读书报告

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目录

PART 1

研究背景和意义

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Apelin-13 analogues show potent in vitro and in vivo insulinotropic and glucose lowering actions

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Apelin-13 analogues
Diabetes
Insulin secretion
Glucose homeostasis

ABSTRACT

Nine structurally modified apelin-13 analogues were assessed for their in vitro and acute in vivo antidiabetic potential. Stability was assessed in mouse plasma and insulinotropic efficacy tested in cultured pancreatic BRIN-BD11 cells and isolated mouse pancreatic islets. Intracellular Ca^{2+} and cAMP production in BRIN-BD11 cells was determined, as was glucose uptake in 3T3-L1 adipocytes. Acute antihyperglycemic effects of apelin analogues were assessed following i.p. glucose tolerance tests (ipGGT, 18 mmol/kg) in normal and diet-induced-obese (DIO) mice and on food intake in normal mice. Apelin analogues all showed enhanced in vitro stability (up to 5.8-fold, $t_{½}$ = 12.8 h) in mouse plasma compared to native apelin-13 ($t_{½}$ = 2.1 h). Compared to glucose controls, stable analogues exhibited enhanced insulinotropic responses from BRIN-BD11 cells (up to 4.7-fold, $p < 0.001$) and isolated mouse islets (up to 5.3-fold) for 10^{-7} M apelin-13 amide (versus 7.6-fold for 10^{-7} M GLP-1). Activation of APJ receptors on BRIN-BD11 cells increased intracellular Ca^{2+} (up to 3.0-fold, $p < 0.001$) and cAMP (up to 1.7-fold, $p < 0.01$). Acute ipGTT showed improved insulinotropic and glucose disposal responses in normal and DIO mice ($p < 0.05$ and $p < 0.01$, respectively). Apelin-13 amide and (pGlu)apelin-13 amide were the most effective analogues exhibiting acute, dose-dependent and persistent biological actions. Both analogues stimulated insulin-independent glucose uptake by differentiated adipocytes (2.9–3.3-fold, $p < 0.05$) and inhibited food intake (26–33%, $p < 0.001$), up to 180 min in mice, versus saline. In contrast, (Ala^{13})apelin-13 and (Val^{13})apelin-13 inhibited insulin secretion, suppressed beta-cell signal transduction and stimulated food intake in mice. Thus, stable analogues of apelin-13 have potential for diabetes/obesity therapy.

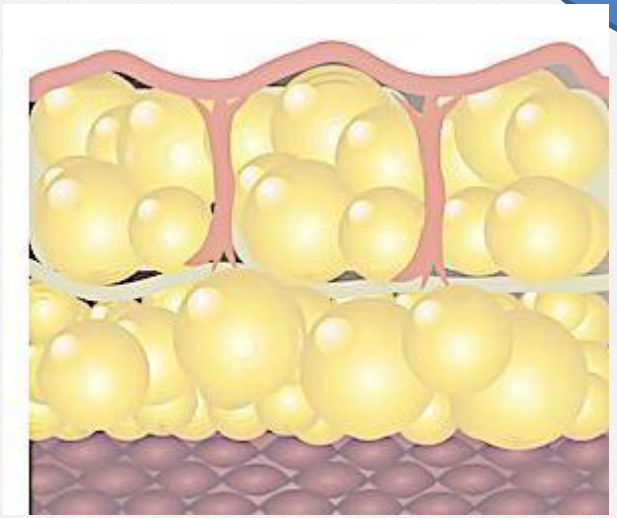
DPP-4抑制剂

GLP-1类似物

SGLT2抑制剂

415,000,000

Apelin/APJ



PART 2

研究内容

Apelin-13 analogues

Apelin-13类似物

ACE2

	Apelin-13	NH_2-Q-*R*-P-*R*-L-*S*-H-K-G-P-M-P-F-COOH
APJ拮抗剂 →	(Ala^{13})apelin-13	NH_2-Q-*R*-P-*R*-L-*S*-H-K-G-P-M-P-**A**-COOH
竞争性抑制剂 →	(Val^{13})apelin-13	NH_2-Q-*R*-P-*R*-L-*S*-H-K-G-P-M-P-**V**-COOH
	(Tyr^{13})apelin-13	NH_2-Q-*R*-P-*R*-L-*S*-H-K-G-P-M-P-**Y**-COOH
	Apelin-13-amide	NH_2-Q-*R*-P-*R*-L-*S*-H-K-G-P-M-P-F-**Amide**
	(pGlu)apelin-13	**pGlu**-*R*-P-*R*-L-*S*-H-K-G-P-M-P-F–COOH
APJ拮抗剂 →	pGlu(Ala^{13})apelin-13	**pGlu**-*R*-P-*R*-L-*S*-H-K-G-P-M-P-**A**-COOH
竞争性抑制剂 →	pGlu(Val^{13})apelin-13	**pGlu**-*R*-P-*R*-L-*S*-H-K-G-P-M-P-**V**-COOH
	pGlu(Tyr^{13})apelin-13	**pGlu**-*R*-P-*R*-L-*S*-H-K-G-P-M-P-**Y**-COOH
	(pGlu)apelin-13-amide	**pGlu**-*R*-P-*R*-L-*S*-H-K-G-P-M-P-F –**Amide**

1. Peptides stability in plasma

血浆中肽的稳定性

4h 后剩余%　半衰期

Table 1
Primary structures, molecular masses and degradation of apelin-13 and related analogues. Half-lives were calculated by constructing a graph of percentage intact peptide against time. Linear regression "best-fit" analysis was used to calculate the time at which half of the peptide was degraded. Values are mean ± SEM for n = 2, where ***p < 0.0001 is compared to native apelin-13 peptide.

