Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 69 (2005) 59–63
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Alterations of insulin secretion following long-term manipulation of ATP-sensitive potassium channels by diazoxide and nateglinide

Andrew J. Ball[a,b,*], Peter R. Flatt[a], Neville H. McClenaghan[a]

[a]*School of Biomedical Sciences, University of Ulster, Coleraine, Northern Ireland, BT52 1SA, UK*
[b]*UCSD Cancer Center, 9500 Gilman Drive, La Jolla, CA 92093-0816, USA*

Received 23 June 2004; accepted 9 September 2004

Abstract

Previous studies have shown that prolonged exposure to drugs, which act via blocking K_{ATP} channels, can desensitize the insulinotropic effects of drugs and nutrients acting via K_{ATP} channels. In this study, effects of prolonged exposure to diazoxide, a K_{ATP} channel opener, on beta cell function were examined using clonal BRIN-BD11 cells. The findings were compared to the long-term effects of K_{ATP} channel blockers nateglinide and tolbutamide. Following 18 h exposure to 200 μM diazoxide, the amounts of insulin secreted in response to glucose, amino acids and insulinotropic drugs were increased. Secretory responsiveness to a variety of agents acting via K_{ATP} channels was retained following prolonged diazoxide exposure. In contrast, 18 h exposure to 100 μM nateglinide significantly attenuated the insulin secretory responses to tolbutamide, nateglinide and BTS 67 582. Glucose- and L-alanine-stimulated insulin release were unaffected by prolonged nateglinide exposure, however responsiveness to L-leucine and L-arginine was diminished. Prolonged exposure to nateglinide had no effect on forskolin- and PMA-stimulated insulin release, and the overall pattern of desensitization was similar to that induced by 100 μM tolbutamide. We conclude that in contrast to chronic long-term K_{ATP} channel blockade, long-term diazoxide treatment is not harmful to K_{ATP} channel mediated insulin secretion and may have beneficial protective effects on beta cell function.
© 2004 Elsevier Inc. All rights reserved.

Keywords: Clonal pancreatic β-cells; Insulin release; K_{ATP} channels; Diazoxide; Nateglinide; Tolbutamide

ATP-sensitive potassium channels are key regulators of insulin secretion by the pancreatic beta cell. Physiologically, these channels are closed as a result of changes in the intracellular ATP/ADP ratio following metabolism of glucose and other nutrients, such as certain amino acids. This elicits membrane depolarisation and elevation of cytoplasmic Ca^{2+} due to increased Ca^{2+} influx through voltage-dependent calcium channels. This, in turn, triggers a complex sequence of intracellular events resulting in insulin secretion [1]. K_{ATP} channels may also be closed pharmacologically. Several classes of insulinotropic drugs used in treatment of type 2 diabetes mellitus act via binding to sites on the K_{ATP} channel, causing channel closure, calcium influx and subsequent insulin secretion [2]. Examples of these drugs are sulphonylureas, such as tolbutamide and glibenclamide, and new drugs, such as the meglitinide analogue nateglinide [3], and the guanidine derivative BTS 67 582 [4].

Another class of drugs, which regulate K_{ATP} channel activity, are the K_{ATP} channel openers, such as diazoxide and pinacidil. By opening K_{ATP} channels they hyperpolarize the beta cell, inhibiting Ca^{2+} entry and thus, glucose-stimulated insulin secretion [5]. Although less widely used clinically than K_{ATP} channel blockers, K_{ATP} channel openers are used to treat excessive insulin secretion in cases of insulinoma and persistent hyperinsulinism and hypoglycaemia of infancy (PHHI) [6,7]. These drugs, particularly diazoxide, have also found use in studies designed to elucidate the complex mechanisms regulating insulin release.

Previous studies from our laboratory and others have shown that prolonged exposure to drugs which close K_{ATP} channels results in reduced responsiveness of these drugs to subsequent acute challenge by drugs acting at the same binding site, a phenomenon known as desensitization. This

Abbreviations: K_{ATP} channels, adenosine triphosphate-sensitive potassium channels; PKA, protein kinase A; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate

* Corresponding author. Tel.: +1 858 822 4178; fax: +1 858 713 6298.
E-mail address: ajball@ucsd.edu (A.J. Ball).

0006-2952/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2004.09.009

observation has been most extensively characterized with regard to the sulphonylureas [8–11], though other studies have extended the findings to more novel insulinotropic drugs such as imidazolines [12,13], nateglinide [14] and BTS 67 582 [4].

