Regulation of Leptin Release by Insulin, Glucocorticoids, G_i-Coupled Receptor Agonists, and Pertussis Toxin in Adipocytes and Adipose Tissue Explants From Obese Humans in Primary Culture

Alie Kanu, John N. Fain, Suleiman W. Bahouth, and George S.M. Cowan Jr

The basal release of leptin by adipocytes from massively obese human subjects incubated for 48 hours in serum-free suspension culture was comparable to that by explants of subcutaneous adipose tissue from the same obese individuals. There was no stimulation due to dexamethasone or insulin alone of leptin release by adipocytes. However, the combination of insulin and dexamethasone doubled leptin release by adipocytes. The release of leptin was also stimulated by agonists of G_i-coupled receptors (prostaglandin E_2 [PGE_2], brimonidine [an α_2 catecholamine agonist] and cyclopentyladenosine [CPA]) in the presence of dexamethasone. Leptin release by these agents was further enhanced by insulin in both adipocytes and adipose tissue. Pertussis toxin, which irreversibly inactivates G_i heterotrimers, inhibited leptin release and abolished the stimulatory effects of G_i-coupled receptor agonists. However, pertussis toxin did not block the stimulation of leptin release by insulin in either adipose tissue or adipocytes. These data indicate that the release of leptin by human adipocytes cultured for 48 hours in a serum-free medium is comparable to that by explants of adipose tissue except that dexamethasone stimulation of leptin release requires the presence of insulin.

PRIOR STUDIES on the regulation of leptin release by human adipose tissue have primarily utilized explants of adipose tissue in primary culture.[1-8] However, some studies have used freshly isolated human adipocytes.[9-12] Explants of human adipose tissue contain a variety of other cells besides adipocytes such as connective tissue and blood vessels. The question arises as to the relative importance of factors released by the non-fat cells of adipose tissue in the regulation of leptin release by adipocytes. It was also of interest to determine whether the digestion of adipose tissue with bacterial collagenases and proteases, which are required to release adipocytes from the tissue matrix, impairs the response of adipocytes to hormones and other factors that regulate leptin release. A major complication in studies on leptin release is the finding that hormones and other factors that regulate leptin release have a prolonged lag period before their effects can be demonstrated on explants of adipose tissue.[1-3]

Leptin release by explants of adipose tissue is quite variable from one individual to the next and a comparison of tissue explants versus adipocytes is best done by paired experiments. Leptin release is 4- to 5-fold greater over a 48-hour incubation in explants of adipose from obese as compared to lean subjects.[7] It is for this reason that adipose tissue was obtained from obese individuals for our studies.

There is general agreement that glucocorticoids stimulate leptin release[1-12] and that agents that elevate cyclic adenosine monophosphate (AMP) and lipolysis inhibit leptin release.[1,2,4,5] There is disagreement with regard to insulin as Considine et al[10] reported that it inhibited leptin release by human adipocytes in the presence of dexamethasone and Halleux et al[7] reported similar effects in adipose tissue explants. However, our laboratory[2] and Russell et al[8] have reported stimulatory effects of insulin on leptin release by human adipose tissue explants. Differences have also been reported for troglitazone which inhibited leptin release by explants[2] or adipocytes[11] in the presence of insulin and dexamethasone while in explants it stimulated leptin release at low concentrations in the absence of dexamethasone and insulin.[2] The present studies were designed to compare the regulation of leptin release by adipocytes to that by explants from the same subjects. We examined the effects of agents that activate the heterotrimeric inhibitory guanine nucleotide binding proteins (G_i) on leptin release, as well as the effects of pertussis toxin, which inactivates G_i and G_o proteins.

MATERIALS AND METHODS

Subjects

Subcutaneous abdominal adipose tissue was obtained from obese subjects undergoing elective open abdominal surgery (gastric bypass) under general anesthesia after an overnight fast. The mean body mass index (BMI) ranged from 36 to 68 and averaged 49 for 43 subjects. The average age of the patients was 38 years and 93% were females. The fasting blood glucose value was greater than 125 mg/dL in 20% of the patients. Each experimental replication involved tissue from a separate individual. The study had the approval of the local institutional review board and all patients involved gave their informed consent. The patients were fasted overnight prior to surgery but had not been on any type of dietary restriction just prior to surgery.

Twenty to eighty grams of abdominal subcutaneous adipose tissue were immediately transported to the laboratory (5 to 10 minutes). The handling of tissue and cells was done under aseptic conditions. The tissue was cut with scissors into small pieces (20 to 30 mg). All of the studies used explants of adipose tissue that had been incubated in buffer plus albumin for approximately 30 minutes to remove diffusible factors and blood cells. At the conclusion of the 30-minute incubation, the tissue explants were centrifuged for 30 seconds at 400 $\times$ *g* in order to remove erythrocytes and pieces of tissues containing insufficient adipocytes to float. The explants were separated from the medium plus the sedimented cells and resuspended in fresh buffer. The explants (80 to 100 mg/mL) were then incubated for 48 hours in suspension culture under aseptic conditions.[1] The release of leptin by explants that had

From the Departments of Molecular Sciences, Pharmacology, and Surgery, College of Medicine, University of Tennessee Health Science Center, Memphis, TN.

