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ADROPIN SUPPRESSES INSULIN EXPRESSION AND SECRETION IN INS-1E CELLS AND RAT PANCREATIC ISLETS

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Adropin is a peptide hormone which is produced in brain and peripheral tissues such as liver. It was found that adropin modulates lipid and glucose homeostasis by interacting with hepatocytes and myocytes. Adropin enhances insulin sensitivity and alleviates hyperinsulinemia in animal models with high-fat diet-induced insulin resistance. However, it is unknown whether adropin regulates insulin secretion and proliferation of beta cells. Therefore, we studied the effects of adropin on insulin secretion in INS-1E cells as well as isolated pancreatic islets. Furthermore, we assessed the influence of adropin on insulin mRNA expression, cell viability and proliferation in INS-1E cells. Pancreatic islets were isolated from male Wistar rats. mRNA expression was evaluated using real-time PCR and cell viability by MTT assay. Cell replication was measured by BrdU incorporation and insulin secretion by RIA. We found that adropin suppresses insulin mRNA expression in INS-1E cells. Moreover, adropin attenuates glucose-induced insulin secretion in INS-1E cells as well as in isolated pancreatic islets. In addition, using INS-1E cells we found that adropin suppresses glucose-induced cAMP production. However, adropin fails to modulate INS-1E cell viability and proliferation. In summary, we found adropin suppresses insulin mRNA expression and secretion, without affecting beta cell viability or proliferation.

Key words: *adropin, energy homeostasis associated gene, insulin, secretion, pancreatic islets beta cells, INS-1E, cell proliferation, cell viability, expression*

INTRODUCTION

Adropin is a peptide hormone composed of 42 amino acids, produced from an 76 amino acids precursor (1). Adropin peptide is encoded by energy homeostasis associated (*Enho*) gene (1). *Enho* mRNA is abundantly expressed in the brain as well as in the liver (1). Initial study showed that Enho expression in the liver is low in mice with genetically induced obesity (1). There is evidence indicating that biological effects of adropin, such as inhibition of water drinking in rats or upregulation of E-catherin expression in breast cancer cells are mediated through G protein-coupled receptor 19 (GPR19) activation (2).

There is growing evidence indicating that adropin controls glucose and lipid metabolism. It was shown that adropin overexpression in mice delays high-fat diet-induced weight gain as well as improves glucose control and lipid parameters (1). Consistently, mice fed high-fat diet and treated with exogenous adropin lose body weight and have improved insulin sensitivity (1). Moreover, adropin depleted animals are obese, dyslipidemic and insulin resistant (3). A recent study provided strong evidence that low levels of adropin in the circulation predict metabolic abnormalities and body weight gain in rhesus macaque, fed carbohydrate-rich diet (4). In addition, adropin suppresses hepatic glucose synthesis and promotes insulin sensitivity in mice (5). Overall, these results collectively indicate that adropin modulates glucose and lipid metabolism.

It is well-known that insulin modulates lipid and carbohydrate metabolism (6). Insulin is produced and secreted in pancreatic beta cells, in a glucose-dependent manner (7). Insulin stimulates glucose uptake in muscle and adipose tissue (8). Furthermore, insulin promotes lipid synthesis in adipocytes and hepatocytes (7). By contrast, insulin attenuates gluconeogenesis in the liver (7).

Although it was found that adropin may contribute to glucose control and lipid metabolism, the direct role of adropin in the modulation of insulin synthesis and secretion remains unknown. Importantly, there is growing evidence indicating that amino acid-derived and peptide hormones are implicated in controlling pancreatic beta cell functions (9-12). Thus, in the present study, we evaluate the effects of adropin on insulin secretion from insulin-producing INS-1E cells and rat pancreatic islets. In addition, we characterize the effects of adropin on insulin mRNA expression and INS-1E cell proliferation, and viability.

MATERIALS AND METHODS

Drugs

Adropin (34-76) (human, mouse, rat) was purchased from Bachem AG (Bubendorf, Switzerland). Media and supplements for cell culture were from Biowest (Nuaille, France). 3-(4,5-

Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was from Calbiochem (Merck, Darmstadt, Germany). BrdU Cell Proliferation kit (#11 647 229001) was from Roche Diagnostic (Manheim, Germany). Rat insulin RIA kit (#RI-13K) was from Merck Millipore (Billerica, MA, USA). cAMP ELISA kit (#KA3388) was from Abnova (Taipei, Taiwan). Other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise stated.

