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Protection of rat pancreatic islets by potassium channel openers against alloxan, sodium nitroprusside and interleukin-1β mediated suppression – possible involvement of the mitochondrial membrane potential

M. Kullin, Z. Li, J. Bondo Hansen, N. Welsh, F. A. Karlsson, S. Sandler

Department of Medical Sciences, Uppsala University, Uppsala, Sweden
Department of Medical Cell Biology, Biomedicum, Uppsala University, Uppsala, Sweden
Novo Nordisk A/S, Måløv, Denmark

Abstract

Aims/hypothesis. We aimed to study the effects of two K_{ATP} channel openers (KCO), diazoxide and the more potent compound NNC 55-0118, on beta-cell suppression and/or toxicity induced by alloxan, sodium nitroprusside and IL-1β.
Methods. Islets from rats were exposed to 0.3 mmol/l diazoxide or NNC 55-0118 for 30 min and either alloxan (0.5 mmol/l), sodium nitroprusside (0.5 mmol/l) or IL-1β (12.5 or 25 U/ml) were added and the incubation continued for 30 min. Islets were then washed and incubated for 24 h before examination.
Results. After exposure to alloxan, islets showed reduced glucose oxidation rate and impaired glucose-stimulated insulin release. NNC 55-0118 counteracted the effects of alloxan, while diazoxide was less effective. After treatment with sodium nitroprusside, islet glucose oxidation rates were reduced and this was prevented by pretreatment with NNC 55-0118. In short-term experiments the potassium channel openers (KCOs) did not influence the IL-1β effect on insulin secretion. However, long-term addition (24 h) of NNC 55-0118 counteracted IL-1β induced inhibition of the glucose oxidation rate. It was shown, using the fluorescent probe JC-1, that the mitochondrial membrane potential was reduced by the potassium channel openers (KCOs), most strongly by NNC 55-0118. Nevertheless culture with KCOs for 72 h did not cause irreversible damage to the islets.
Conclusion/interpretation. Potassium channel openers (KCOs), in particular NNC 55-0118, prevented the toxic effects of alloxan and sodium nitroprusside. IL-1β mediated suppression was reduced by NNC 55-0118 provided the long-term addition of the potassium channel opener (KCO). The protective mechanism of potassium channel openers (KCOs) might involve a decrease of the mitochondrial membrane potential. [Diabetologia (2003) 46:80–88]

Keywords Alloxan, interleukin-1β, mitochondria, nitric oxide, pancreatic islets, potassium channel opener, sodium nitroprusside.

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Corresponding author: S. Sandler, Department of Medical Cell Biology, Biomedicum, Uppsala University, P.O. Box 571, 751 23 Uppsala, Sweden. E-mail: Stellan.Sandler@medcellbiol.uu.se

Abbreviations: CNS Central Nervous System; *COX-2* cyclooxygenase 2; *JC-1* 5,5′6,6′-tetrachloro-1,1′3,3′-tetraethylbenzimidazolylcarbocyanine iodide; *KCO* potassium channel opener; *No* Nitric Oxide; $\Delta\Psi$ mitochondrial membrane potential; *PARP* poly(ADP-ribose) polymerase; *RPMI* Rosewell Park Memorial Institute; *TCA* trichloroacetic acid.

Environmental as well as immunological events are thought to be of importance for the destruction of the insulin-producing beta-cells in the islets of Langerhans in Type 1 diabetes [1]. The mechanisms and mediators of this process are not fully understood. There is evidence suggesting that a period of reduced activity following the start of insulin treatment is beneficial in new onset diabetes [2, 3] and that the cellular activity affects the susceptibility to damage in vitro [4, 5, 6]. Inhibition of insulin secretion by the use of potassium channel openers (KCOs) provides a means of inducing 'beta-cell rest'. The drugs open the ATP-sensitive potassium channel (K_{ATP} channel) and hyperpol-

arise the plasma membrane of the beta cell and prevent Ca^{2+}entry, which is critically needed for the exocytosis of insulin granulae.

We recently observed a protective effect of diazoxide and a new KCO, NNC 55-0118, selective for the SUR1/Kir6.2 K_{ATP} of the beta cell, against the toxic action of streptozotocin on rat islets in vitro [7]. Concentrations of KCOs higher than those needed for inhibition of insulin release provided protection, which could indicate that K_{ATP} channels not only in the plasma membrane, but also in mitochondria were involved. Protection by KCOs against toxins in other tissues, notably the heart and CNS, have shown involvement of mitochondrial K_{ATP} channels in a number of studies [8, 9, 10, 11]. However, the mechanism of protection is not well understood. To explore the protective effects of diazoxide and NNC 55-0118 on beta cells, we exposed rat islets to low concentrations of alloxan, sodium nitroprusside and human recombinant IL-1β, three beta-cell-suppressive substances with different modes of action.

