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ORIGINAL ARTICLE

Intranasal application of the melanocortin 4 receptor agonist MSH/ACTH(4–10) in humans causes lipolysis in white adipose tissue

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Objective: The melanocortin system has a highly significant role in the hypothalamic regulation of body weight and energy expenditure. In animals, intracerebroventricular infusion of melanocortin receptor 4 (MCR-4) agonists increases basal metabolic rate through activation of the sympathetic nervous system and subsequently reduces food intake. In humans, direct access of MCR-4 agonists to the central nervous system can be achieved by a transnasal route, which leads to weight loss with chronic administration. In the present study, we aimed at investigating the effects of intranasally administered MC4-R agonist MSH/ACTH(4–10) on lipolysis and sympathetic nervous system activity in healthy humans.
Design: Healthy normal weight, male volunteers ($n=10$) received either 10 mg MSH/ACTH(4–10) or placebo intranasally in a double-blinded randomized crossover design. Interstitial glycerol release was assessed by microdialysis in abdominal white adipose tissue (WAT) and in skeletal muscle (SM) of the forearm. Local blood flow, systemic blood pressure, heart rate and muscle sympathetic nerve activity (MSNA) within the superficial peroneal nerve were recorded at rest and after nitroprusside infusion.
Results: At 45 min after MSH/ACTH(4–10) administration WAT glycerol concentrations increased by 53.4 ± 19.3% compared with baseline conditions ($P<0.05$) and remained significantly higher throughout the experiment when compared with placebo ($P<0.05$) while local glycerol release in SM was not significantly affected. Resting MSNA was not altered by MSH/ACTH(4–10) administration; however, sympathoexcitation by intravenous nitroprusside was markedly elevated (MSH/ACTH(4–10) 569 ± 69% increase to baseline; placebo: 315 ± 64%; $P<0.01$).
Conclusion: Intranasally administered MCR-4 agonist MSH/ACTH 4–10 increases both subcutaneous WAT lipolysis and MSNA, which suggests a direct central nervous peptide effect in humans on key factors of human energy metabolism.
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Keywords: adipose tissue; melanocortins; sympathetic nerve system; microdialysis; microneurography

Introduction

Body weight is the result of the balance between energy intake and energy expenditure. This balance is under the central nervous control of hypothalamic centers and among the endocrine systems that mediate anorexigenic effects, the hypothalamic pro-opiomelanocortin system has a key role. Neurons within the arcuate nucleus containing melanocortin (MC) are activated by peripheral hormonal signals such as leptin and insulin, all of which lead to increased energy expenditure by sympathetic nervous system-induced lipolysis and diminished food intake through appetite reduction. Among the five subgroups of melanocortin receptors (MC-R) the MC4-R most likely initiates appetite regulation together with sympathoexcitation. MC4-R knockout mice develop hyperphagia, hyperinsulinemia and obesity, and likewise, humans with MC4-R mutations develop morbid obesity.[1] Thus, a promising strategy to reduce body weight would be the administration of MC4-R agonists which indeed has been shown to reduce body weight in animals as well as in humans.[2–4]

MSH/ACTH(4–10) is the core sequence of all melanocortins and binds selectively to MC4-R. In humans, MSH/ACTH(4–10) enters the cerebral fluid compartment after intranasal application[5] and leads to a decrease in total body

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fat after 6 weeks of continuous treatment in a recent study.[2] However, in those experiments MSH/ACTH(4–10) neither affected eating behavior nor cardiovascular sequelae such as heart rate or blood pressure (BP).

In the present study, we investigated the acute effects of intranasally administered MSH/ACTH(4–10) on lipolysis and sympathetic activity in healthy young humans. We used interstitial microdialysis to monitor subcutaneous glycerol release as a measure of lipolytic activity and microneurography of muscle sympathetic nerve activity (MSNA) within the superficial peroneal nerve both at rest and after baroreceptor reflex stimulation.

Methods

Subjects

In all, 10 healthy normal weight men aged between 25 and 30 years were included in the study. Exclusion criteria were a body-mass-index under 20 and over 25 kg m^{-2}, any kind of medication, alcohol or nicotine abuse, any history of depression, sleep disorders, metabolic or endocrine illness or hypertension. Furthermore, all participants had to have a stable body weight for at least 3 months before the experiment. All subjects were asked to refrain from excessive exercise for a period of 24 h before the experiments. A detailed history and a physical examination excluded current illness. The study was approved by the local ethics committee, and all participants gave their written informed consent before the experiments.