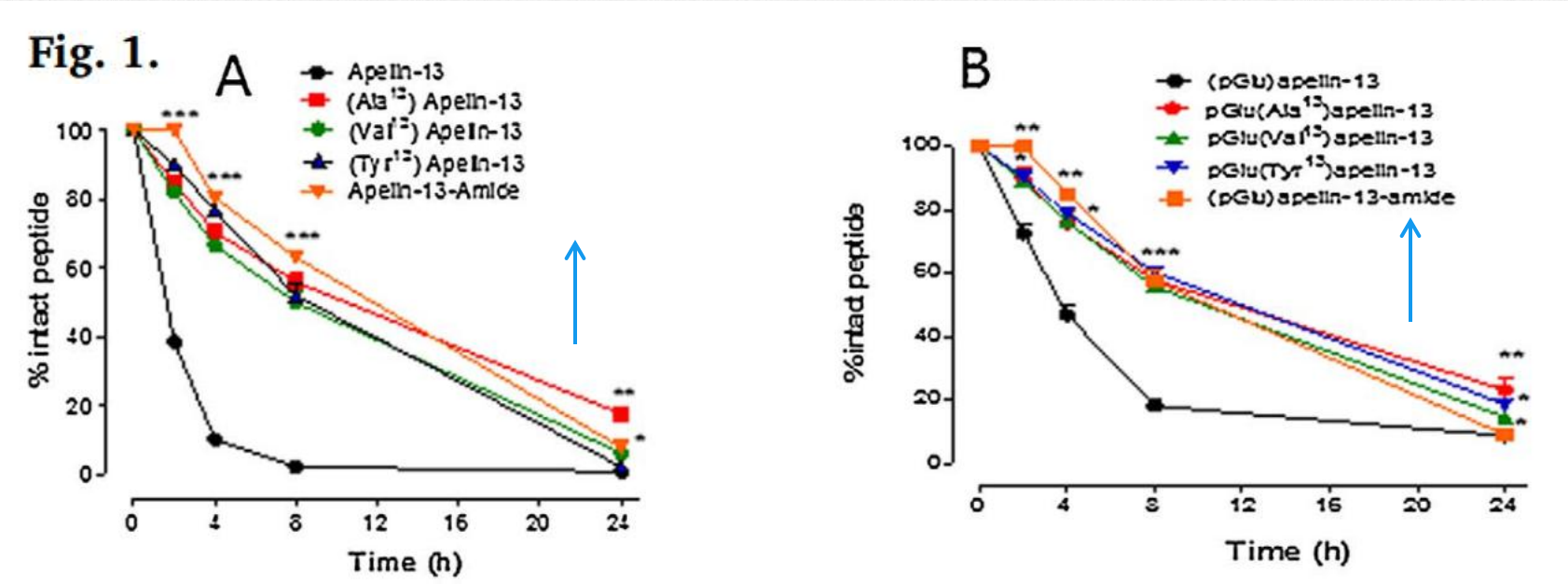
Name	Amino acid sequence	Theoretical molecular mass (observed mass Da)	Degradation	
			% intact peptide (4 h)	Half-life ($t_{1/2}$) (h)
Apelin-13	NH_2-Q-*R*-P-*R*-L-*S*-H-K-G-P-M-P-F-COOH	1551.9 (1551.8)	25.4 ± 1.5	2.1
(Ala^{13})apelin-13	NH_2-Q-R-P-R-L-S-H-K-G-P-M-P-**A**-COOH	1474.8 (1474.8)	70.5 ± 2.2***	10.3
(Val^{13})apelin-13	NH_2-Q-R-P-R-L-S-H-K-G-P-M-P-**V**-COOH	1503.8 (1505.7)	66.5 ± 1.1***	7.7
(Tyr^{13})apelin-13	NH_2-Q-R-P-R-L-S-H-K-G-P-M-P-**Y**-COOH	1566.8 (1569.1)	76.7 ± 1.3***	8.5
Apelin-13-amide	NH_2-Q-R-P-R-L-S-H-K-G-P-M-P-F-**Amide**	1550.9 (1552.3)	80.4 ± 1.9***	11.4
(pGlu)apelin-13	**pGlu**-R-P-R-L-S-H-K-G-P-M-P-F–COOH	1535.8 (1535.7)	47.0 ± 3.2	3.8
pGlu(Ala^{13})apelin-13	**pGlu**-R-P-R-L-S-H-K-G-P-M-P-**A**-COOH	1459.7 (1461.2)	74.4 ± 5.2***	11.1
pGlu(Val^{13})apelin-13	**pGlu**-R-P-R-L-S-H-K-G-P-M-P-**V**-COOH	1487.8 (1488.8)	76.2 ± 1.9***	9.8
pGlu(Tyr^{13})apelin-13	**pGlu**-R-P-R-L-S-H-K-G-P-M-P-**Y**-COOH	1550.7 (1551.5)	79.0 ± 0.6***	12.2
(pGlu)apelin-13-amide	**pGlu**-R-P-R-L-S-H-K-G-P-M-P-F −**Amide**	1534.8 (1536.2)	85.0 ± 1.3***	10.4

焦谷氨酸化提高了apelin-13的稳定性，

结合 C 端修饰后进一步提高其稳定性。

1. Peptides stability in plasma

血浆中肽的稳定性

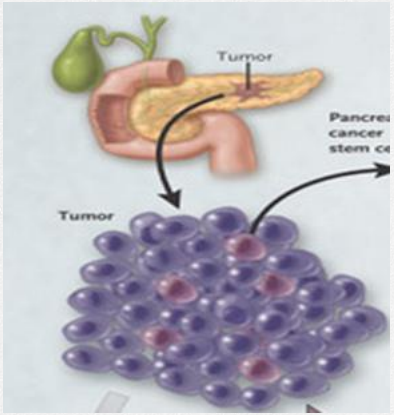


焦谷氨酸化提高了apelin-13的稳定性，

结合 C 端修饰后进一步提高其稳定性。

2. Effects of apelin-13 and its analogues on in vitro insulin release

Apelin-13及其类似物对胰岛素释放的影响



clonal pancreatic beta cells (BRIN-BD11)

Table 2

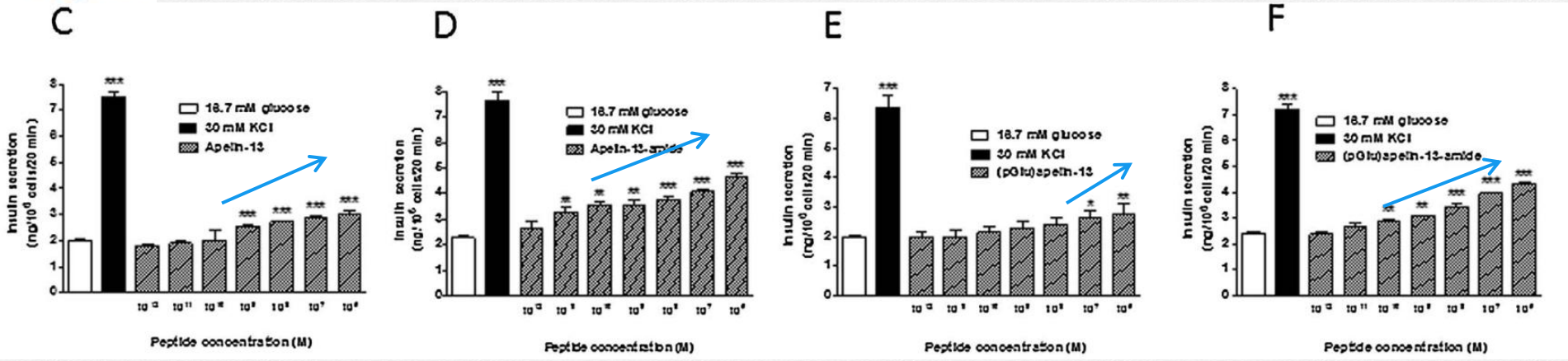
Peptide (10^{-6} M)	*In vitro* insulin secretion (ng/10^6 cells/ 20 min)		
	5.6 mM glucose	16.7 mM glucose	EC_{50} (M)
None (glucose alone)	1.00 ± 0.08	1.99 ± 0.07	----------
Apelin-13	1.37 ± 0.1*	3.03 ± 0.09 ***	6.06e-010
(Ala^{13})apelin-13	0.63 ± 0.02**	1.65 ± 0.06 *	1.74e-009
(Val^{13})apelin-13	0.72 ± 0.04**	1.19 ± 0.04 ***	1.29e-010
(Tyr^{13})apelin-13	1.56 ± 0.06**	3.92 ± 0.07 ***	9.51e-009
Apelin-13 amide	1.92 ± 0.18***	4.66 ± 0.13 ***	2.68e-008
pGlu(apelin-13)	1.28 ± 0.08*	2.76 ± 0.34 **	1.10e-008
pGlu(Ala^{13})apelin-13	0.84 ± 0.07	1.55 ± 0.06	7.68e-009
pGlu(Val^{13})apelin-13	0.62 ± 0.04***	1.19 ± 0.04 ***	1.59e-008
pGlu(Tyr^{13})apelin-13	1.57 ± 0.03**	3.67 ± 0.12 ***	1.48e-008
[illegible]	[illegible]	4.33 ± 0.06 ***	9.92e-009

在5.6和16.7mM 葡萄糖环境中，Apelin-13及其类似物均能促进体外培养胰脏β细胞胰岛素的分泌。

2. Effects of apelin-13 and its analogues on in vitro insulin release

Apelin-13及其类似物对胰岛素释放的影响

Fig. 1.



