KBP-088, a novel DACRA with prolonged receptor activation, is superior to davalintide in terms of efficacy on body weight

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Submitted 15 December 2015; accepted in final form 11 February 2016

Obesity is a consequence of the modern-day lifestyle, and the number of obese people is increasing. Associated with obesity is a number of comorbidities and reduced life expectancy. Of these, type 2 diabetes, nonalcoholic fatty liver disease, osteoarthritis, and cardiovascular disease are prominent, and the obesity-derived insulin resistance is considered a major detrimental event in terms of prognosis (21)(15)(6).

In this study, we studied a novel, highly potent DACRA, KBP-088, with a prolonged receptor activation profile in a long-term in vivo study head to head with davalintide, a potent amylin agonist, to clearly determine peptide properties predictive of in vivo efficacy on body weight.

Research Design and Methods

Peptide therapy. Synthetic KBP-088 and davalintide (American Peptide) were dissolved in saline for subcutaneous delivery. The 5 μg/kg dose chosen for peptide administration in the current investigations was based on previous comparable DACRA studies in animal models of obesity and type 2 diabetes (1, 5) using scT and the potent DACRA KBP-042.

In vitro receptor binding and activity. The receptor specificity and potency at the amylin and calcitonin receptor were determined by the ability of KBP-088 to induce β-arrestin and recruitment in cell lines overexpressing the human calcitonin amylin and calcitonin gene-related peptide receptors, respectively. U20S CALCR cells (DiscoverX cat. no. 93-0566C3), CHO K1 CALCRL RAMP3 (DiscoverX cat. no. 93-0268C2) and CKO-K1 CALCRL RAMP1 (DiscoverX cat. no. 93-0269C2) cells were used to quantify β-arrestin by PathHunter Detection Kit (DiscoverX 93-0001) according to the manufacturer’s instructions. The responses were analyzed and plotted as previously described (1, 2).

Animal experiments. All animal procedures were performed in accordance with guidelines from the Animal Welfare Division of the Danish Ministry of Justice under the institutional license issued to Nordic Bioscience (2012-15-2934-00094). Male Sprague-Dawley rats were obtained at 6 wk of age and housed at the Nordic Bioscience animal facility (21–23°C, 55–65% relative humidity, 12:12-h light-dark cycle) with ad libitum access to food and water.

Animals. Normal-diet age-matched lean rats (ND) were fed a standard pelleted chow, and high-fat-diet-fed rats (HFD) a 60 kcal% fat diet (#858Y1; TestDiet, London, UK). At study start, HFD rats received 10% fructose (#F0127, Sigma-Aldrich, Brøndby, Denmark) in the drinking water, and, in order to avoid bacterial growth, citric acid was added (pH 3.6) in fructose drinking water; ND rats received tap water with an equal amount of citric acid.

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Acute food intake. Acute food intake was tested in overnight-fasted HFD rats. The animals received vehicle, KBP-088 (5, 1.67 μg/kg sc), or davalintide (5, 1.67 μg/kg sc), and food intake was monitored 4, 24, 48, and 72 h postinjection.

Chronic in vivo study. We compared two doses of KBP-088 (1.67 and 5.0 μg/kg) with similar davalintide doses in HFD rats. Following 10 wk of HFD feeding, the rats were randomly (body weight) assigned to treatment groups (n = 8) receiving either vehicle (saline sc), KBP-088 (5, 1.67 μg/kg sc), or davalintide (5, 1.67 μg/kg sc) once daily. Pair-fed rats (saline sc) were food restricted to KBP-088 5 μg/kg and davalintide 5 μg/kg. Food intake and body weight were monitored on days 1–20, 28, 35, 42, 49, 56, and 62. After 3 and 7 wk of treatment, OGTT was performed in overnight-fasted (12 h) rats, with blood glucose measured and EDTA-plasma obtained for hormonal analysis. Rats received glucose gavage (2 g/kg po). Blood samples were collected from the tail vein before drug administration (–30 min) and glucose challenge (0 min) and 15, 30, 60, and 120 min post-glucose challenge.

At study end, the animals were euthanized, anesthetized by inhalation (isoflurane) followed by exsanguination and dissection. Epididymial fat pads were fixed in 4% formaldehyde and then stained with hematoxylin. Sections were randomly and blindly selected and viewed under a microscope (12 sections per group; 6 sections for ND control, ×20 magnification). Pictures were taken and adipocytes counted using Olympus cell imaging software, and the average size of the adipocytes was calculated.