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Address reprint requests to John N. Fain, PhD, Department of Molecular Sciences, College of Medicine, University of Tennessee Health Science Center, Memphis, TN 38163.

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been incubated for 48 hours without preincubation averaged 102 ± 6 ng of leptin/g of tissue as the mean ± SEM of 53 experiments. In contrast, in 31 experiments (each experiment involved fat from a different individual) the release of leptin by washed explants of adipose tissue averaged 144 ± 14 ng of leptin/g of fat. These data indicate that basal release of leptin is enhanced in explants subjected to a prewashing procedure.

Adipocytes were obtained by collagenase digestion and the cells and medium were separated from undigested tissue by filtration through 200 μm nylon mesh at the end of the 90-minute digestion period. The non-fat (stromal cells) were separated from the free adipocytes by centrifugation in 15-mL tubes for 1 minute at 400 × *g*. The adipocytes were resuspended in fresh buffer and centrifuged for 10 seconds at 400 × *g*. The medium was removed and this process repeated 3 times.

The buffer for incubation of adipose tissue and adipocytes was Dulbecco's modified Eagle's medium/Ham's F12 (1:1, Sigma No. 2906; Sigma Chemical, St Louis, MO) containing 17.5 mmol/L glucose, 121 mmol/L NaCl, 4 mmol/L KCl, 1 mmol/L $CaCl_2$, 25 mmol/L HEPES, 2.4 mmol/L sodium bicarbonate, 10 mg/mL bovine serum albumin, 5 μg/mL ethanolamine, 0.1 ng/mL sodium selenite, 90 μg/mL penicillin G, 150 μg/mL streptomycin sulfate, 50 μg/mL gentamicin, and 55 μmol/L ascorbic acid. In some experiments, arachidonic acid (20 μmol/L) was present, which enhances leptin release and prostanoid formation.[3,13] The pH of the buffer was adjusted to 7.4 followed by filtration through a 0.2-μm filter. Aliquots of the medium were taken at 48 hours and stored at −20°C for measurement of leptin or prostaglandin E_2 (PGE_2). The leptin content in 20- to 50-μL aliquots of the incubation medium was determined using radioimmunoassay kits from Linco Research (St Louis, MO). Lipolysis was measured as glycerol release[14] and lactate was assayed as previously described.[1-3] Bovine serum albumin powder (Bovuminar, containing <0.05 mol of fatty acid/mol of albumin) was obtained from Intergen (Purchase, NY). Bacterial collagenase *Clostridium histolyticum* (type 1) was obtained from Worthington Biochemical (Freehold, NJ; lot CLS1-4197-MOB3773-B, 219 U/mg). Other chemicals were from Sigma Chemical.

RESULTS

The release of leptin by explants or adipocytes over a 48-hour incubation in primary culture was quite variable from one individual to the next, although our donor population was primarily obese women. The data in Fig 1 indicate that preexisting factors are responsible for most of the variability from one individual to the next because the trend in leptin release from adipose tissue explants was maintained in adipocytes despite the 90-minute digestion with collagenase. These data also indicate that basal release of leptin by adipocytes over 48 hours does not require factors released by the non-adipocytes in adipose tissue. We have measured the diameter of the adipocytes that are obtained after collagenase digestion and found a rather uniform diameter from one donor to the next averaging 135 μm. It should be noted that larger adipocytes may be more fragile and lost during collagenase digestion.

The basal release of leptin over 48 hours by human adipocytes was comparable to that by explants of adipose tissue when expressed per gram of tissue or cells (Fig 2). Insulin alone had no stimulatory effect on leptin release by adipose tissue or adipocytes. In contrast, dexamethasone, a synthetic glucocorticoid, stimulated leptin release by explants but not by isolated adipocytes. However, the combination of insulin plus dexamethasone stimulated leptin release by human adipocytes (Fig 2).

The data in Fig 2 also demonstrate that the stimulation of

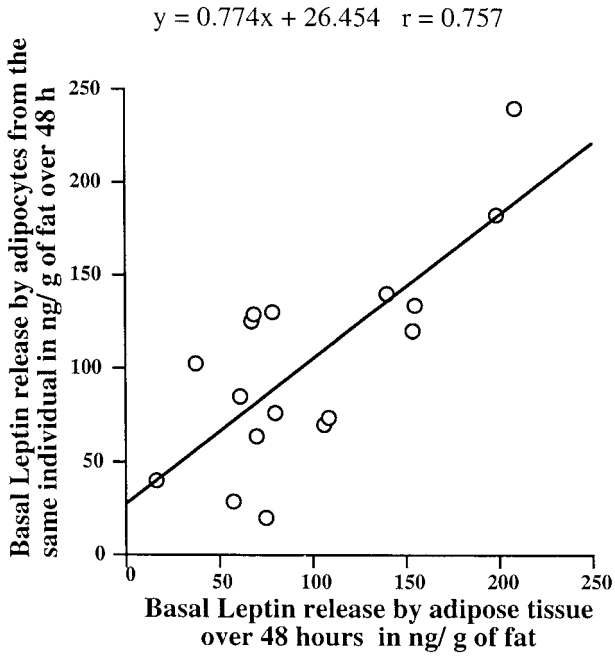

Fig 1. Correlation between release of leptin by adipocytes versus adipose tissue from the same individuals. Explants of human adipose tissue (80 to l00 mg/mL) or adipocytes (80 to 100 mg/mL) from the same individuals were incubated in duplicate for 48 hours. Values are expressed per gram wet weight of tissue or packed cells and are the individual values for the 17 experimental replications. The correlation coefficient was 0.757 and the *P* value was < .01.

