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Biochemical Pharmacology 65 (2003) 1797–1805
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Selection of insulin-producing rat insulinoma (RINm) cells with improved resistance to oxidative stress

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Received 26 August 2002; accepted 13 November 2002

Abstract

The defense system against reactive oxygen species is believed to be crucial for the survival of insulin-producing cells after various injuries. The aim of our study was to select a subpopulation of insulin-producing RINm cells with higher resistance to oxidative stress. The cells resistant to hydrogen peroxide (RINmHP) were obtained by repeated exposure of parental RINm cells to 100 and 200 μM hydrogen peroxide (HP). The increased resistance of RINmHP cells to HP was confirmed by three different cytotoxicity assays. In addition, the selected cells also were resistant to the cytotoxic effect of activated rat splenocytes compared to parental cells. The half-life of HP in the RINmHP cell culture medium was about 2.5 times lower than that of the parental cells, corresponding to the increased level of catalase expression and activity in selected cells. The increased defense property of the selected cells was not associated with any significant changes in insulin content and insulin response to a mixture of glucose with isobutyl methyl xanthine or potassium chloride. In conclusion, repeated exposure to HP induces selection of RINm cells with improved resistance to oxidative stress. This improved defense characteristic probably is due to an increased level of catalase expression and activity in the selected cells.

Keywords: Hydrogen peroxide; Insulin-producing cells; Selection; Catalase; Insulin; Diabetes

1. Introduction

β-Cell lines constitute a potential source of genetically-engineered insulin-producing cells to replace the difficult-to-obtain human tissue for pancreatic islet transplantation [1–3]. Pancreatic β-cells are known to be susceptible to destruction, primarily by autoimmune mechanisms, infectious agents, and by chemical toxins with several features of the destructive process common to all etiological factors [4,5]. Such cell susceptibility is believed to be one of the main causes of post transplantation β-cell failure and death. In our recent publications [6,7] we showed that repeated exposure of the RINm cell line to diabetogenic drugs such as streptozotocin and alloxan, induced the selection of cell populations with increased resistance to both toxins, while conserving functional activity. A selection strategy also was successfully used by Chen *et al*. [8] for isolation of insulinoma cell lines resistant to interleukin-1β, and γ-interferon.

The poor antioxidant system of the β-cells and particularly, the very low level of catalase (CAT), an enzyme involved in HP inactivation, is believed to be responsible for the high sensitivity of these cells to various insults, including that induced by cytokines. In addition, unlike other tissues, insulin-producing cells cannot employ the common defense mechanism against typical stress (e.g. high glucose, heat shock), i.e. the upregulation of CAT gene expression [9]. However, transfection of insulin-producing cells with the CAT gene was able to induce significant protection against reactive oxygen species (ROS) and cytokine-mediated toxicity [9–11]. In contrast to CAT, upregulation of other antioxidant enzymes, including SOD, glutathione peroxidase (GPx), and heat shock protein 70

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Abbreviations: HP, hydrogen peroxide; RINm cells, rat insulinoma cells; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; CAT, catalase; GPx, glutathione peroxidase; HSP70, heat shock protein 70; ECL, enhanced chemiluminescence; FCS, fetal calf serum; KRBB, Krebs-Ringer bicarbonate buffer; Con A, concanavalin A; ILS, islet-like structures; IBMX, 3-isobutyl-1-methylxanthine; ANOVA, analysis of variance; ROS, reactive oxygen species.

0006-2952/03/$ – see front matter
doi:10.1016/S0006-2952(03)00198-9

(HSP70) in insulin-producing cells was achieved by exposing the cells to stressful conditions such as high glucose and interleukin-1β [12–14].

Despite a recent reported describing the successful use of novel immunosuppressive drugs to maintain the function of transplanted pancreatic islets in diabetic patients over a prolonged period [15], efforts continue in the construction of immunoisolation devices that will protect these transplanted cells so as to avoid lifelong immunosuppression [16]. However, although immunoisolation prevents the penetration of immunocompetent cells and immunoglobulins, it is unable to prevent the entry of small molecules such as cytokines, which cause oxidative injury to the fragile β-cell through the generation of oxygen free radicals [17].

