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Extended therapeutic validation of an anti-MC4 receptor antibody in an animal model of anorexia nervosa

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Preliminary data report

Introduction

In addition to the psychosocial disturbances observed in anorexia nervosa patients, self-starvation also comes with a with variety of pathophysiological symptoms related to energy metabolism, such as behavioural hyperactivity and accelerated body weight loss. Energy metabolism is controlled by signals from the body, such as hormones, that give rise to feedback mechanisms in the brain enhancing or decreasing energy intake and energy expenditure. The melanocortin system in the brain is thought to play a central role in these energy homeostasis processes. One of the receptor molecules in this system is the Melanocortin 4 receptor (MC4r). To target energy metabolism processes, an antibody targeting the MC4r was recently created and initial findings showed that infusion of the antibody increased food intake and bodyweight after an intracerebroventricular (i.c.v.) injection in ad libitum fed rats (Peter et al., 2010).

In the Activity-Based Anorexia (ABA) paradigm, animals have limited access to food and have a running wheel in their home cage. When exposed to this paradigm, animals increase their activity in the running wheel and show accelerating body weight loss. Also, body temperature levels are decreasing. ABA studies in rats seem to be an essential next step for profiling the efficacy of anti-MC4r antibodies to further extend our insights into the therapeutic value of these antibodies for anorexia nervosa.

Objective

To extend the validation of the therapeutic potential antibody against the MC4-receptor for the indication of anorexia nervosa. First, we investigate the effects of MC4 receptor antibody administration on body weight, food intake and body temperature during free access of food. Second, we study the effects of this MC4r antibody on body weight, food intake, body temperature and running wheel activity when exposed to the ABA paradigm.

Methods

Female outbred Wistar rats were acclimatized for 14 days in a humidity and temperature controlled room with an 12-hour light/12-hour dark light-dark cycle. Prior to the experiments, animals were housed socially, in a cage with sawdust and a woodpile. Al described procedures were approved by the ethical committee on the use and care of animals, University Utrecht. Animals with body weight loss of 20% were removed from the experiment.

Surgery

An intracerebroventricular (ICV) cannula was implanted in the right lateral ventricle (1.0 mm anterior from Bregma, 1.0 mm lateral from midline and 4.5 mm ventral to the surface of the skull) under fentanyl/fluanisone and midazolam anesthesia. Glass-ionomer cement and stainless steel screws were used to secure the cannula in place. A transmitter was placed in the abdominal cavity. Post-operatively, rats were treated with carprofen at 0, 24 and 48 hour after surgery and were allowed to recover from surgery for two weeks.

MC4r antibody

Monoclonal antibodies were obtained from Applied Pharmacology, Biozentrum, University of Basel, Switzerland (Peter et al., 2010) and dissolved in sterile PBS at concentrations of 1.0 and 0.3 µg/ µl. I.c.v. administrations were performed using a Hamilton syringe and a syringe pump.

Ad libitum food conditions

After recovery from surgery, all animals were moved to their experimental cage in which access to the running wheel was blocked. After three days of baseline measurements an infusion of MC4r antibody (1 µg in 2 µl) was administered 1 hour before the start of the dark phase, the habitual activity phase. Antibody was injected in two doses (1,0 (H) and 0,3 (L) ug in a volume of 2 ul) and a control group received an injection of BSA (0,3 ug in 2 ul). Bodyweight, food intake body temperature and non-specific locomotor activity was registered for 3 days.

ABA conditions

The second experiment started with 17 days habituation of the animals to the same cage in which voluntary running wheel access was offered. Five days of food restriction combined with running wheel activity (the ABA paradigm). At the start of day 0, 1 and 2 of daily scheduled limited food access, animals received an ICV antibody injection. Body weight, food intake, running wheel activity, non-specific locomotor activity and body temperature were assessed. In total, data from 11 rats per group were analyzed; treatment was randomized and counter balanced with respect to the ICV treatments during ad libitum and ABA conditions. Before the start of the ABA experiment animals were allowed to habituate to the running wheel for 17 days. During ABA animals were food restricted to 1.5-hour food access per day for four days, starting at the beginning of dark phase. Bodyweight was assessed before and after food access. Food intake, running wheel activity, body temperature and non-specific locomotor activity was registered during the whole experiment.

