Neuropeptides xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Neuropeptides

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

Acute central effects of alarin on the regulation on energy homeostasis

Alexandra Mikó [a], Nóra Füredi [a], Judit Tenk [a], Ildikó Rostás [a], Szilvia Soós [a], Margit Solymár [a], Miklós Székely [a], Márta Balaskó [a], Susanne M. Brunner [b], Barbara Kofler [b,*], Erika Pétervári [a,**]

[a] Institute for Translational Medicine, Medical School, University of Pécs, Hungary
[b] Laura Bassi Centre of Expertise – THERAPEP, Research Program for Receptor Biochemistry and Tumor Metabolism, Department of Pediatrics, Paracelsus Medical University, Salzburg, Austria

ARTICLE INFO

Article history:
Received 29 June 2016
Accepted 4 September 2016
Available online xxxx

Keywords:
Alarin
Energy balance
Food intake
Body temperature
Metabolic rate
Sickness behavior
Prostaglandin
Meloxicam
Indomethacin

ABSTRACT

Hypothalamic neuropeptides influence the main components of energy balance: metabolic rate, food intake, body weight as well as body temperature, by exerting either an overall anabolic or catabolic effect. The contribution of alarin, the most recently discovered member of the galanin peptide family to the regulation of energy metabolism has been suggested. Our aim was to analyze the complex thermoregulatory and food intake-related effects of alarin in rats.

Adult male Wistar rats received different doses of alarin (0.3; 1; 3 and 15 μg corresponding approximately to 0.1, 0.33, 1, and 5 nmol, respectively) intracerebroventricularly. Regarding thermoregulatory analysis, oxygen consumption (indicating metabolic rate), core temperature and heat loss (assessed by tail skin temperature) were recorded in an Oxymax indirect calorimeter system complemented with thermocouples and Benchtop thermometer. In order to investigate potential prostaglandin-mediated mechanisms of the hyperthermic effect of alarin, effects of intraperitoneally applied non-selective (indomethacin, 2 mg/kg) or selective cyclooxygenase inhibitor (COX-2 inhibitor meloxicam, 1; 2 mg/kg) were tested. Effects of alarin on daytime and nighttime spontaneous food intake, as well as, 24-h fasting-induced re-feeding were recorded in an automated FeedScale system.

Alarin increased oxygen consumption with simultaneous suppression of heat loss leading to a slow coordinated rise in core temperature. Both applied COX-inhibitors suppressed this action. Alarin failed to induce daytime food intake, but suppressed spontaneous nighttime and also fasting-induced re-feeding food intake.

Alarin appears to elicit a slow anorexigenic and prostaglandin-mediated, fever-like hyperthermic response in rats. Such a combination would characterize a catabolic mediator. The potential involvement of alarin in sickness behavior may be assumed.

1. Introduction

Hypothalamic neuropeptides have been shown to influence the main components of energy balance, i.e. metabolic rate (MR), food intake (FI), body weight (BW) and body temperature, by having either an overall anabolic or catabolic effect (Székely et al., 2010, 2012). Anabolic substances increase BW by inducing FI (orexigenic effect) and suppressing MR (hypometabolism), while catabolic mediators decrease BW by suppressing FI (anorexigenic effect) and increasing MR (hypermetabolism) (Székely et al., 2010; Jeanrenaud and Rohner-Jeanrenaud, 2001). Shifts in energy balance (obesity, cachexia, inflammation-induced fever and anorexia) due to altered effects of neuropeptides can lead to serious medical consequences on a population level.

Alarin, a 25 amino-acid peptide, is the newest member of the galanin peptide family found first in gangliocytes of human neuroblastic tumors (Santic et al., 2006). Alarin has also been shown to be localized around the blood vessels with vasoactive actions (Santic et al., 2007) and may have a role in ocular blood flow regulation (Schrödl et al., 2013). In addition, it increases the secretion of luteinizing hormone in male mice (Fraley et al., 2012), has antidepressant-like (Wang et al., 2014, 2015; Zhuang et al., 2016) and antimicrobial (Wada et al., 2013) effects. The potential role of alarin in the regulation of energy balance is raised by its immunoreactivity in the appropriate murine brain regions controlling FI, metabolism and thermoregulation: such as arcuate nucleus (ARC), dorso-medial nucleus (DMH), lateral hypothalamus (LH) and paraventricular nucleus (PVN) of the hypothalamus and the preoptic

Abbreviations: ARC, arcuate nucleus; BW, body weight; COX, cyclooxygenase; DMH, dorso-medial, nucleus; FI, food intake; GALP, galanin-like peptide; ICV, Intracerebroventricular; IP, intraperitoneal; LH, lateral hypothalamus; MR, metabolic rate; NPY, neuropeptide Y; PFS, pyrogen-free saline; PGE_1, prostaglandin E_1; PVN, paraventricular nucleus; Ta, ambient temperature; Tc, core body temperature; Ts, tail skin temperature; VO_2, oxygen consumption.