Microdialysis

Microdialysis was performed using a high-precision pump (CMA 106, CMA Microdialysis, Solna, Sweden) and a microdialysis double lumen catheter (CMA 60, cutoff 20 000 Da), which was inserted via a guidance needle into the adipose tissue 10 cm right or left from the umbilicus in both visits and into the antebrachial muscle of either forearm. The flow rate was set to 5 μl min^{-1}. A washout and equilibration period of 60 min was allowed before intranasal application of placebo or MSH/ACTH(4–10).

Laser doppler flowmetry

Changes in skin and adipose tissue blood flow were quantified by laser doppler flowmetry using a two channel Periflux system (Perimed 6005, Jårsfalla, Sweden). After a warm-up phase of 30 min laser doppler flow probes were calibrated with a motility standard. One probe (needle probe, Perimed) was then inserted at a 45°C angle to insure measurement of ATBF 1 cm in depth next to the microdialysis probe, and fixed to the skin with tape in order to minimize movement artifacts. A second laser doppler probe (skin probe, Perimed) was attached to the skin 10 mm apart from tip of the needle probe to avoid interference.

Hemodynamic monitoring

Heart rate was recorded online using a three-lead ECG, and breathing was monitored by a respiration transducer (Pneumotrace 1130, Adinstruments, Spechbach, Germany). Finger BP was measured continuously using the volume clamp method throughout the whole experiment (Finapres, Ohmeda 2600, Engelwood, CO, USA) and calibrated versus oscillometric BP measured on the contralateral arm (Welch Allyn, Skaneateles Falls, NY, USA).

Microneurography

Microneurography of sympathetic nerve activity within the lateral cutaneous nerve was performed as described previously.[6] ECG, continuous BP curves, respiration and LDF-signals were registered online with a multi-channel analogue/digital converter (Powerlab sp16, Adinstruments, Spechbach, Germany) at a sampling rate of 200 sec^{-1}. BP was calculated as 1-min averages of peak systolic and diastolic measurements, LDF curves were analyzed by calculating the area under the curve in 1-min intervals. Bursts representing sympathetic nerve discharge were identified manually and MSNA was calculated as bursts/minute and burst/heart rate, respectively.

Protocol

The study followed a balanced, randomized and double-blinded crossover protocol and subjects received either MSH/ACTH(4–10) 10 mg (Bachem, Heidelberg, Germany) or placebo (5 ml 0.9% saline). ACTH4-10 was dissolved in 5-ml sterile water shortly before its application by a third researcher who did not directly participate in the experiment. The interval between both experimental sessions was at least 4 weeks.

Subjects arrived at the research unit at 08:00 am after an overnight fast. After emptying their bladder they rested comfortably in a supine position with the upper body slightly (30°) elevated. A 16G venous canula (Becton Dickinson GmbH, Heidelberg, Germany) was inserted into the cubital vein for blood sampling. Microdialysis, laser doppler flowmetry, microneurography and hemodynamic monitoring were prepared, and then the equilibration period for the microdialysis probe was started. After 60 min of equilibration, experiments began with a 30-min baseline period during which microdialysis and blood samples were collected at intervals of 15 min. Then 2.5 ml of the study drug solution was administered into each nostril over a period of 5 min. Thereafter, dialysate samples were collected in 15 min intervals. Blood for analysis of ACTH, cortisol, plasma glycerol and glucose was sampled every 15 min during the first hour and in 30 min intervals, thereafter. All blood samples were immediately centrifuged and stored at −80° until further analysis. For baroreceptor reflex testing a bolus of 100 μg nitroprusside was given intravenously 150 min after administration of the study drug.

Biochemistry

Glycerol and glucose in dialysate and plasma were analyzed using a colorimetric enzymatic method in a CMA/600 analyzer (CMA Microdialysis). Concentrations of plasma free fatty acids were measured with an enzymatic method (Biotrol, Paris, France). Serum cortisol was determined by radioimmunoassay (DPC Biermann GmbH, Bad Nauheim, Germany). Plasma ACTH was determined by immunoluminometric assay (Lumitest, Brahms, Berlin, Germany). The interassay coefficient of variation for the ACTH and cortisol assay was below 10%. All samples were analyzed in duplicate in the same assay. Plasma norepinephrine and epinephrine levels were determined by high-performance liquid chromatography with electrochemical detection. The sensitivity was 15.64 pmol l^{-1} for norepinephrine and 15.46 pmol l^{-1} for epinephrine. The interassay coefficients of variation were 6.1 and 5.6% for norepinephrine and epinephrine, respectively.

Data of BP, heart rate, SNA, skin and adipose tissue perfusion was analyzed off-line using the CHART software (CHART 5, Adinstruments).