16.7mM 葡萄糖
50mM KCL
Apelin-13
10^{-9} M

Apelin-13及其类似物促进体外培养胰脏β细胞胰岛素的分泌，且具有剂量依赖性。

16.7mM 葡萄糖
50mM KCL
(pGlu) Apelin-13-amide
10^{-10}M

3. Effects of apelin analogues on intracellular Ca^{2+} and cyclic AMP production

Apelin-13类似物对细胞内Ca^{2+}和cAMP产生的影响

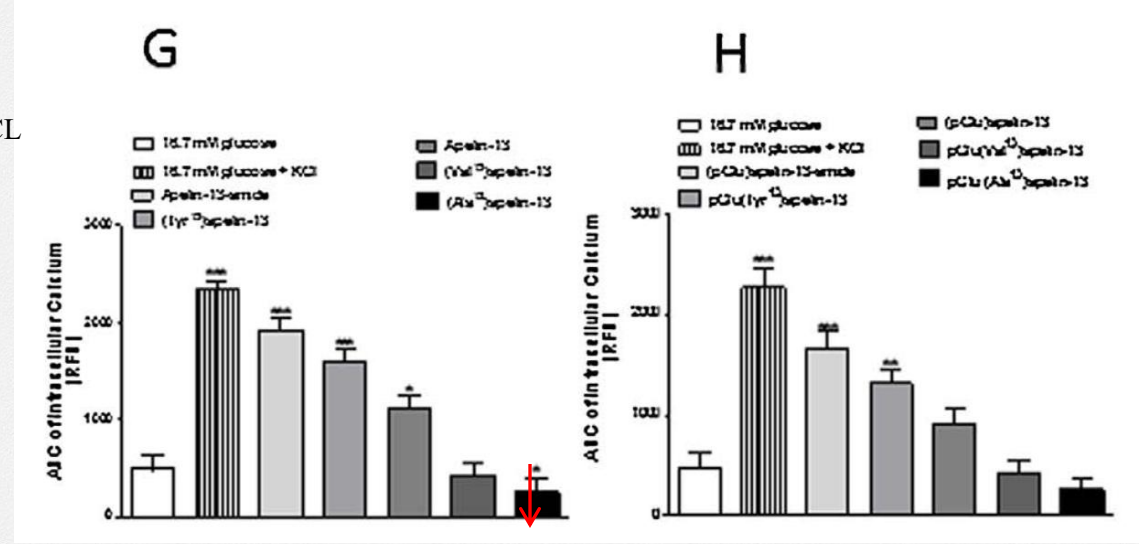
Peptide (10^{-6} M)	*In vitro* insulin secretion (ng/10^6 cells/ 20 min)			Intracellular [Ca^{2+}]	Cyclic AMP
	5.6 mM glucose	16.7 mM glucose	EC_{50} (M)	Change	Change
None (glucose alone)	1.00 ± 0.08	1.99 ± 0.07	----------	----------	----------
Apelin-13	1.37 ± 0.1*	3.03 ± 0.09 ***	6.06e-010	130% ↑ (p<0.05)	75% ↑ (p>0.05)
(Ala^{13})apelin-13	0.63 ± 0.02**	1.65 ± 0.06 *	1.74e-009	48% ↓ (p<0.05)	70% ↓ (p<0.01)
(Val^{13})apelin-13	0.72 ± 0.04**	1.19 ± 0.04 ***	1.29e-010	14% ↓ (p<0.05)	75% ↓ (p<0.01)
(Tyr^{13})apelin-13	1.56 ± 0.06**	3.92 ± 0.07 ***	9.51e-009	171% ↑ (p<0.01)	82% ↑ (p<0.05)
Apelin-13 amide	1.92 ± 0.18***	4.66 ± 0.13 ***	2.68e-008	296% ↑ (p<0.01)	165% ↑ (p<0.01)
pGlu(apelin-13)	1.28 ± 0.08*	2.76 ± 0.34 **	1.10e-008	89% ↑ (p>0.05)	47% ↑ (p>0.05)
pGlu(Ala^{13})apelin-13	0.84 ± 0.07	1.55 ± 0.06	7.68e-009	46% ↓ (p>0.05)	60% ↓ (p<0.01)
pGlu(Val^{13})apelin-13	[illegible]	[illegible]	[illegible]	[illegible]	72% ↓ (p<0.01)
pGlu(Tyr^{13})apelin-13	1.57 ± 0.03***	3.67 ± 0.12 ***	1.48e-008	171% ↑ (p<0.01)	85% ↑ (p<0.05)
(pGlu)apelin-13 amide	1.65 ± 0.03***	4.33 ± 0.06 ***	9.92e-009	244% ↑ (p<0.001)	127% ↑ (p<0.05)

Apelin-13及其类似物能够增加细胞内Ca^{2+}浓度和cAMP的产生。

3. Effects of apelin analogues on intracellular Ca^{2+} and cyclic AMP production

Apelin-13类似物对细胞内Ca^{2+}和cAMP产生的影响

16.7mM 葡萄糖
16.7mM 葡萄糖+50mM KCL
Apelin-13-amide
(Tyr)Apelin-13
Apelin-13
(Val)Apelin-13
(Ala)Apelin-13



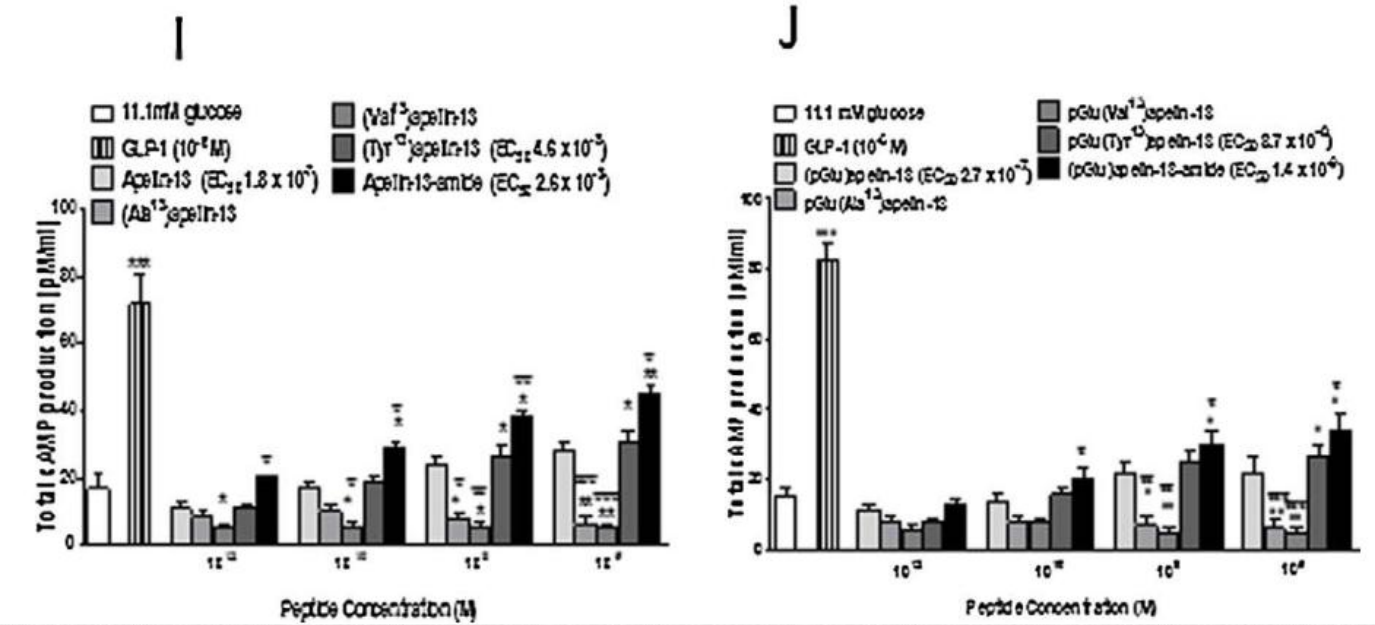
16.7mM 葡萄糖
16.7mM 葡萄糖+50mM KCL
(pGlu)Apelin-13-amide
pGlu(Tyr) Apelin-13
pGluApelin-13
pGlu(Val) Apelin-13
pGlu (Ala) Apelin-13

