β-CELL TROPIN, A PEPTIDE OF THE PITUITARY PARS INTERMEDIA WHICH STIMULATES INSULIN RELEASE

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Received 27 June 1980

1. Introduction

We have shown that the pituitary glands of obese mice perifused in series with isolated pancreatic islets stimulate insulin release. These experiments were carried out with genetically obese mice (ob/ob) [1] and mice rendered obese with goldthioglucose or by dietary manipulation [2]. Experiments with lean mice suggested that the secretion of this insulin releasing factor is partly controlled by high caloric intake and high levels of circulating glucose [2]. It was proposed that the corticotropin-like-intermediate lobe-peptide 'CLIP' [3], (identical to the 18–39 moiety of the ACTH molecule), or a closely related peptide was the insulin releasing peptide secreted by the neurointermediate lobe (NIL) of the ob/ob mouse [4–7]. Further evidence for this conclusion was obtained by experiments in which the insulin releasing action of a pituitary perifusate was inhibited by treatment with a –COOH terminal ACTH antiserum [8]. Now we have done similar experiments using the perfused rat pancreas as the test system.

To establish the nature of the insulin secretagogue, peptide fractions have been separated from the neurointermediate lobe (NIL) of the ob/ob mouse. Two closely related peptide fractions have been isolated by gel chromatography which cross react with –COOH terminal ACTH antiserum and rapidly stimulate insulin release in the perfused rat pancreas. One of these shows greater specific activity and has been called β-cell tropin.

2. Experimental

ob/ob Mice, 2–4 months old were from a strain bred in this Department [9]. The Sprague-Dawley rats used were also bred in this Department.

Purified human serum albumin was from Albumin Kabi, Kabivitrium Ltd, Uxbridge Road, London W5. Dextran T40 was from Pharmacia (Gt. Britain) Ltd, Hounslow. Insulin binding reagent was from the Wellcome Labs., Beckenham and ^{125}I from the Radiochemical Centre, Amersham.

Insulin was measured by the radioimmunoassay technique in [10].

2.1. *Perfusion of rat pancreas*

Sprague-Dawley rats (200–300 g) were anaesthetized with 60 mg sodium pentobarbitone/kg and the pancreas isolated by ligating and severing all relevant blood vessels to surrounding organs and severing all connective tissue except that between the pancreas and the stomach, proximal duodenum and spleen. The pancreas and attached tissue were placed in the well of the warming chamber.

An open 'once-through' perfusion system was used. Buffers were gassed in their reservoirs with 95% O_2, 5% O_2 for ~1 h before use and throughout the perfusion. The perfusion fluid was drawn by means of a multiple channel peristaltic pump through silicone tubing to bubble traps. The perfusion fluid then passed to a junction point consisting of a series of two-way valves, arranged so that rapid switching from buffer in one channel to another occurred prior to the pump. The fluid was then pumped through several coils of silicone tubing in a perspex warming chamber maintained at 38°C by a flow heater. After emerging through the top of the chamber the buffer passed through a T junction, the side arm of which led to a mercury manometer to enable arterial pressure, which must be 60–90 mg Hg, to be monitored. The perfusate

then entered the arterial cannula (fashioned from a 21 gauge needle) in the coeliac artery and was collected from the venous cannula (made from 2.1 mm o.d. nylon) in the portal vein. One minute fractions were collected. The flow rate through the pancreas was 4.5 ml/min. Insulin was determined in the individual fractions by radioimmunoassay. Further details of the perfusion technique will be given in [11].

2.2. *Perifusion technique*

The perifusion technique was as in [1] with modifications indicated in the figure legends.