Results

Under baseline ad libitum food conditions, the low dose of the Mc4r antibody significantly increased body weight (Figure 1). No significant effects were found for food intake, activity levels and body temperature under these conditions. When exposed to the ABA paradigm, the MC4r antibody significantly reduced food intake (Figure 2), body weight (data not shown), and voluntary running wheel activity levels (Figure 3). In addition, under these conditions of limited food access, the high dose of Mc4r antibody prevented the normally observed decline in body temperature levels under ABA conditions (Figure 4).

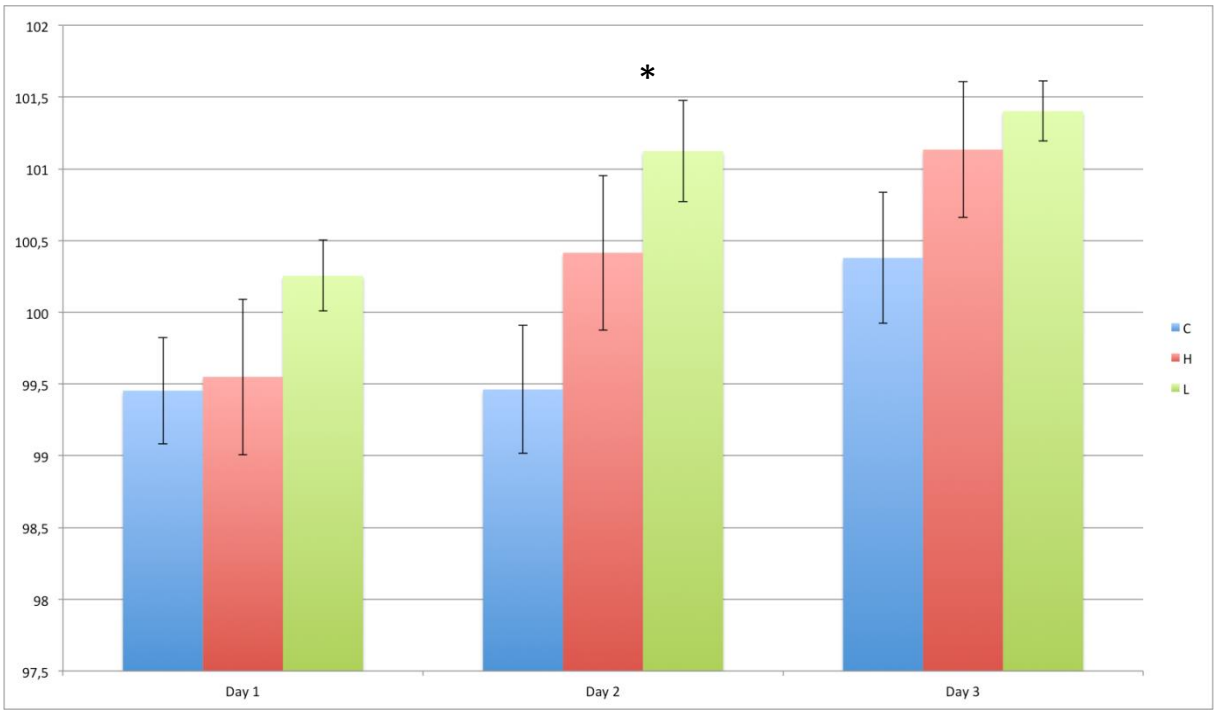

Figure 1. Body weight changes in percentages after a single ICV injection of vehicle (BSA), low (L; 0,3 ug), or high (H; 1,0 ug) doses of MC4r antibody. All animals have ad libitum access to food. Group*time interaction and between-subjects effect for group C vs. group L. At day 2 group L differs significantly from group C (*: $p \leq 0,05$).