(BRIN-BD11)

Apelin-13类似物能够增加细胞内Ca^{2+}浓度，APJ拮抗剂(Val^{13})Apelin-13降低细胞内Ca^{2+}浓度。

3. Effects of apelin analogues on intracellular Ca^{2+} and cyclic AMP production

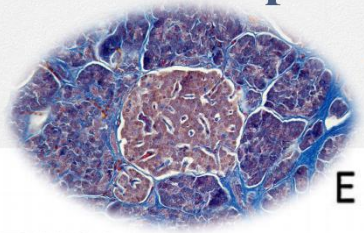
Apelin-13类似物对细胞内Ca^{2+}和cAMP产生的影响



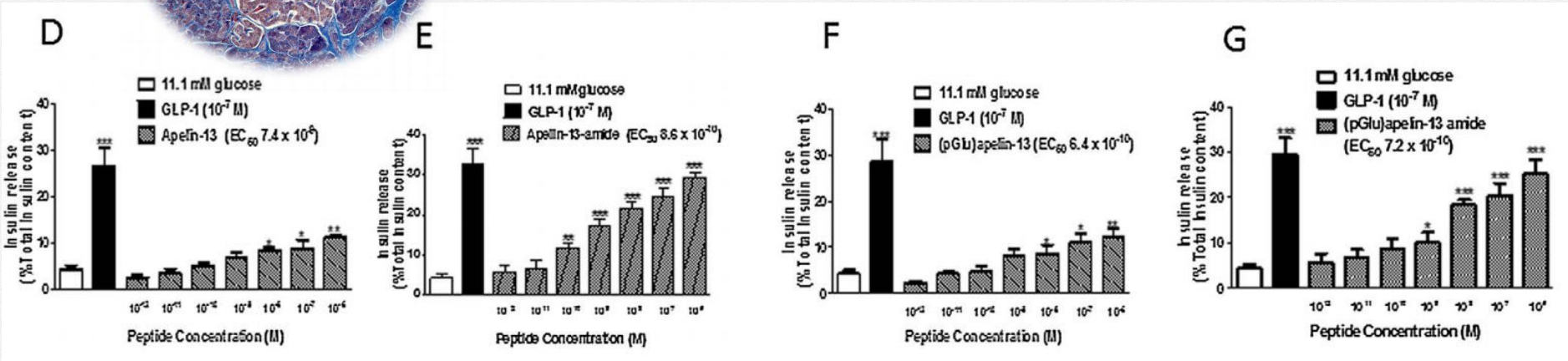
(BRIN-BD11)

Apelin-13类似物能够增加cAMP的产生，并具有剂量依赖性。抑制剂和拮抗剂降低cAMP的产生。

4. Effects of apelin analogues on insulin secretion from isolated islets



Apelin-13类似物对离体胰岛胰岛素分泌的影响



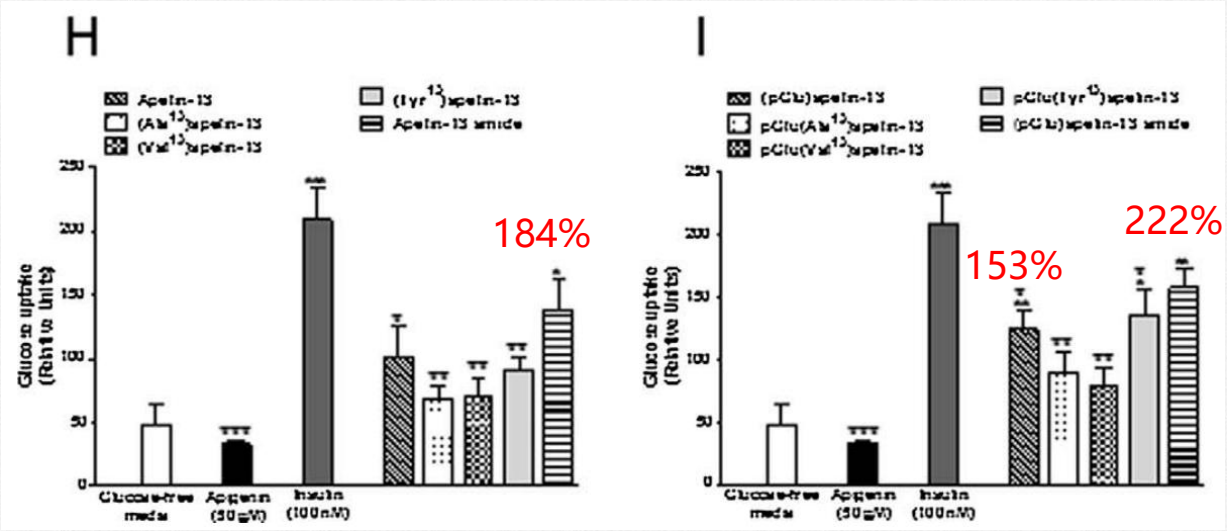
Apelin-13	Apelin-13-amide	pGluApelin-13	pGluApelin-13-amide
10^{-8}M	10^{-10}M	10^{-8}M	10^{-9}M

Apelin-13类似物能够促进离体胰岛胰岛素的分泌，并具有剂量依赖性。拮抗剂抑制胰岛素分泌。

5. Effect of apelin analogues on glucose uptake from 3T3-L1 adipocytes

Apelin-13类似物对3T3-L1脂肪细胞葡萄糖摄取的影响

无葡萄糖
芹菜素
胰岛素
Apelin-13
(Ala) Apelin-13
(Val) Apelin-13
(Tyr) Apelin-13
Apelin-13-amide



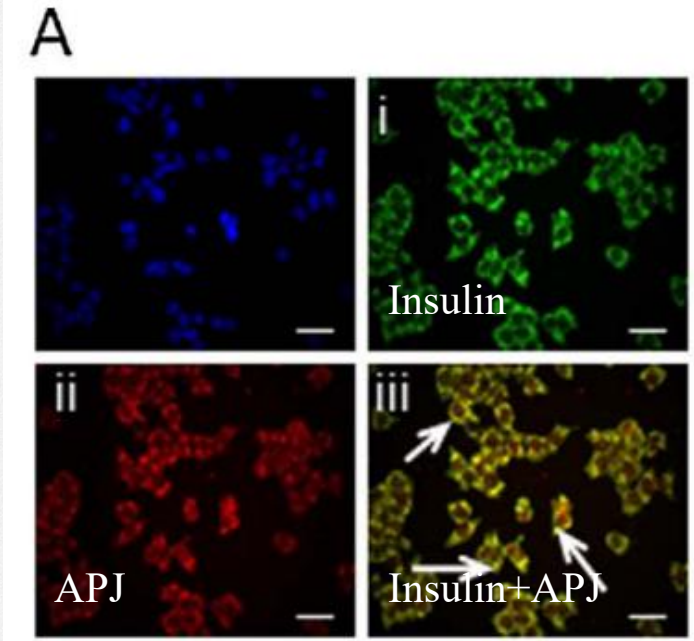
无葡萄糖
芹菜素
胰岛素
(pGlu)Apelin-13
pGlu(Ala) Apelin-13
pGlu(Val) Apelin-13
pGlu(Tyr) Apelin-13
pGluApelin-13-amide

Apelin-13类似物能够促进3T3-L1脂肪细胞摄取葡萄糖。

6. Expression of APJ receptor on BRIN-BD11 cells and pancreatic islets

APJ受体在BRIN-BD11细胞和胰岛上的表达

Fig. 2.

