TITLE

Stable analogues of the dual agonist dogfish glucagon show better therapeutic potential than exendin-4 in diet induced obese diabetic mice

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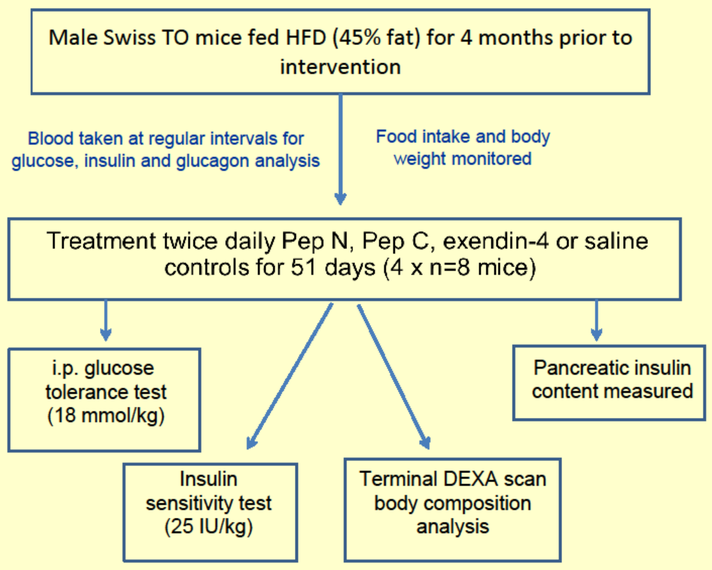
BACKGROUND

- In addition to promoting hepatic glucose output, glucagon has potential in mobilization of fat stores.
- Incretin hormones such as GLP-1 have potent glucose lowering and appetite suppressing activities.
- Novel dual agonist peptides which stimulate both incretin and glucagon receptors have potential in the treatment of Type 2 diabetes
- Dogfish glucagon shares about 50% structural homology with GLP-1 and GIP(1-30).

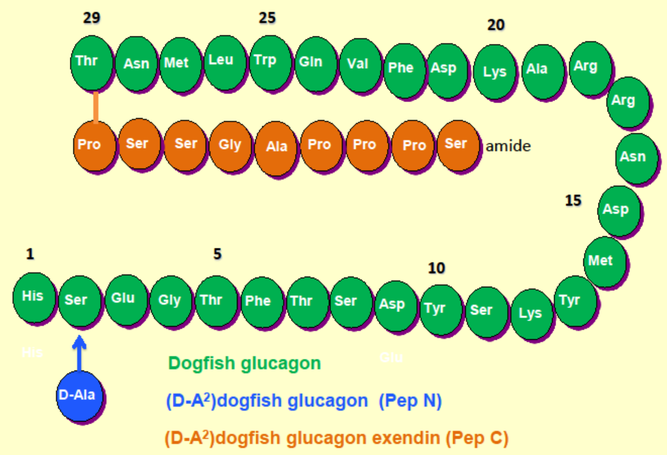
AIM: To test the efficacy of stable dogfish glucagon related analogues Pep N and Pep C, versus exendin-4 treatment in combatting diet induced obesity diabetes in high fat fed mice.

METHODS

Experimental design



Primary structure of dogfish glucagon and D-A^2 analogues



RESULTS

Peptide	Primary sequence
	5 10 15 20 25
Dogfish glucagon	HSEGT FTSDY SKYMD NRRAK DFVQW LMNT
Human glucagon	HSQGT FTSDY SKYLD SRRAQ DFVQW LMNT
GLP-1(7-36)amide	HAEGT FTSDV SSYLE GQAAK EFIAW LVKGR-amide
GIP(1-30)	YAEGT FISDY SIAMD KIHQQ DFVNW LLAQK

Effect of chronic treatment with Pep N, Pep C and exendin-4 on (A) body weight and (B) body weight change in high fat fed mice

Fig. 1A

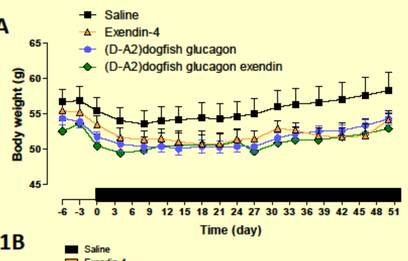
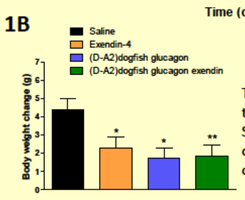


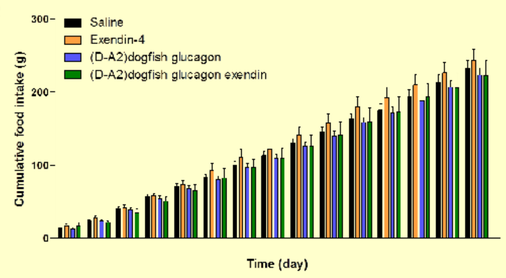
Fig. 1B



The black horizontal bar represents the treatment period. Values represent mean ± SEM for 8 mice. *p<0.05 and **p<0.01 is compared with high fat fed saline treated controls.

Effect of chronic treatment with Pep N, Pep C and exendin-4 on (A) cumulative food intake and (B) body weight change in high fat fed mice

Fig. 2



Values represent mean ± SEM for 8 mice.