2.3. *Fractionation of the perifusate*

Fractionation (based on the technique in [12]) was done on a Biogel P2 column (1.5 × 30 cm) and a Biogel P6 column (1.5 × 90 cm) in series at 4°C. The columns were first equilibrated with 5% (v/v) acetic acid containing 0.02% (w/v) bovine plasma. The flow rate was 3.0 ml/h and 30 min fractions were collected. Fractions so collected were then assayed for ^{14}C content (0.5 ml of each fraction was added to 4.5 ml xylene/Triton X-100 scintillation cocktail). Aliquots of the fractions were diluted 1:10 with mercaptoethanol buffer for immunoassay. Radioimmunoassay was carried out with –COOH terminal ACTH antiserum using a peptide corresponding to the 17–39 moiety of ACTH as standard. Fractions for bioassay were lyophilized and reconstituted in the required medium (see fig.3).

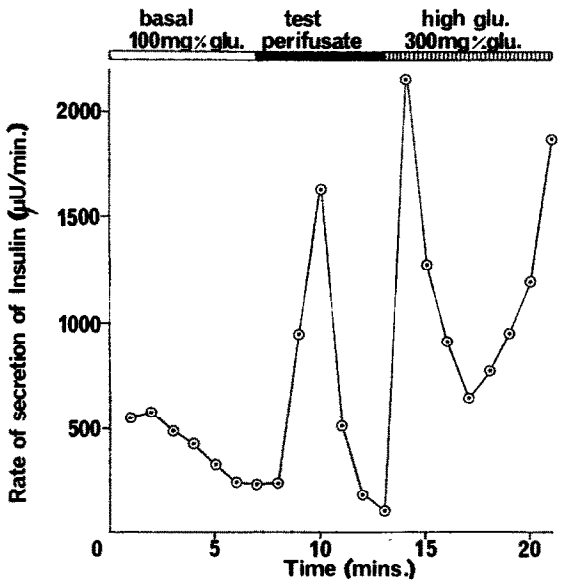

Fig.1. Influence of a perifusate of the pituitary neurointermediate lobe of the ob/ob mouse on insulin release from a perfused rat pancreas. The perfusion buffer contained KCl (4.4 mM), calcium gluconate (2.1 mM), KH_2PO_4 (1.5 mM), $NaHCO_3$ (25 mM), NaCl (115 mM), $MgSO_4 \cdot 7\,H_2O$ (1.2 mM), purified human serum albumin (1%) and Dextran T40 (3%). It was adjusted to pH 7.4 using 0.1 M HCl and filtered. The perifusion buffer was as above but contained only 0.2% albumin, this was then diluted 1:10 with buffer to give a final albumin concentration of 1%.

Table 1
Inhibition of insulin releasing activity of neurointermediate lobe perifusate by –COOH terminal ACTH antiserum

Expt.	(a) Perifusate treated with C-terminal antiserum	(b) Perifusate treated with normal rabbit serum
1	136	500
2	113	583

Two 1 ml aliquots of perifusate from 8 ob/ob mouse neurointermediate lobes were incubated overnight at 4°C with: (a) 200 μl –COOH terminal ACTH antiserum; and (b) 200 μl normal rabbit serum. Each sample was then taken up in 100 ml perfusion buffer. During each experiment normal perfusion buffer was perfused for the first 7 min followed by 5 min perfusion with (a), followed by a further 5 min with normal perfusion buffer and then 5 min perfusion with (b)

Results expressed as % stimulation of insulin secretion: $\frac{(\text{maximal}-\text{basal})}{(\text{basal})} \times 100$

3. Results and discussion

The results given in the figures are based on single experiments, but the findings have been confirmed in 2–4 further experiments in each case. The viability of the perfused pancreas was tested in most experiments by stimulation with high glucose concentrations and this always produced a typical biphasic response [13].

The results in fig.1 show that a perifusate of the NIL of the obese mouse rapidly stimulated insulin secretion in the perfused rat pancreas, reaching a maximum in 1–2 min and returning to basal levels in 4–5 min. It was found that the perfused rat pancreas is a more reliable and sensitive system for measuring insulin stimulation by the pituitary peptide than the microdissected pancreatic islets used in [1,2,4].

