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Short communication

Selective melanocortin MC_4 receptor blockage reduces immobilization stress-induced anorexia in rats

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Abstract

We investigated the effects of selective melanocortin MC_4 receptor blockage on immobilization stress-induced anorexia. Male rats were subjected to immobilization once a day for 4 days. Prior to each of the stress treatments, the rats were injected i.c.v. (intracerebroventricularly) with either saline or the melanocortin MC_4 receptor antagonist HS014 (cyclic [AcCys^{11}, D-Nal^{14}, Cys^{18}, Asp-NH_2^{22}]β-MSH-(11–22) (melanocyte-stimulating hormone). Rats subjected to neither stress nor i.c.v. injections served as controls. The results showed that the cumulative food intake and body weight gain in the stressed group treated with HS014 was significantly higher than in the stressed group and significantly lower than in the control group. Repeated injections of the melanocortin MC_4 receptor antagonist were effective and there were no signs of tachyphylaxis. This is the first report showing that melanocortin MC_4 receptor blockage can relieve an anorectic condition, which may indicate that melanocortin MC_4 receptor blockage is an effective way to treat anorectic disorders.

Keywords: Melanocortin receptor; MSH (melanocyte-stimulating hormone); HS014; Food intake; Immobilization stress; Anorexia

1. Introduction

Exposure of living organisms to any type of stress markedly activates the sympathoadrenal and HPA (hypothalamo–pituitary–adrenocortical) systems. These major stress systems interact at several levels in the periphery and in the brain. Stressful situations bring about complex changes in the physiological status involving several hormones. The HPA axis involves the peptide hormones CRF (corticotropin releasing factor) and ACTH (adrenocorticotropin hormone) and the glucocorticoids in an ordered and well-characterized chain of physiological processes (Assenmacher et al., 1995). It is well known that CRF induces the release of ACTH as well as of other melanocortin peptides, like α-MSH (melanocyte-stimulating hormone), from the pituitary (Eberle, 1988). ACTH acts on the melanocortin MC_2 receptor in the adrenal gland, whereas the other melanocortins (MSH peptides) do not bind to this receptor. ACTH and the other melanocortins may also mediate their effects through the central melanocortin MC_3 and MC_4 receptors or the peripheral melanocortin MC_1 and MC_5 receptors (for review, see Adan and Gispen, 1997).

It was discovered already in the 1980s that melanocortins themselves induce anorexia (Poggioli et al., 1986; Vergoni et al., 1986). Knock-out of the melanocortin MC_4 receptor in mice has demonstrated that this particular melanocortin receptor is a major mediator of weight homeostasis control by MSH peptides and the agouti peptide (Huszar et al., 1997). Recently, we developed the first truly selective melanocortin MC_4 receptor antagonist, termed HS014 (cyclic [AcCys^{11}, D-Nal^{14}, Cys^{18}, Asp-NH_2^{22}]-β-MSH-(11–22) (Schiöth et al., 1998). This peptide, an agouti mimetic, is very potent in increasing food intake in rats (Kask et al., 1998b; Vergoni et al., 1998). On the bases of studies with this peptide, SHU9119 (cyclic [Nle^4, Asp^5, D-Nal^7, Lys^{10}]α-MSH-(4–10)), a non-selective melanocortin MC_4 receptor antagonist, and MTII (cyclic [Nle^4, Asp^5, D-Phe^7, Lys^{10}]α-MSH-(4–10)), a non-selective melanocortin MC_4 receptor agonist (Al-Obeidi et al., 1989; Hruby et al., 1995; Schiöth et al., 1997), it has been postulated that melanocortin MC_4 receptor signalling is downstream to leptin signalling (Seeley et al.,

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1997; Kask et al., 1998c). Moreover, new data suggest that feeding induced by weakening of tonus in melanocortinergic neurons may be mediated through activation of the neuropeptide Y-ergic system (Kask et al., 1998a). Food intake is, however, a very complex behavior involving an increasing number of other central hormones such as CRF, orexins (Sakurai et al., 1998) and CART (catecholamine-amphetamine related transcript) (Kristensen et al., 1998), among others.

In this study we investigated whether immobilization stress-induced anorexia was affected by single and repeated i.c.v. (intracerebroventricularly) administration of the selective melanocortin MC_4 receptor antagonist HS014.