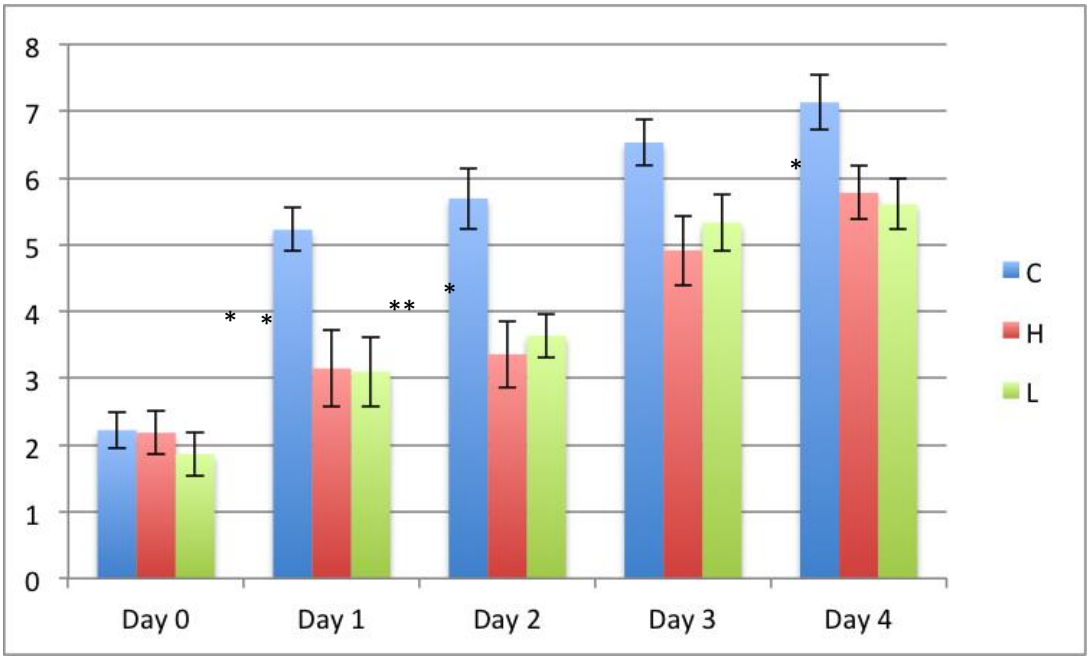

Figure 2. Levels of food intake in grams during ABA conditions. High (H) and Low (L) concentrations of the MC4r antibody did significantly decrease food intake compared to controls (C) during ABA (rmANOVA; p<0,01). Group*time and between-subjects: significant. *: p ≤ 0,05, **: p ≤ 0,01.

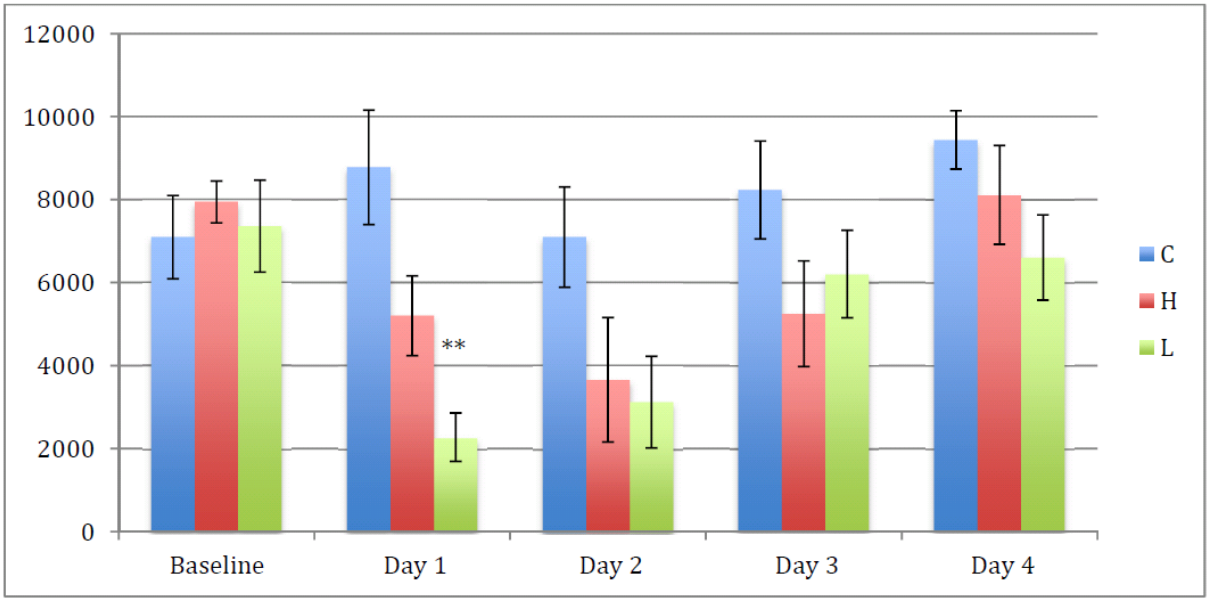

Figure 3. Voluntary running wheel activity levels during the ABA paradigm. Baseline is the average RWA of three days prior to the first injection. Baseline is the average of running wheel activity of three days prior to the first injection. At day 1, Low (L) concentration of MC4r antibody showed a decrease in RWA compared to controls (C) (ANOVA: p<0,01). Group*time: significant. **: p ≤ 0,01.

