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Mechanism of Depletion of Liver Glycogen in Cancer Cachexia

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Mice transplanted with a cachexia-inducing colonic adenocarcinoma (MAC16) show a progressive decrease in liver glycogen in direct proportion to the loss of body weight. Such tumours elaborate a lipid mobilizing factor (LMF), which produces a dose-dependent stimulation, not only of adipocyte adenylate cyclase, but also of heptocyte adenylate cyclase in a GTP-dependent manner. These results suggest that LMF has the capacity to initiate hepatic glycogenolysis through an increase in cyclic AMP.

Cancer cachexia is recognised to be manifested by abnormalities in carbohydrate, lipid and protein metabolism due to distant catabolic effects of the tumor and/or a host-mediated response to the presence of the tumour. This may result in loss of body fat and protein, symptoms of insulin resistance, hypoglycaemia, anemia, muscle weakness, impaired visceral function and immune-defence mechanisms and elevation of basal metabolic rates(1). As a model of the cachexia syndrome we have utilised a transplantable adenocarcinoma of the colon (MAC16), which can produce a 30% loss of body weight of mice with a tumour burden representing just 3% of the host body weight (2). Loss of both adipose tissue and skeletal muscle mass has been shown to be due to the production by the tumour of catabolic factors, which act directly to cause tissue depletion (3, 4). Identical factors can also be isolated from the urine of patients with cancer cachexia (4, 5), but are absent from the urine of normal subjects or patients with weight loss caused by conditions other than cancer. One of these factors proteolysis-inducing factor (PIF) causes wasting of skeletal muscle by decreasing protein synthesis and increasing degradation (6), while a separate factor, a lipid mobilizing factor (LMF) causes triglyceride hydrolysis in adipose tissue through the mediation of cyclic AMP (7). Since glucagon and catecholamines also stimulate hepatic glycogenolysis by raising the intracellular level of cyclic AMP (8) this study investigates the potential role of LMF as an activator of hepatic adenylate cyclase.

MATERIALS AND METHODS

Animals. Pure strain NMRI mice were bred in our own colony and were fed RM3(E) (Lillico and Son, Betchworth, Surrey, U.K.) and water *ad libitum* fragments (1 × 2mm) of either the MAC16 or MAC13 tumour were implanted into the flank of male NMRI mice (starting weight 28 g) by means of a trocar as described (9). Animals bearing the MAC16 tumour developed weight loss, which became apparent 10-12 days after transplantation. They were terminated by cervical dislocation with varying extents of weight loss, the livers were removed onto ice and the glycogen was determined using amyloglucosidase, which hydrolyses α-D-(1 → 4) and α-D-(1 → 6) linkages of glycogen (10). The glucose formed was specifically determined with hexokinase and glucose-6-phosphate dehydrogenase. Animals bearing the MAC13 tumour, which produces no weight loss during growth were terminated when the tumour volumes were comparable with that of the MAC16 and liver glycogen was determined as above.

Purification and evaluation of LMF. LMF was isolated from solid MAC 16 tumours, excised from mice with established weight loss and purified by ion exchange and exclusion chromatography as described (7). Adipocyte plasma membranes were prepared from adipocytes isolated from epididymal adipose tissue of male BKW mice by a modification of the protocol of Belsham *et al.* (11). Essentially plasma membranes were isolated from other components of a cell homogenate using a self-forming Percoll gradient. The membrane fractions were washed in a NaCl buffer, diluted in 10 mM Tris.HCl, pH 7.4, 250 mM sucrose, 2 mM EGTA and 4 μM phenylmethylsulphonyl fluoride at 1-2 mg/ml, snap frozen in liquid nitrogen and stored at −70°C until use. Hepatocyte plasma membranes were purified by a scheme similar to that for adipocytes (11), which had been modified for hepatocytes (12). Adenylate cyclase activity was measured through incorporation of [α-^{32}P]ATP into ^{32}P cyclic AMP using the method of Salomon et al. (13). Briefly, samples were added to an assay mix containing a cyclic AMP regenerating system. The reaction was initiated by the addition of plasma membranes (20-30 μg) and after 10 min at 30°C the reaction was terminated by adding excess ATP, cyclic AMP and SDS. [^{3}H} Cyclic AMP was added to measure recovery and cyclic AMP was separated from other nucleotides using Dowex 50W8-400 columns, which bind cyclic AMP non-specifically and Alumina WN-3 columns, which bind cyclic AMP less avidly than other nucleotides. Samples were collected and the radioactivity was measured on a Tri-carb 2000A scintillation analyser.

