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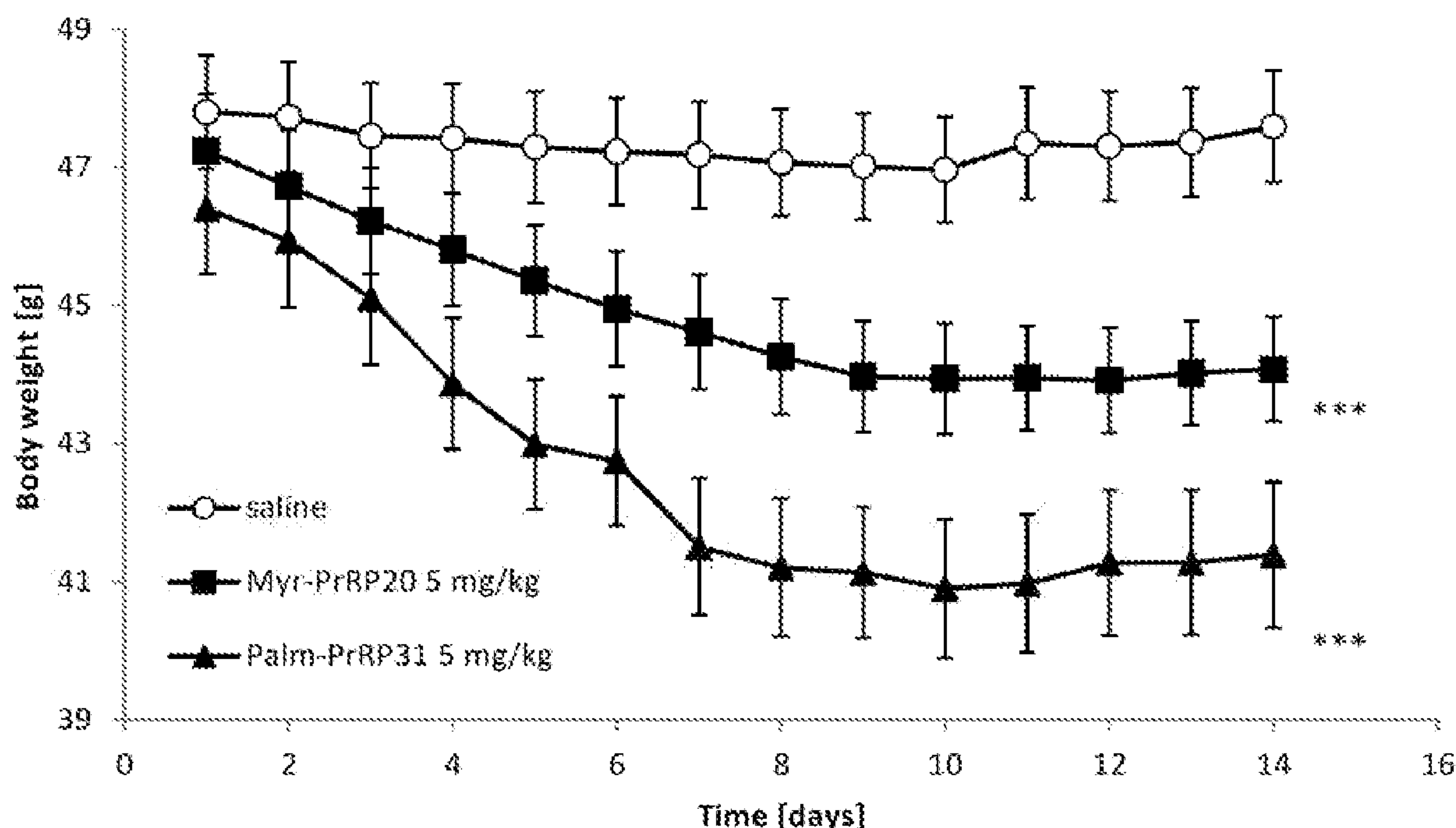
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(54) **Titre : PEPTIDES LIPIDES EN TANT QU'AGENTS ANTI-OBESITE**

(54) **Title: LIPIDATED PEPTIDES AS ANTI-OBESITY AGENTS**



(57) **Abrégé/Abstract:**

Lipidated peptides, analogs of both forms of the prolactin-releasing peptide, PrRP31 and PrRP20, represent anorexigenic compounds that lower food intake and function in the brain after peripheral administration. The analogs PrRP31 and PrRP20 lipidated at the N-terminus by myristic or palmitic acids bind with high affinity to the endogenous receptor GPR10 in the rat pituitary cell line RC-4B/C and CHO cell line with transfected human receptor. These lipidated peptides also significantly decrease, in a dose-dependent manner, the food intake in fasted mice and have similar effects in comparable doses as centrally administered natural PrRP31, these effects are, however, stronger and longer lasting. Lipidation of an effective anorexigenic neuropeptide PrRP induces a central effect after peripheral administration and thus makes the lipidated analogs of PrRP a promising anti-obesity drug.

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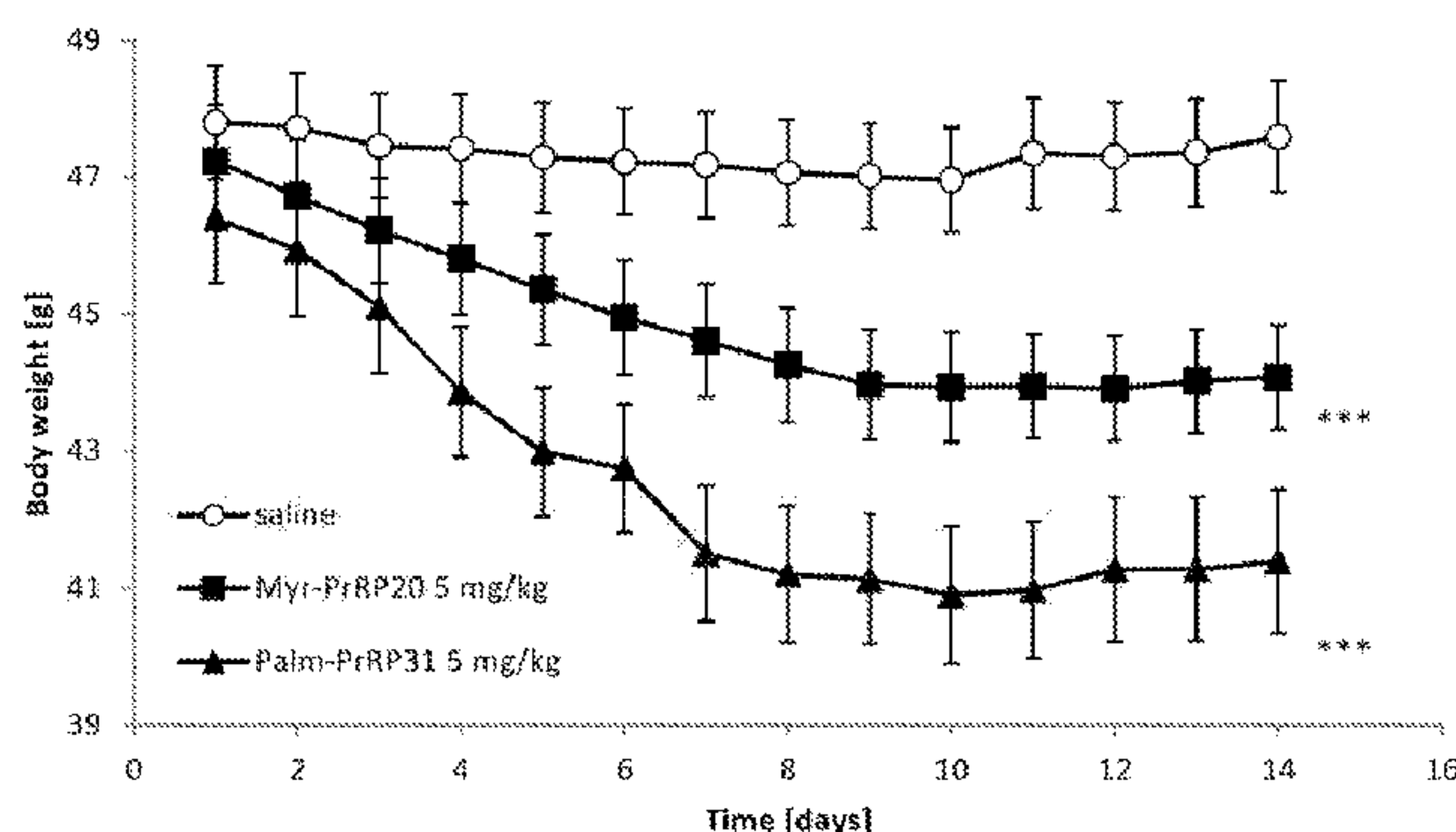


FIG. 9

(57) Abstract: Lipidated peptides, analogs of both forms of the prolactin-releasing peptide, PrRP31 and PrRP20, represent anorexiogenic compounds that lower food intake and function in the brain after peripheral administration. The analogs PrRP31 and PrRP20 lipidated at the N-terminus by myristic or palmitic acids bind with high affinity to the endogenous receptor GPR10 in the rat pituitary cell line RC-4B/C and CHO cell line with transfected human receptor. These lipidated peptides also significantly decrease, in a dose-dependent manner, the food intake in fasted mice and have similar effects in comparable doses as centrally administered natural PrRP31, these effects are, however, stronger and longer lasting. Lipidation of an effective anorexigenic neuropeptide PrRP induces a central effect after peripheral administration and thus makes the lipidated analogs of PrRP a promising anti-obesity drug.



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LIPIDATED PEPTIDES AS ANTI-OBESITY AGENTS

CROSS-REFERENCE

[0001] This application claims the benefit of Czech Republic Application No. CZ 2012-476, filed July 12, 2012.

FIELD OF THE INVENTION

[0002] The embodiments described herein relate to lipidated analogs of prolactin-releasing peptides and the use of these analogs as anorexigenic compounds that lower food intake. The synthesis of these lipidated peptides is described herein, as well as their pharmacological effects *in vitro* and *in vivo*.

BACKGROUND OF THE INVENTION

[0003] In both the developing and developed countries, obesity is an increasingly growing problem, for which no effective therapies are available. Current medications are typically associated with adverse side effects. For example, the only medication available in the Czech Republic is Orlistat, an inhibitor of intestinal lipase which lowers the absorption of lipids from the small intestine. It is, however, associated with adverse effects, such as diarrhoea. Sibutramine, a serotonin-norepinephrine reuptake inhibitor that induces satiety sensation was withdrawn from the market for increased risk of heart disease found during its use (Strader, A., et al., "Gastrointestinal hormones and food intake," *Gastroenterology*, 128:175-191, 2005; Heal, D.J. et al., "What is the prognosis for new centrally-acting anti-obesity drugs?" *Neuropharmacology*, 63:132-146, 2012).

SUMMARY OF THE INVENTION

[0004] Embodiments disclosed herein relate to lipidated analog of prolactin-releasing peptide having a general formula:

$J^1-J^2-rRPsGRt-NH_2$ (I), wherein

J^1 represents a fatty acid C6 to C18 bound by an amide bond to an amino group of a N-terminal amino acid;

J^2 represents a chain of 13 or 24 amino acids;

r is isoleucine, alanine, or phenylglycine;

s is valine or phenylglycine; and

t is phenylalanine or an amino acid with a side chain containing CH_2-Ar or CH_2-S-CH_2-Ar , wherein Ar represents phenyl or naphthyl, optionally substituted by a halogen or nitro group.

[0005] Additional embodiments disclosed herein relate to lipidated analogs of prolactin-releasing peptide having a general formula:

$J^1-k-J^3-rRPsGRt-NH_2$ (II), wherein

J^1 represents a fatty acid C6 to C18 bound by an amide bond to an amino group of a N-terminal amino acid;

k is chosen from serine, threonine or diaminopropionic acid;

J^3 represents a chain of 23 or 12 amino acids;

r is isoleucine, alanine, or phenylglycine;

s is valine or phenylglycine; and

t is phenylalanine or an amino acid with a side chain containing CH_2-Ar or CH_2-S-CH_2-Ar , where Ar represents phenyl or naphthyl, optionally substituted by halogen or nitro group.

[0006] Further embodiments disclosed herein relate to lipidated analogs of prolactin-releasing peptide having a general formula:

$J^1-kRmHnHSqEuRTPDINPAWYmoRprRPsGRt-NH_2$ (III), wherein

k is serine, threonine or diaminopropionic acid;

m is chosen from threonine, alanine or methylalanine;

n is glutamine or arginine;

q is methionine or norleucine;

u is threonine or isoleucine;

o is glycine or serine;

p is glycine, alanine, proline or N-methylglycine;

r is isoleucine, alanine or phenylglycine;

s is valine or phenylglycine;

t is phenylalanine or an amino acid with a side chain containing CH_2-Ar or CH_2-S-CH_2-Ar , where Ar represents phenyl or naphthyl, optionally substituted by halogen or nitro group; and

J^1 represents a fatty acid C6 to C18 bound by the amide bond to the amino group of the N-terminal amino acid, wherein the chain of amino acids **kRmHnHSqEuRTPDINPAWYmoRp** is optionally shortened by eliminating from 1 to 10 amino acids.

[0007] Yet further embodiments herein relate to lipidated analogs of prolactin-releasing peptide, having a general formula

$J^1-kRmHnHSqEuRTPDINPAWYmoRprRPsGRt-NH_2$ (III), wherein

k is serine, threonine or diaminopropionic acid;

m is threonine, alanine or methylalanine;

n is glutamine or arginine;

q is methionine or norleucine;

u is threonine or isoleucine;

o is glycine or serine;

p is glycine, alanine, proline or N-methylglycine;

r is isoleucine, alanine or phenylglycine;

s is valine or phenylglycine;

t is phenylalanine or an amino acid with side chain containing $\text{CH}_2\text{-Ar}$ or $\text{CH}_2\text{-S-CH}_2\text{-Ar}$, where Ar represents phenyl or naphthyl, optionally substituted by halogen or nitro group; and

J¹ represents a fatty acid C6 to C18 bound by the amide bond to the amino group of the N-terminal amino acid, where the chain of amino acids **kRmHnHSqEuRTPDINPAWYmoRp** is optionally shortened from its N-terminus by eliminating from 1 to 10 amino acids.

[0008] Additional embodiments disclosed herein relate to lipidated analogs of prolactin-releasing peptide chosen from the group containing peptides of formula

J¹-kRmHnHSqEuRTPDINPAWYmoRp **prRPsGRt-NH₂** (III), or

J¹-TPDINPAWYmoRp **prRPsGRt-NH₂** (IV), wherein

k is serine, threonine or diaminopropionic acid;

m is threonine, alanine or methylalanine;

n is glutamine or arginine;

q is methionine or norleucine;

u is threonine or isoleucine;

o is glycine or serine;

p is glycine, alanine, proline or N-methylglycine;

r is isoleucine, alanine or phenylglycine;

s is valine or phenylglycine;

t is phenylalanine or an amino acid with side chain containing $\text{CH}_2\text{-Ar}$ or $\text{CH}_2\text{-S-CH}_2\text{-Ar}$, where Ar represents phenyl or naphthyl, optionally substituted by halogen or nitro group; and

J¹ represents a fatty acid C6 to C18 bound by the amide bond to the amino group of the N-terminal amino acid.

[0009] In some embodiments, substitutions of amino acids in positions 1-24 of formula III or in positions 1-13 of formula IV are performed that do not increase the binding affinity value of the resulting lipidated peptide to the receptor GPR10 over K_i 10^{-6} mol.l⁻¹, measured in the rat hypophyseal cell line RC-4B/C grown on 24-well plates, which had their bottom coated with polyethyleneimine, up to the optimal density of 300-450 thousand per well; then the following agents are added: binding buffer containing 20 mmol.l⁻¹ HEPES, pH 7.4, 118 mmol.l⁻¹ NaCl, 4.7 mmol.l⁻¹ KCl, 5 mmol.l⁻¹ MgCl₂, 5.5 mmol.l⁻¹ glucose, 1 mg/ml bovine serum albumin, 0.1 mg/ml bovine pancreatic trypsin inhibitor; unlabelled analogs of PrRP of final concentration within 10^{-11} to 10^{-4} mol.l⁻¹, and ¹²⁵I-PrRP31 of final concentration of 10^{-10} mol.l⁻¹; the plate is incubated for 60 minutes at room temperature and thereafter the cells are solubilized in 0.1 mol/l solution of NaOH and the radioactivity bound to the cell is counted in the γ -counter.

[0010] Additional embodiments relate to lipidated analogs of prolactin-releasing peptide of formula:

J^1 -SRmHnHSqEuRTPDINPAWYmoRprRPsGRt-NH₂ (V), wherein

m is threonine, alanine or methylalanine;

n is glutamine or arginine;

q is methionine or norleucine;

u is threonine or isoleucine;

o is glycine or serine;

p is glycine, alanine, proline or N-methylglycine;

r is isoleucine, alanine or phenylglycine, **s** is chosen from valine or phenylglycine;

t is phenylalanine or an amino acid with side chain containing CH₂-Ar or CH₂-S-CH₂-Ar, where Ar represents phenyl or naphthyl, optionally substituted by halogen or nitro group; and

J^1 represents a fatty acid C6 to C18 bound by an amide bond to the amino group of the N-terminal amino acid.

[0011] In some embodiments, the C-terminal amino acid of any of the lipidated analogs of prolactin-releasing peptide disclosed herein is naphthylalanine, benzylcysteine, benzylhistidine, pentafluorophenylalanine, or nitrophenylalanine.

[0012] In some embodiments, the fatty acid of any of the lipidated analogs of prolactin-releasing peptide disclosed herein is selected from the group consisting of fatty acids with 6 to 18 carbons and fatty acids with 13 to 18 carbon atoms. In some embodiments, the fatty acid is 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 carbon atoms.