leptin release by added agents occurred primarily during the second 24 hours of a 48-hour incubation in both adipocytes and adipose tissue. The question of whether the cells and tissue carry out metabolism at the same rate during the last part of a 48-hour incubation as during the first part was examined in the studies shown in Table 1 where we examined lipolysis, cyclic AMP accumulation, and lactate formation over a 2-hour incubation period in freshly isolated human adipocytes, as well as those previously incubated in primary culture for 48 hours. We incubated adipocytes without and with 50 μmol/L isobutyl methyl xanthine (IBMX) plus 500 nmol/L isoproterenol (ISO) in order to measure cyclic AMP accumulation and hormone-stimulated lipolysis. Lactate formation was measured as an index of glucose metabolism. The amount of lactate formed over 2 hours was elevated by 46% in adipocytes and by 20% to 33% in adipose tissue after a 48-hour incubation (Table 1). Basal lipolysis was not affected by prior incubation for 48 hours, but the increase due to ISO plus IBMX was reduced by 25% in tissue and by 20% in adipocytes (Table 1).

Cyclic AMP accumulation at the end of the 2-hour incubation was not significantly reduced in explants and adipocytes that had been preincubated for 48 hours (Table 1). These data indicated that prior incubation of adipose tissue explants or adipocytes for 48 hours did not have deleterious effects on lactate formation, lipolysis, or cyclic AMP accumulation.

We examined the effects of agonists for G_i-coupled receptors both in the absence and in the presence of insulin (Fig 3). PGE_2, brimonidine (also known as UK14304), and N^6-cyclopentylad-

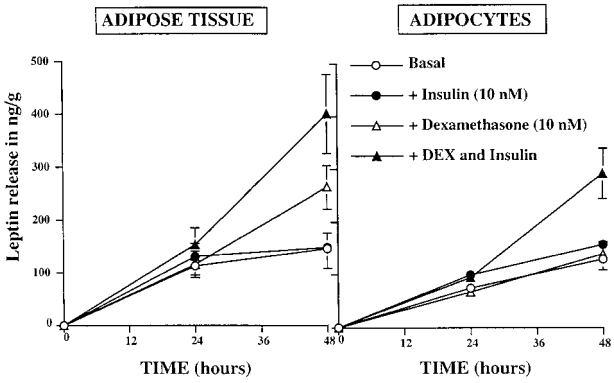

Fig 2. The stimulation of leptin release by insulin is slow in onset and enhanced by dexamethasone. Explants of human adipose tissue as well as adipose from the same individuals were incubated for 48 hours in the absence or presence of 10 nmol/L dexamethasone. Values are the means ± SEM of 8 paired experiments from as many obese humans. Aliquots of the medium were removed at 24 and 48 hours for analysis of leptin release.

enosine (CPA) are antilipolytic agents that stimulate G_i-coupled receptors in adipocytes. We compared the effects of insulin both in the absence and presence of PGE_2 plus brimonidine and CPA. We used the combination of the three G_i agonists since preliminary experiments indicated the effects were greater and more reproducible with all 3 agonists than any single agonist. Lipolysis and leptin release were examined over a 48-hour incubation, while cyclic AMP accumulation was measured by adding ISO and IBMX after 48 hours and continuing the incubation of cells or tissues for 20 minutes.

The data in Fig 3 indicate that basal lipolysis over the 48-hour incubation was inhibited by either insulin or the agonists for G_i-coupled receptors except in tissue explants where insulin alone had no effect. The combination of insulin and the G_i-coupled receptor agonists had no greater effect on lipolysis than was seen with the G_i-coupled receptor agonists alone.

Leptin release was elevated in both adipocytes and adipose tissue by agonists for G_i-coupled receptors (Fig 3). However, there was no significant effect of insulin alone in adipocytes. The combination of insulin plus the G_i-coupled receptor agonists resulted in a greater stimulation of leptin release than was seen with the G_i agonists (Fig 3).

We also examined the effect of a 48-hour exposure to insulin, the G_i-coupled receptor agonists or the combination on the cyclic AMP response. To obtain measurable amounts of cyclic AMP, the adipocytes or tissue were incubated for 20 minutes in the presence of a catecholamine agonist at a high concentration (500 nmol/L isoproterenol) in the presence of a cyclic AMP phosphodiesterase inhibitor (isobutylmethyl xanthine at 50 μmol/L). The effect of a 48-hour incubation with insulin on the subsequent response over 20 minutes to ISO plus IBMX was quite different from that seen with the three G_i-coupled receptor agonists (Fig 3). Prior exposure to insulin enhanced the cyclic AMP response in both adipocytes and adipose tissue while prior exposure to the three G_i-coupled receptor agonists inhibited the cyclic AMP response. The combination of both insulin and the agonists for G_i-coupled receptors had effects intermediate between those of either agent alone (Fig 3).