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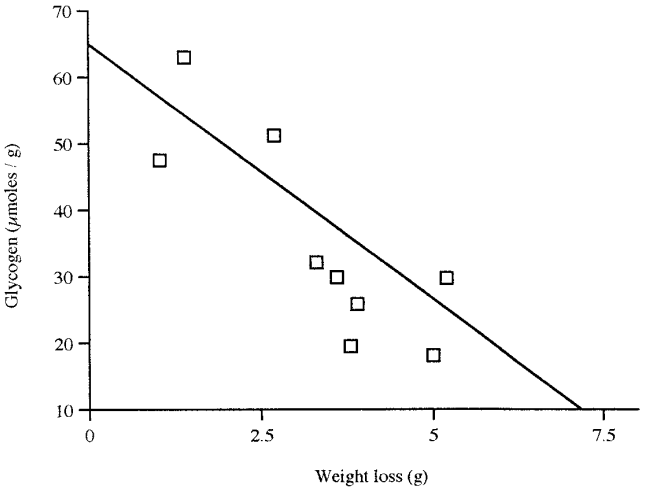

FIG. 1. Relationship between weight loss in mice bearing the MAC16 tumour and liver glycogen concentration. The results were fitted to a linear model by a least squares analysis (r 0.83). Each point represents the results from an individual animal.

Statistical analysis. All values are given as means ± SEM. Differences were determined by Students t-test.

RESULTS

Livers from non tumour-bearing animals and those bearing the MAC13 tumour, which does not produce cachexia, showed a constant amount of glycogen (134 ± 7 and 100 ± 4 μmoles glucose/g wet weight respectively, n=12). In contrast livers of mice bearing the MAC16 tumour showed a progressive decrease in glycogen content (Fig. 1), which was proportional to weight loss (correlation co-efficient 0.83). Since tumour weight has been shown to be proportional to weight loss (2) this suggests that the MAC16 tumour may produce a circulatory mediator initiating glycogenolysis.

We have recently purified a LMF from the MAC16 tumour and from the urine of patients with cancer cachexia, which initiates lipolysis by stimulation of adipocyte adenylate cyclase (7). This material is a glycoprotein of molecular weight 43.kDa. (Fig. 2). Since glycogenolysis utilizes the same intracellular messenger (cyclic AMP) as lipolysis the effect of the LMF on adenylate cyclase activity in heptocyte plasma membranes was investigated. LMF was shown to produce a dose-dependent stimulation of hepatic adenylate cyclase (Fig. 3), with a maximal response occurring with 5 μg LMF. A similar dose-dependency was observed for the stimulation of adipocyte adenylate cyclase (Fig. 4), although the maximum stimulation of cyclic AMP production was approximately three-fold greater than that achieved with hepatocyte plasma membrane preparations. Activation of heptocyte adenylate cyclase was dependent on the presence of GTP, with maximal stimulation occurring at 10 μM GTP (Fig. 5). These results suggest that LMF has the capacity to initiate hepatic glycogenolysis through an elevation of cyclic AMP.

DISCUSSION

In the 'average' man the fuel reserves in the form of liver glycogen represent only 200 k cal and this is readily depleted by overnight starvation, although resynthesis rapidly occurs in the presence of adequate nutrition. An increased energy demand is observed in the tumour bearing state due to an increased operation of futile cycles such as the Cori cycle (14) and an increased glycose flux, which could consume up to 40% of the injested carbohydrate (15). Animals bearing the MAC16 tumour show anomalies in the regulation of carbohydrate metabolism similar to that found in patients with cancer. Thus there is a progressive decrease in blood glucose levels with increasing weight loss (16) and a high glucose consumption by the tumour (17).