An increase of the intracellular Ca^{2+} concentration and metabolic inhibition, which thereby causes an opening of K_{ATP} channels, seems to be a general result of exposing beta cells to alloxan, sodium nitroprusside or nitric oxide. Thus, treatment of rat islet cells with alloxan, or hydrogen peroxide which forms upon alloxan exposure, induced a rapid increase in intracellular Ca^{2+} within 1 to 2 min, probably by an influx of Ca^{2+} from the extracellular space [12]. In studies on rat beta cells using hydrogen peroxide it was shown that hydrogen peroxide induced rapid increases in Ca^{2+} depending on mobilzation of Ca^{2+} both from intracellular and extracellular compartments. This was accompanied by an increased frequency of K_{ATP} channel openings, an effect interpreted to depend on a metabolic inhibition induced by the free radical and not a direct effect on the K_{ATP} channels of the beta cells [13, 14]. In another study aqueous NO was applied to rat islet cells which led to a rapid and transient increase in intracellular Ca^{2+} concentrations probably via a mobilization of Ca^{2+} from the endoplasmic reticulum [15]. Furthermore, acute addition of sodium nitroprusside to rat pancreatic islet cells in patch clamp experiments showed that sodium nitroprusside was accompanied by an opening of K_{ATP} channels in beta cells [16]. This was also considered to depend on an inhibitory effect by sodium nitroprusside of beta-cell glucose metabolism rather than a direct effect by NO on K_{ATP} channel acitivity.

Materials and methods

Islet isolation, culture and incubation. Islets were isolated from male Sprague-Dawley rats by collagenase digestion and hand-picked using a braking pipette.The use of animals was in accordance with international guidelines (NIH publication no. 85-23, revised 1985) and approved by the local animal ethics committee. The islets were precultured free floating in medium RPMI 1640 (Sigma Chemicals, St. Louis, Mo., USA) supplemented with 10% FCS (v/v) (Sigma) and 11 mmol/l glucose for 7 days in 5% CO_2 at 37°C. The medium was changed every second day. Islets were then transferred to sterile petri dishes containing 1 ml KRBH (KRB with 10 mmol/l HEPES) medium with 2 mg/ml BSA and 2 mmol/l glucose for alloxan (Sigma) experiments and 11 mmol/l glucose in experiments carried out with sodium nitroprusside (Sigma) and human recombinant IL-1β (PeproTech, London, UK). Stock solutions of alloxan and sodium nitroprusside in KRBH were prepared immediately before the addition to culture dishes.

Diazoxide (Sigma) and NNC 55-0118 (Novo Nordisk, Copenhagen, Denmark) were prepared as stock solutions of 100 mg/ml in DMSO (final concentration 0.08%) and added to the dishes to a final concentration of 0.3 mmol/l, the concentration at which protective effects against streptozotocin had previously been observed [7]. Islets were incubated for 30 min in air with 5% CO_2 at 37°C with or without KCOs and then either 0.5 mmol/l alloxan, 0.5 mmol/l, sodium nitroprusside or 12.5 or 25 U/ml of IL-1 β were added. The latter incubations continued for another 30 min and were terminated by the addition of 1 ml cold KRBH. The islets were then briefly transferred to culture dishes with fresh KRBH to remove the KCOs and the toxins. After another transfer to new petri dishes, the islets were allowed to recover for 24 h in medium RPMI 1640 with FCS and 11 mmol/l glucose before morphological and biochemical analysis. Concentrations of sodium nitroprusside were tested in a pilot series of experiments, in which 0.5 mmol/l provided a submaximal suppressive effect, without causing major islet loss (data not shown).

In another set of experiments, islets were incubated with 12.5 U/ml of IL-1β for 24 h with addition of KCOs in RPMI 1640 with 10% FCS and possible protective effects were analysed.

To evaluate long-term effects of KCO treatment per se, islets were cultured for 72 h in different concentrations of diazoxide and NNC 55-0118 in RPMI 1640 (11.1 mmol/l glucose) with 10% FCS before analysis. In some of these experiments islets were also cultured up to 48 h in RPMI 1640+10% FCS in the absence of KCOs after preceding 72 h culture with KCOs.

Insulin secretion and islet insulin content. Stimulated insulin release experiments were carried out as previously described [7]. Briefly, triplicates of five islets were transferred to 200 µl of KRBH with 2 mg/ml BSA and 16.7 mmol/l glucose and incubated for 60 min in air with 5% CO_2 at 37°C. Islets from each condition were then pooled and sonicated in 200 µl of redistilled water. The insulin concentrations were then measured with High Range Rat Insulin ELISA (Mercodia, Uppsala, Sweden) according to the manufacturer's protocol.

