PYY peptides and Fc-PYY conjugates selectively agonise Y2R

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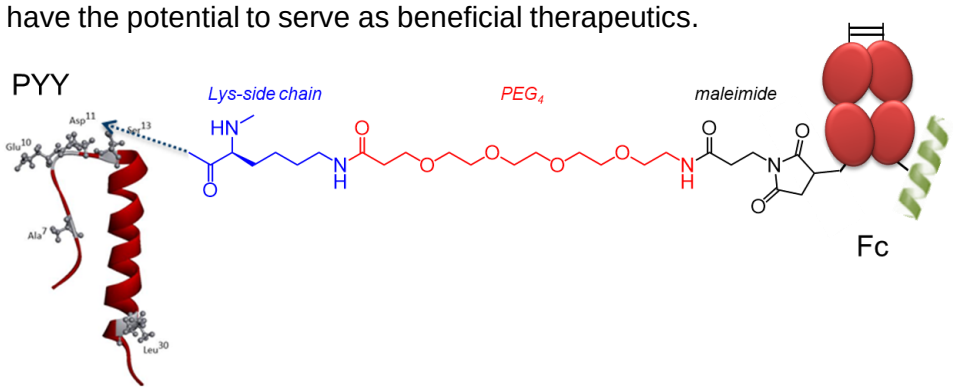
Introduction

- Type 2 Diabetes (T2D) is a disease characterized by hyperglycemia and increased cardiovascular (CV) risk.
- Over 540 million people are afflicted with diabetes worldwide and the International Diabetes Federation projects that this number will increase to 783 million in 2045 [1].
- Bariatric surgery like Roux-en-Y gastric bypass (RYGB) surgery produces long term remission of T2D and reduces CV risk factors [2].
- After diabetic patients undergo RYGB surgery, rapid improvement in blood glucose and insulin levels occur before any significant weight loss is observed. PYY3-36 secretion is also acutely increased after surgery and may act as an effector for reversal of T2D [3].
- PYY peptides and PYY conjugated peptides with improved stability have the potential to serve as beneficial therapeutics.



Methods

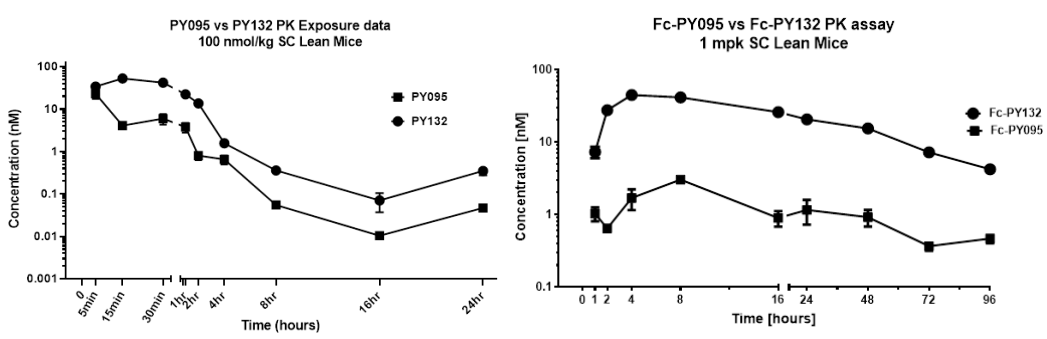
- PYY peptides with a PEG_4 linker on the side chain of a Lys in position 11 are **conjugated to the Fc domain** at amino acid position 442 via maleimide-thiol conjugation.
- For **cAMP activity assays**, forskolin was used to stimulate cAMP production in cells expressing recombinant human neuropeptide Y1, Y2, Y4 or Y5 receptors. Receptor stimulation by agonist ligands results in the inhibition of FSK stimulated cAMP production, which provides a measure of biological activity of the PYY ligands.
- **Pharmacokinetic studies** were conducted with 8-10 weeks old C57BL/6 lean, male mice fed a standard chow diet. They were given a subcutaneous injection of PY095 or PY132 peptide (Figure 1A, dosed at 100nmol/kg) or Fc-PY095 or Fc-PY132 (Figure 1B, dosed at 1 mg/kg) and blood was collected at various time points depending on the expected half-life of the drugs investigated.
- For **BioDaq food intake (FI) study,** on the day of the study mice were fasted for the 6-8 hours preceding lights out. Compounds were dosed approximately 1 hour prior to lights out at the indicated doses at a dose volume of 5-10 mL/kg. Food was given back 30 min prior to lights out and food intake was monitored in real-time in the BioDaq system for at least 48 hours.

Results

Table 1: Potency and selectivity of PYY peptides and Fc-PYY conjugates in the cAMP assay, where unAA is an unnatural amino acid.

