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Possible role of an ischemic preconditioning-like response mechanism in K_{ATP} channel opener-mediated protection against streptozotocin-induced suppression of rat pancreatic islet function

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ABSTRACT

Potassium channel openers (KCOs) decrease insulin secretion from β-cells. Some KCOs also protect against damage to β-cell function and type 1 diabetes in animal models. Previously we have found that the KCO NNC 55-0118 counteracted islet cell dysfunction, and this was associated with a lowering of the mitochondrial membrane potential ($\Delta\psi$). Presently we aimed to explore whether inhibition of insulin secretion *per se* or rather inhibition of mitochondrial function correlates to counteraction of β-cell suppression. For this we used two novel KCOs (NNC 55-0321 and NNC 55-0462), which at certain concentrations have different actions regarding insulin secretion and the $\Delta\psi$, with NNC 55-0321 being a potent inhibitor of $\Delta\psi$ and NNC 55-0462 being a potent inhibitor of insulin secretion. At 10 μM NNC 55-0321, but not with NNC 55-0462, the islet ATP content and ATP/ADP ratio was acutely decreased. This was accompanied by a complete protection against streptozotocin-induced suppression of islet insulin secretion using the former KCO. In cardiac research KCOs have been used to induce an ischemic preconditioning (IPC) response. In line with an IPC-like mechanism we found that NNC 55-0321 induced an initial free oxygen radical formation, PKC-ε isoform activation and a subsequent phosphorylation of the survival promoting factor Akt. Thus, KCOs may elicit mitochondrial events that resemble classical IPC seen in cardiomyocytes, and this could explain the enhanced islet cell function observed. KCOs with this property may be particularly interesting compounds to study as a rescue therapy during acute episodes of β-cell suppression/destruction.

1. Introduction

There are evidences that the vulnerability of the pancreatic β-cell to destructive mechanisms can vary, and that under certain conditions processes involved in cellular defence can be recruited [1,2]. Moreover, data suggest that reduced activity of the insulin-secreting cells is beneficial in newly onset diabetes in human [3–5] and that the activity of β-cell affects

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the susceptibility to damage *in vitro* [6–8]. In this context the β-cell's insulin secretory activity has been believed to be decisive for how these cells will react to an insult. The use of potassium channel openers (KCOs) provides a tool for decreasing insulin secretion [9–11]. The drugs open the ATP sensitive potassium channel (K_{ATP} channel) in the plasma membrane leading to hyperpolarization of the plasma membrane and prevention of Ca^{2+}entry into the β-cell, the latter being crucial for insulin exocytosis.

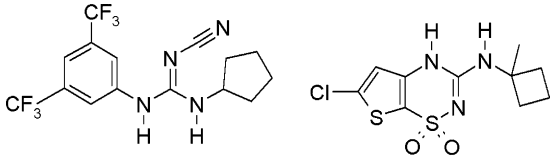
We previously observed that pretreatment with diazoxide or the KCO, NNC 55-0118, selective for the Kir6.2/SUR1 K_{ATP} channel, has a protective effect against the toxic action of streptozotocin (STZ) on rat islets *in vitro* [12]. Furthermore, *in vivo* treatment with diazoxide or NNC 55-0118 was reported to maintain β-cell function in acutely diabetic BB rats [13]. Moreover, the KCO NN414 preserved insulin secretion of human islets exposed to elevated glucose concentrations *in vitro* [14], improved β-cell survival in BB rats [15] and prevented β-cell apoptosis of human islets [16]. In another study we demonstrated that KCOs, in particular NNC 55-0118, also prevented the toxic effects of alloxan and sodium nitroprusside [17]. In addition, IL-1β mediated suppression was reduced in the long-term presence of NNC 55-0118. In our studies the protection against the different noxious agents was provided by higher concentrations of KCOs than those needed for inhibition of insulin release [12,17]. The protection was correlated with a lowering of the mitochondrial membrane potential (Δψ).

In the present study we explored whether inhibition of insulin secretion *per se* or rather inhibition of mitochondrial function is correlated to protection against STZ. For this purpose we used two other KCOs, namely NNC 55-0321 and NNC 55-0462 ([10,11]; Fig. 1), which in certain concentration intervals have different actions regarding effects on insulin secretion and the Δψ. This also prompted us to explore if the protective action could be related to a so-called ischemic preconditioning (IPC)-like mechanism [18,19].

