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Research Paper

Signal transduction mechanism for glucagon-induced *leptin* gene expression in goldfish liver

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金鱼肝脏中胰高血糖素诱导的瘦素基因表达的信号转导机制

IF=4.067

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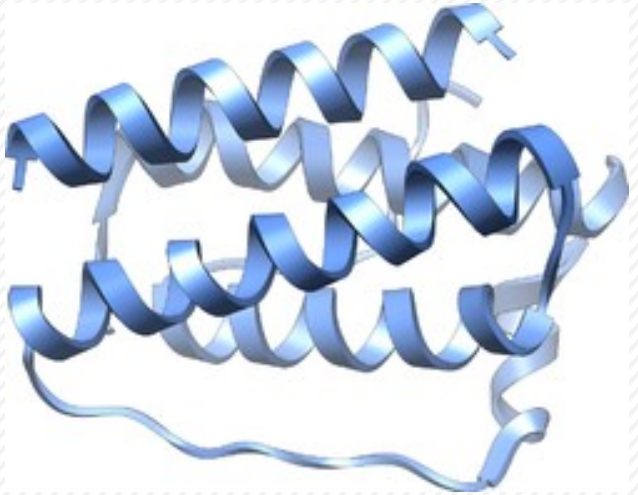
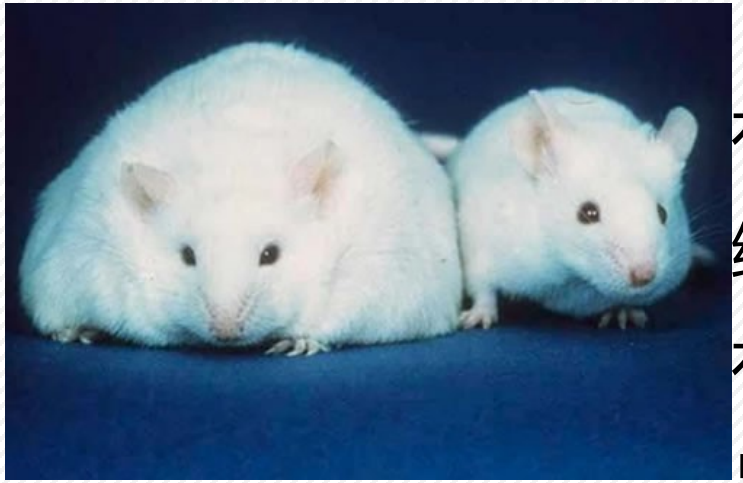


瘦素 由肥胖基因编码，主要由脂肪组织合成，通过受体介导，作用于靶组织，抑制食欲并参与调节能量代谢、神经内分泌和免疫反应等。

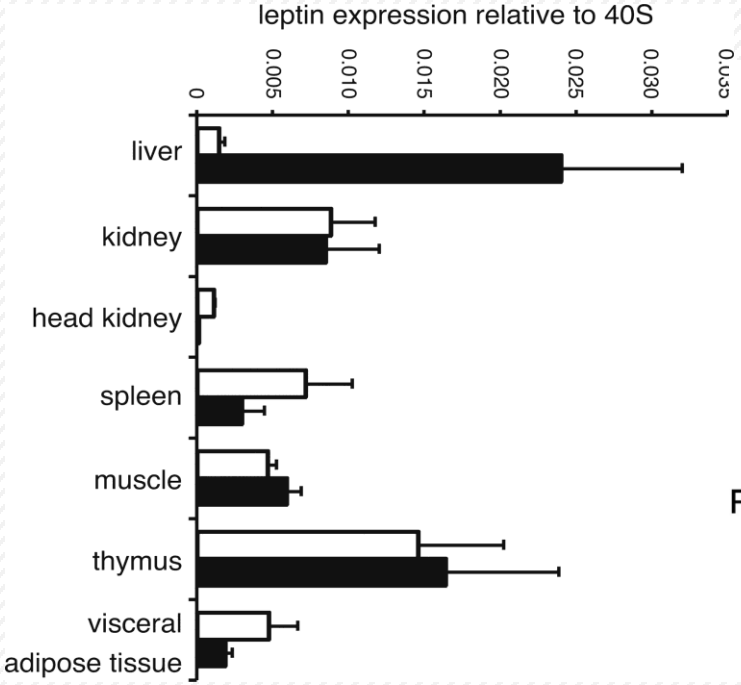


哺乳动物中，mRNA和血清瘦素水平与体脂肪量呈正相关。有研究发现许多肽和非肽激素调节瘦素的产生和分泌。胆囊收缩素和胃饥饿素是两种调节胃肠食欲的激素，对大鼠瘦素分泌有促进作用。胰岛素是大鼠和小鼠脂肪细胞产生和分泌瘦素的另一个主要刺激物，IGF-I有可能抑制大鼠的瘦素分泌。