[0013] Further embodiments relate to a lipidated analog of prolactin-releasing peptide selected from the group consisting of:

Dpr(oct))RmHnHSNleETRTPDINPAWYmoRGrRPsGRF-NH₂;

(Dpr(dodec))RmHnHSNleETRTPDINPAWYmoRGrRPsGRF-NH₂;

(Dpr(tridec))RmHnHSNleETRTPDINPAWYmoRGrRPsGRF-NH₂;

(Dpr(myristic))RmHnHSNleETRTPDINPAWYmoRGrRPsGRF-NH₂;

(Dpr(palm))RmHnHSNleETRTPDINPAWYmoRGrRPsGRF-NH₂;

(myristic)TPDINPAWYmoRGrRPsGRF-NH₂;

(palm)TPDINPAWYmoRGrRPsGRF-NH₂;

(Dpr(oct))RmHnHSNleETRTPDINPAWYmoRGrRPsGR 1-Nal-NH₂;

(Dpr(dodec))RmHnHSNleETRTPDINPAWYmoRGrRPsGR 1-Nal-NH₂;

(Dpr(tridec))RmHnHSNleETRTPDINPAWYmoRGrRPsGR 1-Nal-NH₂;

(Dpr(myristic))RmHnHSNleETRTPDINPAWYmoRGrRPsGR 1-Nal-NH₂;

(Dpr(palm))RmHnHSNleETRTPDINPAWYmoRGrRPsGR 1-Nal-NH₂;

(myr)TPDINPAWYmoRGrRPsGR 1-Nal-NH₂; and

(palm)TPDINPAWYmoRGrRPsGR 1-Nal-NH₂;

wherein **m** is T or A; **o** is G or S; **n** is Q or R; **r** is I, A or phenylglycine; **s** is V or phenylglycine, and wherein at the peptide positions 1 to 24, such substitutions of amino acids may be performed that do not increase the binding affinity value of the resulting lipidated peptide to the receptor GPR10 over K_i 10^{-6} mol.l⁻¹, and its anorexic activity evaluated by the food intake test in fasted mice after both peripheral and central administration is equal or higher as compared to administered PrRP.

[0014] More embodiments relate to a lipidated analog of prolactin-releasing peptide selected from the group consisting of:

(Dpr(oct))RmHnHSNleETRTPDINPAWYmoRGIRPVGRF-NH₂;

(Dpr(dodec))RmHnHSNleETRTPDINPAWYmoRGIRPVGRF-NH₂;

(Dpr(tridec))RmHnHSNleETRTPDINPAWYmoRGIRPVGRF-NH₂;

(Dpr(myr))RmHnHSNleETRTPDINPAWYmoRGIRPVGRF-NH₂;

(Dpr(palm))RmHnHSNleETRTPDINPAWYmoRGIRPVGRF-NH₂;

(myr)TPDINPAWYmoRGIRPVGRF-NH₂;

(palm)TPDINPAWYmoRGIRPVGRF-NH₂;

(Dpr(oct))RmHnHSNleETRTPDINPAWYmoRGIRPVGR 1-Nal-NH₂;

(Dpr(dodec))RmHnHSNleETRTPDINPAWYmoRGIRPVGR 1-Nal-NH₂;

(Dpr(tridec))RmHnHSNleETRTPDINPAWYmoRGIRPVGR 1-Nal-NH₂;

(Dpr(myr))RmHnHSNleETRTPDINPAWYmoRGIRPVGR 1-Nal-NH₂;

(Dpr(palm))RmHnHSNleETRTPDINPAWYmoRGIRPVGR 1-Nal-NH₂;

(myr)TPDINPAWYmoRGIRPVGR 1-Nal-NH₂; and

(palm)TPDINPAWYmoRGIRPVGR 1-Nal-NH₂; wherein

m is T or A;

o is G or S;

n is Q or R, and wherein

at the peptide positions 1 to 24, such substitutions in amino acids may be performed that do not increase the binding affinity value of the lipidated analog to the receptor GPR10 over K_i 10^{-6} mol.l⁻¹, and its anorexic activity evaluated by the food intake test in fasted mice after both peripheral and central administration is equal or higher as compared to administered PrRP.

[0015] Additional embodiments relate to a lipidated analog of prolactin-releasing peptide selected from the group consisting of:

J^a-SRAHQHSMETRTPDINPAWYTGRGIRPVGRF-NH₂;

J^a-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH₂;

(N-palm)-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NHMe;
 (N-palm)-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NOMe;
 (N-myr)-TPDINPAWYTGRGIRPVGRF-NH₂;
 (N-myr)-TPDINPAWYASRGIRPVGRF-NH₂;
 (N-oct)-TPDINPAWYASRGIRPVGRF-NH₂;
 (N-dec)-TPDINPAWYASRGIRPVGRF-NH₂;
 (N-dodec)-TPDINPAWYASRGIRPVGRF-NH₂;
 J^{1'}-SRAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂;
 J^a-SRAHQHS Nle ETRTPDINPAWYTGRGIRPVGR 1-Nal-NH₂;
 J^a-SRAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheCl₂-NH₂;
 J^a-SRAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheNO₂-NH₂;
 J^a-SRAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheF₅-NH₂;
 J^a-SRAHQHS Nle ETRTPDINPAWYTGRGIRPVGRY-NH₂;
 J^a-SRAHRHS Nle EIRTPDINPAWYASRGIRPVGRY-NH₂;
 (N-myr)-Nle-ETRTPDINPAWYTGRGIRPVGRF-NH₂;
 (N-myr)-QHSMETRTPDINPAWYTGRGIRPVGRF-NH₂;
 (N-myr)-QHSMETRTPDINPAWYASRGIRPVGRF-NH₂; and
 (N-palm)SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NOMe; wherein
 J^{1'} is palmitic acid, myristic acid or stearic acid; and
 J^a is palmitic acid or myristic acid.

[0016] Some embodiments described herein relate to the use of lipidated analogs of prolactin-releasing peptide disclosed herein as anorexigenic agents lowering food intake after peripheral administration.

[0017] Some embodiments described herein relate to the use of lipidated analogs of prolactin-releasing peptide disclosed herein as an agent for the treatment of obesity.

[0018] Additional embodiments described herein relate to a lipidated analog of prolactin-releasing peptide selected from:

(palm)-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH₂; or
 (myr)-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH₂.

[0019] In some embodiments, the lipidated analog of prolactin-releasing peptide has the structure:

(palm)-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH₂.

[0020] Additional embodiments described herein relate to lipidated analog of prolactin-releasing peptide according to formula:

J^a-TPDINPAWYmoRGIRPVGRF-NH₂; wherein

J^a is palmitic acid or myristic acid; **m** is T or A; and **o** is G or S. In some embodiments, J^a is myristic acid.

[0021] In some embodiments, the lipidated analog of prolactin-releasing peptide has the structure:

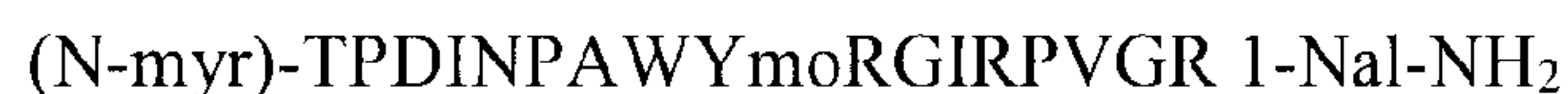


[0022] Further embodiments relate to a method of preventing or treating a subject diagnosed with or susceptible to having obesity comprising administering a lipidated analog of prolactin-releasing peptide disclosed herein. In some embodiments, the lipidated analog is:



In some embodiments, the lipidated analog of prolactin-releasing peptide is administered by a route other than a centrally administered route. In some embodiments, the lipidated analog of prolactin-releasing peptide is administered peripherally (e.g. subcutaneously).

[0022a] In one embodiment, there is provided a lipidated analog of prolactin-releasing peptide selected from the group consisting of:



(N-palm)-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NOMe; wherein

J¹ is a palmitoyl group, a myristoyl group or stearoyl group bound by the amide bond to the amino group of the N-terminal amino acid;

J^a is a palmitoyl group or a myristoyl group bound by the amide bond to the amino group of the N-terminal amino acid; and

m is T or A; and **o** is G or S.

[0022b] In one embodiment, there is provided a lipidated analog of prolactin-releasing peptide selected from the group consisting of:

(N-palm)-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH₂; and

(N-myr)-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH₂.

[0022c] In one embodiment, there is provided a lipidated analog of prolactin-releasing peptide of structure:

(N-palm)-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH₂.

[0022d] In one embodiment, there is provided a lipidated analog of prolactin-releasing peptide according to formula:

J^a-TPDINPAWY**mo**RGIRPVGRF-NH₂; wherein

J^a is a palmitoyl group or a myristoyl group bound by the amide bond to the amino group of the N-terminal amino acid; **m** is T or A; and **o** is G or S.

[0022e] In one embodiment, there is provided a lipidated analog of prolactin-releasing peptide of structure:

(N-myr)-TPDINPAWYASRGIRPVGRF-NH₂.

[0022f] In one embodiment, there is provided a pharmaceutical composition comprising at least one lipidated analog of prolactin-releasing peptide as defined herein, and one or more physiologically acceptable carrier.

[0022g] In one embodiment, there is provided a use of the lipidated analog of prolactin-releasing peptide as defined herein for lowering food intake after peripheral administration in a subject in need thereof.

[0022h] In one embodiment, there is provided a lipidated analog of prolactin-releasing peptide as defined herein for lowering food intake after peripheral administration in a subject in need thereof.

[0022i] In one embodiment, there is provided a use of the lipidated analog of prolactin-releasing peptide as defined herein for the preparation of a medicament for lowering food intake after peripheral administration in a subject in need thereof.

[0022j] In one embodiment, there is provided a use of the lipidated analog of prolactin-releasing peptide as defined herein for the treatment or prevention of obesity in a subject in need thereof.

[0022k] In one embodiment, there is provided a use of the lipidated analog of prolactin-releasing peptide as defined herein for the manufacture of a medicament, for the treatment or prevention of obesity in a subject in need thereof.

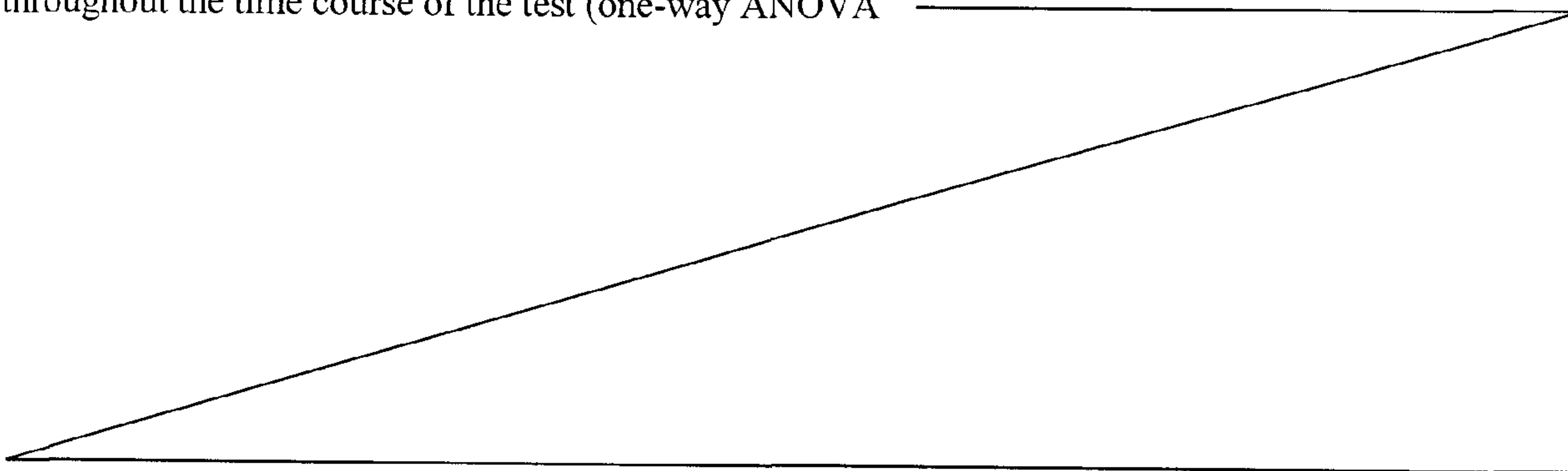
[0022l] In one embodiment, there is provided a lipidated analog of prolactin-releasing peptide as defined herein, for use in the treatment or prevention of obesity in a subject in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0025] **Figure 1** represents the time course of food intake after ICV administration of PrRP31 in the dose of 4 and 10 nmol/mouse to the fasted C57BL/6 mice. On the x axis is time in minutes, on the y axis, the cumulative food intake as a % of food intake in the control group injected with the saline (n=6-7). The significance is **P<0.01, ***P<0.001 versus saline at time points 45 and 75 minutes after injection (one-way ANOVA followed by Dunnett post-hoc test). ■ – saline, ○ – PrRP31 (4 nmol), ● – PrRP31 (10 nmol).

[0026] **Figure 2** represents the time course of food intake after ICV administration of analogs PrRP31 with varying length of the lipid chain to fasted C57BL/6 mice at the dose of 4 nmol/mouse. On the x axis is time in minutes, on the y axis, the cumulative food intake as a % of food intake in the control group injected with the saline (n=6-7). The significance is **P<0.01, ***P<0.001 versus saline throughout the time course of the test (one-way ANOVA



followed by Dunnett post-hoc test). ■ – saline, ● – PrRP31, Δ – analog 1, ◆ – analog 2, ▲ – analog 5.

[0027] Figure 3 represents the time course of food intake after SC administration of analogs of PrRP31 with varying length of the lipid chain to fasted C57BL/6 mice at a dose of 5 mg/kg. On the x axis is time in minutes, on the y axis, the cumulative food intake as a % of food intake in the control group injected with the saline (n=6). The significance is *P<0.05, ***P<0.001 versus saline throughout the time course of the test (one-way ANOVA followed by Dunnett post-hoc test).

■ – saline, Δ - analog 1, ○ – analog 2, ● – analog 3, ◇ – analog 4, ◆ – analog 5, ▲ – analog 6.

[0028] Figure 4 represents the time course of food intake after SC administration of human and rat lipidated analogs of PrRP31 and PrRP20 to fasted C57BL/6 mice at a dose of 5 mg/kg. On the x axis is time in minutes, on the y axis, the cumulative food intake as a % of food intake in the control group injected with the saline (n=6). The significance is ***P<0.001 versus saline throughout the time course of the test (one-way ANOVA followed by Dunnett post-hoc test). ■ – saline, ● – analog 5, Δ – analog 10, ◆ - analog 12, ▲ – analog 13.

[0029] Figure 5 represents the time course of food intake after SC administration of myristoylated analog [Dpr¹]PrRP31 (No.5) to fasted C57BL/6 mice at the doses of 1,2,5 and 10 mg/kg. On the x axis is time in minutes, on the y axis, the cumulative food intake as a % of food intake in the control group injected with the saline (n=6). The significance is ***P<0.001 versus saline throughout the time course of the test (one-way ANOVA followed by Dunnett post-hoc test). ■ – saline, Δ - analog 5: 1 mg/kg, ○ – analog 5: 2 mg/kg, ● – analog 5: 5 mg/kg, ▲ – analog 5: 10 mg/kg.

[0030] Figure 6 represents the time course of food intake after SC administration of myristoylated analog [Dpr¹NaI³¹]PrRP31 (No.9) to fasted C57BL/6 mice at the doses of 1,2,5 and 10 mg/kg. On the x axis is time in minutes, on the y axis, the cumulative food intake as a % of food intake in the control group injected with the saline (n=6). The significance is **P<0.01, ***P<0.001 versus saline (one-way ANOVA followed by Dunnett post-hoc test). ■ – saline, ○ – analog 9: 1 mg/kg, ● – analog 9: 2 mg/kg, Δ – analog 9: 5 mg/kg, ▲ – analog 9: 10 mg/kg.

[0031] Figure 7 represents neural activity showed as Fos immunoreactivity. Fos-immunostained cells in coronal section and number of Fos-immunopositive cells of A/ Arc, B/ PVN, C/ NTS, 90 minutes after SC application of saline, [Dpr¹]PrRP31, [Dpr(oct)¹]PrRP31, [Dpr(myrist)¹]PrRP31 or [Dpr(palm)¹]PrRP31 (dose 5mg/kg, unilaterally per mouse (n =5) and section (n = 5-6)). Significance: A/ * p < 0.05 vs [Dpr(oct)¹]PrRP31, ^x p < 0.05 vs Sal, [Dpr¹]PrRP31, Dpr(oct)¹]PrRP31, B, C/ ^x p < 0.05 vs Sal, [Dpr¹]PrRP31, Dpr(oct)¹]PrRP31. Sal – saline, NTS – solitary tract nucleus, AP – area postrema, PVN –paraventricular nucleus.

[0032] **Fig. 8** represents behavioral activity of fed mice: A/ open field test, total distance traveled for 10 min and B/ analgesic test, hot plate (latency to jump) after administration with SC injection of saline, [Dpr¹]PrRP31, [Dpr(palm)¹]PrRP31 or myr-PrRP20 (dose 5 mg/kg, n=5 mice/group).

[0033] **Fig. 9** represents long-term effect of lipidized PrRP analogs on body weight in a DIO model of mice (SC administration, dose 5 mg/kg, twice per day for 14 days) (n=10). The data were analyzed by two-way ANOVA (treatment x time after injection). ***P<0.001 vs. respective saline-treated group.

DETAILED DESCRIPTION OF THE INVENTION

Prolactin-releasing peptide (PrRP)

[0034] The neuropeptide PrRP, the prolactin-releasing peptide has been shown to fundamentally affect the regulation of energy metabolism (Hinuma, S., et al. "A prolactin-releasing peptide in the brain," *Nature* 393:272-276, 1998). There are two forms of PrRP, one composed of 31 amino acids (PrRP31), the other of 20 amino acids (PrRP20) (Hinuma et al.).

[0035] PrRP has multiple functions in the body. The first described biological effect was the stimulation of prolactin release (Hinuma et al.). Subsequently it was found, however, that this is not affected in male rats and therefore most likely it is not the primary function of PrRP (Jarry, H., et al., "Prolactin-releasing peptides do not stimulate prolactin release in vivo," *Neuroendocrinology*, 71:262-267, 2000). Upon finding the PrRP in hypothalamic paraventricular and dorsomedial nuclei (PVN and DMN) which are important for maintaining the metabolic equilibrium, PrRP was considered as a factor affecting food intake (Lawrence, C., et al., "Alternative role for prolactin-releasing peptide in the regulation of food intake," *Nat. Neurosci*, 3:645-646, 2000).

[0036] The anorexigenic effect of PrRP31 was demonstrated when injected into the third brain ventricle where the front wall and base are formed by the hypothalamus (so called intracerebroventricular administration, ICV). It led to significant lowering of food intake and subsequently to a decrease of body weight in rats (Lawrence et al., 2000; Lawrence, C., et al., "Anorectic actions of prolactin-releasing peptide are mediated by corticotrophin-releasing hormone receptors," *Am J Physiol Regul Integr Comp Physiol*, 286:R101-107, 2004). The decrease in food intake did not affect water consumption or behaviour of the experimental animals. Nausea or anorexia were not demonstrated (Lawrence et al., 2000). It was also found that administration of a shorter peptide, tridecapeptide PrRP13 (containing amino acids 18 to 31 of PrRP31) is not sufficient for maintaining the anorexigenic activity in mice after central administration. (Maixnerová, J., et al., "Characterization of prolactin-releasing peptide: binding,

signaling and hormone secretion in rodent pituitary cell lines endogenously expressing its receptor,” *Peptides*, 32:811-817, 2011).

