

Mechanisms of leptin secretion from white adipocytes

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Cammisotto, Philippe G., and Ludwik J. Bukowiecki. Mechanisms of leptin secretion from white adipocytes. *Am J Physiol Cell Physiol* 283: C244–C250, 2002. First published March 6, 2002; 10.1152/ajpcell.00033.2002.—The mechanisms regulating leptin secretion were investigated in isolated rat white adipocytes. Insulin (1–100 nM) linearly stimulated leptin secretion from incubated adipocytes for at least 2 h. The adrenergic agonists norepinephrine, isoproterenol (two nonselective β -agonists), or CL-316243 (potent β_3) all inhibited insulin (10 nM)-stimulated leptin release. The inhibitory effects of norepinephrine and isoproterenol could be reversed not only by the nonselective antagonist propranolol but also by the selective antagonists ICI-89406 (β_1) or ICI-118551 (β_2), the β_2 -antagonist being less effective than the β_1 . Insulin-stimulated leptin secretion could also be inhibited by a series of agents increasing intracellular cAMP levels, such as lipolytic hormones (ACTH and thyrotropin-stimulating hormone), various nonhydrolyzable cAMP analogs, pertussis toxin, forskolin, methylxanthines (caffeine, theophylline, IBMX), and specific inhibitors of phosphodiesterase III (imazodan, milrinone, and amrinone). Significantly, antilipolytic agents other than insulin (adenosine, nicotinic acid, acipimox, and orthovanadate) did not mimic the acute stimulatory effects of insulin on leptin secretion under these conditions. We conclude that norepinephrine specifically inhibits insulin-stimulated leptin secretion not only via the low-affinity β_3 -adrenoceptors but also via the high-affinity β_1/β_2 -adrenoceptors. Moreover, it is suggested that 1) activation of phosphodiesterase III by insulin represents an important metabolic step in stimulation of leptin secretion, and 2) lipolytic hormones competitively counterregulate the stimulatory effects of insulin by activating the adenylate cyclase system.

lipolytic hormones; phosphodiesterases; β_1 -, β_2 -, and β_3 -adrenoceptors

LEPTIN IS A HORMONE encoded by the *ob* gene and primarily secreted by white adipocytes (for reviews, see Refs. 1, 13, 14, and 38). It stimulates energy expenditure and inhibits food intake by acting via hypothalamic leptin receptors. The expression of leptin in adipocytes and its plasma concentration are both positively correlated with total adiposity. Therefore, it is generally believed that leptin represents a lipostatic factor contributing to the regulation of body weight via a negative feedback loop (10). In addition to total adiposity, plasma leptin concentrations can be acutely modulated by a variety

of physiological conditions (starvation-refeeding and cold exposure) and hormonal factors (insulin, catecholamines, glucocorticoids, thyroid hormones, gonadal steroids, etc.) (26). The observations that starvation decreases both plasma insulin and leptin levels and that obesity is strongly associated with hyperinsulinemia and hyperleptinemia have led many researchers to investigate the effects of insulin on leptin secretion. Although several studies found that insulin stimulates leptin expression and secretion in adipocytes in vitro (3, 6, 11, 15, 30), others found little or no effect of insulin (20, 24, 32). The in vivo effects of insulin on leptinemia are also contradictory; some groups reported that insulin increases plasma leptin levels in rodents or humans (16, 21), whereas others found that insulin does not appear to acutely regulate leptin expression or secretion (8, 36).

In addition to starvation, cold exposure represents another physiological condition known to significantly affect plasma leptin levels, at least in laboratory animals (26). Cold exposure activates the sympathetic nervous system, increases the levels of circulating norepinephrine, and decreases plasma insulin and leptin concentrations (4, 12, 28, 35) as well as leptin gene expression in adipose tissue (34). The effects of cold exposure have been mimicked in vivo by administration of the physiological neurohormone norepinephrine or by treatment with β -agonists in mice (23, 34) and humans (9, 25, 27, 33). However, the nature of the β -adrenoceptor subtypes (β_1 , β_2 , and/or β_3) mediating the effects of norepinephrine on leptin secretion is still not well understood. Although it has been reported that β_3 -adrenergic agonists inhibit leptin secretion in vivo and in vitro, at least in rodents (11), few studies have been performed with selective β -antagonists. One study has claimed that the high affinity β_1/β_2 -adrenoceptors play essentially no role in mediating norepinephrine effects on leptin secretion, because it was unaffected by β_1/β_2 -antagonists (11). However, isoproterenol (a nonselective β -adrenergic agonist) acutely decreases the levels of circulating leptin in humans and decreases leptin expression or secretion in cultured adipocytes (9, 27, 30, 33). Because the β_3 -adrenergic receptor is a low-affinity receptor for β -agonists such as norepinephrine or isoproterenol, these observations suggest that norepinephrine might act, at least

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partially, via the high-affinity β_1/β_2 -adrenoceptors (17). Another observation that prompted the present study is that insulin and catecholamines, the principal hormones acutely regulating energy metabolism, exert antagonistic effects on lipolysis, leptin expression, and leptin secretion from adipose tissues, suggesting the presence of metabolic interactions between insulin activation of phosphodiesterases and the stimulation of the adenylate cyclase system by catecholamines (17, 26). Thus the sympathetic nervous system (catecholamines) and insulin might play a major role in controlling leptin metabolism *in vivo*.