2. Materials and methods

2.1. Animals and surgery

Adult Lister–Hooded male rats (Harlan Nossan, Correzzana, MI, Italy) were individually housed (25 cm × 40 cm × 15 cm Makrolon cages) in climatized colony rooms (21 ± 1°C; 60% humidity) with a 12/12-h light/dark cycle (lights on 2300–1100). The rats were allowed free access to food and water except between 0900 and 1100, when food and water were removed. The 22-h food consumption and body weight were measured during this period. Pre-weighed food was presented at 1100 and measured after 1 h, 2 h and 3 h. After 22-h food intake had stabilized, stainless-steel guide cannulae (23 gauge) (Plastic Products, Roanoke, VA, USA) were stereotaxically implanted in the rats in both lateral ventricles, to a depth of 0.5 mm above the ventricles (measured in millimeters from the bregma: AP = −0.8; $L = 1.4$; $V = 3.25$) (Paxinos and Watson, 1982), under ketamine plus xylazine anesthesia (115 + 2 mg/kg i.p.; Farmaceutici Gellini, Aprilia, Italy and Bayer, Milano, Italy, respectively), and fixed to the skull with screws and dental acrylic. A removable plug, which extended 0.5 mm below the tip of the guide cannula, was kept in place except during the drug injections. Correct placement was verified at the end of the experiment by injecting 2 μl of Toluidine blue dye through an