[0037] The anorexigenic function of PrRP was also shown in additional experiments. It was found that during negative energy balance, encountered for example during breast feeding or starvation, PrRP gene transcription decreased. The reduced body weight after ICV injection of PrRP is not only the consequence of lower food intake but also of the increased body temperature and oxygen consumption which are indirect measures of increased energy output. There is also an increase in uncoupling protein 1 (UCP-1) mRNA production in the brown adipose tissue which is a further indication of an increased energy output (Ellacott, K., et al., “Repeated administration of the anorectic factor prolactin-releasing peptide leads to tolerance to its effects on energy homeostasis,” *Am J Physiol Regul Integr Comp Physiol*, 285:R1005-1010, 2003).

[0038] Genetically modified animals were also used to determine the PrRP function in the body (knock-out (KO) animals with deleted gene coding for PrRP or its receptor GPR10) (Bjursell, M., et al., “GPR10 deficiency in mice results in altered energy expenditure and obesity,” *Biochem Biophys Res Commun*, 363:633-638, 2007; Takayanagi, Y., et al., “Endogenous prolactin-releasing peptide regulates food intake in rodents,” *J Clin Invest*, 118:4014-4024, 2008). Mice with the deleted PrRP gene suffered from hyperphagia. While the frequency of individual meals was not affected, the amount of each meal increased. Lowering of the energy output was not observed in these mice as body temperature and oxygen consumption are comparable to the control animals (Takayanagi et al.; Mochiduki, A., et al., “Stress response of prolactin-releasing peptide knockout mice as to glucocorticoid secretion,” *J Neuroendocrinol*, 22:576-584, 2010).

[0039] Similarly, the mice with a deleted gene for the receptor GPR10 had higher food intake leading to obesity, and this effect was more pronounced in the females (Gu, W., et al., “The prolactin-releasing peptide receptor (GPR10) regulates body weight homeostasis in mice,” *J Mol Neurosci*, 22:93-103, 2004; Bjursell et al.). The food intake in the GPR10 KO mice was not reduced after the administration of cholecystokinin (CCK). This finding supports the hypothesis that the GPR10-PrRP system may have a key role in the signal transfer of CCK, a peripheral peptide that induces satiety sensation during food intake (Bechtold D, et al., “Prolactin-releasing peptide mediates cholecystokinin-induced satiety in mice,” *Endocrinology*, 147:4723-4729, 2006).

[0040] From a structural point of view, the presence of arginine in the position 30 is critical for the proper function of PrRP. Its modification or substitution with a different amino acid leads to a loss of binding to the receptor and of biological activity. Furthermore, for the binding of PrRP

to the receptor GPR10, the position 31 is important and requires phenylalanine or other amino acid with aromatic moiety bound to at least one CH₂ group of the side chain of the amino acid (Boyle, R., et al., "Structure-activity studies on prolactin-releasing peptide (PrRP). Analogues of PrRP-(19-31)-peptide," *J Pept Sci*, 11:161-165, 2005).

[0041] For binding experiments with PrRP analogs, the PrRP with ¹²⁵I on the tyrosine in position 20 is used (Satoh, F., et al., "Characterization and distribution of prolactin releasing peptide (PrRP) binding sites in the rat-evidence for a novel binding site subtype in cardiac and skeletal muscle," *Br J Pharmacol*, 129:1787-1793, 2000). It was confirmed the monoiodation of tyrosine does not affect PrRP binding to the receptor (Maixnerová, J., et al., 2011).

Modifications of the natural peptide PrRP20 which biological activity *in vitro* is comparable to PrRP31 (Langmead, C., et al., "Characterization of the binding of [(125)I]-human prolactin releasing peptide (PrRP) to GPR10, a novel G protein coupled receptor," *Br J Pharmacol*, 131:683-688, 2000; Maixnerová et al., 2011) were used in a recent study (Maletínská, L., et al., "Biological properties of prolactin-releasing peptide analogues with a modified aromatic ring of a C-terminal phenylalanine amide," *Peptides*, 32:1887-1892, 2011). C-terminal phenylalanine was replaced by noncoded amino acids, phenylalanine derivatives PheCl₂, PheF₅ and PheNO₂, or by noncoded amino acids with bulky naphthylalanine 1-Nal and 2-Nal, or by tyrosine. All analogs had conserved C-terminal amide which is necessary for biological activity (Hinuma et al.; Roland, B., et al., "Anatomical distribution of prolactin-releasing peptide and its receptor suggests additional functions in the central nervous system and periphery," *Endocrinology*, 140:5736-5745, 1999) and in addition, they were acetylated at the N-terminus of the peptide chain to increase its stability, especially for the *in vivo* experiments. Biological activity was not dependent on the substitution of amino acids in positions before the C-terminal heptapeptide.

[0042] The analogues [Tyr³¹]PrRP20, [1-Nal³¹]PrRP20, [PheF₅³¹]PrRP20, [PheCl₂³¹]PrRP20 and [PheNO₂³¹]PrRP20 bind to the pituitary cell line RC-4B/C which expresses the receptor GPR10 in the order of tens of thousands of binding sites per cell (Maixnerová et al., 2011) with K_b comparable to values for PrRP31 and PrRP20 (Maletínská et al., 2011). In addition, all the analogs increased phosphorylation of enzymes mitogen activated phosphorylase/ extracellular-regulated kinase (MAPK/ERK1/2) and the cAMP response element-binding protein (CREB), and stimulated the prolactin release into the medium with EC₅₀ comparable to that of PrRP20 (Hinuma et al.). These analogs of PrRP upon ICV administration in the dose of 10 nmol caused statistically significant reduction of food intake in fasted mice (Maletínská et al., 2011). These analogs, therefore, represent very potent anorexigenic peptides which lower food intake when centrally administered, however, not after peripheral administration such as, e.g, subcutaneous, SC, administration.

[0043] Provided herein are analogs of the neuropeptides PrRP31 and PrRP20 lipidated by fatty acids (e.g. by myristoyl or palmitoyl) at the N-terminus of the peptide. The lipidated analogs can further possess modified amino acid compositions as described herein. Both types of neuropeptides with different chain length bound to the PrRP receptor with high affinity in *in vitro* conditions. The lipidated analogs of PrRP20 and PrRP31 significantly increased functionality. The effect was observed in experiments *in vitro* and *in vivo*. Advantageously, the lipidated analogs of PrRP20 and PrRP31 demonstrated increased functionality when administered peripherally (e.g, subcutaneously, SC). In particular, peripheral (e.g., subcutaneous, SC) administration of lipidated analogs of PrRP20 and PrRP31 caused a statistically significant reduction of food intake in fasted mice. In fasted mice the lipidated analogs demonstrated a statistically significant dose-dependent reduction in food intake ($P < 0.001$) not only after central administration but also after peripheral administration (e.g., subcutaneous administration). Furthermore, the effect of lowering food intake was long-lasting and persisted for more than 10 hours after administration. Without being bound by theory, it is thought this is most likely due to increased resistance to degradation by proteases (e.g by introduction of fatty acid and/or noncoded amino acids), and to the binding of lipopeptide to serum albumin. Accordingly, lipidation of PrRP20 and PrRP31 presents a new alternative to deliver these peptides across the blood brain barrier after peripheral administration in order to lower food intake. Furthermore, the lipidated analogs did not induce secretion of prolactin after SC administration to mice and rats in any of the performed tests described herein.

Lipidated analogs of PrRP20 and PrRP31

[0044] Some embodiments disclosed herein include lipidated analogs that are lipidated peptides formed by 20 or 31 amino acids, with the sequence of IRPVGRF-NH₂ at the C-terminus and lipidated with one or more fatty acids. In this sequence, isoleucine may be substituted by phenylglycine or alanine, and valine may be substituted by phenylglycine. The terminal phenylalanine may be replaced by an amino acid with a side chain containing CH₂-Ar or CH₂-S-CH₂-Ar, where Ar represents phenyl or naphthyl, optionally substituted by a halogen or nitro group. The amino group on the N-terminal amino acid can be serine, diaminopropionic acid or threonine and the C6 to C18 fatty acid is bound by the amide bond. In some embodiments, amino acid substitutions can be made in the lipidated analog. For example, the methionine at position 8 in PrRP31 can be replaced by a norleucine, for example, to increase stability.

[0045] In some embodiments, the amino acids in positions before the C-terminal heptapeptide are not essential for the described biological activity of the lipidated analogs. Their substitution by a different amino acid or a slight reduction of the number is possible as long as the resulting lipidated peptide has the binding affinity to the PrRP receptor with K_i in the order of 10⁻⁶ mol.l⁻¹

or lower, (or even better 10^{-8} mol.l⁻¹ or lower), exhibits agonist activity towards PrRP (as determined by MAPK/ERK1/2 signaling in RC-4B/C cells) and the anorexigenic activity, tested by food intake in fasted mice after central and peripheral administration, equal as PrRP or higher.

[0046] In some embodiments, the lipidated analogs of PrRP20 or PrRP31 have the general formula



wherein J^1 represents a fatty acid C6 to C18 bound by an amide bond to an amino group of a N-terminal amino acid, J^2 represents a chain of 13 or 24 amino acids, **r** is chosen from isoleucine, alanine, or phenylglycine, **s** is chosen from valine or phenylglycine, **t** is chosen from phenylalanine or an amino acid with a side chain containing CH₂-Ar or CH₂-S-CH₂-Ar, where Ar represents phenyl or naphthyl, optionally substituted by halogen or nitro group.

[0047] In additional embodiments, the lipidated analogs of PrRP20 or PrRP31 have the general formula



wherein J^1 represents a fatty acid C6 to C18 bound by an amide bond to an amino group of a N-terminal amino acid, **k** is chosen from serine, threonine or diaminopropionic acid, J^3 represents a chain of 23 or 12 amino acids, **r** is chosen from isoleucine, alanine, or phenylglycine, **s** is chosen from valine or phenylglycine, **t** is chosen from phenylalanine or an amino acid with a side chain containing CH₂-Ar or CH₂-S-CH₂-Ar, where Ar represents phenyl or naphthyl, optionally substituted by halogen or nitro group.

[0048] Yet further embodiments relate to lipidated analogs of PrRP20 or PrRP31 that have the general formula



wherein **k** is chosen from serine, threonine or diaminopropionic acid, **m** is chosen from threonine, alanine or methylalanine, **n** is chosen from glutamine or arginine, **q** is chosen from methionine or norleucine, **u** is chosen from threonine or isoleucine, **o** is chosen from glycine or serine, **p** is chosen from glycine, alanine, proline or N-methylglycine, **r** is chosen from isoleucine, alanine or phenylglycine, **s** is chosen from valine or phenylglycine, **t** is chosen from phenylalanine or an amino acid with side chain containing CH₂-Ar or CH₂-S-CH₂-Ar, where Ar represents phenyl or naphthyl, optionally substituted by halogen or nitro group, J^1 represents a fatty acid C6 to C18 bound by the amide bond to the amino group of the N-terminal amino acid, where the chain of amino acids **kRmHnHSqEuRTPDINPAWYmoRp** is optionally shortened by eliminating from 1 to 10 amino acids.

[0049] Additional embodiments relate to lipidated analogs of PrRP20 or PrRP31 that have the general formula



wherein **k** is chosen from serine, threonine or diaminopropionic acid, **m** is chosen from threonine, alanine or methylalanine, **n** is chosen from glutamine or arginine, **q** is chosen from methionine or norleucine, **u** is chosen from threonine or isoleucine, **o** is chosen from glycine or serine, **p** is chosen from glycine, alanine, proline or N-methylglycine, **r** is chosen from isoleucine, alanine or phenylglycine, **s** is chosen from valine or phenylglycine, **t** is chosen from phenylalanine or an amino acid with side chain containing CH₂-Ar or CH₂-S-CH₂-Ar, where Ar represents phenyl or naphthyl, optionally substituted by halogen or nitro group, J¹ represents a fatty acid C6 to C18 bound by the amide bond to the amino group of the N-terminal amino acid, where the chain of amino acids **kRmHnHSqEuRTPDINPAWYmoRp** is optionally shortened from its N-terminal by eliminating from 1 to 10 amino acids.

[0050] Further embodiments relate to lipidated analogs of PrRP20 or PrRP31 that have the general formula



wherein **k** is chosen from serine, threonine or diaminopropionic acid, **m** is chosen from threonine, alanine or methylalanine, **n** is chosen from glutamine or arginine, **q** is chosen from methionine or norleucine, **u** is chosen from threonine or isoleucine, **o** is chosen from glycine or serine, **p** is chosen from glycine, alanine, proline or N-methylglycine, **r** is chosen from isoleucine, alanine or phenylglycine, **s** is chosen from valine or phenylglycine, **t** is chosen from phenylalanine or an amino acid with side chain containing CH₂-Ar or CH₂-S-CH₂-Ar, where Ar represents phenyl or naphthyl, optionally substituted by halogen or nitro group, J¹ represents a fatty acid C6 to C18 bound by the amide bond to the amino group of the N-terminal amino acid.

[0051] Additional embodiments disclosed herein relate to lipidated analogs of PrRP20 or PrRP31 described above where such substitutions of amino acids in positions 1-24 of formula III or in positions 1-13 of formula IV are performed that do not increase the binding affinity value of the resulting lipidated peptide to the receptor GPR10 over K_i 10⁻⁶ mol.l⁻¹, measured in the rat pituitary cell line RC-4B/C grown on 24-well plates, which had their bottom coated with polyethyleneimine, up to the optimal density of 300-450 thousand cells per well; then the following agents are added: binding buffer containing 20 mmol.l⁻¹ HEPES, pH 7.4, 118 mmol.l⁻¹ NaCl, 4.7 mmol.l⁻¹ KCl, 5 mmol.l⁻¹ MgCl₂, 5.5 mmol.l⁻¹ glucose, 1 mg/ml bovine serum albumin, 0.1 mg/ml bovine pancreatic trypsin inhibitor; unlabelled analogs of PrRP of final concentration within 10⁻¹¹ to 10⁻⁴ mol.l⁻¹, and ¹²⁵I-PrRP31 of final concentration of 10⁻¹⁰ mol.l⁻¹;

the plate is incubated for 60 minutes at room temperature and thereafter the cells are solubilized in 0.1 mol/l solution of NaOH and the radioactivity bound to the cell is counted in the γ -counter.

[0052] Further embodiments relate to lipidated analogs of PrRP31 having the general formula



wherein **m** is chosen from threonine, alanine or methylalanine, **n** is chosen from glutamine or arginine, **q** is chosen from methionine or norleucine, **u** is chosen from threonine or isoleucine, **o** is chosen from glycine or serine, **p** is chosen from glycine, alanine, proline or N-methylglycine, **r** is chosen from isoleucine, alanine or phenylglycine, **s** is chosen from valine or phenylglycine, **t** is chosen from phenylalanine or an amino acid with side chain containing CH₂-Ar or CH₂-S-CH₂-Ar, where Ar represents phenyl or naphthyl, optionally substituted by halogen or nitro group, J¹ represents a fatty acid C6 to C18 bound by the amide bond to the amino group of the N-terminal amino acid.

[0053] In some embodiments, the lipidated analogs described herein (e.g, the lipidated analogs of formulas I, II, III, IV, and V) include a C-terminal amino acid selected from naphthylalanine, benzylcysteine, benzylhistidine, pentafluorophenylalanine, and nitrophenylalanine.

[0054] In additional embodiments, the lipidated analogs described herein (e.g, the lipidated analogs of formulas I, II, III, IV, and V) include a fatty acid is selected from a group that includes fatty acids of 6 to 18, and more preferably, of 13 to 18 carbon atoms. In some embodiments, the lipidated analogs described herein include a fatty acid of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18 carbon atoms.

[0055] Additional embodiments include lipidated analogs selected from the group which includes the following:

(Dpr(oct))RmHnHSNleETRTPDINPAWYmoRGrRPsGRF-NH₂;
 (Dpr(dodec))RmHnHSNleETRTPDINPAWYmoRGrRPsGRF-NH₂;
 (Dpr(tridec))RmHnHSNleETRTPDINPAWYmoRGrRPsGRF-NH₂;
 (Dpr(myr))RmHnHSNleETRTPDINPAWYmoRGrRPsGRF-NH₂;
 (Dpr(palm))RmHnHSNleETRTPDINPAWYmoRGrRPsGRF-NH₂;
 (myr)TPDINPAWYmoRGrRPsGRF-NH₂;
 (palm)TPDINPAWYmoRGrRPsGRF-NH₂;
 (Dpr(oct))RmHnHSNleETRTPDINPAWYmoRGrRPsGR 1-Nal-NH₂;
 (Dpr(dodec))RmHnHSNleETRTPDINPAWYmoRGrRPsGR 1-Nal-NH₂;
 (Dpr(tridec))RmHnHSNleETRTPDINPAWYmoRGrRPsGR 1-Nal-NH₂;
 (Dpr(myr))RmHnHSNleETRTPDINPAWYmoRGrRPsGR 1-Nal-NH₂;
 (Dpr(palm))RmHnHSNleETRTPDINPAWYmoRGrRPsGR 1-Nal-NH₂;
 (myr)TPDINPAWYmoRGrRPsGR 1-Nal-NH₂; and

(palm)TPDINPAWYmoRGrRPsGR 1-Nal-NH₂;

where **m** is T or A and **o** is G or S, **n** is Q or R, **r** is I, A or phenylglycine, **s** is V or phenylglycine, Dpr is diaminopropionic acid, *1-Nal* is 1-naphtylalanine, *Nle* is norleucine, myr is myristoyl, palm is palmitoyl, oct is octanoyl, dodec is dodecanoyl, tridec is tridecanoyl. In the peptide positions 1 to 24, such substitutions in amino acids may be performed that do not increase the binding affinity value of the lipidated peptide to the receptor GPR10 over $K_i 10^{-6}$ mol.l⁻¹, and its anorectic activity evaluated by food intake test in fasted mice after both peripheral and central administration is equal or higher as compared to administered PrRP.

