Univerzita Karlova

1. lékařská fakulta

Autoreferát disertační práce

UNIVERZITA KARLOVA
1. lékařská fakulta

Úloha stabilních analogů peptidu uvolňujícího prolaktin při obezitě a hypertenzi

The role of stable analogs of prolactin-releasing peptide in obesity and hypertension

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Disertační práce bude nejméně pět pracovních dnů před konáním obhajoby zveřejněna k nahlížení veřejnosti v tištěné podobě na Oddělení pro vědeckou činnost a zahraniční styky Děkanátu 1. lékařské fakulty.

ABSTRAKT

Peptidy snižující příjem potravy neboli anorexigenní peptidy mají velký potenciál v léčbě obezity a jejích komplikací, jako jsou například vysoké hladiny glukózy nebo vysoký krevní tlak. Nevýhodou těchto látek je neschopnost prostoupit hematoencefalickou bariérou do mozku po periferním podání. V naší laboratoři byly navrženy a syntetizovány lipidizované analogy peptidu uvolňujícího prolaktin (PrRP), u kterých byla prokázána vyšší stabilita v krevní plazmě a centrální biologický účinek po periferním podání. Tento centrální účinek byl navíc potvrzen zvýšenou aktivitou proteinu c-Fos v příslušných hypotalamických jádrech.

Cílem této disertační práce bylo zjistit efektivní dávku palmitovaných PrRP31 analogů v akutních experimentech na příjem potravy a charakterizovat jejich dlouhodobý efekt v myších a potkaních modelech obezity a diabetu. Pro tyto studie byly použity následující modely: dietou indukované obézní (DIO) myši (C57Bl/6J), DIO potkani Sprague Dawley a dva modely s nefunkčním leptinovým receptorem: ZDF (Zucker diabetic fatty) potkani a spontánně hypertenzní obézní (SHROB) potkani.

Příjem vysokotukové diety zvýšil u myší i potkanů tělesnou hmotnost a krevní tlak. Dvoutýdenní intraperitoneální léčba palmitovaným analogem PrRP31 významně snížila příjem potravy i tělesnou hmotnost. Tato léčba dále zlepšila také glukózovou toleranci u DIO potkanů a navrátila krevní tlak k fyziologickým hodnotám u DIO myší. U ZDF a SHROB potkanů intraperitoneální léčba snížila příjem potravy, ale neovlivnila tělesnou hmotnost, pravděpodobně kvůli částečné leptinové rezistenci, která je důsledkem nefunkčního leptinového receptoru. Překvapivě léčba významně zlepšila glukózovou toleranci u SHROB potkanů a byla zde tendence ke zlepšení glukózové tolerance i u ZDF potkanů. Předpokládáme, že funkční leptinová signalizace je důležitá pro anorexigenní účinek a nikoli pro antidiabetický účinek palmitovaného PrRP31.

Lipidizované analogy PrRP31 prokázaly anti-obezitní a anti-diabetické účinky ve zvířecích modelech s obezitou a metabolickými poruchami. Palmitované analogy PrRP jsou díky svým anorexigenním, hmotnost snižujícím a navíc antidiabetickým účinkům atraktivními kandidáty pro léčbu obezity a diabetu.

ABSTRACT

Anorexigenic neuropeptides have the potential to decrease food intake and ameliorate obesity and its complications such as high blood glucose or high blood pressure. However, they are not able to cross the blood-brain barrier after peripheral application. Recently, we have designed and synthesized lipidized analogs of prolactin-releasing peptide (PrRP), which resulted in stabilization of the molecule and allowed us to apply the peptide to the periphery to achieve its central biological effect, as it was demonstrated by increased neuronal activity shown by c-Fos in particular hypothalamic nuclei.

The aim of this study was to choose the effective dose in acute food intake experiments and then to characterize the subchronic effect of palmitoylated PrRP analogs in mouse and rat models of obesity and diabetes. Several animal models were used: diet-induced obese (DIO) mice (C57Bl/6J), DIO Sprague-Dawley rats, and two rat models with leptin receptor-deficiency: Zucker diabetic (ZDF) rats and spontaneously hypertensive (SHROB) rats.

Consumption of a high-fat diet in DIO mice and rats increased their body weight and blood pressure. Two-week intraperitoneal treatment with palmitoylated PrRP31 lowered the food intake, body weight, and returned the blood pressure to normal levels. This treatment also improved glucose tolerance in DIO rats. In contrast, in ZDF and SHROB rats, the same treatment lowered the food intake but did not significantly affect the body weight, probably because of severe leptin resistance that was likely due to a non-functional leptin receptor. However, the treatment improved glucose tolerance and reduced blood insulin levels in the SHROB model and tended to improve glucose tolerance in the ZDF model, suggesting that functional leptin is required for the anorexigenic but not for the antidiabetic effects of palmitoylated PrRP31.

Our data showed a good efficacy of lipidized PrRP31 in animal models of obesity and related metabolic complications. Thus, the strong anorexigenic, body weight-reducing and blood glucose-improving effects make palmitoylated PrRP an attractive candidate for anti-obesity and glucose-lowering treatments.

CONTENT

1. INTRODUCTION

Obesity is the most prevalent health problem worldwide and it is a pre-requisite for metabolic syndrome (MetS), clustering of the risk factors such as insulin resistance, dyslipidemia and hypertension that together culminate in the increased risk of type 2 diabetes mellitus (TDM2) and cardiovascular diseases (O'Neill and O'Driscoll, 2015). Unfortunately, despite tremendous efforts there is still no efficient non-invasive therapy for obesity.

Several antiobesity drugs were withdrawn from the market because of their severe psychiatric or cardiovascular side effects (Bray and Ryan, 2014). The promising tools for antiobesity treatment are analogs of peptides that decrease food intake, so called anorexigenic peptides. Anorexigenic peptides have minimal side effects during the long-term antiobesity treatment (Arch, 2015). Several neuropeptides originating in the brain also have an anorexigenic effect, such as prolactin-releasing peptide (PrRP) (Mikulášková et al., 2016).

Prolactin-releasing peptide is an anorexigenic neuropeptide initially isolated from the hypothalamus as a ligand for the orphan G-protein-coupled receptor GPR10 (Hinuma et al., 1998). There are two biologically active isoforms of PrRP containing 20 amino acids or 31 amino acids (PrRP31), as shown in Fig. 1.

The first described effect of PrRP, the prolactin release was soon questioned (Jarry et al., 2000; Maruyama et al., 1999) and it was established that PrRP has other physiological functions, including food intake regulations (Lawrence et al., 2000) and energy expenditure (Takayanagi et al., 2008).