Blood samples were collected in EDTA tubes and centrifuged at 5,000 rpm for 10 min at 4°C. Blood glucose was monitored by Accu-Check Avia monitoring system (Roche Diagnostics, Rotkreuz, Switzerland). Plasma levels of insulin (Mercodia Rat Insulin ELISA; Mercodia, Uppsala, Sweden) was analyzed according to manufacturer’s instruction.

Statistical analysis. All data are presented as means ± SE. The statistical analyses of various drug effects were conducted using one-way ANOVA, followed by Tukey’s posttest for multiple comparison. ND controls and HFD rats as well as EC_{50} values were compared using Student’s t-test. All analyses were performed using GraphPad Prism software (GraphPad Prism, San Diego, CA). A value of P < 0.05 was considered statistically significant.

RESULTS

KBP-088 induces prolonged receptor activation in vitro, whereas davalintide does not. Table 1 shows the sequence of KBP-088 and davalintide for comparison. In vitro analyses of the potency of KBP-088 and davalintide on the calcitonin, amylin and CGRP receptors showed that both KBP-088 and davalintide are highly potent ligands for the calcitonin and amylin receptors (Fig. 1). EC_{50} values for the CTR were calculated to 4.5 ± (1.4) × 10^{-9} M and 5.2 (± 1.2) × 10^{-9} M for KBP-088 and davalintide (P = 0.68), respectively, whereas the corresponding EC_{50} values for the AMY-R were 4.0 (± 1.7) × 10^{-10} M and 1.3 (± 1.7) × 10^{-9} M for KBP-088 and davalintide (P = 0.38), respectively. In contrast, on the CGRP-R we only observed a response with davalintide, whereas KBP-088 even at very high doses (10^{-7} M) did not induce β-arrestin recruitment (Fig. 1C). However, the mag-

![Fig. 1. Dose-range curves of KBP-088 and davalintide on induction of β-arrestin in calcitonin receptor (CTR; A), amylin receptor (AMY-R; B), and calcitonin gene-related peptide receptor (CGRP-R; C) –expressing cell lines. D: prolonged CT-R-specific β-arrestin response mediated by 100 nM KBP-088 or 100 nM davalintide in CTR-expressing cells for 4–72 h. A–D are pooled data from 3–4 independent experiments.](http://ajpendo.physiology.org/)

Table 1. Amino acid sequences of the dual amylin and calcitonin receptor agonists

<table>
<thead>
<tr>
<th>Sequences</th>
<th>KBP-088</th>
<th>Davalintide</th>
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<tbody>
<tr>
<td>AC</td>
<td>C S N L S T C M L G R L S Q E L H R L Q T F P K T D V G A N A P -NH2</td>
<td></td>
</tr>
<tr>
<td>KS</td>
<td>C N T A T C V L G R L S Q E L H R L Q T Y P R T N G S N T Y -NH2</td>
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</tbody>
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Amino acid sequence comparison of KBP-088 and davalintide. Differences in sequences are highlighted in boldface. KBP-088’s NH2-terminal cysteine has an acetyl modification.
nitude of the CGRP-R induction elicited by davalintide was low compared with the endogenous CGRP-R agonist, H9251-CGRP. An important aspect of the DACRAs is their prolonged interaction with the CTR (2) and while davalintide has been reported to bind irreversibly to the AMY-R (9) it is not clear to what extent this translates into functional receptor activation over time. To address this, we compared davalintide to KBP-088 with receptor to prolonged receptor activation, and as seen in Fig. 1D, KBP-088 induces a potent prolonged receptor activation with activation still observed at 72 h, in line with other DACRAs (2). On the other hand, despite the potent short term activation of the receptors (Fig. 1, A–C), davalintide did not lead to prolonged receptor activation (Fig. 1D).

Both davalintide and KBP-088 attenuate short-term food intake, albeit only KBP-088 shows a prolonged reduction. A single dose of KBP-088 and davalintide resulted in significantly (P < 0.01) reduced food intake 4 h postinjection; however, only KBP-088 significantly reduced food intake 24 (~95%) and 48 (~32%) hours postinjection (Fig. 2A).