Based on our previous experience with the use of streptozotocin and alloxan for cell selection, we employed this strategy for the isolation of insulin-producing cells, highly resistant to oxidative stress. Resistant cells were obtained by the repeated exposure of parental RINm cells to high concentrations of HP. Cell protection potential, the activity and expression of antioxidant enzymes, as well as the effect of the selection procedure on its insulin response is described in the present manuscript.

2. Research design and methods

2.1. Materials

HP, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), neutral red, BSA, and the Bradford reagent were purchased from the Sigma Chemical Co. Alamar blue was obtained from Biosource and the cellular GPx assay kit was from Calbiochem. The RPMI 1640 culture medium, fetal calf serum (FCS), penicillin, streptomycin, and trypsin-EDTA solution were obtained from Biological Industries. The insulin RIA kit was obtained from Sorin Biomedica and the rabbit anti-CAT antibody was purchased from Rockland. Peroxidase-labeled anti-rabbit-IgG was purchased from Jackson Laboratories, Inc. and was used as a secondary antibody. The enhanced chemiluminescence (ECL) detection system was purchased from Amersham and the protein molecular weight standards were from GIBCO Life Technologies.

2.2. Cell culture and selection procedure

RINm cells, described previously [6,7,18], were cultured in RPMI 1640 medium, supplemented with 10% FCS, 2 mM L-glutamine, 100 IU/mL of penicillin and 100 μg/mL of streptomycin. Cells were grown in plastic tissue culture flasks at 37° in humidified air with 5% CO_2. The culture medium was changed every 3 days. Cells free of mycoplasma contamination were used in all experiments. Resistant cell subpopulations (RINmHP) were obtained following repeated exposure of parental RINm cells to 100 and 200 μM HP. Briefly, cells were grown to near confluence in culture medium and then incubated for 2 hr in Krebs-Ringer bicarbonate buffer (KRBB) containing 100 μM HP. Thereafter cells were washed and incubated in culture medium for 24 hr. Following this procedure, approximately 10% of the cells remained attached to the flask wall. When these cells reached semi-confluence they were treated repeatedly with 200 μM HP. After recovery and growth to a semi-confluent state, the cells were re-exposed to 100 and 200 μM HP, as described above. The HP-based selection procedure took about 1 month and then the selected cells were cultivated in regular medium, i.e. without HP supplementation, for an additional month before cryopreservation in liquid nitrogen. After thawing, cells from passage number 10 to 15 were studied, while parental RINm cells at corresponding passage numbers were used as controls.

2.3. Cell sensitivity to HP

Resistance of selected and parental cells to HP was determined by the MTT colorimetric assay, which reflects mitochondrial oxidative processes of living cells [19]. Briefly, cells were cultured for 2 days in 96-well plates (50×10^3 cells/well) and then were exposed to HP as described in the legend to Fig. 1. Thereafter the medium was replaced by KRBB supplemented with 0.5 mg/mL of MTT for a period of 3 hr. The MTT-containing medium then was removed, and the cells were exposed to 0.2 mL of DMSO. The reduction of tetrazolium salt to formazan was quantified by measuring optical density at 540 nm. Results were expressed as percent of MTT reduction. In addition, sensitivity to HP was estimated by the Alamar blue fluorometric method which detects cell metabolic activity in culture medium [20], and by a spectrophotometric assay, based on the incorporation of neutral red, a supravital dye, into the lysosomes of viable cells [21]. All assays were performed in 96-well plate format.

2.4. Splenocyte mediated cytotoxicity

Splenocytes, isolated from Wistar rats (Harlan Laboratories Ltd.), were activated by incubation for 3 days in culture medium supplemented with 10 μg/mL of concanavalin A (Con A). Diluted supernatants (1:2) from activated splenocytes were added for 48 hr to parental and selected RINm cells cultivated in 96-well plates (50×10^3/well). RINm cell viability was measured by the MTT assay. In other experiments, rat splenocytes activated with Con A, as described above, were placed in Nunc 10-mm tissue culture inserts, and co-cultured with target RINm cells in 24-well plates. The ratio of target:effector cells was 1:40. Following a 24-hr incubation, inserts were removed and RINm cell viability was estimated by the MTT assay. Similar experiments were performed with non-activated splenocytes.

