Corticotropin-releasing Factor and Adrenocorticotropic Hormone as Potential Central Mediators of OB Effects

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OB (leptin) has been identified as a factor that suppresses appetite and stimulates metabolism. Attention has focused on the hypothalamus as its potential site of action, but OB could also act on other brain regions. In addition, the paradox of high OB levels in obese humans remains unresolved. Here we show in mice that both the long and short form of the OB receptor are expressed not only in the hypothalamus but also in the amygdala and pituitary. Recombinant murine OB elicited the release of corticotropin-releasing factor from superfused brain slice preparations containing hypothalamus or amygdala. Because corticotropin-releasing factor inhibits appetite and stimulates metabolism, it may be a key mediator of central OB effects. Recombinant OB also induced pituitary release of adrenocorticotropic hormone. Because adrenocorticotropic hormone-induced elevation of plasma glucocorticoid levels can inhibit corticotropin-releasing factor release via negative feedback, the OB effects on pituitary adrenocorticotropic hormone release may be pertinent to human obesity, which combines increased plasma glucocorticoid levels with elevated levels of OB. An imbalance between the effects of OB on corticotropin-releasing factor release from the hypothalamus and on adrenocorticotropic hormone release from the pituitary could contribute to obesity.

Obesity is a cause of serious health problems, and new research on the ob gene, which encodes an adipose tissue-derived hormone (OB or leptin) that suppresses appetite and stimulates metabolism (1, 2), raises hopes for therapeutic intervention. Little is known about the molecular factors mediating OB effects. Increasing evidence supports a central site of action for OB. Infusion of OB into the lateral ventricles of normal or OB-deficient ob/ob mice decreases feeding (3). Although the hypothalamus has been identified as a potential site of action of OB (4), OB may also act on other regions. Short and long forms of the OB receptor (OB-R) differing in the length of their intracellular domain are formed by differential splicing, but only the long OB-R form can activate janus kinase (JAK) and lead to phosphorylation of signal transducers and activators of transcription (STAT) proteins. The JAK/STAT pathway has been proposed to mediate the effects of OB on body weight (5). The function of the short OB-R form has not been identified yet, but this form could also have an important role in mediating central effects of OB.

Although neuropeptide Y has been implicated as a potential central mediator of OB effects (4), recent evidence indicates that additional factors may be involved (6). OB-deficient ob/ob mice treated with OB show strong Fos protein immunoreactivity in the paraventricular nucleus (PVN) (7). It is interesting in this context that corticotropin-releasing factor (CRF) acting at the PVN inhibits food intake and stimulates metabolic rate in genetically obese animals and lean controls (8–10). Furthermore, conditions that increase hypothalamic CRF production also diminish food intake (11–16), and reduction in central CRF activity has been suggested to contribute to the development of obesity (17, 18). This suggests a possible OB-CRF interaction.

The development and maintenance of obesity is associated with profound endocrine disturbances, including increased activity of the hypothalamic-pituitary-adrenal (HPA) axis (19–22). In genetically obese animals, hyperphagia and excessive weight gain are eliminated by adrenalectomy and are restored by treatment with glucocorticoids (23–25). Glucocorticoids inhibit afferent input to the PVN (26), and the inhibitory actions of corticosteroids on hypothalamic CRF synthesis and/or release may contribute to obesity.

The amygdala is involved in stress-related reactions (27) and in the regulation of the HPA axis (28, 29). The amygdala contains high levels of CRF (30) and CRF-containing fibers have been traced from the amygdala to the lateral hypothalamus and may directly innervate CRF-containing neurons within the PVN (31). As yet, no reports have appeared on the expression of OB-R in the amygdala. If expressed, OB-R might mediate CRF release by OB within the amygdala.

The OB receptor is a member of the extended cytokine receptor family and resembles gp130, the common signal-transducing subunit of a group of cytokine receptors that includes receptors for IL-6. Receptors for IL-1, IL-2, and IL-6 have been demonstrated in the pituitary of several species, and direct stimulation of pituitary ACTH release by these cytokines has been reported (32). OB might also directly act at the level of the pituitary to modulate ACTH release.