* Correspondence to: B. Kofler, Laura Bassi Centre of Expertise - THERAPEP, Research Program for Receptor Biochemistry and Tumor Metabolism, Department of Pediatrics, Paracelsus Medical University, Müllner Hauptstrasse 48, 5020 Salzburg, Austria.
** Correspondence to: E. Pétervári, Institute for Translational Medicine, Medical School, University of Pécs, 12. Szigeti út, H-7624 Pécs, Hungary.
E-mail addresses: b.kofler@salk.at (B. Kofler), erika.petervari@aok.pte.hu (E. Pétervári).

http://dx.doi.org/10.1016/j.npep.2016.09.001
0143-4179/

area (Eberhard et al., 2012). In the rat brain alarin was expressed by cells in the posterior hypothalamus (Eberhard et al., 2007) and in locus coeruleus that receives projections from the hypothalamus (Van Der Kolk et al., 2010). Intracerebroventricular (ICV) injection of alarin significantly increased the expression of the immediate early gene *c-fos*, a marker for neuronal activation in different brain regions including the PVN, DMH and the ARC of male rats (Van Der Kolk et al., 2010). In accord with these observations, some earlier in vivo studies raised also the possibility that alarin may participate in the regulation of FI: they described orexigenic effects of alarin (Boughton et al., 2010; Van Der Kolk et al., 2010; Fraley et al., 2013, 2012). However, this orexigenic effect appears to be relatively weak compared to that of a major hypothalamic orexigenic mediator, neuropeptide Y (NPY) (Boughton et al., 2010). Effects of alarin on spontaneous nighttime FI or on fasting-induced refeeding have not been fully investigated (Boughton et al., 2010). Regarding another feature of energy balance, thermoregulatory effects of alarin were also investigated. Although previous reports failed to reveal any change in body temperature in freely moving mice (Fraley et al., 2012, 2013) or any change in oxygen consumption (indicating MR) in freely moving rats upon a central alarin injection (Van Der Kolk et al., 2010), our previous study described a slow, but significant increase in resting MR and in core body temperature upon a similar alarin administration in semi-restrained rats (Mikó et al., 2014). This moderate restraint was applied to avoid interference with locomotion, thus allowed detection of this hyperthermic response. Such a thermoregulatory action would rather characterize a catabolic (hypermetabolic and anorexigenic) neuropeptide. In addition, alarin-induced hyperthermia was further enhanced by simultaneous suppression of heat loss (Mikó et al., 2014). Therefore, this hyperthermic response appears to be coordinated (i.e. similar to a febrile reaction, Myers et al., 1974; Székely and Szelényi, 1979), so the potential involvement of prostaglandins would also be suggested (Mikó et al., 2014; Myers et al., 1974).

Our present study focused on the detailed analysis of the complex effects of alarin on energy homeostasis in Wistar rats with special emphasis on FI and thermoregulation. We hypothesized that alarin that has earlier been shown to elicit hyperthermia would suppress spontaneous or re-feeding FI, i.e. this peptide would prove to be an anorexigenic catabolic mediator. We also assumed that thermoregulatory responses to alarin are mediated by prostaglandins.

2. Materials and methods

2.1. Animals

Young age-groups of male Wistar and Long-Evans rats from the Colony of the Institute for Translational Medicine of the Medical School, University of Pécs, Hungary were used in the experiments of the present study. These rat strains have been chosen in order to obtain result comparable to those of previous studies on alarin. For the investigation of regulatory peptides affecting FI in our laboratory 3 months old young adult male Wistar rats are used. These adult animals have already finished the period of rapid growth. However, earlier studies, investigating the FI-related effects of alarin, tested younger juvenile (6 weeks old) Long-Evans rats (Van Der Kolk et al., 2010). Such animals have not finished the period of rapid growth and they may show altered FI-associated responses to regulatory peptides (Székely et al., 2012). Therefore, we tested FI-related effects of alarin in our automated Feedscale system using 3 months old young adult male Wistar rats as well as juvenile, 6 weeks old Wistar and Long-Evans groups.

