Eicosanoids as endogenous regulators of leptin release and lipolysis by mouse adipose tissue in primary culture

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Abstract **Prostaglandin E_2 (PGE_2) stimulated leptin release over a 24-h incubation of mouse adipose tissue in primary culture. The maximal stimulation of leptin release was seen with 100 nM PGE_2. The role of endogenous eicosanoids in the regulation of lipolysis and leptin formation was examined in the presence of NS-398, a selective cyclooxygenase-2 inhibitor. NS-398 at a concentration of 5 μM enhanced lipolysis by 30% and lowered leptin release by 24%. This concentration of NS-398 almost completely inhibited PGE_2 formation. An inhibition of basal lipolysis by PGE_2 or N^6-cyclopentyladenosine (CPA) was seen in the presence but not in the absence of NS-398. CPA, whose receptor, like that of PGE_2 inhibits cyclic AMP accumulation in adipose tissue, also enhanced leptin release. These data indicate that PGE_2 can stimulate leptin release and suggest that endogenous eicosanoids affect both lipolysis and leptin formation by mouse adipose tissue.** —Fain, J. N., C. W. Leffler, and S. W. Bahouth. **Eicosanoids as endogenous regulators of leptin release and lipolysis by mouse adipose tissue in primary culture.** ***J. Lipid Res.*** **2000.** 41: **1689–1694.**

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The primary factor regulating plasma leptin is the amount of fat (1), but the hormones responsible for the enhanced release of leptin by adipose tissue from obese animals are poorly understood. The synthetic glucocorticoid dexamethasone appears to be the most potent stimulator of leptin release and leptin mRNA accumulation in rodent adipocytes (2). Glucocorticoids activate the transcription of the leptin gene via a nonclassic mechanism (3). Glucocorticoid levels are elevated in obese mice that do not express leptin or the leptin receptor (1). However, there is no evidence that the elevated levels of leptin seen in obese humans are secondary to glucocorticoids (4).

In the course of studies on the regulation of leptin release by pieces of mouse adipose tissue incubated in primary culture for 24 h we found that prostaglandin E_2 (PGE_2) was a potent stimulator of leptin release. PGE_2 is a product of the arachidonic acid cascade that is initiated by breakdown of phospholipids in most cells. However, in adipocytes arachidonic acid can also be derived from the breakdown of triacyglycerols. There are three major pathways for conversion of arachidonic acid to oxygenated metabolites involving prostaglandin H_2 synthase [cyclooxygenase 1 (COX-1) or COX-2], lipoxygenase, and cytochrome *P*-450. The cyclic endoperoxide (PGH_2) can rearrange enzymatically or chemically to generate several prostaglandins as well as prostacyclin and thromboxane. The lipoxygenases are involved in the generation of leukotrienes and the cytochrome *P*-450 epoxygenases generate eicosatetraenoic acids. The present studies are concerned with the products of arachidonic acid generated by COX-2 in adipose tissue. COX-1 is constitutively expressed in many cells while COX-2 is the so-called inducible form of the enzyme located in the perinuclear region (5). There are also drugs that preferentially inhibit COX-2 (6, 7). NS-398 is 165-fold more potent as an inhibitor of COX-2 than of COX-1 activity in human blood assays (6). In the present studies we examined PGE_2 formation as well as the effects of NS-398 and exogenous PGE_2 on leptin release and lipolysis by mouse adipose tissue in primary culture.

MATERIALS AND METHODS

Epididymal adipose tissue in each experiment was obtained from two to eight fed mice weighing 25–35 g unless otherwise noted. Some of the mice had been fed for at least 3 weeks a pelleted high fat diet containing 27% casein, 20% Crisco, 46% sucrose, 2% RP vitamin mix, and 5% PP mineral mix 10 that was supplied by Purina Mills (Richmond, IN). Initially, we used mice fed laboratory chow but many of these mice had small amounts of epididymal fat (less than 500 mg/mouse) and released very little leptin. The mice were either C57-B6, C57-B6 × FVN, C57-B6 × SJL, or C57-B6 × 129SV.

The pooled tissue was cut into small pieces and 100–200 mg was distributed between 13 and 40 tubes and incubated in 5 ml of Dulbecco's modified Eagle's medium/Ham's F12 (8, 9). The adipose tissue was incubated for 24 h and at the end of the incubation, 10- to 50-μl aliquots of the medium were used to mea-

Abbreviations: CPA, N^6-cyclopentyladenosine; COX-1, cyclooxygenase 1; COX-2, cyclooxygenase 2; PGE_2, prostaglandin E_2.