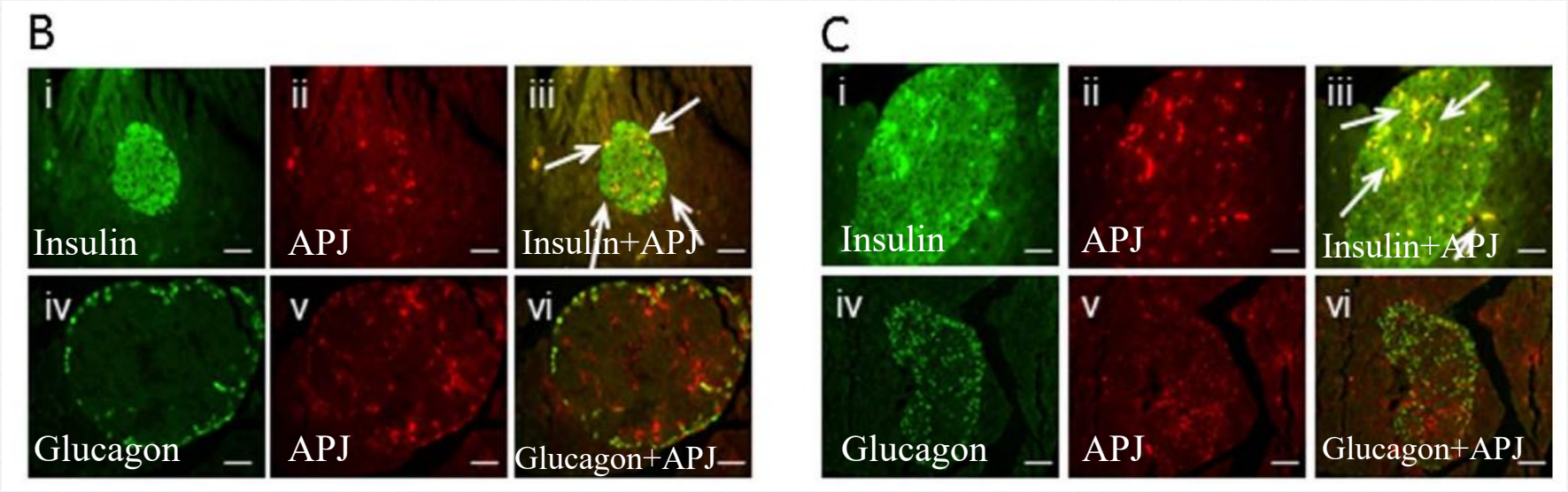


(BRIN-BD11)

APJ与分泌胰岛素的胰岛β细胞实现了共定位。

6. Expression of APJ receptor on BRIN-BD11 cells and pancreatic islets

APJ受体在BRIN-BD11细胞和胰岛上的表达



lean mouse pancreatic islets

DIO mouse pancreatic islets

APJ与分泌胰岛素的胰岛β细胞实现了共定位，但是没有证据表明分泌胰高血糖素的α细胞存在APJ。

7. Acute and persistent glucose-lowering and insulinotropic actions of apelin analogues in lean and DIO mice

apelin类似物在正常和肥胖小鼠中产生的急性的和持续的降低血糖和促胰岛素分泌的作用

Table 3

Integrated glycaemic and insulin (area under the curve, AUC) responses to apelin-13 and related analogues (25 nmol/kg body weight) following i.p. administration to normal mice or high fat fed mice together with 18 mmol/kg glucose. Values are mean ± SEM for n = 8 where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ is compared to the glucose control.

	In vivo response			
	Plasma glucose $AUC_{0\text{-}105}$ (mmol/l.min)		Plasma insulin $AUC_{0\text{-}105}$ (ng/ml.min)	
Peptide (25 nmol/kg body weight)	Normal mice	High fat fed mice	Normal mice	High fat fed mice
Glucose alone	776.9 ± 56.6	1350 ± 86.5	128.9 ± 14.5	167.2 ± 9.6
Apelin-13	716 ± 42.6	1338 ± 100.3	142.6 ± 16.7	182.7 ± 21.2
(Ala^{13})apelin-13	831.2 ± 66.4	1594 ± 73.9 *	87.3 ± 8.1 *	133.3 ± 8.8 *
(Val^{13})apelin-13	968.6 ± 31.3 **	1672 ± 76.5 **	67.1 ± 6.8 **	114.1 ± 3.3 **
(Tyr^{13})apelin-13	596.7 ± 31.5 **	1096 ± 107.1 *	165.8 ± 17.5 *	204.4 ± 12.8 *
Apelin-13 amide	507.0 ± 50.4 **	897.8 ± 87.5 **	196.6 ± 4.9 **	242.3 ± 15.0 ***
pGlu(apelin-13)	763.5 ± 45.2	1127 ± 63.9	121.8 ± 11.2	173.7 ± 16.4
pGlu(Ala^{13})apelin-13	754.3 ± 45.2	1363 ± 57.62 *	101.7 ± 20.6	147.7 ± 6.9 *
pGlu(Val^{13})apelin-13	867.1 ± 51.9	1440 ± 58.9 *	80.4 ± 5.1 **	128.2 ± 5.3 **
pGlu(Tyr^{13})apelin-13	606.6 ± 19.8 *	899.7 ± 117.6*	177.0 ± 17.6 *	215.2 ± 9.9 **
(pGlu)apelin-13 amide	492.4 ± 59.8 *	848.4 ± 92.1 **	203.7 ± 8.2 **	233.8 ± 21.5 **