Effect of chronic treatment with Pep N, Pep C and exendin-4 on non-fasting blood glucose (A) and plasma insulin (B) in high-fat fed mice

Fig. 3A

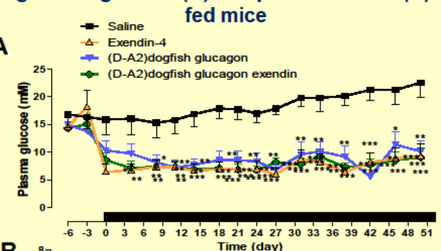
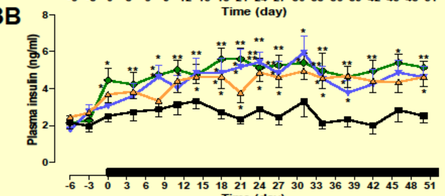


Fig. 3B



The black horizontal bar represents the treatment period. Values represent mean ± SEM for 8 mice. *p<0.05, **p<0.01 and ***p<0.001 compared with high fat saline treated mice

RESULTS

Effect of chronic treatment with Pep N, Pep C and exendin-4 on blood glucose (A) and plasma insulin (B) in response to an ipGGT in high-fat diet fed mice

Fig. 4A

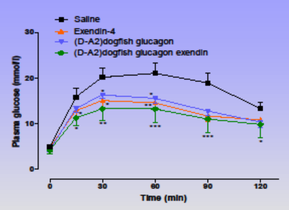
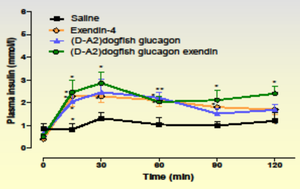


Fig. 4B



Blood glucose and insulin concentrations were measured prior to and after i.p. glucose challenge (18 mmol/kg body weight) in fasted mice. Values represent mean ± SEM for 8 mice. *p<0.05, **p<0.01 and ***p<0.001 compared with saline-treated group.

Effect of chronic treatment with Pep N, Pep C and exendin-4 on insulin sensitivity in high-fat diet fed mice

Fig. 5A

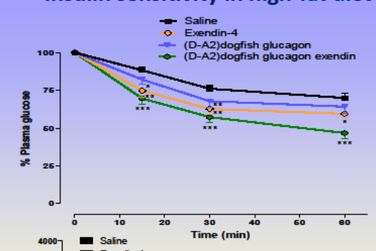
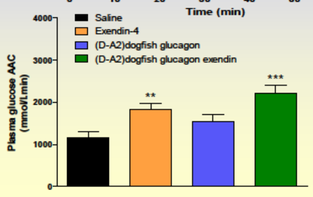


Fig. 5B



Insulin (25 U/kg bw) was administrated by i.p. injection at t=0 min. Values represent mean ± SEM for 8 mice. *p<0.05, **p<0.01 and ***p<0.001 compared with saline-treated group.

Effect of chronic treatment with Pep N, Pep C and exendin-4 on (A) % fat mass as measured by DEXA scan and (B) plasma triglycerides in high-fat diet fed mice

Fig. 6A

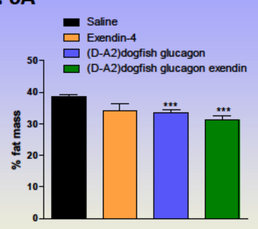
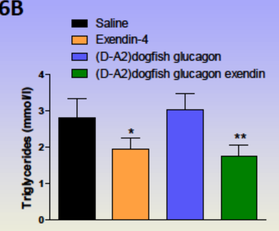


Fig. 6B



Values are mean ± SEM for n=8. *p<0.05, **p<0.01 and ***p<0.001 compared with saline-treated control group.

RESULTS

Effect of chronic treatment with Pep N, Pep C and exendin-4 on (A) pancreatic insulin content and (B) plasma glucagon

Fig. 7A

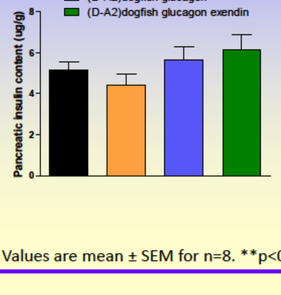
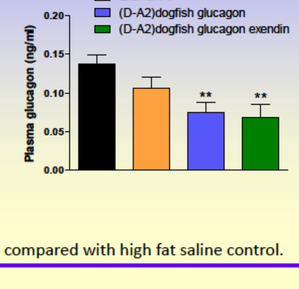


Fig. 7B



Values are mean ± SEM for n=8. **p<0.01 compared with high fat saline control.

Summary

Chronic (D-A^2)dogfish glucagon (Pep N) and (D-A^2)dogfish glucagon exendin (Pep C) administration:

- along with exendin-4 caused a reduction in body weight gain (Fig. 1B) in high fat diet fed (HFD) mice.
- had no significant effect on cumulative food intake in HFD mice (Fig. 2)
- along with exendin-4 significantly decreased non-fasting blood glucose and stimulated plasma insulin by day 3 and this was sustained throughout the study (Fig. 3)
- along with exendin-4 significantly improved glucose tolerance and insulinotropic activities in response to ipGTT in HFD mice (Fig. 4)
- Exendin-4 and Pep C improved tissue insulin sensitivity in HFD mice (Fig. 5)
- reduced the % body fat mass and circulating glucagon concentrations in HFD mice (Fig. 6) and were more effective than exendin-4
- Exendin-4 and Pep C reduced plasma triglyceride concentrations in HFD mice
- along with exendin-4 had no effect upon pancreatic insulin content in HFD mice

Conclusion

- These studies demonstrate the efficacy of (D-A^2)dogfish glucagon and (D-A^2)dogfish glucagon exendin, which share structural homology with incretin hormones, in combatting chronic glucose induced hyperglycaemia and dyslipidaemia in diet induced obese diabetic mice.

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