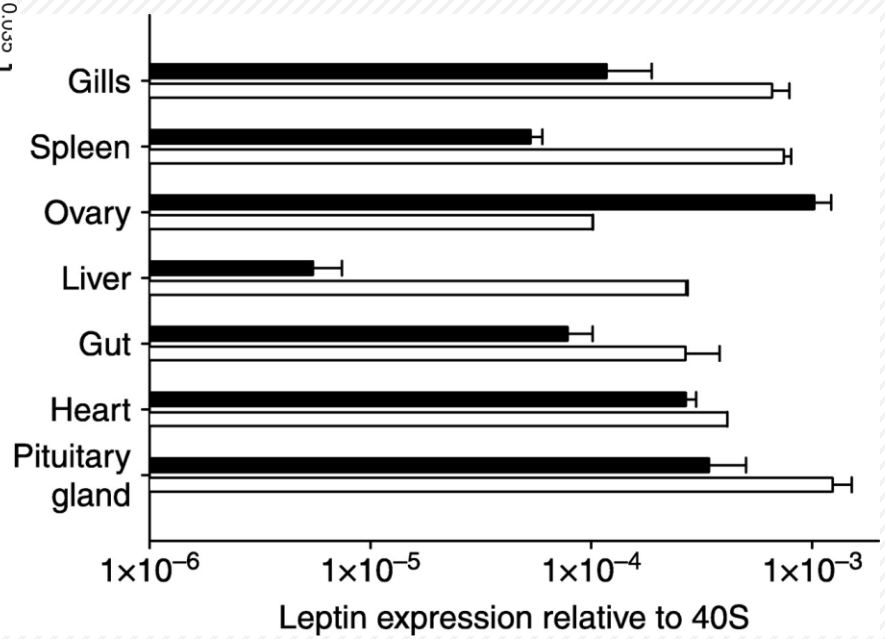
1 研究背景

瘦素在鱼类中的表达与功能

瘦素-a和瘦素-b在所有不同组织中的表达



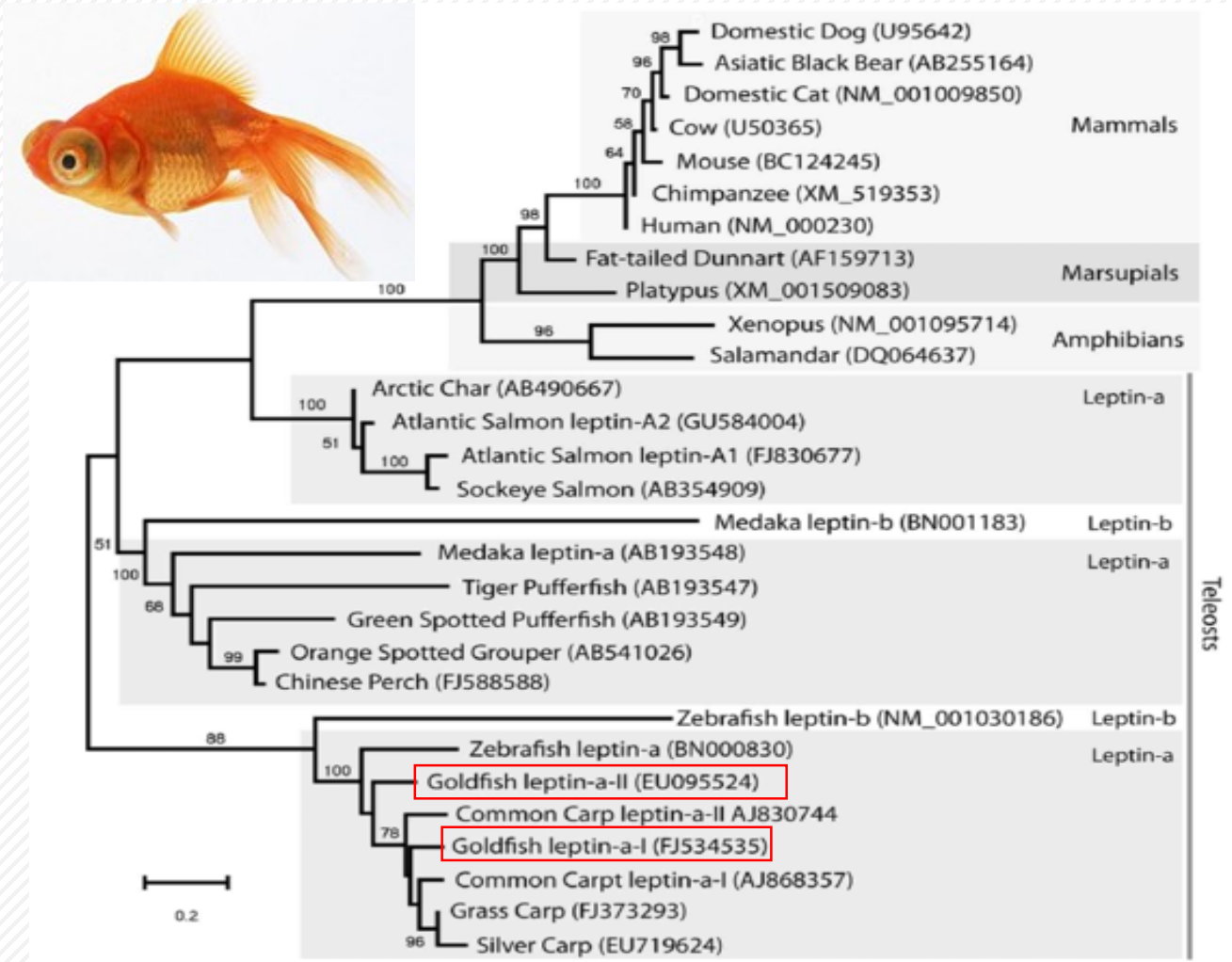
鲤鱼（Mark et al., 2006）



斑马鱼 (Marnix et al., 2009

- 与哺乳动物不同，鱼类的 leptin 在包括肠道、脂肪、大脑等不同组织中都有少量表达, 肝脏是其主要的表达部位之一。
- 不同亚型 leptin 的组织表达情况也有差异在斑马鱼中，leptin-A 主 要在 肝 脏 中 表 达 , 而leptin-B 在脑中大量表达 。
- 瘦素在鱼类中的主要功能有：调控能量代谢；在绝大多数鱼类中可抑制摄食；调节炎症反应等。

1 研究背景



(D.L. Copeland et al.,2011)

- 在金鱼中，已经报道了两个瘦素基因(GenBank: FJ534535和FJ854572)。它们都与其他鲤科鱼类leptin-A聚集在一起，因此分别被命名为leptin-AI和leptin-AII。但在金鱼体内尚未发现瘦素b。