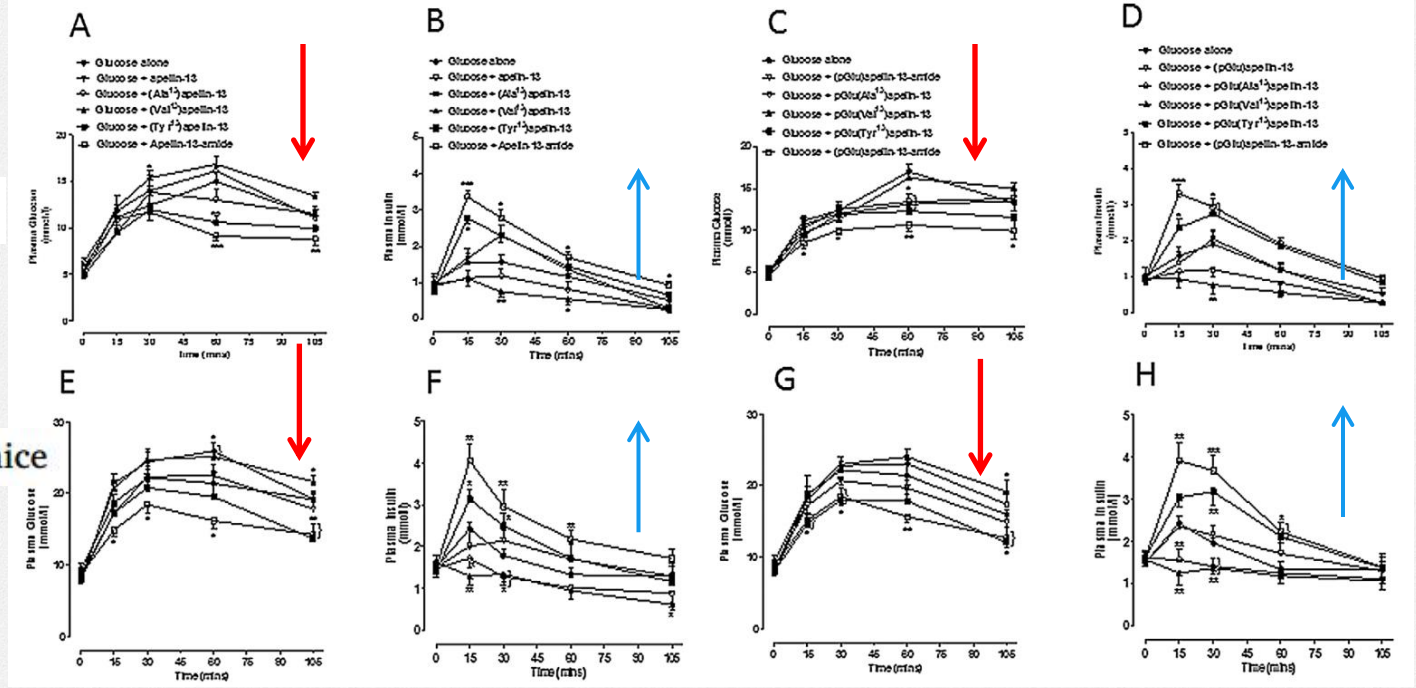
正常和肥胖小鼠腹腔注射apelin类似物，血糖降低和胰岛素分泌增加。且拮抗剂发挥相反的作用。

7. Acute and persistent glucose-lowering and insulinotropic actions of apelin analogues in lean and DIO mice

apelin类似物在正常和肥胖小鼠中产生的急性的和持续的降低血糖和促胰岛素分泌的作用

Normal mice

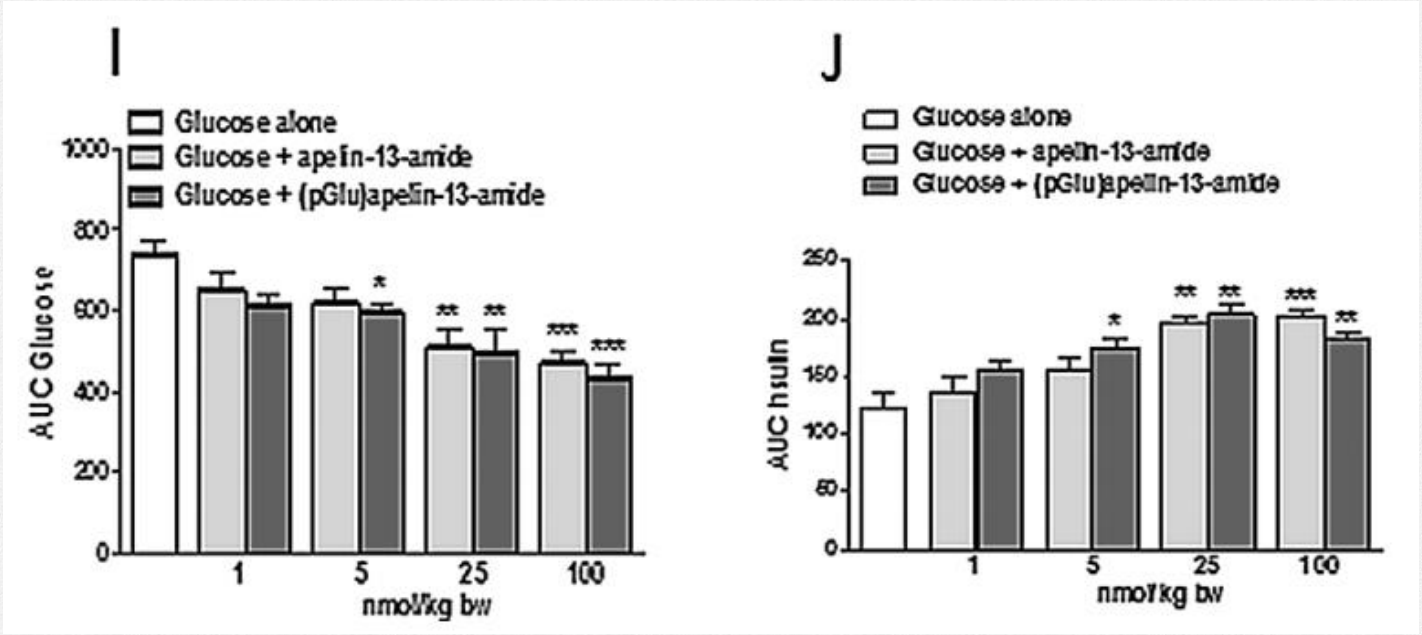
High fat fed mice



正常和肥胖小鼠腹腔注射apelin类似物，血糖降低、胰岛素分泌增加

7. Acute and persistent glucose-lowering and insulinotropic actions of apelin analogues in lean and DIO mice

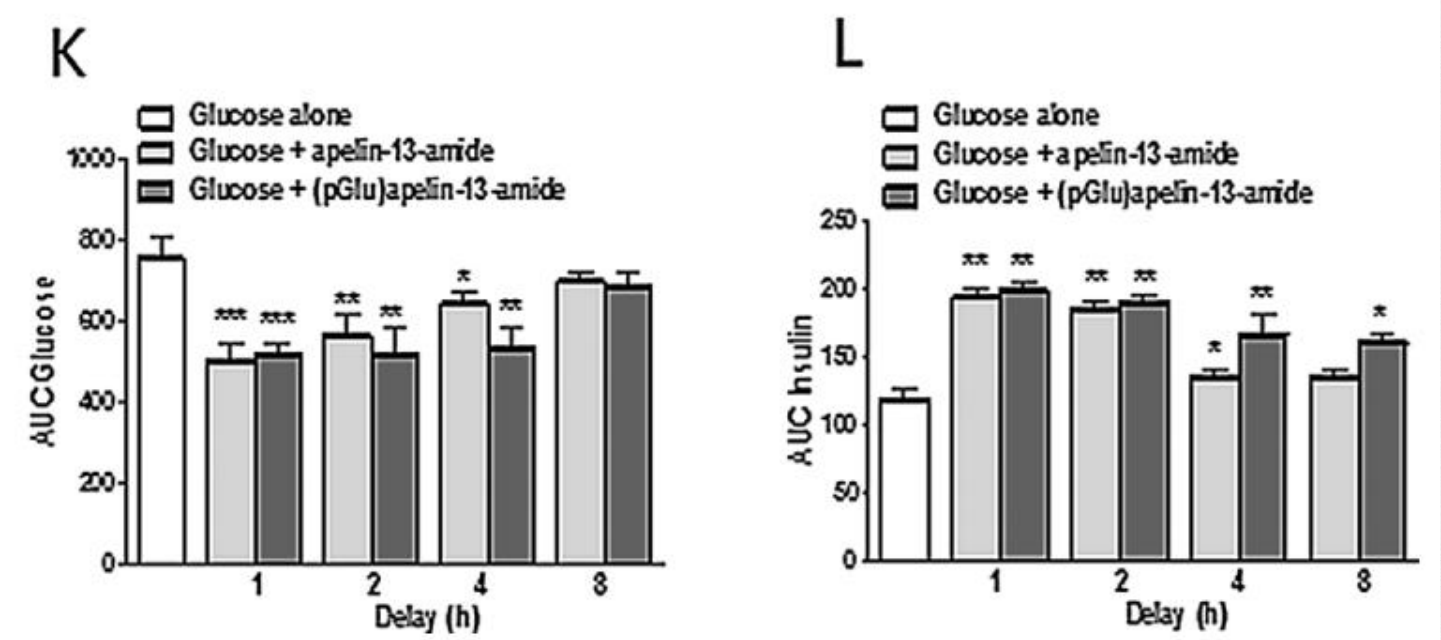
apelin类似物在正常和肥胖小鼠中产生的急性的和持续的降低血糖和促胰岛素分泌的作用