The effects of G_i-coupled agonists are blocked by pertussis toxin after it penetrates cells and adenosine diphosphate (ADP)-ribosylates a cysteine residue on the alpha subunit of G_i. This results in enhanced cyclic AMP accumulation and lipolysis in human adipocytes (Fig 4) confirming the report of Kather et al.[15] The effects of the three G_i-coupled receptor agonists on adipocyte metabolism appear to be mediated through G_i since they were abolished by coincubation with pertussis toxin (Fig 4). This was true with respect to leptin release and lipolysis over 48 hours, as well as inhibition of the cyclic AMP response due to ISO plus IBMX.

Pertussis toxin (100 ng/mL) enhanced lipolysis and inhibited leptin release over a 48-hour incubation (Fig 4). Similar effects were seen in both adipocytes and adipose tissue with regard to the effects of pertussis toxin on leptin release and lipolysis. However, the cyclic AMP response to ISO plus IBMX after a 48-hour incubation with pertussis toxin was different in adipocytes than in adipose tissue. Cyclic AMP formation was 320%

Table 1. Comparison of Cyclic AMP and Formation, Lipolysis, and Lactate Formation by Isolated Human Adipocytes Versus Explants of Human Adipose Tissue During the First 2 and Last 2 Hours of a 50-Hour Incubation Period

		Adipose Tissue		Adipocytes	
	Time (h)	Without	+IBMX & ISO	Without	+IBMX & ISO
Cyclic AMP	0-2	8 ± 4	192 ± 55	12 ± 5	220 ± 86
Cyclic AMP	48-50	2 ± 2	134 ± 88	0 ± 1	171 ± 28
Lipolysis	0-2	1.1 ± 0.5	3.6 ± 1.5	0.5 ± 0.1	2.5 ± 0.4
Lipolysis	48-50	1.0 ± 0.6	2.5 ± 1.0†	0.6 ± 0.1	2.1 ± 0.6*
Lactate	0-2	3.0 ± 0.5	4.0 ± 1.3	1.3 ± 0.1	1.9 ± 0.4
Lactate	48-50	4.0 ± 1.1	4.8 ± 1.4	1.9 ± 0.4	2.8 ± 0.5†

NOTE. Explants of human adipose tissue (100 mg/mL) or adipocytes (100 mg/mL) were incubated in duplicate for 2 hours in the presence of 20 μmol/L arachidonic acid. In the studies where the 2-hour incubation period was preceded by a 48-hour incubation, fresh medium containing arachidonic acid was added at 48 hours. The explants of adipose tissue were preincubated for 30 minutes in medium without arachidonic acid prior to the start of the incubations. The values are the means ± SEM of 6 experimental replications and are expressed in pmol/g wet weight of tissue or packed cells for cyclic AMP or in μmol/g for lactate formation and lipolysis (glycerol release). Each replication involved fat from a different donor.

Statistically significant effects of the 48-hour preincubation based on paired comparison are indicated: *$P < .05$, †$P < .025$.