[0056] Further embodiments include lipidated analogs selected from the group that includes:

(Dpr(oct))RmHnHSNleETRTPDINPAWYmoRGIRPVGRF-NH₂;
 (Dpr(dodec))RmHnHSNleETRTPDINPAWYmoRGIRPVGRF-NH₂;
 (Dpr(tridec))RmHnHSNleETRTPDINPAWYmoRGIRPVGRF-NH₂;
 (Dpr(myristoyl))RmHnHSNleETRTPDINPAWYmoRGIRPVGRF-NH₂;
 (Dpr(palm))RmHnHSNleETRTPDINPAWYmoRGIRPVGRF-NH₂;
 (myristoyl)TPDINPAWYmoRGIRPVGRF-NH₂;
 (palm)TPDINPAWYmoRGIRPVGRF-NH₂;
 (Dpr(oct))RmHnHSNleETRTPDINPAWYmoRGIRPVGR 1-Nal-NH₂;
 (Dpr(dodec))RmHnHSNleETRTPDINPAWYmoRGIRPVGR 1-Nal-NH₂;
 (Dpr(tridec))RmHnHSNleETRTPDINPAWYmoRGIRPVGR 1-Nal-NH₂;
 (Dpr(myristoyl))RmHnHSNleETRTPDINPAWYmoRGIRPVGR 1-Nal-NH₂;
 (Dpr(palm))RmHnHSNleETRTPDINPAWYmoRGIRPVGR 1-Nal-NH₂;
 (myristoyl)TPDINPAWYmoRGIRPVGR 1-Nal-NH₂; and
 (palm)TPDINPAWYmoRGIRPVGR 1-Nal-NH₂;

where **m** is T or A and **o** is G or S, **n** is Q or R, Dpr is diaminopropionic acid, *1-Nal* is 1-naphtylalanine, *Nle* is norleucine, myr is myristoyl, palm is palmitoyl, oct is octanoyl, dodec is dodecanoyl, tridec is tridecanoyl. In the peptide positions 1 to 24, such substitutions in amino acids may be performed that do not increase the binding affinity value of the lipidated peptide to the receptor GPR10 over $K_i 10^{-6}$ mol.l⁻¹, and its anorectic activity evaluated by food intake test in fasted mice after both peripheral and central administration is equal or higher as compared to administered PrRP.

[0057] Some embodiments disclosed herein include lipidated analogs of PrRP20 or PrRP31 selected from the group that includes:

J^a-SRAHQHSMETRTDINPAWYTGRGIRPVGRF-NH₂,
 J^a-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH₂,
 (N-palm)-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NHMe,

(N-palm)-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NOMe,
 (N-myr)-TPDINPAWYTGRGIRPVGRF-NH₂,
 (N-myr)-TPDINPAWYASRGIRPVGRF-NH₂,
 (N-oct)-TPDINPAWYASRGIRPVGRF-NH₂,
 (N-dec)-TPDINPAWYASRGIRPVGRF-NH₂,
 (N-dodec)-TPDINPAWYASRGIRPVGRF-NH₂,
 J^{1'}-SRAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂,
 J^a-SRAHQHS Nle ETRTPDINPAWYTGRGIRPVGR 1-Nal-NH₂,
 J^a-SRAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PhcCl₂-NH₂,
 J^a-SRAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PhcNO₂-NH₂,
 J^a-SRAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PhcF₅-NH₂,
 J^a-SRAHQHS Nle ETRTPDINPAWYTGRGIRPVGRY-NH₂,
 J^a-SRAHRHS Nle EIRTPDINPAWYASRGIRPVGRY-NH₂,
 (N-myr)-Nle-ETRTPDINPAWYTGRGIRPVGRF-NH₂,
 (N-myr)-QHSMETRTPDINPAWYTGRGIRPVGRF-NH₂,
 (N-myr)-QHSMETRTPDINPAWYASRGIRPVGRF-NH₂, and
 (N-palm)SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NOMe

where J^{1'} is chosen from palmitic acid, myristic acid or stearic acid, and J^a is chosen from palmitic acid or myristic acid, wherein N-oct represents N-octanoic acid, N-dec represents N-decanoic acid, N-dodec represents N-dodecanoic acid, N-myr represents N-myristic acid, N-palm represents palmitic acid, wherein each above mentioned fatty acid is always bound by the amide bond to the amino group of the N-terminal amino acid; and wherein Nle represents norleucine, 1-Nal represents naftylalanine, and Me represents methyl.

[0058] Some embodiments disclosed herein include a lipidated analog of PrRP20 or PrRP31 or a modified PrRP20 or PrRP31 (e.g., a peptide with an amino acid substitution or reduction) selected from:

(Dpr) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂;
 (Dpr(oct)) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂;
 (Dpr(dodec)) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂;
 (Dpr(tridec)) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂;
 (Dpr(myr)) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂;
 (Dpr(palm)) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂;
 (Dpr) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR 1-Nal-NH₂;
 (Dpr(oct)) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR 1-Nal-NH₂;
 (Dpr(myr)) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR 1-Nal-NH₂;

(myr)TPDINPAWYTGRGIRPVGRF-NH₂;
 (Dpr) RTHRHS Nle EIRTPDINPAWYASRGIRPVGRF-NH₂;
 (Dpr(myrr)) RTHRHS Nle EIRTPDINPAWYASRGIRPVGRF-NH₂;
 (myr)TPDINPAWYASRGIRPVGRF-NH₂;
 (N-oct) S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂;
 (N-dec) S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂;
 (N-dodec) S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂;
 (N-myr) S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂;
 (N-palm)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂;
 (N-stear)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂;
 (N-myr)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR 1-Nal-NH₂;
 (N-palm)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR 1-Nal-NH₂;
 (N-myr)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheCl₂ -NH₂;
 (N-palm)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheCl₂ -NH₂;
 (N-myr)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheNO₂-NH₂;
 (N-palm)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheNO₂-NH₂;
 (N-palm)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheF₅ -NH₂;
 (N-palm)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR Tyr-NH₂;
 (N-myr)TPDINPAWYTGR Sar IRPVGRF-NH₂;
 (N-myr)TPDINPAWY N-Me-Ala SRGIRPVGRF-NH₂;
 (N-myr)TPDINPAWYTGRGARPFGRF-NH₂;
 (N-myr)TPDINPAWYASRPFRPVGRF-NH₂;
 (N-myr)Nle-ETRTPDINPAWYTGRGIRPVGRF-NH₂;
 (N-myr)QHSMETRTPDINPAWYTGRGIRPVGRF-NH₂;
 (N-oct)TPDINPAWYASRGIRPVGRF-NH₂;
 (N-dec)TPDINPAWYASRGIRPVGRF-NH₂;
 (N-dodec)TPDINPAWYASRGIRPVGRF-NH₂;
 (N-myr)TPDINPAWYASRGIRPVGRF-NH₂;
 (N-myr)SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH₂;
 (N-palm)SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH₂;
 (N-palm)SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NHMe;
 (N-palm)SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NOMe;
 (N-myr)SRAHQHSMETRTPDINPAWYTGRGIRPVGRF-NH₂; and
 (N-palm)SRAHQHSMETRTPDINPAWYTGRGIRPVGRF-NH₂.

[0059] Further embodiments disclosed herein include a myristoylated analog selected from:

(Dpr(myristoyl)) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂,

(Dpr(myristoyl)) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR 1-Nal-NH₂, and

(myristoyl)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂

[0060] Additional embodiments disclosed herein include a palmitoylated analog selected from:

(Dpr(palmitoyl)) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂, and

(palmitoyl)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂,

[0061] Some embodiments include the stearoylated analog:

(stearoyl)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂

[0062] Further embodiments disclosed herein include the myristoylated analog:

(myristoyl)TPDINPAWYTGRGIRPVGRF-NH₂

[0063] Additional embodiments disclosed herein include a lipidated analog selected from:

(N-myristoyl)TPDINPAWYASRGIRPVGRF-NH₂ and

(N-palmitoyl)SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH₂

[0064] Some embodiments relate to the use of the lipidated analogs described herein (e.g, the lipidated analogs of formulas I, II, III, IV, and V) as anorexigenic agents which lower food intake after peripheral administration (e.g, subcutaneous administration).

[0065] Additional embodiments relate to the use of the lipidated analogs described herein (e.g, the lipidated analogs of formulas I, II, III, IV, and V) for the manufacturing of medication for the treatment of obesity.

Methods of Use

[0066] The methods described herein include administering to a subject in need a lipidated analog described herein (e.g., a pharmaceutical composition) containing a therapeutically effective amount of one or more lipidated analogs described herein. Without being bound by theory, the characteristics of the lipidated analogs described herein allow the analogs to be useful for preventing or treating obesity. A mammal can be identified as having or being likely to develop obesity using standard clinical techniques. For example, analysis of a human's family history or eating habits can be used to determine whether or not the human is likely to develop an obesity condition. As described herein, a mammal identified as having or being susceptible to developing an obesity condition can be treated by administering a lipidated analog disclosed herein.

Pharmaceutical Compositions, Routes of Administration, and Dosing

[0067] Provided herein, in certain embodiments, are pharmaceutical compositions comprising at least one lipidated analog as described herein.

[0068] Pharmaceutical compositions are typically formulated using one or more physiologically acceptable carriers including excipients and auxiliaries which facilitate processing of the

therapeutic composition into preparations which are used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. A summary of pharmaceutical compositions is found, for example, in Remington: The Science and Practice of Pharmacy, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y., 1980; and Pharmaceutical Dosage Forms and Drug Delivery Systems, Seventh Ed. (Lippincott Williams & Wilkins, 1999).

[0069] Provided herein are pharmaceutical compositions that include one or more lipidated analogs described herein and one or more pharmaceutically acceptable diluent(s), excipient(s), or carrier(s). In addition, the lipidated analog is optionally administered as pharmaceutical compositions in which it is mixed with other active ingredients, as in combination therapy. In some embodiments, the pharmaceutical composition includes other medicinal or pharmaceutical agents, carriers, adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure, and/or buffers. In addition, the pharmaceutical compositions can also contain other therapeutically valuable substances.

[0070] A pharmaceutical composition, as used herein, refers to a mixture of a lipidated analog with one or more other chemical components, such as carriers, stabilizers, diluents, dispersing agents, suspending agents, thickening agents, and/or excipients. The pharmaceutical composition facilitates administration of the lipidated analog to an organism. In practicing the methods of treatment or use provided herein, a lipidated analog is administered in a pharmaceutical composition to a mammal having a condition, disease, or disorder to be treated. Preferably, the mammal is a human. The dose and dosing regimen varies depending on the severity and stage of the condition, the age and relative health of an individual, the potency of the therapeutic composition used and other factors. The lipidated analog is optionally used singly or in combination with one or more therapeutic agents as components of mixtures.

[0071] The pharmaceutical compositions described herein can be formulated, for example, as aqueous liquid dispersions, self-emulsifying dispersions, solid solutions, liposomal dispersions, aerosols, solid dosage forms, powders, immediate release formulations, controlled release formulations, fast melt formulations, tablets, capsules, pills, delayed release formulations, extended release formulations, pulsatile release formulations, multiparticulate formulations, and mixed immediate and controlled release formulations. The pharmaceutical formulations described herein are optionally administered to a individual by one or more administration routes, including but not limited to, parenteral (e.g., intravenous, subcutaneous, intramuscular, intrathecal), intracerebroventricular, intranasal, buccal, topical, rectal, oral, or transdermal

administration routes. In some embodiments, the pharmaceutical formulations described herein are not centrally administered (e.g., administered directly to the central nervous system). In some embodiments, the pharmaceutical formulations described herein are peripherally administered (e.g., subcutaneously).

[0072] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

EXAMPLES

[0073] The following specific and non-limiting examples are to be construed as merely illustrative, and do not limit the present disclosure in any way whatsoever. Without further elaboration, it is believed that one skilled in the art can, based on the description herein, utilize the present disclosure to its fullest extent.

[0074] The following abbreviations are used herein: analysis of variance (ANOVA); bovine serum albumin (BSA); bovine pancreatic trypsin inhibitor (BPTI); epidermal growth factor (EGF); 4-(2-hydroxyethyl)-1-piperazineethansulfonic acid (HEPES); phosphate buffer saline (PBS); sodium dodecyl sulfate (SDS); Tris-buffered saline (TBS); diaminopropionic acid (Dpr); 1-naphtylalanine (1-Nal); norleucine (Nle); myristoyl (myr); palmitoyl (palm); octanoyl (oct); dodecanoyl (dodec); and tridecanoyl (tridec).

Example 1: Synthesis of the PrRP peptides and PrRPanalogs

[0075] The peptides were synthesized using the method of solid phase synthesis according to Maixnerová et al. (Maixnerová, J., et al., "Structure-activity relationship of CART (cocaine- and amphetamine-regulated transcript) peptide fragments," *Peptides*, 28:1945-1953, 2007) utilizing the Fmoc strategy on the ABI 433A synthesizer (Applied Biosystems, Foster City, CA, USA). Lipidation with the appropriate fatty acid was performed before cleaving the peptide off the resin as reported in Maletínská, L., et al., "Characterization of new stable ghrelin analogs with prolonged orexigenic potency," *J Pharmacol Exp Ther*, 340:781-786, 2012.

[0076] The peptide PrRP31 was iodinated with Na¹²⁵I using Iodo-Gen (Pierce, Rockford, IL, USA) according to the published procedure (Maixnerová et al., 2011). Monoiodinated peptide was stored in aliquots at -20 °C and was used up in binding assays within one month.

[0077] Table 1 describes the structures of PrRP31, PrRP20, and analogs of PrRP31 and PrRP20 synthesized:

Table 1. Structures of PrRP31, PrRP20, and lipidated analogs of PrRP31 and PrRP20

Rat PrRP31 and lipidated PrRP31 analogs	
PrRP31	SRAHQHSMETRTPDINPAWYTGRGIRPVGRF-NH ₂
1	(Dpr) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH ₂
2	(Dpr(oct)) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH ₂
3	(Dpr(dodec)) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH ₂
4	(Dpr(tridec)) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH ₂
5	(Dpr(myristic)) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH ₂
6	(Dpr(palm)) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH ₂
7	(Dpr) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR 1-Nal-NH ₂
8	(Dpr(oct)) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR 1-Nal-NH ₂
9	(Dpr(myristic)) RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR 1-Nal-NH ₂
Rat PrRP20 and lipidated PrRP20 analog	
Analog no.	Sequence
PrRP20	TPDINPAWYTGRGIRPVGRF-NH ₂
10	(myristic)TPDINPAWYTGRGIRPVGRF-NH ₂
Human PrRP31 lipidated PrRP31 and PrRP20 analogs:	
Analog no.	Sequence
hPrRP31	SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH ₂
11	(Dpr) RTHRHS Nle EIRTPDINPAWYASRGIRPVGRF-NH ₂
12	(Dpr(myristic)) RTHRHS Nle EIRTPDINPAWYASRGIRPVGRF-NH ₂
13	(myristic)TPDINPAWYASRGIRPVGRF-NH ₂
Other rat and human PrRP peptides and lipidated PrRP31 and PrRP20 analogs tested:	
Analog no.	Sequence
14	S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH ₂
15	(N-oct) S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH ₂
16	(N-dec) S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH ₂
17	(N-dodec) S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH ₂
18	(N-myristic) S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH ₂
19	(N-palm)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH ₂
20	(N-stear)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH ₂
21	S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR 1-Nal-NH ₂
22	(N-myristic)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR 1-Nal-NH ₂

23	(N-palm)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR 1-Nal-NH ₂
24	S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheCl ₂ -NH ₂
25	(N-myr)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheCl ₂ -NH ₂
26	(N-palm)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheCl ₂ -NH ₂
27	S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheNO ₂ -NH ₂
28	(N-myr)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheNO ₂ -NH ₂
29	(N-palm)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheNO ₂ -NH ₂
30	SHQRPADTHWYPRG Nle FPTIGRITARNGEVS
31	(N-myr)SHQRPADTHWYPRG Nle FPTIGRITARNGEVS
32	S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheF ₅ -NH ₂
33	(N-palm)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheF ₅ -NH ₂
34	S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR Tyr-NH ₂
35	(N-palm)S RAHQHS Nle ETRTPDINPAWYTGRGIRPVGR Tyr-NH ₂
36	f r GVPRIGRGTYWAPNIDPT-NH ₂
37	(N-myr)f r GVPRIGRGTYWAPNIDPT-NH ₂
38	TPDINPAWYTGR Sar IRPVGRF-NH ₂
39	(N-myr)TPDINPAWYTGR Sar IRPVGRF-NH ₂
40	TPDINPAWY N-Me-Ala SRGIRPVGRF-NH ₂
41	(N-myr)TPDINPAWY N-Mc-Ala SRGIRPVGRF-NH ₂
42	TPDINPAWYTGRGARPFGRF-NH ₂
43	(N-myr)TPDINPAWYTGRGARPFGRF-NH ₂
44	TPDINPAWYASRPFRPVGRF-NH ₂
45	(N-myr)TPDINPAWYASRPFRPVGRF-NH ₂
46	Nle-ETRTPDINPAWYTGRGIRPVGRF-NH ₂
47	(N-myr)Nle-ETRTPDINPAWYTGRGIRPVGRF-NH ₂
48	QHSMETRTPDINPAWYTGRGIRPVGRF-NH ₂
49	(N-myr)QHSMETRTPDINPAWYTGRGIRPVGRF-NH ₂
50	SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH ₂
51	TPDINPAWYASRGIRPVGRF-NH ₂
52	(N-oct)TPDINPAWYASRGIRPVGRF-NH ₂
53	(N-dec)TPDINPAWYASRGIRPVGRF-NH ₂
54	(N-dodec)TPDINPAWYASRGIRPVGRF-NH ₂
55	(N-myr)TPDINPAWYASRGIRPVGRF-NH ₂
56	(N-myr)SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH ₂

57	(N-palm)SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH ₂
58	(N-palm)SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NHMe
59	(N-palm)SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NOMe
60	(N-myr)SRAHQHSMETRTPDINPAWYTGRGIRPVGRF-NH ₂
61	(N-palm)SRAHQHSMETRTPDINPAWYTGRGIRPVGRF-NH ₂

Example 2: Competitive binding experiments

[0078] Competitive binding experiments were performed according to Motulsky and Neubig (Motulsky, A., et al., “Analyzing radioligand binding data,” *Curr Protoc Neurosci*, Chapter 7, Unit 7.5, 2002).

[0079] Binding experiments were conducted with rat pituitary cells and CHO cells as follows:

[0080] The rat pituitary cell line RC-4B/C (ATCC, Manassas, USA) was grown on 24-well plates which had their bottom coated with polyethyleneimine. The cells were grown to the optimal density of 300-450 thousand per well. The following agents were used for the experiment: binding buffer (20 mmol.l⁻¹ HEPES, pH 7.4; 118 mmol.l⁻¹ NaCl, 4.7 mmol.l⁻¹ KCl, 5 mmol.l⁻¹ MgCl₂, 5.5 mmol.l⁻¹ glucose, 1 mg/ml BSA, 0.1 mg/ml BPTI), unlabelled analogs of PrRP of final concentration within 10⁻¹¹ to 10⁻⁴ mol.l⁻¹, and rat ¹²⁵I-PrRP31 of final concentration of 10⁻¹⁰ mol.l⁻¹ (Maixnerová et al., 2011).

[0081] CHO cells with transfected human PrRP receptor, GPR10 (Perkin Elmer, USA) were grown on 24-well plates coated with polyethyleneimine. The cells were grown to the optimal density of 40-80 thousand cells per well. The following agents were used for the experiment: binding buffer (25 mmol.l⁻¹ Tris, pH 7.4; 118 mmol.l⁻¹ NaCl, 10 mmol.l⁻¹ MgCl₂, 1 mmol.l⁻¹ CaCl₂, 5.5 mmol.l⁻¹ glucose, 0.5 mg/ml BSA), unlabelled analogs of PrRP of final concentration within 10⁻¹¹ to 10⁻⁴ mol.l⁻¹, and human ¹²⁵I-PrRP31 of final concentration of 5x10⁻¹¹ mol.l⁻¹.

