

# Effect of C-peptide Alone or in Combination with Nicotinamide on Insulin Levels from Pancreatic Islets in Mouse

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## Abstract

**Background:** Both c-peptide and nicotinamide are known to increase blood insulin in diabetes. In the present study, we examined the effect of c-peptide alone or in combination with nicotinamide on insulin levels in pancreatic islets in mice.

**Methods:** This study was conducted with 60 adult male Naval Medical Research Institute (NMARI) mice weighing 25 to 30 g. Pancreatic islets from normal mice were isolated by the collagenase digestion method. Mice were divided into ten groups of six (n = 6): control, glyburide (1 and 10 µM), C-peptide (50 and 100 nM), nicotinamide (10, 25, and 100 mM), nicotinamide + C-peptide (100 mM and 100 nM), and buffer in different glucose concentrations (2.8, 5.6, and 16.7 mM). Insulin secretion was measured using insulin radioimmunoassay method.

**Results:** Insulin secretion significantly increased at 16.7 mM glucose concentration compared with 2.8 and 5.6 mM glucose concentrations. Incubation of islets at 2.8 and 5.6 mM glucose concentrations and nicotinamide + C-peptide, nicotinamide 25 and 100 mM, and C-peptide 100 nM significantly increased insulin secretion compared with the control group. In addition, incubation of islets at 16.7 mM glucose with nicotinamide + C-peptide significantly increased insulin secretion. Glyburide at 10 µM concentration was more effective than nicotinamide at 10 and 100 mM, C-peptide 50 and 100 nM in the presence of 16.7 mM glucose concentration. However, the combination of nicotinamide + C-peptide was more effective than glyburide at a concentration of 10 µM in the presence of a 16.7 mM glucose concentration.

**Conclusions:** This paper suggests that c-peptide, nicotinamide, and the combination of c-peptide and nicotinamide increases insulin secretion from pancreatic islets.

**Keywords:** C-peptide, glyburide, insulin, islets, mice, nicotinamide

## Introduction

Diabetes mellitus is a complex disorder, characterised by high blood glucose and abnormalities in carbohydrate, protein, and lipid metabolism (1). Recent estimates are that diabetes mellitus affects 246 million people worldwide, a figure anticipated to increase to 380 million by 2025 (2). An increase in the production of reactive oxygen species and oxidative stress destroy β-cells in pancreatic tissue and thus play an important role in the induction of diabetes (3). Insulin is a peptide hormone produced

by the pancreas and primarily responsible for maintaining homeostasis of blood glucose (4). Chronic high blood glucose in diabetes may cause serious complications such as retinopathy, hyperlipidemia, hepatopathy, cardiomyopathy, neuropathy, and nephropathy (5). The main goal of diabetes treatment is to control blood glucose in diabetic patients that is suggested as an effective method to impede the complications of diabetes and to improve the quality of life in these patients (6). One of the mechanisms for the reduction of blood glucose is the alteration in insulin release from pancreatic islets. Thus, several studies have

been established for the assessment of various compounds on insulin secretion from pancreatic islets (7). Endocrine cells of pancreatic islets consisted of single islets and are scattered in the acinar parenchyma (8). C-peptide, an effective peptide, is produced in beta cells, released into the blood circulation and has a physiological function in different tissues (9). C-peptide, a valid parameter of beta-cell function, is implicated in type 1 diabetes, distinguishing it from type 2 diabetes (10). C-peptide improves kidney, autonomic, and somatic nervous functions in type 1 diabetic patients, as well as enhancing blood flow and glucose consumption (11). C-peptide could regulate the metabolic functions of insulin, activate p38 protein kinase in lung capillary endothelial cells in the mouse, and ameliorate endothelial dysfunction via an increase of endothelial nitric oxide synthase gene transcription (12,13).