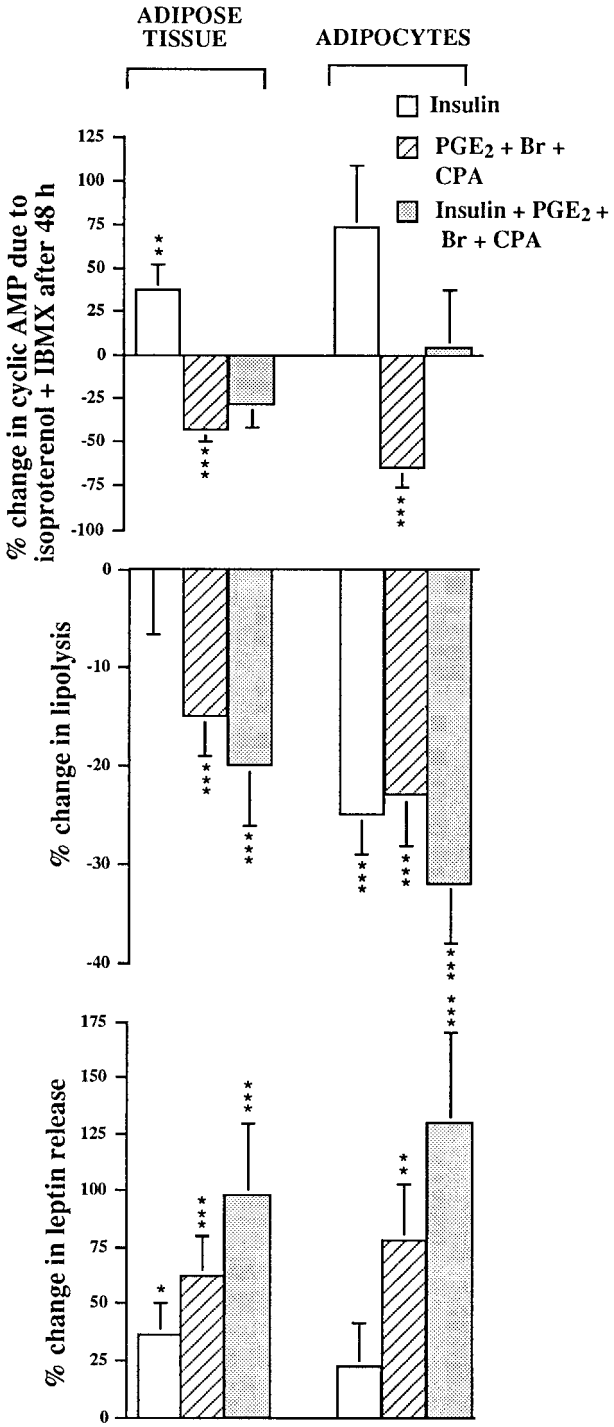

Fig 3. Comparison of insulin effects on lipolysis, leptin release and cyclic AMP accumulation with those of PGE_2, brimonidine, and N^{6}-cyclohexyl adenosine. Adipocytes (l00 mg/mL) or explants of adipose tissue (l00 mg/mL) were incubated for 48 hours in the presence of 10 nmol/L dexamethasone. One-half milliliter of medium was removed after 48 hours for analysis of leptin and glycerol. ISO at 500 nmol/L and IBMX at 50 μmol/L were then added to the cells and remaining medium for a 20-minute incubation to examine cyclic AMP accumulation. The basal values for lipolysis, leptin, and cyclic AMP are shown in Fig 4. The values are the percent change ± SE in 12 paired experimental replications and the percent changes due to l0 nmol/L insulin, the combination of 100 nmol/L each of PGE_2, brimonidine (Br), and CPA, or insulin plus the Gi-coupled receptor agonists. Statistically significant differences are indicated: $*P < .05$, $**P < .025$, $***P < .01$.

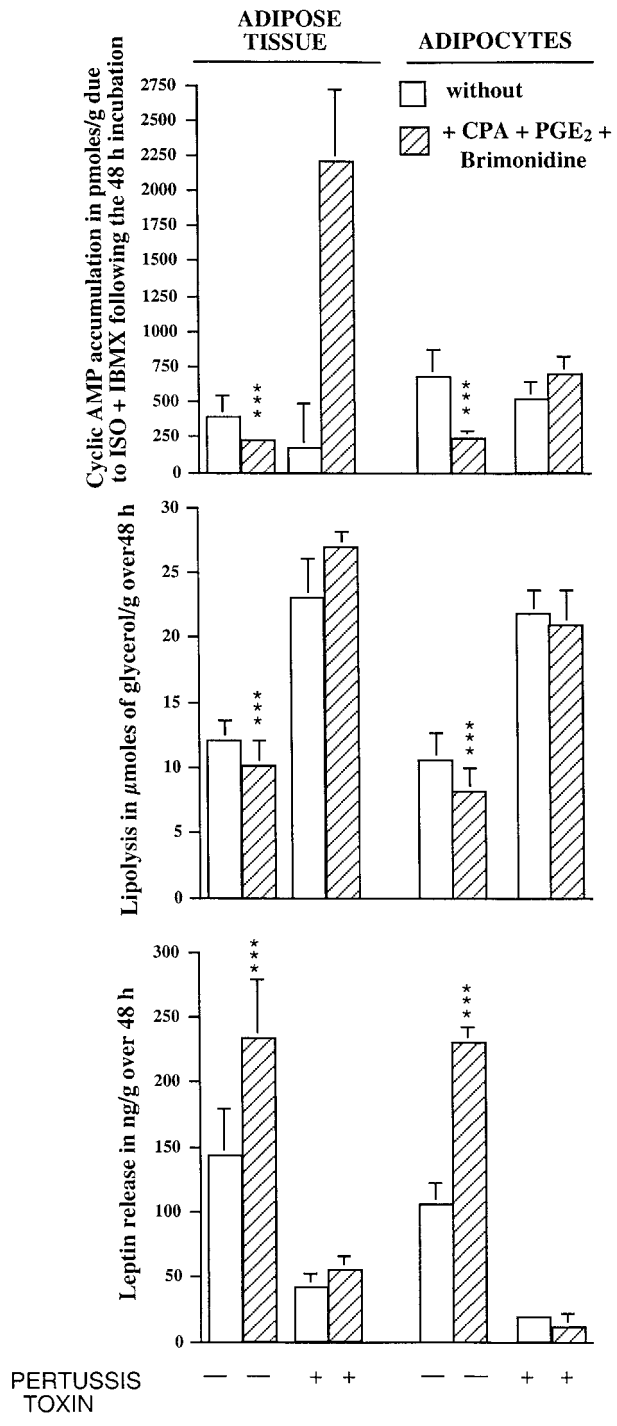

Fig 4. Effect of pertussis toxin on the response of adipocytes and adipose tissue to G_i-coupled receptor agonists. Adipocytes (100 mg/mL) or explants of adipose tissue (100 mg/mL) were incubated for 48 hours in the presence of 10 nmol/L dexamethasone. One-half milliliter of medium was removed for analysis of leptin and glycerol. ISO at 500 nmol/L and IBMX at 50 μmoL/L were then added to the cells and remaining medium for a 20-minute incubation to examine cyclic AMP accumulation. Pertussis toxin (100 ng/mL) was added at the start of the 48-hour incubation, as were PGE_2 brimonidine, and CPA (all at 100 nmol/L), which were added in combination. Values are the means ± SEM of 12 paired replications and significant effects of the combination of PGE_2, brimonidine, and CPA are indicated as follows based on percent changes. $*P < .05$, $**P < .025$, $***P < .01$.

