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CENTRAL ADMINISTRATION OF GHRELIN ALTERS EMOTIONAL RESPONSES IN RATS: BEHAVIOURAL, ELECTROPHYSIOLOGICAL AND MOLECULAR EVIDENCE

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Abstract—The orexigenic and pro-obesity hormone ghrelin targets key hypothalamic and mesolimbic circuits involved in energy balance, appetite and reward. Given that such circuits are closely integrated with those regulating mood and cognition, we sought to determine whether chronic (>2 weeks) CNS exposure to ghrelin alters anxiety- and depression-like behaviour in rats as well as some physiological correlates. Rats bearing chronically implanted i.c.v. catheters were treated with ghrelin (10 μg/d) or vehicle for 4 weeks. Tests used to assess anxiety- and depression-like behaviour were undertaken during weeks 3–4 of the infusion. These revealed an increase in anxiety- and depression-like behaviour in the ghrelin-treated rats relative to controls. At the end of the 4-week infusion, brains were removed and the amygdala dissected for subsequent qPCR analysis that revealed changes in expression of a number of genes representing key systems implicated in these behavioural changes. Finally, given the key role of the dorsal raphe serotonin system in emotional reactivity, we examined the electrophysiological response of dorsal raphe neurons after a ghrelin challenge, and found mainly inhibitory responses in this region. We demonstrate that the central ghrelin signalling system is involved in emotional reactivity in rats, eliciting pro-anxiety and pro-depression effects and have begun to explore novel target systems for ghrelin that may be of importance for these effects.

Key words: emotional reactivity, anxiety, depression, memory, GHS-R1A, serotonin.

Ghrelin, a stomach-derived hormone (Kojima et al., 1999), increases food intake and fat mass (Tschöp et al., 2000; Theander-Carrillo et al., 2006). Apart from the reported metabolic effects that involve hypothalamic actions it is becoming increasingly apparent that ghrelin's neurobiological actions extend to systems involved in memory (Diano et al., 2006), reward (Jerlhag et al., 2006, 2007, 2009; Wellman et al., 2008) and even to systems involved in the cognitive processing of visual food cues (Malik et al., 2008). These effects are consistent with the reported distribution of the growth hormone secretagogue receptor (GHS-R1A), the only identified receptor for ghrelin that is present in, for example, discrete hypothalamic, mesolimbic, tegmental and hippocampal areas (Guan et al., 1997; Zigman et al., 2006). Our discovery that ghrelin targets a mesolimbic circuit, the so-called, "cholinergic dopaminergic reward link" (Jerlhag et al., 2006, 2007), a pathway important for the incentive value of natural and artificial rewards, implicates the central ghrelin signalling system in reward-seeking behaviour.

Given the emerging neurobiology of ghrelin action, surprisingly little is known about its effects on mood. In rodents, acute peripheral, as well as central ghrelin injection (both i.c.v. and site specific: amygdala, dorsal raphe nucleus, hippocampus) induce anxiety-like behaviour (Asakawa et al., 2001; Carlini et al., 2002, 2004). Furthermore, suppression of central ghrelin action by administration of antisense DNA for ghrelin caused a decrease in anxiety- and depression-like behaviour in rats (Kanehisa et al., 2006). In contrast to these findings, Lutter and colleagues reported a decrease in anxiety- and depression-like behaviour in mice after peripheral ghrelin injection as well as after starvation (Lutter et al., 2008). Surprisingly little is known about the effects of chronic ghrelin exposure on these behaviours, forming a key aim of the present study. Even though, genetic and pharmacological studies suggest that ghrelin may be of clinical relevance for psychiatric conditions like mood disorders in man (Schmid et al., 2006; Kurt et al., 2007; Nakashima et al., 2008) some contradictory data have been published (Emul et al., 2007; Kluge et al., 2009).

Here we provide the first description of the effects of chronic stimulation of the central ghrelin signalling system on mood. Thus, in behavioural studies we investigated anxiety- and depression-like behaviour in rats centrally infused with ghrelin for 28 days, and have begun to explore the neurobiological mechanisms underpinning these effects of ghrelin.

EXPERIMENTAL PROCEDURES

Animals

Rats were maintained in a controlled environment (12-h light schedule, 21–22 °C) with food and water available *ad libitum*. For

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Abbreviations: BWB, black and white box; Cnr1, cannabinoid receptor 1; Crhr1, corticotrophin releasing hormone receptor 1; CV, coefficient of variation; EPM, elevated-plus-maze; FST, forced-swim test; Gabra3, gamma-aminobutyric acid A receptor, alpha 3; Gabra5, gamma-aminobutyric acid A receptor, alpha 5; GHS-R1A, growth hormone secretagogue receptor 1A; Grm5, glutamate receptor metabotropic 5; HFD, high fat diet; HPA, hypothalamo-pituitary-adenal; Htr1a, serotonin receptor 1a; IGF-1, insulin-like growth factor 1; OF, open field; qPCR, quantitative polymerase chain reaction; Slc6a3, solute carrier family 6 (neurotransmitter transporter, dopamine), member 3; Syp, synaptophysin.