- 肝脏是鱼类的代谢活动的中心也是瘦素A的主要表达场所，而胰高血糖素是调节糖代谢的主要激素之一。到目前为止，有关体内激素对瘦素的影响的研究还很有限。

1 研究背景

研究目的

本研究以金鱼为研究对象，通过分子生物学，细胞生物学，药理学等实验技术探究胰高血糖素对瘦素基因表达的调控及信号转导机制。

研究意义

这项研究结果可能为瘦素在鱼类模型中作为能量代谢调节网络的中介物质提供新的见解。

2 实验方法

2 实验方法

体重为 25-30g 的金鱼

体内实验

处理组：腹腔注射300 ng/g BW胰高血糖素

对照组：等体积的生理盐水

注射后0, 1.5, 3, 6, 12 and 24 h后取**肝脏**组织

体外实验

分离原代肝细胞，并用胰高血糖素进行处理

用胰高血糖素拮抗剂和cAMP信号通路相关激活剂、抑制剂和类似物孵育原代肝细胞

瘦素的mRNA表达量

检测cAMP的含量，WB检测CREB的磷酸化

胰高血糖素对金鱼瘦素的信号转导的影响

3 结果与讨论

3 结果与讨论—体内注射实验

金鱼注射胰高血糖素后 (300 ng / g bw) 肝脏中leptin-AI (A)和leptin-AII (B) mRNA 在指定时间点的变化

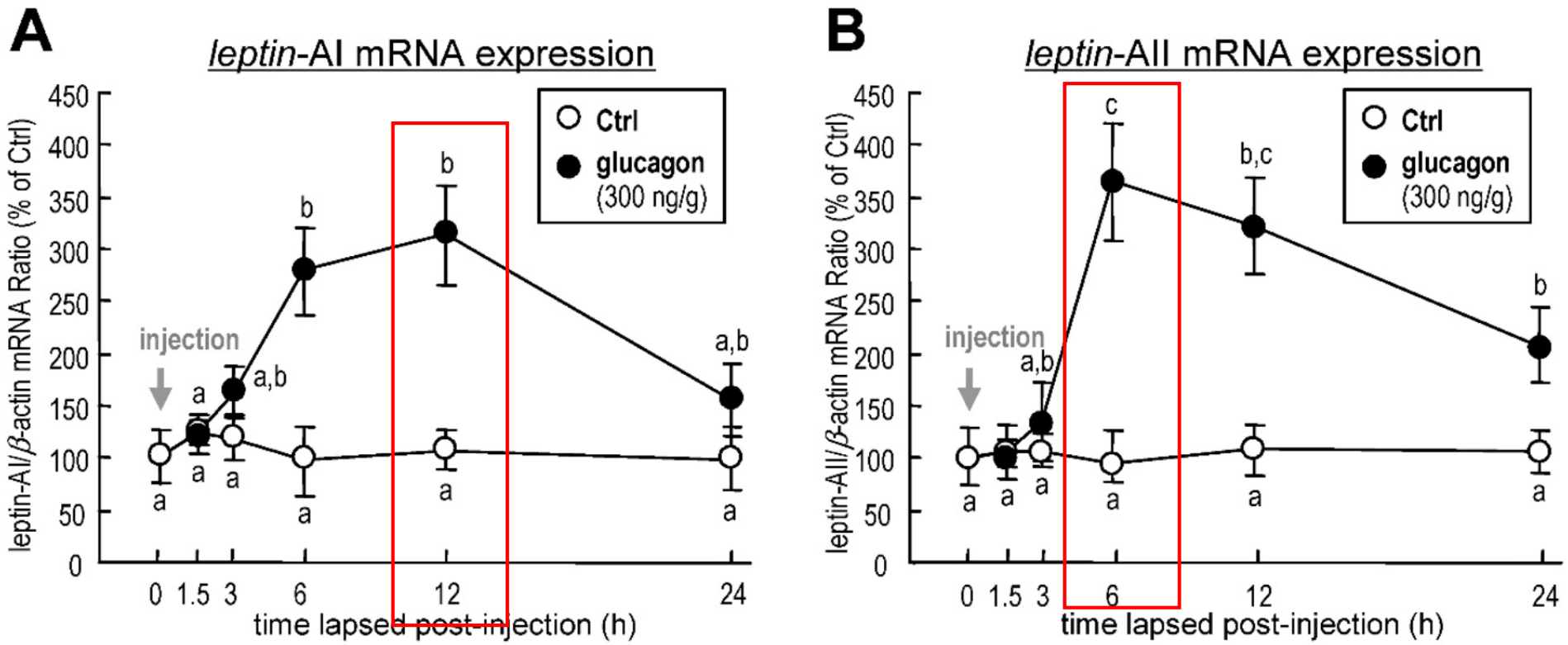
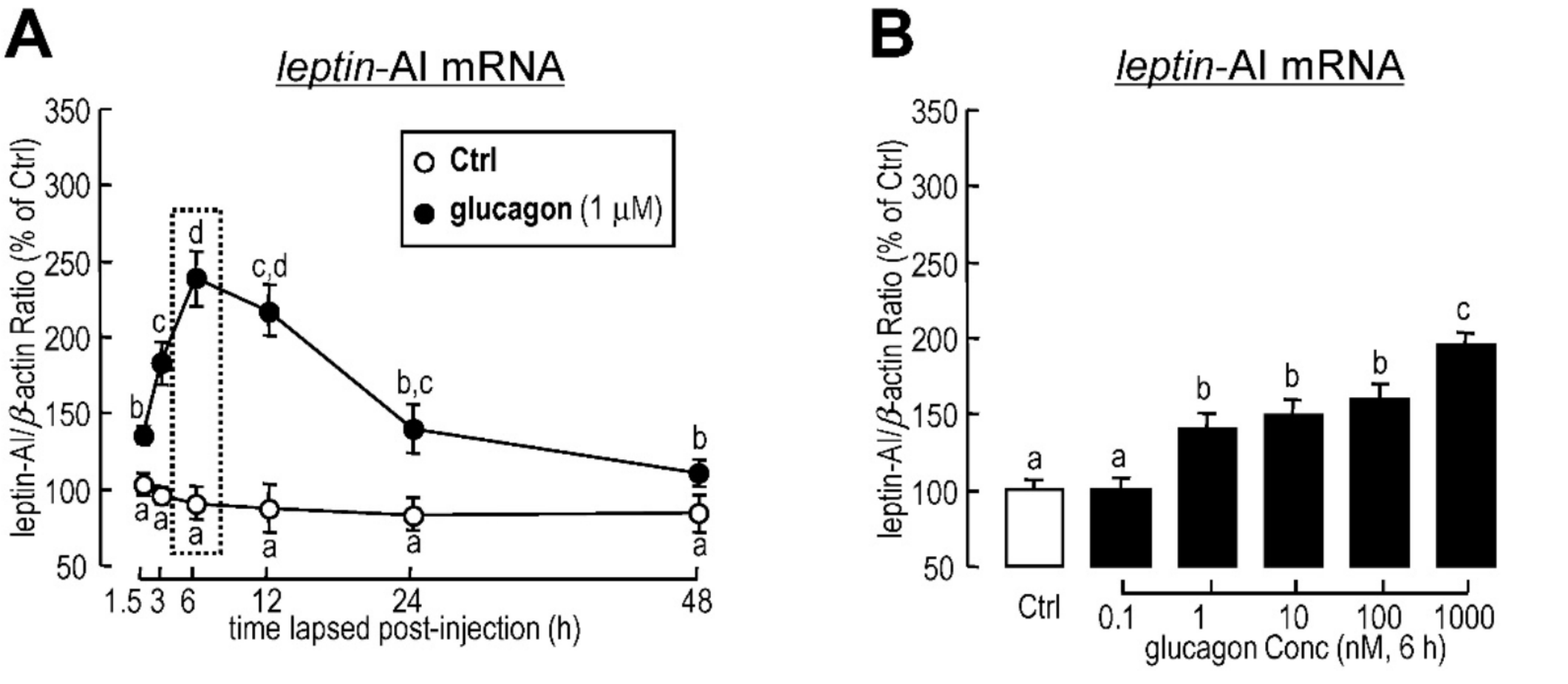


Figure 1. Changes in *leptin*-AI (A) and *leptin*-AII (B) mRNA expression in goldfish liver after IP injection of glucagon (300 ng/g bwt) at selected time points (0, 1.5, 3, 6, 12 and 24 h). In this study, the expression level at time 0 was used as the control group, and real-time PCR for *β-actin* was used as the internal control. The data obtained (n=10) at various time points were then normalized as a percentage of the control group at time 0 h. The same letter represents a similar level of transcriptional expression ($P > 0.05$), and the different letter represents significant difference in levels of transcriptional expression between two groups ($P < 0.05$).