The aim of the present work was to determine whether OB-R mRNA is expressed in the hypothalamus, amygdala, and pituitary and whether OB can modulate the release of CRF from the hypothalamus or the amygdala and of ACTH from the pituitary to identify potential central mediators of OB effects.

MATERIALS AND METHODS

Animals—C57BL/6J mice 8–12 weeks of age (Jackson Laboratories, Bar Harbor, ME) were used in all experiments. All mice were housed at 9100. Tel.: 415-695-3835; Fax: 415-826-6541; E-mail: Jacob_Raber@quickmail.ucsf.edu.

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Expression of the long (OB-RL) (A) and short (OB-RS) (B) form of OB-R mRNA in mouse pituitary, hypothalamus, and amygdala. Because restraint stress has been reported to suppress food intake (47), OB-R mRNA expression before (C) and after (R) 1 h of restraint stress was also examined. 1 h of restraint stress did not alter OB-R mRNA levels in the pituitary, hypothalamus, or amygdala. To determine if additional time was required to detect alterations in OB-R mRNA expression, mice were returned to their cages after restraint stress and killed 2 h (R) and up to 14 h (data not shown). OB-R mRNA expression was not significantly affected either. The L32 signal was used as a control for RNA content/loading.

OB-R RNase Protection Assay—The tissues were snap-frozen in liquid nitrogen and stored at −70 °C until RNA was extracted, and RNase protection assay was performed as described previously (34). OB-R and L32 mRNA levels were determined by RNase protection assay using antisense riboprobes for the short and long forms of the OB-R and murine L32, respectively. cdna templates for the short (1250–1474, GenBank® accession number U42467) and the long (3040–3396, GenBank® accession number U46135) OB-R probes were subcloned from a mouse hypothalamus cDNA library.

Superfusion System—To measure hypothalamic CRF and arginine vasopressin (AVP) release and pituitary ACTH release, dissected tissues were placed onto a Brinkmann tissue chopper, and 300-μm slices were prepared. Slices representing one entire hypothalamus, amygdala, or pituitary were placed into individual chambers and superfused using an in vitro superfusion system (Brandel, Gaithersburg, MD) (33). Basal release of CRF and AVP from hypothalamus stabalizes after 100 min (35), and release of ACTH from the pituitary stabalizes after 180 min (36). These times were chosen as the start of the first 15-min basal period for all subsequent experiments to determine the effect of OB on hypothalamic CRF and AVP and pituitary ACTH release. During the sample collection period, fractions were collected at 15-min intervals, placed on dry ice, and stored at −70 °C until determination of the concentration of CRF and ACTH in the media by radioimmunoassay (33, 35). OB recombinant protein was produced in and purified from Escherichia coli (33, 35). OB recombinant protein was produced in and purified from E. coli (33, 35). OB recombinant protein was produced in and purified from E. coli (33, 35). OB recombinant protein was produced in and purified from E. coli (33, 35).

RESULTS AND DISCUSSION

To determine if OB may act on other regions besides the hypothalamus, we looked for expression of the short and long forms of OB-R mRNA in the amygdala and pituitary, regions that are involved in the regulation of the hypothalamus (28, 39). Both forms of the OB-R were constitutively expressed not only in the hypothalamus, but also in the amygdala and pituitary of mice (Fig. 1).

To determine the role of CRF in the central actions of OB, the effect of recombinant mouse OB on the release of CRF from the hypothalamus and amygdala was investigated in superfused brain slice preparations. Superfusion with purified recombinant OB significantly increased CRF release from hypothalamic slices (Fig. 2A). In contrast to CRF release, hypothalamic AVP release was not affected by superfusion with OB at 100 pM, 1 nM, or 10 nM (data not shown). To ensure that the effect of OB on hypothalamic CRF release was due specifically to OB...
and not to an impurity in the recombinant OB preparation, tissues were superfused with OB in the presence of neutralizing antibodies. Neutralizing OB antibodies blocked the OB-induced hypothalamic CRF release (Fig. 2B), indicating that the effect on CRF release was indeed caused by OB. These findings are consistent with the postulate that CRF may function as a mediator of OB effects.

