Rapid Publication

Gastric Inhibitory Polypeptide Enhanced Lipoprotein Lipase Activity in Cultured Preadipocytes

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SUMMARY

Fat feeding stimulated the release of gastric inhibitory polypeptide (GIP) without concomitant insulin secretion. Since antilipolytic effects of GIP have been demonstrated and the uptake of triglyceride fatty acid by adipose tissue postprandially is a process reciprocally regulated with lipolysis, a stimulatory role of GIP on adipose tissue lipoprotein lipase activity may be present. After cultured preadipocytes were incubated for 2 h with GIP, the release of lipoprotein lipase activity into the culture medium and the total cellular activity present in acetone-ether powders of cells were measured. GIP stimulated significant increases in the lipoprotein lipase activity released into the culture medium and in cells. A dose response relationship was strongest for the effect of GIP on the enzyme activity in extracts of acetone-ether powders of the cells. The increased lipoprotein lipase activity produced by GIP could provide a mechanism for clearance of chylomicron triglyceride after feeding in man. DIABETES *28*:1141–1142, December 1979.

Gastric inhibitory polypeptide (GIP) is considered to play an important role in the enteroinsular axis.[1] Plasma concentrations of GIP increase after glucose feeding. However, GIP enhances insulin secretion only when the plasma glucose concentration is approximately 150 mg/dl or higher.[2] There is also evidence that GIP may participate in lipid metabolism. After fat feeding, a fivefold to sixfold rise in the plasma concentration of GIP occurs,[3] without an increase in plasma glucose or insulin levels. Preliminary studies in rat adipocytes have shown an inhibition by GIP of both glucagon binding and glucagon-mediated cyclic AMP generation.[4] Since lipolysis is reciprocally regulated with hydrolysis of circulating chylomicron or very low density lipoprotein triglyceride mediated by adipose tissue lipoprotein lipase,[5] a direct stimulatory role of GIP on adipose tissue lipoprotein lipase would be of considerable interest.

The 3T3-L1 cell is an established mouse embryo fibroblast cell line that resembles an adipocyte after reaching a confluent stage of growth.[6] Since lipoprotein lipase activity is seen before triglyceride accumulation in these cells, the development of enzyme activity appears to be the earliest manifestation of this differentiation.[7,8] Although it has already been demonstrated that insulin regulates lipoprotein lipase in 3T3-L1 cells,[9] a possible direct role of GIP in lipoprotein lipase regulation was sought in these studies.

METHODS

The 3T3-L1 cells were a gift from Dr. Howard Green, Massachusetts Institute of Technology. Subclones were derived from these cells.[7] Highly purified preparations of GIP were provided by Dr. John C. Brown, University of British Columbia. The 3T3-L1 cells were plated and grown to confluency in 60-mm dishes using Dulbecco-Vogt's modified Eagle's medium (Microbiological Associates) supplemented with 10% (v/v) fetal bovine serum (Gibco), as previously described.[9] Culture media were changed every 2 or 3 days. To reduce the concentration of insulin and other growth factors in the medium, the serum concentration was reduced to 1% overnight, and on the following morning the medium was changed to fresh medium containing 1% serum, and incubations with GIP were carried out over 2 h at 37°C. The culture medium was then removed and the cells were rinsed with phosphate-buffered saline, incubated with 0.25% trypsin in phosphate-buffered saline for 30 s, and collected by centrifugation for subsequent determination of cellular activity. Lipoprotein lipase activity was determined as described previously.[7,9] Enzyme activity in cells extracted with acetone and ether (acetone-ether activity) represents total cellular activity. Lipoprotein lipase activity, present in the culture medium (culture medium activity), represents re-

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lease of enzyme from cells during the incubation period. Lipase activity was expressed as neq FFA/h/10^6 cells.

RESULTS

The experiment was carried out in subclone IX, previously used for the study of insulin regulation of lipoprotein lipase in 3T3-L1 cells.[9] The dose response curves for each of the lipase measurements are shown in Figure 1. Maximum responsiveness occurred at 5.0 ng/ml for each of the activities measured; significant increases were seen in culture medium activity ($r = 0.644$, $P < 0.05$) and in acetone-ether activity ($r = 0.771$, $P < 0.01$).

Over the range of GIP concentrations tested (0.05, 0.5, and 5 ng/ml), the hormone resulted in enhanced enzyme secretion into the culture medium ($P < 0.01$, Figure 2A) and enhanced acetone-ether activity ($P < 0.001$, Figure 2B). As suggested by the data in Figure 1, the effect on total cellular activity was the more significant.