[0082] Plates were incubated for 60 minutes at room temperature. After incubation, the cells were solubilized in 0.1 mol/l solution of NaOH and the radioactivity bound to the cells was counted using a γ -counter. The experiments were performed in duplicate and repeated at least three times.

[0083] All the tested rat and human PrRP analogs (for structures, see Table 1), the lipidated peptides, bound with high affinity to the rat receptor in RC-4B/C cells (Table 2, 5) and the human receptor GPR10 in CHO cells (Table 5) (on the order of nmol.l⁻¹). With the lengthening of the fatty acid chain, the value of K_i decreased, indicating the affinity was stronger with the lipidated PrRP analogs, up to an order of magnitude as compared to the original unlipidated peptide (see Table 2, 5).

[0084] The results indicate the lipidation of the tested peptides led to the increase in receptor binding and the lipidated analogs lowered the K_i in binding to rat RC-4B/C cells and human GPR10 receptor in correlation with the chain length of the fatty acid present on the lipidated analog. The addition of fatty acid not only preserved receptor binding (as a result of N-terminal lipidation, whereas the receptor binding depends on the C-terminal RF-amide), it increased the binding affinity by up to an order of magnitude.

[0085] Human lipidated PrRP analogs (for structures, see Table 1, analog nos: 11-13) displaced to a similar extent the binding of both the rat and human ^{125}I -PrRP to the RC-4B/C cells with the rat PrRP receptor and human GPR10 receptor in the nmol.l^{-1} range (see Table 3). Lipidized shortened PrRP31 analogs (21-30 amino acids, C-terminal heptapeptide preserved) showed binding affinities similar to both native forms of PrRP.

[0086] For evaluation of competitive binding experiments, the program Graph Pad Prism Software (San Diego, CA, USA) was used. A method of nonlinear regression assuming one-site binding was used. The value of K_i was calculated using the equation of Cheng and Prusoff (Cheng, Y., et al., "Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (I_{50}) of an enzymatic reaction," *Biochem Pharmacol*, 22:33099-3108, 1973), using the value of K_d 4.21 nmol.l^{-1} and concentration of radioligand was 0.1 nmol.l^{-1} (Maixnerová et al., 2011).

Example 3: Cellular signaling.

[0087] RC-4B/C cells, which were used to determine if lipidated PrRP20 analogs are able to initiate the MAPK/ERK1/2 signaling pathway, were grown in 6-well plates up to the optimal density of 700-900 cells per well. Seventeen hours before sample harvest, the growth medium was replaced by a serum-free medium lacking epidermal growth factor (EGF). The respective lipidated PrRP analog was added to each well in the final concentration of $10^{-6} \text{ mol.l}^{-1}$. After a 5 minute incubation at 37°C , the plate was placed on ice and each well was rinsed three times with PBS pH 7.4 ($137 \text{ mmol.l}^{-1} \text{ NaCl}$, $2.7 \text{ mmol.l}^{-1} \text{ KCl}$, $8 \text{ mmol.l}^{-1} \text{ Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ a $1.76 \text{ mmol.l}^{-1} \text{ KH}_2\text{PO}_4$) and cooled to 4°C . The cells were then solubilized in a sample buffer ($62.5 \text{ mmol.l}^{-1} \text{ Tris-HCl}$ pH 6.8, 10% glycerol, 2% SDS, 0.01% bromophenol blue, 5% merkaptoethanol, $50 \text{ mmol.l}^{-1} \text{ NaF}$ and $1 \text{ mmol.l}^{-1} \text{ Na}_3\text{VO}_4$), and collected into microtubes and frozen at -20°C . Samples were collected in at least three independent experiments.

[0088] To determine if the lipidated PrRP20 analogs initiated the MAPK/ERK1/2 signaling pathway, Western blot analysis was used. Electrophoresis was performed on 5% / 12% polyacrylamide gel in the presence of SDS (SDS-PAGE) on the instrument MiniProtean 3 (BioRad, Herkules, CA, USA). Samples that were loaded on the gel were first disintegrated by ultrasound, then heated at 100°C for 2 minutes, and centrifuged for 5 minutes at $500 \times g$ at room

temperature. PrRP20, which was shown to initiate MAPK/ERK1/2 signaling (Maixnerová et al., 2011), was used as a positive control. Electrophoresis was performed at a constant voltage of 100 V for 10 minutes, or for 60 minutes at 150 V.

[0089] To show the presence of phosphorylated proteins in the samples, the proteins from the SDS-PAGE gel were transferred to the ImmobilonTM-P PVDF (polyvinylidene difluoride) membrane (Sigma-Aldrich, USA). The transfer was performed in blotting buffer at pH 8.3 (25 mmol.l⁻¹ Tris, 192 mmol.l⁻¹ glycine and 20% methanol) for 20 hours at 4 °C at a constant voltage of 30 V.

[0090] After the protein transfer to the PVDF membrane, the membranes were washed for 5 minutes in TBS washing buffer (20 mmol.l⁻¹ Tris, 140 mmol.l⁻¹ NaCl and 0.1% Tween-20) and then incubated for 1 hour in blocking buffer (TBS with 5% non-fat milk powder and 5 mmol.l⁻¹ Na₃VO₄ and 50 mmol.l⁻¹ NaF) at room temperature. Further, the membranes were washed three times for 5 minutes using the TBS washing buffer. The membranes were then incubated with the primary antibody against phospho-p44/42 MAPK(Thr202/Tyr204) (Cell Signaling Technology, Beverly, USA) which was diluted in blocking buffer at 1:1000. After the next three washes with the TBS washing buffer for 5 minutes, the membranes were incubated for 1 hour with rabbit secondary antibody labeled with peroxidase (Sigma, St. Louis, USA) which was diluted in blocking buffer at 1:12,000.

[0091] The membranes were then washed three times in TBS washing buffer for 5 minutes and then the solution Femto (Pierce SuperSignal, Thermo Fisher Scientific, Rockford, IL, USA) was applied. The induced chemiluminescence was detected by a CCD camera (LAS 3000, Fuji Photo Film GmbH, Düsseldorf, Germany).

[0092] To assess the initiation of signaling pathways, a densitometric analysis using the program Quantity One (BioRad, Hercules, CA, USA) was used. For statistical evaluation of the statistically significant response of the cellular signaling, one-way ANOVA with subsequent Dunnett post-hoc test was used. Data was statistically significant at P<0.05.

[0093] The results of the MAPK/ERK1/2 signaling analysis are shown in Table 2.

Table 2

The affinity of lipidated peptidic analogs of the rat PrRP31 and PrRP20 to the rat receptor, and biological activity in RC-4B/C cells.

Analogue	¹²⁵ I-rat PrRP31	ERK signaling
	K _i (nM)	% of PrRP31 binding
PrRP31	4.93 ± 0.61	100
1	3.74 ± 0.29	132
2	2.86 ± 0.17	172
3	1.12 ± 0.12	440
4	0.51 ± 0.03	967
5	0.82 ± 0.13	601
6	0.46 ± 0.06	1072
7	60.07 ± 2.10	8
8	39.24 ± 2.71	13
9	2.95 ± 0.62	167
PrRP20	3.44 ± 0.85	143
10	0.78 ± 0.45	632

[0094] All of the tested lipidated peptides displayed a positive result for signaling in RC-4B/C cells which was comparable or higher to the effect of PrRP31. All of the lipidated peptides were therefore agonists *in vitro*.

Table 3.

Affinity of lipidated analogs of rat and human PrRP to the rat receptor in RC-4B/C cells

Analogue	¹²⁵ I-rat PrRP31	¹²⁵ I-human PrRP31
	K _i (nM)	K _i (nM)
hPrRP31	1.54 ± 0.42	3.01 ± 0.70
11	2.28 ± 1.30	2.00 ± 1.26
12	4.13 ± 2.57	3.16 ± 0.83
13	0.41 ± 0.13	0.66 ± 0.14

[0095] Table 2 and 3: The values of the inhibition constant K_i were calculated from IC₅₀ using Cheng and Prusoff's equation (for K_d the value 4.21 nmol.l⁻¹ found in the saturation binding experiments was used and the concentration of the radioligand was 0.1 nmol.l⁻¹ with relevant values of S.E.M.

[0096] Example 4. Test of food intake after SC or ICV administration of lipidated PrRP analogs

[0097] Male C57BL/6 mice (An Lab, Prague 4) were bred in the accredited animal facility in the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, at the Institute of Molecular Genetics, Prague 4, at the temperature of $22 \pm 2^\circ\text{C}$ with access to food and water *ad libitum*. The day/night cycle was 12/12 hours (light turned on at 6:00 am). Animals were treated according to the Animal Protection Act (Act No.246/1992). The males were fed standard chow diet St-1 (Mlýn Kocanda, Praha, Czech Republic) which contained 66% saccharides, 25% proteins and 9% lipids with a caloric value of 3.4 kcal/g.

[0098] The food was withdrawn 17 hours prior to injection of lipidated peptide and free access to water was maintained. The administration of saline, PrRP or lipidated analogs was performed either by SC injection at doses of 1-10 mg/kg (volume 0.2 ml/mouse), or by ICV using infusion pump (cannulae were inserted into the third brain ventricle 4 days before the experiment following the procedure of Maixnerová et al. (Maixnerová et al., 2011)). The single dose of peptide was 4 nmol/mouse (5 μl /mouse).

[0099] Fifteen minutes after the lipidated peptide administration, the mice were given pre-weighed food. The food was then weighed every 30 minutes for 5-10 hours. The administration of every dose was performed at least twice and one group of mice consisted of at least 6 mice. The results were presented as % of food intake as compared with the control group injected with saline.

[00100] Statistical evaluation was performed using program Graph-Pad Prism Software (San Diego, CA, USA), and the one-way ANOVA with a subsequent Dunnettovým post-hoc test. The difference in food intake between mice injected with saline and mice injected with the examined peptide were considered statistically significant at $P < 0.05$.

[00101] The results are summarized in Table 4 and 5 and Figures 1-6.

Table 4. Biological activity of lipidated peptides – rat PrRP analogs *in vivo*

Analog	SC administration				ICV administration			
	45 minutes		300 minutes		45 minutes		300 minutes	
	food intake (g)	% control	food intake (g)	% control	food intake (g)	% control	food intake (g)	% control
PrRP31	0.32	100	1.15	105	0.42	75	1.37	104
1	0.38	117	1.05	97	0.39	70	0.71	53
3	0.12	50	0.61	60	NT			
4	0.07	30	1.04	101	0.38	71	1.4	105

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5	0.05	25	0.18	21	0.35	62	0.96	73
6	0	0	0.14	14	0.38	71	1.3	100
7	0.16	49	0.88	81		NT		
8	0.17	76	0.78	90	0.34	60	1.05	79
9	0.01	2	0.22	25	0.28	51	1.03	78
PrRP20	0.32	100	1.1	100	0.32	68	1.29	100
10	0.11	32	44	44	0.28	51	1.1	86

[00102] The food intake was evaluated at 300 minutes after administration of the compound. At 45 minutes after administration, the effect was at the maximum. Compounds 1, 2, 5, 7, 9 were administered at the dose of 10 mg/kg, and compounds 3, 4, 6, 10 were administered at the dose of 5 mg/kg (SC administration). NT indicates not tested.

Table 5

Competitive binding to rat RC-4B/C cells and CHO cells with transfected human GPR10 receptor and food intake in fasted C57BL/6 mice (5 mg/kg SC)

Analog no.	C-4B/C cells ²⁵ I-rat PrRP31	GPR10 receptor in CHO cells	Food intake in mice (5mg/kg SC)
	K _i (nM)	K _i (nM)	% saline-treated group (45 min)
14	1.28 ± 0.2	1.92 ± 0.43	100
15	0.98 ± 0.22	1.53 ± 0.07	94
16	0.65 ± 0.41	1.46 ± 0.57	57
17	0.39 ± 0.14	1.19 ± 0.36	22
18	0.69 ± 0.09	0.71 ± 0.09	3
19	0.51 ± 0.16	3.03 ± 0.34	2
20	0.96 ± 0.10	5.39 ± 0.58	3.5
21	102 ± 19.1	34.52 ± 16.00	NT
22	3.96 ± 1.22	1.33 ± 0.22	NT
23	2.17 ± 1.29	4.27 ± 0.77	13
24	3.84 ± 0.93	2.27 ± 0.48	NT
25	1.79 ± 1.02	0.95 ± 0.33	13
26	1.30 ± 0.29	1.05 ± 0.41	5

27	19.90 ± 5.9	4.80 ± 0.88	NT
28	1.97 ± 1.26	1.59 ± 1.05	NT
29	0.58 ± 0.10	0.77 ± 0.28	0.7
30	1317000 ± 1259000	$> 10^7$	NT
31	123 ± 16.1	262.0 ± 55.4	100
32	9.69 ± 1.86	8.59 ± 1.20	NT
33	0.51 ± 0.08	1.92 ± 0.53	41
34	3.25 ± 0.15	2.02 ± 0.09	NT
35	0.44 ± 0.22	1.57 ± 0.39	22
36	5200 ± 420	6290 ± 3970	NT
37	364 ± 83	$> 10^7$	100
38	7.94 ± 3.74	4.51 ± 1.49	NT
39	0.32 ± 0.07	3.12 ± 0.37	NT
40	49.4 ± 9.35	8.72 ± 2.22	NT
41	0.46 ± 0.05	2.10 ± 0.77	NT
42	655 ± 164	583 ± 293	NT
43	7.21 ± 0.71	8.47 ± 3.18	NT
44	2.14 ± 0.53	1.73 ± 0.22	NT
45	0.84 ± 0.17	0.58 ± 0.26	NT
46	2.94 ± 0.62	3.44 ± 0.48	NT
47	0.45 ± 0.04	5.48 ± 0.64	NT
48	2.61 ± 0.15	5.92 ± 0.76	NT
49	0.37 ± 0.11	6.93 ± 4.74	NT
50	1.78 ± 0.46	8.70 ± 1.56	100
51	2.38 ± 0.19	4.53 ± 0.80	100
52	NT	3.02 ± 0.49	100
53	NT	2.41 ± 0.26	100
54	NT	2.41 ± 0.26	85
55	0.48 ± 0.18	4.33 ± 0.25	10
56	0.95 ± 0.35	NT	35
57	0.28 ± 0.80	4.71 ± 0.56	11
58	NT	3.16 ± 0.51	NT
59	NT	4.21 ± 0.55	NT

60	0.75 ± 0.10	NT	20
61	0.41 ± 0.12	NT	2

NT – not-tested

[00103] Lipidated analogs of PrRP31 and PrRP20 lowered food intake in fasted mice after ICV administration to a comparable degree as PrRP31 (Figure 1) but the effect was prolonged (see, e.g., Figure 2). The effect of lipidated analogs of PrRP after ICV administration was not dependent on the length or presence of the fatty acid.

[00104] Lipidated analogs of the rat PrRP31 and PrRP20 with bound myristic or palmitic acid (Figure 3) significantly lowered (and for a prolonged period of time) the food intake in fasted mice after SC administration. The analog with tridecanoic acid partially lowered the food intake, however. The analogs lacking fatty acid or containing octanoyl or dodecanoyl had no effect or did not significantly lower food intake. This was most likely caused by a poor penetration through the hematocephalic barrier.

[00105] Myristoylated analogs of human PrRP31 and PrRP20 significantly lowered food intake in fasted mice, comparable to the effect of corresponding rat analogs (Figure 4). The lowering of food intake in mice after SC administration of the lipidated PrRP analogs was dose dependent (Figure 5 demonstrates the effect of compound 5 and Figure 6, the effect of compound 9). Doses of 2-10 mg/kg significantly lowered food intake.

Example 5. Fos Immunohistochemistry

Tissue Processing

[00106] For Fos immunohistochemical processing, overnight fasted mice (n=5 mice/group) were treated SC with saline, [Dpr¹]PrRP31, [Dpr(oct)¹]PrRP31, [Dpr(myristic)¹]PrRP31 or [Dpr(palm)¹]PrRP31 (dose 5mg/kg). Ninety minutes after SC injection, the mice were deeply anesthetized with pentobarbital (50 mg/kg, IP) and perfused transcardially with 0.1 M phosphate buffer (PB, pH 7.4) containing 4 % paraformaldehyde, 0.1 % glutaraldehyde, and 10 % picric acid (w/w). Then the brains were removed, postfixed in the same fixative overnight at 4 °C, and infiltrated with 30 % sucrose in 0.1 M PB for 48 h at 4 °C. Before sectioning, the brains were rapidly (20 sec) frozen in cold isopentane (-30/-40 °C) and placed into a Reichert cryocut device adjusted to -16 °C for 1 h. 30 µm coronal sections were cut from the brains and collected as free floating in cold (4°C) PB.

Immunohistochemistry

[00107] Free floating sections were repeatedly washed in cold PB followed by preincubation in 3 % H₂O₂ for 40 min at room temperature. They were incubated with polyclonal Fos protein antiserum (1:2000), diluted in 0.1 M PB containing 4 % normal goat serum (Gibco, Grand Island, NY, USA), 0.5 % Triton X-100 (Koch-Light Lab. Ltd., Colnbrook,

Berks, England), and 0.1 % sodium azide for 48 h at 4 °C. After several rinses in PB, the sections were incubated with biotinylated goat-anti-rabbit IgG (1:500, VectorStain Elite ABC, Vector Lab., Burlingame, CA, USA) for 90 min at room temperature. Next PB rinses were followed by incubation with the avidin–biotin peroxidase complex (1:250) for 90 min at room temperature. PB washing was followed by washing in 0.05 M sodium acetate buffer (SAB, pH 6.0). The Fos antigenic sites were visualized with 0.0266 % 3,3'-diaminobenzidine tetrahydrochloride (DAB) dissolved in SAB containing 0.0042 % H₂O₂ and 2.5 % nickel ammonium sulfate, for 7 min. The metal-intensification of DAB produced black staining in the labeled nuclei. Finally, the sections were rinsed in 0.05 M SAB, mounted into 0.1 % of gelatine dissolved in 0.0125 M SAB, air-dried and coverslipped with Permount (Sigma, St. Louis, MO, USA). Immunostaining of negative controls, which did not show any antiserum immunolabeling, included substitution of the primary antiserum with normal rabbit serum, and sequential elimination of the primary or secondary antibody from the staining series.