Relatively little is known about the effects of long-term exposure to K_{ATP} channel openers upon beta cell function and insulin secretion. A recent study using human islets [15] has shown that prolonged exposure to 11.1 mM glucose and diazoxide results in changes to cellular insulin content and glucose-induced insulin secretion, and other studies have suggested that diazoxide treatment may improve beta cell function in models of diabetes and in human subjects [16–18]. The BRIN-BD11 cell line has been extensively characterized as a model of normal insulin secretion [12,14,19], and has been utilized previously to examine the effects of prolonged administration of a range of insulinotropic drugs on insulin secretion and cellular insulin content [4,10,11,13]. This study uses the BRIN-BD11 cell line [19] to examine the effects of prolonged exposure to diazoxide upon cellular insulin content, insulin secretion, and responsiveness to a range of insulin secretagogues representing several distinct insulin secretory pathways. The long-term effects of diazoxide are contrasted with those of recently developed and well-established antidiabetic K_{ATP} channel blocking agents, namely nateglinide and tolbutamide.

1. Materials and methods

1.1. Chemicals

Reagents of analytical grade and deionised water (Purite) were used. RPMI-1640 tissue culture medium, foetal bovine serum and antibiotics were from invitrogen, rat insulin standard was from Novo-Nordisk, and [^{125}I]-bovine insulin was from Lifescreen. BTS 67 582 was obtained from Knoll Pharmaceuticals and nateglinide was a gift from Novartis Pharmaceuticals Corporation. All other chemicals were from Sigma and BDH Chemicals Ltd.

1.2. Cell culture and measurement of insulin release

Clonal pancreatic BRIN-BD11 cells (passage numbers 20–30) were used for this study. BRIN-BD11 cells were grown in RPMI-1640 tissue culture medium containing 11.1 mmol/l glucose and 0.3 g/l L-glutamine, and supplemented with 10% (v/v) foetal calf serum, 100 IU/ml penicillin and 0.1 g/l streptomycin at 37 °C with 5% CO_2 and 95% air. Tissue culture media were removed and replaced with fresh media every 24 h. Cells were washed with Hanks' balanced saline solution (HBSS) prior to detachment from tissue culture flasks with the aid of 0.025% trypsin (v/v) containing 1 mM EDTA and seeded at 1.5×10^5 cells/well into 24-multiwell plates. Monolayers of cells were then cultured for 18 h at 37 °C. This time period was selected on the basis of previous culture experiments using sulphonylureas [11,13]. Culture medium was then replaced with 1 ml of a Krebs ringer bicarbonate (KRB) buffer, consisting of (in mM) 115 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.28 $CaCl_2$, 1.2 KH_2PO_4, 25 Hepes and 8.4% (w/v) $NaHCO_3$ (pH 7.4) supplemented with 0.1% (w/v) bovine serum albumin and 1.1 mmol/l glucose. After 40 min preincubation at 37 °C, the buffer was replaced with 1 ml of KRB test buffer containing glucose and test agents as detailed in the table and legends to figures. After 20 min incubation at 37 °C, aliquots of test buffer were removed and stored at −20 °C for insulin radioimmunoassay [20].

1.3. Determination of cellular insulin content

After harvesting, BRIN-BD11 cells were resuspended in tissue culture medium, seeded at a density of 1.5×10^5 cells/well, and allowed to attach overnight, forming monolayers in 24-well multiplates. The culture medium was then completely removed and 500 μl of acid–ethanol solution (1.5% (v/v) HCl, 75% (v/v) ethanol, 23.5% (v/v) H_2O) was added. The cells were disrupted with the aid of a Pasteur pipette and incubated overnight at 4 °C prior to centrifugation (900 rpm) and storage at −20 °C for subsequent determination of cellular insulin content by radioimmunoassay [20].

1.4. Statistical analysis

Results are presented as mean ± standard error of the mean (S.E.M.) for a given number of observations (n). Groups of data were compared by two-way ANOVA in conjunction with Bonferroni's modified t-statistics. Differences were considered significant if $P < 0.05$.

2. Results

2.1. Effects of long-term drug exposure upon cellular insulin content

Following 18 h standard culture conditions (RPMI-1640 medium; 11.1 mM glucose), BRIN-BD11 cells had mean cellular insulin content of 64.3 ± 3.1 ng/10^6 cells (Table 1). Prolonged exposure to 200 μM diazoxide for 18 h resulted in a significantly increased (1.2-fold, $P < 0.05$) cellular insulin content. Cells which had been previously exposed to 100 μM tolbutamide or nateglinide exhibited in no significant changes in cellular insulin content (Table 1).

2.2. Responsiveness to insulinotropic drugs following long-term drug exposure

As shown in Fig. 1A, tolbutamide, nateglinide and BTS 67 582 (each at 200 μM) elicited respective 1.9-, 2.0- and

Download English Version:

https://daneshyari.com/en/article/9002478

Download Persian Version:

https://daneshyari.com/article/9002478

Daneshyari.com