Regulatory Peptides 179 (2012) 84–90

Contents lists available at SciVerse ScienceDirect

Regulatory Peptides

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

Prokineticin 2 is involved in the thermoregulation and energy expenditure

Wenbai Zhou [a,b], Jia-Da Li [a,c,*], Wang-Ping Hu [a,d], Michelle Y. Cheng [a], Qun-Yong Zhou [a,**]

[a] Department of Pharmacology, University of California, Irvine, CA 92697, USA
[b] Department of Endocrinology and Metabolism, Huashan Hospital, Shanghai, China
[c] The State Key Laboratory of Medical Genetics, Central South University of China, Changsha, Hunan, China
[d] Department of Pharmacology, Xianning College, Xianning, Hubei, China

ARTICLE INFO

Article history:
Received 21 March 2012
Received in revised form 28 June 2012
Accepted 27 August 2012
Available online 4 September 2012

Keywords:
Prokineticin 2
Torpor
Fasting
Arousal

ABSTRACT

Animals have developed adaptive strategies to survive tough situations such as food shortage. However, the underlying molecular mechanism is not fully understood. Here, we provided evidence that the regulatory peptide prokineticin 2 (PK2) played an important role in such an adaptation. The PK2 expression was rapidly induced in the hypothalamic paraventricular nucleus (PVN) after fasting, which can be mimicked by 2-deoxy-D-glucose (2-DG) injection. The fasting-induced arousal was absent in the PK2-deficient ($PK2^{-/-}$) mice. Furthermore, $PK2^{-/-}$ mice showed less energy expenditure and body weight loss than wild-type (WT) controls upon fasting. As a result, $PK2^{-/-}$ mice entered torpor after fasting. Supply of limited food (equal to 5% of body weight) daily during fasting rescued the body weight loss and hypothermal phenotype in WT mice, but not in $PK2^{-/-}$ mice. Our study thus demonstrated PK2 as a regulator in the thermoregulation and energy expenditure.

1. Introduction

Prokineticins, including prokineticin 1 (PK1) and prokineticin 2 (PK2), are a pair of regulatory peptides with a similar molecular weight of ~10 kDa [1]. PK1 and PK2 are the cognate ligands for two closely related G-protein-coupled receptors (PKR1 and PKR2) [2–4]. In the central nervous system, PK2 is expressed in the suprachiasmatic nucleus (SCN), islands of Calleja, medial preoptic area (MPA), olfactory bulb, nucleus accumbens shell, hypothalamic arcuate nucleus and amygdale, while PKR2 is widely expressed throughout the brain [5]. PK2 is reported to be an output molecule for the SCN clock. In the SCN, the circadian pacemaker, PK2 expression is highly circadian and regulated by the central clock machinery [6]. Lacking of PK2 genes in the mice results in reduced circadian rhythmicity in a variety of behavior and physiology, including locomotion, sleep/wake cycle, body temperature, food intake, hormone level, emotional conditions, and energy metabolism [7,8]. The circadian system may affect thermoregulation depending on the time of day and feeding condition [9,10]. During the food deprivation, the SCN is activated, associating with activation of other hypothalamic areas like the hypothalamic paraventricular nucleus (PVN), which plays important roles in the regulation of the sympathetic nervous system (SNS). SNS regulates the heat production through general activity, shivering, and brown fat thermogenesis [11]. Thus, both of the SCN and PVN are involved in energy expenditure. Recently, Jethwa et al. [12] demonstrated that prokineticin receptor 2 (PKR2) signaling was crucial in the thermoregulation. They observed that null mutation of PKR2 ($PKR2^{m/m}$) mice showed sporadic bouts of torpor under *ad libitum* feeding at constant temperature. During the torpor, $PKR2^{m/m}$ mice showed behavioral hyporesponsive, as decreasing locomotor activities, oxygen consumption and respiratory quotient (RQ). As they removed food for a period, $PKR2^{m/m}$ mice and their littermates all displayed low body temperature, in which $PKR2^{m/m}$ mice had a deeper and longer hypothermia associated with greater decreased oxygen consumption and RQ compared to their littermates. They suggested that PKR2 signaling pathway is involved in the regulation of energy balance and thermoregulation. As PK2 and PKR2 are likely a pair in the central nervous system, we investigated the role of PK2 in the thermoregulation using PK2-deficient ($PK2^{-/-}$) mice in the study.