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sure the leptin content, using radioimmunoassay kits with antibody raised against mouse leptin and with mouse leptin standards from Linco Research (St. Charles, MO).

Total RNA was extracted from adipose tissue at the start and at the end of the incubation (10, 11). Leptin mRNA was analyzed by Northern blot analysis, using a ^{32}P-labeled mouse leptin cDNA probe and the radioactivity in leptin mRNA in each experiment was corrected for recovery of 18S RNA (10, 11).

The insulin was bovine insulin obtained from Sigma (St. Louis, MO), as were the other hormones and reagents. Glycerol was analyzed in 10- to 50-μl aliquots of the medium by applying glycerokinase (12) and lactate by the same fluorometric procedure using lactate dehydrogenase. PGE_2 was measured in aliquots of the medium as previously described (13, 14). PGE_2 was obtained from Cayman Chemical (Ann Arbor, MI) and stock solutions were prepared in 100% ethanol. PGE_2 was added in a volume of 5 μl or less to 5 ml of medium just before the addition of adipose tissue. Alcohol at the same concentration was added to control tubes.

The effects of added agents are generally shown as the percentage change from the incubation control in each experiment. This transformation resulted in a more normal distribution of the data because the basal value for leptin release at 24 h was quite variable. Part of the variability in leptin release was due to the amount of fat, which ranged from 300 to 2,600 mg per mouse. Statistical comparisons were made by applying Student's *t*-test to the paired differences.

RESULTS

The accumulation of leptin in culture medium containing 25 nM dexamethasone was 540 ± 85 (mean ± SEM) ng/g over a 24-h incubation (n = 5). The leptin content of the tissue was 18 ± 12 ng/g at the start and 12 ± 6 ng/g after 24 h, indicating that there was no significant change in tissue leptin over a 24-h incubation. Therefore leptin accumulation in the medium at 24 h represented new synthesis of leptin. Most experiments were performed in the presence of 25 nM dexamethasone because the basal release of leptin as well as the level of leptin mRNA were maintained between 6 and 24 h of incubation of rat adipose tissue (10). In pieces of mouse adipose tissue the level of leptin mRNA was 136 ± 26% (mean ± SEM of nine experiments) of the initial value after a 24-h incubation in the presence of 25 nM dexamethasone, while it was 42 ± 11% in the absence of dexamethasone.

The data in **Fig. 1** demonstrate that 0.1–10 μM PGE_2 enhanced leptin release by adipose tissue incubated for 24 h in the presence of 25 nM dexamethasone. Release of leptin in the same experiments at 6 h was 32% of that seen at 24 h.

We also examined the effect of 100 nM PGE_2 in the presence of 25 nM dexamethasone on the leptin mRNA content of mouse adipose tissue at the end of a 24-h incubation in nine separate experimental replications. The leptin mRNA content as a percentage of that present at the start of the incubation was 118 ± 46% (mean ± SEM) in tissue incubated without as compared with 112 ± 21% in tissue incubated with 100 nM PGE_2. The stimulation of leptin release without a similar stimulation of leptin mRNA content by PGE_2 is similar to the effects of insulin on rat adipose tissue. Insulin stimulated leptin release over a 24-h incubation, but reduced leptin mRNA in both rat adipocytes (11) and intact pieces of rat epididymal adipose tissue (9, 10). In mouse adipose tissue 10 nM insulin increased leptin release over a 24-h incubation by 42 ± 9% (mean ± SEM of 20 experiments), which was comparable to the 40 ± 7% (mean ± SEM of 25 experiments) increase in leptin release due to 100 nM PGE_2 in the presence of 25 nM dexamethasone.