Cell culture

Rat insulin-producing INS-1E cells were purchased from AddexBio (San Diego, CA, USA). INS-1E cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 2 mmol/l glutamine, 10 mmol/l HEPES buffer, 1 mmol/l sodium pyruvate, 50 μmol/l mercaptoethanol and antibiotics (100 kU/l penicillin and 100 mg/l streptomycin). Cells were cultured at 37°C in humidified atmosphere, containing 5% CO_2 and 95% air.

Isolation of pancreatic islets

Male Wistar rats (body weight 300 – 350 g) were obtained from the Department of Toxicology (Poznan University of Medical Sciences, Poznan, Poland). Pancreatic islets were isolated as described in our previous study (13). Briefly, pancreas was injected with 8 ml of ice-cold Hank's balanced salt solution (HBSS) supplemented with HEPES (25 mmol/l) and collagenase type XI (0.35 mg/ml). Then, pancreas was excised and digested for 30 min in a water bath at 37°C. Isolated islets were purified with Histopaque 1077 discontinuous gradient. Next, islets were repeatedly washed with HBSS supplemented with 0.2% bovine serum albumin (Biowest) and maintained overnight in RPMI 1640 supplemented with 10% (v/v) FBS for regeneration.

All applicable international, national and/or institutional guidelines for the care and use of animals were followed, according to The Act on The Protection of Animals used for Scientific or Educational Purposes in Poland adopted on 15 January 2015 and according to earlier regulations. Those experiments focused on analysis of tissues obtained from dead animals, which did not undergo any experimental procedures did not require any permission of The Local Ethical Commission for Investigation on Animals.

Real-time PCR

Total RNA was extracted using Extrazol (DNA Gdansk, Gdansk, Poland). RNA (1 μg) was reverse transcribed to cDNA by FIREScript RT cDNA Synthesis Mix (Solis BioDyne, Tartu, Estonia). A real-time quantitative PCR reaction with primer/probes and TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) was performed using a QuantStudio 12K Flex (Thermo Fisher Scientific, Waltham, MA, USA). The following primers/probes (Applied Biosystems) were used: *Enho*, Rn01436637_m1; *Gpr19*, Rn02758803_s1; *Ins1*, Rn02121433_g1; *Ins2*, Rn01774648_g1; *Hprt1*, Rn01527840_m1. Gene expression was calculated by the $2^{-\Delta\Delta}$ Ct method. The *Hprt1* was used as the reference gene.

Insulin secretion

INS-1E cells were seeded into 24-well plates at the density of 1.5×10^5 cells/well and cultured for 48 hours. Thereafter, cells were incubated (1 h) in a glucose-free Krebs-Ringer-HEPES buffer (KRHB) containing in mmol/l: 136 NaCl, 4.7 KCl, 1 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_2, 2 $NaHCO_3$, 10 HEPES (pH 7.4), 0.1% free fatty acid BSA. Next, cells were washed with the same buffer and exposed to 2.8 mmol/l or 16.8 mmol/l glucose in KRHB with or without adropin (1, 10 or 100 nmol/l) for 60 min. Then, the incubation medium was collected and centrifuged (250 × g for 5 min). Insulin content was determined in the supernatants using Rat Insulin RIA RI-13K (Merck Millipore). Data were normalized to protein concentrations, determined by BCA Protein Assay kit (Thermo Scientific).

In experiments with pancreatic islets, following 1 h preincubation with glucose-free KRHB supplemented with 0.1% free fatty acid BSA, groups of 5 pancreatic islets per well were exposed to 2.8 mmol/l or 16.8 mmol/l glucose in KRHB with or without adropin (1, 10 or 100 nmol/l) for 60 min. Medium was collected and stored at –20°C for further analysis.

INS-1E cell proliferation and viability INS-1E cells were seeded into 96-well plate at the density 4×10^4 cell/well. After 48 h, cells were incubated in a serum-free medium for additional 24 hours. Thereafter, cells were incubated with serum-free medium containing 0.1% free fatty acid BSA and 0, 1, 10 or 100 nmol/l adropin for 24 or 48 hours. Cell viability was studied by MTT assay, cell proliferation was detected using Cell Proliferation ELISA BrdU kit (Roche Diagnostics) according to the manufacturer's procedure, as previously described in our earlier studies (14, 15).

cAMP assay

INS-1E cells were seeded into 12 well plate (3×10^5 cells/well) for 48 hours. Next, cells were incubated in Krebs-Ringer-HEPES buffer without glucose for 1 h and exposed to 2.8 mmol/l or 16.8 mmol/l glucose in KRHB with or without adropin (1, 10 or 100 nmol/l) for 60 min. Thereafter, cAMP intracellular content was measured using cAMP ELISA Kit (Abnova) according to the manufacturer's protocol.

