 100 mg% glucose. The low rate was 370 μl/min, and the temperature was maintained at 37°C throughout the perifusion. In each experiment 2 isolated pituitary glands were used in each preparation and islets were perifused in groups of 5. (•————•) Insulin secretion from islets stimulated at 0 time with one preparation of pituitary glands (a) and after 35 min with a fresh preparation of pituitary glands (b). (○————○) Control islets stimulated at 35 min ↓ with first preparation of pituitary glands (a). Average of 5 experiments ± S.E.M.

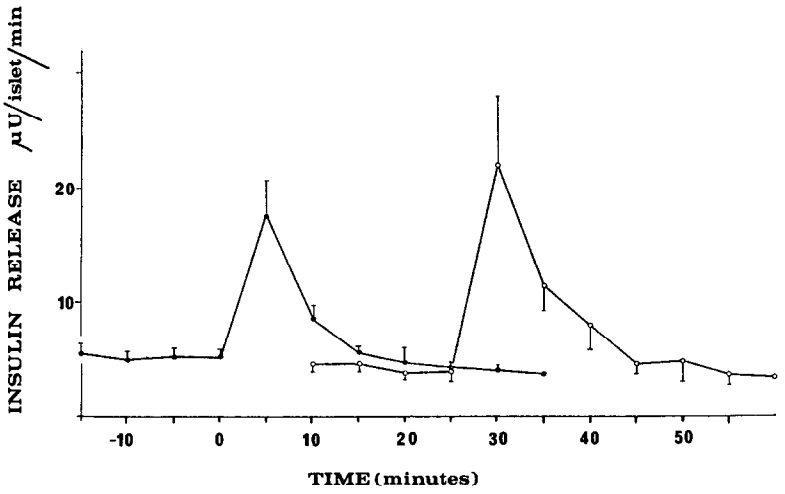

Fig.2. Influence of the perifusate from pituitary glands of ob/ob mice on insulin secretion from two lots of isolated lean mouse pancreatic islets. Experimental conditions as in fig.1. (•————•) Insulin secretion from first lot of islets. Perifused pituitaries transferred to fresh lot of islets at 25 min. (○————○) Insulin secretion from second lot of islets. Average of 5 experiments ± S.E.M.

Krebs–Ringer bicarbonate buffer containing 100 mg% glucose, the glucose concentration was then increased to 300 mg% and insulin secretion measured for another 40 min.

3. Results and discussion

The results given in fig.1 show that the islets failed to respond a second time to stimulation of insulin secretion by the pituitary gland. The results show that the response of the control islets to the pituitary stimulation after 55 min of perifusion was less marked than when the islets were stimulated after 35 min, however, the unstimulated islets showed a significantly greater response than those which had already been stimulated. These results suggest that it is more probable that a pool of rapidly released insulin has been exhausted by the initial pituitary stimulation than that the stimulatory factor of the pituitary becomes depleted in this time period. Confirmation of this conclusion was made by the results of the experiments given in fig.2. These results show that the pituitaries are still active in stimulating insulin secretion after 35 min perifusion, as the second lot of islets introduced into the system responded quantitatively in the same way as the first lot. It has been suggested that some insulin secretagogues, as for example the sulphonylureas, may produce a refractory state in the pancreas following the rapid period of insulin secretion so that there is no furhter response to other stimuli [3]. These results would imply that stimulation of insulin secretion by the pituitary produces a similar refractory state. Although the pituitary and other insulin secretagogues may stimulate insulin secretion from the same labile insulin pool, [3,4] the mechanism of stimulation could be different. Thus in the present work it is shown that pancreatic islets from obese mice have a reduced response to stimulation of insulin secretion by the pituitary whereas they have an increased response to high glucose concentration (fig.3). The increased response of the obese mouse pancreas to high glucose concentration has also been reported using incubated and perifused small pieces of pancreas [5]. It is probable that the pituitary stimulation of insulin secretion is due to a humoral factor and the decreased response of the obese islets could reflect a resistance to this factor. This could be analogous to the well established insulin resistance in the muscle and adipose tissue of obese mice [6,7]. Further investigation on the mechnism of action of the pituitary factor on insulin secretion will be required to interpret the lack of response of the obese mouse islets.

