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Sulfonylurea and glinide reduce insulin content, functional expression of K_{ATP} channels, and accelerate apoptotic β-cell death in the chronic phase

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Abstract

We previously found that chronic exposure to glibenclamide inhibits acute glibenclamide-induced insulin secretion by reducing the number of functional ATP-sensitive K^+ (K_{ATP}) channels on the plasma membrane of pancreatic β-cells. In the present study, we compared sulfonylurea-induced and glinide-induced insulin secretion in pancreatic β-cells chronically exposed to these widely used oral hypoglycemic agents. Chronic exposure of pancreatic β-cells to sulfonylureas (glibenclamide or tolbutamide) and glinide (nateglinide) similarly impaired their acute effectiveness by reducing the insulin content and the number of functional K_{ATP} channels on the plasma membrane. Functional expression of the voltage-dependent Ca^{2+} channels (VDCCs), ion channels that play a critical role in the K_{ATP} channel dependent insulin secretory pathway, was similar to that in drug-untreated cells. Chronic exposure to each of the three agents similarly accelerated apoptotic β-cell death. Thus, reduction of the insulin content, reduction of the number of functional K_{ATP} channels on the plasma membrane, and acceleration of apoptotic β-cell death all are involved in impaired insulinotropic agent-induced acute insulin secretion in the chronic phase of sulfonylurea and glinide treatment. These findings help to clarify the mechanism of secondary failure after long-term therapy by these hypoglycemic agents, and should have important clinical implications regarding pharmacotherapy for type 2 diabetes.

Keywords: Insulinotropic agents; Chronic exposure; K_{ATP} channel; Insulin secretion; Secondary sulfonylurea failure

1. Introduction

Sulfonylureas and glinide drugs stimulate insulin secretion from pancreatic β-cells, and are widely used oral hypoglycemic agents in the treatment of type 2 diabetes [1,2]. Their principal target is the ATP-sensitive potassium (K_{ATP}) channels in pancreatic

Abbreviations: K_{ATP}, ATP-sensitive K^+; VDCCs, voltage-dependent Ca^{2+} channels; DMEM, Dulbecco's modified Eagle's medium; KRBB, Krebs–Ringer bicarbonate buffer; RIA, radioimmunoassay; TUNEL, TdT-mediated dUTP nick end labeling; PBS, phosphate buffered saline; DMSO, dimethylsulfoxide

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β-cells [3], a key regulator of glucose- and sulfonylurea-induced insulin secretion [4]. By cloning members of the inwardly rectifying K^+ channel subfamily and the receptors for sulfonylureas, we have clarified their molecular structure [5–7]. We also have investigated the physiological roles of K_{ATP} channels in vitro and in vivo [5–8]. The K_{ATP} channel is an octamer formed by the physical association of inwardly rectifying K^+ (Kir) channel subunits (Kir6.1 or Kir6.2) and regulatory sulfonylurea receptor subunits (SUR1, SUR2A, or SUR2B) [7]. Different combinations of these subunits comprise the K_{ATP} channels in pancreatic β-cells (Kir6.2 plus SUR1), cardiac myocytes (Kir6.2 plus SUR2A), vascular smooth muscles (Kir6.1 plus SUR2B), and substantia nigra pars reticulata in brain (Kir6.2 plus SUR1) [7,8]. In pancreatic β-cells, inhibition of the K_{ATP} channels by glucose, and by sulfonylurea and glinide drugs, causes depolarization of the β-cell membrane. This triggers opening of the voltage-dependent Ca^{2+} channels (VDCCs), eliciting Ca^{2+} influx and a rise in intracellular Ca^{2+} that stimulates exocytosis of the insulin-containing secretory granules [9]. In treatment by insulinotropic agents including the sulfonylureas (e.g., glibenclamide and tolbutamide) and glinides (e.g., nateglinide), the drug is usually taken orally every day with a long-range plan for the patient. Karam et al. reported that exposure of pancreatic β-cells to sustained stimulation by therapeutic doses of sulfonylureas can selectively abolish their responsiveness to acute stimulation with tolbutamide in type 2 diabetes [10]. Treatment of diabetic patients with sulfonylureas often causes secondary failure for reasons that might be linked either to evolution of the disease or to effects of the drugs [11,12]. It also has been suggested that perturbations in glucose-induced insulin secretion provoked by sulfonylureas might result from dysfunction of the K_{ATP} channels [13–16], a target that the drugs share with glucose. Although the transient effects of sulfonylureas and glinides on insulin secretion have been widely investigated, the mechanism of the decreasing effect of acute stimulation on insulin secretion after chronic exposure to the drugs, so-called secondary failure, is not fully understood. In this study, we investigated the mechanisms of inhibited acute sulfonylurea-induced and glinide-induced insulin secretion in pancreatic β-cells exposed chronically to stimulus by the drugs. We found reduced insulin content and a reduced number of functional K_{ATP} channels on the plasma membrane, as well as accelerated apoptotic β-cell death, which together might contribute to the progressively impaired acute insulinotropic action of these agents.

2. Materials and methods

2.1. Culture of MIN6 cells

MIN6 is a mouse pancreatic β-cell line with an insulin secretory response to glucose, sulfonylureas, and other secretagogues [17,18]. MIN6 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Sigma, St. Louis, U.S.A.) containing 25 mM glucose supplemented with 13% heat-inactivated fetal bovine serum (Bio-Whittaker, Maryland, U.S.A.), 65.5 mg/l U/ml penicillin G (Sigma, St. Louis, U.S.A.), 100 mg/l streptomycin (Meiji Seika, Tokyo, Japan), and 5 μl/l β-mercaptoethanol (Nacalai Tesque, Kyoto, Japan) in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C.

2.2. Long-term exposure of insulinotropic agents

MIN6 cells were plated on 48-well plate at a density of 1×10^5 cells/well in DMEM medium containing 25 mM glucose and the test agent (100 nM glibenclamide, 300 μM tolbutamide, or 100 μM nateglinide) and cultured for 3 days (72 h).

2.3. Measurement of insulin secretion

After long-term exposure, MIN6 cells were washed with HEPES-balanced Krebs–Ringer bicarbonate buffer (KRBB: NaCl 129.4 mM, KCl 5.2 mM, $CaCl_2$ 2.8 mM, KH_2PO_4 1.3 mM, $MgSO_4 \cdot 7H_2O$ 1.3 mM, $NaHCO_3$ 24.8 mM, 10 mM HEPES with 2.8 mM glucose, 0.2% bovine serum albumin adjusted to pH 7.4) before the experiments. For batch incubation, MIN6 cells were preincubated for 30 min in KRBB containing 2.8 mM glucose without test agent, and the cells then were stimulated in KRBB containing 2.8 mM glucose with various concentrations of the agents for 2 h. The supernatant was removed and insulin released into the medium was measured by radioimmunoassay (RIA). To determine insulin content, MIN6 cells were homogenized in 400 μl acid–ethanol (37% HCl in 75% ethanol, 15:1000 (v/v)) and extracted at 4 °C overnight. The acidic extracts were dried by vacuum, reconstituted, and insulin was measured by RIA.

2.4. Detection of β-cell apoptotic cell death

The TdT-mediated dUTP nick end labeling (TUNEL) method was used to detect DNA strand breaks formed during apoptosis [5]. The MIN6 cells were plated on a slide glass in a 100 mm dish at a