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Repeated peripheral administration of lipidized prolactin-releasing peptide analog induces c-fos and FosB expression in neurons of dorsomedial hypothalamic nucleus in male C57 mice

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ABSTRACT

Previous studies indicate that hypothalamic prolactin-releasing peptide (PrRP), signaling via GPR10 and neuropeptide FF2 receptor, is involved in energy homeostasis, stress responses, and cardiovascular regulation. Energy homeostasis depends on the balance between food intake regulation and energy expenditure, in which the hypothalamus plays a key role. The lipidization of PrRP31 with palmitoyl acid allows it to produce its anorexigenic effect after repeated peripheral administration and to reduce body weight and improve metabolic parameters in diet-induced obese (DIO) mice. The aim of this study was to reveal the transient and long-lasting changes in neuronal activity via c-Fos and FosB immunohistochemistry in brain nuclei related to food intake regulation and energy homeostasis during the first days of treatment with a newly designed lipidized analog of PrRP31 (palm^{11}-PrRP31) with promising antiobesity effects. The data revealed that the anorexigenic effect of repeated application of palm^{11}-PrRP31 was associated with delayed but gradually significantly reduced cumulative food intake in mice as well as with a significant reduction in their body weight. Moreover, while the repeated application of palm^{11}-PrRP31 was associated with a significant reduction in acute cell activity in the paraventricular hypothalamic nucleus (PVN) and nucleus of the solitary tract (NTS) compare to its acute treatment, both acute and long-lasting cell activity in the dorsomedial hypothalamic nucleus (DMN) were increased. The data indicate that DMN neurons might be tonically activated after repeated administration of lipidized PrRP analogs that may be associated with the process of long-term adaptation to modified energy homeostasis.

1. Introduction

Appetite is a highly regulated phenomenon in vertebrates and is essential for maintenance of energy homeostasis and control of body weight (Kalra et al., 1999). Abnormalities in the onset, periodicity, duration and magnitude of eating episodes can be involved in augmented appetite (Kalra, 1997). While increased appetite invariably culminates in an increased rate of body weight gain and obesity (Bray, 1996; Wadden, 1993), anorexia due to psychobiological causes (Crips, 1984) or in response to infections, inflammation, or trauma is followed by severe loss of body weight (Moldawer et al., 1988; Grunfeld and Feingold, 1992). The neuroanatomical substrate associated with appetite control was localized in the hypothalamus, where the gentle and progressive disturbances in neurochemical signaling by environmental, genetic and hormonal factors may lead to hyperphagia or anorexia (Kalra, 1997; Kalra et al., 1996, 1999). Several hypothalamic nuclei, such as the paraventricular hypothalamic nucleus (PVN), arcuate hypothalamic nucleus (Arc), dorsomedial hypothalamic nucleus (DMN), ventromedial hypothalamic nucleus (VMN) and lateral hypothalamic area (LHA), are crucial in the regulation of daily energy homeostasis via neural mechanisms affecting appetite (Kalra et al., 1999; Berthoud, 2002).

The DMN mainly appears to be an intrahypothalamic relay station for receiving information from most other hypothalamic nuclei, including the PVN, Arc, VMN and LHA (Berthoud, 2002). There are comparatively few inputs from the forebrain, including a few projections from the ventral subiculum (hippocampal complex), infralimbic prefrontal cortex (visceromotor cortex), lateral septum, and the bed

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nucleus of the stria terminalis (Thompson and Swanson, 1998). Furthermore, neurons in the DMN may receive different hormonal inputs through the circulation. In the DMN, a high concentration of leptin receptors was noted, mainly in the caudal regions of the nucleus, ventral to the compact formation, and many of these neurons in turn project to the PVN (Elmquist et al., 1998). In the compact formation of the DMN, there is a population of neuropeptide Y (NPY) neurons (Lewis et al., 1993), which express orexigenic NPY under conditions of increased energy demand such as lactation (Parker and Bloom, 2012) and in obesity of various etiologies (Guan et al., 1998; Bi et al., 2001). The efferent projections from the DMN are mainly intrahypothalamic and are directed to the periventricular zone, mainly the PVN. A few efferent projections are sent towards other nuclei in the medial zone, including the VMN and anterior hypothalamus (Berthoud, 2002). DMN neurons express neuropeptide FF receptor type 2 (NPFF2), which is expressed in all hypothalamic nuclei except the PVN (Gouardères et al., 2004), and G protein-coupled receptor 10 (GPR10), which is also localized in the PVN (Ibata et al., 2000). Both of these receptors are activated by prolactin-releasing peptide (PrRP), an anorexigenic peptide produced by neurons of the nucleus of the solitary tract (NTS), the ventrolateral medulla, and the dorsomedial hypothalamus (Maruyama et al., 1999; Ibata et al., 2000). In the medulla oblongata, PrRP immunoreactivity was demonstrated in A1 and A2 noradrenergic neurons (Chen et al., 1999).