2. Materials and methods

2.1. Animals

$PK2^{-/-}$ mice were generated by homologous recombination as previously described [13]. $PK2^{-/-}$ mice and their littermate wild-type

Abbreviations: PK2, prokineticin 2; PVN, hypothalamic paraventricular nucleus; 2-DG, 2-deoxy-D-glucose; SCN, suprachiasmatic nucleus; SNS, sympathetic nervous system; RQ, respiratory quotient; $PK2^{-/-}$, PK2-deficient; NREM, non-rapid eye movement; REM, rapid eye movement; DMH, dorsomedial hypothalamus; VLPO, ventrolateral preoptic nucleus; CNS, central nervous system; MPA, medial preoptic area; Arc, arcuate nucleus.

* Correspondence to: J.-D. Li, The State Key Laboratory of Medical Genetics, Central South University of China, Changsha, 410078, Hunan, China. Fax: +86 731 84805339.
** Correspondence to: Q.-Y. Zhou, Dept of Pharmacology, Univ. of California, Irvine, CA92697, USA. Fax: +1 9498244855.
E-mail addresses: lijiada@sklmg.edu.cn (J.-D. Li), qzhou@uci.edu (Q.-Y. Zhou).

0167-0115/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.regpep.2012.08.003

(WT) mice in a C57BL/6×129/Ola hybrid background were used in all experiments. We have monitored the body weight change of WT and PK2$^{-/-}$ mice under regular chow feeding. There is dramatic body weight difference between male WT and PK2$^{-/-}$ mice, whereas the body weight in female mice is quite similar (data not shown). To avoid potential confound caused by the basal body weight, we only used females of 3–5 months of age in this study. We did not observe difference in the food intake of WT and PK2$^{-/-}$ mice under basal conditions [8]. Before the experiments, the mice were group-housed (3–5 animals per cage) under controlled conditions [temperature, 20± 2 °C; relative humidity, 50–60%; 12:12-h light–dark (LD) cycle, lights on at 7:00 AM and lights off at 7:00 PM] and had free access to food and water [14]. During experiments, mice were individually housed. All procedures regarding the care and use of animals were approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

2.2. In situ hybridization

Mice were food-deprived starting from the lights off and killed at 0, 4, 8, 16 and 24 h after food deprivation. For re-feeding experiments, an independent group of mice was supplied with food pellets after 24-h food deprivation, and mice were killed at 15, 30, 60, 120 and 240 min after re-feeding. For 2-deoxy-D-glucose (2-DG) experiment, mice were injected with 2-DG (250 mg/kg) or saline at 9 am, and returned to their home cages without food. These mice were killed at 0, 2, 4 and 8 h after injection. Brains were sectioned and processed for *in situ* hybridization as described previously [15]. This DNA sequence used for PK2 probe is nucleotides 1–528 of mouse Prok2 (GenBank accession no. AF487280). We have used this probe in a variety of studies [6,14,16–18]. We verified this probe by hybridizing with brain slices with a sense cRNA, which make no signal. Antisense and sense cRNA probes were generated by *in vitro* transcription in the presence of ^{35}S-labeled UTP (1200 Ci/mmol). The PK2 mRNA distributions were analyzed in autoradiograms. Specific hybridization signals were quantitatively analyzed using a video-based computer image analysis system (MCID, Imaging Research, St. Catharine's, Ontario, Canada). A calibration curve of optical density *versus* radioactivity (dpm/mg tissue wet weight) was constructed using ^{14}C-standards. Specific hybridization signals in PVN were obtained by subtracting background values obtained from adjacent brain areas that have no hybridization signal.

2.3. Recording and analysis of sleep/wake

The electroencephalogram (EEG) and electromyogram (EMG) signals from mice were recorded and analyzed as described [7]. The mice were connected to a wire tether system (Plastics One, Roanoke, VA) for the collection of EEG and EMG signals. This swivel system allowed the animal unrestricted movement throughout the recording cage. After at least 5 days of adaptation to the recording environment, a 48-h baseline EEG/EMG recording was collected on a LD cycle with lights on at 7:00 AM and off at 7:00 PM. Mice were recorded concurrently in matched littermate pairs of PK2$^{-/-}$ and WT mice. EEG/EMG signals were amplified using a Grass Telefactor Model 15LT with 15A94 amplifier (Grass Instruments, West Warwick, RI) and filtered (EEG: 0.3–100 Hz, EMG: 30–300 Hz) before being digitized at a sampling rate of 128 Hz and stored on a computer.

