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INFLUENCE OF SOME GASTROINTESTINAL HORMONES ON ADIPOSE TISSUE LIPOPROTEIN LIPASE ACTIVITY IN VITRO : EVIDENCE OF A STIMULATORY EFFECT OF PANCREOZYMIN AND GASTRIN.

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SUMMARY : At concentrations corresponding to the levels usually reported in the blood of different species in the fed state, gastrin and pancreozymin but not secretin and vasoactive intestinal peptide, stimulate the lipoprotein lipase activity of adipose tissue from fasted rats. The enzyme response to gastrin is, like that to insulin, dependent on the presence of glucose and is not additive with the enzyme response to insulin. On the contrary, the effect of pancreozymin on lipoprotein lipase is glucose-independent and is additive with the enzyme response to insulin. Both the effects of gastrin and pancreozymin depend on protein synthesis as shown by their suppression by cycloheximide. With isolated fat cells, gastrin increases both the releasable and non-releasable lipase activities whereas pancreozymin increases almost exclusively the non-releasable activity. The mechanisms and the possible physiological significance of these findings are discussed in relationship with the influence of insulin and the nutritional state on adipose tissue lipoprotein lipase.

INTRODUCTION:

While it is well established that the nutritional status plays an important role in the regulation of adipose tissue lipoprotein lipase (LPL) activity (1), the mechanisms underlying its increase in the fed state and, conversely, its decrease in the fasting state (1) are still incompletely understood. To explain these modifications, efforts were made to find, among the factors which are increased in the blood by feeding, those which could directly enhance LPL activity in adipose tissue "in vitro". These investigations have provided evidence that glucose in combination with insulin could be one of the factors responsible for the LPL variations occurring in fat cells in response to nutritional changes (2 - 5).

Beside insulin, however, there are other hormones (gastrin, secretin, vasoactive intestinal peptide, pancreozymin), the secretion of which is also considerably and specifically increased by feeding (6). Thus and because the hormonal regulation of

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Abbreviations : LPL, lipoprotein lipase.

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LPL is poorly understood, we have attempted to determine whether some of these so-called gastrointestinal hormones could also intervene in the regulation of LPL. This was achieved by investigating "in vitro" the effects of different physiological levels of these hormones on LPL activity of rat fat pad homogenates and on both the LPL activity remaining in and released from isolated fat cells.

MATERIAL AND METHODS :

Porcine insulin (Actrapid, 40 units/ml) was from Novo ; porcine gastrin (B grade), a mixture of gastrin I and II, was from Calbiochem. Highly purified porcine vasoactive intestinal peptide was a generous gift of Dr. Laburthe (INSERM U55, Paris) and was obtained from Dr. Mutt from the Karolinska Institut (Stockholm, Sweden). Porcine secretin (grade II) containing 25-30 units pancreozymin per 100 units secretin and porcin pancreozymin (grade II) were from Sigma. Tri [-1^{14}C] oleylglycerol (spec. act. : 55 mCi/mmol) was purchased from the Radiochemical Centre (Amersham). All other chemicals were of analytical grade.

Male Wistar rats (120-140g) were fasted overnight before sacrifice. After decapitation, epididymal fat pads were excised and isolated fat cells prepared as previously described (7). In studies using adipose tissue, fragments from one pad from each animal served as control while fragments from the other pad were used as the test. Adipose tissue (200 mg/ml) or isolated fat cells (500 µl packed cells/ml) were incubated under O_2/CO_2 (95 : 5 v/v), in the absence or presence of the hormones to be tested, in Krebs-Ringer (8) bicarbonate buffer (pH 7.4) containing 1.25 mM calcium, 5 mM glucose (unless otherwise stated), 20 mg/ml dialysed bovine albumin (fraction V), and an amino-acid mixture (final concentration 390 nM), the composition of which corresponded to the amino-acid concentration of rat plasma (9). In studies performed with isolated fat cells, the basic incubation medium described above was supplemented with 3.8 % (v/v) fresh rat serum. After a two hour incubation at 37°C (adipose tissue) or 26°C (fat cells), adipose tissue fragments or fat cells were separated from the incubation buffer, homogenized at 4°C in 50 mM NH_4 OH-NH_4Cl buffer (pH 8.6) and centrifuged at 4°C. The clear supernatant was used for the assay of LPL.