The results in table 1 show that if the perifusate is pretreated with –COOH terminal ACTH antiserum the stimulation of insulin release is markedly inhibited as compared to the second challenge with a control perifusate pretreated with normal rabbit serum. (Experiments submitted for publication have shown that when the pancreas was challenged twice with untreated NIL perifusate the islets were refractory to the second challenge.) These experiments confirm the original conclusion discussed above that the active insulin releasing factor in NIL perifusate from ob/ob mice cross reacts with –COOH terminal ACTH antiserum.

We decided to fractionate the peptides of the NIL perifusates following incubation with [^{14}C]proline (see legend fig.2) and to test those fractions which cross-reacted with –COOH terminal ACTH antiserum for insulin releasing activity. The chromatogram obtained from the NIL perifusate of an ob/ob mouse (fig.2) showed that in addition to the peaks A and B (CLIP) observed in [12] using rat pars intermedia, there were 3 more small molecular weight fractions C, D and E which also cross-reacted with the antiserum. The distribution of ^{14}C in these chromatograms is at present under investigation with further purified peptide samples. The results in fig.3 show that neither CLIP (B) nor fraction C stimulated insulin release. Fractions D and E both stimulated insulin release, the pattern being very similar to that obtained with the whole perifusate.

Recent experiments involving the further purification and elucidation of the structure of these peptides have shown that D, although present in approximately equal quantities to E as measured by immunoassay, is