On the basis of these observations, we decided to investigate the nature of β -adrenergic pathways regulating insulin stimulation of leptin secretion in adipocytes isolated from rat epididymal adipose tissue using selective β -adrenergic agonists/antagonists, lipolytic hormones, phosphodiesterase inhibitors, hydrolyzable and nonhydrolyzable cAMP analogs, and other drugs known to affect lipolysis or the adenylate cyclase complex.

MATERIALS AND METHODS

Chemicals. Fatty acid-free bovine serum albumin, norepinephrine, isoproterenol, dobutamine, procaterol, propranolol, cAMP analogs 8-bromo-cAMP (8-BrcAMP), N^6 -monobutyl cAMP (MbcAMP), and N^6 -dibutyl cAMP (DbcAMP), IBMX, forskolin, pertussis toxin, adrenocorticotrophic hormone, thyrotropin-stimulating hormone (TSH), collagenase (type II, lot 107H8649), caffeine, theophylline, adenosine, N_6 -(2-phenylisopropyl)adenosine (PIA), and adenosine deaminase were all obtained from Sigma Chemical (St. Louis, MO). Insulin (Humulin R) was purchased from Eli Lilly (Toronto, Canada). ICI-89406 was a generous gift from ICI pharmaceuticals. ICI-118551 was purchased from RBI Sigma-Aldrich (Oakville, Canada). CL-316243 was obtained from American Cyanamid. Imazodan was purchased from Warner-Lambert. Amrinone and milrinone were obtained from Sterling-Winthrop Research Institute.

Animals. Male Wistar rats were obtained from Charles River and were housed in individual cages at 24°C with a 12:12-h light-dark cycle. The rats received standard Purina chow and water *ad libitum*. The mean body mass of the rats used in the present experiments was 290 ± 15 g.

Adipocyte isolation. Adipocytes were isolated from epididymal fat pads by a slight modification of Rodbell's method (29). Briefly, rats were killed by decapitation, and their epididymal fat pads were removed and placed in Krebs-Ringer bicarbonate (KRB) buffer of the following composition (in mM): 120 NaCl, 4.75 KCl, 2.5 CaCl_2 , 1.2 KH_2PO_4 , 1.2 MgSO_4 , 25 NaHCO_3 , 5.5 glucose, 20 HEPES, and 1% fatty acid-free bovine serum albumin, pH 7.4 (KRB 1%). The minced tissue was incubated in KRB 1% containing 0.5 mg/ml collagenase at 37°C for 15–20 min with a shaking frequency of 150 cycles/min. At the end of incubation, the cells were filtered through a 500- μm nylon filter (Nitex) and diluted with 5 ml of KRB 1%. The floating cells were washed four times with KRB 1% and preincubated at 37°C for 15 min in KRB 1% (shaking frequency of 40 cycles/min) and washed twice with warm (37°C) KRB containing 4% fatty acid-free bovine serum albumin (KRB 4%). Finally, the cells were incubated under the same conditions for 2 h (unless otherwise specified) in the presence of hormones or drugs at a concentration of $3\text{--}5 \times 10^5$ cells/ml KRB 4%. The adipocytes

were then allowed to float, and the infranatants were frozen at -20°C for leptin and glycerol measurements.

Leptin and glycerol assays. Leptin concentrations were determined by radioimmunoassay using a kit available from Linco Research (St Charles, MO). Glycerol was measured using an enzymatic method (37).

Statistics. The data were analyzed using analysis of variance. Values represent the means \pm SE of a number of individual experiments performed on separate occasions (n), as indicated in the text. The responsiveness and sensitivity of adipocytes for the stimulation of leptin secretion or lipolysis (V_{max} and EC_{50}) and the half-effective concentration for inhibition of these parameters (IC_{50}) were determined by computer analysis (SigmaPlot program) of concentration-response curves.

RESULTS

Insulin stimulation of leptin secretion. Concentration-response experiments revealed that insulin approximately doubled (from 4.1 ± 0.2 to 7.9 ± 0.3 ng leptin $\cdot 10^{-6}$ cells $\cdot 2$ h $^{-1}$, $P < 0.01$) the basal rates of leptin release from white adipocytes isolated and incubated as described in MATERIALS AND METHODS (Fig. 1). Insulin acted with an EC_{50} value of 0.7 nM, which is in the physiological range of plasma insulin concentrations in the rat. Insulin stimulated leptin secretion in a linear manner for at least 4 h (Fig. 1, *inset*). Therefore, all subsequent incubations were carried out for a 2-h period.