Assay of LPL was performed according to a modification of the procedure of Greten and Walter (10) as follows : in a total volume of 1 ml, each vial contained 0.6 ml of substrate emulsion consisting of 1.2 nmol tri [-1^{14}C] oleylglycerol (spec. act. : 55 mCi/mmol), 21.8 µmol of unlabeled trioleylglycerol, 40 mg bovine albumin, 50 µl of a 1 : 100 diluted Triton X-100 solution all in 0.2 M Tris-HCl buffer (pH 8.6), 150 µl of rat serum as cofactor and 0.4 ml of enzyme solutions. Incubations were performed for 60 min at 37°C. Lipids were then extracted (11) and ^{14}C-labeled free fatty acids isolated by the ion exchange procedure of Kelley (12). LPL activity is calculated as µmol FFA released per gram fat pad or gram cell lipid per hour.

RESULTS AND DISCUSSION :

The effects of various concentrations of gastrin, pancreozymin, secretin and vasoactive intestinal peptide on rat adipose tissue lipoprotein lipase are shown in Table I. The range of the hormonal concentrations tested were chosen to cover the hormonal levels usually reported in the blood of different species in both the fasting and postprandial states (6, 13-16). As can be seen, vasoactive intestinal peptide failed to

TABLE I : Effects of gastrin, pancreozymin, secretin, vasoactive intestinal peptide and insulin on the lipoprotein lipase activity of rat epididymal adipose tissue.

	Lipoprotein lipase activity (μmol FFA x h^{-1} x g^{-1} wet weight)			
Gastrin (pM)	0	54	270	540
	11.7 $\pm$ 1.4	14.8 $\pm$ 1.2**	15.3 $\pm$ 1.7**	15.5 $\pm$ 0.9**
Pancreozymin (pM)	0	6	12	24
	15.1 $\pm$ 1.6	18.0 $\pm$ 0.8**	22.7 $\pm$ 1.6***	22.6 $\pm$ 1.4***
Secretin (pM)	0	0.2	2	20
	15.1 $\pm$ 1.6	17.0 $\pm$ 0.7*	15.2 $\pm$ 1.1*	18.2 $\pm$ 0.8**
Vasoactive intestinal peptide (pM)	0	10	50	100
	11.7 $\pm$ 1.4	12.7 $\pm$ 0.4*	11.3 $\pm$ 0.6*	13.3 $\pm$ 1.4*
Insulin (mU/ml)	0	3	6	9
	11.6 $\pm$ 0.3	14.4 $\pm$ 0.2***	17.1 $\pm$ 0.7***	18.7 $\pm$ 1.1***

Incubations were performed as described under Material and Methods. Each value represents the mean $\pm$ S.E. of 5 determinations. *, non significant ; **, $0.01 < P < 0.02$; ***, $P < 0.001$.

induce any significant modification of LPL activity in adipose tissue of fasted rats after two hours incubation. Under the same conditions, however, gastrin, pancreozymin and secretin increased this enzyme activity. This effect, although being slight (+ 26 %), was already maximal and significant with 54 pM gastrin. In the case of pancreozymin, stimulation of LPL was already significant at 6 pM (+ 19 %, $0.01 < P < 0.02$) and maximal at 12 pM (+ 50 %, $P < 0.001$). In the case of secretin, a slight (+ 20 %) but significant ($0.01 < P < 0.02$) stimulation of LPL activity was observed with 20 pM of this hormone. However, considering the fact that the secretin preparation used in these experiments contained 25-30 % pancreozymin, it appears more than likely that the observed stimulatory effect of secretin is due to the presence of pancreozymin rather than to secretin "per se".

For purpose of comparison, insulin was also tested under the same experimental conditions. As shown in Table I, adipose tissue LPL activity was increased by 61 % after 2 hours incubation with insulin (9 mU/ml).

