ACTION OF β-CELL TROPIN ON INSULIN SECRETION IN VIVO AND ON LIPID SYNTHESIS

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1. Introduction

A peptide of the pituitary pars-intermedia (β-cell tropin) from genetically obese mice (ob/ob) stimulates insulin secretion in vitro [1,2]. β-Cell tropin (BCT) crossreacts with a -COOH terminal ACTH antiserum.

Experiments have now been carried out in which purified samples of BCT prepared by Bio-Gel chromatography [2] have been tested for in vivo stimulation of insulin release and of lipid synthesis in rats. The influence of BCT on lipid synthesis in adipocytes has also been investigated.

The results show a significant rise in plasma insulin levels 2 min following the injection of BCT into the jugular vein of rats. A significant increase in total lipid synthesis in the subcutaneous tissue of rats treated with BCT was demonstrated, 45 min following an injection of ^3H_2O, as measured by ^3H incorporation into saponified fatty acids. BCT was also shown to stimulate total lipid synthesis in vitro in adipocytes incubated for 1 h.

2. Materials and methods

Male Sprague Dawley rats bred in this department, fed ad libitum (~200 g body wt) were used.

2.1. *β-Cell tropin*

BCT was prepared from the neurointermediate lobes of obese mice (ob/ob) and purified on Bio-Gel columns as in [2]. The amounts of BCT were determined by radioimmunoassay using a -COOH terminal ACTH antiserum and synthetic 17–39 ACTH as standard [2].

2.2. *Procedure for in vivo experiments*

Rats were anaesthetized with Hypnorm (Janssen Pharmaceut., Crown Chemicals, Kent) and Valium (Roche Products, Welwyn Garden City). A cannula containing saline and heparin (Pularin, Evans Medical, Speke, Liverpool) was introduced into the heart through an incision in the right jugular vein. The same cannula served for collecting blood samples and for injecting saline + ^3H_2O or saline containing BCT + ^3H_2O. Precautions were taken to prevent the mixing of blood samples with saline and to ensure that the cannula was precisely emptied of blood or test substances. After cannulation the animal was left to recover for 10 min. Three blood samples of 350 μl were drawn through the cannula into a heparised syringe at 5 min intervals. Immediately after the third sample had been taken experimental rats were injected with 25 ng BCT and 6.25 mCi ^3H_2O (Radiochemical Centre, Amersham) in 0.5 ml saline. The control rats were injected with 0.5 ml saline and an equivalent amount of ^3H_2O. Further blood samples were collected from all the animals at 2,5,15,30 and 45 min intervals, following the injections.

Animals were killed after 45 min and samples of liver, mesenteric fat and subcutaneous fat were frozen on dry ice, to be used for lipid analysis.

2.3. *Analytical techniques for in vivo experiments*

Insulin was measured in aliquots of plasma samples by radioimmunoassay [3] and plasma glucose by an enzymatic colorimetric method (assay kit from Sigma, Dorset). The specific activity of the ^3H_2O in plasma collected 45 min after the injection of ^3H_2O was determined by liquid scintillation counting.

The total rate of lipid synthesis in vivo was determined by measuring incorporation of ^3H_2O into

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saponified fatty acids extracted from the tissue samples collected and the specific activity of the plasma ^3H_2O [4–6].

2.4. *Experimental procedure in vitro*

Adipocytes were prepared from epididymal fat pads using the method in [7] with minor modifications. Adipocytes ($0.7–1 \times 10^6$) were incubated in 1 ml Krebs Ringer bicarbonate buffer containing 1 mg glucose, 4% albumin and 1 mCi ^3H_2O. Incubations were done in plastic vials at 37°C for 1 h in the absence and presence of BCT at concentrations indicated in the results.

Adipocyte incubations (500 μl) were saponified and the saponified fatty acids extracted. The ^3H content was determined by liquid scintillation counting and lipogenesis calculated by the relative activity in the fatty acids and the specific activity of the ^3H_2O in the incubation medium [8].

2.5. *Statistics*

The statistical significance of the results was calculated using Student's *t*-test.

3. Results and discussion

The results in fig.1A show that BCT rapidly increased the plasma insulin levels, reaching a maximum in 2 min and falling to values which did not differ significantly from basal levels after 5 min. This effect was not due to hyperglycaemia as no significant change in plasma glucose levels was observed at any of the time periods measured. The control rats injected with saline showed no significant change in plasma insulin or glucose concentrations (fig.1B).

The possible significance of the pituitary control of insulin secretion in obesity has been discussed [9,10]. BCT is elevated in the ob/ob mouse [10] as well as in other forms of obesity [9]. Evidence for the hormonal nature of BCT has been obtained by demonstrating its presence in the plasma of the ob/ob mice (Billingham, Beloff-Chain and Cawthorne, unpublished) and its in vivo action confirms this conclusion.

The transitory action of BCT on insulin secretion found in this study might suggest that it is unlikely to be of biological significance. However, it has been demonstrated in the perfused rat pancreas [10] that BCT not only produces a rapid monophasic stimulation of insulin release at physiological glucose concentrations, but potentiates the biphasic action of high glucose stimulation of insulin secretion. It is therefore probable that in animals which are hyperphagic and hyperglycaemic (as in obesity) the action of BCT on insulin levels could be more prolonged. It is of interest that a similar monophasic insulin releasing activity has been reported [11] in vivo by a low M_r factor (or factors) in hypothalamic extracts of rats. Whether this activity is due to the presence of BCT can only be ascertained by further purification of the hypothalamic insulin secretagogue.