The main physiological roles of PrRP are the negative regulation of food intake (Lawrence et al., 2000; Takayanagi et al., 2008) and the central control of the cardiovascular system (Kónyi et al., 2010; Samson et al., 2000). Intracerebroventricular (i.c.v.) PrRP administration increases hypothalamic release of anorexigenic corticotropin-releasing hormone (Seal et al., 2002) and consequently the plasma levels of adrenocorticotropic hormone and corticosterone in rats (Akana et al., 1994). PrRP can achieve its anorexigenic effect only after its i.c.v. administration (Lawrence et al., 2004) because natural PrRP does not cross the blood-brain barrier (BBB) after peripheral administration. The lipidization of the N-terminal amino acid of PrRP31 with palmitoyl (palm-PrRP31) allows its use as a prototypical molecule suitable for further study to verify its anorexigenic effect after repeated peripheral administration, which has been associated with reduced body weight and improvement of worsened metabolic parameters in diet-induced obese (DIO) mice (Maletínska et al., 2015). Activation of c-Fos in specific brain nuclei that are involved in food intake regulation and contain GPR10 and NPFF2 and the ability of palm-PrRP31 analogs to act as an agonist toward both of those receptors support suggestions that such lipidized PrRP molecules cross the BBB and can have a central anorexigenic effect after their peripheral administration (Maletínska et al., 2015; Pirnik et al., 2015; Pražienková et al., 2017). Moreover, although there are PrRP binding sites in peripheral tissues (Satoh et al., 2000), the idea mentioned above may also be supported by the small bioactive amounts of PrRP in extracts of peripheral tissues (Fujii et al., 1999; Matsumoto et al., 1999) and the lack of an anorexigenic effect after peripheral administration of non-lipidized PrRP molecules accompanied by unchanged Fos immunoreactivity in the brain nuclei involved in food intake regulation (Maletínska et al., 2015).

The cellular expression of Fos family proteins is widely accepted as a marker of neuronal activation for mapping functional changes in neural networks such as those controlling food intake (Morgan and Curran, 1991; Herdegen and Leah, 1998; Allingham et al., 1998; Vrang et al., 1999; Watts et al., 2006). The low level of c-Fos transcription under basal conditions in various brain structures (Herdegen and Leah, 1998; Okuno, 2011) and its rapid inducibility by a wide range of *trans*-synaptic/transcriptional stimuli make c-Fos a powerful and validated marker of acute neuronal activation after physiological, environmental, pharmacological and other stress challenges (Herdegen and Leah, 1998; Kovács, 1998, 2008; Watts et al., 2006). On the other hand, the transience of c-Fos expression after stimulation (especially if the stimulus is persistent) complicates the design of experiments to assess the function of c-Fos and makes c-Fos inadequate as a marker for long-term changes in neuronal activity (Hoffman and Lyo, 2002). In contrast to rapid c-Fos kinetics with a peak of c-fos mRNA at approximately 30 min and c-Fos protein between 90 and 120 min, the very long half-lives of FosB/delta FosB in tens of hours, with gradual accumulation in cell nuclei, enable the use of FosB/delta FosB expression as a marker of chronic neuronal stimulation (Herdegen and Leah, 1998; Kovács, 1998; Nestler, 2015). FosB/delta FosB has a crucial role in long-term adaptive changes in the brain associated with the development and maintenance of behavior and neuronal plasticity (McClung et al., 2004; Ruffle, 2014), including those that are associated with neuronal changes upon chronic drug treatments (Herdegen and Leah, 1998; Nestler, 2015; Majercikova et al., 2016).

Recently, the promising antiobesity effect of a newly designed lipidized analog of PrRP31 palmitoylated through a linker of gamma-glutamic acid to Lys^{11} ($palm^{11}$-PrRP31) in DIO mice was studied under chronic treatment (Pražienková et al., 2017; Holubová et al., 2018) with no observed yo-yo effect on feeding and leptin-related hypothalamic signaling even 14 days after the termination of its repeated treatment (Holubová et al., 2018). For this reason, the aim of this study was to reveal the acute and long-lasting changes in neuronal activity of brain nuclei related to food intake regulation and energy homeostasis, especially the DMN, during the first days of $palm^{11}$-PrRP31 treatment.

2. Materials and methods

2.1. Animals

Male C57 mice (Charles River, Sulzfeld, Germany) were housed at 23 °C under a 12-hr light-dark cycle (lights on at 6:00). The mice (m = 23.8 ± 0.9 g) were provided water and a standard chow diet (Ssniff Spezialdiaten GmbH, Soest, Germany) ad libitum. All experiments followed the ethical guidelines for animal experiments of the European Union Council (86/609/EEC) and the Act of the Czech Republic Nr. 246/1992 and were approved by the Committee for Experiments with Laboratory Animals of the Academy of Sciences of the Czech Republic.

2.2. Synthesis of $palm^{11}$-PrRP31

Human palmitoylated PrRP31 (SRTHRHSMEI K (N-γ-E (N-palm)) TPDINPAWYASRGIRPVGRF-NH2), $palm^{11}$-PrRP31, was synthesized and purified as described previously (Maletínska et al., 2015). Palmitoylation at position 11 of the PrRP31 analog was performed on a fully protected peptide on resin as the last step (Pražienková et al., 2017). The purity and identity of the peptide were determined by analytical high-performance liquid chromatography and using a Q-TOF micro MS technique (Waters, Milford, MA, USA).

2.3. Experimental design of the study

The experimental design of the study included food intake monitoring followed by immunohistochemical analysis after acute (A) and repeated (R) administration of Saline (Sal) or lipidized PrRP analog ($palm^{11}$-PrRP31) dissolved in Sal to mice (Table 1).

2.3.1. Food intake monitoring

To minimize within- or between-group variability in the food intake of animals, the food intake in all intact mice (n = 24) was analyzed 36 h before the treatment in every 12 h interval (covering both the light and dark phases of the day, Table 1). After subsequent randomization of animals, mice (n = 16, Table 1) were injected with Sal or $palm^{11}$-PrRP31 (5 mg/kg) in a volume of 0.15 ml (s.c.) per mouse (n = 8 mice per group). The first s.c. administration to mice was applied 1 h before the light was turned off and was repeated twice daily for 4 consecutive days (1 h after the light was turned on and 1 h before the light was