Statistical analysis

Data are presented as the mean ± standard error of the mean. Statistical analysis were performed using GraphPad Prism version 8.0 (GraphPad Software, Inc.). Student's t-test for comparison between two groups or one-way ANOVA followed by the Bonferroni *post hoc* test for comparison between more than two groups were used. Each experiment was repeated independently at least two times. $P < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

Expression of Enho and Gpr19 mRNA in INS-1E cells and rat pancreatic islets.

First, we investigated mRNA expression of *Enho* and putative adropin receptor *Gpr19* in rat tissues, INS-1E cells and rat isolated pancreatic islets. *Enho* mRNA was detected in rat hypothalamus (positive control (1)) (*Fig. 1A*). Approximately 88-fold lower expression was detected in INS-1E cells in comparison to that detected in the hypothalamus (*Fig. 1A*). *Enho* mRNA was not detectable in rat pancreatic islets (*Fig. 1A*). *Gpr19* mRNA was expressed in rat hypothalamus (positive control (2)) as well as in INS-1E cells and rat pancreatic islets (*Fig. 1B*). Expression of *Gpr19* in pancreatic islets and INS-E cells was approximately 13- and 2-fold higher as compared to that detected in the brain (*Fig. 1B*). Taken together, these results indicate that *Enho* mRNA is not expressed in rat pancreatic islets, while very low *Enho* levels are detectable in INS-1E cells. *Gpr19* mRNA is present in INS-1E cells and pancreatic islets.

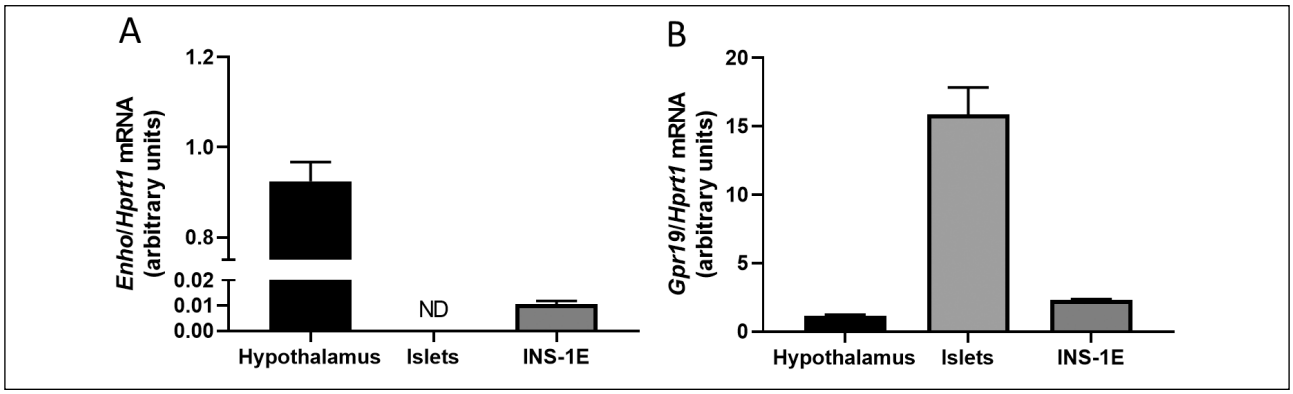

Fig. 1. Enho and *Gpr19* expression in rat pancreatic islets and INS-1E cells. (A): Real-time PCR detection of *Enho* in the hypothalamus (positive control), rat pancreatic islets and INS-1E beta cells. (B): *Gpr19* mRNA expression in the hypothalamus, rat pancreatic islets and INS-1E beta cells. ND, not detected. Results are mean ± SEM, (n = 6).