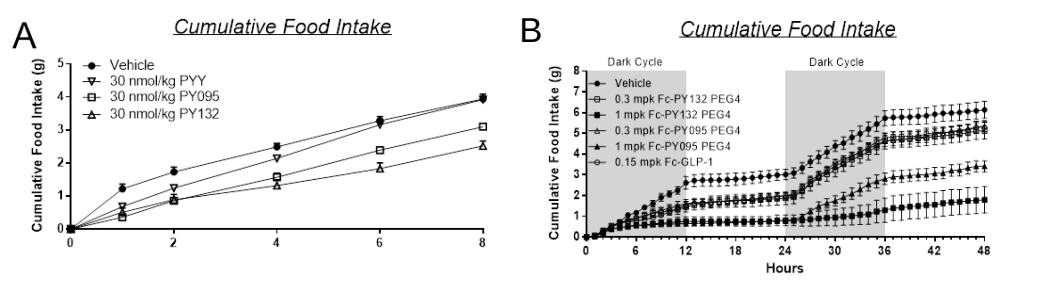
Alias	Sequence	cAMP EC_{50} nM			
		Y2R	Y1R	Y4R	Y5R
PYY 3-36	IKPEAPGEDASPEELNRYYASLRHYLNLVTRQRY-amide	**0.15**	83	98	25
Fc-PYY3-36	IKPEAPGEK[PEG_4-MAL-442C]ASPEELN RYYASLRHYLNLVTRQRY-amide	**0.143**	>1000	>1000	183
PY095	PKPEAPGEDASPEELARYYASLRAYINLITRQRY-amide	**0.029**	490	198	37
Fc-PY095	PKPEHPGE K[PEG_4-MAL-442C] ASPEELARYYASLRAYINLITRQRY- amide	**0.039**	>1000	>1000	113
PY132	PKPEAPGEDASPEELARYYASLRAYINLITRQ(unAA)Y-amide	**0.022**	>1000	>2000	19.9
Fc-PY132	PKPEHPGE K[PEG_4-MAL-442C] ASPEELARYYASLRAYINLITRQ(unAA)Y- amide	**0.005**	>1000	>1000	161

Figure 1: plasma concentrations of (A) unconjugated PYY peptides PY095 and PY132, and (B) Fc-PYY molecules Fc-PY095 and Fc-PY132 from PK studies in lean mouse



- Selectivity of the PYY peptides and Fc-PYY conjugates: PYY3-36, PY095 and PY132 were conjugated to the Fc domain at position 442C and all constructs were tested for potency in the cAMP Y2R assay and for selectivity in the cAMP Y1R, Y4R and Y5R assays (Table 1).
- All PYY peptides conjugated to Fc gained Y2R selectivity over Y1R, Y4R and Y5R versus the unconjugated PYY peptide.
- Unconjugated peptides PY095 and PY132 were more selective than PYY3-36 across all the receptors.
- The unconjugated PY132 showed similar selectivity for Y2R over Y1R and Y4R as observed for conjugated molecules. However, the peptide lost selectivity over Y5R compared to PY095: this loss in selectivity over Y5R was gained on conjugation and the unnatural amino acid containing conjugate Fc-PY132 shows the best Y2R selectivity profile.

Figure 2: *in vivo* data from a single dose food intake study in mouse comparing unconjugated PYY peptides PYY3-36, PY095 and PY132 (fig. 2A) and Fc-PYY molecules Fc-PY095 and Fc-PY132 (fig. 2B). Data for Fc-GLP-1 is also provided.



Conclusions

- PYY peptides and Fc-PYY conjugates activate Y2R receptor and reduce food intake.
- All Fc-PYY conjugates retained potency and gained Y2R selectivity upon conjugation to Fc.
- Peptide-conjugates proved to be better than peptides alone on stability and half-life but modifying Arg35 gave an unconjugated peptide with an equivalent if not better selectivity on Y2R receptor.
- Substitution of Arg 35 with the unnatural amino acid clearly resulted in more stable molecules.
- For these peptides, conjugation improves selectivity toward Y5R.

- Biological stability of PYY analogue molecules: mouse PK and FI studies were carried out using both conjugated and unconjugated PYY peptides.
- PK studies showed that when dosed at 100 nmol/kg PY132 was more stable than PY095 which has previously been shown to be more stable than PYY3-36 (Fig. 1A). PYY3-36 efficacy was lost after 4 hrs, while PY095 and PY132 showed efficacy for up to 8 hrs (Fig. 2A).
- When conjugated to Fc, the PK data clearly indicate that Fc-PY132 is more stable than Fc-PY095 (Fig. 1B). The food intake studies in lean mice showed efficacy up to 48 hrs for Fc- PY132 and Fc-PY095. Fc-PY132 showed more durable effects than Fc-PY095 (Fig. 2B).
- Conjugation to Fc domain provides 20 times more plasma stability than keeping the naked PYY sequence.

References

1. https://idf.org/about-diabetes/diabetes-facts-figures/
2. Brethauer et al., *Ann. Surg.*, **2013**, 258(4), 628-636.
3. Ramracheya et al. *Cell Reports*, **2016**, 15, 944-950.

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