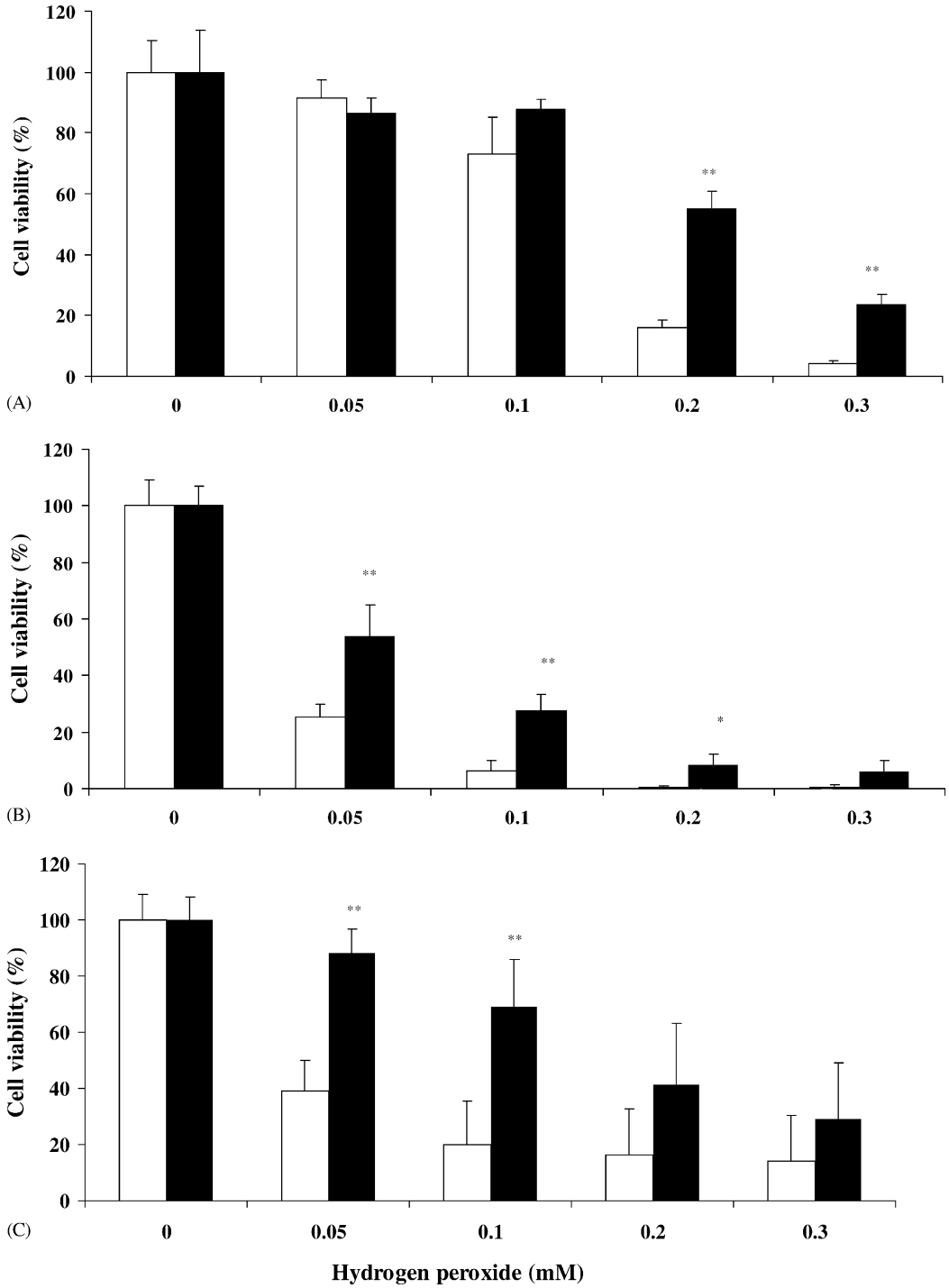

Fig. 1. Viability of RINm (white bars) and RINmHP (black bars) cells exposed to HP. The cells were treated with various concentrations of HP for 2 hr at 37° in KRBB. Following this procedure, cells were cultured for 20 hr in medium without HP. Viability was measured by the MTT assay (A) Alamar blue method (B) and neutral red assay (C) and expressed as percent of untreated controls. Data are given as means ± SD from four or five independent experiments, each performed in triplicate. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. parental RINm cells.

