Regulatory Peptides 182 (2013) 19–27

Contents lists available at SciVerse ScienceDirect

Regulatory Peptides

journal homepage: www.elsevier.com/locate/regpep

Genetic and pharmacological mouse models of chronic melanocortin activation show enhanced baroreflex control of heart rate

Petteri Rinne [a,b], Janne Harjunpää [a], Satu Mäkelä [a], Eriika Savontaus [a,c,*]

[a] Department of Pharmacology, Drug Development and Pharmaceutics, and Turku Center for Disease Modeling, Itäinen Pitkäkatu 4b, 20014 University of Turku, Finland
[b] Drug Discovery Graduate School, University of Turku, Finland
[c] Unit of Clinical Pharmacology, Turku University Hospital, Turku, Finland

ARTICLE INFO

Article history:
Received 12 June 2012
Received in revised form 5 September 2012
Accepted 17 December 2012
Available online 14 January 2013

Keywords:
Melanocortin
Autonomic nervous system
Baroreflex

ABSTRACT

The central melanocortin system is an important regulator of energy homeostasis and cardiovascular functions. Although the acute effects of melanocortins on central blood pressure regulation are well-established, their long-term effects on autonomic balance and baroreflex function remain largely unexplored. Here we investigated the impact of chronic melanocortin activation on cardiovascular and autonomic nervous system functions by studying α- and γ-MSH overexpressing (MSH-OE) mice and, as pharmacological model, mice treated with the stable α-MSH analogue melonotan-II (MT-II, 0.3 mg/kg/day for 7 days, i.p.). Mean arterial pressure (MAP) and heart rate (HR) were measured in conscious mice by radiotelemetry. MSH-OE mice did not differ from their wild-type littermates in terms of MAP, but displayed reduced HR under physiological baseline conditions. To evaluate the relative activities of sympathetic and parasympathetic nervous systems, we applied autonomic receptor blockers and found an enhanced HR response to atropine in MSH-OE mice, indicating increased cardiac vagal activity. The compensatory increase in HR after drug-evoked vasodilatation was also augmented in MSH-OE mice. Exposure to a high-sodium diet (8% NaCl) markedly reduced HR in MSH-OE mice without concomitant changes in blood pressure, suggesting improved reflex regulation of HR. Chronic treatment with MT-II did not change 24-h MAP or HR regardless of the acute pressor and tachycardic actions of MT-II. Consistent with the finding in MSH-OE mice, MT-II-treated mice showed an enhanced HR response to vasodilatation. These observations suggest that chronic melanocortin activation may provide cardioprotective regulation by enhancing vagal nerve activity and baroreflex control of heart rate.

1. Introduction

The melanocortin system regulates important physiologic functions such as body energy balance, inflammation and sexual behavior making the system an attractive target for drug discovery across multiple therapeutic areas [1–3]. Melanocyte stimulating hormones, α-, β- and γ-MSH that result from the proteolytic cleavage of prohormone proopiomelanocortin (POMC) are essential components of this system by acting through G-protein-coupled melanocortin receptors (MC1–MC5) [4]. α-MSH, in particular, has caught wide attention as it promotes energy expenditure and suppresses appetite by stimulating MC3 and MC4 receptors in the central nervous system (CNS) [5,6]. A wealth of evidence indicates that α-MSH and central MC receptors also regulate blood pressure and heart rate by controlling sympathetic nervous system activity [7–10]. α-MSH and other melanocortins are known to acutely influence the central regulation of cardiovascular functions, but the long-term effects of melanocortins on autonomic balance and baroreceptor-mediated responses have not been thoroughly explored.

Centrally mediated effects of melanocortins on the cardiovascular system seem to be complex and involve multiple signaling pathways. In the brain, the predominant sites of POMC expression originate in the hypothalamus and in the brainstem [11–14]. These two brain areas are thought to differentially mediate the effects of α-MSH on cardiovascular homeostasis. According to current understanding, activation of hypothalamic MC4 receptors by α-MSH leads to sympathetic activation and an increase in blood pressure and heart rate [9,10,15–17]. In contrast, microinjections of α-MSH into the brainstem have been reported to elicit bradycardia and hypotension, responses that are likely to be dependent on activation of brainstem MC4 receptors and an increase in cardiac vagal activity [18–22]. Although the acute effects of centrally administered α-MSH on hemodynamics are well-established, it remains to be determined how melanocortins affect the cardiovascular system and autonomic nervous system in the long-term. Chronic effects of α-MSH on systemic hemodynamics have been studied in rats by administering the parent compound or its stable analogue melanotan-II (MT-II) into the cerebroventricular

* Corresponding author at: Department of Pharmacology, Drug Development and Therapeutics, Itäinen Pitkäkatu 4b, 20014 University of Turku, Finland. Tel.: +358 2 333 7362; fax: +358 2 333 7216.
E-mail address: eriika.savontaus@utu.fi (E. Savontaus).

0167-0115/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.regpep.2012.12.002

system [9,15,23]. However, considering the diverse biology of the melanocortin system and the wide distribution of MC receptors in the CNS and in the periphery [3,24,25], it is highly relevant to investigate the cardiovascular effects in such models where the increase in melanocortin activity is more generalized rather than localized around the cerebroventricular system. Furthermore, from a drug development and therapeutic perspective, the systemic effects of melanocortins should be characterized to increase our understanding of the overall effects of chronic melanocortin activation on cardiovascular health.

