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The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress

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Abstract

We found that increasing ghrelin levels, through subcutaneous injections or calorie restriction, produced anxiolytic- and antidepressant-like responses in the elevated plus maze and forced swim test. Moreover, chronic social defeat stress, a rodent model of depression, persistently increased ghrelin levels, whereas *growth hormone secretagogue receptor* (*Ghsr*) null mice showed increased deleterious effects of chronic defeat. Together, these findings demonstrate a previously unknown function for ghrelin in defending against depressive-like symptoms of chronic stress.

Chronic stress induces changes in mood, feeding and metabolism by a poorly understood neurobiological mechanism. Recent studies have suggested that key metabolic signals may interact with CNS circuits to regulate reward and mood[1]. To further explore these links, we investigated the potential role of ghrelin, an important feeding peptide, in the development of depressive symptoms. Ghrelin is a hormone synthesized predominantly by specialized gastrointestinal endocrine cells and is released during periods of negative energy balance[2]. In response to energy insufficiency, ghrelin induces a potent feeding response via activation of the growth hormone secretagogue receptor (GHSR, ghrelin receptor)[2,3].

To determine whether ghrelin can affect mood symptoms, we physiologically increased ghrelin levels by restricting the food intake of mice with a diet containing 60% of normal calories for

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AUTHOR CONTRIBUTIONS

M.L. and J.M.Z. conceived, designed and performed these studies, analyzed the data and wrote the manuscript. I.S. assisted on all pharmacologic experiments. S.O.-L., S.A.R. and J.G.A. maintained the mouse colony and genotyped the mice. S.B. and S.J. assisted with the behavioral testing. M.Y. provided the orexin-deficient mice. J.K.E. and E.J.N. helped supervise and fund these studies, and critiqued the manuscript.

Note: Supplementary information is available on the Nature Neuroscience website.

ten days (60% calorie restriction; Fig. 1). This resulted in a fourfold increase in circulating levels of acylated ghrelin (calorie restricted wild-type mice: 7.93 ± 1.59 pg mL^{-1}, $n = 6$; wild-type mice fed *ad libitum*: 1.98 ± 0.37 pg mL^{-1}, $n = 5$; $P < 0.01$). Calorie-restricted wild-type mice showed robust anxiolytic- and antidepressant-like behavior in the elevated plus maze (EPM) and forced swim test (FST), respectively, as compared with wild-type mice fed *ad libitum* (controls; Fig. 1a,c). In contrast, genetic blockade of ghrelin signaling in *Ghsr*$^{-/-}$ mice negated these calorie restriction–associated anxiolytic- and antidepressant-like effects. Further analyses demonstrated that the observed differences between the two genotypes cannot be attributed to differences in sensorimotor coordination, general locomotor activity or body weight (Supplementary Figs. 1–3 online).

We used a pharmacologic approach to extend our food-restriction results. We subcutaneously injected C57BL6/J mice with a dose of ghrelin that induces potent feeding (Fig. 1f) and tested them in the EPM and FST 45 min later. Mice receiving ghrelin demonstrated significantly less anxiety- and depression-like symptoms in these tests compared with saline-injected controls (Fig. 1b,d).

Next, we determined whether ghrelin signaling regulates depressive symptoms in a mouse model of chronic stress. We used the chronic social defeat stress (CSDS) procedure, which subjects mice to ten daily bouts of social defeat by aggressive CD1 male mice[1,4] (Fig. 2). Mice subjected to CSDS showed lasting behavioral deficits, including social avoidance (Supplementary Fig. 4 online), which can be reversed by chronic, but not acute, antidepressant treatment[4]. Following CSDS, defeated C57BL6/J mice had significantly elevated levels of ghrelin that persisted for at least 4 weeks after the last defeat ($P < 0.02$; Fig. 2a). This finding is consistent with previous studies that have demonstrated increases in gastric ghrelin mRNA or total plasma ghrelin after acute stress[5,6].

We then tested *Ghsr*$^{-/-}$ mice and their wild-type littermates in CSDS to determine the role of these elevated ghrelin levels in the development of depressive symptoms. *Ghsr*$^{-/-}$ mice showed significantly greater social avoidance than wild-type littermates, thus indicating an exacerbation of the depressive-like symptoms normally induced by CSDS ($P < 0.002$; Fig. 2c). Moreover, although there was no difference in body weight following CSDS between the two genotypes (Fig. 2b), food intake was significantly elevated in wild-type, but not *Ghsr*$^{-/-}$, mice (Fig. 2d). These findings suggest that activation of ghrelin signaling pathways in response to chronic stress may be a homeostatic adaptation that helps an individual cope with stress, but at the expense of increased caloric intake.

Ghrelin's antidepressant- and anxiolytic-like actions might involve the engagement of neurons in the ventral tegmental area or hippocampus, both of which express GHSRs, experience ghrelin-induced modulation of synapse formation and are important sites of mood regulation[4,7,8]. Ghrelin's antidepressant actions also may include direct and/or indirect activation of orexin-containing neurons in the lateral hypothalamic area, as we have recently shown that orexin neurons are required for the antidepressant-like effect of calorie restriction[1]. Consistent with this hypothesis, ghrelin can induce c-Fos in orexin neurons and can stimulate the activity of isolated orexin neurons[9,10]. We found that ghrelin's antidepressant-like effects in the FST were blocked in mice lacking orexin (Fig. 1e).