Nicotinamide, niacin derivative, is a water-soluble vitamin and its main metabolite is nicotinamide adenine dinucleotide (NAD). Nicotinamide is used for the induction of type 2 diabetes in animals (15). Nicotinamide increases energy production and has antioxidant and antiapoptotic effects, as well as participating in signal transduction (16). Nicotinamide has an important role in the protection of B-cells and the prevention of type 1 diabetes (17). Previous studies have demonstrated that nicotinamide can prevent interleukin-1 beta or macrophage-induced beta-cell injury and decrease the production of IL-12 and TNF-alpha. A decrease of IL-12 production by nicotinamide could play a critical role in the regulation of the immune response in type 1 diabetes (18).

It is increasingly evident that diabetes needs to be treated. New diabetes treatment methods should thus be explored. Metabolic regulators and antioxidant agents such as C-peptide and nicotinamide have been recommended for the improvement and prevention of diabetes; however, there is a lack of empirical studies on the effects of C-peptide and nicotinamide (alone and in combination) on insulin secretion from pancreatic islets. The present study aims to evaluate the effects of C-peptide, alone and in combination with nicotinamide, on the insulin levels of pancreatic islets in mice.

## Material and Methods

### *Animals*

Adult male Naval Medical Research Institute (NMRI) mice weighing 25 to 35 g (aged 5 weeks) were bought from the animal facility of the Ahvaz

university (Iran) and housed in polypropylene cages at a regulated temperature of 21 to 23°C, humidity of 65 ± 10%, and 12-h simulated light term for one week. They were provided with water and pellet food. Protocol comprises mice, and their handling was in accordance with accepted guidelines for the care of laboratory animals.

### *Chemicals*

NaCl, CaCl<sub>2</sub>, MgSO<sub>4</sub>, KCl, NaH<sub>2</sub>PO<sub>4</sub>, NaHCO<sub>3</sub>, glucose, Hepes, collagenase, nicotinamide, and glyburide were bought from Sigma-Aldrich Co. (St. Louis, MD, USA). C-peptide was purchased from the Bachem Company (Germany). IRMA kit for the measurement of insulin was bought from a Belgian company (DiaSource Belgium, INS).

### *Isolation of pancreatic islets and assay of insulin secretion*

All tools and material were prepared before killing the mice. Buffer solution was prepared by 140 mM NaCl, 4.5 mM KCl, 2.5 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 20 mM Hepes, and the glucose concentration according to each experimental condition with pH at 7.4. A total of 100 mL of buffer solution was combined with 5% bovine serum albumin (Sigma, USA) (19). Isolation of pancreatic islets was conducted using the Lacy and Kostainovsky method (20). The mice were killed by cervical dislocation. The abdomen was wet with 70% alcohol (Simin tak toos, Iran) to reduce the risk of infection by hairs in the abdominal cavity during the later stages of investigation. The abdomen was opened with scissors in a V-shape incision beginning from the lower abdomen and stretching to the external parts of the diaphragm forward, to expose all organs in the abdominal cavity. The pancreas is annexed to the spleen. After cannulation into the common bile duct and an injection of 4 to 5 mL of buffer solution and collagenase (1 mg/mL), the spleen was located and raised by forceps. The pancreas was detached from visceral tissues with miniature scissors, then placed in a 15 mL buffer solution for approximately ten minutes at 37°C, and shaken to separate the tissue. Then the cold buffer was used to retard the digestion caused by the collagenase and was twice centrifuged for five minutes at 1200 rpm. Subsequently, the supernatant was disposed of and the remaining solution returned to a black petri dish for purification of the islets by a stereomicroscope (Kyowa optical, SDZ-TR-PL, Japon) (8). Islets were divided into ten groups of six, each placed in a 1 mL/dish, in 2.8 mM, 5.6 mM, and 16.7 mM glucose concentrations. Cell viability after the isolation of the pancreatic islets

was evaluated by assay of insulin secretion in the presence a 2.8 mM glucose concentration (21).