The fact that glucose is required for the "in vitro" expression of the insulin-mediated stimulation of adipose tissue LPL (2,4,17,18) raised the possibility that the action of pancreozymin and gastrin were also dependent on the presence of this hexose in the incubation buffer. To evaluate this, fat pads were incubated as above but

TABLE II : Influence of glucose on the stimulating effect of gastrin and pancreozymin on adipose tissue lipoprotein lipase activity.

	Lipoprotein lipase activity (μmol FFA x h^{-1} x g^{-1} wet weight)	
	Glucose (5 mM)	No Glucose
Control	11.7 ± 1.0	9.6 ± 1.0
Gastrin (54 pM)	14.8 ± 1.2**	11.5 ± 1.4*
Pancreozymin (12 pM)	16.8 ± 0.6***	13.7 ± 1.3***

Incubations were performed in the presence or absence of glucose, as described under Material and Methods. Each value represents the mean ± S.E. of 5 determinations. *, non-significant ; **, $0.01 < P < 0.02$; ***, $P < 0.001$.

in a glucose-free medium, with or without gastrin (54 pM) or pancreozymin (12 pM). Under these conditions (Table II), the stimulatory effect of gastrin on LPL was no more statistically significant, whereas the enzyme response to pancreozymin remained unchanged whether glucose was present (+ 44 ± 5 %) or absent (+ 43 ± 13 %) in the incubation medium. These data thus suggest that the mechanism underlying the gastrin- and insulin- mediated stimulation of adipose tissue LPL may be different from that involved in the LPL response to pancreozymin.

This hypothesis was further supported by the following experiments in which the effects of pancreozymin, gastrin or insulin alone were compared with the combined effects of either insulin and pancreozymin or insulin and gastrin. As shown in Table III, the effect of pancreozymin was additive with that of insulin, indica-

TABLE III : Influence of insulin on the stimulating effect of gastrin and pancreozymin on adipose tissue lipoprotein lipase activity.

	Lipoprotein lipase activity (μmol FFA x h^{-1} x g^{-1} wet weight)	
	No insulin	Insulin (9 mU/ml)
Control	11.1 ± 0.7	17.9 ± 1.0
Gastrin (54 pM)	14.0 ± 0.9***	17.8 ± 1.1*
Pancreozymin (12 pM)	16.0 ± 1.4***	22.7 ± 1.4***

Incubations were performed in the presence or absence of insulin, as described under Material and Methods. Each value represents the mean ± S.E. of 10 to 15 determinations. *, non-significant ; **, $0.01 < P < 0.02$; ***, $P < 0.001$.

TABLE IV : Influence of cycloheximide on the stimulating effect of gastrin, pancreozymin and insulin on adipose tissue lipoprotein lipase activity.

	Lipoprotein lipase activity (μmol FFA x h^{-1} x g^{-1} wet weight)	
	No cycloheximide	Cycloheximide (36 μM)
Control	11.3 ± 0.7	11.7 ± 1.0
Gastrin (54 pM)	13.8 ± 0.9**	11.3 ± 0.9*
Pancreozymin (12 pM)	16.5 ± 0.8***	11.4 ± 1.0*
Insulin (9 mU/ml)	19.1 ± 0.8***	10.9 ± 0.7*

Incubations were performed in the presence or absence of cycloheximide, as described under Material and Methods. Each value represent the mean ± S.E. of 10 to 15 determinations. *, non-significant ; ** , $0.001 < P < 0.01$; *** $P < 0.001$.

ting thus that the site of action of pancreozymin on adipose tissue LPL is different indeed from the site of action of insulin. On the contrary, the effects of gastrin and insulin were not additive, a result which suggests the existence of a common site of action for these two hormones.