KBP-088 potently reduces appetite, body weight, and fat depots. Ten weeks of high-fat feeding resulted in a phenotype with significantly (P < 0.001, ~30%) increased body weight (HFD), hyperinsulinemia, impaired glucose control without hyperglycemia, but impaired insulin sensitivity (HOMA-IR) compared with the lean age-matched controls (ND) (Table 2) resembling an obese and prediabetic phenotype.

To investigate the anti-obesity potential of KBP-088 in vivo we treated HFD rats for 8 wk, and compared the metabolic effects with equivalent davalintide dosing. Previously, DACRAs have shown a hypophagic effect (5); therefore, we included a pair-fed group to both KBP-088 and davalintide treated rats exploring impact of food restriction regarding body weight. KBP-088 and davalintide were subcutaneously administered (1.67 and 5 μg/kg sid) throughout 62 days. During the study period food intake was transiently attenuated by KBP-088 (Fig. 2, B and C) treatment, although cumulative food intake after the initial 2 wk of treatment was not significantly different from control.

**Table 2. Model characterization**

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<thead>
<tr>
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<th>ND Control</th>
<th>HFD</th>
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<tr>
<td>Body weight (g)</td>
<td>466 ± 33</td>
<td>607 ± 23***</td>
</tr>
<tr>
<td>Fasting plasma glucose (ng/ml)</td>
<td>6.2 ± 0.1</td>
<td>5.6 ± 0.1**</td>
</tr>
<tr>
<td>Fasting plasma insulin (ng/ml)</td>
<td>1.0 ± 0.1</td>
<td>2.2 ± 0.2***</td>
</tr>
<tr>
<td>HOMA-IR (mM x μU/ml)</td>
<td>6.3 ± 0.6</td>
<td>14.8 ± 1.1***</td>
</tr>
<tr>
<td>Glucose tAUC in OGTT after 7 wk of treatment (mmol/l·min)</td>
<td>1,253 ± 20</td>
<td>1,422 ± 31***</td>
</tr>
<tr>
<td>Insulin tAUC in OGTT after 7 wk of treatment (ng/ml·min)</td>
<td>260 ± 17</td>
<td>358 ± 33*</td>
</tr>
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Values are means ± SE; n = 8 rats per group. Model characterization of normal-diet lean (ND Control) and high-fat diet-fed (HFD) rats. HOMA-IR, homeostasis model assessment of insulin resistance; tAUC, total area under the curve; OGTT, oral glucose tolerance test. Statistical tests performed with Student’s t-test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. ND Control.

Fig. 2. A: short-term treatment effect on food intake (4–48 h) by 2 different concentrations of KBP-088 and davalintide in high-fat diet-fed (HFD) rats (n = 8 rats per group). Weekly food intake (B), cumulative food intake days 1–14 (C), and days 15–62 (D) in HFD rats dosed with davalintide and KBP-088 (1.67 and 5 μg/kg) for 62 days (n = 8 rats per group; KBP-088 5 μg/kg n = 4). *P < 0.05, **P < 0.01, ***P < 0.001 vs. KBP-088 1.67 μg/kg; # vs. 1.67 μg/kg davalintide, $ vs. 5 μg/kg davalintide g. * vs. vehicle. Statistical analysis between groups was evaluated by one-way ANOVA post hoc analyses. All data are means ± SE.

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compared with davalintide and vehicle treated rats (Fig. 2D). At study end, body weight of KBP-088-treated animals was significantly lowered compared with vehicle- and davalintide-treated rats and associated pair-fed rats (Fig. 3B). The reduced food intake in the initial phase of the study (Fig. 2A) corresponds well with the significant weight loss observed in both KBP-088-treated groups and the KBP-088-associated pair-fed group compared with vehicle- and davalintide-treated rats (Fig. 3A).

Based on food intake and body weight change, food efficacy was calculated. We used the total food intake from days 15–62, as there were no significant difference in food consumption between the groups in this period. Expectedly, KBP-088 treatment (1.67 and 5 µg/kg) resulted in a marked attenuation of food efficiency (Fig. 3C), which was significantly different from vehicle- and davalintide-treated rats and the pair-fed group associated with the KBP-088 5 µg/kg group.