1- Saline, 2- glyburide (1 and 10  $\mu$ M) [22], 3- C-peptide (50 and 100 nM) [23], 4- nicotinamide (10, 25, and 100 mM) [24], 5- C-peptide + nicotinamide (100 nM and 100 mM), and 6- control (buffer). All groups were exposed for 30 min at 37°C and 95 % O<sub>2</sub> and 5% CO<sub>2</sub>. C-peptide, nicotinamide, and glyburide were dissolved in saline. These stages were repeated eight times. Finally, the supernatant was removed and the released insulin examined using an IRMA kit (DiaSource, Belgium, INS). Intra- and inter-assay coefficients of variation were 2/1% and 4/2% respectively, and low-end sensitivity of insulin was 1  $\mu$ U/mL. IRMA kit had 100% cross-reactivity with the insulin of a mouse. Dithizone (DTZ, Sigma-Aldrich, USA) was used to identify islets in the material obtained by collagenase digestion of mouse pancreas.

### Statistical analysis

Data are expressed as a mean  $\pm$  standard deviation (SD). The one sample Kolmogorov–Smirnov test was used to test whether a sample was derived from a specific distribution. We concluded that there was no significant difference between the experimental data and normally distributed data. Thus, data were analyzed using SPSS version 15. One- and two-way analysis of variance (ANOVA) and a post hoc (LSD) test were conducted. *p* was significant at the level of 0.05.

## Results

Glucose at 5.6 mM and 16.7 mM significantly increased insulin secretion from pancreatic islets ( $P < 0.01$ ,  $P < 0.001$ , respectively), compared with the 2.8 mM group. Saline in the presence of 2.8, 5.6, and 16.7 mM glucose concentrations significantly decreased insulin secretion from pancreatic islets ( $P < 0.001$ ,  $P < 0.01$ ,  $P < 0.001$ , respectively), compared with the control group (Figure 1).

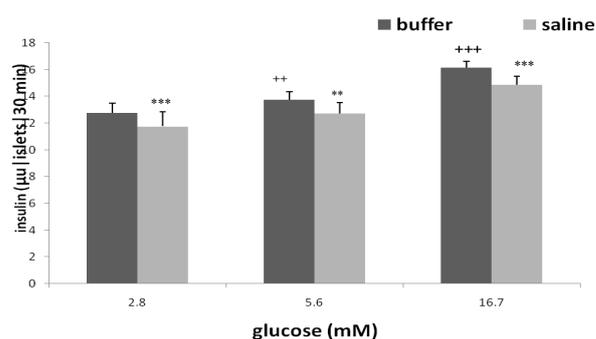
The effect of C-peptide, nicotinamide, c-peptide + nicotinamide, and glyburide on insulin secretion from pancreatic islets was as follows. In the presence of a 2.8 mM glucose concentration, C-peptide at 100 nM ( $P < 0.01$ ), nicotinamide at 100 mM ( $P < 0.001$ ), and C-peptide (100 nM) + nicotinamide at 100 mM ( $P < 0.001$ ) significantly increased insulin release from pancreatic islets, compared with the saline group (Figure 2).

In the presence of a 5.6 mM glucose concentration, nicotinamide at 25 and 100 mM ( $P < 0.01$ ,  $P < 0.01$  respectively), C-peptide at

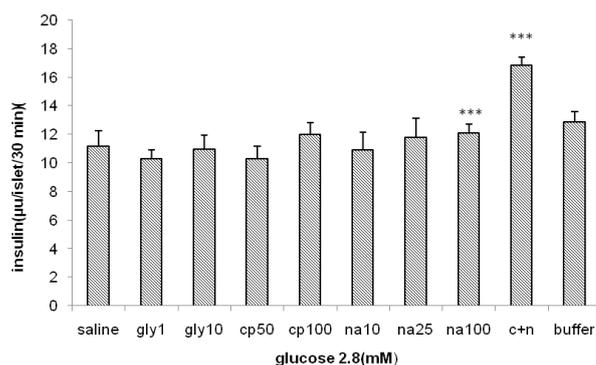
100 nM + nicotinamide at 100 mM ( $P < 0.001$ ), and glyburide at 10  $\mu$ M ( $P < 0.01$ ) significantly increased insulin release from pancreatic islets compared with the saline group (Figure 3).

In the presence of 16.7 mM glucose concentration, C-peptide (100 nM) + nicotinamide (100 mM) ( $P < 0.001$ ) significantly increased insulin release from pancreatic islets, compared with the saline group (Figure 4).

