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Leptin affects food intake via CRF-receptor-mediated pathways

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Leptin is an important regulator of energy balance, which is thought to provide negative feedback to the brain to inhibit body fat accumulation. This protein is produced by adipose tissue in proportion to fat mass and released into circulation, and it subsequently acts on the brain to inhibit food intake and stimulate thermogenesis[1,2]. Leptin expression can also be induced by inflammatory stimuli such as bacterial endotoxin and cytokines[3,4], which could result in loss of body weight. Here we show that leptin's effects on food intake and body weight are blocked by co-infusion of an antagonist to the corticotrophin-releasing-factor (CRF) receptor.

There is some evidence that leptin's actions in the brain may involve CRF or a related neuropeptide. Expression of *c-fos* (a measure of neuronal activation) increases in the paraventricular nucleus of the hypothalamus in response to intracerebroventricular (icv) injection of leptin[5]. Leptin also induces expression of CRF mRNA in the paraventricular nucleus[6], and leptin receptors are present on CRF-containing neurons[7]. Although CRF is best known for its involvement in stress responses, it also potently inhibits food intake and mediates several actions of cytokines in the brain[8,9]. The regulation of energy balance probably involves CRF because it inhibits food intake and stimulates energy expenditure and sympathetic nervous system activity[9]. In addition, the CRF-like neuropeptide urocortin, which also acts at CRF receptors, is a more potent suppressor of appetite than CRF[10].

The experiments described here test the hypothesis that CRF or urocortin mediates the actions of leptin on food intake. The CRF-receptor antagonist D-Phe CRF_{12-41} has similar affinity for type I and type II CRF receptors[11]. Intracerebroventricular administration of leptin (Insight Biotechnology, UK; 4 µg per rat at 18:00 h) to individually housed, free-feeding male Sprague-Dawley rats (250–300 g) inhibited food intake, measured 1 h prior to the injection and again 14 h afterward, by 50% ($p<0.001$) compared to vehicle-treated animals (**Fig. 1a**). Administration of the CRF-receptor antagonist D-Phe CRF_{12-41} (Bachem, UK; 1–5 µg per rat) alone did not significantly affect food intake. Co-administration of the lowest dose (1 µg per rat) of D-Phe CRF_{12-41} with leptin had no significant effect on food intake, but higher doses (2.5 and 5 µg per rat) attenuated effects of leptin on food intake by 58% ($p<0.001$), such that food intake of these animals was restored to 80% of the intake of vehicle-treated controls. Leptin injection also caused marked loss of body weight (12 ± 3 g) over 14 h, which was attenuated by cotreatment with all doses of CRF receptor antagonist used (**Fig. 1b**).

The antagonist D-Phe CRF_{12-41} has been used widely at doses similar to those used here to inhibit actions of CRF[12]. This antagonist (5 µg, icv; $n = 5$) failed to influence food intake or body weight gain in vehicle-treated rats ($n = 10$) and did not influence the hypophagia or reduced body-weight gain induced by icv injection of prostaglandin E_2 (500 ng per rat, icv; $n = 6$), suggesting that its effects are specific to the CRF receptor. (Food intake: vehicle, 28 ± 1 g; D-Phe CRF_{12-41}, 28 ± 1 g; PGE_2, 20 ± 1 g; PGE_2 and D-Phe CRF_{12-41}, 21 ± 1 g. Body weight gain: vehicle, 19 ± 1 g; D-Phe CRF_{12-41}, 18 ± 2 g; PGE_2, 11 ± 1 g; PGE_2 and D-Phe CRF_{12-41}, 11 ± 1 g).

Because effects of leptin were attenuated but not abolished by the CRF receptor antagonist, CRF or urocortin may act in conjunction with other mediators, such as melanocortin, which have been implicated in the regulation of appetite[13,14]. Nevertheless, these results indicate that CRF or another CRF-like neuropeptide, such as urocortin, is important for the actions of leptin on food intake.

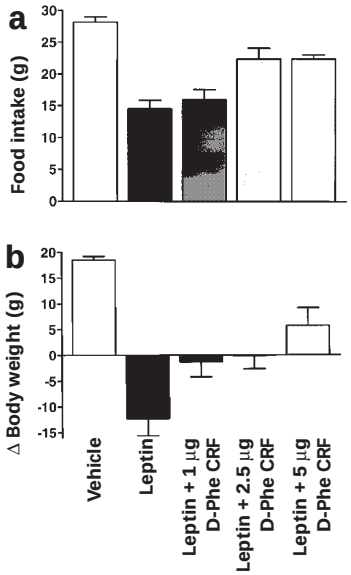

Fig. 1. Leptin-induced reduction in food intake and body weight is attenuated by D-Phe CRF. **(a)** Injection of leptin (4 µg, icv; $n = 10$) reduced food intake by 50% over 14 h ($p<0.001$) compared with vehicle-treated animals. Co-injection of D-Phe CRF_{12-41} (2.5 or 5 µg/rat, icv; $n = 6,7$) significantly attenuated this effect ($p<0.001$) to 80% of control food intake. **(b)** Injection of leptin (4 µg, icv; $n = 10$) reversed the gain in body weight seen in vehicle-treated rats over 14 h ($p<0.001$). Co-injection of D-Phe CRF_{12-41} (1, 2.5 or 5 µg/rat, icv; $n = 6,7$) significantly attenuated this effect in a dose-dependent manner ($p<0.001$). Animals were housed under a 12 h light-dark cycle (08:00 to 20:00) and had free access to food (pelleted rat chow, Beekay International, UK) and water. All drugs were made up in sterile water for injection. Data were analyzed using ANOVA followed by Newman-Keuls post test.

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