PrRP and its receptor were detected in several hypothalamic nuclei as well as in the brainstem, suggesting an involvement of PrRP in the control of food intake and body weight (BW) regulation (Ibata et al., 2000; Lawrence et al., 2000; Roland et al., 1999).

The effects of PrRP are considered to be associated with the effects of leptin, a long-term acting regulator of energy balance. Moreover, PrRP acting through its receptor may be a key mediator in the central satiating action of cholecystokinin (CCK) (Kuneš et al., 2016).

PrRP is suggested to be involved in the central control of blood pressure (BP) and to play a role in the regulation of cardiovascular homeostasis; for review see (Mikulášková et al., 2016).

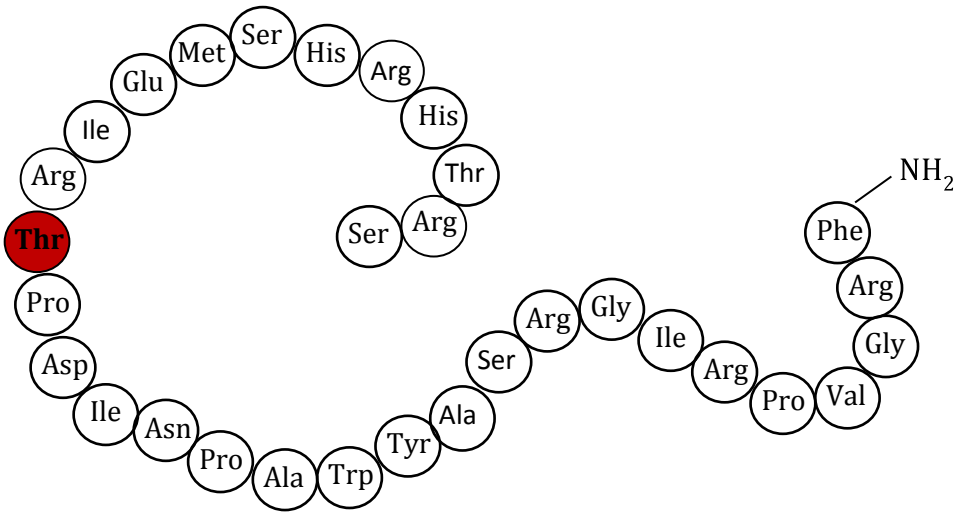

Fig. 1 Structure of human prolactin-releasing peptide *(threonine in red marks the beginning of PrRP20)*

One of the frequently used strategies for peptidic drug development is lipidization, i.e., attachment of fatty acid to the peptide through an ester or amide bond (Malavolta and Cabral, 2011). Lipidization allows neuropeptides reach their target brain receptors and exert their central effect and moreover increases stability and prolongs half-life of neuropeptide in organism.

We have previously shown that analogs of lipidized PrRP designed in our laboratory revealed high affinities for PrRP receptor GPR10 as well as for neuropeptide FF2 receptor *in vitro* (Prazienkova et al., 2016; Pražienková et al., 2017). These analogs also showed prolonged anorexigenic effects in different animal models (for review see (Kuneš et al., 2016)).

The currently used pharmacological therapy for obesity has been shown to have great potential to improve or prevent some cardiovascular risks and diseases. To prevent these serious health problems, it is crucial to determine the mechanisms that regulate both food intake and blood pressure (Mikulášková et al., 2016).

The regulation of food intake involves mutual integration of signals from both the central nervous system and the periphery. Hypothalams plays the key role in the regulation of food intake. The hypothalamus is a brain region containing several nuclei: nucleus arcuatus (ARC), paraventricular nucleus (PVN), lateral hypothalamic area (LHA), ventromedial nucleus (VMN) and dorsomedial nucleus (Schwartz et al., 2000). One of the major regions of the hypothalamus considered as involved in feeding and satiety is ARC (Sobrino Crespo et al., 2014). Another

important brain area involved in regulating food intake is nucleus tractus solitarii (NTS) in the brainstem (Yu and Kim, 2012). NTS receives short-term signals derived from the gastrointestinal tract and also receives neuronal projections from other hypothalamic areas, mainly PVN, indicating that there is communication between the hypothalamus and brainstem.