Inhibition of the stimulatory effects of insulin by β -adrenergic agonists. Because insulin is a potent antilipolytic hormone, we tested whether lipolytic agents such as β -adrenergic agonists would reverse the stimulatory effects of insulin on leptin release. Adipocytes

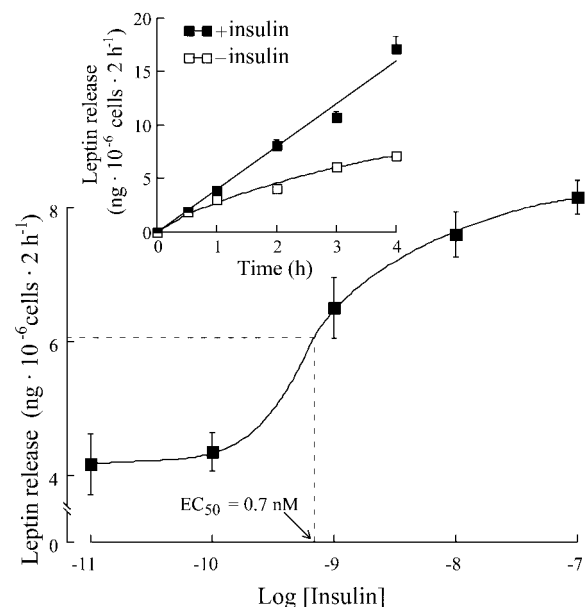


Fig. 1. Concentration-response curve of the effects of insulin on leptin secretion. Freshly isolated adipocytes were incubated in the presence of various concentrations of insulin for 2 h at 37°C as described in MATERIALS AND METHODS ($n = 6$). *Inset*: time course of leptin release from adipocytes incubated in the presence (■) and absence (□) of insulin (100 nM) ($n = 4$).

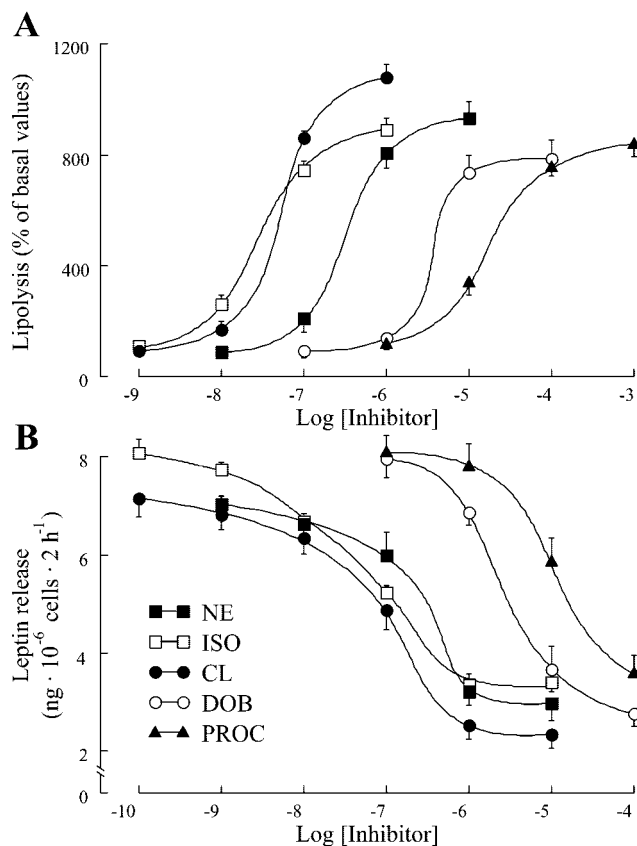


Fig. 2. Effects of norepinephrine (NE), isoproterenol (Iso), CL-316243 (CL), dobutamine (Dob), and procaterol (Proc) on lipolysis (A) and leptin release (B). Adipocytes were incubated in the presence of a near-maximal concentration of insulin (10 nM) (see Fig. 1) and various concentrations of β -adrenergic agonists. Incubation conditions were the same as in Fig. 1. At the end of the incubations, extracellular samples were removed for glycerol and leptin determinations ($n = 8$).

were incubated in the presence of insulin (added at a concentration of 10 nM that induces a near-maximal stimulation of leptin release) (see Fig. 1) and increasing concentrations of β -adrenergic agonists. Extracellular glycerol (an index of lipolysis) and leptin concentrations were measured in parallel in the same cellular preparations (Fig. 2, A and B). Norepinephrine, isoproterenol (two nonselective β -agonists), dobutamine, procaterol, and CL-316243 (selective β_1 -, β_2 -, and β_3 -agonists, respectively) all inhibited insulin-stimulated leptin secretion with an order of potency (IC_{50} values: CL-316243 = 10 nM > isoproterenol = 56 nM > norepinephrine = 219 nM > dobutamine = 3 μ M > procaterol = 14 μ M) (Fig. 2) that was similar to that by which these agents stimulated lipolysis (EC_{50} values: CL-316243 = 33 nM > isoproterenol = 47 nM > norepinephrine = 350 nM > dobutamine = 3 μ M, procaterol = 19 μ M) (Fig. 2). This provided a first indication that leptin secretion might be metabolically associated with the stimulation of lipolysis via cAMP-dependent protein kinases.