At termination, we isolated epididymal, inguinal, and perirenal fat depots. Interestingly and in conjunction with the significant body weight reduction, the weight of epididymal white adipose tissue was significantly reduced after treatment with 1.67 and 5 µg/kg KBP-088 (Fig. 4A). This reduction was not observed in the pair-fed control or in davalintide-treated rats. There was a trend toward reducing inguinal and perirenal adipose tissue (Fig. 4, B and C). Furthermore, the size of the adipocytes (Fig. 4D, I–6, E) in KBP-088-treated HFD rats was markedly reduced compared with vehicle- and davalintide-treated rats and corresponding pair-fed controls.

**KBP-088 enhances glucose tolerance and potentially insulin sensitivity.** As expected, the basal insulin levels were markedly increased in HFD rats compared with ND rats; however, hyperinsulinemia was significantly reduced in KBP-088 groups compared with vehicle (data not shown). To investigate the effect of KBP-088 on glucose tolerance, we performed an OGTT in weeks 3 and 7. Glucose tolerance was significantly improved by KBP-088 (1.67 and 5 µg/kg) and davalintide (5 µg/kg) to a similar extent (Fig. 5, A and B), evidenced by the ~12% decreases in the blood glucose AUC values for both treatment doses of 5 µg/kg (Fig. 5, C and D). Pair feeding did not improve glucose tolerance in either test. The glucose-induced insulin hypersecretion observed in vehicle- and pair-fed groups was markedly suppressed during OGTT by the two concentrations of KBP-088 and by davalintide at 5 µg/kg, which resulted in significantly reduced insulin AUC values in KBP-088- (1.67 and 5 µg/kg) and davalintide- (5 µg/kg) treated rats (Fig. 5, E and F).

Overall, these findings suggest that KBP-088 exerts a pronounced anorectic effect in HFD rats, a reduction of body weight, and an improvement in energy homeostasis in conjunction with alleviation of hyperinsulinemia, which is in line with previous findings for injectable DACRAs (5), and illustrates the need for prolonged receptor activation to induce these effects.

**DISCUSSION**

Amylin receptor agonists are highly interesting as candidates for the treatment of type 2 diabetes and obesity (4). However, despite the approval of the amylin receptor agonist pramlintide for the treatment of diabetes as adjunct to mealtime insulin, these ligands are notoriously limited in terms of efficacy both on glucose homeostasis and on weight control. Recent studies have indicated that DACRAs, dual amylin and calcitonin receptor agonists, have potency extending far beyond classical amylin agonists such as pramlintide, although the explanation for this remained to be elucidated.

In this study, we compared a novel DACRA, called KBP-088, to the amylin mimic davalintide, an amylin, calcitonin, and calcitonin gene-related peptide receptor agonist, using a series of in vitro and in vivo tests to elucidate the mechanism underlying the superior activity of the DACRAs. By use of short-term in vitro assays, davalintide was roughly equipotent to KBP-088; however, when their ability to elicit long-term receptor activation was tested, davalintide did not induce this. On the other hand, KBP-088 activated the receptor for up to 72 h, demonstrating a superior receptor activation profile. Furthermore, these effects manifested directly in a prolonged ability to control appetite by KBP-088, which was not seen for davalintide. This was somewhat surprising, as davalintide previously had been shown to bind irreversibly to the AMY-R (9); however, due to some yet to be identified mechanism, this does not translate into prolonged receptor activation or prolonged suppression of appetite.

In this study, KBP-088 induced a marked weight loss. The drastic reduction in body weight observed at study start could be explained by the initial anorectic effect of KBP-088, as the food-restricted pair-fed controls lowered their body weight simi-

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**Fig. 3.** Weekly (A), study end body weight (B), and food efficacy (days 15–62; C) in HFD rats dosed with davalintide and KBP-088 (1.67 and 5 µg/kg) for 62 days and pair-fed (n = 8 rats per group; KBP-088 5 µg/kg n = 4). *P < 0.05, **P < 0.01, ***P < 0.001; * vs. vehicle, # vs. 1.67 µg/kg davalintide, $ vs. 5 µg/kg davalintide, π vs. pair-fed 5 µg/kg KBP-088.
Importantly, five animals were subtracted from the 5 μg/kg group due to a too-large weight loss. The maximal dose of KBP-088 was selected based on previous findings using salmon calcitonin, an amylin and calcitonin receptor agonist, and DACRAs (5); however, this peptide exerts a very potent anorectic effect, and in future studies the maximal doses will be of lower concentration. Interestingly, the food intake returned to normal within 3 wk, and the pair-fed group regained lost body weight, whereas the KBP-088-treated groups maintained the weight loss achieved, 16% throughout the study in the highest-concentration KBP-088 group. We speculate that the nonexisting prolonged response of davalintide underlay the lack of ability to suppress body weight at the doses chosen, although it transiently suppressed food intake. These are consistent with the need for infusion pumps and thereby continuous exposure to davalintide in order for it to exert a weight-reducing effect (9).