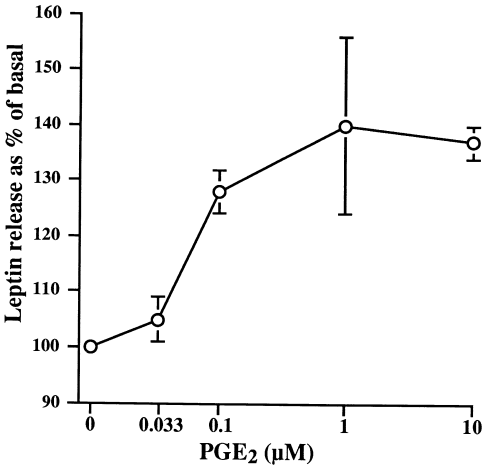

Fig. 1. PGE_2 at a concentration of 0.1 μM near maximally stimulates leptin release. Pieces of mouse adipose tissue (165 mg) were incubated for 24 h in 5 ml of medium containing 25 nM dexamethasone either without or with PGE_2 at the indicated concentrations. Leptin release is shown as the percentage of the basal release at 24 which was 275 ng/g of fat. The values represent means ± SE and are from four paired experimental replications.

The effect of blocking endogenous PGE_2 formation was examined with NS-398, which is a specific cyclooxygenase 2 (COX-2) inhibitor (6, 7). NS-398 (5 μM) inhibited leptin accumulation by 24% but enhanced lipolysis by 30% in the presence of 25 nM dexamethasone (**Table 1**). These data suggest that part of the basal release of leptin (~25%) is due to endogenous formation of eicosanoids such as PGE_2. The finding that 5 μM NS-398 inhibited leptin release while enhancing lipolysis suggests that endogenous eicosanoids formed via COX-2 also regulate lipolysis (Table 1).

TABLE 1. A specific COX-2 inhibitor stimulates lipolysis and inhibits leptin release by mouse adipose tissue

Basal	% Change Due to NS-398
Lipolysis (μmoles/g) 31 ± 2	+30 ± 8
Leptin release (ng/g) 300 ± 50	−24 ± 9
PGE_2 formation (pmol/g) 113 ± 18	−84 ± 17

Pieces of mouse adipose tissue (200 mg) were incubated for 24 h in 5 ml of medium containing 25 nM dexamethasone either without or with 5 μM NS-398. The data represent the means of eight paired replications and effects of NS-398 are shown as the percent change ± SEM.

TABLE 2. NS-398 enhances the stimulation of leptin release by PGE_2 in mouse adipose tissue

NS-398	Basal Leptin Release	% Change due to 0.1 μM PGE_2
μM	*ng/g*	
0 [12]	256	+24 ± 15
0.5 [12]	189	+98 ± 25
0 [4]	338	+23 ± 9
5 [4]	230	+96 ±22

Pieces of mouse adipose tissue (200 mg) were incubated for 24 h in 5 ml of medium containing 25 nM dexamethasone. The values are from two different series of experiments and the number of paired replications in each series is shown in brackets. The effects of PGE_2 are shown as the percent change ± SEM.

Further support for the hypothesis that endogenous eicosanoids are physiological regulators of leptin release is shown in **Table 2**. The stimulation of leptin release by 0.1 μM PGE_2 was markedly enhanced in the presence of either 0.5 or 5 μM NS-398, which reduced the basal formation of leptin (Table 2).

The dose-response relationships for effects of NS-398 on lipolysis and leptin release in the absence or presence of 25 nM dexamethasone are shown in **Fig. 2**. Maximal inhibition of leptin release was seen with only 0.05 μM NS-398 in the presence of 25 nM dexamethasone. However, in the absence of dexamethasone there was no statistically significant effect of NS-398 on leptin release at any concentration (Fig. 2). In contrast, the ability of NS-398 to stimulate lipolysis at a concentration of 1 or 5 μM was little affected by the presence or absence of dexamethasone (Fig. 2).

PGE_2 is known to interact in adipose tissue with G protein-linked receptors that are associated with an elevation of the activity of the α subunit of the inhibitory regulatory GTP-binding protein G_i, resulting in inhibition of adenylyl cyclase activity and of lipolysis (15). We therefore compared the effects of PGE_2 with those of another antilipolytic agent. Activation of adenosine receptors in adipose tissue results in a similar activation of G_i resulting in inhibition of lipolysis (16, 17). N^6-Cyclopentyladenosine (CPA) is an analog of adenosine that is a potent activator of A_1 adenosine receptors in adipose tissue (16). We compared the effects of PGE_2 and CPA, both at a concentration of 0.1 μM, in the studies shown in **Fig. 3**. The two compounds were equivalent as inhibitors of lipolysis in the presence of NS-398 (5 μM) but had little effect in its absence (Fig. 3). In contrast to PGE_2, there was a smaller effect of CPA on leptin release (+49% for CPA vs. +97% increase for PGE_2). In the presence of 5 μM NS-398 the increase in leptin release by CPA in mouse adipose tissue was reduced from 49% to an insignificant 11% while the increase due to PGE_2 was increased from 97 to 150% above basal. These results indicate that there is something unique about the stimulation of leptin release by CPA because the increase in leptin release, unlike the inhibition of lipolysis, was virtually abolished by NS-398 (Fig. 3).