Reversal of the inhibitory effects of adrenergic agonists on insulin-stimulated leptin secretion by propranolol.

To determine whether the inhibitory effects of norepinephrine, isoproterenol, and CL-316243 were reversible, we first tested the effects of propranolol, a potent β_1/β_2 - and weak β_3 -antagonist. Adipocytes were incubated in the presence of insulin (10 nM), norepinephrine (1 μ M), isoproterenol (0.1 μ M) or CL-316243 (0.1 μ M), and various concentrations of propranolol. The β -agonists were added at concentrations that nearly maximally inhibited insulin-stimulated leptin secretion (see Fig. 2). It can be seen in Fig. 3 that propranolol reversed the effects of norepinephrine and isoproterenol when added at concentrations that were 1–100 times greater than the agonist concentrations. However, propranolol only partially reversed the action of CL-316243, as expected for a weak β_3 -antagonist.

Reversal of the inhibitory effects of norepinephrine on insulin-stimulated leptin secretion by selective β_1 - and β_2 -antagonists. To further assess the contribution of β_1 -, β_2 -, and/or β_3 -adrenoceptors to the inhibitory effects of norepinephrine, adipocytes were incubated in the presence of insulin (10 nM), norepinephrine, and various concentrations of the selective β_1 -antagonist ICI-89406 or β_2 -antagonist ICI-118551 (Fig. 4). Norepinephrine was added at a concentration that nearly

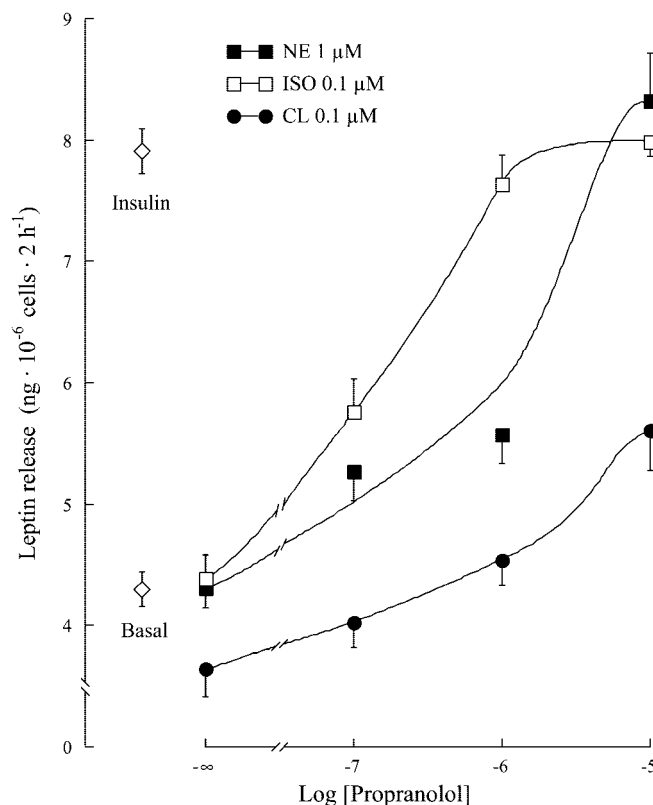


Fig. 3. Reversal of the inhibitory effects of adrenergic agonists (NE, Iso, and CL) on insulin-stimulated leptin secretion by propranolol. Adipocytes were incubated in the presence of insulin (10 nM), NE, Iso, CL, and various concentrations of propranolol for 2 h. For the purpose of comparison, the 2 lozenges at left indicate basal and insulin-stimulated leptin levels measured in the absence of NE, Iso, CL, and propranolol. The incubation conditions were the same as in Fig. 1 ($n = 8$).