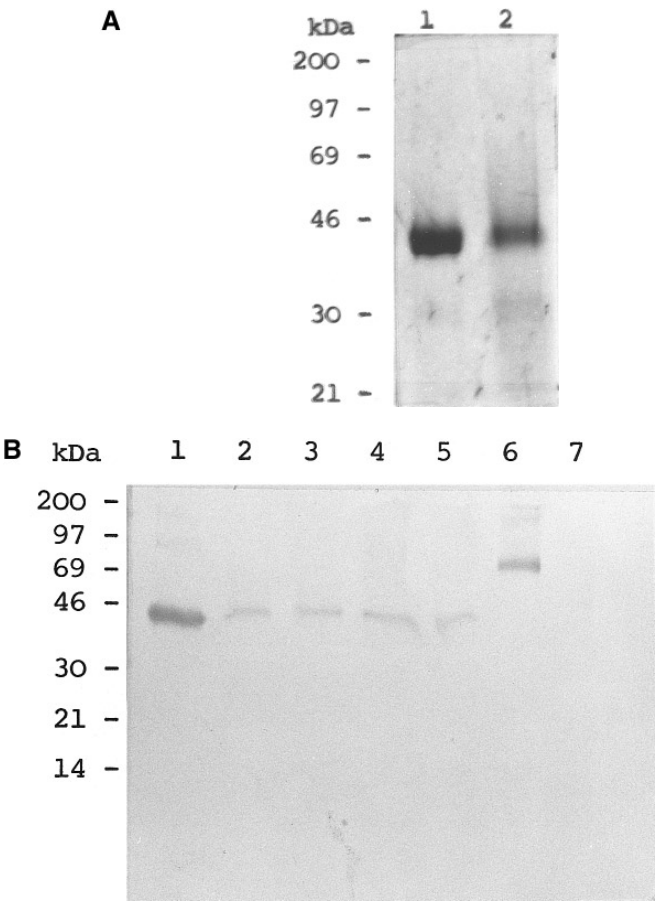

FIG. 2. A. 15% SDS-PAGE of: lane 1, human LMF, lane 2 murine LMF. Detection was by Coomassie staining. B. 15% SDS-PAGE of: lane 1, human LMF; lanes 2-5, different batches of murine LMF; lane 6, transferrin (positive control); lane 7, creatinase (negative control). The gel was stained for carbohydrate using the DIG glycan detection kit (Boehringer Mannhein, East Sussex, U.K.), according to the manufacturer's instructions.

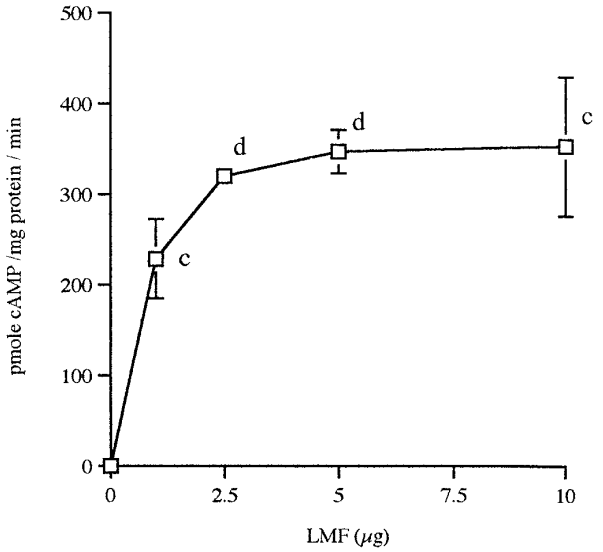

FIG. 3. Dose-dependency of stimulation of adenylate cyclase in isolated hepatocyte plasma membranes in the presence of 10 μM GTP by murine LMF. Cyclic AMP formation in the absence of LMF (basal values) has been subtracted from the figures given. The figure is representative of two separate experiments where n=3. Differences from basal values are indicated as c, $p \leq 0.005$ and d, $p \leq 0.001$.