Glucose oxdation rate. Groups of 10 islets in triplicate samples were transferred to glass vials containing 100 µl KRBH supplemented with D-[U-^{14}C]glucose (Amersham Pharmacia Biotech, Uppsala, Sweden) and nonradioactive glucose to a final concentration of 16.7 mmol/l glucose. The vials were suspended in scintillation flasks, gassed with 95% O_2 + 5% CO_2 and sealed air tight. The flasks were shaken for 90 min at 37°C and metabolism was stopped by injection of 100 µl of 0.05 mmol/l antimycin A (Sigma) into the center vial. Then, 250 µl hyamine hydroxide (Packard Instruments, Downers Grove, Ill., USA) was injected into the outer flask. CO_2 was released from the incubation medium by injecting 100 µl 0.4 M Na_2HPO4 solution (pH 6.0) into the center vial. To trap CO_2 with hyamine hydroxide, vials were incubated for another 120 min at 37°C. Scintillation fluid was then added to each flask and the radioactivity counted in a liquid scintillation counter.

Table 1. Islet retrieval and insulin content in islets allowed to recover for 24 h after 30 min to alloxan (0.5 mmol/l) in presence or absence of diazoxide (0.3 mmol/l) or NNC 55-0118 (0.3 mmol/l)

	Control	Diazoxide	NNC 55-0118	Alloxan	Diazoxide + Alloxan	NNC 55-0118 + Alloxan
Retrieval (%)	99±1	100±1.0	98±1.5	98±1.5	99±2.5	101±2.2
Insulin Content (ng/10 islets)	2080±400	2070±440	2470±500	1770±330	2120±440	1990±380

Values are means ± SEM for nine observations

Proinsulin and protein biosynthesis. For each condition duplicate samples of 10 islets were incubated in 100 µl KRBH with L-[4.5-^{3}H]leucine (1850 MBq/ml; Amersham Pharmacia Biotech), 2 mg/ml BSA and 16.7 mmol/l glucose for 120 min in 5% CO_2 at 37°C. Islets were then washed in Hanks' solution supplemented with 10 mmol/l nonradioactive leucine and sonicated in 200 µl of redistilled water. A 10 µl fraction of the homogenate was incubated for 90 min with insulin antibodies to separate proinsulin from other labelled proteins [17]. Total protein biosynthesis was obtained by precipitating the labelled proteins with 1 mol/l trichloroacetic acid (TCA). The antibody bound and TCA precipitable radioactivity were assessed in a liquid scintillation counter.

Assessment of mitochondrial membrane potential. In order to assess the mitochondrial membrane potential $\Delta\Psi$), 5,5′6,6′-tetrachloro-1,1′3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1; Molecular Probes Europe, Leiden, the Netherlands) fluorescence was measured [18]. Islets in groups of 50 were incubated in 100 µl RPMI 1640+10% FCS in the absence or addition of different concentrations of diazoxide or NNC 55-0118 (0.003, 0.03 and 0.3 mmol/l) at 37°C for 30 min. The islets were then incubated for 20 min at room temperature in the same medium as that used during the preincubation with the addition of 10 µg/ml JC-1 (solubilized in N,N-dimethylformamide; 1% vol/vol). This was followed by dispersion of the islet cells by trypsin incubation (0.5%, wt/vol) for 8 min at 37°C. The dispersed islet cells were resuspended in KRBH containing 5.6 mmol/l glucose and then the fluorescence was measured using a Becton Dickinson Facscalibur flow cytometer (Becton Dickinson, San Jose, Calif., USA) with respect to their FL_2 (590 nm) and FL_1 (530 nm) fluorescence, using an excitation wavelength at 488 nm. Data were analysed using the CellQuest software (Beckton Dickinson).

Islet viability. Long term effects of NNC 55-0118 on islet cell viability was assessed after 72 h exposure to 0.03 mmol/l or 0.3 mmol/l diazoxide or NNC 55-0118 in 11 mmol/l glucose RPMI 1640 with 10% FCS. Fifty islets were stained with Viaprobe (BD Biosciences Pharmingen, Stockholm, Sweden) in culture medium for 15 min and then washed and trypsinised (0.5% trypsin, w/v) for 5 min at 37°C in 200 µl of Ca^{2+}-free Hanks' solution. Islet cell viability was examined on Facscalibur (Becton Dickinson) and the data analysed by CellQuest software (Becton Dickinson).

Statistics. Values are expressed as means ± SEM and when applicable means were calculated from each duplicate or triplicate group and considered as one observation. Every observation relates to different islet donors. Student's *t* test or ANOVA for repeated measures including the Bonferroni t-test, were used. A *p* value of less than 0.05 was considered statistically significant.

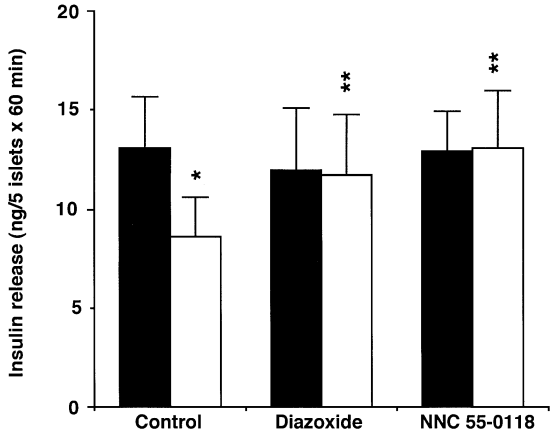

Fig. 1. Glucose-stimulated insulin release of islets after 24 h of recovery after a 60 min incubation with medium alone, or with 0.3 mmol/l diazoxide or 0.3 mmol/l NNC 55-0118 without (black bars) or with 0.5 mmol/l alloxan (open bars) during the last 30 min. Triplicates of five islets were subsequently incubated in KRBH with 2 mg/ml of BSA and 16.7 mmol/l glucose. Bars are means ± SEM for eight observations and * denotes $p<0.05$ compared to control and ** denotes $p<0.05$ vs. treatment with 0.5 mmol/l alloxan only