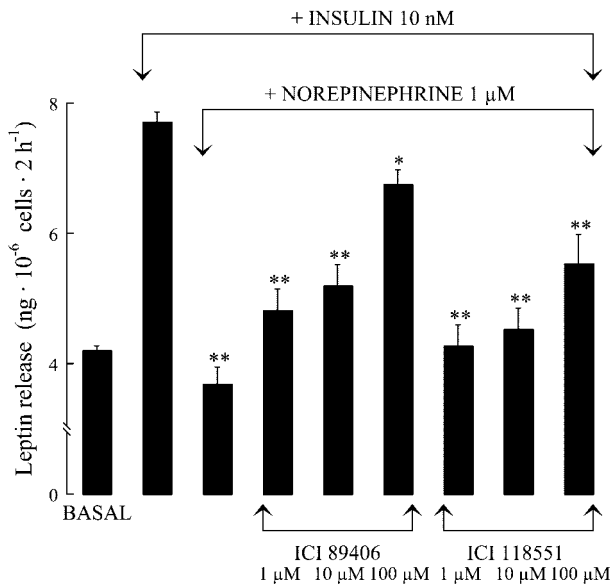


Fig. 4. Reversal by selective β_1 - and β_2 -antagonists of the inhibitory effects of NE on insulin-stimulated leptin secretion. Adipocytes were incubated in the presence of fixed concentrations of insulin (10 nM) and NE (1 μ M) as indicated. The effects of the selective β_1 -antagonist ICI-89406 and β_2 -antagonist ICI-118551 were studied at concentrations that were 1–100 times greater than (1–100 μ M) that of NE (1 μ M). Incubation conditions were the same as in Fig. 1 ($n = 6$). Bars and vertical lines indicate means \pm SE. Agonist and antagonist effects were compared with those induced by insulin alone. * $P < 0.05$, ** $P < 0.01$.

totally inhibits insulin (10 nM)-stimulated leptin secretion (Fig. 2), whereas ICI-89406 and ICI-118551 were studied at concentrations that were 1–100 times greater (1–100 μ M) than that of norepinephrine (1 μ M). Both β -antagonists partially reversed the inhibitory effects of norepinephrine on insulin-stimulated leptin release. However, the β_1 -antagonist ICI-89406 was more effective than the β_2 -antagonist ICI-118551. These results indicate that β_1 - and β_2 -adrenoceptors mediate norepinephrine action, at least in part. Selective β_3 -antagonists were not tested because they are not yet available, at least to our knowledge. However, two observations strongly indicate that β_3 -adrenoceptors also mediate norepinephrine effects: 1) the reversal by β_1 - and β_2 -antagonists was only partial (Fig. 4), and 2) the selective β_3 -agonist CL-316243 effectively inhibited insulin-stimulated leptin secretion (Fig. 2).

Reversal of the inhibitory effects of isoproterenol on insulin-stimulated leptin secretion by selective β_1/β_2 -antagonists. Experiments similar to those described in Fig. 4 were carried out with isoproterenol (Fig. 5). This agent is a potent inhibitor of leptin secretion in humans and acts at much lower concentrations than norepinephrine (Fig. 2). Comparable results to those obtained with norepinephrine (1 μ M) (Fig. 4) were obtained with isoproterenol (0.1 μ M) but at lower antagonist concentrations (0.1, 1, or 10 μ M). The β_1/β_2 -antagonists completely reversed the effects of isoproterenol, as expected from a potent β_1/β_2 -agonist.

Inhibition of the stimulatory effects of insulin by lipolytic hormones, adenylate cyclase activators, meth-

ylxanthines, cAMP analogs, and phosphodiesterase inhibitors. The goal of the next experiments was to test whether there is a cause-effect relationship between the stimulation of lipolysis via the adenylate cyclase-phosphodiesterase system and inhibition of extracellular leptin release. If such a relationship exists, then one should expect that all agents stimulating lipolysis (increasing intracellular cAMP levels) would inhibit insulin stimulation of leptin secretion. To test this hypothesis, adipocytes were incubated in the presence of insulin (10 nM) and a variety of agents that increase cAMP levels via different mechanisms (Figs. 6 and 7). In addition to catecholamines and adrenergic agents (Figs. 2), we tested a dozen other agents: 1) lipolytic hormones such as ACTH and TSH, which activate the adenylate cyclase-G protein complex by acting via their specific membrane receptors, 2) the adenylate cyclase activator forskolin, 3) pertussis toxin, which is known to inactivate G_i protein and promote cAMP increment in fat cells, 4) the phosphodiesterase-hydrolyzable analog 8-Br-cAMP as well as the nonhydrolyzable cAMP analogs MBcAMP and DBcAMP, 5) the methylxanthines caffeine, theophyllin, and IBMX, which inhibit nonspecifically phosphodiesterases, and 6) specific inhibitors of phosphodiesterase III (imazodan, milrinone, and amrinone). Each of these agents was tested at a concentration that maximally stimulates lipolysis and that was determined in preliminary concentration-response experiments. Comparison of Figs. 6A and 7A (lipolysis) with Figs. 6B and 7B (leptin secretion) reveals that all agents that maximally stimulated lipol-