Considering the fact that KBP-088 significantly suppresses body weight compared with the pair-fed controls emphasizes...
that KBP-088 has some beneficial effect on the weight reduction besides suppressed food intake. Furthermore, the decreased food efficiency of the KBP-088-treated rats, and the difference between treated and pair-fed rats, suggest an increased energy expenditure. Davalintide has enhanced pharmacological properties over rat amylin (8), albeit a short-lasting effect compared with KBP-088. Under normal conditions the rats will lower energy expenditure during weight loss; however, continuous infusion of amylin prevents this reduction (11, 18), and similar effects were observed with davalintide (9).

Interestingly, amylin increases energy expenditure only when given as a continuous infusion or icv (3, 7, 18), a finding likely related to the short-lived activation of the AMY-R. However, the energy expenditure needs to be formally assessed in the future.

Fig. 5. A and B: plasma glucose during oral glucose tolerance test (OGTT) in HFD rats dosed with davalintide and KBP-088 (1.67 and 5 μg/kg) after 3 and 7 wk, respectively. Total AUC for glucose (C and D) and insulin (E and F) during OGTT after 3 and 7 wk, respectively (n = 8 rats per group; KBP-088 5 μg/kg n = 4). *P < 0.05, **P < 0.01, ***P < 0.001 vs. pair-fed 5 μg/kg davalintide, □ vs. pair-fed 5 μg/kg KBP-088. Statistical analysis between groups was evaluated by one-way ANOVA post hoc analyses. All data are means ± SE.
Additionally, KBP-088 reduced overall adiposity as well as decreasing the size of adipocytes in the epididymal white adipose tissue over the study period. Weight loss has on multiple occasions been associated with beneficial effects on adipokynes (14) and leptin metabolism – leptin sensitivity, which has previously been shown to be improved by DACRAs (5). Furthermore, weight loss and reduction in adipose cell size are involved in restoring plasma insulin concentration toward normal, concomitant with the return of normal tissue insulin sensitivity (13), and whether KBP-088 has a direct effect on adipocyte hypertrophy needs further investigations.

Short- and long-term treatment with KBP-088 improved glucose tolerance compared with both vehicle and pair-fed groups in accord with previous studies performed with DACRAs (5) and davalistide. The previously described effect of davalintide on glucose tolerance was performed in rats receiving a continuous infusion of davalintide (8). Notably, glucose tolerance was also improved in davalintide-treated rats even though there was a lack of prolonged receptor activation. This was probably due to the predosing of the rats with the peptides 30 min prior to an OGTT, which confirms the ability of davalintide to improve glucose tolerance short-term, as previously described. However, to evaluate the overall treatment effect of KBP-088 and davalintide on glucose tolerance, the OGTT must be performed without predosing. As KBP-088 has a prolonged response and reduces body weight, the glucose-regulatory and insulinosynthetic effects would undoubtedly be present; however, as davalintide did not elicit long-term receptor activation, the glucose-lowering effect might have been lost.

In line with previous DACRA findings, albeit in contrast to other glucose-lowering agents such as sultfonylureas and GLP-1 analogs, the enhanced glucose disposal was achieved with an attenuated insulin secretion. This could imply an enhanced insulin sensitivity; however, this needs further investigations addressing insulin sensitivity and circumventing gastric emptying, as amylin agonism lowers the gastric emptying rate (19, 20).

In conclusion, the novel DACRA KBP-088 has prolonged receptor activation, and furthermore, KBP-088 induces and sustains a marked weight loss over 62 days in obese rats, which concomitantly leads to a reduced amount of adipose tissue. In addition, KBP-088 improves glucose tolerance and implies improved insulin action, underscoring the potential of KBP-088 as an antiobesity agent with additional benefits on glucose control.

REFERENCES