Results

Alloxan exposure. Islet retrieval was not affected by 0.5 mmol/l alloxan, when examined after the 24-h recovery period (Table 1). Insulin content was not affected by 0.5 mmol/l alloxan at that time point (Table 1). Moreover, diazoxide or NNC 55-0118 did not change the islet insulin content. Glucose-stimulated insulin release was reduced after treatment with 0.5 mmol/l alloxan (Fig. 1). This was prevented by pretreatment with 0.3 mmol/l diazoxide as well as by 0.3 mmol/l NNC 55-0118. The glucose oxidation rate in islets exposed to 0.5 mmol/l alloxan was decreased (Fig. 2A). Islets treated with NNC 55-0118 before the addition of alloxan had a normal glucose oxidation rate, whereas the glucose oxidation rate in islets treated with diazoxide + alloxan did not differ from that of islets treated with alloxan alone.

Sodium nitroprusside exposure. Glucose-stimulated insulin release was strongly diminished in islets exposed to 0.5 mmol/l sodium nitroprusside (Fig. 3). Diazoxide could not prevent this effect, whereas NNC 55-0118 fully counteracted the sodium nitroprusside

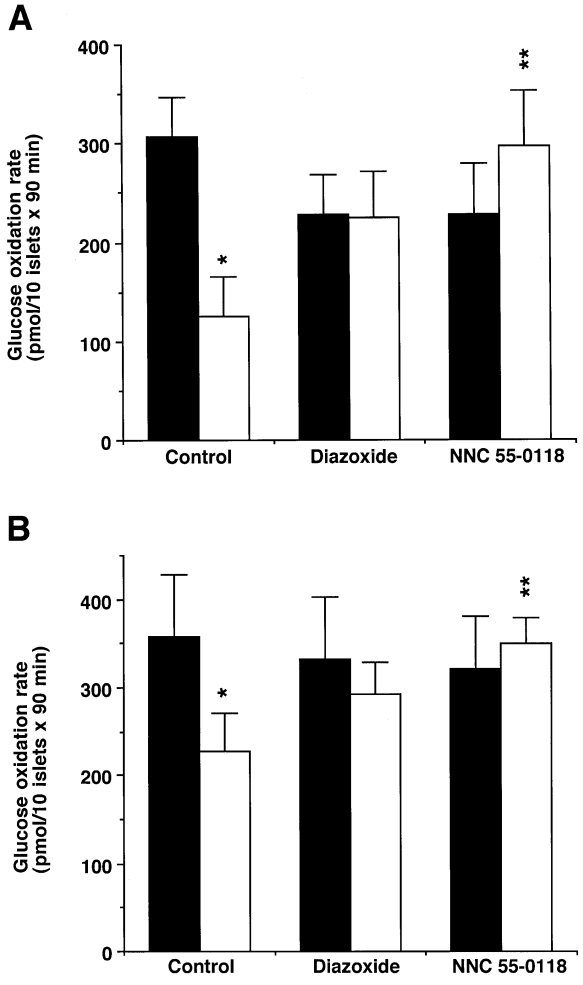

Fig. 2 A, B. Glucose oxidation rates after 24 h in islets incubated for 60 min in KRBH with 2 mg/ml BSA and 0.3 mmol/l of KCOs; (**A**) 2 mmol/l glucose without (black bars) and with (white bars) 0.5 mmol/l alloxan or (**B**) 11 mmol/l glucose without (black bars) or with (white bars) 0.5 mmol/l sodium nitroprusside. Bars represent means ± SEM of eight observations for alloxan and seven for sodium nitroprusside. * means $p<0.05$ vs. control and ** denotes $p<0.05$ compared to islets treated with alloxan (**A**) or sodium nitroprusside (**B**)

induced inhibition of islet insulin release. The glucose oxidation rate was reduced by 36% after treatment with sodium nitroprusside (Fig. 2B). When 0.3 mmol/l NNC 55-0118 was added before sodium nitroprusside, islets showed normal glucose oxidation, however, pretreatment with diazoxide failed to show a different glucose oxidation rate compared to islets treated with sodium nitroprusside only.