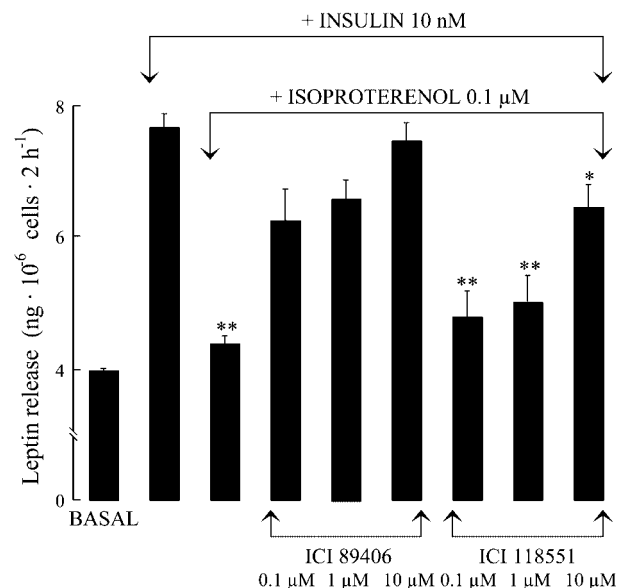


Fig. 5. Reversal by selective β_1 - and β_2 -antagonists of the inhibitory effects of isoproterenol on insulin-stimulated leptin secretion. Adipocytes were incubated in the presence of fixed concentrations of insulin (10 nM) and Iso (0.1 μ M) as indicated in the figure. The effects of the selective β_1 -antagonist ICI-89406 and β_2 -antagonist ICI-118551 were studied at concentrations that were 1–100 times greater than that of Iso (0.1 μ M). Incubation conditions were the same as in Fig. 1 ($n = 5$). Bars and vertical lines indicate means \pm SE. Agonist and antagonist effects were compared with those induced by insulin alone. * $P < 0.05$, ** $P < 0.01$.

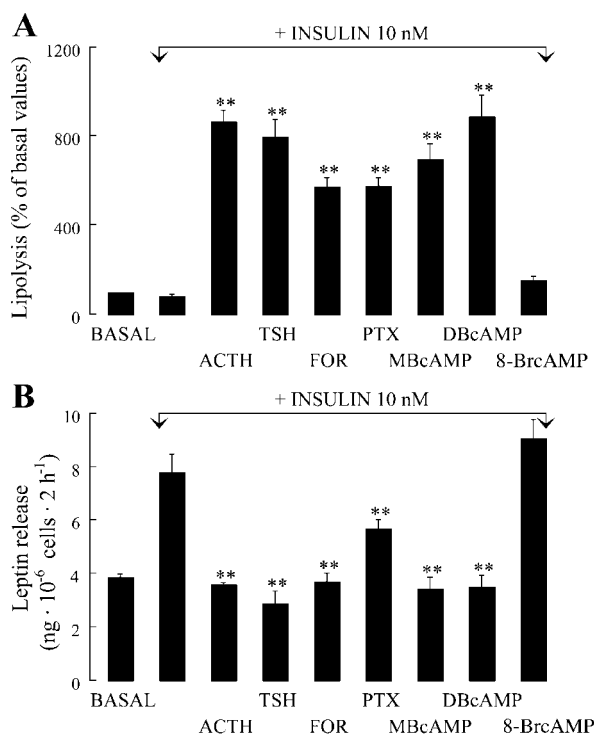


Fig. 6. Comparison of the effects of lipolytic hormones, adenylate cyclase activators, and cAMP analogs on lipolysis (A) and leptin release (B) in the presence of insulin. Adipocytes were incubated in the presence of insulin (10 nM) and fixed concentrations of ACTH (10 nM), thyrotropin-stimulating hormone (TSH; 0.2 μ M), forskolin (For; 10 μ M), pertussis toxin (PTX) (1 μ g/ml), *N*⁶-monobutyl cAMP (MBcAMP; 1 mM), and *N*⁶-dibutyl cAMP (DBcAMP; 1 mM) or 8-bromo cAMP (8-BrcAMP; 1 mM). Incubation conditions were the same as in Fig. 1. ($n = 5-6$). Bars and vertical lines indicate means \pm SE. Effects of the above agents were compared with basal lipolysis (A) or to insulin-stimulated values (B) * $P < 0.05$, ** $P < 0.01$.

ysis totally inhibited insulin-stimulated leptin secretion. Significantly, the hydrolyzable cAMP analog 8-BrcAMP did not stimulate lipolysis (in the presence of insulin) and did not inhibit leptin secretion. However, the two cAMP analogs that are resistant to phosphodiesterase-dependent hydrolysis, MBcAMP and DBcAMP, both stimulated lipolysis and inhibited leptin secretion. On the whole, these data show that insulin-stimulated leptin secretion can be totally inhibited by lipolytic agents known to increase intracellular cAMP concentration, independently of the mechanisms by which they act.