greater in tissue after 48 hours incubation with pertussis toxin, whereas this increase was not seen in adipocytes (Fig 4).

In both adipocytes and adipose tissue, the effects of insulin on leptin release, cyclic AMP accumulation, and lipolysis were enhanced by pertussis toxin (Fig 5). This was striking with regard to cyclic AMP accumulation by adipocytes, where in the presence of insulin the cyclic AMP response to pertussis toxin was more similar to that seen in adipose tissue in the absence of insulin (Fig 4). These data suggest that the enhancement of a subsequent cyclic AMP response by pertussis toxin in adipocytes is dependent on either insulin carried over in adipose tissue explants and/or the release of insulin-like factors by the other cells present in the adipose tissue explants.

DISCUSSION

The role of the heterotrimeric G proteins in insulin action appears to be minimal,[16,17] even though some groups have claimed that heterotrimeric G_i proteins are involved in insulin action on adipocytes.[18-20] Pertussis toxin inactivates the alpha subunits of both G_i and G_o heterotrimers in cells and this results in a marked stimulation of lipolysis in rat[21-23] and human adipocytes.[15] The ability of antilipolytic agents that activate receptors linked to G_i is markedly impaired in adipocytes after exposure to pertussis toxin while the response to insulin is unaffected.[21-24] The ability of insulin to stimulate glucose metabolism by adipocytes is also unaffected by pertussis toxin.[16] Our results indicate that the ability of insulin to enhance leptin release over a 48-hour incubation of adipocytes is actually enhanced in the presence of pertussis toxin (Fig. 5). Similar effects were seen with regard to insulin effects on cyclic AMP accumulation and lipolysis (Fig 5), whereas the effects of the G_i-coupled receptor agonists on leptin, lipolysis, and cyclic AMP accumulation were abolished in the presence of pertussis toxin (Fig 4).

We used the combination of PGE_2, brimonidine, and CPA to maximally activate their respective G_i-coupled receptors. CPA is a potent stimulator of leptin secretion in rats[24] and of leptin release by explants of human adipose tissue in primary culture.[25] The receptor activated by adenosine and its analogs in adipose tissue appears to be the A_1 receptor that is linked to G_i.[26] PGE_2 activates receptors of the EP3 type in adipocytes that are linked to inhibition of adenylate cyclase and lipolysis.[27] PGE_2 stimulated leptin release by explants of mouse,[28] rat,[29] and human[24] adipose tissue in primary culture. Brimonidine, an