Animals were kept individually in plastic cages with wood-chip bedding at an ambient temperature of 23–26 °C, on a 12:12 h light-dark cycle. The lights were turned on at 06:00 h. Standard laboratory rat chow (CRLT/N rodent chow, Szindbád Kft., Gödöllő, Hungary, 11 kJ/g) and water were available ad libitum, except for the 24-h fasting period when only water was provided for the appropriate groups. All animals were habituated to regular handling.

All experimental interventions and procedures were undertaken according to general rules of the University of Pécs Ethical Committee for the Protection of Animals in Research. In general, the rules of this Committee are in accord with the main directives of the National Ethical Council for Animal Research and those of the European Communities Council (86/609/EEC, Directive 2010/63/EU of the European Parliament and of the Council). Special permission: BA 02/2000-11/2011, valid for 5 years.

2.2. Surgeries

After about 1 week adaptation period to the experimental settings, surgical interventions were performed under intraperitoneal (IP) ketamin + xylazine [78 mg/kg (Calypsol, Richter) + 13 mg/kg (Sedaxylan, Eurovet)] anesthesia. Animals received also 2 mg intramuscular gentamycin for the prevention of infections. A 22-gauge metal cannula was stereotaxically implanted into the right lateral cerebral ventricle (parameters: 1 mm posterior and 1.5 mm right lateral to bregma, 3.5 mm ventral to dura; coordinates according to the Rat Brain Atlas, Paxinos and Watson, 2006). This metal leading cannula was fixed in position with the help of two miniature screws and dental cement, then the lumen of the guide cannula was closed by a stylet. For the testing of its appropriate location, the stylet was removed and replaced by a fitting 28-gauge injection cannula a few days after the implantation and angiotensin II (Sigma, A9525, 20 ng/5 μl) was injected ICV through a pp10 polyethylene tube attachment and the subsequent water consumption was measured. The test passed the required standards, if at least 5 ml water was consumed by the animal within 30 min, and the location of the cannula was presumed to be right.

For IP injections, before the experiments, a polyethylene tube was inserted under ether anesthesia through a needle into the abdominal cavity. After withdrawal of the needle, the tube tunneled under the skin was fixed at the nape.

Following experiments, the animals were euthanized by IP urethane (3–5 g/kg, Reanal). Brains were removed and fixed, the injection sites were checked macroscopically by coronal sections. Rats, where cannulas were found to be inappropriately placed, were excluded from the analysis.

2.3. Substances applied

Synthetic full-length alarin (alarin 1–25, MW: 2820.19) was custom-synthesized by GL Biochem (Shanghai, China). In order to test dose dependence, alarin [dissolved in pyrogen-free saline (PFS)] was administered at doses of 0.3, 1, 3 or 15 μg. In the experiments different doses of the peptide filled a 5 μl volume of the proximal end of the 20–25 cm-long pp10 polyethylene tube that was attached to the injection cannula, while the rest of the tube was filled with PFS. A small bubble separated the substance from the PFS in the distal part of the tube. Injecting 5 μl saline at the distal end of the tube resulted in ICV delivery of the substance without causing any discomfort to the animal. Control animals received the same amount of PFS.

In order to investigate the potential prostaglandin-mediated mechanism of the hyperthermic effect of alarin, indomethacin (Sigma, I7378), a non-selective COX inhibitor and meloxicam, a selective COX-2 inhibitor were applied through an IP inserted polyethylene tube 30 min prior to ICV alarin injection. Indomethacin was dissolved in Tris-HCl (0.2 M, pH 8.2), diluted in PFS and injected at a dose of 2 mg/kg (Werner et al., 2006). Meloxicam (Sigma, M3935) was dissolved in 10% ethanol and administered at two different doses: 1 or 2 mg/kg. The prostaglandin-synthesis attenuating effect of subcutanously (5 mg/kg) or intramuscularly (0.25 mg/kg) injected meloxicam has been described in earlier studies (Knorr et al., 2010; Mohn et al., 2001).

In thermoregulatory tests, the successful ICV administration of alarin was checked by an injection of prostaglandin E_1 (Sigma, P-5515, 100 ng

Please cite this article as: Mikó, A., et al., Acute central effects of alarin on the regulation on energy homeostasis, Neuropeptides (2016), http://dx.doi.org/10.1016/j.npep.2016.09.001