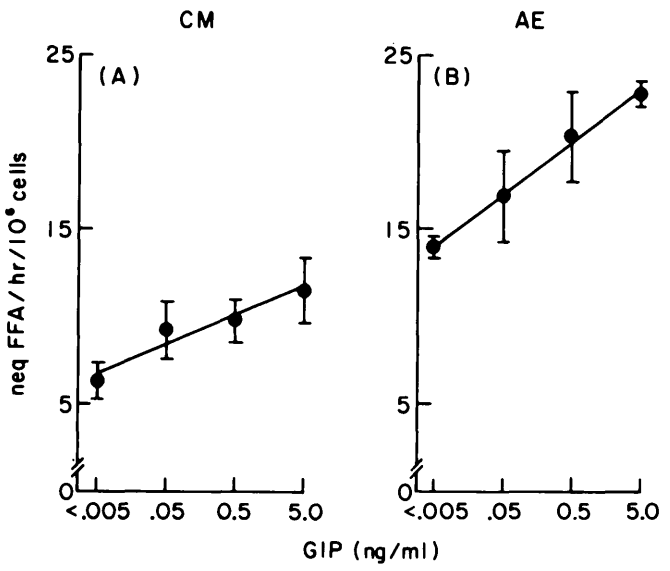
DISCUSSION

The stimulation of lipoprotein lipase activity by GIP in 3T3-L1 cells suggests a mechanism by which dietary fat could be assimilated and a possible role for the rise seen in plasma GIP concentration after fat feeding in man.

The most substantial effect of GIP was to stimulate total cellular lipoprotein lipase activity (Figures 1 and 2). Since this activity reflects protein synthesis (inhibited 80% by a 2-h incubation in 1.5×10^{-8} M cycloheximide, data not shown), the predominant role of GIP could be to increase lipoprotein lipase through an effect on protein synthesis. Whether or not the increase in culture medium activity is a reflection of the increase in the cell cannot be determined from these studies.

The role of GIP in regulating adipose tissue lipoprotein lipase activity needs to be confirmed in animal and human studies, but the implications of the findings in 3T3-L1 cells to the physiology of lipid metabolism and the application to such disease states as obesity and diabetes mellitus, where alterations in insulin, GIP, and adipose tissue lipoprotein lipase have all been described, are of interest.

FIGURE 1. Dose response for the effect of GIP on lipoprotein lipase activity in cultured 3T3-L1 fibroblasts. Enzyme activity is measured after a 2-h incubation of cells in fresh Dulbecco's modified medium supplemented with 1% fetal bovine serum and GIP. Each point represents the mean ± SEM of three measurements. (A) CM is the lipase activity secreted into the culture medium, $r = 0.644$, $P < 0.05$; (B) AE is the lipase activity present in acetone-ether powders of cells, $r = 0.771$, $P < 0.01$.



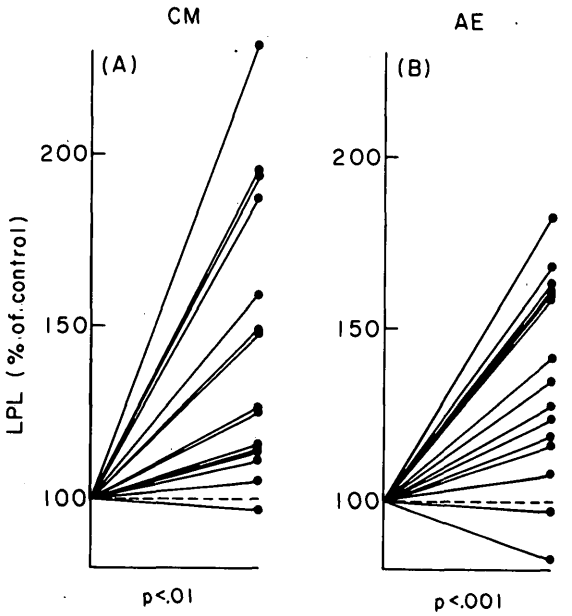


FIGURE 2. The effect of 0.05, 0.5, and 5.0 ng/ml GIP on lipoprotein lipase activity in three experiments using 3T3-L1 subclones. The data are represented by percentage of control activity (< 0.005 ng/ml GIP) and are shown for (A) CM, the activity secreted into the culture medium, and for (B) AE, the activity present in acetone-ether powders of cells, all after 2 h of incubation in GIP. Discrimination between different GIP concentrations is not shown. Each point represents a single measurement. The nonpaired *t* test was used to compare the enzyme activity after GIP to the non-GIP–treated control group (represented by a mean of 100% of control).

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