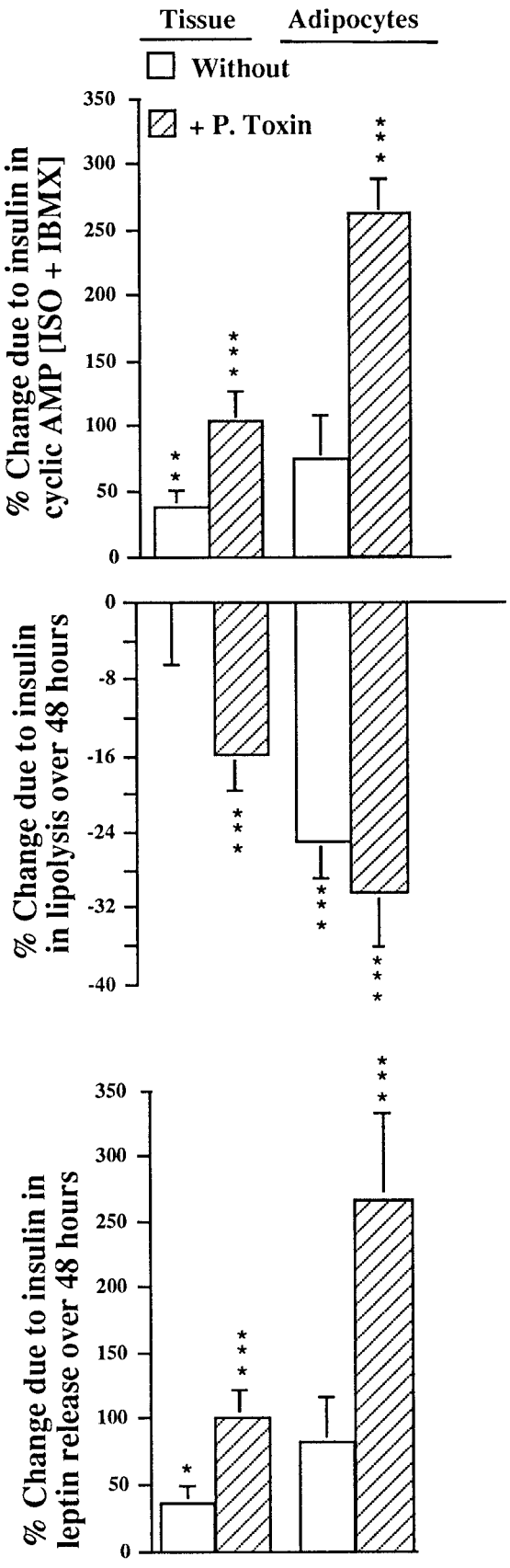

Fig 5. Effect of insulin on lipolysis, leptin release, and cyclic AMP accumulation due to pertussis toxin. Adipocytes (l00 mg/mL) or explants of adipose tissue (l00 mg/mL) were incubated for 48 hours in the presence of 10 nmol/L dexamethasone either without, with 10 nmol/L insulin or with 100 ng/mL of pertussis toxin. One-half milliliter of medium was removed after 48 hours for analysis of leptin and glycerol. ISO at 500 nmol/L and IBMX at 50 μmol/L were then added to the cells and remaining medium for a 20-minute incubation to examine cyclic AMP. The basal values for lipolysis, leptin release, and cyclic AMP are shown in Fig 4. Values are shown as the percent changes due to insulin ± SEM in 12 paired experimental replications in the absence or presence of 100 ng/mL of pertussis toxin. Significant effects are indicated as follows: *$P < .05$, **$P < .025$, ***$P < .01$.

α_2-adrenergic receptor agonist, is a potent inhibitor of lipolysis and cyclic AMP accumulation in human adipocytes.[30,31]

The combination of insulin plus the three G_i-coupled receptor agonists in the presence of dexamethasone consistently results in a release of leptin that is twice as large as seen with only dexamethasone in adipocytes over a 48-hour incubation (Fig 3). However, the increase in leptin release due to insulin did not require the activation of G_i, since the largest percentage increase due to insulin was seen in the presence of pertussis toxin, where it markedly reduced the inhibitory effects of pertussis toxin on insulin release (Fig 5).

We conclude that adipocytes isolated by collagenase digestion of human adipose tissue are suitable for studies on leptin release over a 48-hour incubation in suspension culture. These adipocytes are insulin-responsive with respect to leptin release, lipolysis, and cyclic AMP accumulation despite the fact that they were obtained from massively obese humans (average BMI of 49). It remains to be demonstrated that the results are applicable to subcutaneous adipose tissue from non-obese individuals. It is accepted that the release of adipocyte-derived hormones such as leptin is altered in obesity and this may contribute to the cardiovascular-renal dysfunction seen in massively obese individuals.[32]

The studies using adipocytes incubated in primary culture for 48 hours demonstrate that factors released by the non-fat cells in and around the adipocytes are not required to sustain leptin release. The amount of leptin released by adipocytes was quite variable between subjects but correlated with the release of leptin by explants of tissue from the same individual (Fig 1). The primary factors needed to sustain leptin release over the second 24 hours of a 48-hour incubation appear to be the presence of glucocorticoids, insulin, and antilipolytic agents that act through G_i-coupled receptors, as well as the absence of lipolytic agents. The one major difference between explants of adipose tissue and adipocytes was the inability of glucocorticoids in the absence of insulin to enhance leptin release. These data suggest that the physiologic regulation of leptin release is multihormonal and slow in onset, and is a complex process involving hormones as well as factors that remain to be isolated.

REFERENCES

1. Fain JN, Cowan GSW, Buffington C, et al: Synergism between insulin and low concentrations of isoproterenol in the stimulation of leptin release by cultured human adipose tissue. Metabolism 49:804-809, 2000

2. Fain JN, Cowan GSW, Buffington C, et al: Regulation of leptin release by troglitazone in human adipose tissue. Metabolism 49:1485-1490, 2000

3. Fain JN, Leffler CW, Cowan GSW, et al: Stimulation of leptin release by arachidonic acid and prostaglandin E_2 in adipose tissue from obese humans. Metabolism 50:921-928, 2001

4. Ricci MR, Fried SK: Isoproterenol decreases leptin expression in adipose tissue of obese humans. Obes Res 7:233-240, 1999

5. Scriba D, Aprath-Husmann I, Blum WF, et al: Catecholamines suppress leptin release from in vitro differentiated subcutaneous human adipocytes in primary culture via beta1- and beta2-adrenergic receptors. Eur J Endocrinol 143:439-445, 2000

6. Casabiell X, Pineiro V, Peino R, et al: Gender differences in both spontaneous and stimulated leptin secretion by human omental adipose tissue in vitro: Dexamethasone and estradiol stimulate leptin release in women, but not in men. J Clin Endocrinol Metab 83:2149-2155, 1998

7. Halleux CV, Servais I, Reul BA, et al: Multihormonal control of ob gene expression and leptin secretion from cultured human visceral adipose tissue: Increased responsiveness to glucocorticoids in obesity. J Clin Endocrinol Metab 83:902-910, 1998