We examined the accumulation of PGE_2 in the medium

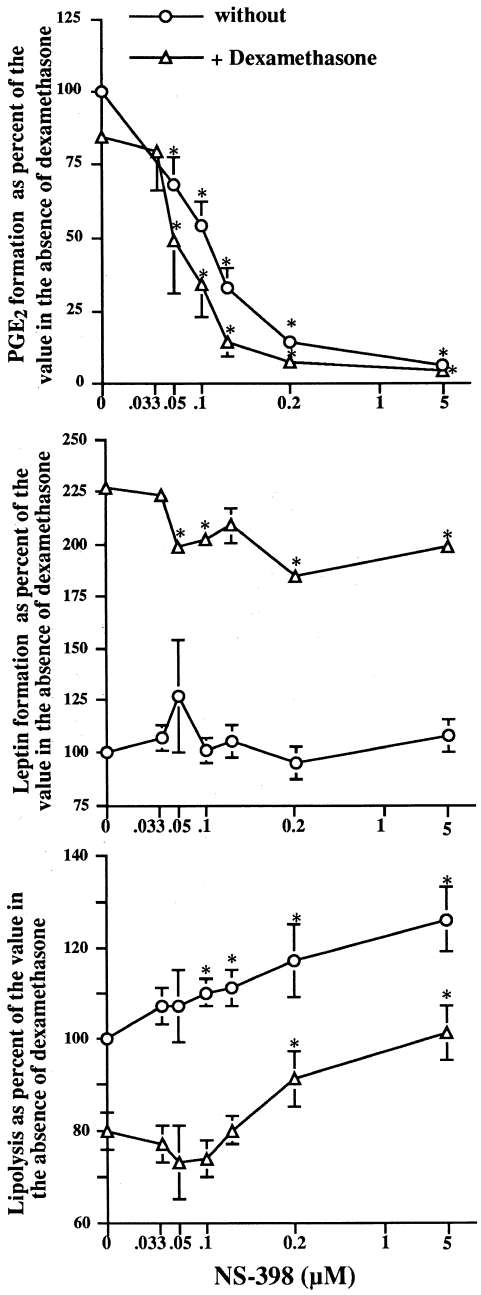

Fig. 2. Effect of NS-398 on lipolysis and leptin release by mouse adipose tissue. Pieces of mouse adipose tissue (100 mg) were incubated for 24 h in 5 ml of medium without (circles) or with 25 nM dexamethasone (triangles) and the indicated concentrations of NS-398. The effects of dexamethasone and NS-398 are shown as the percentage ± SEM of the basal value at 24 h in the absence of dexamethasone for the paired differences from 14 experimental replications. Basal leptin release was 123 ng/g of fat while basal lipolysis in micromoles per gram of glycerol over 24 h was 33 in the absence of dexamethasone. Basal PGE_2 formation in pmol/g over 24 h was 633 pmol/g in the absence of dexamethasone. Statistically significant effects of NS-398 based on paired comparisons are indicated by an asterisk ($P < 0.05$). The decrease in lipolysis and the increase in leptin release due to dexamethasone were both statistically significant ($P < 0.05$) but not the decrease in PGE_2 ($P < 0.4$).

over a 24-h incubation of mouse adipose tissue. PGE_2 release averaged 633 ± 235 pmol/g in the absence of dexamethasone in the 14 experiments shown in Fig. 2. Half-