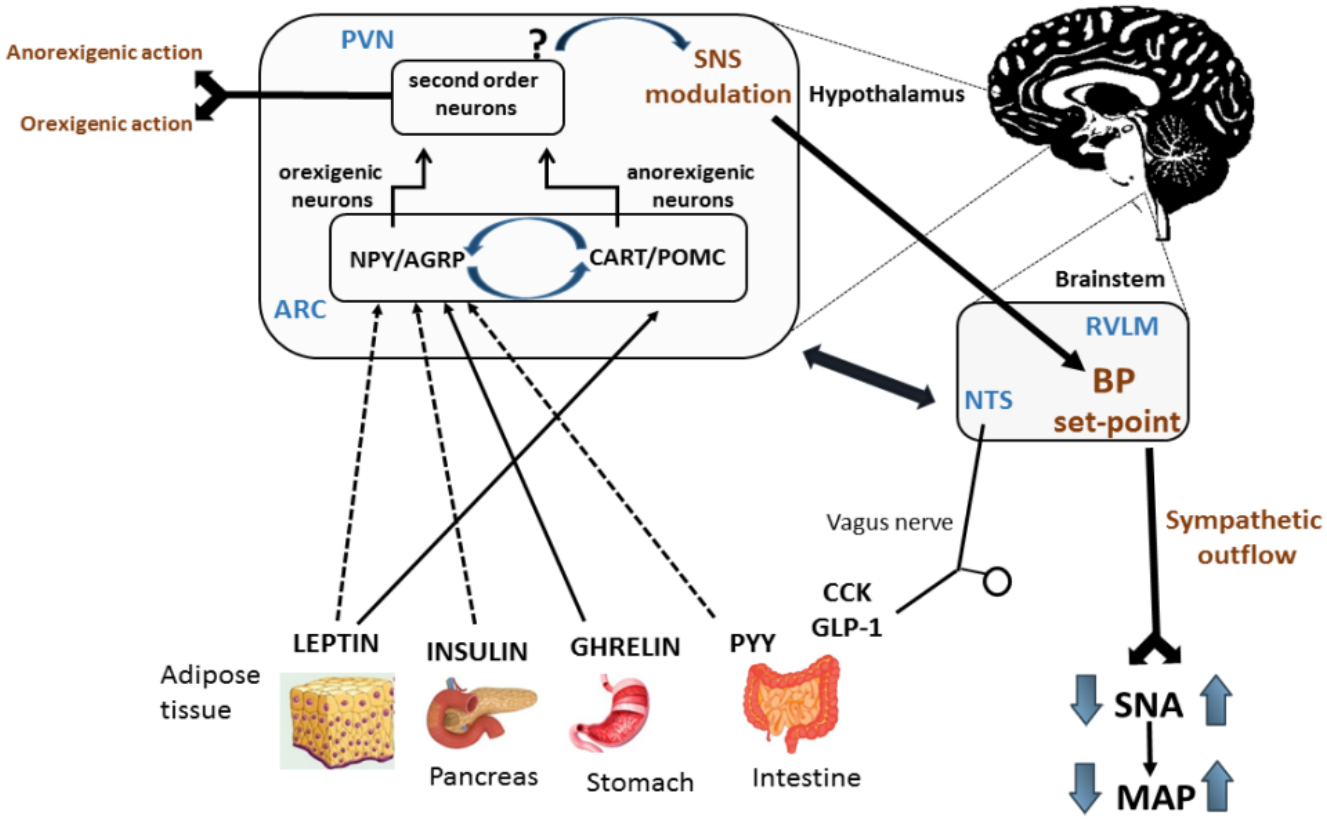

Fig. 2 Potential mechanism by which food-regulating peptides could modulate SNA and thus blood pressure

PVN - paraventricular nucleus, ARC - arcuate nucleus, NTS - nucleus tractus solitarii, RVLM - rostral ventrolateral medulla, SNS - sympathetic nervous system, BP - blood pressure, MAP - mean arterial pressure, SNA - sympathetic nervous activity, CCK - cholecystokinin, GLP-1 - glucagon-like peptide-1, NPY - neuropeptide Y, AgRP - agouti-related peptide, CART - cocaine- and amphetamine-regulated transcript peptide, POMC - pro-opiomelanocortin (Mikulášková et al., 2016)

It is evident that several peptides participating in the regulation of appetite or feeding behavior also cause cardiovascular effects that could be mediated through

the activation or inactivation of the sympathetic nervous system (SNS) (Mikulášková et al., 2016). Increasing evidence points to central nervous system activation as a key contributor to the development of both obesity and hypertension (Hall et al., 2010). As demonstrated in Fig. 2, we can hypothesize that peptides released from second-order neurons in PVN can modulate SNS in the hypothalamus. This leads to activation of a specific BP set-point in rostral ventrolateral medulla (RVLM) of the brainstem and finally to the modulation of sympathetic outflow (Osborn, 2005).

However, the link between the pathways involved in food intake regulation and BP regulation is not completely known. Undoubtedly, the central sympathetic nervous system is a common denominator in this process (Vaněčková et al., 2014). However, further research is needed to enable more precise identification of the hypothalamic signaling mechanisms of food intake regulating peptides that might be considered as therapeutic targets for obesity treatment and maybe even for influencing blood pressure as well (Mikulášková et al., 2016).

Since metabolic syndrome is a multifactorial disorder, it is difficult to find suitable experimental model to study this pathology. A representative rat model to study MetS seems to be Zucker diabetic fatty (ZDF) rats, which is a model of impaired leptin receptor signaling (Fellmann et al., 2013). ZDF rats are mainly used for studying obesity, but they also present changes similar to those seen in human MetS and they are frequently used for studying potential antiobesity and antidiabetic peptidic drugs (Skarbaliene et al., 2015). Another rat model with impaired leptin receptor signaling used for studying MetS are spontaneously hypertensive obese rats (SHROB), so called Koletsky rats (Koletsky, 1973). SHROB rats are considered an animal model with phenotypic features of MetS. They develop obesity, hyperinsulinemia, hyperlipidemia, and spontaneous hypertension.

One of the major risks for development of cardiovascular and metabolic dysfunction including obesity and hypertension is high dietary fat intake. Hypercaloric diets, rich in lipids are widely used in experimental studies to induce metabolic disorders commonly found in humans (Buettner et al., 2007; Dourmashkin et al., 2005; Oliveira et al., 2009). Most rodents tend to become obese on specific diets, and diet-induced obese rodents are therefore suitable for

study of pathologies of MetS (Bergman et al., 2006; Shafrir et al., 1999; Varga et al., 2010).

2. AIMS OF THE THESIS

Anorexigenic neuropeptides have the potential to decrease food intake and ameliorate obesity. However, they are not able to cross the blood-brain barrier after peripheral application. Recently, in our laboratory at IOCB AS CR we have designed and synthetized lipidized analogs of prolactin-releasing peptide, which resulted in stabilization of the molecule and allowed us to apply the peptide to the periphery to achieve their central biological effect. The aims of my thesis were:

Investigation of the acute effect of palmitoylated analogs of prolactin-releasing peptide on food intake in free-fed rats The first aim of my thesis was to evaluate the acute anorexigenic effect of different doses and different routes of administration of palmitoylated analogs of prolactin-releasing peptide *in vivo* in free-fed lean Wistar rats.

Characterization of palmitoylated analogs of prolactin-releasing peptide in chronic *in vivo* experiments – mice and rats with metabolic disorders

The second aim, based on the data from acute food intake experiments, was to determine the long-term effect of palmitoylated prolactin-releasing peptide analogs in obese mice and rats with associated metabolic complications by monitoring the metabolic parameters related to food intake regulation and cardiovascular regulation.

Blood pressure measurement after administration of prolactin-releasing peptide - investigation of the acute effect in free-fed rats and the chronic effect in mice and rats on high-fat diet

High blood pressure is related to obesity. Despite the fact that obesity-related hypertension is a very serious health problem, no clear evidence yet exists as to which occurs first, obesity or hypertension, because both diseases can also exist separately. The third aim of my thesis was to evaluate the acute and chronic effect of anorexigenic prolactin-releasing peptide on blood pressure in normotensive Wistar rats and also to determine the effect of high-fat diet on the blood pressure in mice and rats.

3. METHODS

Human palmitoylated PrRP31 analogs (SRTHRHSMEI K (N-γ-E (N-palm)) PDINPAWYASRGIRPVGRF-NH2) (palm^{11}-PrRP31) and (N-palm-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH2) (palm-PrRP31) were synthesized and purified at the Institute of Organic Chemistry and Biochemistry, Prague, as described in (Maletínská et al., 2015; Mikulášková et al., 2018).

