Background and aim

Amylin is a peptide co-secreted with insulin from pancreatic β-cells in response to meal ingestion. Amylin plays important roles in the control of food intake and in the regulation of postprandial glucose levels, and has inhibitory effects on gastric emptying, and pancreatic glucagon secretion. Here we investigated the anti-diabetic effects of long-acting amylin analogues ZP4982 and ZP5461 in Zucker Diabetic Fatty (ZDF) rats.

Materials and methods

The study was conducted with 94 male ZDF rats (20-10 weeks of age). The rats were divided into 7 groups (n = 13). Animals from group 2 were pair-fed to group 5 (ZP5461, 30 nmol/kg) in order to compare between the contribution made by reduced food intake, and drug-induced effects on glycemic control and body weight. Rats were treated for 4 weeks with long-acting amylin analogues ZP4982 (30 nmol/kg, s.c., every 5th day), ZP4981 (L, 30 and 100 nmol/kg, s.c., every 3rd day), and the GLP-1 analogues, liraglutide (40 nmol/kg, s.c., bd). The effects of treatments on glycemic control were investigated by measurements of non-fasting and fasting glucose, HbA1c, and glucose tolerance during an intra-peritoneal glucose tolerance test (IPGTT). Additionally, measurements on body weight, food- and water intake were conducted in order to monitor the metabolic state of the animals.

Results: Efficacy on human calcitonin receptor (CTR), CTR/receptor activity-modifying protein 1 (RAMP1), and CTR/RAMP3

Table 1. EC50 on the human CTR, CTR/RAMP1, and CTR/RAMP3, measured on JAMP formation in CDE-7 cells, stably expressing the human CTR, CTR/RAMP1, or CTR/RAMP3. Data are mean ± SD.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>CTR (nM)</th>
<th>CTR/RAMP1 (nM)</th>
<th>CTR/RAMP3 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZP4982</td>
<td>0.038 ± 0.012 (n=3)</td>
<td>0.250 ± 0.003 (n=2)</td>
<td>0.120 ± 0.015 (n=2)</td>
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<tr>
<td>ZP5461</td>
<td>0.072 ± 0.033 (n=2)</td>
<td>0.41 ± 0.05 (n=2)</td>
<td>0.120 ± 0.015 (n=2)</td>
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</tbody>
</table>

Results: Effect on food- and water intake

Figure 2. Effect of treatment with vehicle, ZP4982, ZP5461, or liraglutide on food and water intake. A) Accumulated food intake (g/rat); B) Accumulated water intake (g/rat).

Results: Effect on glucose tolerance

Figure 3. Effect of treatment with vehicle, ZP4982, ZP5461, or liraglutide on glucose tolerance (day 21). A) Blood glucose profiles during the IPGTT; B) Blood glucose AUC; C) Plasma insulin profiles during the IPGTT; D) Plasma insulin AUC. Data are mean values ± SEM (n=5/group). Data were analyzed by 2-way ANOVA followed by Bonferroni post tests. The lines below the graphs represent significant differences between the groups. Significance differences were considered statistically significant at p < 0.05.

Results: Effect on body weight

Figure 1. Effect of treatment with vehicle, ZP4982, ZP5461, or liraglutide on body weight. Data are mean values ± SEM (n=5/group). Data were analyzed by 2-way ANOVA followed by Bonferroni post tests. The bias lines below the graphs indicate days during which statistically significant differences were observed. Significance differences were considered statistically significant at p < 0.05. Black arrows indicate dosing days for ZP4982 and ZP5461.

Conclusions

We have demonstrated that novel long-acting amylin analogues ZP4982 and ZP5461 improved glycemic control in ZDF rats by a decrease in non-fasting and fasting blood glucose levels, and reduced HbA1c levels (termination, %). Significantly improved body weight, reduced food intake, and decreased postprandial glucose and insulin levels were observed by ZP4982 and ZP5461 treatments compared to vehicle control. ZP4982 and ZP5461 treatments significantly improved glucose tolerance compared to the vehicle treated rats after an intra-peritoneal glucose injection.

We conclude that ZP4982 and ZP5461 may provide an attractive option for the treatment and/or prevention of type 2 diabetes.