Because both glycerol release and MSNA critically depend on the position of the microdialysis probe and microneurography electrode, respectively, data were normalized and expressed as percentage of baseline. The average of the baseline period was set 100%.

We calculated a sample size of at least eight volunteers to reach a statistical power of 80% (significance level alpha 0.05, two tailed) to determine a 20% difference in interstitial glycerol concentrations between the two treatment conditions (NQuery Advisor sample size software 4.0, Statistical Solutions Limited, Cork, Ireland). We performed experiments in 10 subjects to allow for dropouts or experimental problems. Statistical analysis of our results relied on analysis of variance with the repeated measures factor 'time' and the group factor 'treatment' (GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego, CA, USA). When analysis of variance showed significant treatment effects, subsequent *post hoc* pairwise comparisons were performed. A Greenhouse Geisser-corrected *P*-value of <0.05 was considered significant. Results are presented as mean ± s.e.m.

Results

Intranasal (i.n.) administration of MSH/ACTH(4–10) resulted in a significant increase of interstitial glycerol in white adipose tissue (WAT) both with regard to baseline as well as compared with placebo administration (Figure 1). The treatment effect became significant 45 min after i.n. administration (difference to baseline 156.0 ± 17.6 vs 120.1 ± 11.7%, $P<0.05$) and interstitial glycerol levels remained higher thereafter throughout the experimental period (185.3 ± 22.8 vs 142.5 ± 19.1%, $P<0.05$). Glycerol in skeletal muscle (SM) was not significantly affected by MSH/ACTH(4–10) (difference to baseline 109.5 ± 14.9 vs 94.6 vs 6.1%, $P=0.12$). Similarly, plasma glycerol levels and FFA measured in 30 min intervals did not increase over time and were not affected by treatment. Neither did serum levels for cortisol, ACTH and insulin at baseline 60 and 120 min differ after MSH/ACTH(4–10) treatment. Plasma epinephrine was 22.3 ± 5.6 pmol l^{-1} at baseline and after 60/120 min 18.5 ± 1.3/20.4 ± 1.8 pmol l^{-1} in the MSH/ACTH(4–10) condition and 25.6 ± 3.6/28.1 ± 5.2 pmol l^{-1} after placebo ($P=0.18$). Similarly, norepinephrine concentrations were unaffected by MSH/ACTH(4–10) treatment.

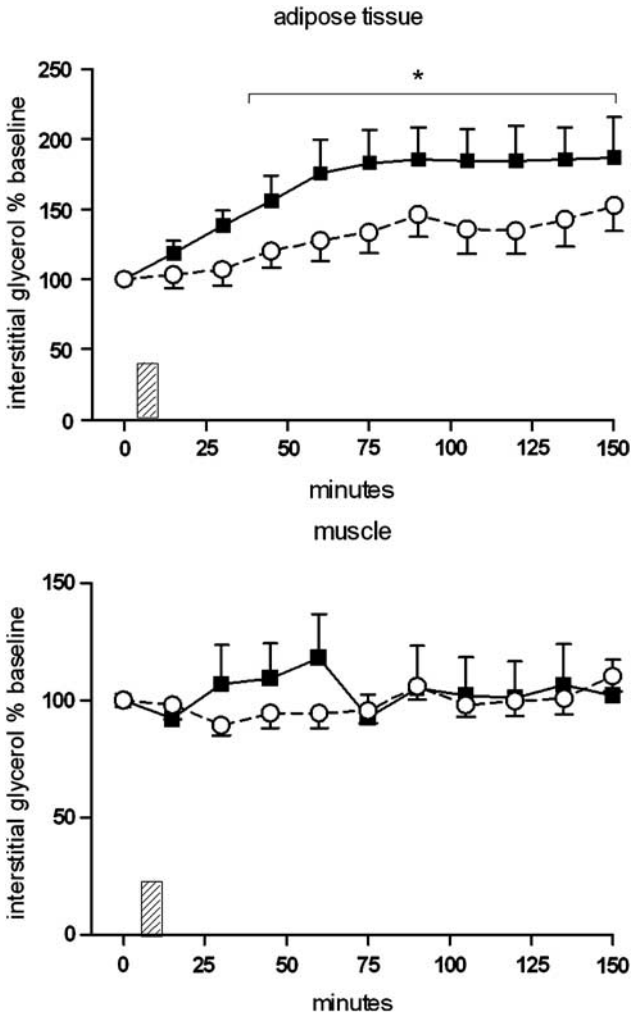

Figure 1 Interstitial glycerol in adipose tissue (upper panel) and in the forearm muscle (lower panel) after intranasal administration of 10 mg MSH/ACTH(4–10) (solid squares) and placebo (open circles) (*$P<0.05$ for factor treatment). The hatched bars mark the time of verum/placebo application.

