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INGAP-related pentadecapeptide: Its modulatory effect upon insulin secretion

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Abstract

We examined the effects of a pentadecapeptide having the 104–118 aminoacid sequence of islet neogenesis-associated protein (INGAP-PP) on insulin secretion, and the morphological characteristics of adult and neonatal pancreatic rat islets cultured in RPMI and 10 mM glucose for 4 days, with or without different INGAP-PP concentrations (0.1–100 μg/ml). A scrambled 15 aminoacid peptide was used as control for the specificity of INGAP-PP effect. Cultured neonatal and adult islets released insulin in response to glucose (2.8–16.7 mM) in a dose-dependent manner, and to leucine and arginine (10 mM). In all cases, the response was greater in adult islets. INGAP-PP added to the culture medium significantly enhanced glucose- and aminoacid-induced insulin release in both adult and newborn rats; however, no changes were observed with the scrambled peptide. Similar results were obtained incubating freshly isolated adult rat islets with INGAP-PP. Whereas INGAP-PP did not induce significant changes in islet survival rate or proportion/number of islet cells, it increased significantly β-cell size. This first demonstration of the enhancing effect of INGAP-PP on the β-cell secretory response of adult and newborn islets opens a new avenue to study its production mechanism and potential use to increase the secretory capacity of endogenous islets in intact animals or of islets preserved for future transplants.

Keywords: Islets; Islet culture; INGAP-PP; Islet growth factor; Insulin release

1. Introduction

An appropriate secretion of insulin is necessary to provide a sufficient supply of glucose and other metabolic fuels to body tissues, and to maintain serum glucose concentrations within normal range. Pancreatic β-cells can cope with these functions by coupling insulin secretion to differing conditions of fuel availability, such as the fasting and postprandial states, as well as to the uneven response of different tissues to insulin. To assure such precision, β-cell function is controlled by an ever increasing list of complex regulatory factors [1].

During the last years we have been studying one of these molecules – islet neogenesis-associated protein (INGAP) – and its relationship with the changes induced by chronic sucrose feeding to normal hamsters and rats on β-cell function and islet cell populations [2–6]. In hamsters, we have consistently found a simultaneous and significant increase in β-cell mass [3], INGAP-positive cell mass [7], glucose-induced insulin secretion, and the appearance of precursor cells co-expressing INGAP/Pdx-1 [4].

INGAP was identified as part of a pancreatic protein complex originally isolated from normal hamsters, whose pancreas heads were previously wrapped in cellophane (CW) [8]. Although it was later identified that the *INGAP* gene expressed only in the exocrine sector of the CW

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pancreas [9], we have recently demonstrated its transcription and expression both in normal hamster islets and exocrine cells [10].

It has been shown that a pentadecapeptide having the 104–118 aminoacid sequence of INGAP (INGAP-PP) reproduces the effect of the intact molecule upon thymidine incorporation into ductal cells and a ductal cell line [9]. This peptide also enhances neurite outgrowth from dorsal root ganglia in vitro in a time- and dose-dependent manner [11]. This effect correlates with an increase in [^{3}H]thymidine incorporation and mitochondrial activity. Rosenberg et al. have recently reported an increase in β-cell mass and signs of neogenesis after intraperitoneal injection of this peptide to either normal or streptozotocin-induced diabetic mice [12].

The aim of this study was to test the effect of a pentadecapeptide with the 104–118 aminoacid sequence of INGAP upon insulin secretion and islet morphology. Hence, we measured (1) glucose- and aminoacid-induced insulin secretion and morphological cell changes in islets isolated from newborn and adult normal rats previously cultured with different concentration of the peptide, and (2) glucose-induced insulin secretion of freshly isolated islets in the presence or absence of INGAP-PP. Using this approach, we have shown that INGAP-PP significantly enhances the release of insulin in response to glucose and aminoacids, and increases β-cell size in islets isolated from normal adult rats.

2. Materials and methods

2.1. Chemicals and drugs

Collagenase was obtained from Boehringer Mannheim, Indianapolis; bovine serum albumin (BSA) fraction V and other reagents of the purest available grade were from Sigma Chemical Co (St. Louis, MO). INGAP-PP was synthesized at the Laboratory of Peptide Synthesis, School of Pharmacy and Biochemistry, Buenos Aires University, Argentina.

2.2. Pentadecapeptide synthesis

Peptide synthesis was performed on a 431A Applied Biosystems peptide synthesizer using Fmoc solid-phase methodology on HMP (*p*-hydroxymethylphenoxymethyl polystyrene) resin [13]. Aminoacids, previously activated with HOBt/DCC (1-hydroxybenzotriazole/dicyclohexyl-carbodiimide), were incorporated using trityl (Asn, His) and *tert*-butyl (Thr and Ser) as side-chain protecting groups. The peptide was cleaved off the resin with 2% ethanedithiol, 5% thioanisole, 5% phenol and 5% water in trifluoroacetic acid; it was then precipitated by adding cold diethyl ether, and finally lyophilized. Final purification was achieved by high-performance liquid-chromatography (HPLC) on a C18 Vydac 218TP 510 semi preparative column eluted with an acetonitrile gradient (12% to 80%). Quality control of the peptide (aminoacid analysis and mass spectrometry) indicated >95% purity and a molecular weight of 1501.63. As control for the specificity of the INGAP-PP effect, we used a peptide with the same 15 aminoacids in its molecule, but arranged in a completely different sequence (Ser-Ser-Thr-Gly-Gly-Gly-Asp-Ile-Pro-Pro-His-Leu-Leu-His-Asn). This "scrambled peptide" (95.3% purity and a molecular weight of 1501.63) had been previously reported to be inactive by Rosenberg et al. [12].

2.3. Animals and islet isolation

Male adult (100–150 g) and neonatal (1–2 days old) Wistar rats were used as source of pancreas. Adult rats were housed in a room at 21±1 °C and 50±5% humidity with a 12-h light/12-h dark cycle, and fed a commercial diet and tap water ad libitum. The animals were sacrificed by cervical dislocation and the entire pancreas was removed and digested with collagenase to isolate islets [14]. To obtain islets from neonatal rats, 20–25 pancreases were minced in 1 ml sterile Hanks' solution and digested with collagenase. Animal experiments were approved by the Committee for Ethics in Animal Experimentation (CEEA/IB/UNICAMP).

2.4. Islet culture

After digestion, islets from adult or neonatal rats were maintained at 37 °C during 4 days in RPMI 1640 (Gibco BRL, California, USA) pH 7.4, containing 2 g/L NaHCO3, 5% (v/v) fetal calf serum, 1% penicillin/streptomycin, and 10 mM glucose in a humid atmosphere (5% CO_2/95% O_2), with or without the addition of differing INGAP-PP concentrations (0.1, 0.25, 0.5, 1.0 and 100 μg/ml) or scrambled peptide, as summarized in the figure and table legends.

2.5. DNA content

Islets from adult and neonatal rats cultured with or without INGAP were homogenized and stored at −70 °C for subsequent measurement of DNA content by the fluorometric assay [15].

2.6. Insulin secretion studies

Cultured islets from adult or neonatal rats were rinsed in Krebs–Ringer bicarbonate buffer (KRB), pH 7.4, previously gassed with a mixture of CO_2/O_2 (5/95%), and preincubated in 1.0 ml of KRB containing 1.5% (w/v) BSA and 5.6 mM glucose at 37 °C for 45 min. After this period, groups of 5 islets were incubated in 0.6 ml KRB with the addition of 2.8, 8.3 or 16.7 mM glucose for 90 min [16]. In other experiments, the incubation was performed under the