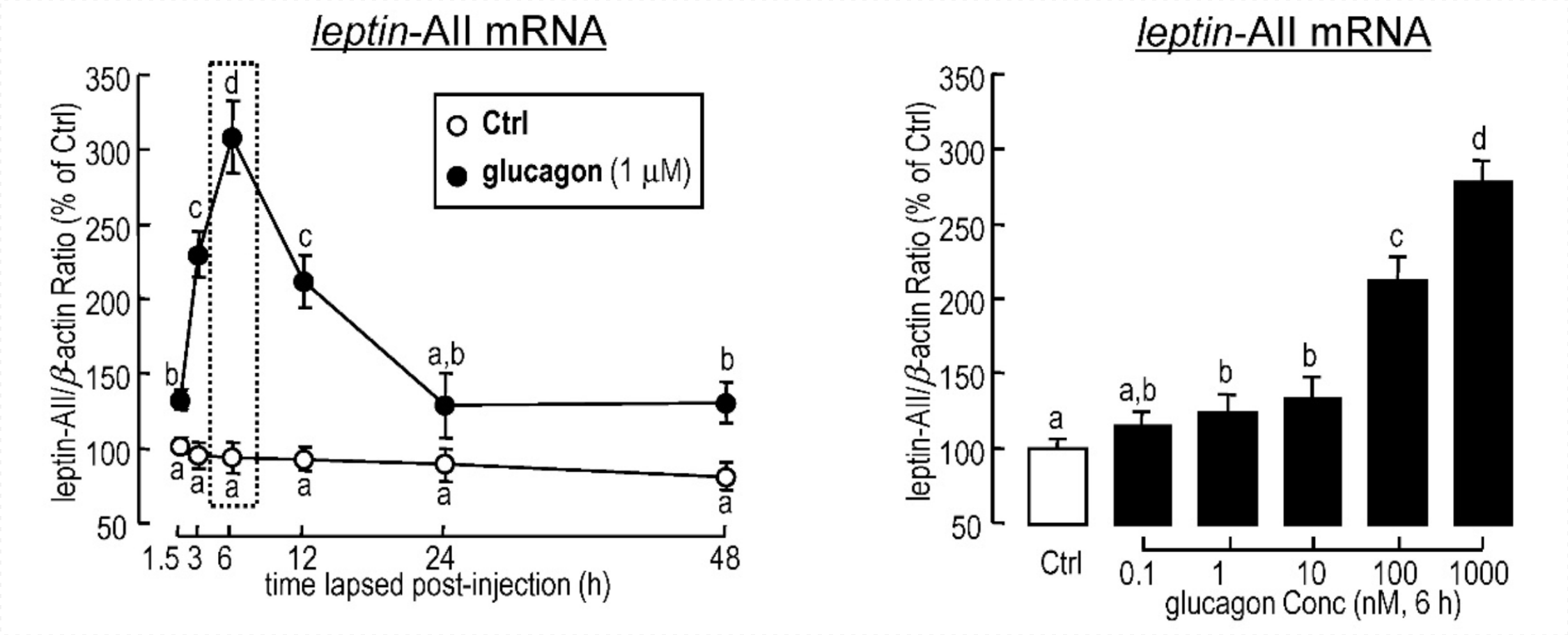
3 结果与讨论

胰高血糖素对金鱼原代肝细胞leptin-AI mRNA表达的影响



3 结果与讨论

胰高血糖素对金鱼原代肝细胞leptin-AII mRNA表达的影响



胰高血糖素可能诱导的leptin-AI和leptin-AII的表达

3 结果与讨论

胰高血糖素抑制剂验证胰高血糖素对金鱼原代肝细胞瘦素表达的影响

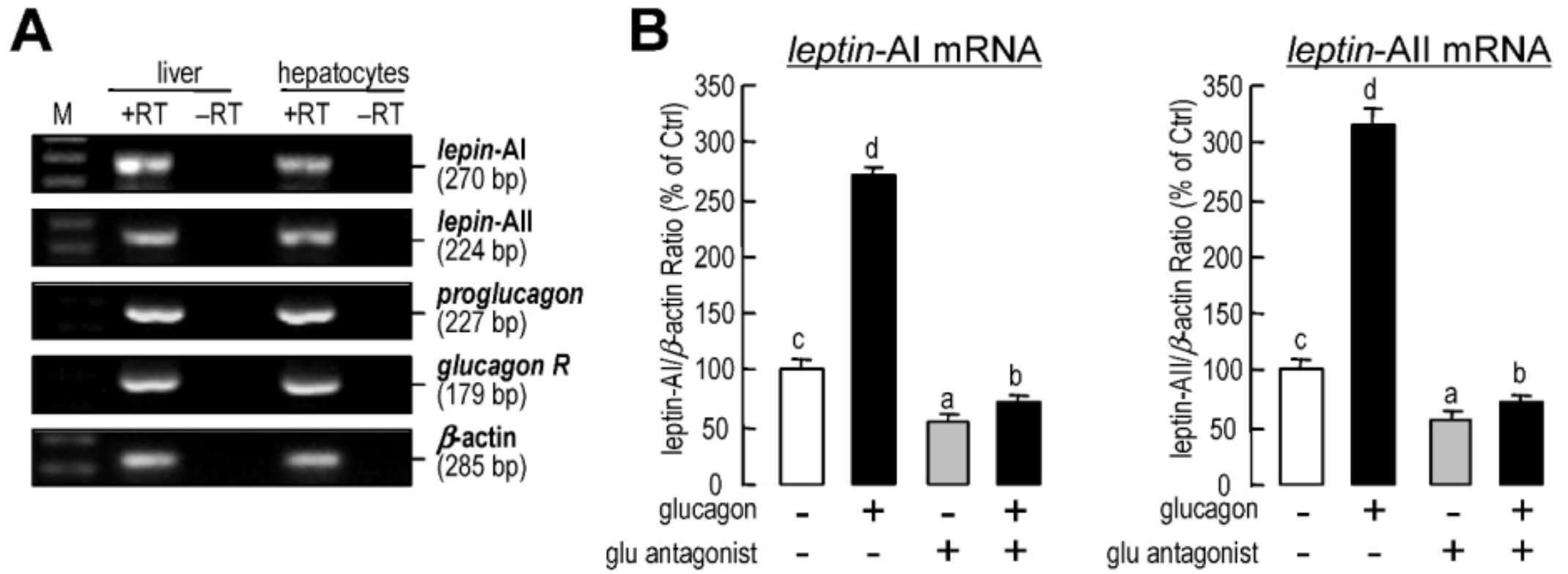
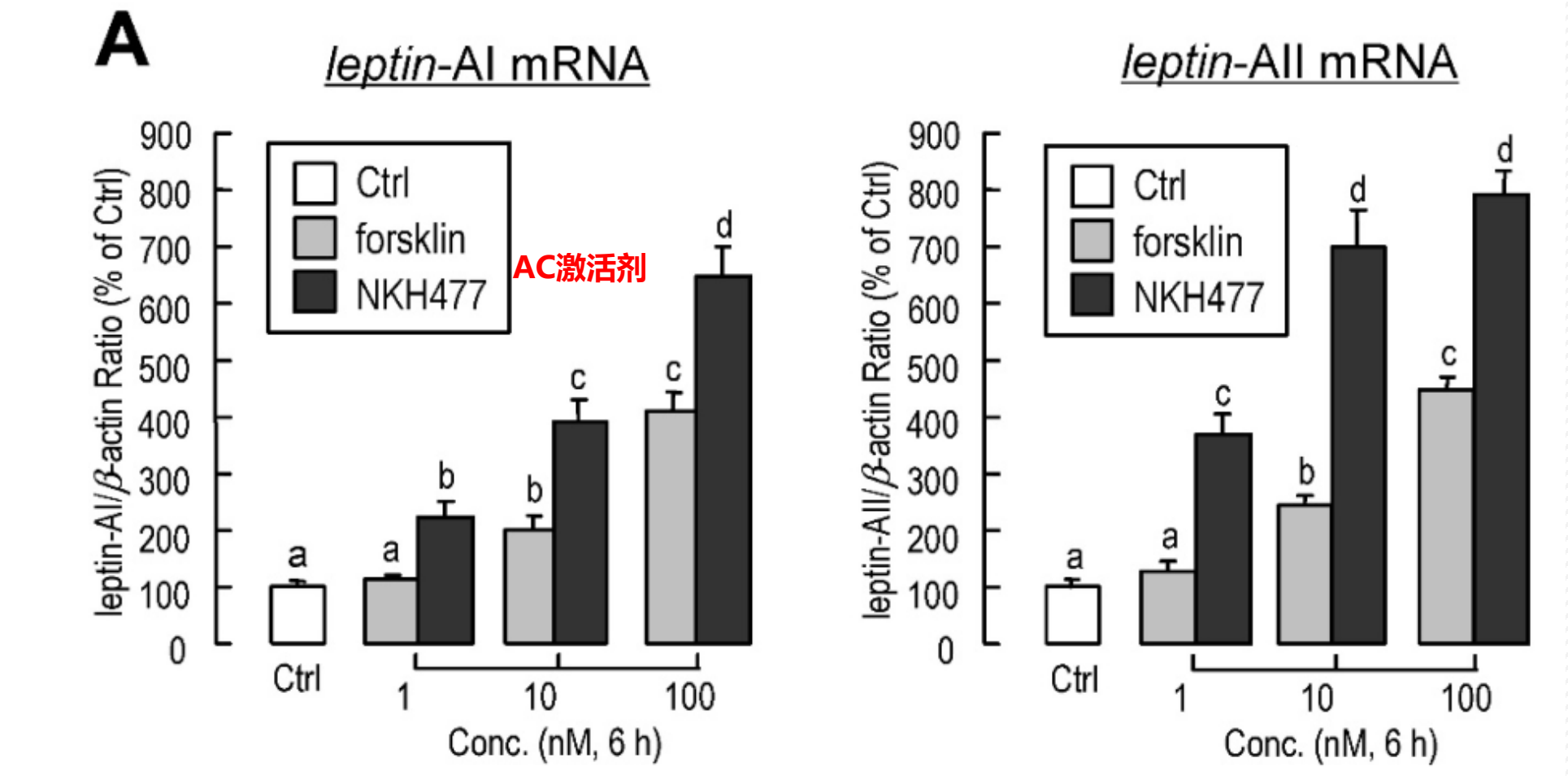