These data reveal a unique and previously unrecognized function for ghrelin in the regulation of mood symptoms. Our data suggest that chronic stress, such as repeated social defeat, can elevate ghrelin levels. Although unclear, stress may act to increase circulating ghrelin via direct stimulation of ghrelin cells by catecholamines following activation of the sympathetic nervous system[11]. The ghrelin response then helps the animal cope with the stress by generating anxiolytic- and antidepressant-like behavioral adaptations. These results may also be relevant

in the psychopathology of conditions with known alterations of ghrelin, such as anorexia nervosa[12].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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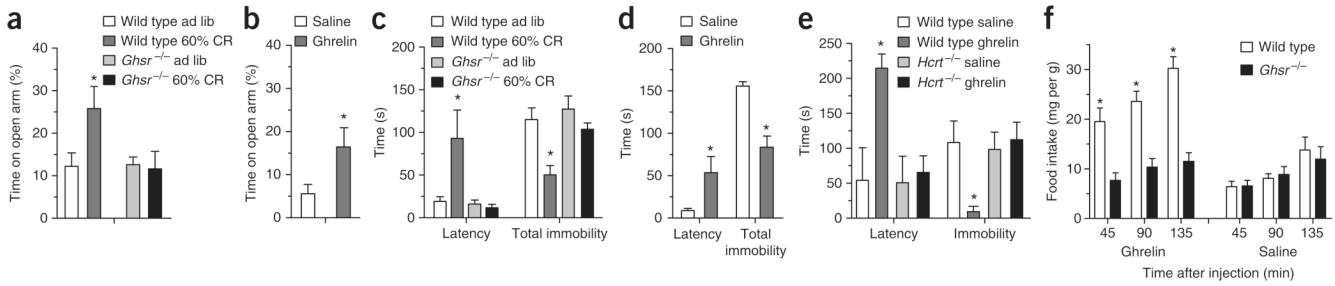

Figure 1.

Anxiolytic- and antidepressant-like effects of ghrelin signaling. (**a**–**d**) Calorie restriction (CR) induced an anxiolytic-like effect in the EPM (**P* < 0.02, **a**) and an antidepressant-like effect in the FST in wild-type, but not *Ghsr*$^{-/-}$, mice (latency to immobility, **P* < 0.002; total immobility, **P* < 0.02; **c**). Wild type ad lib indicates wild-type mice fed *ad libitum*. Administration of ghrelin (2 μg per g of body weight subcutaneously), but not saline, produced an anxiolytic-like effect in the EPM (**P* < 0.05; **b**) and an antidepressant-like effect in the FST in wild-type mice 45 min after injection (latency to immobility, **P* < 0.04; total immobility, **P* < 0.0002; **d**). (**e**) The ghrelin-induced antidepressant-like effect that we observed in wild-type mice in the FST was absent in orexin-deficient (*Hcrt*$^{-/-}$) mice (*n* = 5 for both wild-type groups, *n* = 6 for the saline-treated *Hcrt*$^{-/-}$ group, *n* = 7 for the ghrelin-treated *Hcrt*$^{-/-}$ group). (**f**) Food intake responses of *Ghsr*$^{-/-}$ and wild-type littermates following subcutaneous administration of ghrelin (2 μg per g) or saline in a crossover fashion at 10–11 weeks of age and again 1 month later. Statistically significant differences between food intake of ghrelin-injected wild-type mice and that of similarly treated *Ghsr*$^{-/-}$ littermates and littermates treated with saline are indicated (**P* < 0.001, *n* = 6 per group). Data are mean ± s.e.m. See Supplementary Methods online for detailed methods.

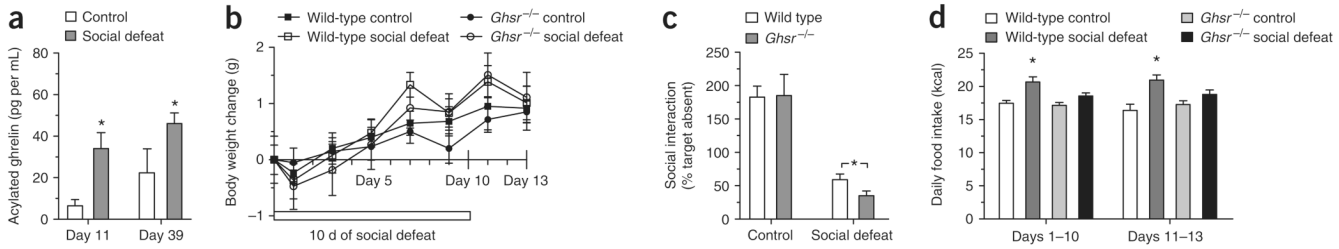

Figure 2.

Ghrelin signaling regulates social isolation after CSDS. (**a**) Acylated ghrelin was persistently elevated after CSDS in wild-type C57BL6/J mice (significant effect of treatment, **P* < 0.02; *post hoc* analysis showed no significant effect of day in the control mice; *n* = 5 in the control group and 10 in the CSDS group). (**c**) *Ghsr*$^{-/-}$ mice showed increased social avoidance after CSDS (**P* < 0.05). (**b,d**) Although there is no effect on body weight in any group either during or shortly after CSDS (**b**), increased food intake was induced during the 10 d of CSDS and maintained for at least 3 d following CSDS in wild-type, but not *Ghsr*$^{-/-}$, mice (during CSDS (days 1–10), **P* < 0.002; after CSDS (days 11–13), **P* < 0.002; *n* = 6 in each control group, 9 in the wild-type defeated group and 10 in the *Ghsr*$^{-/-}$ defeated group; **d**). Data are mean ± s.e.m.