Blood flow

Adipose tissue blood flow was not affected by MSH/ACTH (4–10), but increased independently from treatment over the

whole experimental period in most subjects ($P<0.05$ for repeated measures factor 'time').

MSNA

In all, eight recordings in the MSH/ACTH(4–10) condition and nine recordings in the placebo group were suitable for analysis of MSNA. In these sessions MSH/ACTH(4–10) had no effect on basal MSNA within the superficial peroneal nerve. Baroreceptor reflex testing with nitroprusside led to an increase in burst frequency (bursts/min) of 569 ± 69% in MSH/ACTH(4–10) conditions, but only 315 ± 64% after placebo. This difference was statistically significant ($P< 0.01$ for the factor treatment) (Figure 2).

Discussion

In humans intranasal (i.n.) administration of neuropeptides allows their direct access to the brain. After 30-min intranasal application of 10 mg MSH/ACTH(4–10), the peptide has been detected in the spinal fluid in previous experiments.[5] The major finding of the present study is that this nose–brain pathway for the MC4-R agonists MSH/ACTH(4–10) can be utilized to directly affect lipid metabolism in subcutaneous WAT of the abdomen in humans. Specifically, nasal application of the MC4-R agonist MSH/ACTH(4–10) leads to interstitial glycerol release indicating increased lipolysis in the abdominal WAT. Blood flow was not specifically affected by the peptide that excludes that the observed effects were caused by a vasoconstrictive effect of MSH/ACTH(4–10). A reduction of tissue perfusion would have increased the level of glycerol in the extracellular space of adipose tissue due to decreased metabolite transport.[7]

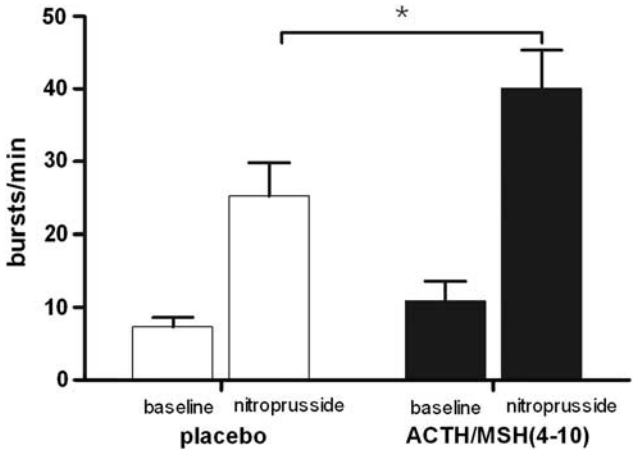

Figure 2 MSNA recordings of the peroneal nerve. Burstrate/minute under baseline conditions and after sympathoexcitation with nitroprusside bolus injection (100 μg) ($P<0.05$ ACTH/MSH(4–10) vs placebo conditions after nitroprusside).

The findings of the present study resemble results of previous experiments in rodents in which intracerebroventricular administration of the MCR agonist MT II caused lipid mobilization in WAT and increased sympathetic nerve activity in a dose-dependent manner[4] by a yet undetermined mechanism. We cannot exclude a direct effect of MSH/ACTH(4–10) on adipocyte function. However, human adipose tissue only expresses MC1-R to a relevant extent and binding to MC1-R does not induce lipolysis.[8,9] Additional experiments are needed to examine the dose dependence of the MSH/ACTH(4–10) effect. In a pilot experiment with five volunteers and 5 mg MSH/ACTH(4–10) i.n., we could not detect any changes in subcutaneous lipolysis in any of the individuals. However, we cannot exclude that higher doses than 10 mg i.n. would have a stronger effect.

In our experiments the lipolytic effects of MSH/ACTH (4–10) occurred 45 min after i.n. administration, which represents a delay of 15 min compared with the previously described increase of MSH/ACTH(4–10) in the cerebrospinal fluid.[5] This rapid response of adipocytes favors a non-genomic MCR agonist effect on adipocytes, and an activation of the autonomic innervation to adipose tissue would be an attractive explanation for our findings.

