CALCYCLIN, A CALCIUM-BINDING PROTEIN, WHICH REGULATES INSULIN SECRETION FROM THE PERMEABILIZED PANCREATIC \( \beta \)-CELL

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INTRODUCTION

Although it is widely accepted that an increase in intracellular \( \text{Ca}^{2+} \) is a crucial event in the stimulus-secretion coupling in the pancreatic \( \beta \)-cell [1], we have limited knowledge on the later steps in the signal transduction pathway. Calcium binding proteins are considered to be involved in the control of \( \text{Ca}^{2+} \)-dependent cellular functions including hormone release [2]. Various types of calcium-binding proteins such as calmodulin, calbindin and S-100b protein are identified in the islet cells biochemically or immunohistochemically [3,4]. Calmodulin, the best characterized calcium binding protein, was demonstrated to exist in the pancreatic islet cells by Sugden et al [5]. The pancreatic \( \beta \)-cell possesses the kinase activity which is dependent on \( \text{Ca}^{2+}/\text{calmodulin} [6] \). Involvement of calmodulin and calmodulin-dependent protein kinases in insulin release has been suggested mainly based on the inhibitory effect of calmodulin antagonists on the release [7,8,9], albeit there is the argument against this idea [10].

Calcyclin is a 10.5 kDa Ca-binding protein with two EF-hands. It was reported that expression of calcyclin is dependent on cell cycle [11]. There is a report that a calcyclin-like mouse decidual protein may be involved in the secretion of lactogen-II [12]. In this study, we aimed to elucidate the role of calcyclin in insulin release from the rat pancreatic islets.

METHODS AND MATERIALS

Immunoblot Analysis

The polyclonal antibodies against calcyclin were raised in a guinea pig by injecting rabbit lung calcyclin. For immunoblot analysis, pancreatic islets were isolated from
female rabbits of the Japanese White strain using collagenase digestion. The islets were homogenated in 1mM EGTA and 20mM Tris-HCl (pH 7.5), and centrifuged at 100,000g. The supernatant was loaded onto SDS-PAGE and transferred to PVDF membranes. The procedure of Burnette [13] was then followed with minor modifications.

**Light-Microscopic Immunohistochemistry**

The sections of rabbit pancreas were incubated in 10% goat serum for 30min and then in anti-calcyclin or anti-insulin antibodies for 12hrs. After washing in phosphate-buffered saline (PBS) with 5mM Tris-HCl (pH 7.6), the sections were incubated in the biotinylated goat anti-guinea pig antibody for 30min. The tissue was washed in PBS, incubated in avidin horseradish-peroxidase for 30min and washed again in PBS. Finally, the bound antibody was visualized by peroxidase reaction.

**Permeabilization of Pancreatic Islets with Streptolysin-O (STLO)**

Pancreatic islets were isolated from male Wistar rats by collagenase digestion. The solution used during the isolation was Hepes-buffered Krebs-Ringer bicarbonate buffer containing 119mM NaCl, 4.75mM KCl, 5mM NaHCO₃, 2.54mM CaCl₂, 1.2mM MgSO₄, 1.2mM KH₂PO₄ and 20mM Hesp (pH 7.4 with NaOH) supplemented with 3mM glucose. Groups of size-matched 5 islets were preincubated at 37°C for 1hr in the same buffer with 5mg/ml bovine serum albumin gassed with 95% O₂/5% CO₂. The islets were then washed twice with 0.8ml of the glutamate buffer containing 100mM K-glutamate, 42mM Na-glutamate, 16mM Hesp, 5mg/ml bovine serum albumin, 1mM EGTA and 3mM Mg-ATP. The islets were permeabilized by incubation with 0.125 IU/ml STLO (Eiken, Tokyo, Japan) in the same glutamate buffer with various additions. After the 45min incubation, the amount of released insulin was measured by enzyme-linked immunoassay.

**Others**

Rabbit calcyclin was isolated from rabbit lungs as described previously [14]. For the secretion experiments, calcyclin was dialyzed against the glutamate buffer with 10⁻⁷ M Ca²⁺. Calgizzarin was prepared from chicken gizzard as described elsewhere [15]. W-77 ((S)-P-(2-aminoethoxyloxy)-N-[2-(4-benzyloxy-carbonyl))piperazine]-1-(P-methoxy-benzoyl)ethyl]-N-methylbenzene-sulfonamide dihydrochloride) was synthesized by Hidaka et al. [16].

**RESULTS AND DISCUSSION**

**Immunological Identification of Calcyclin in the Pancreatic Islet Cells**

Fig. 1a demonstrates the presence of calcyclin-like immunoreactivity in the pancreatic islet cells. Immunohistochemical experiments revealed the presence of calcyclin-like immunoreactivity with some heterogenous distribution in the rabbit islet cells. The activity was partly co-localized with the insulin-like immunoreactivity, but not found in the pancreatic acinar cells. Fig. 1b shows immunoblot analysis of the rabbit islet homogenate using the anti-calcyclin antibody. It resulted in two immunopositive bands at 10.5 and 21kDa in the positive control (purified rabbit lung calcyclin; lane 1) and the islet homogenate (lane 2). The higher band at approx. 98kD, however, was observed only in purified