In this thesis various animal models with metabolic complications were used for chronic studies: **ZDF rats and diet-induced obese (DIO) Sprague-Dawley rats** (Holubová et al., 2016) **and SHROB and spontaneously hypertensive (SHR) rats** (Mikulášková et al., 2018). At the end of the experiments, fasted blood plasma was colected for determination of metabolic parameters and oral glucose tolerance test (OGTT) was performed. Hypothalami samples were processed and western blotting was performed as described in our previous papers (Mikulášková et al., 2018; Špolcová et al., 2015).

Acute food intake experiments were performed with **male Wistar rats** (Mikulaskova et al., 2016).

Diet-induced obese mice: Inbred C57BL/6 male mice were obtained from Charles River Laboratories (Sulzfeld, Germany). Beginning at nine weeks of age, mice were fed with high-fat diet containing 60% of fat for four months (Maletínská et al., 2015) to induce obesity (HFD groups). The control group was fed with standard rodent chow diet Ssniff1 R/M-H (Ssniff Spezialdiäten GmbH, Soest, Germany) (STD group). Mice were subcutaneously (SC) injected twice a day with saline (HFD and STD vehicle) or palm^{11}-PrRP31 (5 mg/kg) (HFD palm^{11}-PrRP) for 21 days. Food intake and BW was monitored every day. Arterial blood pressure was measured at the end of the experiment by direct puncture of the carotid artery under pentobarbital anesthesia.

Acute blood pressure measurement: Acute BP experiments were performed with conscious male Wistar rats (250-300g) (Harlan Laboratories, Correzzana, Italy). Rats were housed under standard laboratory conditions. Water and pelleted diet Ssniff were provided *ad libitum*. The carotid artery was cannulated for BP and heart rate (HR) monitoring and the jugular vein for intravenous (IV) drug administration, under isoflurane anaesthesia. Eighteen hours after the surgery, palm^{11}-PrRP31 or palm-PrRP31 dissolved in saline was administred IV,

intraperitoneally (IP) and SC at doses, that had decreased food intake in previous experiments – 0.1 mg/kg IV and 5 mg/kg IP and SC. Basal BP values were monitored first. Thereafter, in the IP and SC experiments saline was injected IP or SC to receive control BP. After saline injection palm^{11}-PrRP31 or palm-PrRP31 was injected IP or SC. Blood pressure and HR was monitored online (n=8-9) using PowerLab system (AD Instruments Ltd., Bella Vista, NSW, Australia) in the following intervals for IP and SC experiments: 10 minutes – basal BP; 10 minutes – after saline injection; 60 minutes – after palm^{11}-PrRP or palm-PrRP31 administration; for IV experments: 10 minutes – basal BP, 10 minutes after the treatment.

Telemetry: To allow BP and HR monitoring, telemetry probes (Data Sciences International, St. Paul, MN) were implanted to the abdominal cavity and connected with the abdominal aorta to Wistar male rats (250-300g) (Harlan Laboratories, Correzzana, Italy), under isoflurane anaesthesia. After the recovery BP and HR were monitored. Rats were housed under standard laboratory conditions. Water and pelleted diet Ssniff were provided ad libitum. Animals were treated SC or IP with palm^{11}-PrRP31 at a dose of 5 mg/kg once daily, one hour before lights were turned off (n=5-6). Treatment started seven days after implantation of the telemetric probe and blood pressure was measured continuously for 15 folowing days.

Statistics: The results are expressed as the means ± S.E.M. Data were evaluated by unpaired t-test, one-way or repeated measures analysis of variance (ANOVA) with Bonferroni or Dunnett´s post hoc test as indicated in the tables and figures using the GraphPad software (Graph-Pad Software, San Diego, CA, USA). $P < 0.05$ was considered statistically significant.

4. RESULTS

4.1. Palm-PrRP31 analog decreased food intake in free-fed rats

The results obtained in this experiments were published in Peptides (Mikulaskova et al., 2016).

Figure 3A,B shows food intake in free-fed Wistar rats after repeated SC and IP (dose 5 mg/kg) and IV (dose 0.01 mg/kg) administration of palm-PrRP31. The cumulative food intake was monitored after three consecutive injections. Food intake was significantly decreased on the first day of the experiment after administration by all routes, SC, IP and IV, and this effect lasted after the second and third injection and on the following days of the experiment without injection. The most significant effect was observed after the IV injection because the dose of palm-PrRP was five hundred times lower than in IP and SC.

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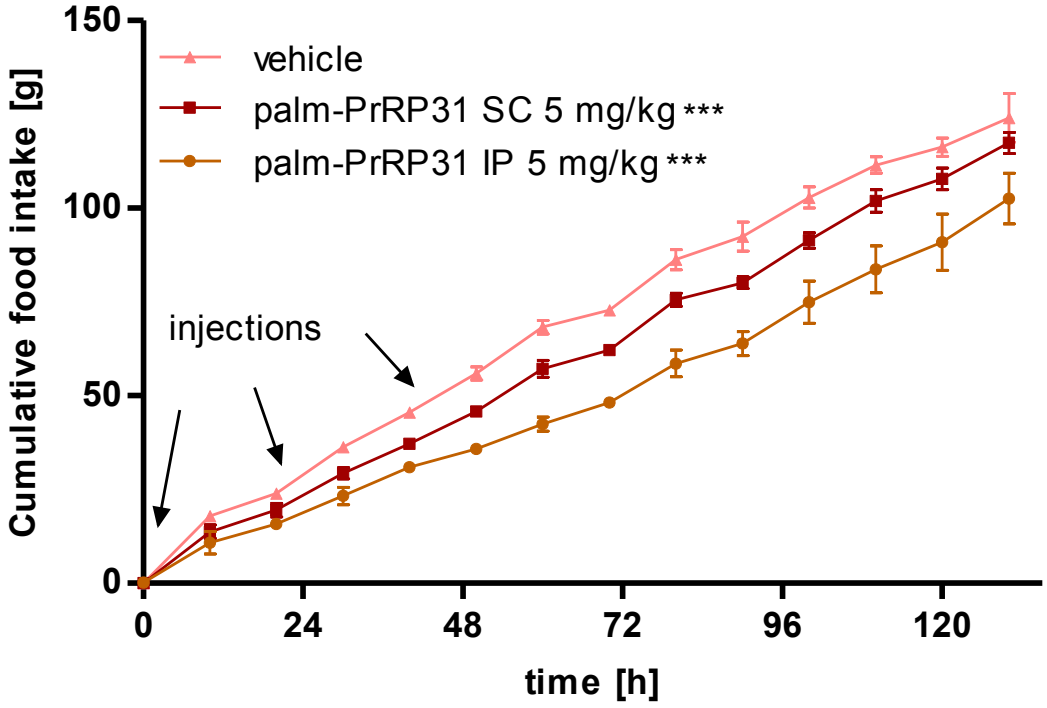

