available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

# Antagonism of the insulinotropic action of first generation imidazolines by openers of $K_{ATP}$ channels

*Antje Wienbergen, Claudia Bleck, Timm Grosse Lackmann, Ingo Rustenbeck* *

*Institute of Pharmacology and Toxicology, Technical University of Braunschweig, D-38106 Braunschweig, Germany*

ARTICLE INFO

*Article history:*
Received 17 July 2006
Accepted 15 September 2006

*Keywords:*
Pancreatic islets
B-cells
Cytosolic calcium concentration
$K_{ATP}$ channel
Efaroxan
Imidazolines
Tolbutamide

*Abbreviations:*
$K_{ATP}$ channels, ATP-dependent $K^+$ channels
$[Ca^{2+}]$, cytosolic calcium concentration

ABSTRACT

The antagonism between $K_{ATP}$ channel-blocking insulinotropic imidazolines – phentolamine, alinidine, idazoxan and efaroxan – and $K_{ATP}$ channel openers, diazoxide and nucleoside diphosphates, was studied in mouse pancreatic islets and B-cells. In inside-out patches from B-cells, 500 μM MgGDP abolished the inhibitory effect of the imidazolines. 300 μM diazoxide further increased channel activity. The depolarizing effect of all imidazolines (100 μM) on the B-cell membrane potential was practically completely antagonized by 300 μM diazoxide. In contrast, diazoxide was unable to decrease the cytosolic $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) which was elevated by phentolamine, whereas the $[Ca^{2+}]_i$ increases induced by the other imidazolines were promptly antagonized. The effects on $[Ca^{2+}]_i$ were reflected by the secretory activity in that the stimulatory effects of alinidine, idazoxan and efaroxan, but not that of phentolamine were antagonized by diazoxide. Metabolic inhibition of intact B-cells by 250 μM NaCN, most likely by a decrease of the ATP/ADP ratio, significantly diminished the $K_{ATP}$ channel-blocking effect of a low concentration of alinidine (10 μM), whereas efaroxan proved to be susceptible even at a highly effective concentration (100 μM). This may explain the oscillatory pattern of the $[Ca^{2+}]_i$ increase typically produced by efaroxan in pancreatic B-cells. In conclusion, the inhibitory effect of imidazolines on $K_{ATP}$ channels, which is exerted at the pore-forming subunit, Kir6.2, is susceptible to the action of endogenous and exogenous $K_{ATP}$ channel openers acting at the regulatory subunit SUR, which confers tissue specificity. With intact cells this antagonism can be obscured, possibly by intracellular accumulation of some imidazolines.



## 1. Introduction

Imidazolines are investigated as potential oral antidiabetic drugs with an antihyperglycemic, but not a hypoglycemic mode of action [1], because several compounds of this group enhance insulin secretion only in the presence of a stimulatory glucose concentration [2,3]. Sulfonylureas, in contrast, stimulate insulin secretion in the absence or near absence of glucose [4,5]. Thus, insulin secretion can be inappropriately stimulated by sulfonylureas and the inherent risk of hypoglycemias is limiting their therapeutic use [6].

Early on, it was shown that, similar to sulfonylureas, the stimulation of insulin secretion by imidazolines involves the closure of ATP-dependent potassium channels ($K_{ATP}$ channels) [7–9]. Later, it was found that the imidazolines RX871024 and efaroxan have additional sites of action at a late step in stimulus-secretion coupling [10,11]. The contribution of these effects to the insulinotropic characteristics in general and to the glucose-dependency of imidazoline-stimulated secretion in particular is still unclear. In fact, the imidazoline RX 871024 stimulated insulin secretion from $K_{ATP}$-deficient B-cells but did so at stimulatory and at non-stimulatory glucose

* *Corresponding author*. Fax: +49 531 3918287.
E-mail address: i.rustenbeck@tu-bs.de (I. Rustenbeck).
0006-2952/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2006.09.017

concentrations [12]. Imidazolines which do not inhibit $K_{ATP}$ channels were presented recently (second generation imidazolines), but appear to be less effective as insulin secretagogues [13,14].

It is well known that $K_{ATP}$ channel-blocking imidazolines antagonize the channel-opening effect of diazoxide and increase diazoxide-inhibited insulin secretion. In fact, the antagonism of a diazoxide-induced suppression of insulin secretion has been used as test system to identify insulinotropic imidazolines [8,15]. However, it was shown that diazoxide had only a very small opening effect on a pre-existent $K_{ATP}$ channel block induced by phentolamine [7,16]. On the other hand, diazoxide significantly reduced the $K_{ATP}$ channel-blocking activity of the imidazoline analogue guanabenz in inside-out patches from B-cells [17] and reduced the ability of phentolamine to raise the cytosolic $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) [15,18].

Two questions led to this investigation: firstly, can the B-cell $K_{ATP}$ channel still respond to changes in cellular energy metabolism and to pharmacological $K^+$ channel openers in the presence of $K_{ATP}$ channel-blocking imidazolines, which is of obvious relevance for a potential clinical use? Secondly, are there differences within the group of $K_{ATP}$ channel-blocking imidazolines, which correspond to the different glucose dependencies of the insulinotropic effect of phentolamine and efaroxan [19], and to the different pattern of drug-induced desensitization of insulin secretion [20]?