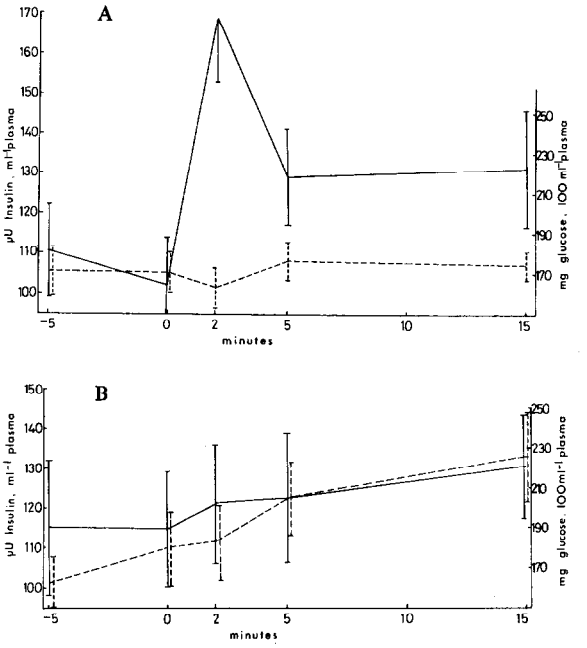

Fig.1. Influence of BCT on plasma insulin and glucose concentrations in rats: (A) 0.5 ml saline containing 25 ng BCT; (B) 0.5 ml saline (controls). Injections at 0 time. Results are the mean of: (A) 7 rats; (B) 6 rats. Vertical bars indicate SEM: (——) insulin; (– – –) glucose. Differences between insulin levels at 0 min and 2 min, significant in animals treated with BCT $p < 0.001$.

BCT increased significantly the incorporation of ^3H from ^3H_2O into the saponifiable fatty acids of subcutaneous fat (fig.2). There appeared to be an effect on mesenteric fat but this was not statistically significant ($0.1 > P > 0.05$) due to the large variation in the treated animals. There was no action of BCT on hepatic fatty acids.

In view of the transitory nature of the elevated circulating insulin under the experimental conditions

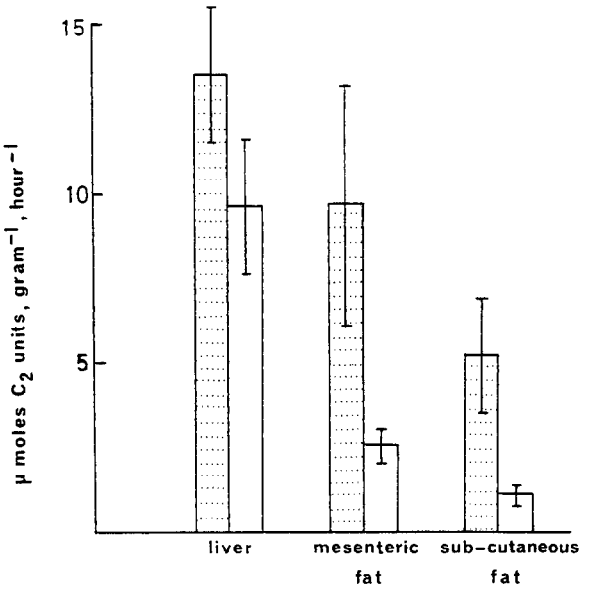

Fig.2. Influence of BCT in fatty acid synthesis. Rats treated as in fig.1; samples for determining ^3H content of saponifiable fatty acids taken at 45 min: (open bars) rats injected with saline (controls); (stippled bars) rats injected with 25 ng BCT. Both groups injected with 6.25 mCi ^3H_2O at 0 time. Results are the mean of 6 rats in each group; vertical bars indicate ±SEM. Differences between control and treated groups significant in subcutaneous fat $p < 0.05$.

used, it appeared unlikely that the effect of BCT on lipid synthesis was indirectly due to its action on insulin secretion. To clarify this point it was decided to investigate whether BCT had a direct in vitro action on lipid synthesis using an adipocyte preparation (table 1): BCT had a marked effect at both concentrations studied.

That BCT stimulated lipogenesis in adipose tissue both in vivo and in vitro suggests that this hormone may contribute to obesity both by its lipogenic action as well as through its action on insulin secretion. The rate of lipogenesis is elevated in the adipose tissue of genetically obese mice [5]; this has been confirmed in this laboratory with 3-week-old ob/ob mice compared with homozygote (+/+) lean mice of this age group (Carr and Beloff-Chain, unpublished). In earlier work on young ob/ob mice (3-week-old) it was suggested that BCT may play a role in the early development of obesity before the adipose tissue becomes insulin resistant [9], and it was assumed that excessive insulin secretion could produce the increased lipogenesis. However, it was shown [5] that increased lipogenesis occurs in adipose tissue of mice at 3 months of age in spite of the fact that in this age group insulin resistance in adipose tissue is very marked [12, 13]. A hormone such as BCT may be contributing to increased lipogenesis in these animals. Investigations to establish with greater precision the role of BCT in the obesity syndrome are underway.

Table 1
Effect of BCT on the rate of lipogenesis in isolated rat adipocytes

Incubation medium	Rate of lipogenesis (μmol C_2 units . 10^6 cells^{-1} . h^{-1})
Control	0.81 ± 0.13 (10)
+5 ng BCT/ml	2.63[a] ± 0.60 (13)
+0.5 ng BCT/ml	1.98[a] ± 0.34 (5)

[a] Significantly different from controls $p < 0.01$

Results expressed as mean values ± SEM (no. expt)

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