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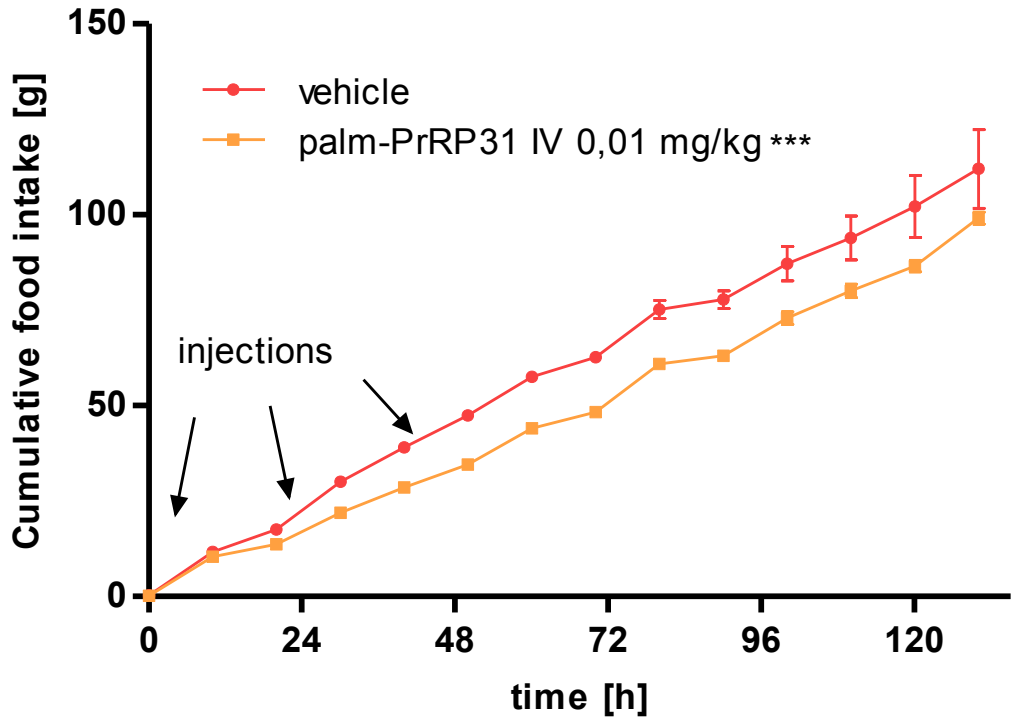

Fig. 3 Food intake after peripheral administration of palm-PrRP31 for three consecutive days, by different routes of administration to Wistar rats

*Data are presented as means ± S.E.M. Statistical analysis was performed by two-way ANOVA followed by Bonferroni post hoc test. The significance level was *P < 0.05, **P < 0.01 and***P < 0.001 vs the respective vehicle-treated group (n = 5–6).*

4.2. Palm^{11}-PrRP31 analog decreased food intake, body weight and mean arterial pressure in DIO mice

The results obtained in the following experiments have not yet been published.

Food intake after the 21-day treatment was significantly decreased (not shown). Consumption of HFD for four months significantly increased BW compared to the STD group; the average BW of the STD group was 27.6 ± 0.5 g, while the average BW of the HFD group was 44.9 ± 1.1 g. Palm^{11}-PrRP31 markedly decreased BW (38.3 ± 1 g) in the HFD group in comparison with the HFD group treated with vehicle. Mean arterial pressure (MAP) was significantly increased in the HFD group in comparison to the STD group and treatment with palm^{11}-PrRP31 returned MAP in the HFD group to normal levels compared to the STD group (Fig. 4).

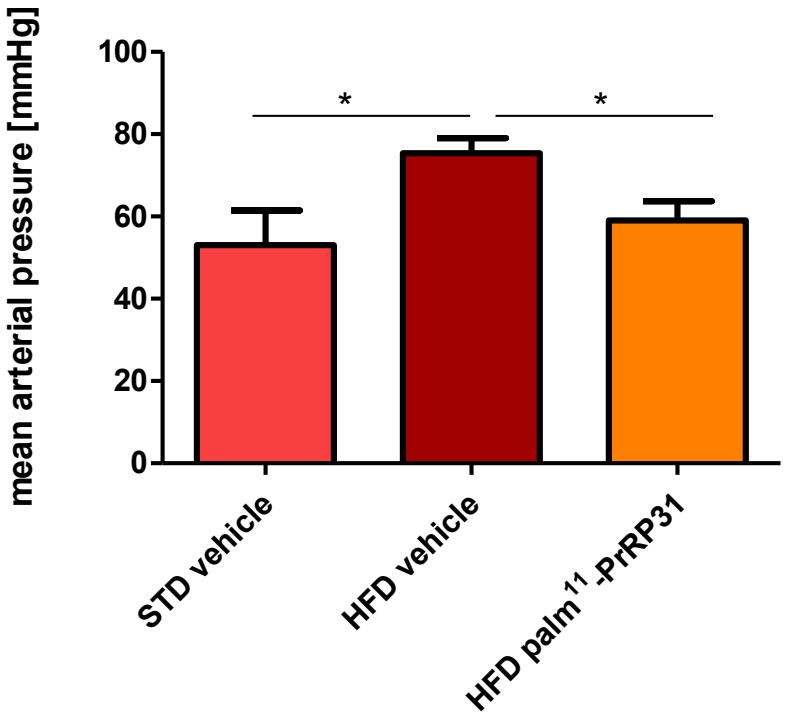

Fig. 4 Chronic effect of palm^{11}-PrRP31 on mean arterial pressure in DIO mice

*Data are presented as means ± S.E.M. Statistical analysis was performed by unpaired t-test, significance is *P < 0.05, **P < 0.01, ***P < 0.001 vs the respective group (n = 15).*

4.3. Palm-PrRP31 analog decreased BW and improved glucose tolerance in Sprague-Dawley DIO rats but not in diabetic ZDF rats

The results obtained in the experiments performed in DIO and ZDF were published in the Journal of Endocrinology (Holubová et al., 2016).

4.3.1. DIO rats

The consumption of HFD for 25 weeks – start of the treatment (31-33 weeks of age) resulted in a significantly affected body weight gain; the average BW of the STD diet-fed control group was 581.4 ± 4.4 g, whereas the average BW of the HFD fed control group was 638.6 ± 10.4 g ($P < 0.001$).