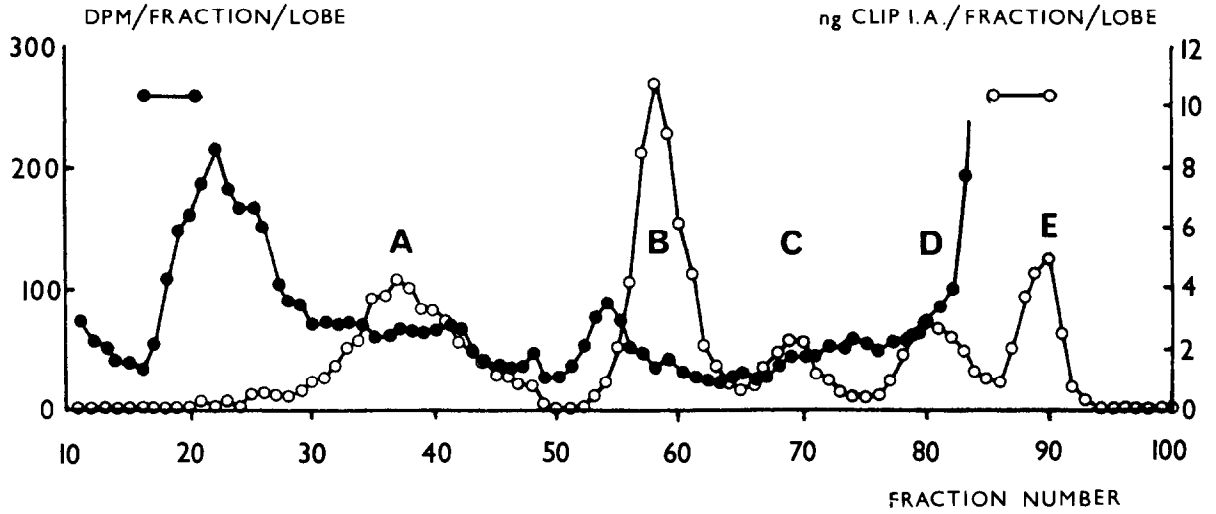

Fig.2. Fractionation of peptides from a perifusate of neurointermediate lobes of obese mice. Three groups of 5 neurointermediate lobes were incubated for 4 h in 200 μl of an incubation medium comprising 100 μl Trowell's medium and 100 μl 0.9% (w/v) saline containing 2.5 μCi [^{14}C]proline. The incubation was carried out in plastic disposable 5 ml tubes in a shaking water bath at 37°C. The tubes were thoroughly gassed with 95% O_2:5% CO_2 and securely stoppered. The lobes were then placed into 2 channels of a perifusion block, 8 into one channel and 7 into another and perifused with Krebs-Ringer bicarbonate buffer, containing 0.1% (w/v) glucose and 0.2% (w/v) plasma albumin, at 0.67 ml . min^{-1} . channel^{-1} until 100 ml perifusate had been collected. This was divided into 4 × 25 ml aliquots and each was placed into a siliconized 250 ml round-bottomed flask and lyophilized. The lyophilized solid was then taken up in 5.0 ml 5% (v/v) acetic acid and fractionated on Biogel columns.

in fact present in considerably higher concentration than E when estimated by ultraviolet absorption. As the stimulation of insulin release above basal levels was consistently greater with E, it appears possible that the activity of D is due to some contamination with the extremely potent insulin secretagogue, peptide E. The latter has therefore been called β-cell tropin. Work is in progress on the further purification