Effects of adenosine and other antilipolytic agents on insulin-stimulated leptin secretion. We also investigated whether antilipolytic agents other than insulin would mimic its effects on leptin secretion. We tested several insulin mimetics and/or antilipolytic agents acting via different mechanisms on adenylate cyclase: orthovanadate, adenosine, PIA (a nonmetabolizable analog of adenosine), nicotinic acid, and acipimox, a stable analog of nicotinic acid. Sodium orthovanadate is an insulin mimetic that stimulates glucose uptake in isolated adipocytes and inhibits lipolysis, presumably by the inhibiting protein phosphotyrosine phosphatases (18). Adenosine, PIA, nicotinic acid, and acipimox

are all antilipolytic G_i-coupled adenylate cyclase inhibitory agonists (5, 7, 19). Although all these agents completely inhibited norepinephrine-stimulated lipolysis under the present experimental conditions, none of them mimicked the stimulatory effects of insulin on leptin secretion (not shown), at least at short term (within 2 h of incubation). This suggests that an inhibition of lipolysis consequent to an intracellular decrease in cAMP levels does not represent a metabolic event that may, per se, trigger leptin secretion. However, it has been shown that the antilipolytic agent neuropeptide Y is able to stimulate leptin secretion after longer periods of incubation (≥ 22 h) (31).

Finally, we tested the effects of insulin and lipolytic (norepinephrine, isoproterenol, and CL-316243) and antilipolytic (orthovanadate, nicotinic acid, and acipimox) agents on leptin secretion in the presence of a mixture of adenosine deaminase (0.5 U/ml) and PIA (100 nM), i.e., under the conditions suggested by Londos et al. (19) to stabilize basal cAMP production. These incubation conditions did not significantly alter the concentration-response curves of insulin and other agents cited above on leptin secretion (not shown).

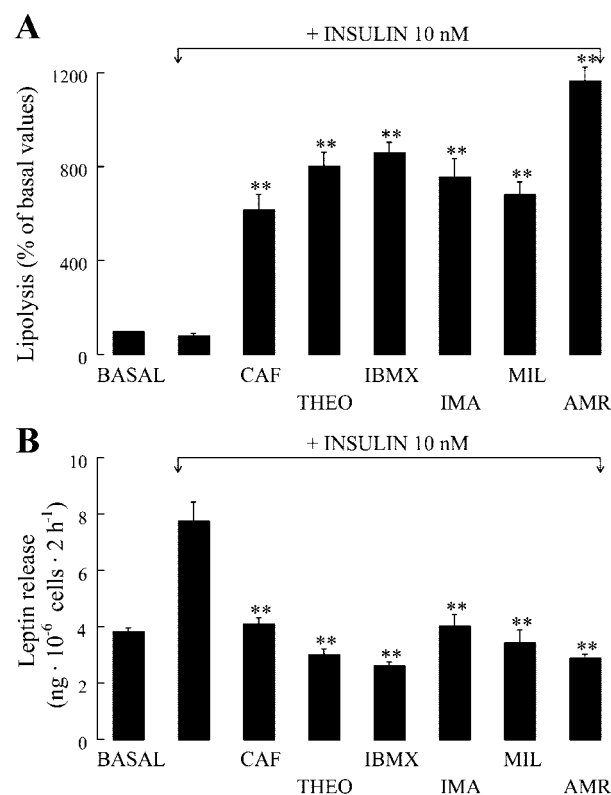


Fig. 7. Comparison of the effects of specific and nonspecific phosphodiesterase inhibitors on lipolysis (A) and leptin release (B) in the presence of insulin. Adipocytes were incubated in the presence of insulin (10 nM) and fixed concentrations of caffeine (Caf; 1 mM), theophylline (Theo; 1 mM), 3-isobutyl-1-methyl-xanthine (IBMX; 0.1 mM), imazodan (IMA; 0.1 mM), milrinone (Mil; 0.1 mM), or amrinone (AMR; 1 mM). Incubation conditions were the same as in Fig. 1 ($n = 5$). Bars and vertical lines indicate means \pm SE. Effects of the above agents were compared with basal lipolysis (A) or insulin-stimulated values (B). * $P < 0.05$, ** $P < 0.01$.

Such observations were expected because we observed a very good interexperimental reproducibility.