Thus, we characterized the ability of nucleoside diphosphates and diazoxide to antagonize the insulinotropic action of first generation ($K_{ATP}$ channel-blocking) imidazolines. In contrast to sulfonylureas, which inhibit $K_{ATP}$ channel activity by binding to the regulatory subunit of the B-cell $K_{ATP}$ channel, SUR1 [21,22], imidazolines block $K_{ATP}$ channels by interaction with the pore-forming subunit, Kir6.2 [23]. Both channel-opening nucleotides and pharmacological potassium channel openers exert their effect by binding to SUR [21,22].

For comparison, the antagonism of the effects of tolbutamide and quinine was also measured. Tolbutamide is the prototypical first generation sulfonylurea which closes $K_{ATP}$ channels by binding to the SUR subunit. In concentrations above 500 μM tolbutamide also exerts a blocking effect directly on Kir6.2 [22]. Quinine stimulates insulin secretion by inhibiting $K_{ATP}$ channel activity [24,25] and, similar to imidazolines, this effect is exerted at the pore-forming subunit Kir6.2 [26,27].

## 2. Materials and methods

### 2.1. Materials

Phentolamine was kindly donated by Novartis/Ciba-Geigy (Lörrach, Germany) and alinidine by Boehringer Ingelheim (Ingelheim, Germany). Idazoxan and efaroxan were from Tocris (Bristol, UK). Idazoxan was kept tightly sealed and in the dark because of the chemical instability of the dioxane ring structure. Quinine and diazoxide were from Sigma (Taufkirchen, Germany) and tolbutamide from Serva (Heidelberg, Germany). Collagenase P and GDP (dilithium salt) was supplied by Roche Diagnostics (Mannheim, Germany), UDP (trisodium salt) by Sigma and Fura-2/AM by Molecular Probes (Leiden, The Netherlands). Cell culture medium RPMI 1640 was purchased from Gibco BRL (Gaithersburg, MD, USA) and fetal calf serum from Biochrom (Berlin, Germany). ATP was measured using reagent kits from Sigma. All other reagents of analytical grade were from E. Merck (Darmstadt, Germany). Diazoxide was dissolved in dry dimethylsulfoxide (DMSO) to prepare stock solutions of various concentrations. Tolbutamide stock solutions were prepared in 0.1 N NaOH. When nucleoside diphosphates (0.5 mM) were present in the test media 0.2 mM $MgCl_2$ was added to keep the free $Mg^{2+}$ concentration constant.

### 2.2. Tissues

Islets were isolated from the pancreas of NMRI mice by a conventional collagenase digestion technique. Islets were hand picked under a stereomicroscope. Single cells were obtained by incubation of the islets for 10 min in a $Ca^{2+}$-free medium and subsequent vortex mixing for 2 min. Islets and single islet cells were cultured in cell culture medium RPMI-1640 with 10% fetal calf serum in a humidified atmosphere of 95% air and 5% $CO_2$ at 37 °C.

### 2.3. Measurement of insulin secretion

Batches of 50 freshly isolated NMRI mouse islets were introduced into a purpose-made perifusion chamber thermostated at 37 °C and perifused with a HEPES-buffered Krebs–Ringer medium containing the respective secretagogue. The insulin content in the fractionated effluate (1 ml/min) was determined by ELISA (Mercodia, Uppsala, Sweden).

### 2.4. Electrophysiological recordings

$K_{ATP}$ channel activity was measured by the patch-clamp technique using the cell-attached and inside-out configurations. The membrane potential was measured using the conventional whole-cell configuration under current clamp condition [28]. Pipettes were pulled from borosilicate glass (2 mm o.d., 1.4 mm i.d., Hilgenberg, Malsfeld, Germany) by a two-stage vertical puller (List Electronic, Darmstadt, Germany) and had resistances between 3 and 6 MΩ when filled with solution. Currents were recorded by an EPC 7 patch-clamp amplifier (List Electronic), low pass-filtered by a 4-pole Bessel filter at 2 kHz and stored on a video tape. The pipette holding potential was 0 mV in cell-attached and +50 mV in inside-out recordings.

The composition of the bath solution for inside-out experiments (intracellular solution) was: 140 mM KCl, 1.0 mM $MgCl_2$, 10 mM EGTA, 2.0 mM $CaCl_2$ and 5 mM Hepes, pH 7.15. The pipette solution in these experiments consisted of 146 mM KCl, 2.6 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM Hepes and 3 mM glucose, pH 7.4. An ATP-containing solution (bath solution, supplemented with 1.0 mM MgATP and, additionally, 0.8 mM $MgCl_2$) was used to close the $K_{ATP}$ channels completely and to inhibit channel run-down. The composition of the bath solution in cell-attached and whole-cell experiments (extracellular solution) was: 140 mM NaCl, 5.6 mM KCl, 1.2 mM $MgCl_2$, 2.6 mM $CaCl_2$, and 10 mM Hepes, pH 7.40. The pipette solution in the cell-attached mode consisted of 146 mM KCl,