8. Russell CD, Ricci MR, Brolin RE, et al: Regulation of the leptin content of obese human adipose tissue. Am J Physiol Endocrinol Metab 280:E399-E404, 2001

9. Fawcett RL, Waechter AS, Williams LB, et al: Tumor necrosis factor-alpha inhibits leptin production in subcutaneous and omental adipocytes from morbidly obese humans. J Clin Endocrinol Metab 85:530-535, 2000

10. Considine RV, Nyce MR, Kolaczynski JW, et al: Dexamethasone stimulates leptin release from human adipocytes: Unexpected inhibition by insulin. J Cell Biochem 65:254-258, 1997

11. Williams LB, Fawcett RL, Waechter, AS, et al: Leptin production in adipocytes from morbidly obese subjects: stimulation by dexamethasone, inhibition with troglitazone, and influence of gender. J Clin Endocrinol Metab 85:2678-2684, 2000

12. Gottschling-Zeller H, Birgel M, Scriba D, et al: Depot-specific release of leptin from subcutaneous and omental adipocytes in suspension culture: Effect of tumor necrosis factor-alpha and transforming growth factor-beta1. Eur J Endocrinol 14:436-442, 1999

13. Fain JN, Kanu A, Bahouth SW, et al: Comparison of PGE_2, prostacyclin and leptin release by human adipocytes versus explants of adipose tissue in primary culture. Prostaglandins Leukot Essent Fatty acids (in press)

14. Boobis LH, Maughan RL: A simple one-step enzymatic fluorometric method for the determination of glycerol in 20 μl of plasma. Clin Chim Acta 132:173-179, 1983

15. Kather H, Bieger W, Michel G, et al: Human fat cell lipolysis is primarily regulated by inhibitory modulators acting through distinct mechanisms. J Clin Invest 76:1559-1565, 1985

16. Dalle S, Ricketts W, Imamura T, et al: Insulin and insulin-like growth factor I receptors utilize different G protein signaling components. J Biol Chem 275:15688-15695, 2001

17. Joost HG, Schmitz-Salue C, Hinsch KD, et al: Phosphorylation of G-protein alpha-subunits in intact adipose cells: Evidence against a mediating role in insulin-dependent metabolic effects. Eur J Pharmacol 172:461-469, 1989

18. Goren HJ, Northup JK, Hollenberg MD: Action of insulin modulated by pertussis toxin in rat adipocytes. Can J Physiol Pharmacol 63:1017-1022, 1985

19. Green a, Walters DJ, Belt SE: Insulin resistance in adipocytes after downregulation of G_i subtypes. Am J Physiol 273:E254-E261, 1997

20. Song X, Zheng X, Malbon CC, et al: $G\alpha_{i2}$ enhances in vivo activation of and insulin signaling to GLUT4. J Biol Chem 276:34651-34658, 2001

21. Mills, I, Fain JN: Pertussis toxin reversal of the antilipolytic action of insulin in rat adipocytes in the presence of forskolin does not involve cyclic AMP. Biochem Biophys Res Commun 130:1059-1065, 1985

22. Kather H, Aktories, K, Schulz G, et al: Islet-activating protein discriminates the antilipolytic mechanism of insulin from that of other antilipolytic compounds. FEBS Lett 161:149-152, 1983

23. Wesslau C, Eriksson JW, Smith U: Cellular cyclic AMP levels modulate insulin sensitivity and responsiveness—Evidence against a significant role of Gi in insulin signal transduction. Biochem Biophys Res Commun 196:287-293, 1993

24. Rice AM, Fain JN, Rivkees SA: A_1 adenosine receptor activa-

tion increases adipocyte leptin secretion. Endocrinology 141:1442-1445, 2000

25. Fain JN, Bahouth SW: Regulation of leptin release by mammalian adipose tissue. Biochem Biophys Res Commun 274:571-575, 2000

26. Linden J: Structure and function of A_1 adenosine receptors. FASEB J 5:2668-2678, 1991

27. Strong P, Coleman RA, Humphrey PPA: Prostanoid-induced inhibition of lipolysis in rat isolated adipocytes: Probable involvement of EP3 receptors. Prostaglandins 43:559-566, 1992

28. Fain JN, Leffler CW, Bahouth SW: Eicosanoids as endogenous regulators of leptin release and lipolysis by mouse adipose tissue in primary culture. J Lipid Res 41:1689-1694, 2000

29. Fain JN, Leffler CW, Bahouth SW, et al: Regulation of leptin release and lipolysis by PGE_2 in rat adipose tissue. Prostaglandins Other Lipid Mediat 62:343-350, 2000

30. Vikman H-L, Savola J-M, Raasmaja A, et al: α_2A-adrenergic regulation of cyclic AMP accumulation and lipolysis in human omental and subcutaneous adipocytes. Int J Obes 20:185-189, 1996

31. Lafontan M, Berlan M: Fat cell α_2-adrenoceptors: The regulation of fat cell function and lipolysis. Endocr Rev 16:716-738, 1995

32. Engeli S, Sharma AM: Role of adipose tissue for cardiovascular-renal regulation in health and disease. Horm Metab Res 32:485-499, 2000