Evaluation of the immunostaining

[00108] An identical set of mice was used for determination of Fos immunoreactivity in NTS, PVN, and Arc. Counting of Fos immunoreactive cells within the NTS, from Bregma -7.48 mm to Bregma -7.32 mm, PVN, from Bregma -0.7 mm to -0.94 mm, and DMH, from Bregma -1.46 mm to -1.82 mm according to the mouse brain atlas (Franklin KBJ, Paxinos G: *The mouse brain in stereotaxic coordinates*. New York: Academic Press; 1997), was performed separately in each side of the sections. Quantitative assessment was performed from the images captured with a Canon digital camera (PowerShot S40) and Leica DMLS light microscope in a computer screen obtained from 5-6 brain sections per animal. Representative sections were captured by the same computerized system. The counting of Fos-positive neurons was done under blinded conditions (the counted slides from each animal were analyzed independently and randomly and encoded by other person).

[00109] Results are shown in Fig. 7. Neural activity presented in Fos immunoreactivity was significantly increased in a case of [Dpr(my¹)]PrRP31 and [Dpr(palm)¹]PrRP31 analogs in all brain nuclei involved in food intake regulation tested (i.e. Arc, PVN and NTS). These two analogs also significantly decreased food intake.

Example 6. Open field locomotor activity and hot-plate analgesic test

[00110] Locomotor activity was measured using the VideoMot system (TSE Systems, Bad Homburg, Germany). *Ad libitum* fed mice were placed individually in the open field (1x1 m) and their locomotor activity, total distance traveled, was measured for 10 min. Mice were administered with SC injection of saline, [Dpr¹]PrRP31, [Dpr(palm)¹]PrRP31 or myr-PrRP20 (dose 5 mg/kg, n=5 mice/group).

[00111] Analgesia was measured by a hot-plate analgesia meter (TSE Systems, Bad Homburg, Germany) set at 53°C after completion of the locomotor activity test. The end-point was the latency to jump, after which the animals were immediately removed from the plate.

[00112] Results of behavioral tests in mice are described in Fig. 8. Administration of lipidized PrRP analogs caused mild sedative effect similar as an effect accompanying satiety induced by, for example, cholecystokinin. PrRP analogs also showed some analgesic effect.

Example 7. 14-day administration of lipopeptides in model of diet-induced (DIO) mice

[00113] Inbred C57BL/6 male mice (AnLab, Prague, Czech Republic) were housed at a temperature of 23°C under a daily cycle of 12 hours light and dark (light from 6:00 a.m.) with free access to water and food pellets (St-1, Mlýn Kocanda, Jesenice, Czech Republic).

[00114] At 8 weeks of age, mice were divided into a standard (St) diet-fed group (the energy content of the St-1 diet was 3.4 kcal/g, containing 25, 9, and 66% of calories from protein, fat, and carbohydrate, respectively (St-1, Mlýn Kocanda, Jesenice, Czech Republic)) and a high fat (HF) diet-fed group (the energy content of the HF diet was 5.3 kcal/g, containing 13, 60, and 27% of calories from protein, fat, and carbohydrate, respectively). The HF diet consisted of 40% standard St-1 diet, 34% powdered cow milk for human neonates, 25% lard, and 1% corn starch w/w (Kopecký, J., et al., "Reduction of dietary obesity in aP2-Ucp transgenic mice: physiology and adipose tissue distribution," *Am J Physiol*, 270:E768-75, 1996). Food intake and body weight were monitored weekly from 9 to 20 weeks of age. Mice resistant to the HF diet were withdrawn from the experiment (approximately 10% of mice).

[00115] At the age of 20 weeks, mice were placed into separate cages with free access to food and water. The following week, mice were subjected to a 14-day food intake experiment. They were injected with palm-PrRP31 or myr-PrRP20 dissolved in saline at doses of 5 mg/kg (n=10) or saline twice a day (at 8:00 and 18:00) for 14 days. Consumption of the St or HF diet and the weight of the mice were simultaneously followed.

[00116] After 14 days of treatment, mice were fasted overnight and sacrificed the following morning. Their blood sera were isolated, and the white adipose tissue (subcutaneous, abdominal, and gonadal), and the liver of all mice were dissected, weighed, and stored at -70°C. Fat to body weight ratio was calculated as a ratio of adipose tissue weight to body weight.

Determination of hormonal and biochemical parameters

[00117] Serum insulin and leptin concentrations were measured by RIA assays. Serum glucose levels were measured using a Glucocard glucometer (Arkay, Kyoto, Japan). Serum triglyceride levels were measured by quantitative enzymatic reactions (Sigma, St. Louis, USA). Statistical analysis

[00118] The data are presented as means $\pm$ SEM for the number of animals indicated in the Figures and Tables. They were analyzed by a one-way ANOVA followed by a Dunnett *post-hoc* test (metabolic parameters, behavioral study) or two-way ANOVA followed by a Bonferroni *post-hoc* test (body weight evaluation) using Graph-Pad Software (San Diego, CA, USA). $P < 0.05$ was considered statistically significant.

[00119] Results of long-term administration of myr-PrRP20 and palm-PrRP31 into DIO mice are shown in Fig. 9 (body weight change) and Table 6 (metabolic parameters). Body weight of DIO mice significantly decreased both after myr-PrRP20 and palm-PrRP31 treatment. Fat/body weight, liver weight, leptin and insulin were also significantly lowered, especially after palm-PrRP31 administration.

Table 6

Metabolic parameters after 14-days SC administration of lipidized PrRP analogs in 17h fasted DIO male mice

Treatment	Fat/body weight [%]	Liver/body weight [%]	Leptin [ng/ml]	Glucose [mmol/l]	Insulin [ng/ml]	Triglycerides [mg/dl]
Saline	16.15 $\pm$ 0.40	4.07 $\pm$ 0.19	53.26 $\pm$ 3.49	6.94 $\pm$ 0.28	4.09 $\pm$ 0.55	72.25 $\pm$ 3.20
Myr-PrRP20	15.30 $\pm$ 0.42	3.60 $\pm$ 0.18*	39.56 $\pm$ 3.47***	7.52 $\pm$ 0.16	3.54 $\pm$ 0.47	68.71 $\pm$ 4.16
Palm-PrRP31	12.69 $\pm$ 0.71***	3.56 $\pm$ 0.07**	24.71 $\pm$ 3.39***	7.26 $\pm$ 0.29	2.37 $\pm$ 0.47**	66.81 $\pm$ 8.74

All values are expressed as mean $\pm$ SEM (n=10 per group).

Significance (one-way ANOVA following by Dunnett post-hoc test) is * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs saline.

Example 8: Test of food intake after administration of analog no. 55 to Rhesus monkeys

[00120] Food intake (amount of pellets consumed) was measured in Rhesus monkey (n=4) after a single subcutaneous administration of myristoylated human PrRP20 (analog no. 55).

[00121] The study was conducted on overnight fasted animals in Meditox breeding facility (Konárovice, Czech Republic). Each animal received a subcutaneous injection of saline, or of analog 55 in a dose of 1.0 mg/kg or 2.0 mg/kg. Test item formulation was prepared in saline solution as a fresh solution, just before dosing.

[0122] All of the animals were observed for clinical signs, morbidity or mortality once a day during acclimatisation and frequently after the administration. Food intake monitoring was performed 2, 4, 8 and 24 hours after single administration.

[0123] All of the animals were in good health throughout the acclimatisation, treatment period, no clinical symptoms of toxicity were observed after the administration of analog 55. Significantly lower food intake after both injected doses of the peptide in comparison to the saline-treated animals was observed between 1-6 hours after the single subcutaneous administration of analog 55.

[0124] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. The scope of the claims should not be limited by the preferred embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description as a whole.

WHAT IS CLAIMED IS:

1. A lipidated analog of prolactin-releasing peptide selected from the group consisting of:

J^a-SRAHQHSMETRTPDINPAWYTGRGIRPVGRF-NH₂;
J^a-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH₂;
(N-palm)-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NHMe;
(N-palm)-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NOMe;
(N-myr)-TPDINPAWYTGRGIRPVGRF-NH₂;
(N-myr)-TPDINPAWYASRGIRPVGRF-NH₂;
J^{l'}-SRAHQHS Nle ETRTPDINPAWYTGRGIRPVGRF-NH₂;
J^a-SRAHQHS Nle ETRTPDINPAWYTGRGIRPVGR 1-Nal-NH₂;
J^a-SRAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheCl₂-NH₂;
J^a-SRAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheNO₂-NH₂;
J^a-SRAHQHS Nle ETRTPDINPAWYTGRGIRPVGR PheF₅-NH₂;
J^a-SRAHQHS Nle ETRTPDINPAWYTGRGIRPVGRY-NH₂;
J^a-SRAHRHS Nle EIRTPDINPAWYASRGIRPVGRY-NH₂;
(N-myr)-Nle-ETRTPDINPAWYTGRGIRPVGRF-NH₂;
(N-myr)-QHSMETRTPDINPAWYTGRGIRPVGRF-NH₂;
(N-myr)-QHSMETRTPDINPAWYASRGIRPVGRF-NH₂;
(N-myr)-TPDINPAWYmoRGIRPVGR 1-Nal-NH₂;
(N-palm)-TPDINPAWYmoRGIRPVGR 1-Nal-NH₂; and
(N-palm)-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NOMe; wherein

J^{l'} is a palmitoyl group, a myristoyl group or stearoyl group bound by the amide bond to the amino group of the N-terminal amino acid;

J^a is a palmitoyl group or a myristoyl group bound by the amide bond to the amino group of the N-terminal amino acid; and

m is T or A; and **o** is G or S.

2. A lipidated analog of prolactin-releasing peptide selected from the group consisting of:

(N-palm)-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH₂; and

(N-myr)-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH₂.

3. A lipidated analog of prolactin-releasing peptide of structure:

(N-palm)-SRTHRHSMEIRTPDINPAWYASRGIRPVGRF-NH₂.

4. A lipidated analog of prolactin-releasing peptide according to formula:

J^a-TPDINPAWY**m****o**RGIRPVGRF-NH₂; wherein

J^a is a palmitoyl group or a myristoyl group bound by the amide bond to the amino group of the N-terminal amino acid; **m** is T or A; and **o** is G or S.

5. The lipidated analog of prolactin-releasing peptide of claim 4, wherein J^a is a myristoyl group bound by the amide bond to the amino group of the N-terminal amino acid.

6. A lipidated analog of prolactin-releasing peptide of structure:

(N-myr)-TPDINPAWYASRGIRPVGRF-NH₂.

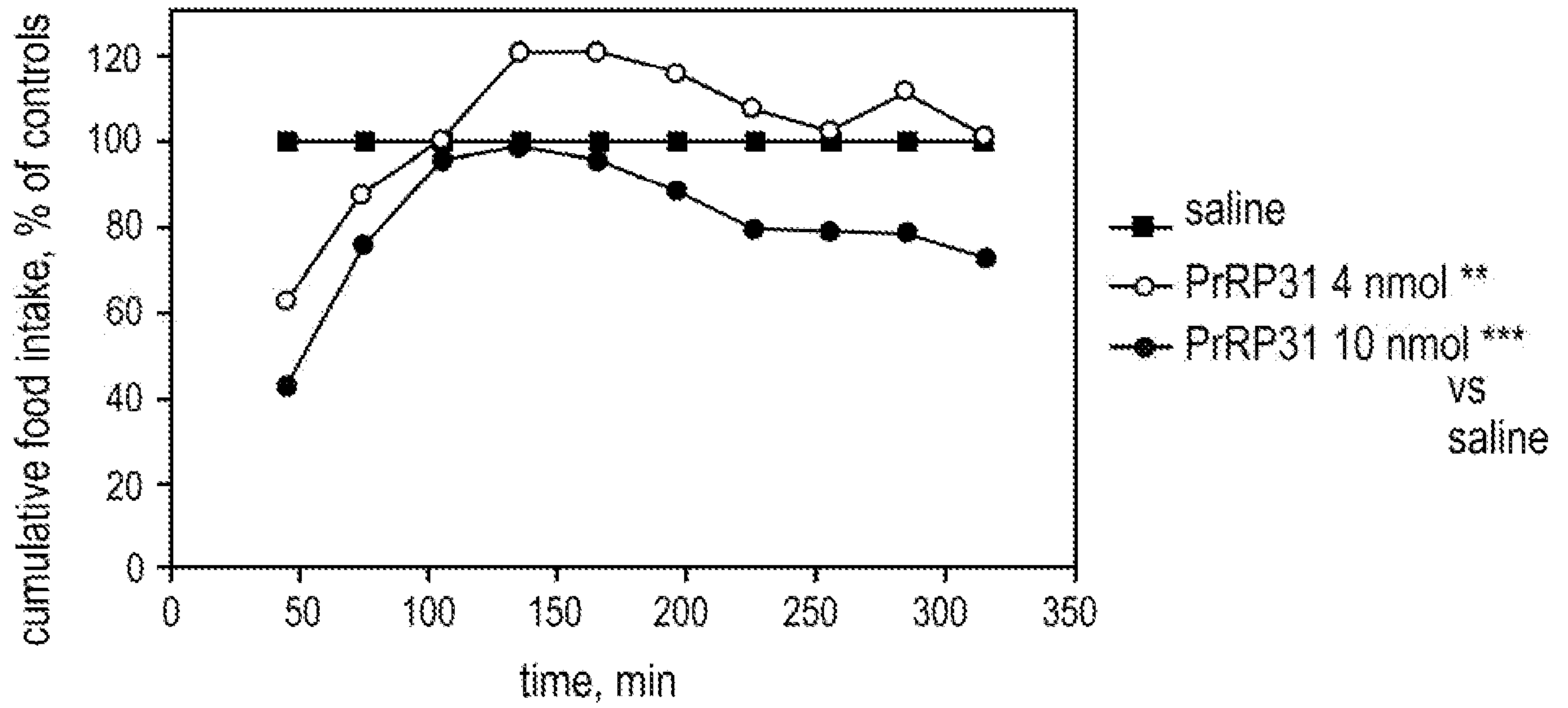
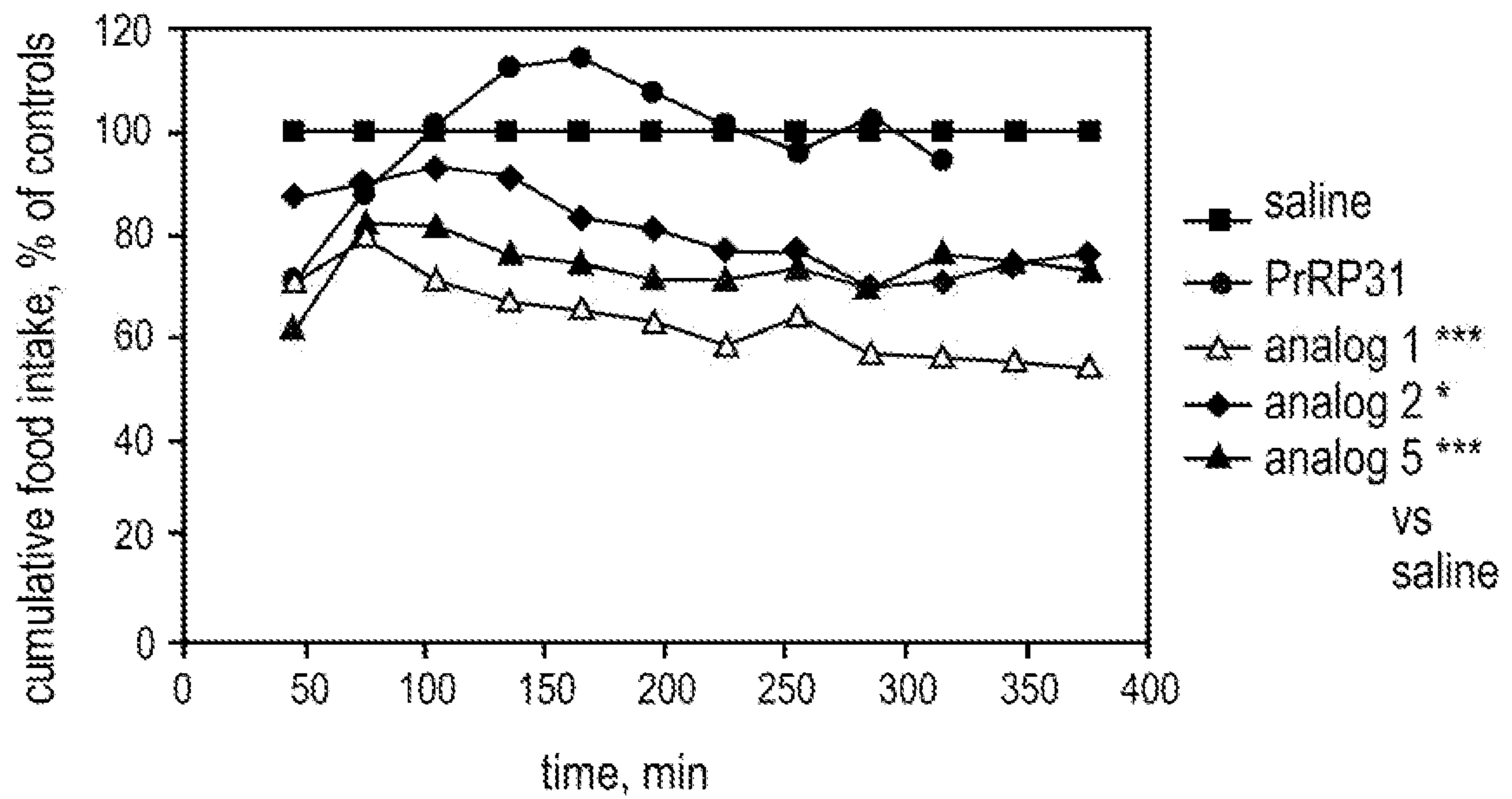
7. A pharmaceutical composition comprising at least one lipidated analog of prolactin-releasing peptide as defined in any one of claims 1 to 6, and one or more physiologically acceptable carrier.

8. Use of the lipidated analog of prolactin-releasing peptide as defined in any one of claims 1 to 6 for lowering food intake after peripheral administration in a subject in need thereof.