Rats on HFD were divided into four groups (n=8) and IP injected with vehicle or palm-PrRP31 at doses 0.2, 1 or 5 mg/kg for 17 days twice a day. Food intake and BW after the treatment was lowered in a dose-dependent manner, with the effect being more pronounced at week 1 and significant at 1 and 5 mg/kg doses. The highest tested dose of palm-PrRP31 lowered BW by 8 %. OGTT blood glucose

levels were lowered in a dose-independent manner. This decrease was significant compared with the vehicle-treated obese control group only after treatment with the 1 mg/kg dose of palm-PrRP31 (results not shown).

4.3.2. Diabetic ZDF rats

The IP treatment of diabetic ZDF rats with palm-PrPR31 significantly lowered food intake at a dose of 5 mg/kg. All rats were gaining weight during the dosing period and the body weight gain was not significantly lowered after the palm-PrRP31 treatment. The treatment with palm-PrRP31 resulted in a nonsignificant dose-dependent decrease in blood glycemia during OGTT (results not shown).

4.4. Palm^{11}-PrRP31 improved glucose tolerance in SHROB rats

The results obtained in the experiment performed in SHROB rats presented in the this chapter were published in Nutrition & Diabetes (Mikulášková et al., 2018).

Treatment with palm^{11}-PrRP31 decreased food intake in both, SHROB and SHR rats. However, the effect was more pronounced in the SHR rats compared to SHROB rats (results not shown). Similarly, BW was lowered significantly in the SHR rats (−13%, $P < 0.001$), but not in the SHROB rats after palm^{11}-PrRP31 treatment (Fig. 5A). However, palm^{11}-PrRP31 administration improved tolerance to glucose measured by OGTT in both genotypes (Fig. 5B).

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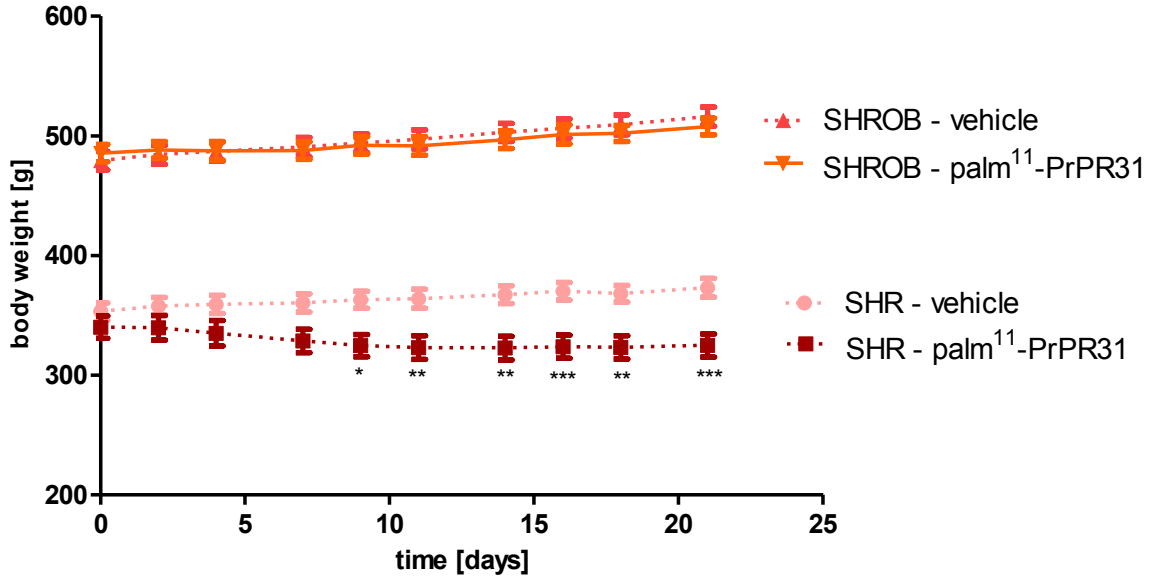

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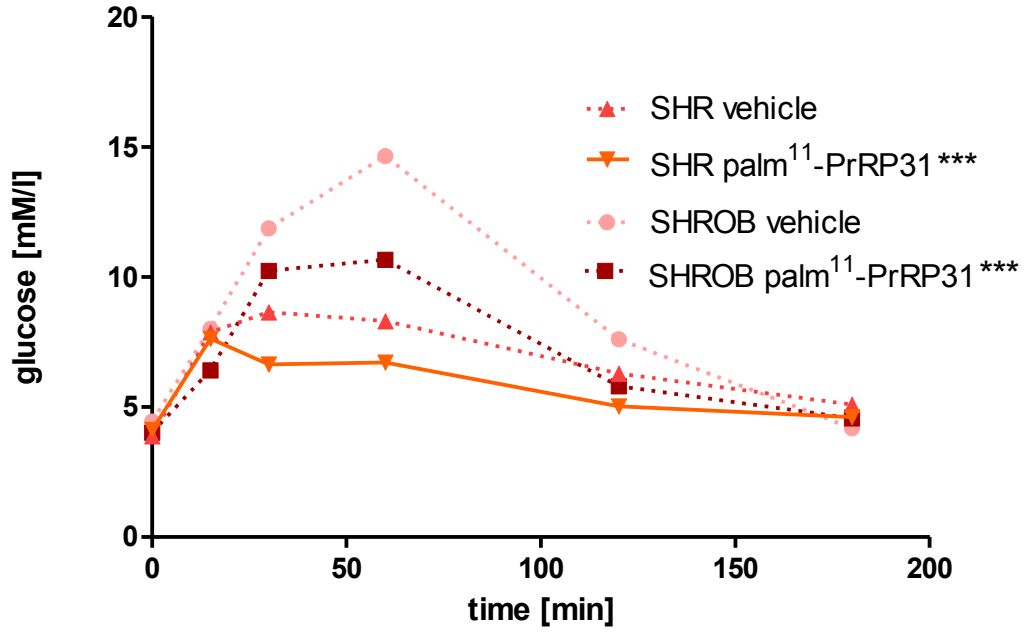

Fig. 5 Chronic effect of palm^{11}-PrRP31 in SHR and SHROB on body weight (A) and OGTT response (B)

*Data are presented as means ± S.E.M. Statistical analysis was performed by repeated measures ANOVA with Bonferroni post hoc test, significance is *P < 0.05, **P < 0.01, ***P < 0.001 vs the respective control group (n = 8)*

4.5. Blood pressure experiments in Wistar rats

The results obtained in the following experiments have not yet been published.

