Insulin and Leptin Combine Additively to Reduce Food Intake and Body Weight in Rats

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Leptin and insulin are distinct adiposity signals that regulate food intake and body weight. Because recent evidence suggests that the central catabolic action of each is mediated by the hypothalamic melanocortin system, we tested the hypothesis that leptin and insulin interact within the brain, either additively or synergistically, to suppress food intake and reduce body weight. Using a within-subjects design, we co-administered leptin and insulin into the 3rd cerebral ventricle (i.c.v.) over a wide range of doses, and compared the combined effects to what occurred following the administration of each peptide alone. The data suggest that leptin and insulin interact sub-additively to regulate food intake and body weight over the first few hours. That is, the ability of combinations of leptin and insulin to reduce food intake and body weight was less than what would be predicted by the sum of their independent actions. Over 24 hours, however, the combined doses fit a strictly additive model. These data therefore imply a redundancy in the functional intracellular pathways or neuronal circuits that leptin and insulin utilize in the acute regulation of food intake and body weight, and they further imply that over time, the redundancy dissipates.

LEPTIN and insulin are peptide hormones that provide important signals to the central nervous system (CNS). When energy balance is stable, such as during fasting, plasma levels are directly proportional to body fat (1, 2), and each peptide therefore provides a signal informing the CNS of the amount and distribution of peripheral energy stores. More acutely, fluctuation of the secretion of these hormones varies with changes in energy balance and is especially influenced by the macronutrient content of meals (3, 4). Hence, leptin and insulin provide short-term, as well as long term, signals related to metabolism and energy balance. Each peptide is transported into the CNS via receptor-mediated uptake (5, 6), and each interacts with its own receptors in the hypothalamus and elsewhere in the brain (7, 8). Considerable evidence suggests that activation of these hypothalamic receptors is responsible for the suppression of food intake and reduction of body fat stores elicited by leptin or insulin, thus completing a key negative feedback loop in the regulation of caloric homeostasis (9, 10).

Leptin is secreted by adipocytes in direct proportion to the amount of adipose tissue (2). While leptin receptors are located throughout the CNS, it is the long-form of the receptor (Ob-Rb) that is most important in the regulation of energy balance since mice and rats with mutated receptors (db/db and fafa mutations, respectively) are hyperphagic and obese (11, 12). Ob-Rb is heavily expressed in the arcuate nucleus of the hypothalamus (ARC) by pro-opiomelanocortin (POMC)-synthesizing cells that secrete α-melanocyte stimulating hormone (α-MSH) as a transmitter. Leptin stimulates POMC synthesis and α-MSH release (13), and α-MSH in turn interacts with melanocortin 3 and 4 receptors (MC3/4r) leading to a reduction in food intake and body weight (14). Antagonizing these receptors prevents the decrease of food intake elicited by exogenous leptin (15). Both also interact with Neuropeptide Y (NPY) (16).

Pancreatic insulin, in addition to its role as a regulator of plasma glucose, also functions as an adiposity signal to the central nervous system (17), and, analogous to leptin, insulin receptors are concentrated in the ARC (8). Our lab has recently demonstrated that insulin interacts with the hypothalamic melanocortin system in ways similar to leptin. First, insulin receptors are localized to POMC neurons. Second, centrally administered insulin stimulates POMC expression in fasted rats. Third, a non-selective MC3/4r antagonist blocks insulin-induced hypophagia (18). Hence, both leptin and insulin reduce food intake and body weight in part by stimulating the ARC melanocortin system. Because of the apparent redundancies in their effects on hypothalamic melanocortins and on energy balance, we sought to assess whether leptin and insulin would act synergistically or additively when co-administered into the ventricular system of the brain.

Material and Methods

Animals and housing

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) were maintained in tub cages at constant temperature (25° C) in an AAALAC accredited vivarium. They had ad lib access to pelleted food and water except as noted below. Rats (250 - 300 g) were implanted with 21-gauge canulas aimed at the third cerebral ventricle (13vt, 2.2 mm caudal to bregma and 7.5 mm ventral to dura with bregma and lambda at the same vertical coordinate) and secured to the skull with screws and dental acrylic as previously described (19). Following recovery of body weight, cannula placement was confirmed by i.c.v. injection of 10 ng angiotensin. Only those rats which consumed at least 5 ml water within one hour of
injection were determined to have correct placement and were included in the studies. All procedures were approved by the University of Cincinnati IACUC.