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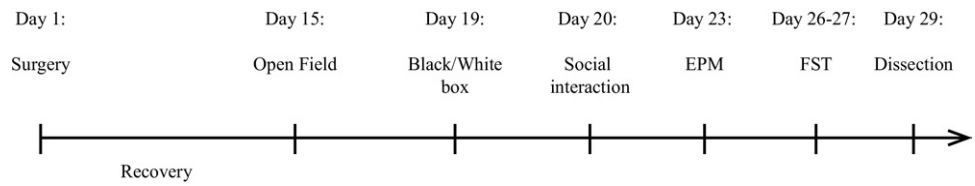

Fig. 1. Timeline of the experimental schedule.

behavioural/molecular studies, male Sprague-Dawley rats (270–300 g; B&K Universal, Sollentuna, Sweden) were used. After surgery, they were housed individually. Behavioural tests were carried out during the light phase (0800 h–1300 h). Rats were tested in a balanced order and all behavioural analyses were done by an experimenter who was unaware of the drug treatment. The behaviour was recorded via a camera directly linked to a computer located in the adjacent room. For *in vitro* electrophysiological studies male Sprague-Dawley rats (80–150 g; B&K Universal, Sollentuna, Sweden and Charles River, Germany) were used. All procedures were approved by the Göteborg Animal Experiment Ethics Committee.

Surgical procedure for central ghrelin administration

Rats were anesthetised (60–75 mg/kg Ketalar® and 0.5 mg/kg Domitor® IP; Pfizer, Sweden; Orion Co, Finland) and placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). An i.c.v. cannula (Alzet Brain Infusion Kit II, Durect Corporation, Cupertino, Canada) was inserted into the lateral ventricle using the following coordinates from bregma: AP −0.6, ML −1.4, V −2.3. The cannula was anchored to the skull with two jeweller screws (EICOM, Kyoto, Japan) and Paladur dental cement (Heraeus Kulzer, Hanau, Germany). The cannula was connected via polyethylene catheter to an osmotic minipump (Alzet Mini-Osmotic Pump Model 2004, Durect Corporation, Cupertino, Canada, flow rate, 0.25 μl/h for 28 days) implanted s.c. in the back of the animals. The catheter and the osmotic pump were filled with saline (NaCl 0.9%) or with saline containing ghrelin (10 μg/animal/d; gift from Rose Pharma A/S, Copenhagen, Denmark). The selected i.c.v. dose of ghrelin was based on previous publications that reported effects on peripheral metabolic parameters (Theander-Carrillo et al., 2006). After surgery, rats were allowed a 15-day recovery period before commencing the behavioural testing.

Metabolic parameters and hormone measurement

Regular measurements of food intake and body weight were made throughout the study period, and were limited to every fourth day during the behavioural testing period. At the end of the 28 days of treatment, rats were killed by decapitation and fat tissues (mesenteric, retroperitoneal, inguinal, reproductive adipose tissue and brown adipose tissue) were dissected and weighed. Trunk blood was collected in order to measure plasma IGF-1 and corticosterone, hormones that could provide a link between ghrelin action and CNS effects on emotional reactivity. IGF-1 was measured using a radioimmunoassay kit from Mediagnost (Reutlingen, Germany; assay sensitivity was 0.02 nmol/L). Corticosterone levels were determined in 50 μl plasma samples using a radioimmunoassay kit from MP Biomedicals (Orangeburg, NY, USA).

Behavioural testing

Anxiety-related behaviour was investigated in four widely used tests in rodents: the open field test, the black and white box, the social interaction test and the elevated plus maze (Ramos and Mormede, 1998; File and Seth, 2003; Cryan and Holmes, 2005). Depression-like behaviour was assessed in the forced swim test (Porsolt et al., 1977). The order of the tests was chosen to reduce as much as possible the influence of the successive testing on the outcomes of each test. Thus behavioural tests were arranged such that there was a progressive increase in the stress evoked by the test. A timeline of the experimental schedule is given in Fig. 1.

Open field test. The open field consisted of a wood box ($60\times60\times60$ cm^3) in which the open field was divided into a 25×25 cm^2 central zone and a border zone surrounding it. The illumination at floor level was 300 lux. In order to evaluate locomotor activity, the floor was divided in 16 equal squares. At the beginning of the test, rats were individually placed in the open field, and their behaviour was analysed during the 10-min test period: time spent in the central zone, the number of entries into the central zone, the number of groomings, the time spent grooming, the number of rearings in the central and peripheral zone and the number of line crossings. The arena was cleaned between each test session.

Black and white box. The box was made of wood and divided into two compartments, connected by an opening (5×5 cm^2 wide). The first compartment ($18\times27\times27$ cm^3 high) was painted in black and covered by a black top giving an illumination of 0 lux. The other compartment ($27\times27\times27$ cm^3 high) was painted in white and lit by a white incandescent bulb (100 lux). The floor of the box was cleaned before each trial. At the beginning of each 10-min trial, the rat was placed in the centre of the white compartment facing the opening. The behavioural parameters scored were: the latency until the first entry into the black compartment and the number of entries into it, the latency until the first entry into the white compartment (after the first entry into the black compartment) and the time spent there as well as the number of rearing into the white compartment.

Elevated-plus maze. Two open arms (50×10 cm^2) surrounded by a 1 cm high Plexiglas and two closed arms ($50\times10\times38$ cm^3 high walls) emerged from a central platform. The apparatus was made from dark grey PVC and the arms were elevated 73 cm above the floor. A white incandescent bulb provided a light intensity over the open arms of 100 lux and of 60 lux over the closed arms. The behavioural parameters scored were: the number of entries into the closed arms and in all arms, the number of open arm entries (expressed as percentage of the total number of entries; an entry was counted when both forepaws were placed on the respective arm), the time spent there (expressed as the percentage of the total time spent in all arms), the total time spent on all arms, the number of partial entries, the number of stretched attends in closed and open arms, the number of head dips.

Social interaction test. Two rats from the same treatment group were placed together in the experimental cage in a wood box that was used previously for the open field test under bright light (700 lux) for a 10-min observation period. Interaction time was recorded manually and consisted of active behaviours such as grooming, chasing and playing. The number of entries into the central zone and the time spent there, the number of rearing, the number of line crossings, the latency until the first self-grooming, the number of grooming and the time spent grooming were analysed. Five rat pairs were tested per treatment group.