Apelin-13-amide和(pGlu)Apelin-13-amide分别从25nmol/kg和5nmol/kg浓度开始发挥降血糖和促进胰岛素分泌的作用。

7. Acute and persistent glucose-lowering and insulinotropic actions of apelin analogues in lean and DIO mice

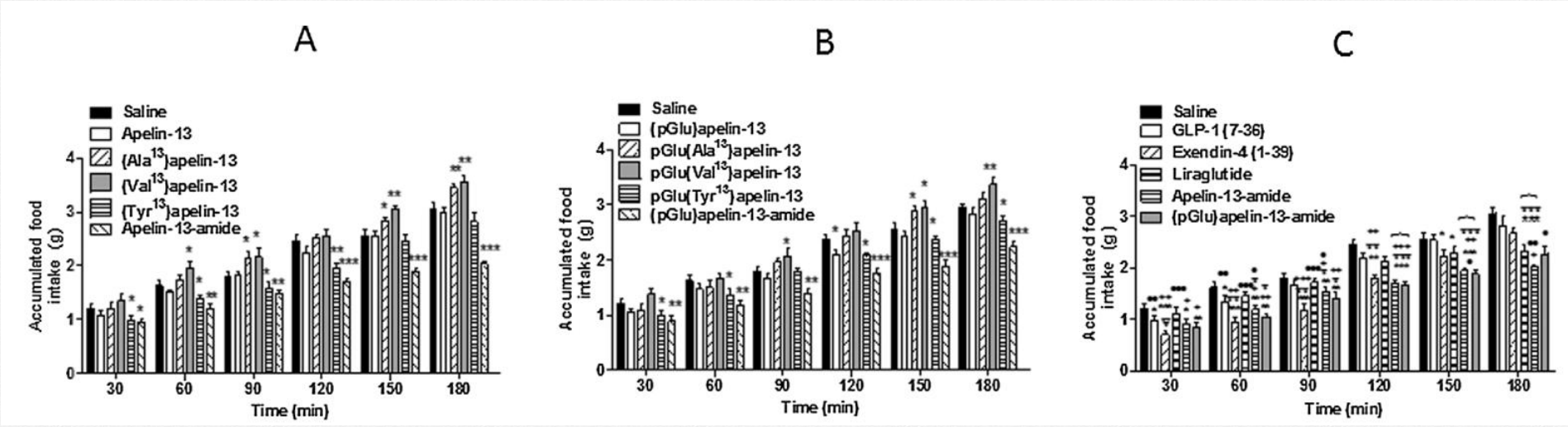
apelin类似物在正常和肥胖小鼠中产生的急性的和持续的降低血糖和促胰岛素分泌的作用



Apelin-13-amide和(pGlu)Apelin-13-amide发挥降血糖作用时间均持续 4h，促进胰岛素分泌的作用时间分别持续 4h 和 8h。

8. Acute in vivo food-intake studies

摄食研究



腹腔注射25nmol/kg， 部分Apelin-13类似物降低了摄食量，拮抗剂和抑制剂均提高了摄食量。

8. Acute in vivo food-intake studies

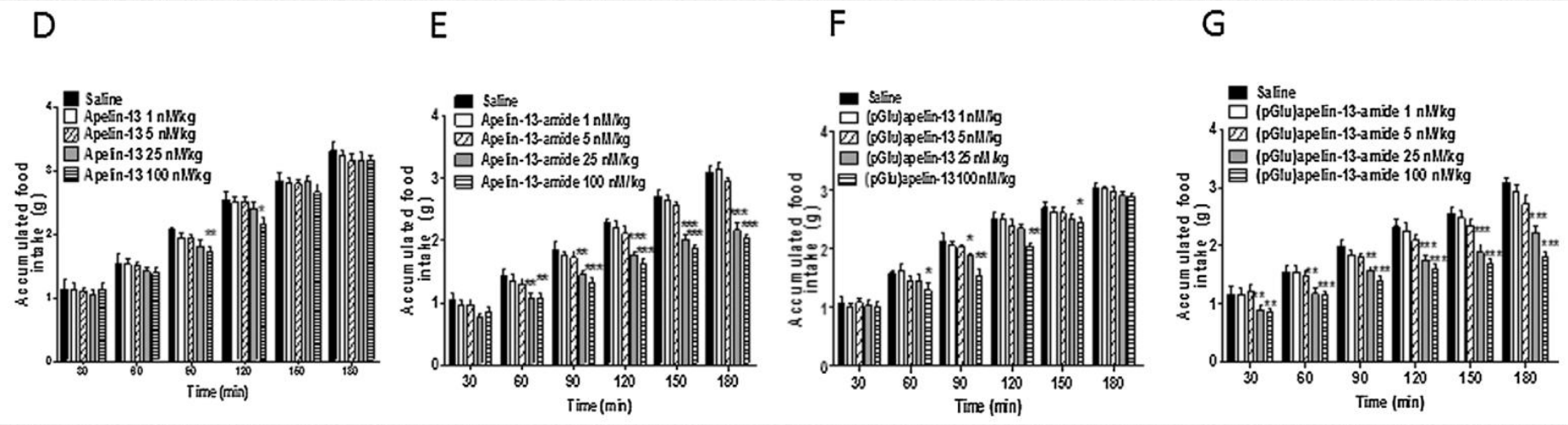
摄食研究

腹腔注射25nmol/kg

肽	有效时间
(Tyr^{13})apelin-13	0-120min
Apelin-13-amide	0-180min
pGlu(Tyr^{13})apelin-13	0-180min，除了90min
(pGlu)apelin-13-amide	0-180min

8. Acute in vivo food-intake studies

摄食研究



Apelin-13　　Apelin-13-amide　　(pGlu)Apelin-13　　(pGlu)Apelin-13-amide

8. Acute in vivo food-intake studies

摄食研究

肽	有效剂量	有效时间
Apelin-13	100 nmol	90-120min
Apelin-13-amide	25 nmol	60-180min
(pGlu)apelin-13	100 nmol	60-150min
(pGlu)apelin-13-amide	25 nmol	0-180min

Apelin-13及其类似物对摄食的抑制作用有剂量依赖性。

PART 3

结果与讨论

结果

1. Apelin-13N端进行焦谷氨酸化修饰可以提高其稳定性，结合 C 端修饰会进一步提高其稳定性。

2. 无论是体外（ in *vitro* ）培养的胰脏β细胞，还是离体（ Ex *vivo* ）胰脏，用Apelin-13及其类似物孵育均能促进其胰岛素的分泌。

3. Apelin-13及其类似物能够增加细胞内Ca^{2+}浓度和促进cAMP的产生。这说明Apelin-13及其类似物可能是通过细胞内Ca^{2+}浓度所形成细胞膜的去极化以及cAMP第二信使通路在机体内发挥作用的。

结果

4. Apelin-13类似物能够促进3T3-L1脂肪细胞摄取葡萄糖。且(pGlu)Apelin-13-amide从5nmol/kg浓度开始发挥降血糖和促进胰岛素分泌的作用。作用时间分别持续 4h 和 8h。

5. Apelin 受体APJ存在于胰岛β细胞，但不存在于胰岛α细胞。

6. 正常和肥胖小鼠腹腔注射apelin类似物，血糖降低、胰岛素分泌增加。

7. 腹腔注射Apelin-13类似物降低了正常和肥胖小鼠的摄食量，且对摄食的抑制作用有剂量依赖性。(pGlu)apelin-13-amide最小有效剂量为25 nmol，可以持续发挥作用180min。