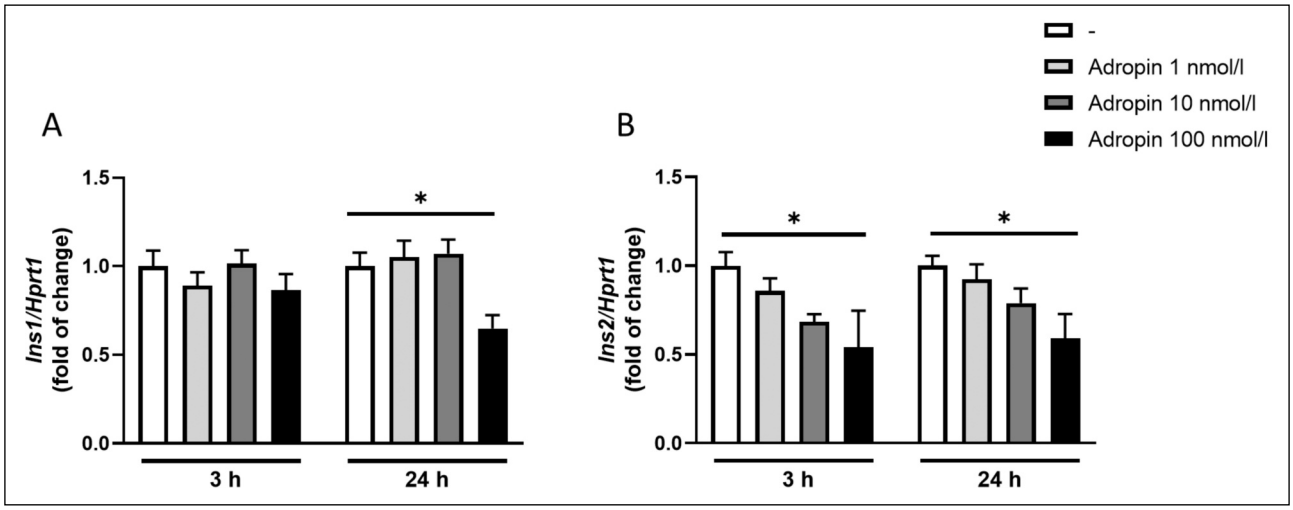

Fig. 2. Effects of adropin on insulin expression in INS-1E cells. The expression of *Ins1* (A) and *Ins2* (B) in cells exposed to adropin (1, 10 and 100 nmol/l) for 3 and 24 hours. Results are mean ± SEM, (n = 5 – 6). *P < 0.05.

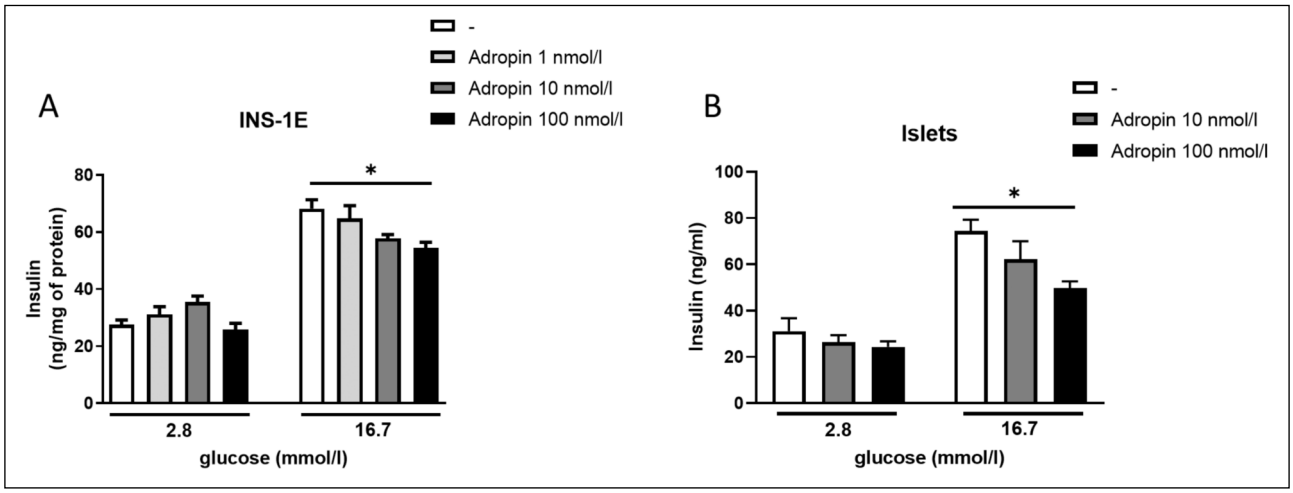

Fig. 3. Effects of adropin on insulin secretion in INS-1E cells and pancreatic islets. Insulin secretion evaluated in cells (A) and rat pancreatic islets (B) treated with or without adropin (1 and/or 10 and 100 mmol/l) in the presence of 2.8 or 16.7 mmol/l glucose for 60 min. Results are mean ± SEM, (n = 5 – 7). *P < 0.05.

Adropin suppresses insulin mRNA expression in INS-1E cells

As demonstrated in *Fig. 2*, adropin at the concentration of 100 nmol/l failed to modulate *Ins1* mRNA expression after 3 hours (*Fig. 2A*). By contrast, adropin (100 nmol/l) suppressed *Ins2* mRNA expression after 3 hours (*Fig. 2B*). In addition, adropin (100 nmol/l) attenuated expression of *Ins1* and *Ins2* after 24 h (*Fig. 2A* and *2B*).