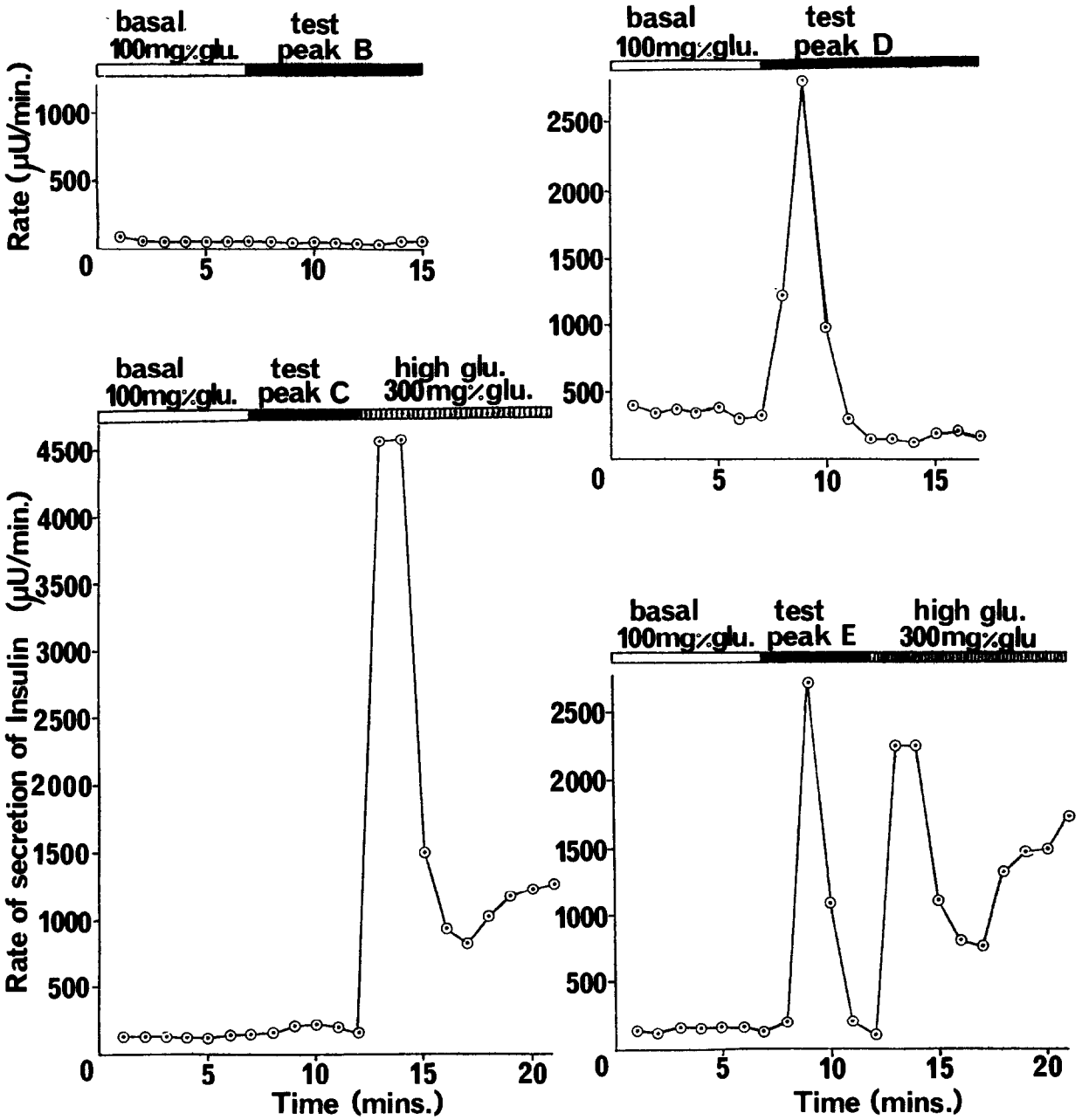

Fig.3. Fractions separated from perifusate of neurointermediate lobes of obese mouse tested on the perfused rat pancreas. The lyophilized fractions B–E (see fig.2) (corresponding to 25% of the total prepared from 15 neurointermediate lobes from obese mice) were dissolved in 100 ml perfusion buffer and tested on the perfused rat pancreas.

of E and the elucidation of its chemical composition.

β-Cell tropin could play a role in the complex control of insulin secretion which leads to the hyperinsulinaemia associated with obesity and insulin resistance [6,14].

Acknowledgement

The work was supported by NIH grant no. AM 19488-02 and the Herbert E. Dunhill Trust. We thank Dr J. A. Edwardson for the –COOH terminal ACTH antiserum and the 17–39 ACTH (a gift from Ciba-Geigy Ltd). We are also grateful to Dr N. R. Lazarus of the Wellcome Foundation for valuable help to S.D. in the setting up of the perfused rat pancreas.

References

[1] Beloff-Chain, A., Edwardson, J. A. and Hawthorn, J. (1975) J. Endocrinol. 65, 109–116.
[2] Beloff-Chain, A., Bogdanovic, S. and Cawthorne, M. A. (1979) J. Endocrinol. 81, 271–279.
[3] Scott, A. P., Ratcliffe, J. G., Reese, L. H., Landon, J., Bennett, H. P. J., Lowry, P. J. and McMartin, C. (1973) Nature New Biol. 244, 65–67.
[4] Beloff-Chain, A., Edwardson, J. A. and Hawthorn, J. (1976) J. Endocrinol. 73, 28P–29P.
[5] Beevor, S., Beloff-Chain, A., Donaldson, A. and Edwardson, J. A. (1978) J. Phys. 275, 55P.
[6] Beloff-Chain, A. (1979) in: Animal Models of Obesity (Festing, M. F. W., ed) pp. 91–106, Macmillan, London.
[7] Edwardson, J. A. and Donaldson, A. (1979) in: Animal Models of Obesity (Festing, M. F. W., ed) pp. 177–190, Macmillan, London.
[8] Bogdanovic, S., PhD thesis, London.
[9] Beloff-Chain, A., Hawthorne, J. and Green, D. (1975) FEBS Lett. 76, 290–293.
[10] Hales, C. N. and Randle, P. J. (1963) Biochem. J. 88, 137–146.
[11] Dunmore, S. and Beloff-Chain, A. (1980) submitted.
[12] Scott, A. P., Lowry, P. J. and Van Wimersma Greidanus, Tj. B. (1976) J. Endocrinol. 70, 197–205.
[13] Grodsky, G. M. (1970) Vitam. Horm. 28, 37–101.
[14] Trowell, O. A. (1959) Exp. Cell Res. 16, 118–147.