讨论：

Apelin是会被ACE2（血管紧张素转换酶2）降解，ACE2移除Apelin-13和Apelin-36 C-末端苯丙氨酸，并造成进一步的降解，最终导致Apelin失去生物活性。

在本研究中，自然产生的肽，apelin-13和（pGlu）apelin-13，在体外培养的小鼠血浆在4小时内降解50-75%。与此相反，C-末端进行氨基酸取代或进行酰胺化修饰，新和成的类似物表现出明显的代谢稳定性方面的改善（75-85%完整性。4h，table1），说明这种修饰通过阻断进一步降解来增强apelin在体内的生物活性。

讨论：

氨基酸的替代和化学修饰产生了显著的药理学变化，包括提高细胞内Ca^{2+}浓度和促进cAMP的产生。这表明本文中C-末端修饰对受体结合有积极而非消极的影响。这些Apelin-13类似物对胰岛素分泌的作用可以归因于APJ受体存在于胰岛细胞中。

讨论：

早期的研究显示，天然存在的肽apelin-13会抑制葡萄糖诱导的胰岛素的分泌，随后抑制cAMP水平。这与本研究是相反的。

本研究清楚地表明，apelin-13和（pGlu）apelin-13增加葡萄糖刺激的胰岛素分泌，特别是在高血糖条件下。本文也首次证明，Apelin的类似物均可以促进胰岛素分泌，且具有葡萄糖敏感性和Apelin浓度依赖性。其中Apelin-13-amide和（pGlu）apelin-13酰胺是最有效的。

这些β细胞似乎是通过激活多个信号传导通路来进行调节的。包括提高细胞内Ca^{2+}浓度和促进cAMP的产生。

讨论：

肥胖促进胰岛素抵抗，一些降糖药物也降低食欲，如GLP-1类似物特别适用于2型糖尿病治疗。本研究中，apelin-13-amide和（pGlu）apelin-13-amide能抑制小鼠摄食量，且比GLP-1，Exendin-4或利拉鲁肽更有效。

抑制食欲的效果可能因为APJ受体与下丘脑弓状核（ARC）的POMC神经细胞共定位有关。 POMC 会分泌α-MSH，这是一种强烈的食欲抑制因子。另外，Apelin可以通过APJ受体诱导下丘脑迷走神经的激活，这可能对食欲中枢产生间接的抑制效应。

讨论：

Apelin-13类似物可以促进胰岛素分泌、促进组织对葡萄糖的摄取、降低血糖并降低食欲，而高血糖和胰岛素分泌不足正是T2D的典型特征。说明Apelin-13类似物可以作为治疗糖尿病的药物进行开发。

PART 4

结论

结论

总之，这项研究表明：新的类似物（Tyr^{13}）apelin-13 、pGlu （Tyr^{13}）apelin-13，apelin-13-amide和（pGlu）apelin-13-amide是稳定的，通过多种生理途径对葡萄糖稳态产生积极作用。有望成为抗糖尿病肽家族的一员。

PART 4

启发和思考

一

分清楚in *vitro* 和Ex *vivo* 。

二

研究性论文的语言要严谨。

三

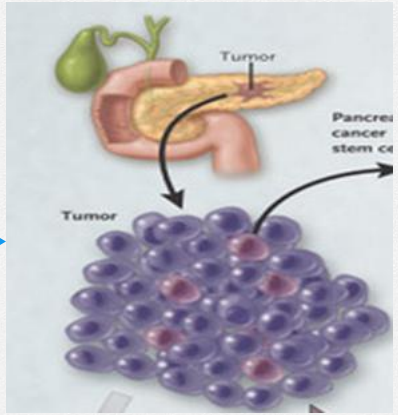
一个简单的结论需要大量的实验结果来支撑。

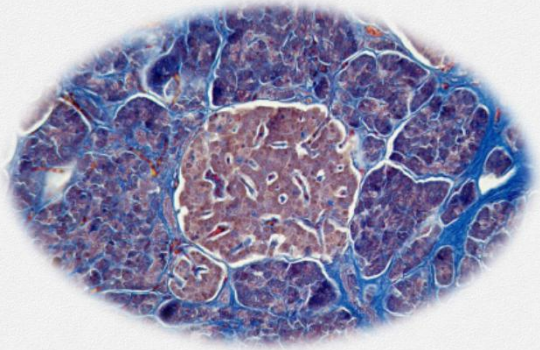
四

个人认为不足的地方在于，论文思路略显零散，不够系统，讨论里面每段之间语言上缺乏联系，逻辑关系不明确。

2.3. *in vitro insulin secretion*

The effects of apelin peptide analogues on insulin secretion in vitro were examined using clonal pancreatic BRIN-BD11 β-cells [31]. Briefly, cells were seeded into 24 well plates (150,000 cells/well) and allowed to attach overnightat 37 °C. Following pre-incubation (1.1 mmol/L glucose, 40 min; 37 °C) cells were treated with various concentration of peptides (10^{-12} to 10^{-6} M) in the presence of 5.6 and 16.7 mmol/L glucose. After 20 min incubation, the supernatant was removed from each well and aliquots (200 µl) stored at −20 °C prior to determination of insulin release by radioimmunoassay [32].





2.6. *Ex vivo insulin secretion from isolated islets*

Pancreatic islets were isolated from adult male C57BL/6 mice (8–10 weeks old, Harlan Ltd., Blackthorne, UK) by digestion with collagenase P obtained from *Clostridium histolyticum* (Sigma-Aldrich, Poole, Dorset, UK) as described previously [34,35]. Following 48 h culture, groups of 10 islets were pre-incubated with 500 µl KRB buffer containing 1.1 mmol/L glucose for 1 h at 37 °C. Test incubations with peptides and GLP-1 (10^{-7} M) were carried out in KRB buffer supplemented with 11.1 mmol/L glucose for 1 h at 37° C. Insulin release and insulin content of islets treated overnight with acidified ethanol [36] were determined by radioimmunoassay.

3.6. *Expression of APJ receptor on BRIN-BD11 cells and pancreatic islets*

Distribution of APJ receptors are displayed as double immunofluorescence showing insulin (green) and APJ (red) and co-localization (yellow) in BRIN-BD11 cells (Fig. 2A), suggesting that APJ receptors co-localize with insulin-secreting beta cells. A similar staining pattern was observed in pancreatic islets of normal (Fig. 2B) and DIO mice (Fig. 2C). Glucagon was stained on the periphery of the islet (Fig. 2B,C) though no evidence of the receptor co-localisation with glucagon-secreting alpha cells (no yellow staining) was observed.

A 剂量和时间

考虑到剂量问题，所以设置了从低到高的剂量。

B 对比研究

空白对照、阳性对照、阴性对照。

正常小鼠和糖尿病小鼠。

低糖负荷和高糖负荷。

抑制剂和拮抗剂从反向证明。

感谢聆听！

请各位老师同学批评指正！