As anticipated, all islets stained with DTZ in the culture medium became crimson red,



**Figure 1:** The effects of saline and buffer with different concentrations of glucose on insulin secretion from pancreatic islets (mean  $\pm$  SD).  $n = 6$ ,  $**P < 0.01$ ,  $***P < 0.001$  when compared with buffer group,  $++ P < 0.01$ ,  $+++ P < 0.001$  when compared with 2.8 group.

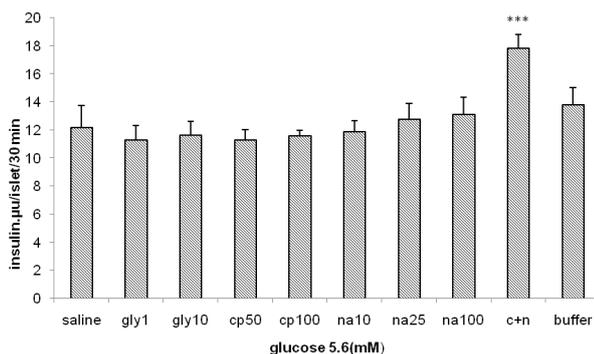


**Figure 2:** The effects of C-peptide (cp) at 50 and 100 nM, nicotinamide (na) at 10, 25, and 100 mM, nicotinamide + C-peptide [c(100nM) + n(100mM)] and glyburide (gly) at 1 and 10  $\mu$ M with 2.8 glucose concentration on insulin secretion.  $N = 6$ ,  $*P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$  whereas compared with saline group, F ratio 12.27.

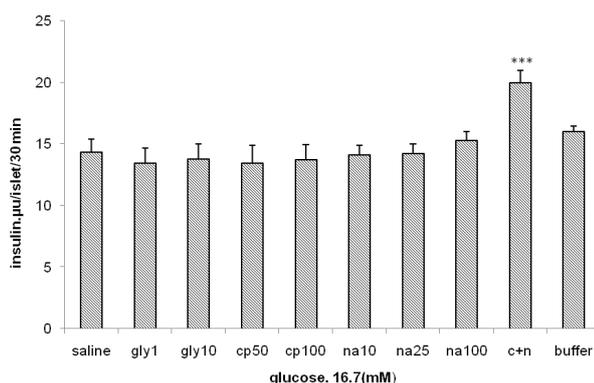
indicating that only islets were used in the investigation (Figure 5).

## Discussion

In the present study, insulin secretion at 5.6 mM and 16.7 mM glucose concentrations increased significantly, compared with secretions at the 2.8 mM glucose concentration. These results



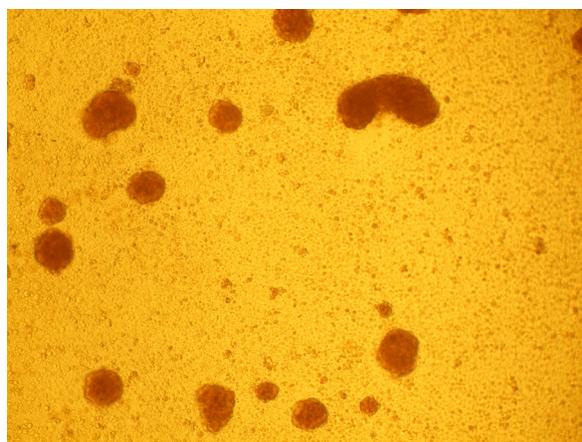
**Figure 3:** The effects of C-peptide (cp) at 50 and 100 nM, nicotinamide (na) at 10, 25, and 100 mM, nicotinamide + C-peptide [c(100nM)+n(100mM)] and glyburide (gly) at 1 and 10 µM with 5.6 glucose concentration on insulin secretion. N = 6, \*\**P* < 0.01, \*\*\**P* < 0.001 when compared with saline group, F ratio 7.80.