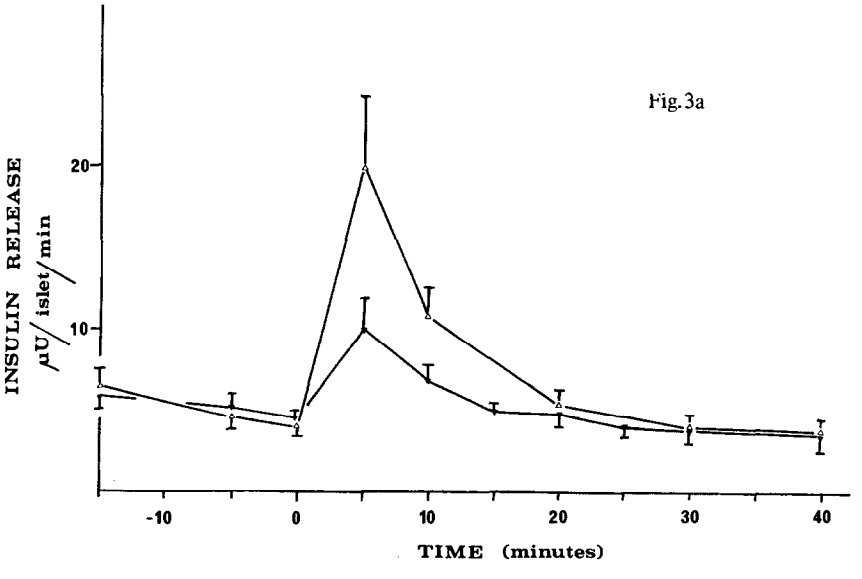

Fig.3a

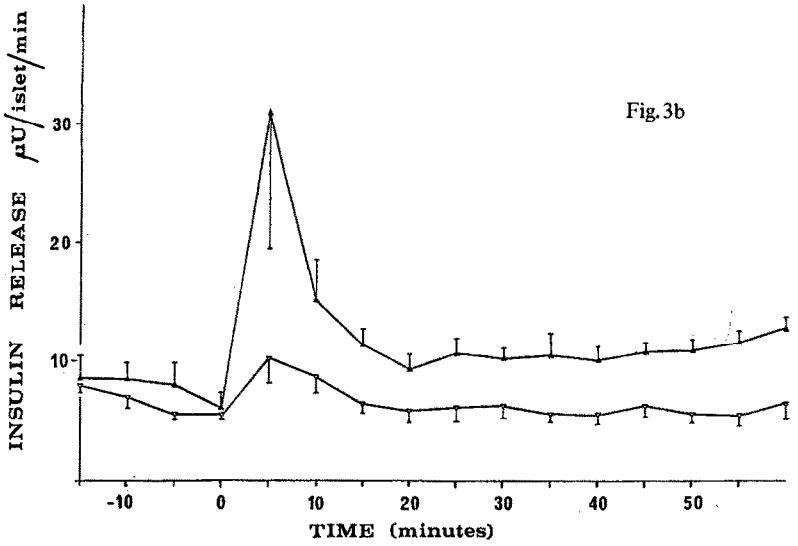

Fig.3. Influence of (a) perifusate from pituitary glands of ob/ob mice and (b) glucose concentration, on insulin secretion from pancreatic islets of lean and obese mice. Experimental conditions as in fig.1. (a) Pituitary glands perifused in series with islets at time zero. (b) Glucose concentration increased from 60 mg% to 300 mg% at time zero. Insulin secretion from lean mouse islets (△————△); obese mouse islets (▲————▲).

Acknowledgements

We are grateful to the Herbert E. Dunhill Trust for a grant which supported this investigation and to H. Dalal for valuable technical assistance.

References

[1] Beloff-Chain, A., Edwardson, J. A., Hawthorn, J. (1975) J. Endocrin. 65, 109–116.
[2] Beloff-Chain, A., Hawthorn, J. and Green, D. (1975) FEBS Lett. 55, 72–74.
[3] Grodsky, G. M. (1970) Vitamins and Hormones 28, 37.
[4] Burr, I. M., Taft, H. P. and Stauffacher, W. and Renold, A. E. (1971) Ann. of the New York Acad. Sci. 185, 245–262.
[5] Beloff-Chain, A., Newman, M. E. and Mansford, K. R. L. (1973) Diabetologia 9, 447–452.
[6] Chlouverakis, C. and White, P. A. (1969) Metabolism 18, 998–1009.
[7] Abraham, R. R. and Beloff-Chain, A. (1971) Diabetes 20, 522–534.