IL-1β exposure. Glucose-stimulated insulin release was reduced in islets exposed to 12.5 U/ml or 25 U/ml of IL-1β by about 35% and 70%, respectively. Short-term addition of diazoxide and NNC 55-0118 failed to prevent this reduction (Fig. 4). Islet insulin content was not affected by any concentration of IL-1β (not shown). Since no protective action by the KCOs was observed against inhibition of insulin release by the

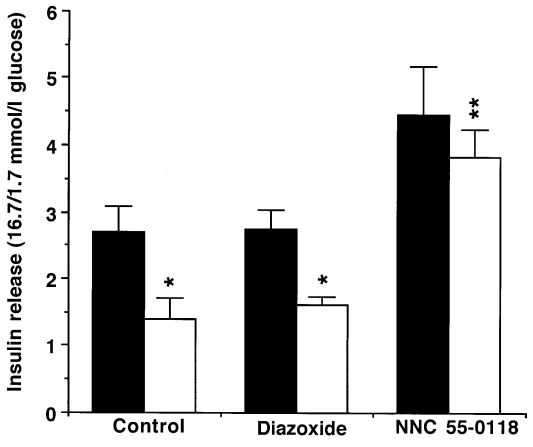

Fig. 3. Glucose-stimulated insulin release after 24 h of islets incubated for 60 min in KRBH with 2 mg/ml BSA and 0.3 mmol/l of KCOs without (black bars) and with 0.5 mmol/l sodium nitroprusside (white bars) during the last 30 min. Insulin release experiments were carried out in triplicate groups of five islets at high glucose (16.7 mmol/l) and at low glucose (1.7 mmol/l). Data are expressed as relative stimulation of insulin release (16.7/1.7). Bars represent means ± SEM of four experiments. * denotes $p<0.05$ vs. corresponding islets not exposed to sodium nitroprusside and ** denotes $p<0.05$ vs. control islets exposed to sodium nitroprusside

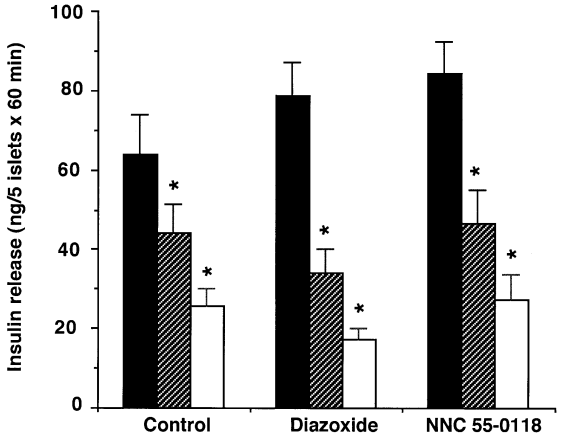

Fig. 4. Glucose-stimulated insulin release after 24 h of islets exposed to medium (KRBH with 2 mg/ml BSA and 11 mmol/l glucose) or 0.3 mmol/l of KCOs for 60 min and different concentrations of IL-1β (0, black bars; 12.5 U/ml hatched bars; 25 U/ml white bars) during the latter 30 min. Bars represent means ± SEM for six experiments and * denotes $p<0.05$ compared to corresponding islets not exposed to IL-1β

cytokine, similar glucose oxidation experiments were not performed.

However, in order to examine if a prolonged exposure to KCOs, which thus differs from the experimental design in Fig. 4, would affect the extent of IL-1β induced suppression, glucose oxidation experiments were carried out on islets incubated for 24 h with 12.5 U/ml of IL-1β with and without KCOs (Fig. 5). Islets incubated with IL-1β had an impaired glucose oxidation rate compared to control islets. This was not

Table 2. Characteristics of islets cultured for 72 h in medium RPMI 1640 with 10% FCS and different concentrations of diazoxide or NNC 55-0118

	Control	Diazoxide (0.03 mmol/l)	Diazoxide (0.3 mmol/l)	NNC 55-0118 (0.3 mmol/l)	NNC 55-0118 (0.3 mmol/l)
Retrieval (% of day 0)	98±2.8 (3)	98±0.3 (3)	103±3.6 (3)	96±4.5 (3)	99±2.5 (3)
Cell Death (% of all islet cells)	3.6±0.1 (3)	3.4±0.3 (3)	3.7±0.6 (3)	3.7±0.6 (3)	3.5±0.9 (3)
Insulin Biosynthesis (10^3 dpm/10 islets × 2 h)	8.4±2.3 (7)	8.3±2.2 (7)	8.7±1.9 (7)	7.7±1.2 (7)	1.7±0.6 (7)*
Protein Biosynthesis (10^3 dpm/10 islets × 2 h)	116±9.6 (7)	100±9.3 (7)	107±14 (7)	88.5±9.4 (7)	48.3±6.0 (7)*
Insulin Biosynthesis/Protein Biosynthesis (%)	7.1±1.6 (7)	5.9±0.9 (7)	8.3±1.2 (7)	8.8±1.0 (7)	3.3±0.9 (7)*
Insulin Release (% of control)	100 (6)	140±19 (6)*	128±21 (6)	105±19 (6)	38±9.7 (6)*
Insulin Content (% of control)	100 (6)	124±16 (6)	128±8.0 (6)*	130±8.0 (6)*	85.4±7.3 (6)

Values are means ± SEM for the number of observations given within parentheses. The glucose-stimulated insulin release rate and insulin content of the control group was 27.5±5.2 ng/5 islets x 60 min and 2020 ± 300 ng/10 islets, respectively. * denotes $p<0.05$ or less vs control.