Figure 3. Autocrine/paracrine actions of glucagon on *leptin*-AI and *leptin*-AII transcripts in goldfish liver. A: Detection of *leptin*-AI, *leptin*-AII, *proglucagon* and *glucagon receptor* transcripts in goldfish livers. cDNA samples from goldfish livers and primary hepatocytes were used for detection and RNA samples reverse transcribed without the enzyme were used as negative controls. B: Effects of glucagon antagonist on glucagon-stimulated *leptin*-AI and *leptin*-AII mRNA expression in goldfish hepatocyte cultures. The hepatocytes were incubated for 6 h with glucagon (1 μM) in the presence or absence of glucagon antagonist (1 μM). In this study, the data presented are expressed as the mean ± SE (n=4). The same letter represents a similar level of transcriptional expression ($P > 0.05$), and the different letter represents significant difference in levels of transcriptional expression between two groups ($P < 0.05$).

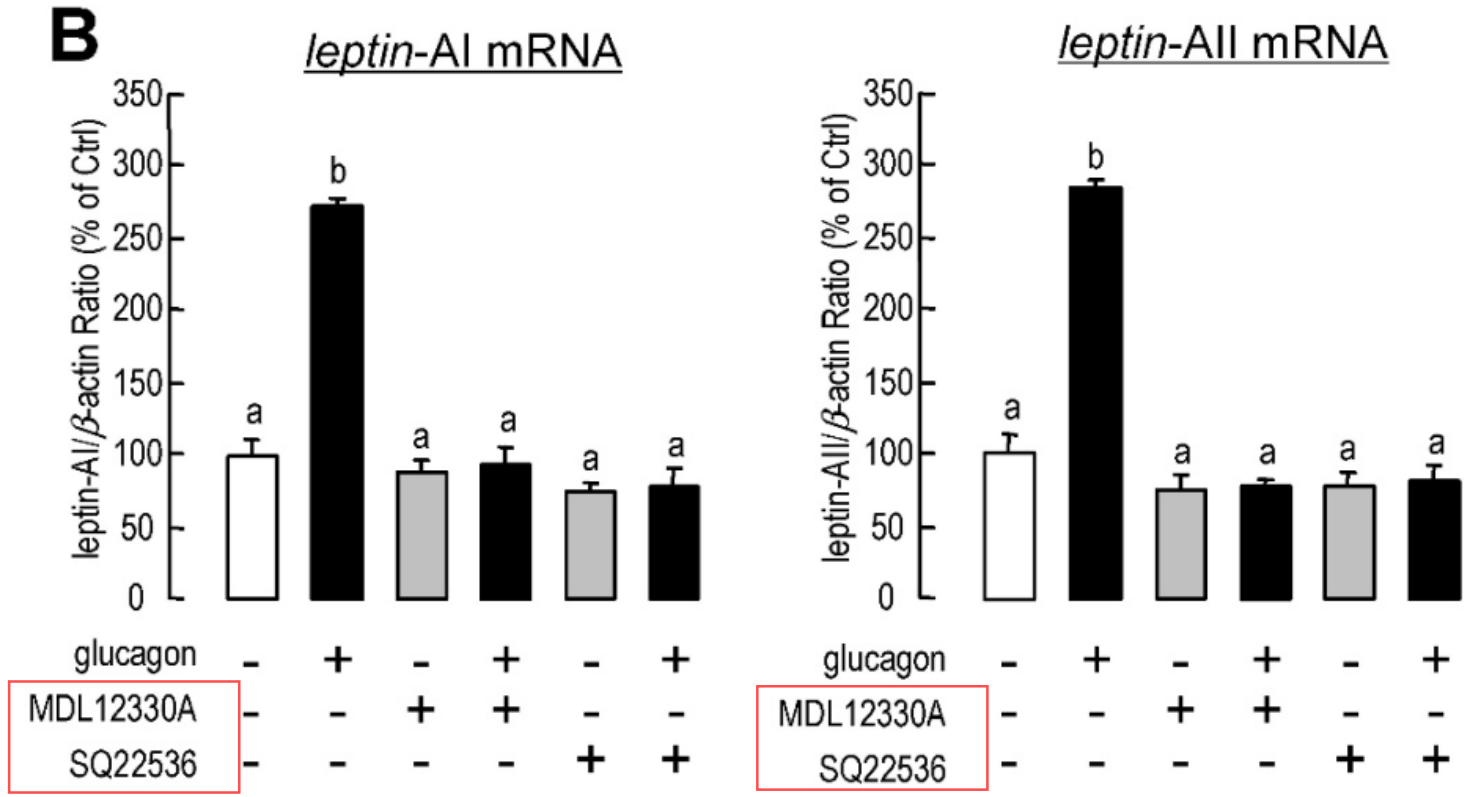
胰高血糖素确实可以诱导的leptin-AI和leptin-AII的表达

3 结果与讨论

瘦素mRNA的升高与cAMP的水平有关吗?