4.5.1. The effect of acute injections of palm^{11}-PrRP31 and palm-PrRP31

Basal MAP and HR was measured in Wistar rats at the begginig of the experiment for 10 minutes. Thereafter, rats were injected IP or SC with saline, to receive control MAP and HR. Then palm^{11}-PrRP31 or palm-PrRP31 at a dose of 5 mg/kg was injected IP or SC. There were no changes after the injection of saline (control MAP) in both, SC and IP injections. A temporary significant increase of MAP after IP treatment was observed 10, and 20 minutes after injection of palm^{11}-PrRP31 and a temporary significant decrease of MAP after IP treatment was observed 20, and 30 minutes after injection of palm-PrRP31. HR was temporarily increased 10, 20, and 30 minutes after IP injection of palm^{11}-PrRP31. After SC treatment there were no significant changes in MAP and HR after injections of both analogs. In the IV administration experiment, the basal MAP and HR were

measured for the first 10 minutes and then the rats were injected with palm-PrRP31 or palm^{11}-PrRP31 at a dose of 0.1 mg/kg. We did not obsereve any significant changes in MAP and HR after IV administration of both compounds (results not shown).

4.5.2. The effect of palm^{11}-PrRP31 on telemetry-measured MAP

Continous measurement started one week after implantation of telemetric probe, and BP and HR was monitored for 15 consecutive days. For the first two days basal MAP was recorded. As shown in Fig. 6, on the 3rd, 4th and 6th day rats were injected SC with palm^{11}-PrRP31 at a dose of 5 mg/kg. From the 10th to the 14th day palm^{11}-PrRP31 was injected IP at a dose 5 mg/kg. Neither way of treatment (SC and IP) with palm^{11}-PrRP31 did significantly change the mean arterial pressure, or the heart rate (not shown).

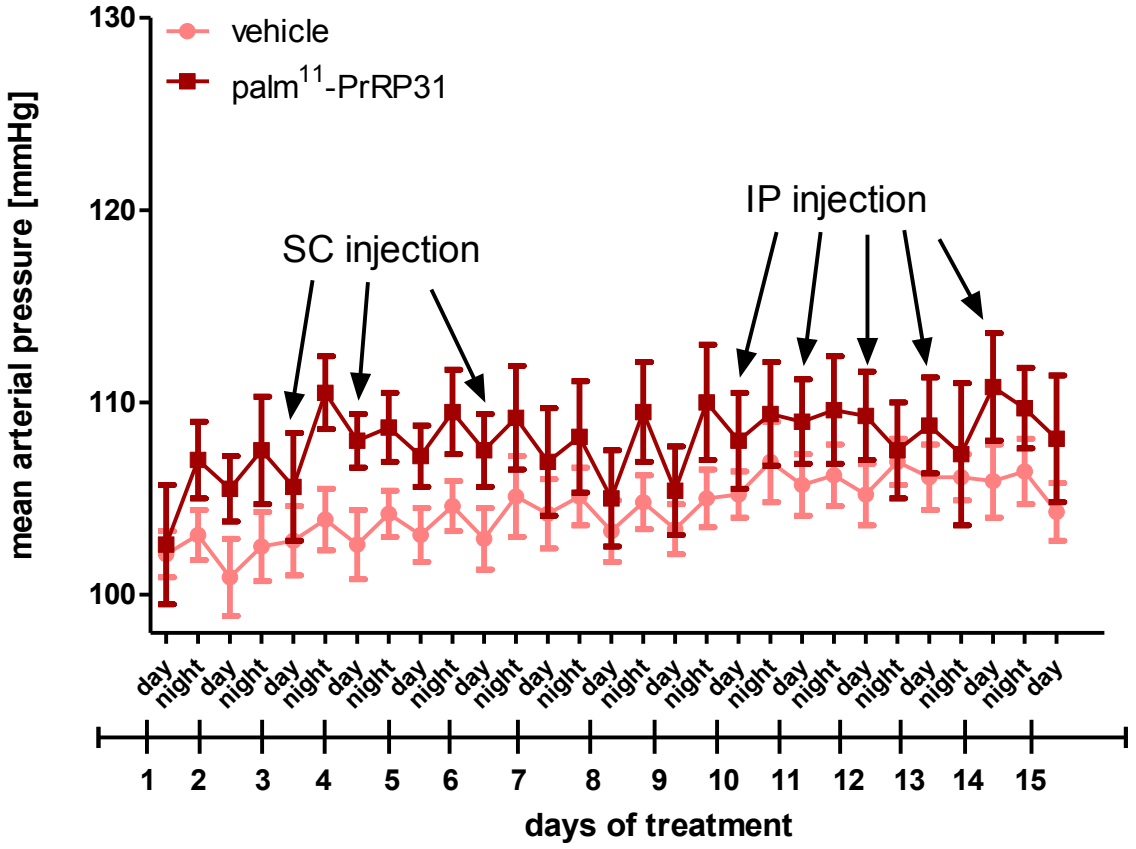

Fig. 6 Effect of palm^{11}-PrRP31 on telemetry-measured MAP in Wistar rats

Data are presented as means ± S.E.M. Each point represents the average value of daily or nightly values of BP/HR. Statistical analysis was performed by two-way ANOVA with Bonferroni post hoc test (n=5-6).

5. DISCUSSION

Obesity and related type 2 diabetes are serious health problems with increasing prevalence. Moreover, obesity together with other symptoms of metabolic syndrome play an important role in cardiovascular morbidity and mortality. Since mortality of cardiovascular diseases is one of the biggest problems around the world, the individual risk factors of MetS should be solved in order to prevent these cardiovascular diseases.

The treatment of obesity has proven to be challenging. Despite that pharmacotherapy for weight loss has progressed extensively over the past decade (for review see (Jackson et al., 2015)) a large medical need exists for new weight loss drugs, with higher efficacy and improved side-effect profile compared to the current antiobesity therapeutics.

Native anorexigenic neuropeptides have the potential to decrease food intake and ameliorate obesity. However, they are not able to cross the blood-brain barrier after peripheral application. Recently, we have designed and synthetized lipidized analogs of prolactin-releasing peptide, which resulted in stabilization of the molecule in plasma and allowed us to apply the peptide to the periphery to achieve its central biological effects (Kuneš et al., 2016).