2.5. Degradation of HP

Cells were seeded in 96-well plates (50×10^3 cells/well) for 24 hr and then exposed to 0.1 mL of 200 μM HP for a period of 60 min at 37° in KRBB. The HP concentration was measured in KRBB at 0-, 5-,10-, 20-, 40-, and 60-min time points according to Tiedge *et al.* [9]. Briefly, 0.05 mL aliquots of supernatant were added to equal volumes of test buffer consisting of 300 mM citric acid, 130 mM Na_2HPO_4, and 3 mM *O*-phenylenediamine at pH 5. After mixing, 0.05 mL of substrate solution consisting of 100 mU/mL of horseradish peroxidase was added. Following a 30-min

incubation at room temperature, the reaction was terminated with 0.05 mL of 0.5 M H_2SO_4. The absorbance then was measured at 492 nm in a micro-plate reader. The concentration of HP in the medium, in the absence of RINm cells, was measured under the same experimental conditions.

2.6. CAT and GPx activities

Cells were grown to semi-confluence and then trypsinized. The cell pellet was washed and homogenized in 50 mM phosphate buffer (pH 7.8). The samples were frozen at −70°, thawed, and sonicated on ice for 1 min in 10-s bursts with an ultrasonic processor (Misonix Inc., model XL 2020). The homogenate was centrifuged at 10,000 *g* for 30 min at 4° and the protein content was measured using the Bradford reagent. CAT activity was determined by measuring the initial rate of decay of HP absorbance at 240 nm with a Uvikon 860 spectrophotometer (Kontron Instruments). One unit of activity corresponds to the loss of 1 μmol of peroxide per min at 25° [22]. Cellular glutathione peroxidase (c-GPx) activity was measured in a photometric assay using a commercially available kit (Calbiochem). The assay is based on the oxidation of NADPH to $NADP^+$, which is accompanied by a decrease in absorbance at 340 nm. The rate of decrease in absorbance is directly proportional to the c-GPx in the sample.

2.7. Western blot analysis of CAT expression

Parental and selected cells were homogenized, on ice, in medium containing 20 mM HEPES, 210 mM mannitol and 70 mM sucrose (pH 7.4), and then were centrifuged to pellet insoluble material. The protein concentration in the supernatant was determined using the Bradford reagent. Thirty micrograms, per well, of total protein was resolved in a 4–12% NuPAGE™ Bis-Tris Gel System (Invitrogen), and the proteins were electroblotted to nitrocellulose membranes. The membranes were blocked using 1% gelatin for 1 hr at room temperature and then were incubated overnight at 4° with a 1:10,000 dilution of a rabbit anti-CAT antibody. The following day the membranes were incubated with a 1:20,000 dilution of a peroxidase-conjugated goat anti-rabbit (IgG) antibody at room temperature. The specific protein bands were visualized by chemiluminescence using the ECL detection system. Data were quantified using a soft laser scanning densitometer.

2.8. Insulin secretion in a static assay

Seventy-two hours prior to the experiments, 2×10^5 cells/well were seeded in 24-well plates. After being washed, cells were preincubated for 1 hr in glucose-free KRBB with 0.25% BSA, and re-incubated in the buffer at 37° for an additional 2 hr with 5 or 20 mM glucose. Supernatants were centrifuged at 1500 *g* for 10 min at 4° and collected for insulin determination by RIA.