3 结果与讨论



AC抑制剂

Figure 4. Involvement of AC in glucagon-induced *leptin*-AI and *leptin*-AII expression in goldfish livers. A: Effects of AC activators on *leptin*-AI and *leptin*-AII mRNA expression in goldfish hepatocyte cultures. The hepatocytes were incubated for 6 h with increasing doses of forskolin (1-100 nM) or NKH477 (1-100 nM). B: Effects of AC inhibitors on glucagon-stimulated *leptin*-AI and *leptin*-AII mRNA expression in goldfish hepatocyte cultures. The hepatocytes were incubated for 6 h with glucagon (1 μM) in the presence or absence of MDL12330A (20 μM) or SQ22536 (50 μM). In this study, the data presented are expressed as the mean ± SE (n=4). The same letter represents a similar level of transcriptional expression ($P > 0.05$), and the different letter represents significant difference in levels of transcriptional expression between two groups ($P < 0.05$).

cAMP可能参与胰高血糖素诱导的leptin-AI和leptin-AII的表达

3 结果与讨论

cAMP确实参与胰高血糖素诱导的leptin-AI和leptin-AII的表达

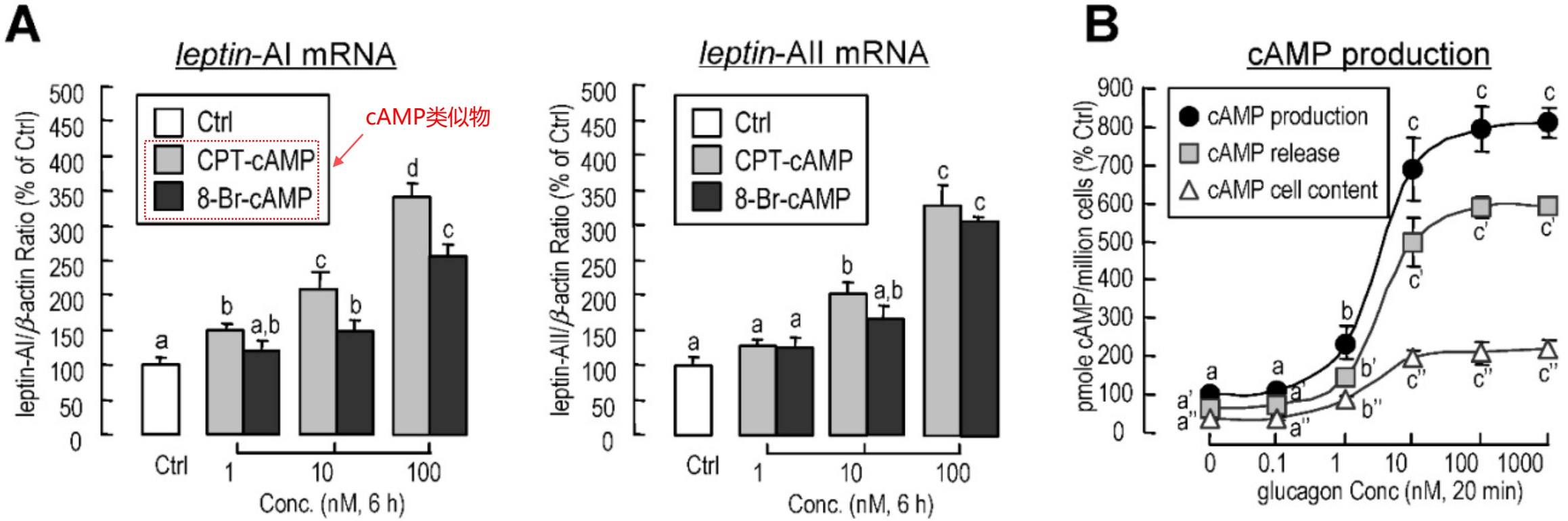
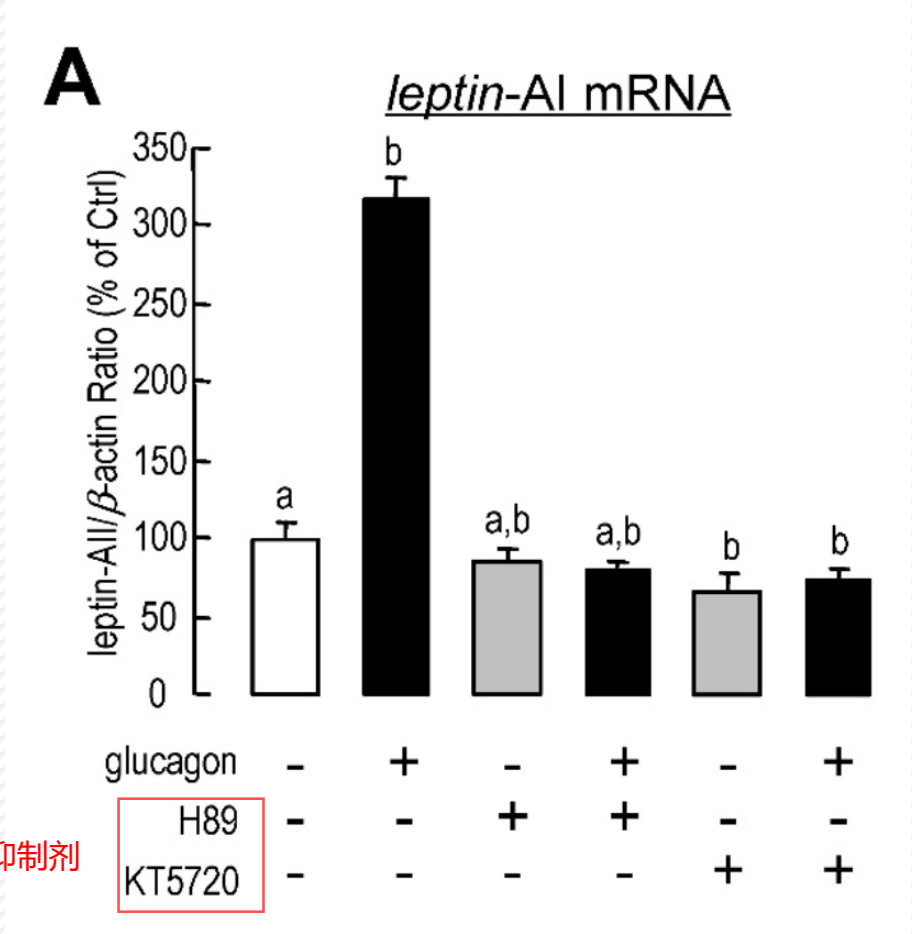