Adropin inhibits insulin secretion in INS-1E cells and rat pancreatic islets

Next, we evaluated the effects of adropin on insulin secretion from INS-1E cells and rat pancreatic islets. We found that adropin (100 nmol/l) suppresses insulin secretion from INS-1E cells (*Fig. 3A*). Inhibition of insulin secretion by 100 nmol/l adropin was also detectable in isolated rat pancreatic islets (*Fig. 3B*). These results show that adropin suppresses insulin secretion from INS-1E cells and rat pancreatic islets.

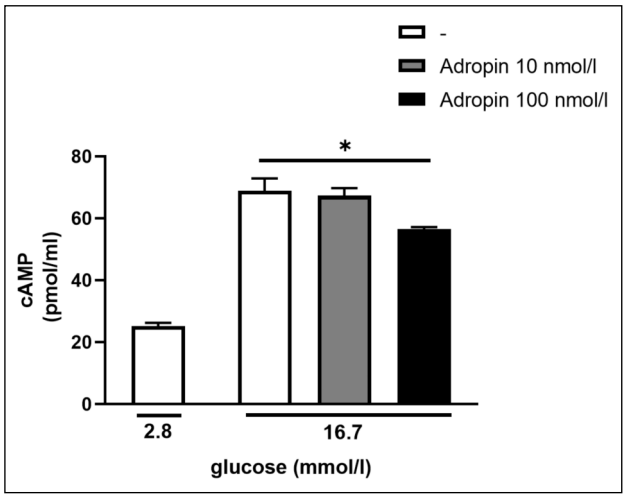

Fig. 4. Effects of adropin on cAMP synthesis in INS-1E cells. The cAMP production in cells exposed to adropin (10 and 100 nmol/l) in the presence of 2.8 or 16.7 mmol/l glucose for 60 min. Results are mean ± SEM, (n = 6 – 7). *P < 0.05.

Adropin suppresses glucose-induced cAMP production in INS-1E cells

cAMP signalling modulates insulin secretion in pancreatic beta cells (16, 17). Thus, we next assessed the effects of adropin on cAMP levels in INS-1E cells. As shown in *Fig. 4*, glucose at 16.7 mmol/l increased intracellular level of cAMP as compared with cells exposed to 2.8 mmol/l glucose. Moreover, stimulation of cAMP production by glucose was attenuated by adropin (100 nmol/l) (*Fig. 4*).

Adropin does not modulate INS-1E cell proliferation and viability

As shown in *Fig. 5A* and *5B*, adropin failed to modulate INS-1E cell proliferation assessed after 24 or 48 hours. Moreover, cell viability was not affected in cells exposed to adropin for 24 (*Fig. 5C*) or 48 h (*Fig. 5D*).

DISCUSSION

Our study shows that adropin suppresses insulin expression and secretion, while it fails to affect INS-1E cell replication and viability. First of all, we found that adropin's putative receptor *Gpr19* is expressed in INS-1E cells and rat pancreatic islets. By