2.9. Insulin secretion in a perifusion assay

Cells grown in tissue culture flasks to semi-confluence were trypsinized and transferred to bacteriological grade petri dishes. After 24 hr in culture medium the cells formed islet-like structures (ILS) with a diameter of 50–100 μm. The ILS, comprised of about 40×10^6–50×10^6 cells, were placed on filters made from Kimwipes™ EX-L (Kimberly-Clark) in a Swinnex filter holder of 25 mm (Millipore). The perifusion device equipped with TYGON® R-3603 tubing (Cole-Parmer) and a peristaltic pump was placed in a temperature regulated plastic box at 37°. ILS were placed in glucose-free KRBB containing 0.25% BSA for 45 min before undergoing periperfusion. Basal secretion in the absence of secretagogues was estimated in the same medium and then the cells were switched to medium containing secretagogues (glucose alone and glucose with 3-isobutyl-1-methylxanthine (IBMX) or KCl). The cells then were switched back to the basal condition. The medium was bubbled with 95% air and 5% carbon dioxide. Perifusion flow was 0.3 mL/min and samples were taken every 3 min.

2.10. Determination of intracellular insulin content

Following trypsinization, cells were harvested, counted, and their intracellular insulin content was determined, after cell sonication for 15 s and acid ethanol extraction of insulin overnight at 4°. The level of insulin in the samples was estimated using a radioimmunoassay kit (INSIK-5, DiaSorin) that is 100% specific for rat insulin.

2.11. Cell growth and morphology

The morphological and growth characteristics of parental and selected cells were studied in regular culture medium. Briefly, cells were seeded into 24-well plates in culture medium at a cell density of 1×10^5/well. Following trypsinization, the cell number, per well, was determined at 3, 5, 7, and 10 days after seeding using a counting chamber and a light microscope.

2.12. Statistical analysis

ANOVA was used to evaluate the statistical significance of differences between groups. The results are presented as mean values ± SD of independent, repeated experiments. A $P < 0.05$ was considered statistically significant.

3. Results

3.1. Cell morphology and growth characteristics

Following cell selection and culturing, the morphological and growth characteristics of parental and selected cells

were studied. We found that the growth curve of selected cells was similar to parental cells (data not shown). Morphological analysis indicated that when cells were grown beyond confluency, small increases in the frequency of hillocks and islet-like cell clusters were found in monolayer cultures of selected cells. Additional morphologic characteristics such as cell shape and size were similar in both cell populations.

3.2. Cell resistance to different toxins

Estimation of cell viability with three different cytotoxicity assays indicated that selected RINmHP cells were more resistant to HP than were the parental RINm cells (Fig. 1A–C). Some differences in HP cytotoxicity found between the MTT-, neutral red- and Alamar blue-assays probably reflected assay sensitivity.

As ROS are involved in cell-mediated cytotoxicity, we estimated the sensitivity of parental and selected RINm cells to Con A-activated splenocytes and to culture supernatants obtained from these splenocytes. Unlike parental RINm cells, selected cells incubated in the presence of activated rat splenocytes, were resistant to splenocyte-generated soluble toxins (Fig. 2). Similar results were obtained following treatment with supernatants of activated splenocytes, as only $65 \pm 4\%$ of the parental cells were viable following exposure compared to $100 \pm 10\%$ of the RINmHP cells ($P < 0.05$). Non-activated splenocytes had no effect on RINm cell viability.

3.3. HP degradation in cell culture

To estimate the capacity of RINm and RINmHP cells to inactivate HP in the extracellular medium, we used a colorimetric assay with *O*-phenylenediamine as the chromogenic substrate for horseradish peroxidase. In order to ascertain the extent of HP metabolism by RINm and RINmHP cells the inactivation of HP was measured in KRBB in the presence and absence of cells (Fig. 3). HP (200 μM) had a half-life of 49 min in the presence of the parental cells, while in selected cells the HP half-life was about 2.5 times shorter, i.e. 20 min. The concentration of HP measured under the same experimental conditions but without cells remained unchanged over a 60-min incubation period (data not shown).