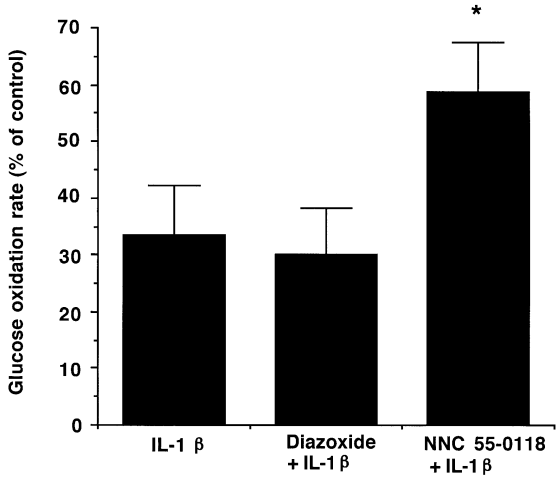

Fig. 5. Glucose oxidation rates at 16.7 mmol/l glucose in islets exposed to 12.5 U/ml of IL-1β for 24 h in the presence or absence of KCOs (0.3 mmol/l). Incubations were carried out in medium RPMI 1640 with 10% FCS. Bars are means ± SEM of seven experiments and data are expressed in % of corresponding non-cytokine-treated control islets. * denotes $p<0.05$ compared to islets exposed to diazoxide + IL-1β

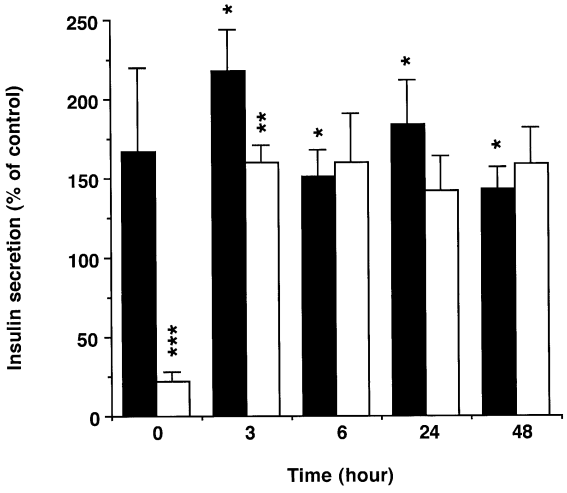

Fig. 6. Insulin secretion of pancreatic islets at 16.7 mmol/l glucose at different time points (0–48 h) after a preceding 72 h exposure to 0.3 mmol/l diazoxide (black bars) or 0.3 mmol/l NNC 55-0118 (white bars) and subsequent withdrawal of the KCOs. The data are expressed in % of insulin secretion of non-KCO exposed control islets examined on corresponding time points. Values are means ± SEM of six experiments. *, ** and *** denote $p<0.05$, $p<0.01$ and $p<0.001$, respectively, compared to control islets

influenced by 0.3 mmol/l diazoxide. Islets incubated with 0.3 mmol/l NNC 55-0118 + IL-1β were less suppressed than islets exposed to 0.3 mmol/l diazoxide + IL-1β. Islets incubated with NNC 55-0118 + IL-1β also tended to be less suppressed than islets incubated with IL-1β alone ($p=0.051$). Culture with the KCOs in the absence of the cytokine did not change the glucose oxidation rate as compared to control islets not exposed to KCOs (data not shown). We did not carry out glucose-stimulated insulin release experiments after long-term exposure to KCOs and IL-1 β, because in such an experiment the inhibitory effect of the KCOs on insulin secretion would persist and mask a possible protective effect against IL-1β.

Long-term exposure to KCOs (72 h). Prolonged exposure to the KCOs (0.03 or 0.3 mmol/l) did not affect islet retrieval or islet cell death in any of the treatment groups compared to control (Table 2). (Pro)insulin and total protein biosynthesis was reduced after 72-h culture with 0.3 mmol/l NNC 55-0118. A larger reduction of proinsulin biosynthesis at the higher concentration indicates a beta cell specific effect of NNC 55-0118 (Table 2). Insulin release in these islets was reduced, but insulin content was not different from control islets not exposed to KCOs. The other concentrations of diazoxide or NNC 55-0118 examined had no effects on proinsulin and total protein biosynthesis, but insulin content was about 30% higher in islets treated with 0.3 mmol/l diazoxide and 0.03 mmol/l NNC 55-0118.