**Procedure**

On a test day, rats received an i.c.v. injection of saline (the vehicle for the peptides), leptin (human, Calbiochem, San Diego, CA), insulin (leitin regular pork insulin, Lilly, Indianapolis, IN), or leptin plus insulin in a total volume of 2 μl. Doses of leptin were 0.5, 1, 3.5, 7 and 10 μg, and doses of insulin were 0.5, 1, 2, 4 and 8 μl. A total of 6 to 10 rats was used for each condition. Injections were administered 4 hours prior to lights off at which time food was removed from the cages. Food was returned just prior to lights–off, and food intake was recorded after 1, 2, 4 and 24 hours. Body weight was measured daily. At least 2 days occurred between tests for any subject.

**Statistical analyses**

Cumulative food intake at each time point for each experimental condition (i.e., each dose of leptin, insulin, and leptin plus insulin) was compared to that of the saline-only condition and expressed as percent suppression. Additivity versus synergism was determined by plotting the percent suppression caused by combined doses of leptin and insulin against the summed percent suppression of those doses administered alone (Figure 2 below). ANCOVA was used to assess the slope of the resulting regression line and to evaluate the hypothesis that the observed slope was significantly different from 1.0.

**Results**

As has been observed in many previous studies, both leptin and insulin suppressed food intake and reduced body weight when administered i.c.v. individually (representative data are depicted in Figure 1). Doses of 0.5 and 1 μg leptin, and 0.5 and 1 μl of insulin were subthreshold in that they did not reduce food intake or body weight significantly.

Combinations of leptin and insulin were also effective at reducing food intake (Figure 1a), and body weight change over 24 hours (Figure 1b). Figure 2a depicts the "predicted" percent suppressions for combined doses of leptin and insulin over 4 hours with a slope of 1 (dashed line). The actual percent suppressions of the same combinations are also depicted (●). Regression analysis of the observed suppressions (solid line) yielded a slope of 0.41 ± 0.10 (r = +0.88) which was significantly less than 1 (F(1,10) = 35.97, p < .0005), suggesting that leptin and insulin combine subadditively when given in combination relative to what would be anticipated from their individual effects. A comparable analysis was made after 20 hours (Figure 2b). The slope of the observed percent suppressions was 0.53 ± 0.18, (r = +0.80) and was also significantly less than 1 (F(1,10) = 7.01, p < .05). A comparable analysis for the percent reduction of body weight over 24 hours is depicted in Figure 2c. The

![Fig 1](https://example.com/fig1.png)

**Fig 1.** A. Mean cumulative food intake from 1 to 20 hours following lights off. B. Mean 24-hour change in body weight following the injections. Error bars = SEM.

![Fig 2](https://example.com/fig2.png)

**Fig 2.** Effect of combined injection of leptin and insulin versus the sum of their individual effects on (A) 4-hour and (B) 20-hour food intake, and (C) the 24-hour change in body weight. Results from individual trials are indicated by the symbols (●), while the regression line of these points is indicated by the solid line (—). The dashed line (—) represents simple additivity with a slope of 1.0.
slope of the observed percent suppressions was 0.59 ± 0.08 (r = +0.95) and was significantly less than 1 (F(1,10) = 23.35, p < 0.001). The regression slopes were also calculated after 1 (slope = 0.18 ± 0.06; r = +0.79) and 2 (slope = 0.37 ± 0.18; r = 0.67) hours, and both were also significantly less than 1. As depicted in Figure 3a, the slope of the regression line increased steadily from 1 to 20 hours, suggesting that an additive relationship between leptin and insulin developed after an interval of several hours. To assess this, an independent analysis was made for each condition for the interval from 4 to 20 hours following the injections. As depicted in Figure 3b, the slope was equal to 0.60 ± 0.29 (r = +0.69) and was not significantly different from a slope of 1 (F(1,10) = 1.94, p > 0.05).

Fig 3. A. Change in the slope of the regression line over time. B. Effect of combined injection of leptin and insulin versus the sum of their individual effects on food intake from 4 to 20 hours following lights off. Results from individual trials are indicated by the symbols (●), while the regression line of these points is indicated by the solid line (—). The dashed line (—) represents a slope of 1.0.