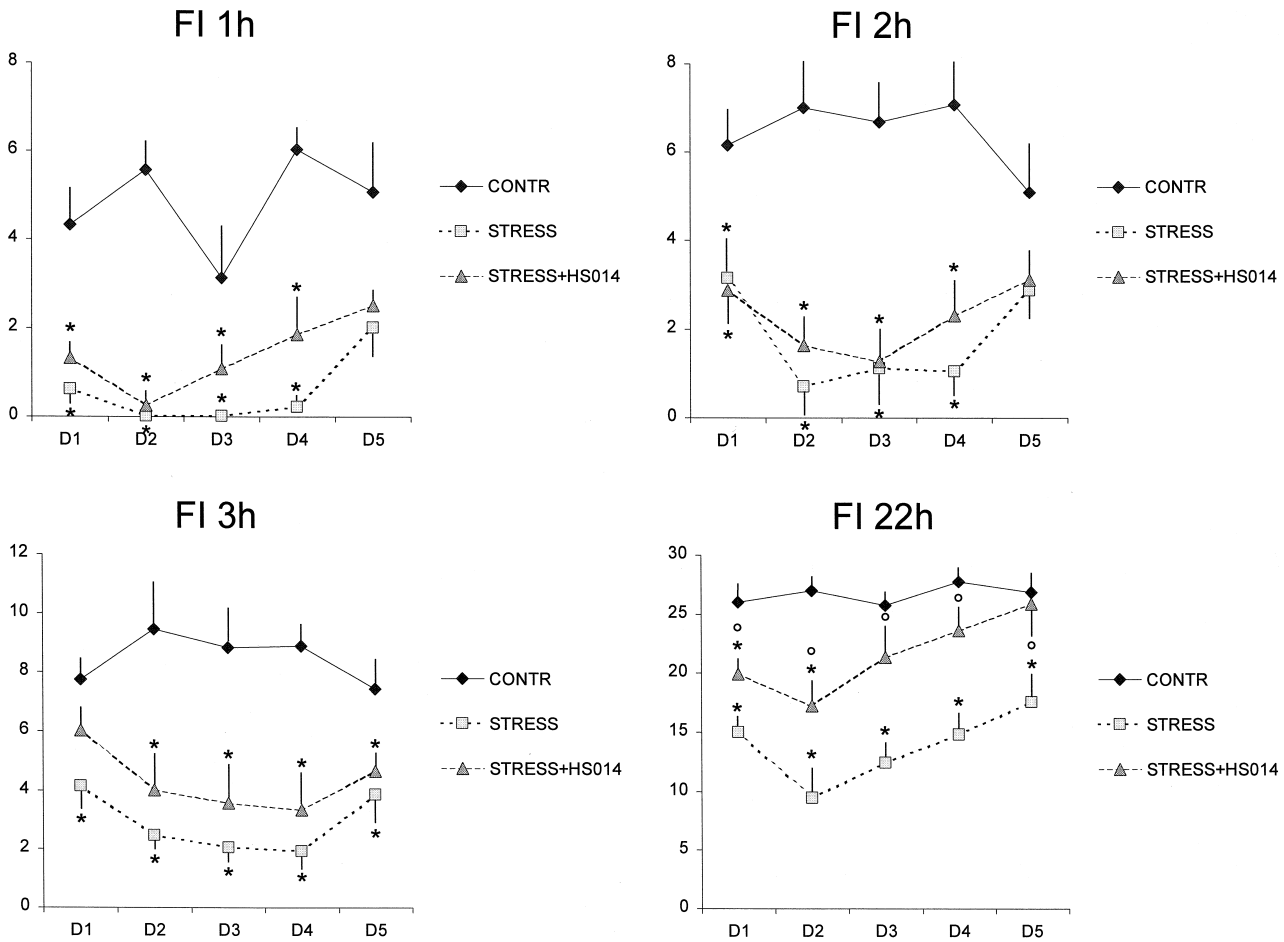

Fig. 1. Influence of HS014 on food intake following immobilization stress in Lister–Hooded rats from day 1 (D1) to day 5 (D5). CONTR: control group; STRESS: immobilized group; STRESS + HS014: immobilized group i.c.v. treated with HS014 (10 μg/rat) 5 min before stress. Values (means ± S.E.M.) represent cumulative food intake (FI) 1 h, 2 h, 3 h and 22 h after stress. $N = 5–6$ per group. Data were analyzed by means of ANOVA followed by the Student–Newmann–Keuls test for individual comparisons. $* P < 0.05$ vs. SALINE; $^{8}P < 0.05$ vs. STRESS.

internal cannula used for drug (or saline) injection (which extended 0.5 mm below the tip of the implanted guide cannula), followed by decapitation under ethyl ether anesthesia and dissection of the brain. Data obtained from improperly implanted animals were discarded.

2.2. Drugs and treatments

HS014 was synthesized using a solid-phase approach and purified by HPLC (high-performance liquid chromatography) as described earlier (Schiöth et al., 1998). The molecular weight of the peptide was confirmed by mass spectrometry. The peptide was dissolved in saline and injected into a brain lateral ventricle (i.c.v.), in a volume of 5 μl, at the rate of 1 μl/20 s, via the i.c.v. internal cannula connected by polyethylene tubing to a 50-μl Hamilton syringe driven by a micrometric screw.

2.3. Immobilization experiments

The rats were immobilized by strapping their paws to restraining grids with plastic clamps. Immobilization was started at 10:15 and lasted 30 min (day 1 and day 2) or 15 min (day 3 and day 4). From day 1 to day 4, the animals were treated with i.c.v. injections of HS014 (10 μg/rat) 5 min before the beginning of the immobilization period. The rats were also treated 24 h after the last stress (day 5).

2.4. Statistics

Data from each day were submitted to an overall analysis of variance followed by the Student–Newmann–Keuls test for individual comparisons between groups, when F values indicated a significant difference among treatments.

2.5. Animal ethics

Experimental procedures were carried out in accordance with guidelines of the European Community, local laws and policies (D.L.vo 116/92).

3. Results

Rats were subjected to immobilization stress for 4 days. The food intake data showed that the stressed rats ate significantly less than the control group at all observation points (Fig. 1). The relative influence of the stress treatment was greatest after 1 h, less and similar for 2 h and 3 h and lower, but still very significant after 22 h. The rats which were stressed and treated with HS014 had, in all cases, a higher food intake than the stressed untreated rats. This difference was however, only significant at the 22 h observation point from day 1 to day 4. The 22 h data show that the stressed HS014-treated rats ate significantly less than the control rats but significantly more than the stressed untreated rats on days 1 and 2. On days 3, 4 and 5, the stressed HS014-treated animals had a significantly higher food intake than the stressed untreated rats, and a not significantly lower food intake than the control rats.

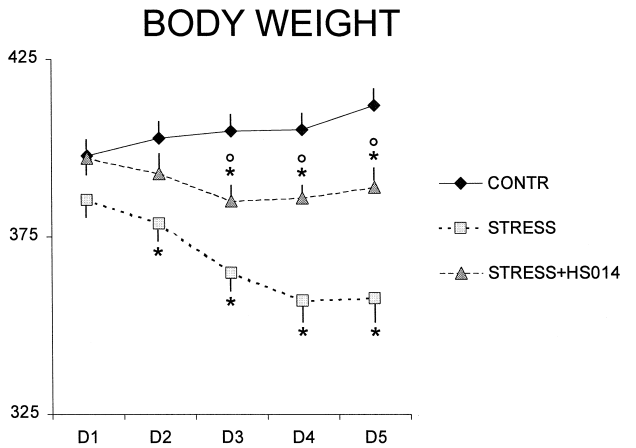

Fig. 2. Influence of HS014 on body weight gain following immobilization stress in Lister–Hooded rats from day 1 (D1) to day 5 (D5). CONTR: control group; STRESS: immobilized group; STRESS + HS014: immobilized group i.c.v. treated with HS014 (10 μg/rat) 5 min before stress. Values are means ± S.E.M. $N = 5–6$ per group. Data were analyzed by means of ANOVA followed by the Student–Newmann–Keuls test for individual comparisons. $* P < 0.05$ vs. SALINE; $^{8}P < 0.05$ vs. STRESS.