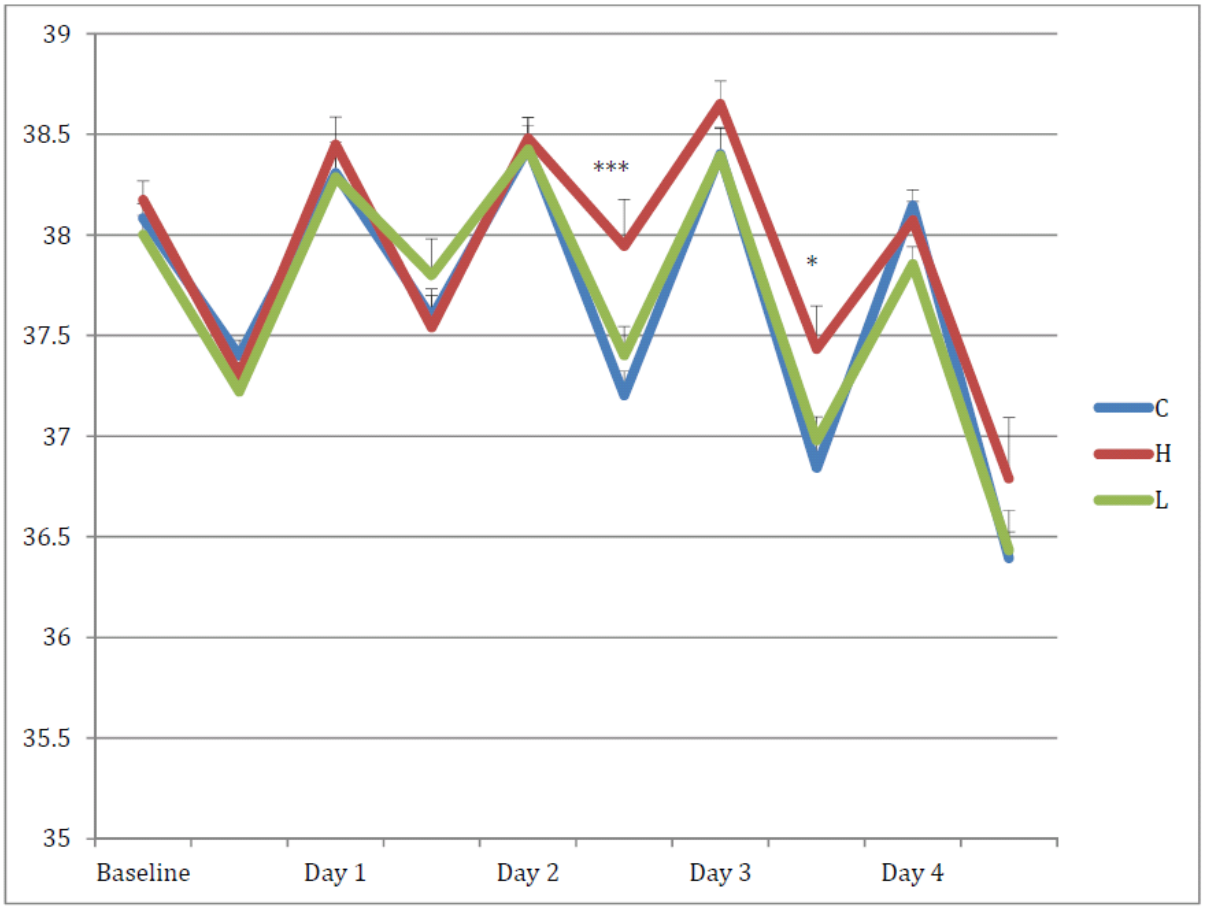

Figure 4. Body temperature levels (in grades Celsius) during ABA. Baseline is the average body temperature of three days prior to start ABA. Significant difference in body temperature at day 2 of the animals receiving the high concentration (H) (ANOVA: p=0,001). Group*time: significant. *: p ≤ 0,05, ***: p≤ 0,001.

Conclusions

The effects of the MC4r antibody on energy metabolism parameters under ad libitum food conditions are remarkable different from those under ABA conditions. Antibody administration during ad libitum food conditions stimulates food intake. Under ABA conditions the MC4r antibody seems to induce beneficiary effects by reducing hyperactive behavior and by promoting maintenance of body temperature. However, under ABA conditions, the MC4r antibody also decreased food intake, suggesting that this antibody has both beneficiary and worsening effects on rats exposed to ABA.

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