Discussion

Leptin and insulin fill distinct niches in the endocrine system. Although leptin has been implicated in several systemic processes, such as angiogenesis (20), the primary role of leptin appears to be to act as a negative feedback adiposity signal that acts in the brain to suppress food intake and net catabolic effector (21). Consistent with this, animals lacking leptin or functional leptin receptors are grossly obese. Insulin, in contrast, has a primary action in the periphery to regulate blood glucose and stimulate glucose uptake by most tissues. Analogous to leptin, however, deficits in insulin signaling are also associated with hyperphagia in humans, and animals that lack normal insulin signaling in the brain are also obese (22-24).

Based upon these common actions, and the fact that both leptin and insulin enter the brain and interact with cells in the arcuate nucleus, we predicted that they would act in concert, each complementing the actions of the other. We in fact hypothesized that leptin and insulin would interact synergistically to reduce food intake and body weight. We addressed this by comparing the effect of a co-administration of leptin and insulin to the summed effects of the same doses of leptin and insulin when given separately. In such a paradigm, if leptin and insulin combine additively, then the sum of the individual effects of leptin and insulin should be approximately equal to the effect of the combined injection over a range of combinations. In other words, there would be a one-to-one correlation between the summed single-effects and the effects of combined administrations. However, if leptin and insulin combine synergistically, then the effect of the combined injections will be greater than the sum of the effects of the individual injections and regression analysis of their relationship would reveal a slope greater than 1.0. Analysis of the data revealed that neither hypothesis explained the results. At each time point, the effect of the combined injections was significantly less than that which was predicted from the individual injections. Consistent with this conclusion, regression analyses revealed that the relationship between the combined effect and the sum of the individual effects had a slope significantly less than 1.0 (Figures 2 and 3a). This was also true for body weight (Figure 2c).

These data therefore imply that leptin and insulin combine sub-additively. A sub-additive relationship is consistent with the concept that leptin and insulin utilize redundant signaling pathways to regulate energy homeostasis, and that activation of the pathway by one peptide attenuates the efficacy of the second when both are present at the same time. Alternatively, these data may reflect a floor effect in that leptin and insulin, given individually, maximally suppress food intake during the first four hours such that the combination of the two cannot further suppress intake. However, in none of the trials did leptin or insulin alone result in zero food intake during the first two hours, arguing against this explanation. Finally, since leptin and insulin reach a more limited region of the brain when administered i.c.v. as compared to uptake from the periphery, it is possible that leptin and insulin are only sub-additive in the ARC and may be additive or synergistic when all brain regions are exposed.

We also observed that the slope of the relationship for combined administration of leptin and insulin increased over time. This may indicate that, in the short-term (i.e., the first 4 hours), leptin and insulin provide redundant signals, but that over a time course of several hours, their combined actions approach a more additive model. We evaluated this
hypothesis through analysis of food intake during the 4 to 20-
hour period after food presentation. The resultant slope,
though less than the predicted 1.0, was no longer significantly
different (Figure 3b). These results are consistent with the
possibility that the nature of the relationship between leptin
and insulin, while sub-additively acutely, may be additive over
the longer term.

The potential for such redundancy between leptin and
insulin has been highlighted by several recent studies in
which leptin and insulin have been found to share both
intracellular and neuronal signaling pathways. While the
melanocortin system has long been thought to mediate the
central actions of leptin, recent studies in which insulin
significantly stimulated POMC expression in fasted rats and
insulin-induced hypophagia was blocked by a non-specific
melanocortin receptor antagonist (18) strongly support a role
for the melanocortin system in the regulation of energy
balance by insulin as well. Conversely, both peripherally and
centrally administered leptin reduced streptozotocin-induced
hyperphagia. Conversely, both peripherally and centrally
administered leptin reduced streptozotocin-induced
hyperphagia (25). Furthermore, phosphatidylinositol-3-OH
kinase (PI(3)K), an enzyme which is an intracellular mediator
of insulin signaling (26), appears to play a crucial role in the
leptin-induced anorexia signal transduction pathway (27). While
these data are consistent with the concept that leptin
and insulin share such pathways, they also suggest that over
time, this redundancy dissipates and their pathways diverge.

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