Several studies clearly demonstrated autonomic innervation of the adipose tissue in animals,[10–12] and recently we reported that stimulation of efferent nerves to the subcutaneous tissue increases local subcutaneous lipolysis.[13] However, lipomotor neuronal signals are not subject to recording by microneurography; thus, direct measurement of MC-R agonist effects on sympathetic nerve activity to WAT in humans is not yet feasible. We therefore decided to record MSNA together with muscular interstitial glycerol concentrations in addition to subcutaneous microdialysis. It is known that intramuscular fat depots could have an important role in energy homeostasis, while also SM might be differently affected by lipomotor signals than adipose tissue.[14–17] Neither baseline sympathetic neuronal outflow to the muscle vascular bed nor glycerol release within the muscle was significantly affected by MSH/ACTH(4–10) under baseline conditions. However, our negative findings in the forearm muscle do not necessarily apply to all SM in general, as there is a marked heterogeneity between different SM groups regarding lipolysis.[18] Furthermore, the MSH/ACTH (4–10) had significant effects on the sympathoexcitability in response to nitroprusside. Thus, although the MSH/ACTH (4–10) effects on sympathetic outflow to the subcutaneous tissue remains undetermined, the peptide obviously had specific effects on sympathetic baroreflex centers within the central nervous system. This finding clearly supports the use of highly specific methods to determine peptide effects on sympathetic nervous activity. More global and less specific measures like plasma catecholamine measurements or end organ responses were not able to reveal the MSH/ACTH(4–10) effects on baroreflexive sympathetic activation in our study. However, while being an interesting hypothesis, MSH/ACTH(4–10) induced sympathoexcitation as explanation

for the observed lipolysis, this idea cannot be conclusively proofed by the present experiment. This needs an additional battery of experiments using techniques, which are aiming to block the effects of catecholamines like blockade with propranolol or injection of local anesthetics. Those experiments again need a reliable method to monitor blood flow that is likely to be increased by either method, which would make interpretation of changes in metabolite concentration in the dialysate difficult.

Intranasal application of MSH/ACTH(4–10) over a period of 6 weeks leads to a moderate decrease of body fat in healthy humans which is not caused by a reduction of caloric intake.[2] The results of our experiments suggest an increase of lipolysis in WAT as one possible mechanism for the catabolic effect of MSH/ACTH(4–10). In the search of a therapy of obesity-targeting central melatonin receptors by intranasal administration of specific agonist might be a part of a multimodal concept. Unfortunately, overweight humans do not appear to be susceptible to the weight reducing effects of intranasally applied MSH/ACTH(4–10)[19] and likewise, in another study the melanocortin receptor 4 (MCR-4) antagonist MK 0493 did not lead to weight loss in overweight humans after chronic administration.[20] As a body mass index >25 was an exclusion criterion for participation in our experiments, our findings can only be applied to normal weight individuals. Furthermore, we have learned from *in vitro* experiments that desensitization of MCR could occur after prolonged administration due to internalization of MCR-4 after binding to agonists, which would limit its therapeutical use.[21–23] Interestingly, obese individuals with MCR-4 mutations show a markedly reduced sympathetic outflow to the vasculature.[24]

The observed effects of i.n. MSH/ACTH(4–10), might not only have been due to an increased sympathetic outflow to WAT. Natriuretic Peptide (ANP) is locally expressed in adipocytes suggesting a paracrine system for ANP in human adipose tissue.[25] ANP in pharmacological doses stimulates lipid mobilization at plasma concentrations that are encountered in conditions such as heart failure and might contribute to cardiac cachexia.[26] This effect is independent of catecholaminergic induction of lipolysis[27] and is only observed in adipose tissue but not in SM,[28] which would fit with our observations showing effects of the intranasally administered peptide only in the subcutaneous fat. In our experiment, the effect of intranasal ACTH 4–10 is significant but small. With respect to animal studies subtle activation of the sympathetic nerve system is an attractive but not exclusive explanation for an increased lipolysis. We cannot exclude other mechanisms such as a yet unknown activation of local ANP. However, we are not aware of experimental data that link activation of central MCR to local or systemic ANP release. We did not measure ANP in plasma or in the microdialysis effluent and so we cannot exclude this possibility.

In summary, intranasal intake of MSH/ACTH(4–10) acutely affected both the subcutaneous lipolysis and sympathetic regulation in healthy humans. The peptide has specific lipolytic effects on WAT metabolism in humans, which can be induced after intranasal administration. Furthermore, the peptide evoked an induced baroreflex-mediated sympathoexcitability clearly suggesting that this pro-opiomelanocortin-derived peptide is involved in the regulation of sympathetic functions. Thus, the study demonstrates the important physiological effects of MSH/ACTH(4–10) on fat tissue metabolism and sympathetic regulation which may be of use in the development of therapeutic strategies in obesity.

Conflict of interest

The authors declare no conflict of interest.

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