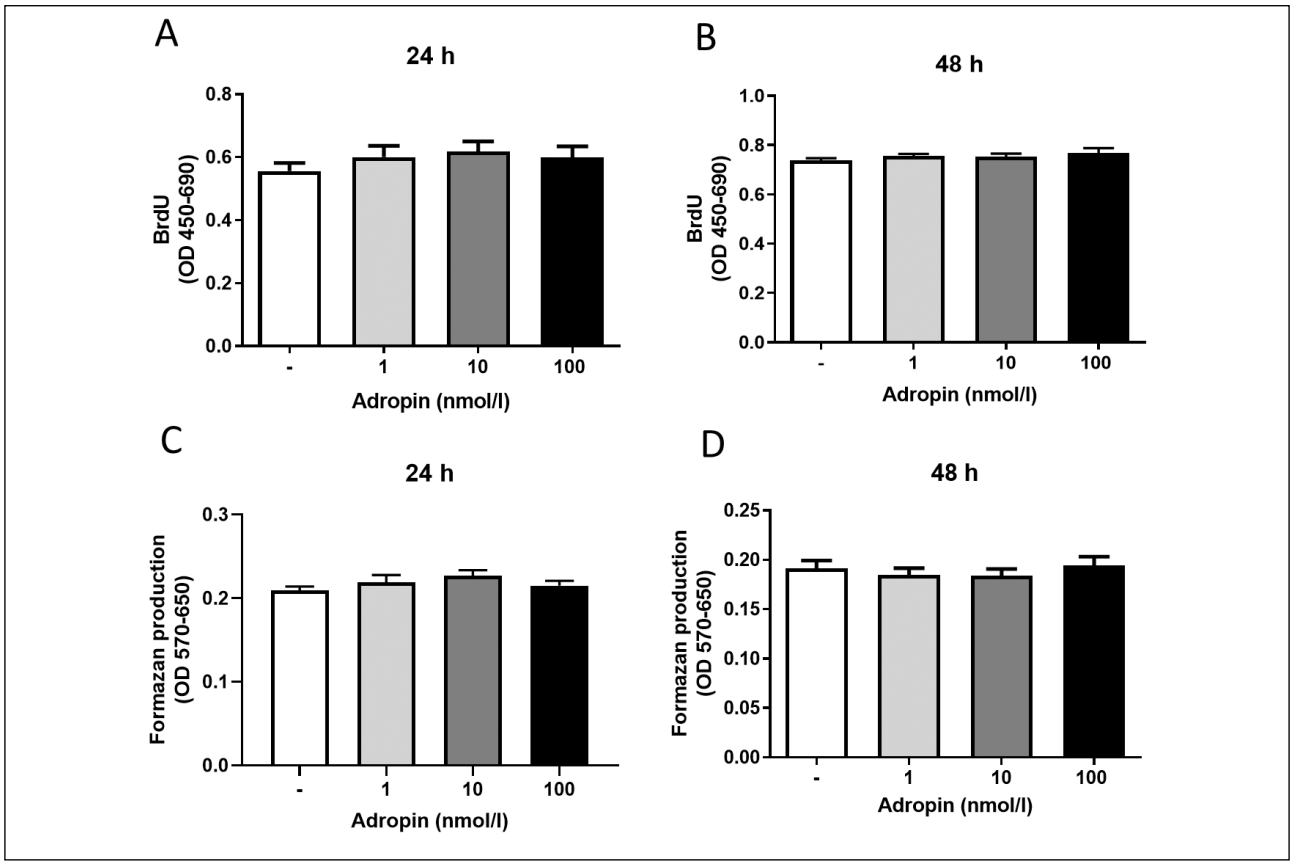

Fig. 5. Effects of adropin on proliferation and viability of INS-1E cells. Cell proliferation and viability measured by BrdU incorporation and formazan production, respectively, in cells exposed to adropin (1, 10 or 100 nmol/l) for 24 (A, C) or 48 hours (B, D). Results are mean ± SEM, (n = 8).

contrast, we found very low levels of *Enho* mRNA expression in INS-1E cells and the absence of *Enho* mRNA in rat pancreatic islets. These results indicate that adropin is rather not produced by endocrine cells in rat pancreas. It is worth to note that an earlier study showed that adropin is present in rat pancreas and its immunoreactivity is increased in animals with type 2 diabetes mellitus (18). Nevertheless, adropin immunoreactivity was found in the acini, thereby supporting our finding with respect to the lack of adropin production in the endocrine pancreas. Overall, these results suggest that adropin rather does not modulate endocrine cells of islets *via* autocrine mechanism. However, since adropin is present in the circulation (1, 18, 19), it cannot be excluded that adropin may affect pancreatic islets *via* endocrine mode of action.

Another finding in our study is the ability of adropin to suppress insulin mRNA expression and glucose-induced insulin secretion from both INS-1E cells as well as rat pancreatic islets. It is worth to note that adropin suppressed more potently *Ins2* mRNA expression. Importantly, *Ins2* is the orthologue of the human insulin gene (20) which may suggest a potential role of adropin in controlling human insulin mRNA expression. Nevertheless, the effect of adropin on insulin mRNA expression in human beta cells remains still unknown. An *in vivo* study showed that adropin treatment suppressed PKA phosphorylation and cAMP production in the liver (5). Moreover, the same work showed that adropin is able to attenuate forskolin-induced cAMP production in human liver cancer HepG2 cells (5). It is well known that insulin exocytosis is caused by an increase in intracellular calcium levels (21). Nevertheless, there is convincing evidence that glucose-induced insulin secretion is additionally mediated *via* cAMP and its downstream targets protein kinase A and Epac (22, 23). Thus, since adropin suppressed insulin exocytosis in our current study, we assessed the effects of adropin on glucose-induced cAMP levels in INS-1E cells. We found that adropin suppressed cAMP levels in INS-1E cells. These results suggest that adropin suppresses insulin secretion *via* downregulation of glucose-induced cAMP production in beta cells. In this context, it is also important to consider that cAMP is implicated in promoting insulin mRNA expression (24, 25). Therefore, these results suggest that adropin may attenuate insulin mRNA expression *via* downregulation of cAMP production.