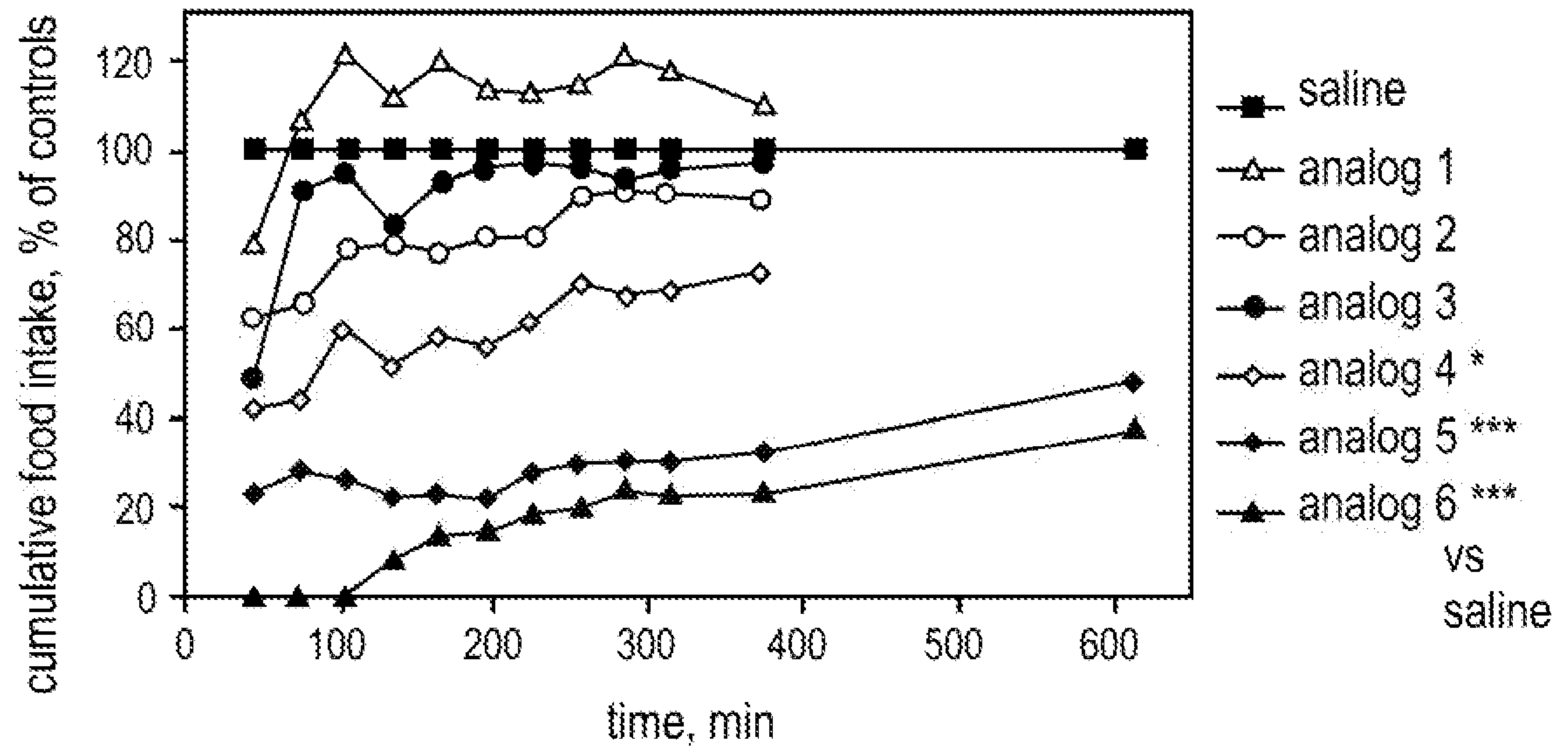
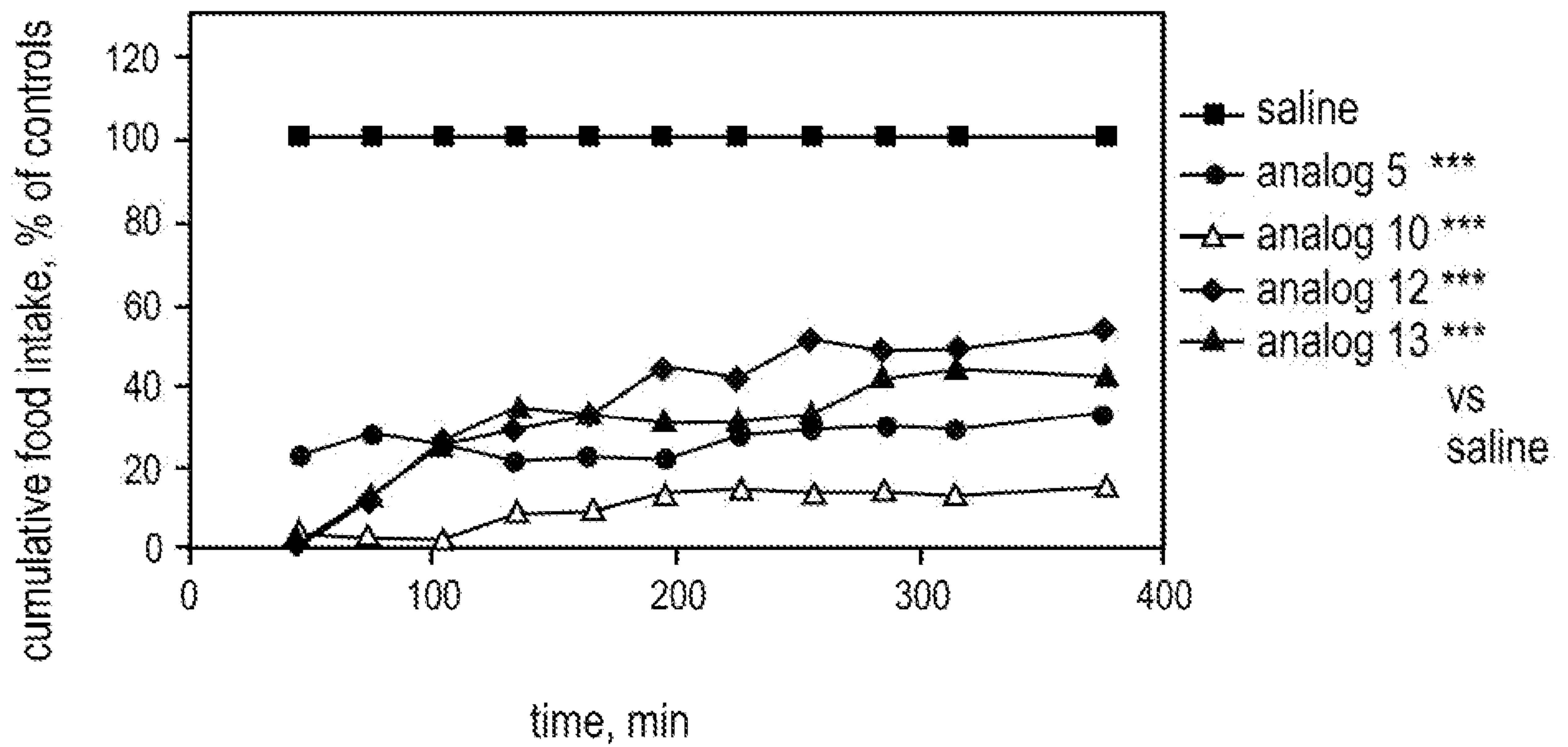
9. The lipidated analog of prolactin-releasing peptide as defined in any one of claims 1 to 6 for lowering food intake after peripheral administration in a subject in need thereof.

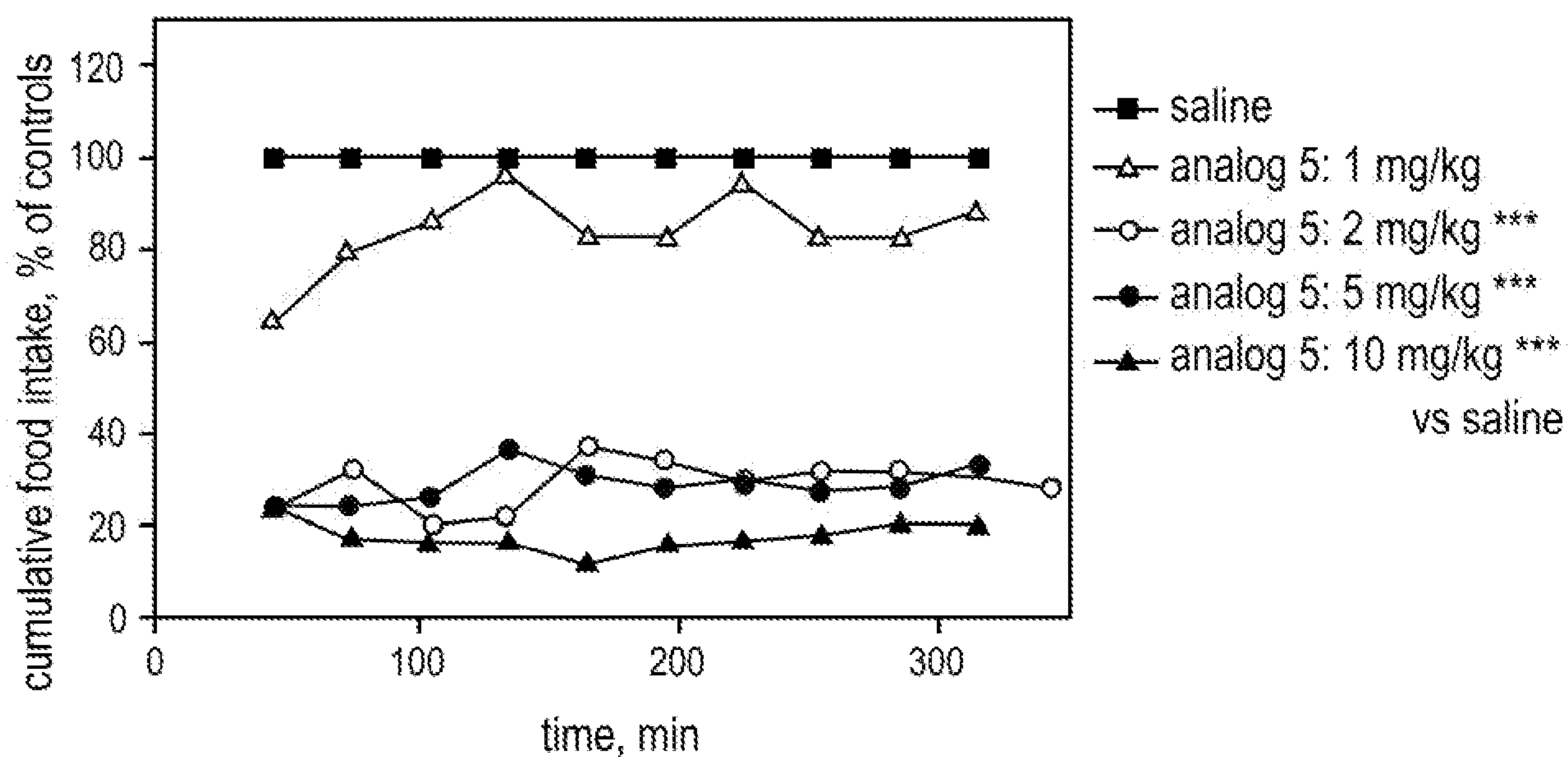
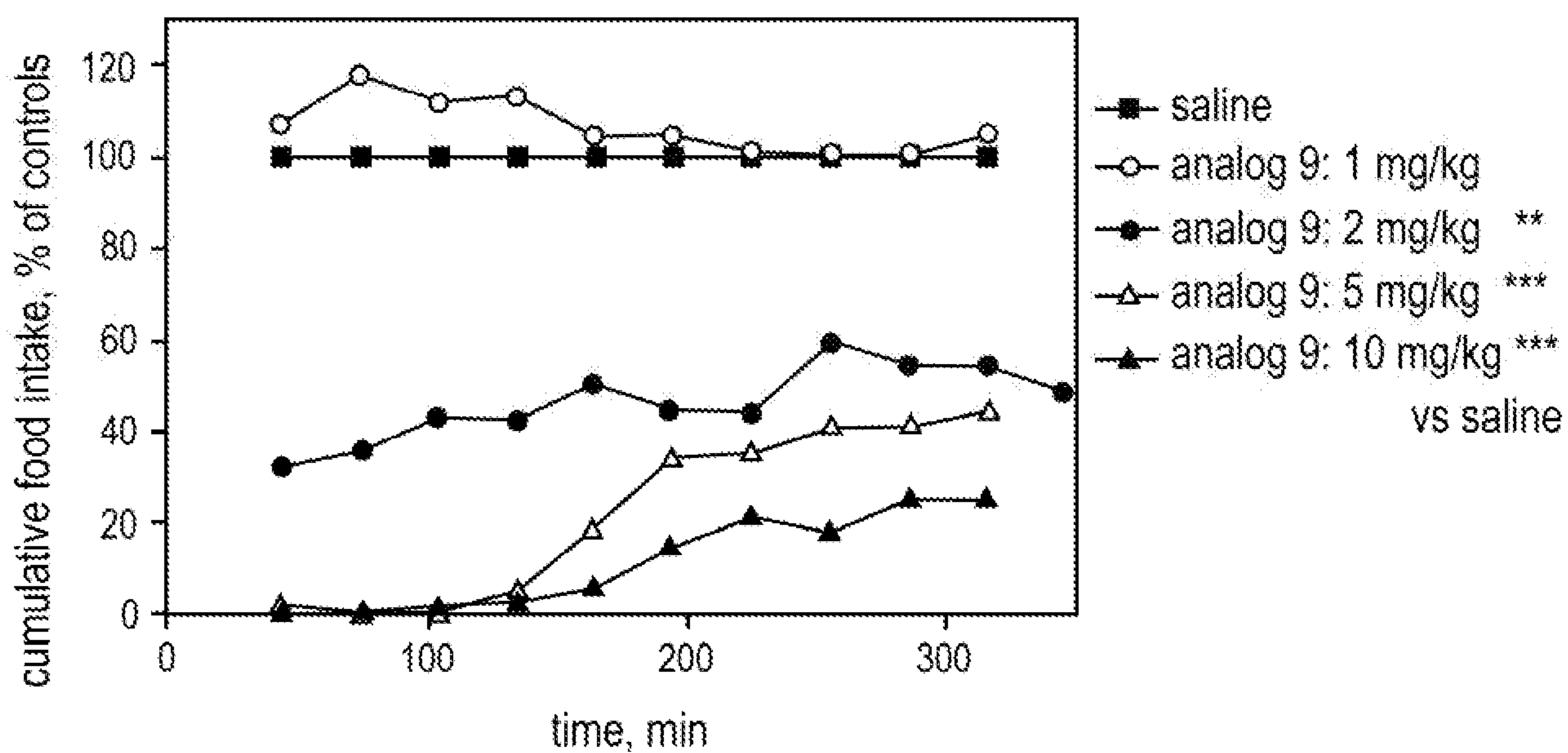
10. Use of the lipidated analog of prolactin-releasing peptide as defined in any one of claims 1 to 6 for the preparation of a medicament for lowering food intake after peripheral administration in a subject in need thereof.

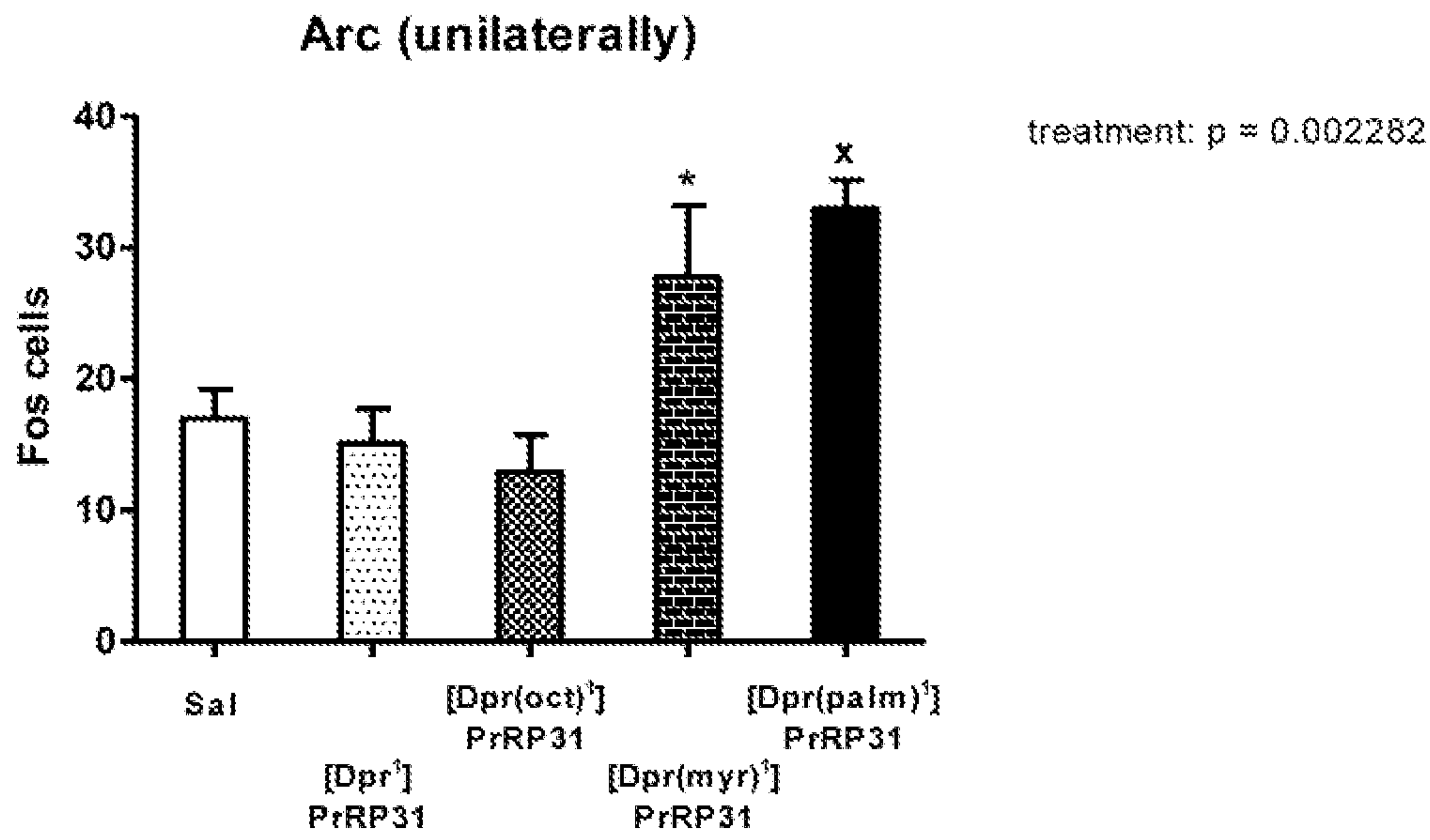
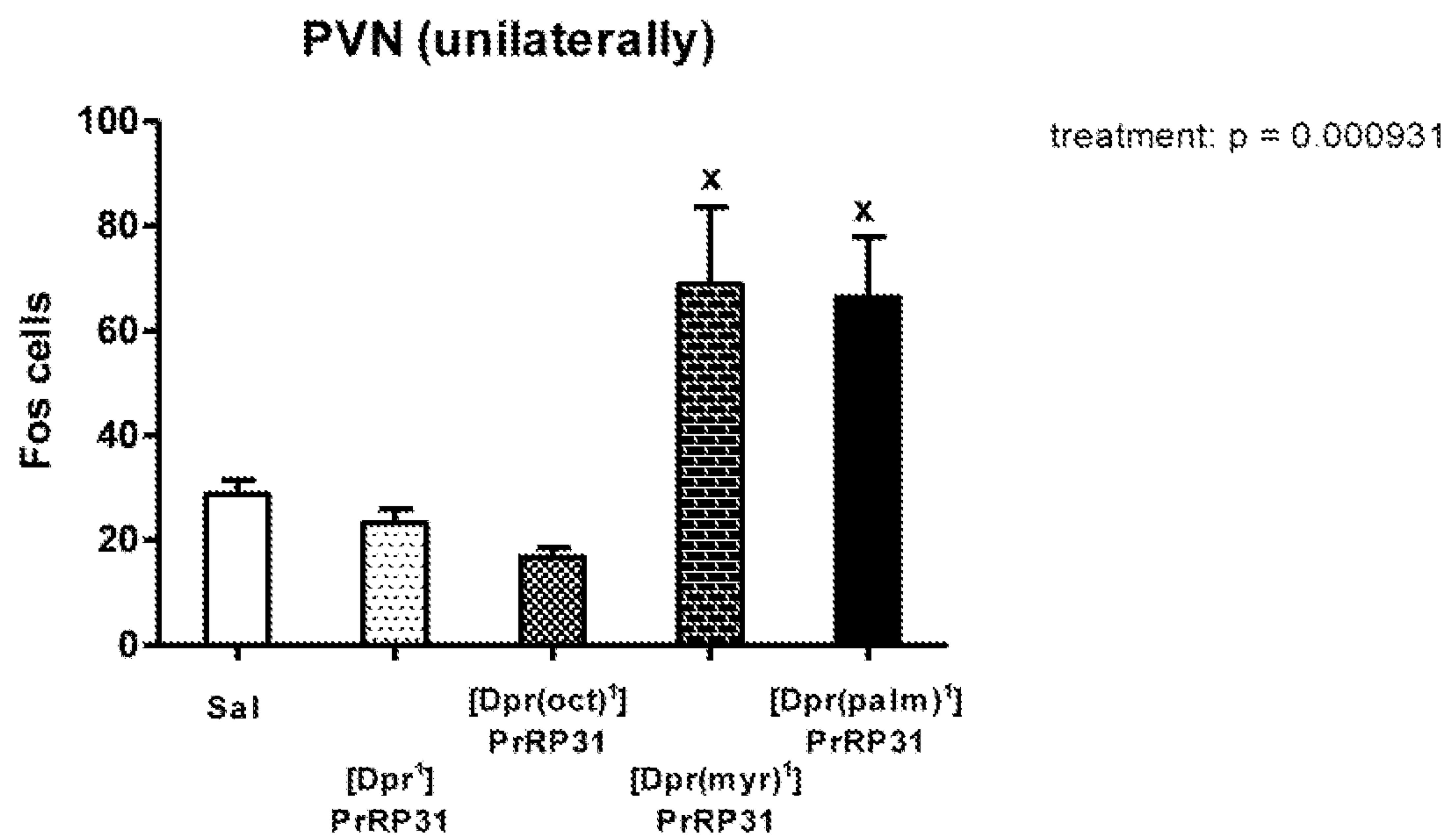
11. Use of the lipidated analog of prolactin-releasing peptide as defined in any one of claims 1 to 6 for the treatment or prevention of obesity in a subject in need thereof.
12. Use of the lipidated analog of prolactin-releasing peptide as defined in any one of claims 1 to 6 for the manufacture of a medicament, for the treatment or prevention of obesity in a subject in need thereof.
13. The lipidated analog of prolactin-releasing peptide of any one of claims 1 to 6, for use in the treatment or prevention of obesity in a subject in need thereof.