The above observations support a key role for CRF in OB biology and confirm the hypothalamus as an important site of OB actions. In addition, our study revealed that OB has specific effects on other brain regions. OB stimulated CRF release also from the amygdala (Fig. 3). In combination with the expression of OB-R mRNA in the amygdala (Fig. 1), this finding suggests that the amygdala may play a role in centrally mediated OB effects. The amygdala, which contains high levels of CRF, is involved in the expression of fear and anxiety (40) and could mediate behavioral effects of OB.

The development and maintenance of obesity is associated with an increased activity of the HPA axis (22). To determine if pituitary OB-R might play a role in HPA axis activation by inducing ACTH release, the effect of OB on ACTH release from the pituitary was assessed. Superfusion with recombinant OB significantly increased ACTH release from pituitary slices (Fig. 4A). This effect of OB on ACTH release was specific, because it was blocked by anti-OB antibodies (Fig. 4B).

In humans, serum OB concentrations correlate positively with the percentage of body fat (41, 42). The expression of the short and long forms of the OB-R in the pituitary and the OB-induced ACTH release from the pituitary identified here may play an important role in the development and maintenance of obesity. OB-induced increases in ACTH release could be directly responsible for the elevated glucocorticoid levels often found in obesity (22). Glucocorticoid receptors are colocalized in CRF cells in the PVN of the hypothalamus (26), and glucocorticoids can inhibit hypothalamic CRF synthesis/release via negative feedback (43). Thus, in the presence of severely elevated plasma OB, as found in obese individuals, two major effects could prevent OB from reducing body weight via enhanced CRF release (Fig. 5). First, because the pituitary is not shielded from the systemic circulation by the blood-brain barrier, blood-derived OB would enhance pituitary ACTH release, increase glucocorticoid levels and, via negative feedback, decrease hypothalamic CRF release. Second, direct stimulatory OB effects on hypothalamic CRF release depend on efficient transport of OB across the blood-brain barrier, and there is evidence that this process may become increasingly inefficient as plasma OB levels rise above a critical level. A saturable transport system for OB from blood to brain has been described (44), and the ratio of cerebrospinal fluid to plasma OB levels shows also signs of saturability when plasma OB levels rise to levels seen in obesity (45, 46).

In addition to the data presented here, a number of other studies also support the disturbed negative feedback circuitry proposed in Fig. 5. In obese fa/fa rats with high corticosterone levels, hypothalamic CRF content and portal secretion of CRF are reduced, and adrenalectomy in these animals results in enhanced portal CRF secretion (17, 43). Adrenalectomy also eliminates hyperphagia and excessive weight gain in obese fa/fa rats (23) as well as in obese rats with ventromedial hypothalamic lesions (24) and ob/ob mice (25). Lastly, treatment of such adrenalectomized models with glucocorticoids restores the obese phenotype (25–27).

In conclusion, we have demonstrated expression of both the short and long form of the OB-R in the hypothalamus, as well as in the amygdala and pituitary. In addition, OB was shown to induce CRF release from the hypothalamus and amygdala and ACTH release from the pituitary. These data indicate that CRF and ACTH may function as key mediators of OB effects and
CRF and ACTH Potential Central Mediators of OB Effects

**Fig. 5. Potential neuroendocrine interactions involved in OB effects and obesity (see text for further details).** The effect of CRF on adipose tissue might be mediated by the sympathetic nervous system, whose activity is increased by central CRF treatment (48). Note that this diagram focuses primarily on effects immediately pertinent to the current study. Because obesity is likely multifactorial in etiology, many additional factors and interactions may be involved.

emphasize the need to consider the differential effects of OB on multiple regions of the central nervous system in the design of OB-targeted treatments for obesity.

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**REFERENCES**


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