Several studies showed that adropin is able to affect cell proliferation. For example, it was shown that adropin stimulates proliferation of endothelial or 3T3-L1 cells (26, 27). Since stimulation of beta cell replication is considered as a novel therapeutic strategy in diabetic patients (28), we assessed the effects of adropin on INS-1E cell proliferation. We failed to find any effect of adropin on cell vitality and replication. Overall, these data show that adropin's influence on mitogenesis is cell-specific. On the other hand, it needs to be pointed out that our observations are restricted to INS-1E cells, only. Thus, more experiments with a different beta cell line and/or primary beta cells are needed to confirm our findings.

To our best knowledge, this is the first report indicating that adropin may be involved in the modulation of endocrine activities of pancreatic beta cells. A previous study showed that mice over-expressing adropin display low levels of fasting insulin (1). In line with these results it was also found that intraperitoneal injection of adropin for 14 days significantly reduced fasting insulin levels in mice challenged to high-fat diet (1). Furthermore, another animal study showed that adropin deficiency is associated with increased fasting insulin levels (3). Consistently, others demonstrated that adropin treatment for 3 days attenuates fasting hyperinsulinemia in mice fed high-fat diet (29). These data may indicate that adropin may be considered as an insulinostatic factor. Nevertheless, it needs to be pointed out that *in vivo* adropin improves insulin resistance and ameliorates obesity-induced metabolic dysfunctions such increased triacylglycerol level (1). Thus, it remains unknown whether adropin prevents from hyperinsulinemia by acting on beta cells or whether lower levels of insulin in adropin-treated animals result from improved insulin sensitivity or lipid metabolism. More experiments are needed to study whether adropin suppresses insulin synthesis and/or secretion *in vivo*.

Our study has several limitations. First of all, all experiments were performed *in vitro* using either permanent cell model of beta cells or isolated islets. Thus, *in vivo* experiments should confirm insulinostatic action of adropin in the living organism. Moreover, although adropin suppresses cAMP production, more experiments are needed to answer the question regarding the role of adropin in calcium signalling in beta cells which plays an essential role in insulin exocytosis (30).

In summary, our study shows that adropin suppresses insulin mRNA expression in INS-1E cells but not cell proliferation. Furthermore, we demonstrated that adropin attenuates insulin mRNA expression in INS-1E cells and isolated rat pancreatic islets. In conclusion, these results suggest that adropin may contribute to glucose and lipid metabolism through suppression of insulin neogenesis and secretion from pancreatic beta cells.

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Conflict of interests: None declared.

REFERENCES

1. Kumar KG, Trevaskis JL, Lam DD, *et al.* I dentification of adropin as a secreted factor linking dietary macronutrient intake with energy homeostasis and lipid metabolism. *Cell Metab* 2008; 8: 468-481.
2. Stein LM, Yosten GL, Samson WK. Adropin acts in brain to inhibit water drinking: potential interaction with the orphan G protein-coupled receptor, GPR19. *Am J Physiol Regul Integr Comp Physiol* 2016; 310: R476-R480.
3. Ganesh Kumar K, Zhang J, Gao S, *et al.* Adropin deficiency is associated with increased adiposity and insulin resistance. *Obesity (Silver Spring)* 2012; 20: 1394-402.
4. Butler AA, Zhang J, Price CA, *et al.* Low plasma adropin concentrations increase risks of weight gain and metabolic dysregulation in response to a high-sugar diet in male nonhuman primates. *J Biol Chem* 2019; 294: 9706-9719.
5. Thapa D, Xie B, Manning JR, *et al.* Adropin reduces blood glucose levels in mice by limiting hepatic glucose production. *Physiol Rep* 2019; 7: e14043. doi: 10.14814/phy2.14043.
6. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 2001; 414: 799-806.
7. Wilcox G. Insulin and insulin resistance. *Clin Biochem Rev* 2005; 26: 19-39.
8. Tokarz VL, MacDonald PE, Klip A. The cell biology of systemic insulin function. *J Cell Biol* 2018; 217: 2273-2289.
9. Favaro E, Granata R, Miceli I, *et al.* The ghrelin gene products and exendin-4 promote survival of human pancreatic islet endothelial cells in hyperglycaemic conditions, through phosphoinositide 3-kinase/Akt, extracellular signal-related kinase (ERK)1/2 and cAMP/protein kinase A (PKA) signalling pathways. *Diabetologia* 2012; 55: 1058-1070.
10. Vilsboll T. The effects of glucagon-like peptide-1 on the beta cell. *Diabetes Obes Metab* 2009; 11 (Suppl. 3): 11-18.