2.4. Core body temperature monitoring

A radio transmitter device (G2 E-mitter; Mini-Mitter, Sunriver, OR) used to measure body temperature and locomotion activity simultaneously was implanted in the abdominal cavity by sterile technique under general anesthesia [19]. Body temperature and locomotion activity were recorded by a receiver board (ER-4000 energizer receiver; Mini-Mitter) underneath the cage and were stored in a personal computer every 5 min. The mice were allowed to recover for at least 2 weeks before the experiments. The ambient temperature was constant at 21 °C unless otherwise indicated.

2.5. Energy expenditure and oxygen consumption

A comprehensive Lab Animal Monitoring System (CLAMS; Columbus Instruments, Columbus, OH) was used to monitor oxygen (O_2) and carbon dioxide (CO_2) gas fractions at both the inlet and outlet ports to each of 4 test chambers, which were supplied with corn cob bedding. The airflow was 0.65 L/min, and air from each chamber was sampled and analyzed every 10 s. Experimental mice were either fasted or had free access to food. The mice were allowed to familiar the cages for 2 days, and then were recorded.

2.6. Statistical analyses

A repeated-measures ANOVA followed by an unpaired *t* test was used to analyze the data for differences between genotypes or treatments. All statistical analysis was performed using Prism 4.4 (GraphPad Software, San Diego, CA).

3. Results

3.1. Fasting induced PK2 expression in the PVN

We previously showed that light induced PK2 expression in the SCN [16]. To study the effect of food deprivation on PK2 induction, we subjected wild-type (WT) mice to fasting and detected the PK2 mRNA expression throughout the brain using *in situ* hybridization. Under *ad lib* feeding conditions, PK2 was barely detectable in the PVN (Fig. 1A); however, the expression of PK2 was significantly induced in the PVN after fasting (Fig. 1B). The induction occurred as early as 4 h after fasting (Fig. 1C). The fasting-induced PK2 expression was restricted to the PVN as no significant induction was found in the other brain areas (data not shown). When food pellets were supplied to the fasted mice (*e.g.* re-feeding), the PK2 level in the PVN rapidly fell to the basal undetectable level within 4 h (Fig. 1C). Moreover, 2-deoxy-D-glucose (2-DG), an inhibitor of glucose utilization, also rapidly induced PK2 expression in the PVN (Fig. 1D), implying that the induction was a response to the dwindling energy reserves.

3.2. PK2$^{-/-}$ mice displayed torpor upon fasting

Torpor is generally defined as reduction in body temperature below 31 °C [20]. Previously, Jethaw et al. [12] reported a portion of PKR2$^{m/m}$ mice showed spontaneous torpor under normal feeding conditions. However, no spontaneous torpor was observed in PK2$^{-/-}$ mice under *ad lib* feeding and constant ambient temperature conditions (21 °C). We did not even observe torpor in *ad lib*-fed PK2$^{-/-}$ mice at 4 °C (data not shown). Jethaw et al. [12] also reported that a severe torpor occurred in PKR2$^{m/m}$ mice during fasting. Thus, we subjected PK2$^{-/-}$ mice to fasting and monitored their body temperature using telemetries. Both WT and PK2$^{-/-}$ mice showed a decreased body temperature during fasting; however, the body temperature of PK2$^{-/-}$ mice was much lower (genotype: $F(1)=19.13$, $P<0.0001$, two-way ANOVA). PK2$^{-/-}$ mice entered torpor after fasting, *i.e.* the core body temperature dropped below 30 °C, whereas WT mice were still able to keep the core body temperature above 33 °C (Fig. 2A). After prolonged fasting (>36 h), WT mice displayed a transitional body temperature dipping close to 30 °C, whereas PK2$^{-/-}$ mice showed a period of ~4 h of torpor, when the body temperature dropped to as low as 25 °C (Fig. 2A). Interestingly, most of the torpors occurred at the transition from the active phase to the inactive phase (night to day).