Recent immunotitration studies (19) have shown that the increase in adipose tissue LPL which occurs in the fed state is due to an increased enzyme level. To assess whether the LPL-response to pancreozymin and to gastrin involves new protein synthesis, the effect of cycloheximide (36 μM), an inhibitor of this process, was examined. Table IV shows that the increase in LPL activity induced by pancreozymin and gastrin were completely blocked by cycloheximide. Cycloheximide also suppressed the insulin stimulation of LPL (Table IV), a result which was not surprising, considering a recent report (20) in which the effect of insulin on LPL activity was correlated with its concurrent stimulation of protein synthesis in adipose tissue

Several authors (21-23) have recently reported that, when fat cells are isolated from adipose tissue of starved rats and incubated at 25-30°C, the total LPL activity of the incubation system increases progressively over a period of several hours. All of this increase in activity is accounted for by the appearence of enzyme in the incubation medium (21-23), a phenomenon which does not involve new protein synthesis (24). Moreover, under appropriate conditions, the rate of this LPL release is also increased by the addition of insulin (4), which furthermore increases

TABLE V : Effects of gastrin and pancreozymin on fat cell lipoprotein lipase and release of enzyme into the medium.

	Lipoprotein lipase activity (μmol FFA $\times h^{-1} \times g^{-1}$ cell lipid)	
	Cell enzyme activity	Medium enzyme activity
Exp. 1 : Control	46.7 $\pm$ 2.5	12.2 $\pm$ 1.7
Gastrin 54 pM	49.7 $\pm$ 1.5 (N.S.)	12.1 $\pm$ 1.1 (N.S.)
" 270 pM	58.3 $\pm$ 5.4 ($0.001 < P < 0.01$)	15.1 $\pm$ 0.9 ($0.02 < P < 0.05$)
" 540 pM	59.2 $\pm$ 3.3 ($0.001 < P < 0.01$)	16.9 $\pm$ 1.3 ($0.01 < P < 0.02$)
Exp. 2 : Control	47.9 $\pm$ 5.8	15.8 $\pm$ 0.4
Pancreozymin 6 pM	63.3 $\pm$ 2.5 ($0.001 < P < 0.01$)	16.9 $\pm$ 0.7 (N.S.)
" 12 pM	87.2 $\pm$ 2.9 ($P < 0.001$)	18.4 $\pm$ 0.6 ($0.001 < P < 0.01$)

Incubations were performed as described under Material and Methods. Each value represents the mean $\pm$ S.E. of 3 incubations. Statistical significance is given in parentheses. N.S. = non-significant.

also the LPL activity remaining in the cell (25). Considering these data, further experiments were undertaken to determine whether gastrin and pancreozymin may alter the releasable and/or the non-releasable from of LPL in isolated rat fat cells. As shown in Table V, gastrin (270 pM) increased slightly (+ 25 %) but significantly ($0.001 < P < 0.01$) both the releasable and non-releasable LPL activities. In contrast, pancreozymin increased almost exclusively the intracellular LPL activity (+ 87 % in the cell vs. + 16 % in the medium at 12 pM pancreozymin). These results provide thus an additional support to the hypothesis put forward above, according to which the mechanisms through which gastrin increases adipose tissue LPL activity may be common with those involved in the action of insulin, i.e. the induction of LPL through both hexose- and amino acid transport-independent and -dependent mechanisms and the release of the enzyme from the cell (18).

From this study it is clear that among the four gastrointestinal hormones tested, only pancreozymin and to a lesser extent gastrin are able to stimulate "in vitro" the LPL activity in adipose tissue and isolated fat cells from fasted rats. Expression of this LPL stimulation does not require prolonged incubation since the present results

were observed after a two hour-exposure of adipose tissue or isolated fat cells to the hormones. As these effects were obtained with hormone concentrations similar to those found in the blood of different species during feeding (6,13-16), this would suggest that these hormones may contribute, together with insulin, to the enhancement of adipose tissue LPL activity which occurs after feeding (1). However, the fact that the LPL response to gastrin was slight and not additive with the LPL response to insulin does not argue in favour of a possible physiological role of gastrin in the hormonal regulation of adipose tissue LPL in the fed state. On the contrary, the fact that the LPL responses to pancreozymin and to insulin were similar and additive is an argument supporting a possible important role of pancreozymin in the "in vivo" control of the uptake of triacylglycerol from chylomicrons and very low density lipoproteins by adipose tissue. Further experiments are currently in progress to substantiate this hypothesis.

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