In the present study, we investigated how increased melanocortin activity, obtained either by a genetic or pharmacological approach, affects systemic cardiovascular and autonomic nervous system functions. As a genetic model, we studied transgenic mice overexpressing α- and γ-MSH. To mimic this model by pharmacological means, we subjected wild-type C57Bl/6N mice to chronic treatment with a stable α-MSH analogue. Autonomic nervous system and baroreflex functions were characterized in these models by challenging the mice with pharmacological and dietary interventions. Here we show that a universal and long-term increase in melanocortin activity has prominent effects on cardiovascular regulation by increasing cardiac vagal activity and by enhancing baroreceptor-mediated responses.

2. Methods

2.1. Animals and treatments

All experiments were approved by the national Animal Experiment Board in Finland and conducted in accordance with the European Union Directive. Animals were housed on a 12 h light/dark cycle and fed *ad libitum* a regular chow diet, unless otherwise indicated.

As a chronic model of increased melanocortin activity, transgenic mice overexpressing α- and γ_3-MSH under the universal CMV promoter (C57BL/6J-A^{wj} background after 8 backcrosses) were studied. All experiments were performed in male wild-type (WT) and transgene homozygous (MSH-OE) mice. MSH-OE mice display two-fold increased peptide levels in tissues where POMC is normally processed to α- and γ_3-MSH, including the hypothalamus and the brainstem [26]. Similarly, plasma levels of biologically active peptide levels are elevated in MSH-OE mice. To evoke a hemodynamic challenge, 12-month-old WT and MSH-OE mice were placed on a high-sodium diet (8% NaCl, Research Diets Inc.) for 4 weeks. To pharmacologically mimic increased melanocortin activity, 3-month-old male C57Bl/6N mice were treated with the stable α-MSH analogue, melanotan-II (MT-II, 0.3 mg/kg/day) for 7 days. Control mice received physiological saline solution (0.9% NaCl). Treatments were given as daily intraperitoneal (i.p.) injections.

2.2. Arterial pressure measurements

Blood pressure (BP) and heart rate (HR) were measured in conscious, unrestrained mice using a radiotelemetry system (TA11PA-C10 and Dataquest software, Data Sciences International) as previously described [27,28] or by a nonivasive tail-cuff method (TSE Systems) in conscious, restrained mice. For tail-cuff measurements, the mice were trained for at least 2 consecutive days before the actual data collection. In each recording session, the mice were placed on a heated pad (35 °C) and allowed to settle for at least 5 min before data acquisition. The average of 10 readings from each mouse was recorded.

2.3. Urine analyses

Twenty-four hour urine samples were collected in metabolic cages. Urine sodium and potassium concentrations were measured by flame photometry.

2.4. Pharmacological testing of autonomic balance

To evaluate autonomic control of BP and HR, mice were randomly (Latin square-based block design) assigned to one of the following treatment sessions: saline (10 ml/kg), muscarinic blockade by atropine (2 mg/kg), β_1-adrenergic blockade by metoprolol (4 mg/kg) or α_1-adrenergic blockade with prazosin (1 mg/kg) [29–31]. All substances were given i.p. The treatment sessions were conducted during morning hours and separated by at least 24 h. Each session included a control 30-min recording and a 60-min recording after drug injection. A separate session with an i.p.-injection of saline was conducted to validate the recovery period needed to eliminate the stress-induced BP and HR changes associated with handling and injection. The values from 45 to 60 min after drug injection were used to analyze the respective drug responses.

2.5. Evaluation of baroreflex control of heart rate

Spontaneous baroreflex sensitivity (BRS) was investigated using the sequence technique as previously described but with some modifications [27]. Briefly, blood pressure signals were recorded on a beat-by-beat basis for 30 min (9–10 *am*). Up sequences of 3 or more beats, with a delay of 3 beats were analyzed. Up sequence was defined as baroreflex operation in which an increase in systolic blood pressure (SBP) was paralleled by a lengthening in pulse interval (PI). No thresholds for the coefficient of correlation, SBP and PI changes were used as recommended by Laude et al. [32,33]. BRS was assessed as the slope (ms/mm Hg) of the linear regression lines between SBP and PI values. BRS for each mouse was calculated as the mean value of all valid slopes obtained. The proportion of valid baroreflex sequences with respect to the total number of SBP ramps was applied as an index of baroreflex effectiveness [27,32]. In addition, the mean proportion of valid baroreflex sequences in the recordings was computed using the following equation: Baroreflex power (%) = (Number of heart beats in valid baroreflex sequences) / (Total number of heart beats) * 100 [34].

2.6. Drugs and solutions

For *in vivo* pharmacological testing, all drugs except prazosin were dissolved in a 0.9% NaCl (10 μl/g body weight). Prazosin was dissolved in a 10% glucose solution containing 5% PEG. All drugs were purchased from Sigma-Aldrich.

2.7. Statistics

Statistically significant differences between experimental groups were evaluated using the Student's t test for unpaired data or two-way ANOVA followed by Bonferroni *post hoc* tests. ANOVA for repeated measures was used to analyze circadian BP and HR rhythms. A two-tailed p value of less than 0.05 was considered statistically significant. All data are expressed as mean ± SEM.

3. Results

3.1. MSH-OE mice show lower heart rate in physiological baseline conditions

To study the effects of transgenic MSH overexpression on blood pressure regulation, hemodynamic parameters were monitored by radiotelemetry in wild-type (WT) and MSH-OE mice at 3 and 6 months of age in physiological baseline conditions. The regain of circadian blood pressure and HR rhythm occurred within 5 days after the implantation of pressure transmitters in both age groups and strains of mice (data not shown). Baseline values of hemodynamic parameters and locomotor activity were analyzed between days 10 and 12 after the implantation surgery. The general pattern of circadian rhythm in