**FIG. 1****FIG. 2**

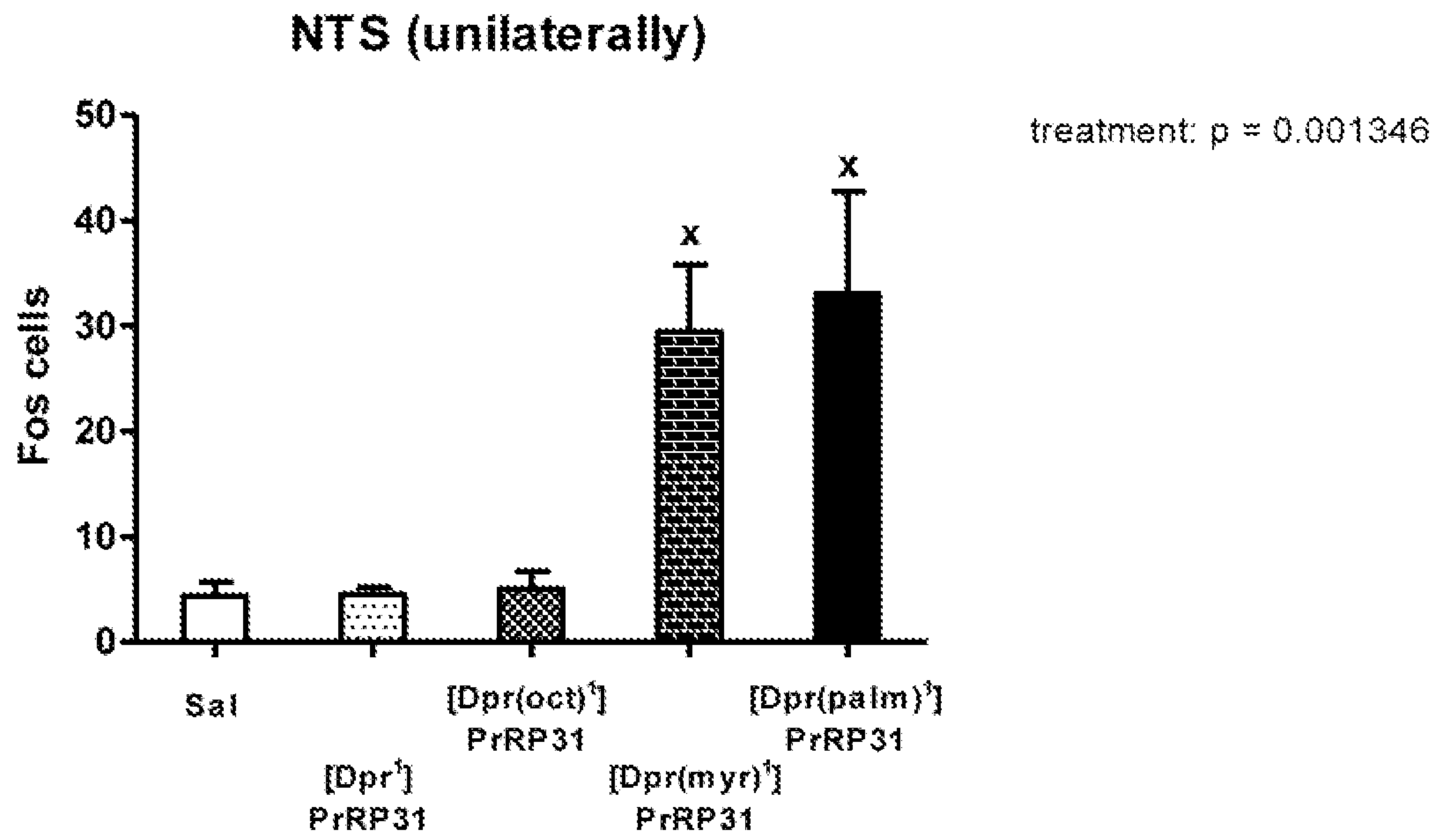
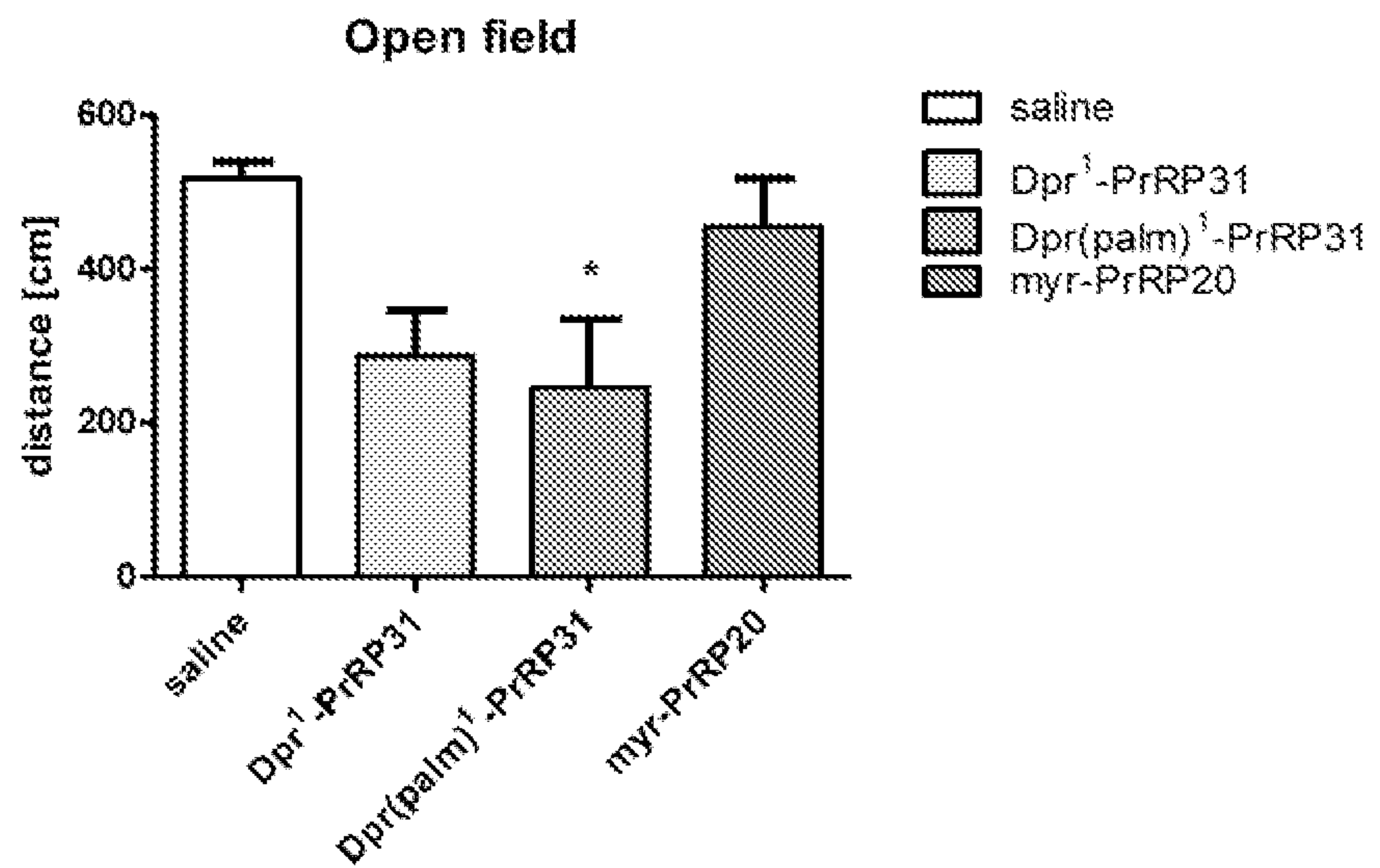
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**FIG. 3****FIG. 4**

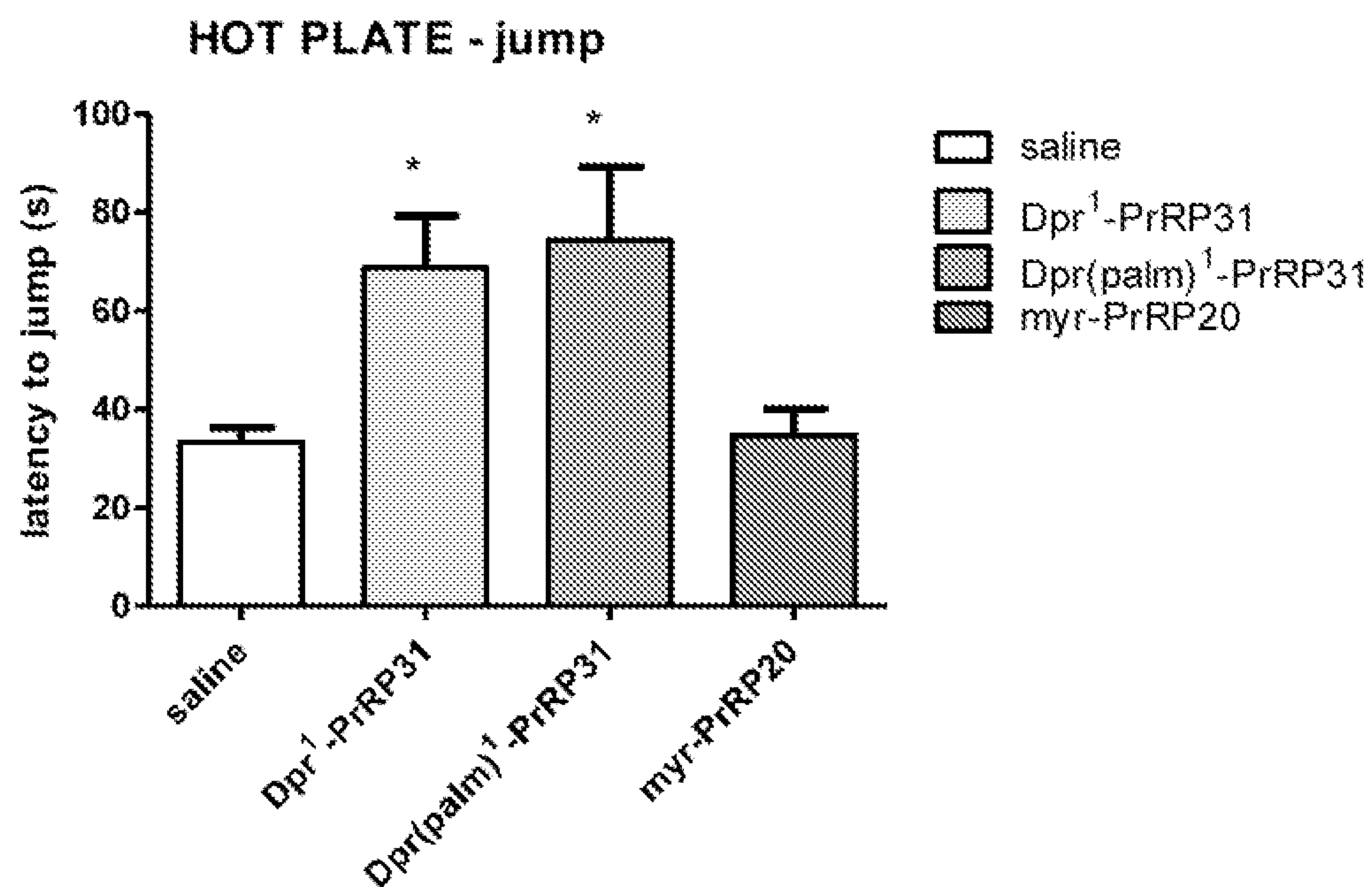
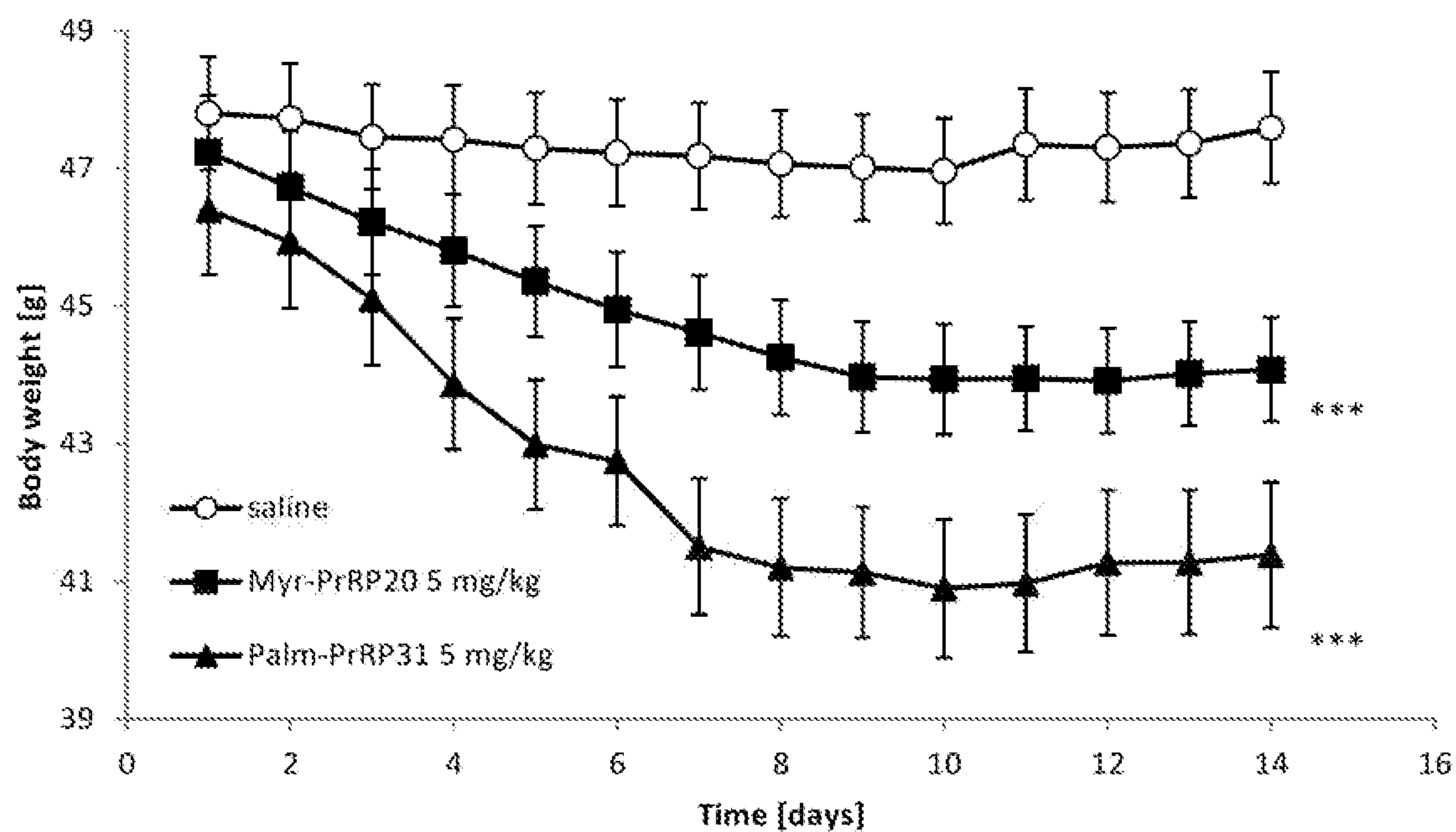
**FIG. 5****FIG. 6**

**FIG. 7A****FIG. 7B**

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**FIG. 7C****FIG. 8A**

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**FIG. 8B****FIG. 9**