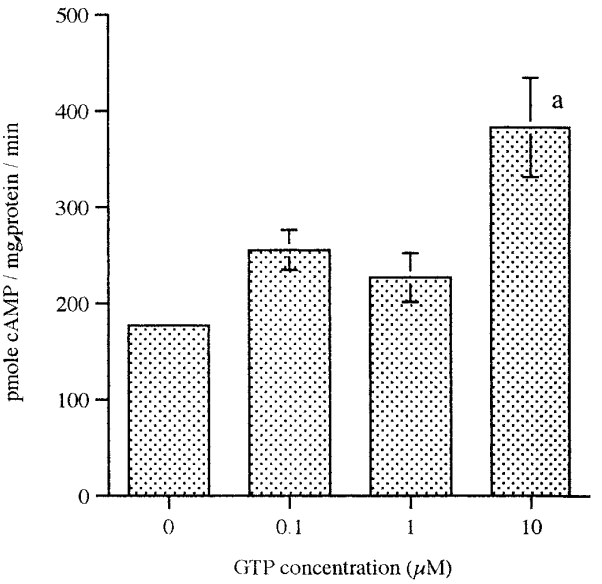

FIG. 5. GTP-dose dependency for stimulation of adenylate cyclase in isolated hepatocyte plasma membranes by murine LMF (7.5 μg). Cyclic AMP formation in the absence of LMF (basal values) has been subtracted from the figures given where n = 3 and is representative of two separate experiments. Differences from values in the absence of GTP are indicated as a, $p \leq 0.05$.

Such an increased demand for glucose would correlate with the present findings of a linear decrease in liver glycogen with increasing weight loss. A similar relationship was found for the depletion of carcass fat and skeletal muscle mass and weight loss (2) and is suggestive of tumour-produced factors initiating glycogen catabolism.

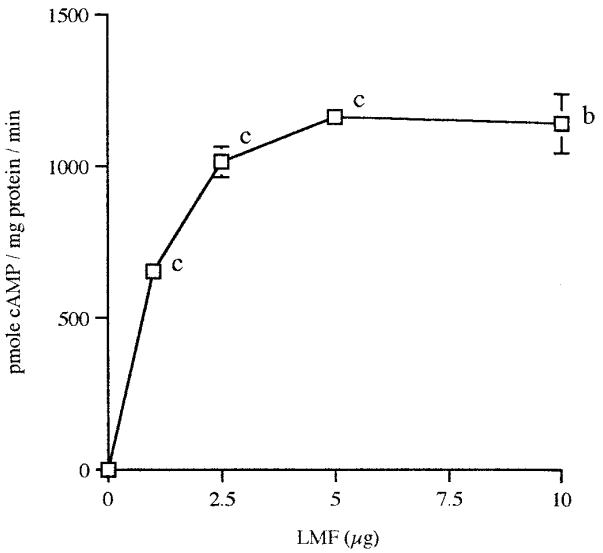

FIG. 4. Dose-dependency of stimulation of adenylate cyclase in isolated adipocyte plasma membranes in the presence of 10 μM GTP by murine LMF. Cyclic AMP formation in the absence of LMF (basal values) has been subtracted from the figures given where n = 4. Differences from basal values are indicated as b, $p \leq 0.01$ and c, $p \leq 0.005$.

Glycogenolysis is the result of hormonal stimulation of hepatic adenylate cyclase leading to an increase in cyclic AMP (8). This study shows that a tumour-produced LMF, which we have previously shown to be indentical with Zn-α_2-glycoprotein in amino acid sequence, electrophoretic mobility, immunoreactivity and biological activity (7) is capable of activation of hepatic adenylate cyclase in a GTP-dependent manner, with a maximal response about one-third of that of adipocyte adenylate cyclase. The nature of the hepatic receptor for LMF is not known, nor indeed whether the effect is receptor mediated or is a result of direct stimulation of adenylate cyclase.

These results suggest that cachexia-inducing tumours produce a catabolic factor capable of initiating the production of an increased flux of glucose and fatty acids, which are needed to supply the energy requirements of the host in the tumour-bearing state.

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