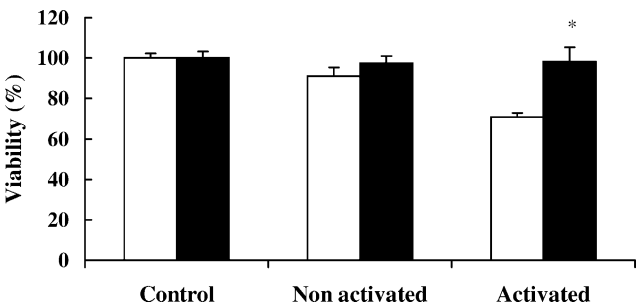

Fig. 2. Viability of RINm (white bars) and RINmHP (black bars) cells following treatment with activated rat splenocytes. Splenocytes were activated with Con A (10 μg/mL). Non-activated splenocytes were used as an additional control. RINm cells were seeded in 24-well plates and then treated for 24 hr with rat splenocytes placed in well inserts. The ratio of target effector cells was 1:40. Thereafter, the inserts with these cells were removed and viability of target cells was estimated by the MTT assay and expressed as percent of untreated controls. Data are given as means ± SD from three independent experiments, each performed in triplicate. $^{*}P < 0.05$ vs. parental RINm cells.

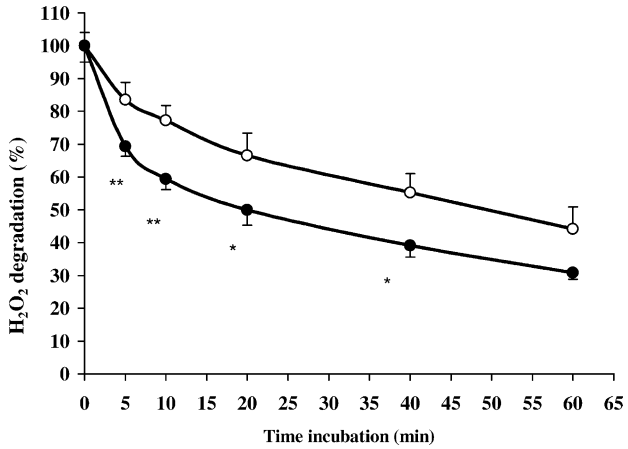

Fig. 3. Degradation of HP by RINm (white circles) and RINmHP (black circles) cells. The cells were exposed to 200 μM HP for up to 60 min at 37° in KRBB. Degradation was measured using a colorimetric assay. Data are given as means ± SD from five independent experiments, each performed in triplicate. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. parental RINm cells.

3.4. Cellular GPx and CAT expression

To determine the effects of the selection procedure on the cellular antioxidant enzymes responsible for the decomposition of HP, both GPx and CAT activities were measured. In both parental and selected cells the activity of GPx was barely detectable. Very low or barely detectable levels of GPx activity and expression previously were reported in RINm5F cells [9]. In contrast to GPx, the level of CAT activity was detectable in parental and selected cells. As shown in Fig. 4A, the CAT activity in parental cells was about 20 Units per mg of total protein, with a 2-fold higher activity in selected RINmHP cells. Elevated expression of CAT (about 50% increase) also was shown by western blot analysis (Fig. 4B).

3.5. Insulin content and secretion

We found that the intracellular insulin content was insignificantly reduced ($P > 0.05$) in selected RINmHP cells compared with the parental cells (82.8 ± 15.7 μU vs. 118.2 ± 20.5 μU per one million cells). The static assays of insulin-response to glucose alone indicated that both cell populations were able to secrete insulin in the absence of this secretagogue. However, neither parental nor selected cells demonstrated a statistically significant elevation of insulin secretion in response to 5 mM vs. 20 mM glucose