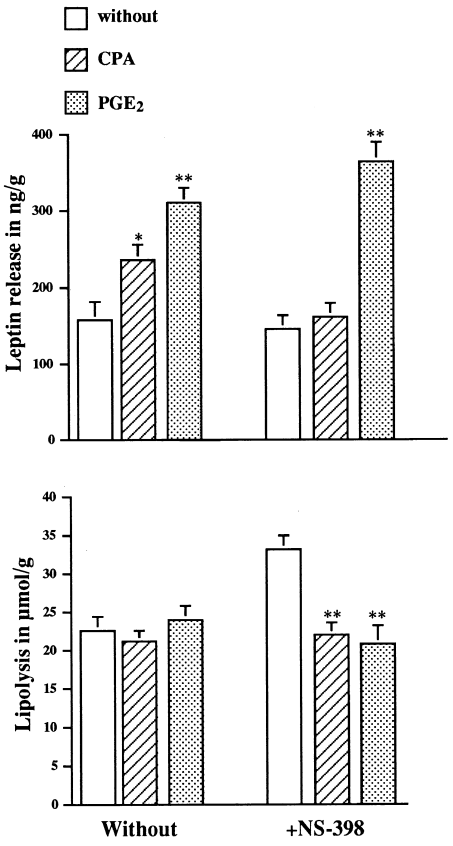

Fig. 3. Comparison of effects of PGE_2 versus CPA, an A_1 adenosine agonist. Pieces of mouse adipose tissue (90 mg) were incubated for 24 h in 5 ml of medium without or with 5 μM NS-398. Values represent the means ± SEM of 20 experiments. CPA and PGE_2 were added at a concentration of 0.1 μM. Statistically significant effects of CPA or PGE_2 based on paired comparisons are indicated as follows: * $P < 0.05$, ** $P < 0.01$.

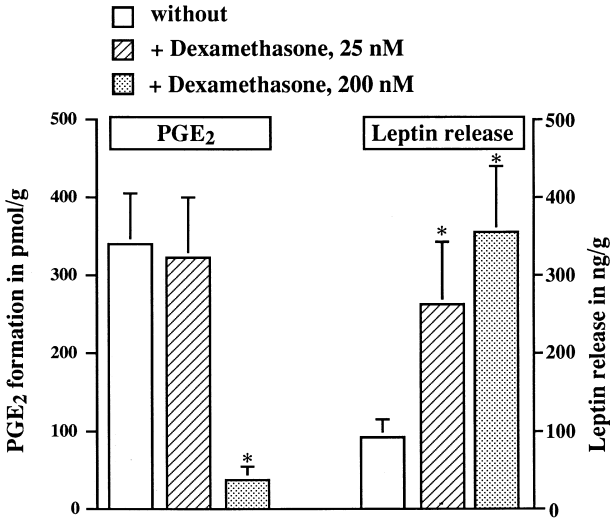

Fig. 4. Effects of dexamethasone on leptin and PGE_2 accumulation in mouse adipose tissue. Pieces of mouse adipose tissue (165 mg) were incubated for 24 h in 5 ml of medium without or with 25 nM or 200 nM dexamethasone. Values represent the means ± SEM of nine paired experimental replications. Statistically significant effects of dexamethasone are indicated by an asterisk ($P < 0.05$).

maximal inhibition of PGE_2 formation was seen with approximately 0.1 μM NS-398 while 90% inhibition was seen with 1 μM NS-398 (Fig. 2). There was no statistically significant effect of 25 nM dexamethasone on PGE_2 release or the inhibition of PGE_2 formation by NS-398 (Fig. 2).

The PGE_2 content in mouse adipose tissue at the start and end of the 24-h incubation was examined to confirm that PGE_2 accumulation in the medium represented new synthesis versus release from tissue stores. In three experiments the decrease in PGE_2 content of the tissue over 24 h was 10 pmol/g while accumulation in the medium was 190 pmol/g of fat. These data indicate that most of the PGE_2 released to the medium represented net synthesis of PGE_2 over a 24-h incubation.

Dexamethasone at a concentration of 25 nM had no significant effect on PGE_2 release by mouse adipose tissue (Fig. 2). However, if the concentration was increased to 200 nM there was a marked inhibition of PGE_2 release (**Fig. 4**). It is known that dexamethasone reduces COX-2 expression in other tissues (18, 19). Leptin release was near-maximally stimulated by 25 nM dexamethasone but 25 nM dexamethasone had little effect on PGE_2 formation (Fig. 4).

We also examined the stimulation of leptin release in the absence of dexamethasone by exogenous addition of 0.1 μM PGE_2 in 15 experiments and found that the percent increase in leptin release was 65 ± 21% (mean ± SEM). In the same experiments the percent increase in leptin release due to PGE_2 in the presence of 25 nM dexamethasone was +97 ± 19%. However, the difference was not statistically significant (+32 ± 25% for 15 paired comparisons without and with dexamethasone). These data indicate that exogenous PGE_2 can stimulate leptin release in the absence of dexamethasone while NS-398 inhibits leptin release only in the presence of 25 nM dexamethasone (Fig. 2).