The data concerning body weight gain are shown in Fig. 2. Body weight was significantly lower in the stressed group than in the control group on days 2, 3, 4 and 5. The stressed group treated with HS014 had a significantly lower body weight than the control group on days 3, 4 and 5 and significantly larger body weight then the stressed and untreated group at day 3, 4 and 5. On day 5, the stressed untreated group had a 13% lower body weight than the control group, while the stressed HS014-treated group had a 5.6% lower body weight than the control group.

4. Discussion

There are several neuroregulators found at various brain sites which are involved in the control of food intake. These include neuropeptide Y, leptin, CRF, oxytocin, orexins, CART and melanocortins among others. Food intake is a behavior that occurs for many reasons and different neuroregulators may participate in different stimuli. For example, neuropeptide Y may initiate feeding for energy needs, opioid peptides may provide the rewarding aspects of eating, and CRF may affect stress-induced eating.

In this study we took the advantage of using the first selective melanocortin MC_4 receptor antagonist HS014 to investigate whether it influenced food intake under stressed conditions. HS014 has been shown to block α-MSH-induced anorexia and also to reduce food intake and body weight in rats (Kask et al., 1998a,b,c; Vergoni et al.,

1998). Our data showed that food intake was very low in the first hour after immobilization stress and slightly increased during the second and the third hours. The anorectic effect of immobilization stress was also evident from the total daily food intake. The treatment with HS014 seemed to have a slight, not significant effect on food intake for the first three hours after the stress treatment. However, after 22 h, the cumulative food intake was significantly higher for the stressed HS014-treated rats compared with the stressed untreated rats. It is thus evident that melanocortin MC_4 receptor blockage can reduce stress-induced anorexia. Good pharmacokinetic data on HS014 are not available but earlier experiments indicate that the orexigenic effect of HS014 lasts at least 4 h (Kask et al., 1998b). Interestingly, our data show that repeated injections of HS014 have a repeated effect on food intake without there being any sign of tachyphylaxis. Looking at the cumulative food intake data after 22 h, it is interesting to note that the difference between food intake in the stressed rats treated or not with HS014 seemed to be consistent for each of the treatment days and that the stress tolerance which was observed was independent of HS014 treatment.

The data on body weight gain confirmed what was observed for food intake. The treatment with HS014 reduced the stress-induced body weight loss by more than a half. The stressed rats that received HS014 did not significantly lose weight during the test period, whereas the control rats gained about 4% (14 g). The body weight data confirmed also the notion that HS014 is effective after repeated injections. The data provide a further indication that the melanocortin MC_4 receptor may be a target for long-term treatment of weight disorders.

The underlying mechanism of how melanocortinergic neurons influence food intake is not known. Proopiomelanocortin (POMC)-synthesizing neurons are located in the arcuate nucleus from where they send their projections to the paraventricular nucleus and the dorsomedial hypothalamus. It is in these structures that endogenous ligands for the melanocortin MC_4 receptor, like α-MSH, β-MSH (Schiöth et al., 1996) and other melanocortins, may act. Our data may indicate that the inhibition of food intake which is induced by stress can be relieved by melanocortin MC_4 receptor blockage. However, the inhibition of stress-induced anorexia was only partial, which clearly indicates that stress-induced anorexia is not solely mediated through the melanocortin MC_4 receptor. The involvement of the melanocortin MC_4 receptor may be linked to the stress-induced release of melanocortins or be secondary due to the stress-induced release of CRF, although the latter seems to be unlikely as our unpublished data show that CRF-induced inhibition of food intake in rats is not affected by HS014. Recently, it was shown that the melanocortin receptor antagonist SHU9119 inhibited the melanocortin-induced activation of the HPA axis (Von Frijtag et al., 1998). It is thus possible that the rats treated with HS014 may have experienced the immobilization as less stressful than the controls.

In summary, we have for the first time demonstrated that melanocortin MC_4 receptor blockage can relieve an anorectic condition. The data indicate that melanocortin MC_4 receptor blockage may be an effective way to treat anorectic disorders.

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