**Figure 4:** The effects of C-peptide (cp) at 50 and 100 nM, nicotinamide (na) at 10, 25, and 100 mM, Nicotinamide + C-peptide [c(100nM) + n(100mM)] and glyburide (gly) at 1 and 10 µM with 16.7 glucose concentration on insulin secretion., n = 6, \*\*\**P* < 0.001 when compared with saline group, F ratio 29.79.

indicate that glucose-induced insulin secretion is dose-dependent, consistent with our previous study on mouse beta cells (25). In the presence of different glucose concentrations, saline reduced insulin secretion from pancreatic islets, compared with a buffer group. The mechanism of this is proposed to be activation of the Na<sup>+</sup>/Ca<sup>2+</sup> exchanger. Glucose-induced insulin secretion in the presence of saline was significantly decreased, which might also relate to the increase in Ca<sup>2+</sup> extrusion and subsequent decrease in cellular Ca<sup>2+</sup> content and insulin secretion. previous study reported that saline decreases insulin secretion in pancreatic islets (26).

Glyburide is an effective antidiabetic drug used to treat type 2 diabetes (27). Glyburide at 10 µM concentration increased insulin secretion, compared with the saline group in the presence of a 5.6 mM glucose concentration; at 1 µM, this concentration had no effect on insulin secretion. Previous studies suggest that this drug leads to an increase in insulin release from pancreatic islets at 10 µM concentration (28). The best response of glyburide to insulin release is in the presence of 5.6 mM glucose concentration, which is the same fasting plasma glucose concentration in mouse (29). Glyburide at high glucose concentration (16.7 mM) reduced insulin secretion, consistent with previous studies (30,31). Glyburide has no effect on insulin secretion in the presence of 16.7 mM glucose concentration, which might relate to glucose toxicity and the presence of high glucose concentrations as a potent activator of insulin secretion, interfering with glyburide (32). Incubation of pancreatic islets at 2.8 mM glucose concentration and C-peptide at 100 nM significantly increased insulin secretion,



**Figure 5:** Staining isolated islets by Dithizone in the culture medium.

although the effect of C-peptide on insulin release was concealed in the 5.6 and 16.7 mM glucose concentrations. C-peptide exerts no direct effect on insulin secretion in human islets (33). Previous studies have demonstrated that C-peptide increased blood flow in pancreatic microcirculation and stimulated insulin secretion. C-peptide increases protein kinase c activity, intracellular calcium of the kidney proximal tubular cells, and Na<sup>+</sup>, K<sup>+</sup>-ATPase activity of pancreatic islets (23). The effect of C-peptide at 100 nM concentration on insulin release in this experiment can probably be related to the increase protein kinase c, Na<sup>+</sup>, K<sup>+</sup>-ATPase activity, and intracellular calcium in pancreatic islets. Nicotinamide at 25 mM concentration in present 5.6 mM glucose concentration increased insulin secretion from isolated islets (17). Nicotinamide is known to have antiapoptotic effects on pancreatic islets. Previous studies have demonstrated that nicotinamide can increase plasma insulin levels in diabetic rats (16). One of the possible mechanisms for the excitatory effect of nicotinamide on insulin secretion is its major metabolite, nicotinamide adenine dinucleotide (NAD) (34). NAD participates in glycolyse pathways and increases ATP production, leading to the closing of the ATP-sensitive potassium channels and stimulation of insulin secretion. C-peptide (100 nM) + nicotinamide (100 mM) in the present range of glucose concentrations increased insulin release from pancreatic islets, compared with C-peptide and nicotinamide alone. In contrast, C-peptide + nicotinamide have an additive effect on insulin secretion. Nicotinamide + C-peptide were more effective than glyburide at 10 μM concentration in present 16.7 mM glucose concentration. Further studies are required to understand the exact mechanisms by which C-peptide, nicotinamide, and C-peptide + nicotinamide enhance insulin secretion from pancreatic islets.

## Conclusions

This paper suggests that C-peptide, nicotinamide, and the combination of C-peptide and nicotinamide may increase insulin secretion from pancreatic islets.

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## Conflict of Interest

None.

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## Authors' Contributions

Conception and design: AA

Analysis and interpretation of the data, drafting of the article, final approval of the article, provision of study materials or patients, statistical expertise, obtaining of funding, administrative, technical, or logistic support, collection and assembly of data: AA, FRAA

Critical revision of the article for important intellectual content: AA, FRAA, HFM

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