DISCUSSION

The role of β_1 - and β_2 -adrenoceptors in mediating norepinephrine action. The present study revealed that insulin-stimulated leptin secretion is mediated not only via the low-affinity β_3 -adrenoceptors but also via the high-affinity β_1/β_2 -adrenoceptors with β_2 -adrenoceptors playing a lesser role than β_1 -adrenoceptors. This conclusion is based on the following observations: 1) selective β_1 - and β_3 -agonists (dobutamine and CL-316143, respectively) and nonselective agonists (norepinephrine and isoproterenol) all completely inhibited insulin-stimulated leptin secretion (Fig. 2); 2) the selective β_2 -antagonist procaterol, in contrast, partly inhibited insulin-stimulated leptin secretion and only at high concentrations (0.1 mM); and 3) the selective β_1 -antagonist ICI-89406, likewise, reversed more effectively than the β_2 -antagonist ICI-118551 the inhibitory effects of norepinephrine or isoproterenol on insulin-stimulated leptin secretion (Figs. 4 and 5). Contrary to the present observations, it has been claimed that the norepinephrine effects were essentially mediated by β_3 -adrenoceptors because they were unaffected by selective β_1/β_2 -antagonists (11). However, these results are difficult to interpret because the concentrations of the antagonists were either too low or not specified, as in the case of the β_1/β_2 -blocker CGP-12177. Furthermore, CGP-12177 may also act as a β_3 -agonist, depending on its concentration (22). Another observation that supports a role for β_1 -adrenoceptors in regulating leptin secretion is the fact that propranolol inhibited the effects of norepinephrine and isoproterenol when added at slightly higher concentrations than the two agonists (Fig. 3). Direct binding studies have demonstrated that the affinity of propranolol, norepinephrine, and isoproterenol for the β_1 -adrenoceptors is about 10^4 – 10^5 greater (pK_d or pK_i values varying between 8 and 9) than the one for β_3 -adrenoceptors (pK_d or pK_i values varying between 4 and 5). Thus it is likely that low concentrations of propranolol reverse the effects of norepinephrine and isoproterenol by competing for the high-affinity β_1/β_2 binding sites. However, at higher concentrations, propranolol may also affect β_3 -adrenoceptors. These observations suggest that circulating catecholamines, levels of which rarely exceed 25 nM, would regulate leptin secretion mainly via the high-affinity β_1 -adrenoceptors, whereas the low-affinity β_3 -adrenoceptors would mainly be activated when norepinephrine concentrations in the synaptic cleft reached higher concentrations, such as after cold exposure or other intensive stress (2).

The role of the adenylate cyclase-phosphodiesterase system. In addition to β -adrenergic agonists, insulin-stimulated leptin secretion could also be inhibited by a wide variety of agents known to increase intracellular cAMP levels by stimulating its production at the cyclase level (ACTH, TSH, pertussis toxin, or forskolin), inhibiting its degradation by phosphodiesterases (caf-

feine, theophylline, IBMX, imazodan, milrinone, or amrinone), or mimicking its action (MbcAMP or DBcAMP). Without exception, all these agents stimulated lipolysis in the range of concentrations at which they inhibited insulin-stimulated leptin secretion. Although cAMP levels were not directly measured in the present experiments, these results strongly indicate that cAMP plays a fundamental role in regulating insulin stimulation of leptin secretion. However, the role of cAMP appears to be merely modulatory because, in the absence of insulin, all the above lipolytic agents either did not alter or slightly inhibited basal leptin secretion.

Perspectives

The present study has shown that catecholamines, β -agonists, lipolytic hormones, cAMP analogs, or other agents activating adenylate cyclase inhibit insulin-stimulated leptin secretion. Norepinephrine, the physiological effector of lipolysis, inhibits leptin secretion not only via the low-affinity β_3 -adrenoceptors, as previously shown (11), but also via the high-affinity β_1/β_2 -adrenoceptors. Significantly, the neurohormone modulates insulin-stimulated leptin secretion in a reversible manner, most probably by stimulating adenylate cyclase activity. Experiments with phosphodiesterase inhibitors revealed that activation of phosphodiesterase III by insulin represents an important metabolic step in stimulation of leptin secretion. Thus it is likely that, under physiological conditions, norepinephrine and other lipolytic hormones competitively counterregulate the stimulatory effects of insulin on leptin secretion by activating the adenylate cyclase system. In rodents, several studies have shown that central administration of leptin increases the activity of the sympathetic nervous system, norepinephrine turnover in various tissues, and glucose uptake in brown adipose tissue and striated muscles, but not in white adipose tissue (26). By inhibiting leptin secretion from white adipose tissue, norepinephrine may decrease plasma leptin levels and consequently reduce the activity of the sympathetic nervous system. Thus norepinephrine may participate in a negative feedback loop preventing excessive activation of the sympathetic nervous system and energy expenditure. The fact that selective β_1 - and β_2 -adrenergic antagonists entirely reverse the effects of norepinephrine may help develop new drugs for controlling leptin levels in humans where β_3 -adrenoceptors play a minor role in modulating lipolysis and leptin secretion (17, 30). Indeed, in humans, dysregulated leptin levels have been associated not only with obesity and diabetes but also with a series of pathologies, such as leptin-induced anorexia, cardiovascular diseases, and hematopoietic dysregulation (14).

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