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Effect of palmitoylated prolactin-releasing peptide on food intake and neural activation after different routes of peripheral administration in rats

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ABSTRACT

Obesity is an escalating epidemic, but an effective non-invasive therapy is still scarce. For obesity treatment, anorexigenic neuropeptides are promising tools, but their delivery from the periphery to the brain is complicated by their peptide character. In order to overcome this unfavorable fact, we have applied the lipidization of neuropeptide prolactin-releasing peptide (PrRP), whose strong anorexigenic effect was demonstrated. A palmitoylated analog of human PrRP (h palm-PrRP31) was injected in free-fed Wistar rats by three routes: subcutaneous (s.c.), intraperitoneal (i.p) (both 5 mg/kg) and intravenous (i.v.) (from 0.01 to 0.5 mg/kg). We found a circulating compound in the blood after all three applications with the highest concentration after i.v. administration. This corresponds to the effect on food intake, which was also strongest after i.v. injection. Moreover, this is in agreement with the fact that the expression of c-Fos in specific brain regions involved in food intake regulation was also highest after intravenous application. Pharmacokinetic data are further supported by results obtained from dynamic light scattering and CD spectroscopy. Human palm-PrRP31 analog showed a strong tendency to micellize, and formation of aggregates suggested lower availability after i.p. or s.c. application. We have demonstrated that palm-PrRP influenced food intake even in free fed rats. Not surprisingly, the maximal effect was achieved after the intravenous application even though two orders of magnitude lower dose was used compared to both two other applications. We believe that palm-PrRP could have a potential as an antiobesity drug when its s.c. application would be improved.

1. Introduction

Obesity has been recently described as an "escalating epidemic". As obesity exacerbates chronic diseases such as cardiovascular diseases, type 2 diabetes, or hypertension, high incidence of obesity has significant implications for population morbidity and mortality. The etiology of obesity is multifactorial. Genetic, environmental, metabolic and other issues may all contribute to the development and progression of this disease [8]. In experimental conditions, the physiological and genetic animal models of human obesity can be studied in several species, mostly in mice and rats [36,38,45].

Non-pharmacologic therapy such as proper nutrition, regular physical activity and changes in eating behavior should be used

Abbreviations: ANOVA, analysis of variance; AP, area postrema; ARC, nucleus arcuatus; BBB, blood brain barrier; i.c.v., intracerebroventricular; i.p., intraperitoneal; i.v., intravenous; NTS, nucleus tractus solitarius; PBS, phosphate buffered saline; PVN, paraventricular nucleus; PrRP, prolactin-releasing peptide; s.c., subcutaneous.

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after the first indicators of obesity occur. Moreover, proper lifestyle therapy can be supplemented by pharmacologic therapy. Only a limited number of new anti-obesity pharmacotherapeutics have been introduced until now (for reviews, see Refs. [9,31,42]), and not all of them were proved safe. Recently, two high-profile antiobesity drugs (sibutramine and rimonabant) were suspended from the market after they negatively impacted clinical treatment of obesity. New potent anti-obesity substances are therefore needed, and among them, analogs of centrally acting anorexigenic neuropeptides seem to be promising.

An important aspect of developing a new drug based on a neuropeptide with central action is the ability to cross the blood–brain barrier (BBB). It was recently demonstrated that a promising strategy for designing peptide drugs is lipidization of peptides, i.e., attachment of a fatty acid to the peptide chain through an ester or amide bond, leading to an increased stability and half-life in the organism and potency to cross the BBB [5,27,30]. Palmitoylation or myristoylation through amide bond at Lys have been used in insulin analog detemir [16] or analog of glucagon-like peptide 1 (GLP-1) liraglutide [14].

The neuropeptide of the interest, prolactin-releasing peptide (PrRP), was isolated from the hypothalamus as an endogenous ligand of a human orphan G-protein coupled receptor GPR10 [18]. Later, it was established that PrRP does not actually affect prolactin secretion [19], but its main physiological functions are negative regulation of food intake [23,47] and the central control of the cardiovascular system [22,43,46]. Both PrRP or PrRP receptor-deficient mice became obese in adulthood [7,15,47]. Anorexigenic properties of PrRP came to light after its intracerebroventricular (i.c.v.) administration [25,26,47].

Natural PrRP does not cross the BBB and does not exert its central anorexigenic effect after its peripheral administration. In order to overcome this problem, PrRP was modified by fatty acid to increase stability of the neuropeptide and its penetration through BBB [30]. Our previous study [30] showed the attenuating effects of palmitoylated PrRP31 (palm-PrRP31) on food intake, body weight and fat mass in diet-induced obese mice after its subcutaneous administration.

The aim of this study was to prove if such effects on food intake could be achieved after peripheral administration to rats as they are an animal species frequently used to develop the obesity model as well as in preclinical study of anti-obesity drug action. Human palm-PrRP31 that was proved to possess the identical affinity to both human and rat GPR10 was administered by three routes: subcutaneous (s.c.), intraperitoneal (i.p.) and intravenous (i.v.) in order to find out impact of specific route on central effect of palm-PrRP31. Pharmacokinetics of palm-PrRP31 in rat blood plasma as well as the effect on the expression of c-Fos in specific brain regions related to food intake regulation were also investigated.