We then studied if a prolonged exposure to KCOs (0.3 mmol/l for 72 h) followed by withdrawal of the KCOs, would lead to a recovery of glucose-stimulated insulin secretion (Fig. 6). For this purpose we studied

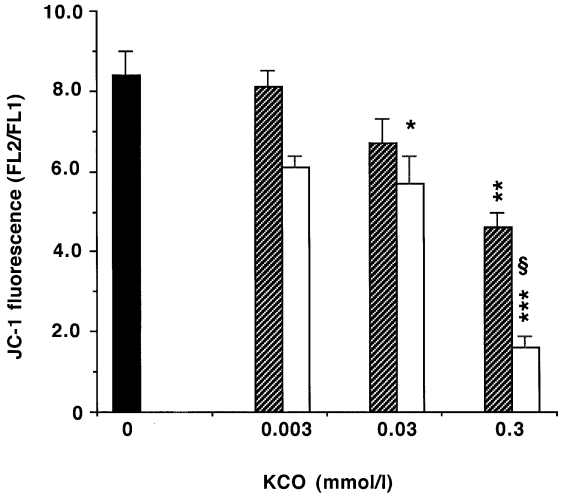

Fig. 7. Mitochondrial membrane potential ($\Delta\Psi$), in pancreatic islets exposed at 37°C for 30 min in the absence (black bar) or presence of diazoxide (hatched bars) or NNC 55-0118 (white bars). The islets were then incubated with JC-1, trypsinised, and analysed in a flow cytometer in which the fluorescense was measured at 588 nm (FL2) and 490 nm (FL1), The $\Delta\Psi$ was expressed as a ratio FL2/FL1. Values are means ± SEM for six experiments. *, ** and *** denote $p<0.05$, $p<0.01$ and $p<0.001$, respectively, compared to control islets not exposed to KCOs. § denotes $p<0.001$ compared to 0.3 mmol/l diazoxide

the islets at different time points (0–48 h) after drug withdrawal. In comparison to non-KCO exposed islets studied in parallel, NNC 55-0118 caused a pronounced inhibition of insulin secretion at time 0 i.e. immediately after the 72 h. However, already after 3 h this inhibition had disappeared and at that time point there was an even increased rate of insulin secretion. At other time points studied (6 h, 24 h, 48 h) there was no difference compared to control islets. In line with our previous experiments (Table 2), we could not observe an inhibition of glucose-stimulated insulin secretion when diazoxide was added for 72 h (Fig. 6; time 0). In contrast, after withdrawal of diazoxide there was an enhanced insulin response on all time points during the subsequent 48 h.

Mitochondrial membrane potential. JC-1 aggregate fluorescence was measured as a quantitative measurement of mitochondrial membrane potential ($\Delta\Psi$) after exposure to diazoxide or NNC 55-0118 for 30 min (Fig. 7). Diazoxide at 0.3 mmol/l decreased $\Delta\Psi$ by about 45%, whereas at lower concentrations of diazoxide no effects were observed. NNC 55-0118 induced a decline of $\Delta\Psi$ at 0.03 mmol/l and this became even further pronounced at 0.3 mmol/l (decline by about 80% compared to control islets).

Discussion

In this study we showed that short-term pretreatment with two KCOs, diazoxide and NNC 55-0118, protected rat islets from alloxan and sodium nitroprusside toxicity. In long-term, but not in short-term exposures, NNC 55-0118 reduced IL-1β induced suppression and/or toxicity.

Glucose has structural similarities to alloxan and the uptake of alloxan has been reported to be liver and islet specific [19, 20]. Alloxan is rapidly converted in the cytoplasm to dialuric acid, which is a source of free oxygen radicals during reconversion to alloxan [21]. The toxic action of alloxan is probably through oxidative stress in the beta cell, which unlike the hepatocyte, has a poor oxygen radical scavenging mechanism [19]. Alloxan could induce DNA strand breaks and poly(ADP-ribose) polymerase (PARP) activation [22], although islet protection against alloxan by the use of nicotinamide, a PARP inhibitor, has not been conclusive [23].

Both sodium nitroprusside and IL-1β are thought to suppress rodent beta cells through the generation of nitric oxide (NO) [24]. The half-life of sodium nitroprusside is short and the release of NO essentially instantaneous. IL-1β in contrast, binds to the type I IL-1 receptor in the plasma membrane and activates the transcription factor NF-KB [25]. This induces transcription of inducible nitric oxide synthase (iNOS) in the beta cell [26], leading to increased NO formation after 4 to 6 h. The exact mechanism of NO toxicity remains to be clarified. NO release from sodium nitroprusside is known to cause cytochrome c release from mitochondria and caspase 3 activation, a key step in apoptosis, in RINm5F cells [27] and chelation of mitochondrial hemoproteins [28]. Perturbation of the the mitochondria [29, 30, 31] and DNA damage [32, 33], have been shown following IL-1β exposure of rodent beta cells.

Apart from activation of iNOS transcription, IL-1β also generates prostaglandin E_2 (PGE_2) through the induction of the enzyme cyclooxygenase 2 (COX-2) [34]. Moreover, it has been reported that PGE_2 inhibits insulin secretion in rat islets and protection against IL-1β was observed using specific COX-2 inhibition [35]. However, other studies did not find a clear relationship between IL-1β induced activity of cyclooxygenase pathways and suppression of beta-cell function [30, 34].