DISCUSSION

There was a stimulation of lipolysis by 5 μM NS-398 in both the absence and presence of dexamethasone, which suggests that endogenous eicosanoids regulate basal lipolysis. Our data in mouse adipose tissue are in contrast to prior reports using indomethacin; these reports failed to provide any evidence of a role of endogenous eicosanoids in the regulation of lipolysis by rat adipose tissue (20). There appear to be species differences because in cut pieces of rat adipose tissue incubated for 24 h with 0.05 to 5 μM NS-398 we could see no enhancement of basal lipolysis (J. N. Fain, C. W. Leffler, and S. W. Bahouth, unpublished experiments).

There was no significant effect of NS-398 on leptin release in the absence of 25 nM dexamethasone, thus dissociating the inhibition of leptin release from stimulation of lipolysis. It is unclear why the low rate of leptin release seen in the absence of dexamethasone was not further reduced by inhibition of endogenous eicosanoid formation.

There was an inhibition of leptin release by 0.05 μM NS-398 in the presence of 25 nM dexamethasone. This concentration of dexamethasone (25 nM) had no significant effect on PGE_2 formation (Figs. 2 and 4). Furthermore NS-398 had the same effects on lipolysis and PGE_2 formation whether 25 nM dexamethasone was absent or present. However, if the concentration of dexamethasone was increased to 200 nM there was a marked inhibition of PGE_2 formation (Fig. 4), confirming prior reports that dexamethasone is a potent inhibitor of COX-2 (18, 19).

It is well established that adipocytes as well as stromal-vascular cells of rat adipose tissue can form PGE_2 (20–22). While the nonfat cells in rat adipose tissue have little effect on lipolysis, their addition to adipocytes enhances the formation of PGE_2 (20–22). There is little formation of PGE_2 by the stromal-vascular cells of rat adipose tissue in the absence of adipocytes, suggesting that formation of PGE_2 is dependent on arachidonic acid released by adipocytes (20–22). Possibly eicosanoids made the stromal-vascular cells act as paracrine regulators and interact with the receptors for PGE_2 on the surface of adipocytes (15). In contrast, PGE_2 may be formed in the nucleus of mouse adipocytes, because COX-2 is a nuclear enzyme (23, 24) and interacts with intracellular receptors.

We did not see an increase in leptin mRNA content due to PGE_2 after 24 h of incubation, but it is possible that there was a transient increase at an earlier time period. It is unclear how PGE_2 increases leptin release but its effects are similar to those we have previously reported with regard to effects of insulin on leptin release and leptin mRNA content after a 24-h incubation of rat adipose tissue (11). The relatively slow stimulation of leptin release by adipocytes in response to insulin occurs by mechanisms that are incompletely understood (25).

The activation of EP_3 receptors in adipose tissue by PGE_2 results in an elevation of $G_{i\alpha}$ activity just as is seen with the activation of A_1 adenosine receptors (16). The finding that CPA and PGE_2 inhibited the increase in lipolysis seen only in the presence of 5 μM NS-398 but not basal lipolysis (Fig. 4) suggests that endogenous eicosanoids inhibit lipolysis by mouse adipose tissue in primary culture. In contrast, exogenous CPA or PGE_2 stimulated leptin release in the absence of NS-398, indicating that leptin release was not maximally activated by endogenous eicosanoids (Fig. 3). However, the effect of 100 nM PGE_2 on leptin release was greater in the presence of NS-398 than in its absence (Table 2 and Fig. 3). In contrast, no significant effect of CPA was seen on leptin release in the presence of NS-398 (Fig. 3). These data suggest that endogenous eicosanoids are required for stimulation of leptin release by CPA. Furthermore, it appears that PGE_2 and CPA effects on leptin release by adipocytes involve something more than activation of $G_{i\alpha}$ activity and inhibition of lipolysis. The finding that CPA inhibited lipolysis to the same extent as PGE_2 in the presence of NS-398 without affecting leptin (Fig. 3) suggests that the stimulation of leptin release by PGE_2 is not secondary to its antilipolytic effect.

We conclude that exogenous PGE_2 can stimulate leptin release and inhibit lipolysis in mouse adipose tissue. Endogenous eicosanoids also appear to affect lipolysis and leptin release under appropriate conditions.

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