2. Experimental procedures

2.1. Synthesis of PrRP analogs

Rat PrRP31 (SRAHQHSMETRTPDINPAWYTGRGIRPVGRF-NH_2), rat palmitoylated PrRP analog r palm-PrRP31 ((N-palm)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH_2), human PrRP31 (SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH_2) and human palmitoylated PrRP analog h palm-PrRP31 (N-palm-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH_2) were synthesized and purified in the Institute of Organic Chemistry and Biochemistry, Prague, as described previously [30]. Lipidization of PrRP was performed on fully protected peptide on resine as the last step. The purity and identity of the substances was determined by analytical HPLC and by using a Q-TOF micro MS technique (Waters,

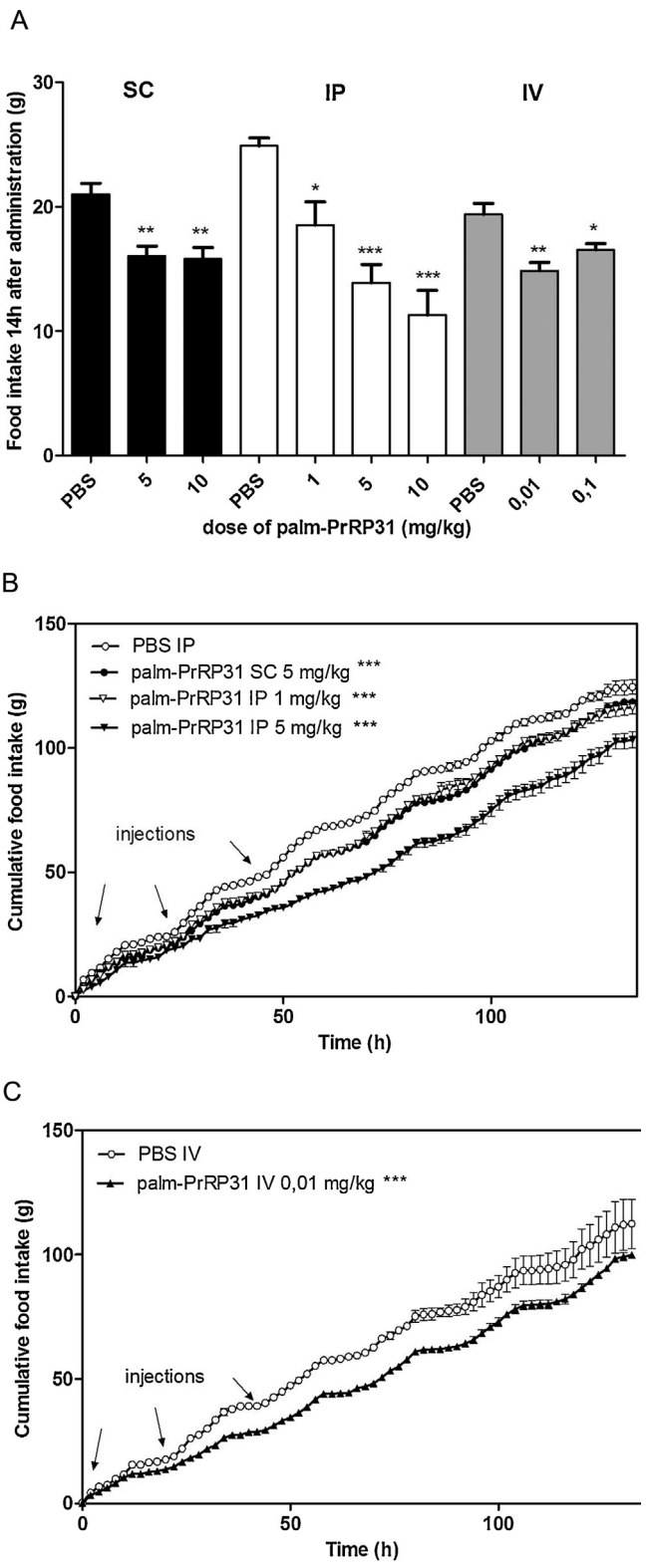

Fig. 1. Food intake of free fed Wistar rats after (A) single injection of vehicle (PBS) and h palm-PrRP31 in doses and routes indicated in figure (food intake monitored after 14 h) and (B) repeated administration of vehicle (PBS) and h palm-PrRP31 at a dose of 5 mg/kg s.c. and 1 and 5 mg/kg i.p., (C) vehicle (PBS) and h palm-PrRP31 at dose of 0.01 mg/kg i.v. into jugular vein for three consecutive days. Peptide was dissolved in PBS. Cumulative food intake was monitored continuously for one week using automatic feeding system. The significance level was $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus the respective PBS-treated group ($n = 5$–6).