In our study we found protection against alloxan and sodium nitroprusside, both compounds with a very short half-life. Using the same experimental design with short-term addition of KCOs, which conveyed protection against alloxan and sodium nitroprusside, IL-1β mediated beta-cell suppression could not be prevented. However, long-term simultaneous addition of NNC 55-0118, but not diazoxide, partially counteracted IL-1β induced inhibition of the glucose oxidation rate. It is thus possible that the long-term addition of NNC 55-0118 ameliorated NO effects following IL-1β stimulation of iNOS activity, but that additional IL-1β effects might not be prevented.

KCOs, such as diazoxide and NNC 55-0118, block insulin secretion by opening the ATP-sensitive potassium channels in the plasma membrane and thus preventing depolarisation and entry of extracellular Ca^{2+}. In clinical studies, treatment with diazoxide at the onset of Type 1 diabetes has shown improved C-peptide concentrations up to 18 months after disease onset [2], supporting a beneficial effect of beta-cell rest. These beneficial effects in vivo in humans might be related to a decreased beta-cell activity and down-regulation of putative beta-cell autoantigens recognized by the immune system [3]. Whether the activity of the beta cell affects the extent of damage in vitro, an experimental condition where the immune system is absent, is debatable. Some studies have shown protective effects by high glucose from alloxan toxicity claiming that increased metabolism and production of reduced pyridine nucleotides like NADH and NADPH could keep the content of glutathione high in the beta cell and thereby counteract the oxidative actions [36]. Studies with cytokines, indicate that lower glucose concentrations are protective in cells exposed to IL-1β [4, 5, 37], suggesting that insulin secretion and beta-cell activity is important for the extent of this type of beta-cell damage. In contrast, also very high ambient concentrations of glucose, i.e. during enhanced stimulation of insulin secretion, conveyed a relative protection against IL-1β [38, 39]. In this study we used a glucose concentration of 2 mmol/l with alloxan. At 2 mmol/l glucose the beta-cell activity is low with respect to insulin secretion and yet a high concentration of KCOs was needed to protect from alloxan toxicity. This might suggest that the secretory activity is of little importance with regard to protection against alloxan.

In our experiments we used concentrations of KCOs known to act on mitochondrial K_{ATP} channels of beta cells [40]. It can be assumed that an opening of mitochondrial K_{ATP} channels would reduce the membrane potential, increase the electron flow in the respiratory chain and lead to uncoupling of the oxidative phosphorylation. Indeed, we found that especially NNC 55-0118 effectively reduced the $\Delta\Psi$. It is difficult to explain the exact mechanism of how such action would protect the beta cells against toxins, but similar protective effects by opening of mitochondrial K_{ATP} channels have been seen in heart [8,9], and brain [10, 11] preparations. In myocytes, the opening of mitochondrial K_{ATP} ('preconditioning') during hypoxia is thought to be preceded by the MAP kinase pathway activation and preconditioning could lead to cytoskeleton stability [41]. Moreover, recently it was reported that transfected clonal beta cells (INS-1) overexpressing the gene for the mitochondrial uncoupling protein UCP-2, which reduces ATP production, showed an increased survival after exposure to oxidative stress induced by hydrogen peroxide [42]. It could be anticipated that a prolonged effect on the mitochondrial membrane potential would lead to mitochondrial permeability transition, reduced ATP concentrations and cell death [43]. However, high concentrations of KCOs up to 72 h did not affect the viability of islets according to our data, and the glucose-stimulated insulin release was readily recovered.

In conclusion, we found that the KCOs diazoxide and to a larger extent NNC 55-0118 could protect rat pancreatic islets exposed to alloxan, sodium nitroprusside and IL-1β. Our results suggest that the decreased vulnerability when KCOs were present might involve a decreased mitochondrial membrane potential during a situation of increased free radical generation in the beta- cells. Possible protective mechanisms by the KCOs could be related to cell membrane hyperpolarization which would decrease Ca^{2+} entry from the extracellular space and suppression of Ca^{2+}-dependent toxic cascades leading to cell death. Furthermore, a decreased mitochondrial activity would reduce the ATP supply available for activating energy requiring cell destructive pathways such as apoptosis. To further study if mitochondrial K_{ATP} channels are involved in the protective mechanism of KCOs will be of importance, not least for the understanding of clinical 'beta-cell rescue'. Treatment of autoimmune Type 1 diabetes with a drug that reduces insulin biosynthesis and insulin release during a situation of reduced capacity for insulin production is questionable. We hypothesize that this type of drug which down-regulates beta-cell function and perhaps has protective properties against noxious compounds, can help to preserve beta-cell function during an aggressive stage of the disease process. We also anticipate that such a drug would be given in addition to intensive insulin therapy [2]. Before this might be attempted, we suggest the candidate drug is tested in relevant animal models for Type 1 diabetes. It could also be worthwhile to experimentally test other drugs with a similar profile of action as that of NNC 55-0118.

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