11. Kaczmarek P, Skrzypski M, Pruszynska-Oszmalek E, *et al.* Chronic orexin-A (hypocretin-1) treatment of type 2 diabetic rats improves glucose control and beta-cell functions. *J Physiol Pharmacol* 2017; 68: 669-681.
12. Jung EM, Yoo YM, Jeung EB. Melatonin influences the expression and oligomerization of amylin in rat INS-1E cells. *J Physiol Pharmacol* 2019; 70: 695-703.
13. Billert M, Sassek M, Wojciechowicz T, *et al.* Neuropeptide B stimulates insulin secretion and expression but not proliferation in rat insulinproducing INS1E cells. *Mol Med Rep* 2019; 20: 2030-2038.
14. Skrzypski M, Khajavi N, Mergler S, *et al.* Orexin A modulates INS-1E cell proliferation and insulin secretion via extracellular signal-regulated kinase and transient receptor potential channels. *J Physiol Pharmacol* 2016; 67: 643-652.
15. Skrzypski M, Khajavi N, Mergler S, *et al.* TRPV6 channel modulates proliferation of insulin secreting INS-1E beta cell line. *Biochim Biophys Acta* 2015; 1853: 3202-3210.
16. Seino S, Takahashi H, Fujimoto W, *et al.* Roles of cAMP signalling in insulin granule exocytosis. *Diabetes Obes Metab* 2009; 11 (Suppl. 4): 180-188.
17. Zywert A, Szkudelska K, Szkudelski T. Inhibition of phosphodiesterase 3B in insulin-secreting cells of normal and streptozocin-nicotinamide-induced diabetic rats: implications for insulin secretion. *J Physiol Pharmacol* 2014; 65: 425-433.
18. Aydin S, Kuloglu T, Aydin S, *et al.* Expression of adropin in rat brain, cerebellum, kidneys, heart, liver, and pancreas in streptozotocin-induced diabetes. *Mol Cell Biochem* 2013; 380: 73-81.
19. Lian W, Gu X, Qin Y, Zheng X. Elevated plasma levels of adropin in heart failure patients. *Intern Med* 2011; 50: 1523-1527.
20. Shiao MS, Liao BY, Long M, Yu HT. Adaptive evolution of the insulin two-gene system in mouse. *Genetics* 2008; 178: 1683-1691.
21. Fu Z, Gilbert ER, Liu D. Regulation of insulin synthesis and secretion and pancreatic beta-cell dysfunction in diabetes. *Curr Diabetes Rev* 2013; 9: 25-53.
22. Tengholm A and Gylfe E. cAMP signalling in insulin and glucagon secretion. *Diabetes Obes Metab* 2017; 19 (Suppl. 1): 42-53.
23. Prentki M, Matschinsky FM. Ca2+, cAMP, and phospholipid-derived messengers in coupling mechanisms of insulin secretion. *Physiol Rev* 1987; 67: 1185-1248.
24. Nielsen DA, Welsh M, Casadaban MJ, Steiner DF. Control of insulin gene expression in pancreatic beta-cells and in an insulin-producing cell line, RIN-5F cells. I. Effects of glucose and cyclic AMP on the transcription of insulin mRNA. *J Biol Chem* 1985; 260: 13585-13589.
25. Melloul D, Marshak S, Cerasi E. Regulation of insulin gene transcription. *Diabetologia* 2002; 45: 309-326.
26. Jasaszwili M, Wojciechowicz T, Billert M, Strowski MZ, Nowak KW, Skrzypski M. Effects of adropin on proliferation and differentiation of 3T3-L1 cells and rat primary preadipocytes. *Mol Cell Endocrinol* 2019; 496: 110532.
27. Lovren F, Pan Y, Quan A, *et al.* Adropin is a novel regulator of endothelial function. *Circulation* 2010; 122: S185-S192.
28. Jiang WJ, Peng YC, Yang KM. Cellular signaling pathways regulating beta-cell proliferation as a promising therapeutic target in the treatment of diabetes. *Exp Ther Med* 2018; 16: 3275-3285.
29. Thapa D, Xie B, Zhang M, *et al.* Adropin treatment restores cardiac glucose oxidation in pre-diabetic obese mice. *J Mol Cell Cardiol* 2019; 129: 174-178.
30. Gilon P, Chae HY, Rutter GA, Ravier MA. Calcium signaling in pancreatic beta-cells in health and in Type 2 diabetes. *Cell Calcium* 2014; 56: 340-361.

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