In my PhD thesis, two potent analogs of prolactin-releasing peptide palmitoylated either at the N-terminus (palm-PrRP31) or at position 11 (palm^{11}-PrRP31) were used for metabolic studies in different animal models.

To sum up, lipidized analogs of PrRP31 designed in our laboratory showed strong anorexigenic, body weigh-reducing and glucose tolerance-improving effects. However, the mechanism of their action is still unclear. In this work we used various animal models with different metabolic disorders to clarify the mechanisms how lipidized PrRP analogs act *in vivo*. The models with impaired leptin receptor signaling, the ZDF and SHROB rats, and two models of rodents fed high fat diet, DIO mice and DIO rats were used in this study. All these models are frequently used for studying obesity and its complications.

Firstly, we investigated the effect on food intake in free-fed rats and based on these data we selected the dose for chronic experiments (Mikulaskova et al., 2016). Palm-PrRP31 analog influenced food intake even in free-fed rats; however, the degree of food intake reduction was partially dependent on the route of

administration and on the dose of the peptide. The most sufficient food intake decreasing effect was achieved after IV injections and a less pronounced significant effect was shown after SC administration. In spite of the fact that subcutaneous drug administration should provide an important route for its delivery, the exact mechanism underlying SC absorption is not completely understood. Future study towards a better formulation of the drug could help to decrease the effective dose and increase the release of the compound both after intraperitoneal and subcutaneous administrations.

Secondly, the long-term treatment with these analogs decreased food intake, body weight and improved metabolic parameters in DIO mice and DIO rats with metabolic complications. In ZDF rats with severe leptin resistance due to a nonfunctional leptin receptor (Ishizuka et al., 1998), two-week treatment with palm-PrRP31 decreased only food intake and failed to decrease BW and only tended to improve glucose tolerance (Holubová et al., 2016). Similarly, three-week treatment with palm^{11}-PrRP31 decreased food intake and did not decrease BW in SHROB rats with impaired leptin receptor signaling. Surprisingly, glucose tolerance in SHROB rats was significantly improved after the treatment (Mikulášková et al., 2018). Taken together, these data suggest that improving glucose tolerance could be mostly independent of antiobesity effects. Moreover, as the effects of palm^{11}-PrRP31 were observed in the leptin receptor-deficient SHROB rats, improvements in the glucose metabolism appear to be completely independent of the leptin signaling.

In SHROB and SHR rats (Mikulášková et al., 2018), palm^{11}-PrRP31 increased PI3K levels and additionally ERK activation in the hypothalamus, both pathways that are known to be activated by insulin. We can hypothesize that the anorexigenic effect of the PrRP31 analog was mediated by PI3K through the insulin receptor IR activation. Increased MAPK/ERK1/2 phosphorylation in the hypothalamus could also be the result of PrRP effects, because ERK1/2 is the main activation pathway through its GPR10 receptor (Maixnerová et al., 2011).

It is known that peptides that regulate food intake also play an important role in cardiovascular regulation. Therefore, in my PhD. thesis the blood pressure was measured after acute and chronic administration of PrRP31 analogs to normotensive Wistar rats. Acute IP administration of palm^{11}-PrRP31 at a dose that was used in metabolic studies (food intake decreasing dose – 5 mg/kg)

temporarily increased blood pressure and the same administration of palm-PrRP31 temporarily decreased blood pressure. On the other hand, SC and IV administration of both analogs in respective food intake-decreasing doses did not cause any effect on the blood pressure. Chronic SC and IP administration of palm^{11}-PrRP31 had no effect on the blood pressure in Wistar rats.

Last but not least, as the high blood pressure is one of the obesity-related complications, the effect of PrRP31 palmitoylated analog (palm^{11}-PrRP31) on BP was investigated. High-fat diet significantly increased blood pressure in DIO rats and DIO mice, and chronic administration of palm^{11}-PrRP31 (5 mg/kg) decreased the blood pressure in DIO mice to the normal level. Therefore, lipidized PrRP analogs may be important tools even for the treatment of obesity complications such as increased blood pressure.

6. CONCLUSION

In general, peptides are key regulators of physiological processes with low risk of toxicity and side effects. Despite their clinical potential, natural peptides have several limitations such as poor bioavailability, low stability in the organism, and difficulties to cross the blood-brain barrier after peripheral application. One of the recently used strategies for peptide drug development is lipidization of peptides. These lipidized peptides are more stable with a high effectiveness and potential to act centrally after peripheral application.

In our laboratory we designed palmitoylated PrRP31 analogs that exhibited strong anorexigenic and antidiabetic effects after peripheral administration to several rat and mouse models of obesity and diabetes. Moreover palm^{11}-PrRP31 was able to return increased blood pressure in DIO mice to the normal levels.

Based on the results from my PhD thesis, we would like to hypothesize that the full effect of the lipidized PrRP analogs on food intake and hence on body weight but not improvement in the glucose metabolism might be related to intact leptin signalization.

To conclude, our data suggest a good efficacy of lipidized PrRP in rat and mouse models of obesity and diabetes. Thus, the strong anorexigenic, body weight-reducing and blood glucose-improving effects make palmitoylated PrRP analogs attractive candidates for antiobesity and glucose-lowering treatment.

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LIST OF MY PUBLICATIONS

Publications related to PhD thesis:

1. **Mikulášková, B.**, Maletínská, L., Zicha, J., & Kuneš, J. 2016. The role of food intake regulating peptides in cardiovascular regulation. *Mol Cell Endocrinol*, 436: 78-92. **IF = 3.754**

2. Kuneš, J., Pražienková, V., Popelová, A., **Mikulášková, B.**, Zemenová, J., & Maletínská, L. 2016. Prolactin-releasing peptide: a new tool for obesity treatment. *J Endocrinol*, 230(2): R51-58. **IF = 4.706**

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1. Špolcová, A., **Mikulášková, B.**, Kršková, K., Gajdošechová, L., Zórad, Š., Olszanecki, R., Suski, M., Bujak-Giżycka, B., Železná, B., & Maletínská, L. 2014. Deficient hippocampal insulin signaling and augmented Tau phosphorylation is related to obesity- and age-induced peripheral insulin resistance: a study in Zucker rats. *BMC Neurosci*, 15: 111. **IF = 2.312**

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EP15729749. Lipidized peptides as neuroprotective agents. Maletínská, L., Zělezná, B., Blechová, M., Popelová, A., Neprašová, B., Kuneš, J. (granted 2018)

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Based on the results of this PhD. thesis the research collaboration and license agreement between IOCB AS CR and IP AS CR and Novo Nordisk A/S was signed in August 2017.