2. Materials and methods

2.1. β-cell preparations and culture

Pancreatic islets were isolated from adult male Sprague–Dawley rats (BK-Universal, Sollentuna, Sweden) by collagenase digestion and hand-picked. The islets were precultured free-floating in RPMI 1640 (Sigma–Aldrich, St. Louis, MO, USA) supplemented with 10% FCS (v/v) (Sigma–Aldrich) and antibiotics for 5–6 days in air + 5% CO_2 at 37 °C. Medium was changed every second day. In some experiments mouse insulinoma βTC-6 cells ([20]; ATCC, Manassas, VA, USA) were used and maintained in Dulbecco's modified Eagle medium + 10% FCS and antibiotics. The passage number was 20–30 and the cells were used when 70% cell confluent. The use of rats was approved by the local Animal Ethics Committee (Tierp, Sweden).



NNC 55-0321 NNC 55-0462

Fig. 1 – Chemical structures and correct chemical names of NNC 55-0321 and NNC 55-0462. NNC 55-0321 = *N*-cyano-*N*′-(3,5-bis(trifluoromethyl)phenyl)-*N*″-(cyclopentyl)guanidine and NNC 55-0462 = 6-chloro-3-(1-methylcyclobutyl)amino-4*H*-thieno[3,2-*e*]-1,2,4-thiadiazine 1,1-dioxide; cf. Refs. [9,10].

2.2. Insulin secretion and dose–response experiments

The potency of NNC 55-0321 and NNC 55-0462 to affect glucose-stimulated insulin secretion was examined in short-term incubations of islets at 16.7 mM glucose. Triplicates of five islets were transferred to 200 μl Krebs-Ringer bicarbonate buffer supplemented with 10 mM Hepes (KRBH; Sigma–Aldrich), 2 mg/ml BSA and 16.7 mM glucose in the absence or presence of NNC 55-0321 and NNC 55-0462 (0.01, 0.1, 1.0, 10 and 100 μM; Novo Nordisk Company, Copenhagen, Denmark) and incubated for 60 min at 37 °C. The insulin concentrations in the media were then determined with High-Range Rat Insulin ELISA (Mercodia, Uppsala, Sweden).

NNC 55-0321 and NNC 55-0462 were prepared as stock solutions in DMSO and diluted in KRBH 100–1000 times when added to the incubation media. In separate control experiments it was ascertained that the attained levels of DMSO did not affect islet insulin secretion (data not shown).

2.3. STZ experiments

The design of these experiments was adopted from [12]. Thus, cultured islets in groups of 40 were incubated in 1 ml KRBH (5.6 mM glucose +2 mg/ml BSA). Stock solutions of NNC 55-0321 and NNC 55-0462 were added to a final concentration of none, 1 or 10 μM for 30 min at 37 °C. Then STZ (Sigma) was dissolved in 0.9% saline, and 10 μl of saline or STZ solution was added for another 30 min (final STZ concentration 0.5 or 1.8 mM). The incubation was terminated by the addition of 2 ml cold KRBH and the islets transferred to culture dishes and allowed to recover for 24 h in medium RPMI 1640 + 10% FCS. Insulin secretion was then examined at 16.7 mM glucose, as described above. The data were expressed as % of the insulin secretory rate of control islets incubated in parallel, but not treated with STZ.

2.4. Assessment of mitochondrial membrane potential

To assess Δψ, 5,5′6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1; Molecular Probes Europe, Leiden, the Netherlands) fluorescence was measured [21]. Islets in groups of 300 were incubated in 4.5 ml RPMI 1640 + 0.5 ml FCS and 50 μl of JC-1 solution (4 μg/ml) for 20 min at 37 °C. Then islets were washed and dispersed into free cells by trypsin incubation (0.5%, w/v) in Ca^{2+}- and Mg^{2+}-free Hanks' medium (Sigma–Aldrich) ≈5 min at 37 °C. The dispersed cells were centrifuged and the cell pellet resuspended in culture medium and subsequently subdivided into five groups. After this NNC 55-0321 or NNC 55-0462 at different