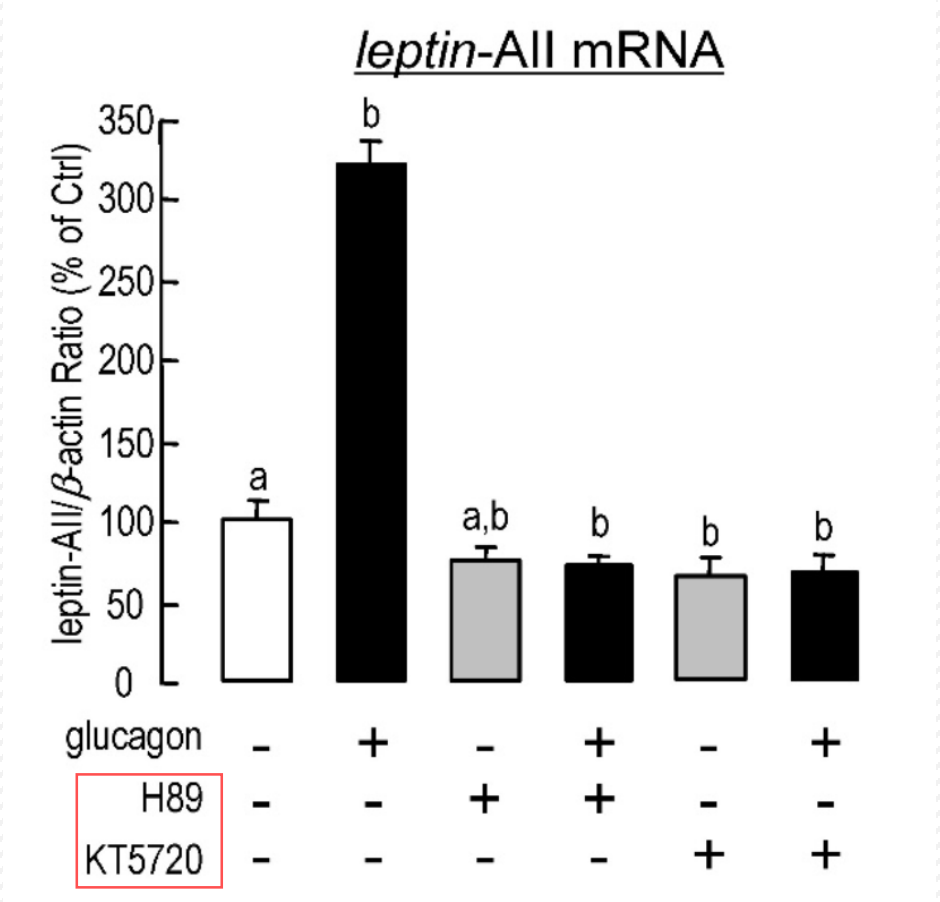
Figure 5. Involvement of cAMP in glucagon-induced *leptin*-AI and *leptin*-AII expression in goldfish livers. A: Effects of cAMP analogs on *leptin*-AI and *leptin*-AII mRNA expression in goldfish hepatocyte cultures. The hepatocytes were incubated for 6 h with increasing doses of CPT-cAMP (1-100 nM) or 8-Br-cAMP (1-100 nM). B: Effects of glucagon on cAMP release, cellular cAMP content, and total cAMP production in goldfish primary hepatocytes. The hepatocytes pretreated with IBMX (0.1 mM) were then incubated for 20 min with increasing doses of glucagon (0.1-1000 nM). In this study, the data presented are expressed as the mean ± SE (n=4 and 3 for mRNA and cAMP measurement, respectively). The same letter represents a similar level of transcriptional expression ($P > 0.05$), and the different letter represents significant difference in levels of transcriptional expression between two groups ($P < 0.05$).

3 结果与讨论

PKA参与胰高血糖素诱导的leptin-AI和leptin-AII在金鱼肝脏中的基因表达



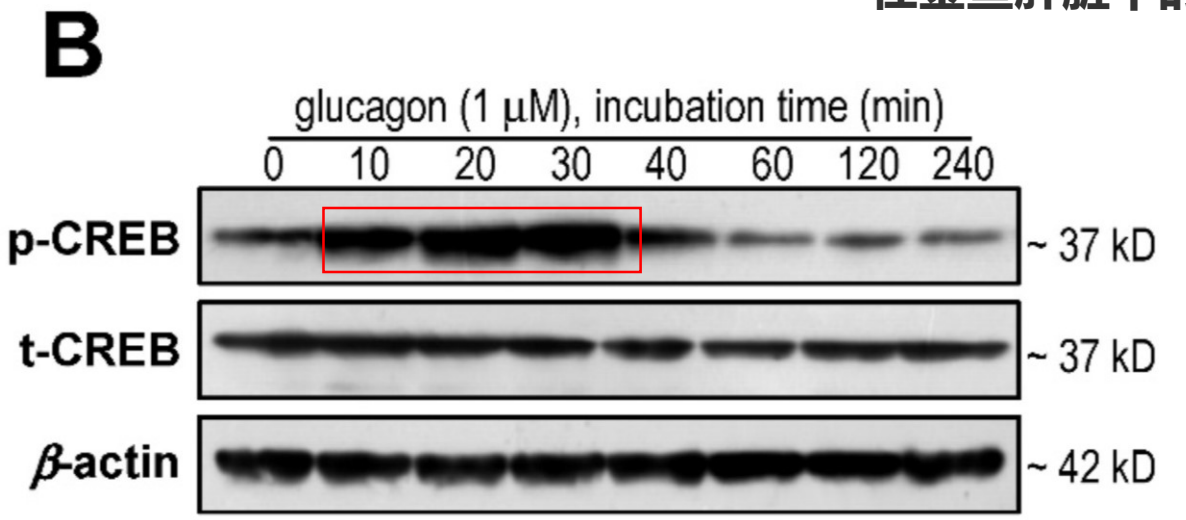
PKA抑制剂

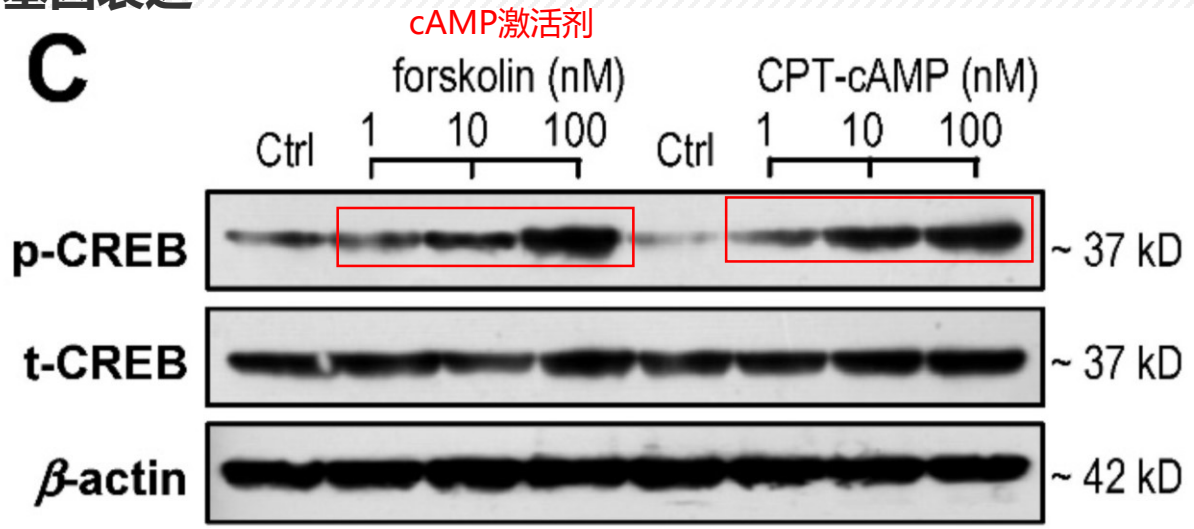


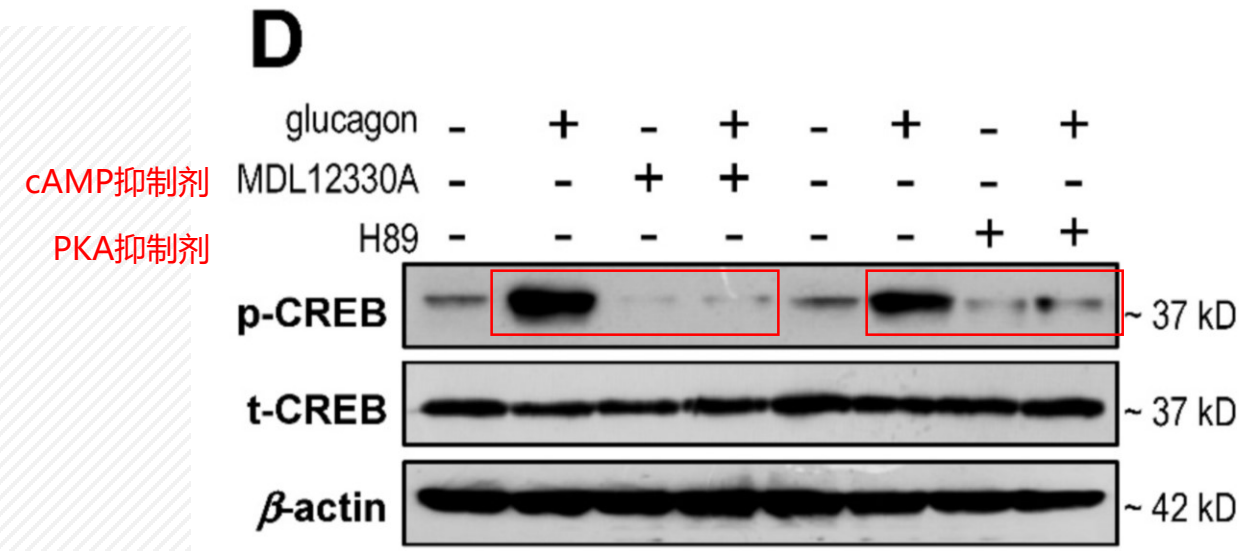
基因表达水平

3 结果与讨论

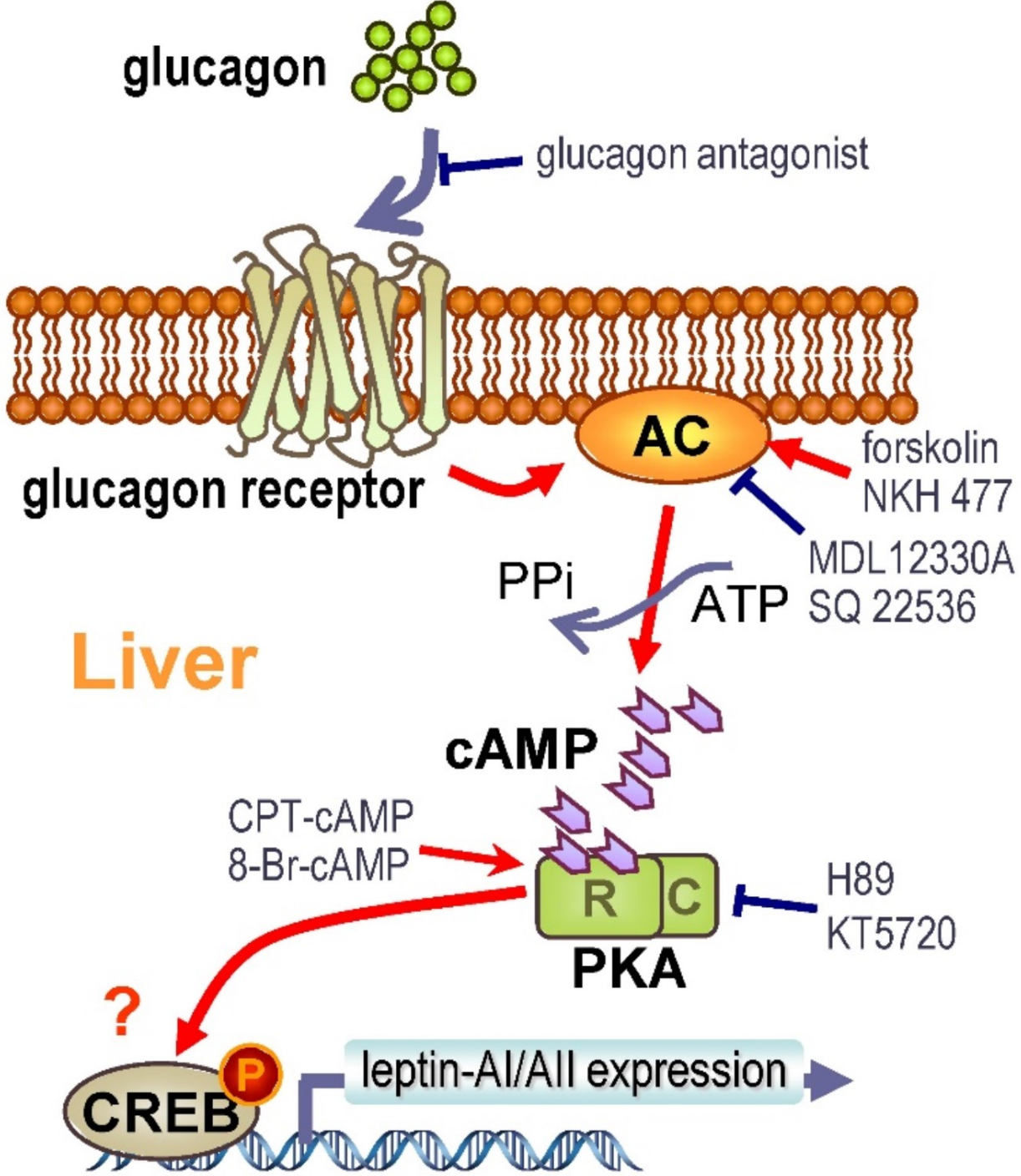
PKA和CREB参与胰高血糖素诱导的leptin-AI和leptin-AII在金鱼肝脏中的基因表达







孵育30min



综上所述，通过体内外实验证明，

- 在金鱼肝脏中，胰高血糖素可以刺激leptin-AI和leptin-AII的表达。
- leptin-AI和leptin-AII对胰高血糖素处理的反应具有高度相似性，阻断局部胰高血糖素作用可降低诱导的leptin-AI和leptin-AII mRNA的表达。
- PKA和CREB参与胰高血糖素诱导的leptin-AI和leptin-AII在金鱼肝脏中的基因表达。

敬请各位老师同学批评指正！

报告人：赵文丽

时间：2019年12月29日