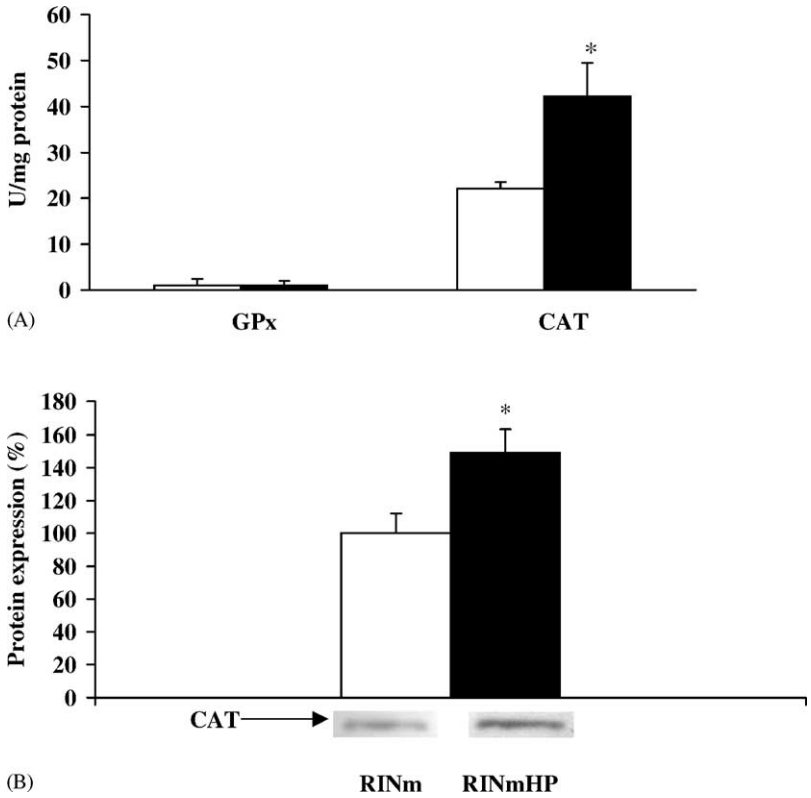

Fig. 4. Activity and expression of antioxidant enzymes in RINm (white bars) and RINmHP (black bars) cells. (A) Activity of GPx and CAT in cell homogenates. Values are means ± SD from three independent experiments. (B) Western blot analyses of CAT. Thirty micrograms of protein, per well, was resolved on a 4–12% gradient gel and electroblotted onto a nitrocellulose membrane. The specific protein bands were visualized using the ECL detection system. Band intensity was expressed as a percentage of the corresponding band in the parental RINm cells (100%). Data are representative of blots from three independent experiments. $^{*}P < 0.05$ vs. parental RINm cells.

(24 ± 4 μU/2 hr/10^6 RINm cells vs. 33 ± 4 μU/2 hr/10^6 RINm cells and 26 ± 2 μU/2 hr/10^6 RINmHP cells vs. 27 ± 5 μU/2 hr/10^6 RINmHP cells). A lack of glucose-stimulated insulin secretion is a well-known phenomenon in RINm cells following cultivation *in vitro*. To potentiate the effect of glucose, 0.1 mM IBMX or 30 mM KCl was added to 5 mM glucose, and the insulin response was studied in perifusion assays. As shown, IBMX (Fig. 5A) and KCl (Fig. 5B) significantly increased insulin secretion in response to glucose in both parental and selected cells, to approximately equal levels.

4. Discussion

The low defense characteristic of β-cells is believed to be responsible for their high sensitivity to different diabetogenic factors. In our recent publications [6,7], we have shown that repeated exposure of RINm cells to a high dose of diabetogenic compounds, such as streptozotocin and alloxan, induced the selection of cell populations with increased resistance to both chemical toxins, and an unimpaired insulin response. The improved defense property of the selected cells can be partially explained by their lower expression of GLUT 2, which acts as a transporting substrate for both streptozotocin and alloxan [23,24]. In the present study, we demonstrated that the selection of cell subpopulations with improved defense properties against oxidative stress can be obtained by repeated exposure of tumoral insulin-producing β-cells to high doses of HP. The improved resistance of the selected cells to HP also was accompanied by an increased protection potential against Con A-activated rat splenocytes. Cell-mediated cytotoxicity is known to be a crucial step in autoimmune destruction of pancreatic β-cells, involving cytokine secretion by inflammatory cells, and generation of ROS by cytokine-stimulated β-cells [25–28]. The higher resistance of RINmHP cells to activated splenocytes probably reflects the cells improved defense properties against oxidative stress, induced by a mixture of cytokines and toxins, generated by activated splenocytes.

To better understand the mechanisms leading to the improved antioxidative defense capacity following the selection procedure, we studied the cellular potential to inactivate HP. The finding that selected cells are both more resistant to various oxidative stresses, and more efficient in inactivating HP, can partially explain RINmHP cell survival during the selection procedure. We found GPx activity to be barely detectable in RINm and RINmHP cells. These findings correspond with reports showing a very low level of cytoprotective enzymes in insulin-producing cells [29,30]. In contrast to GPx, the level of CAT activity was significantly higher in RINmHP cells. In these cells,

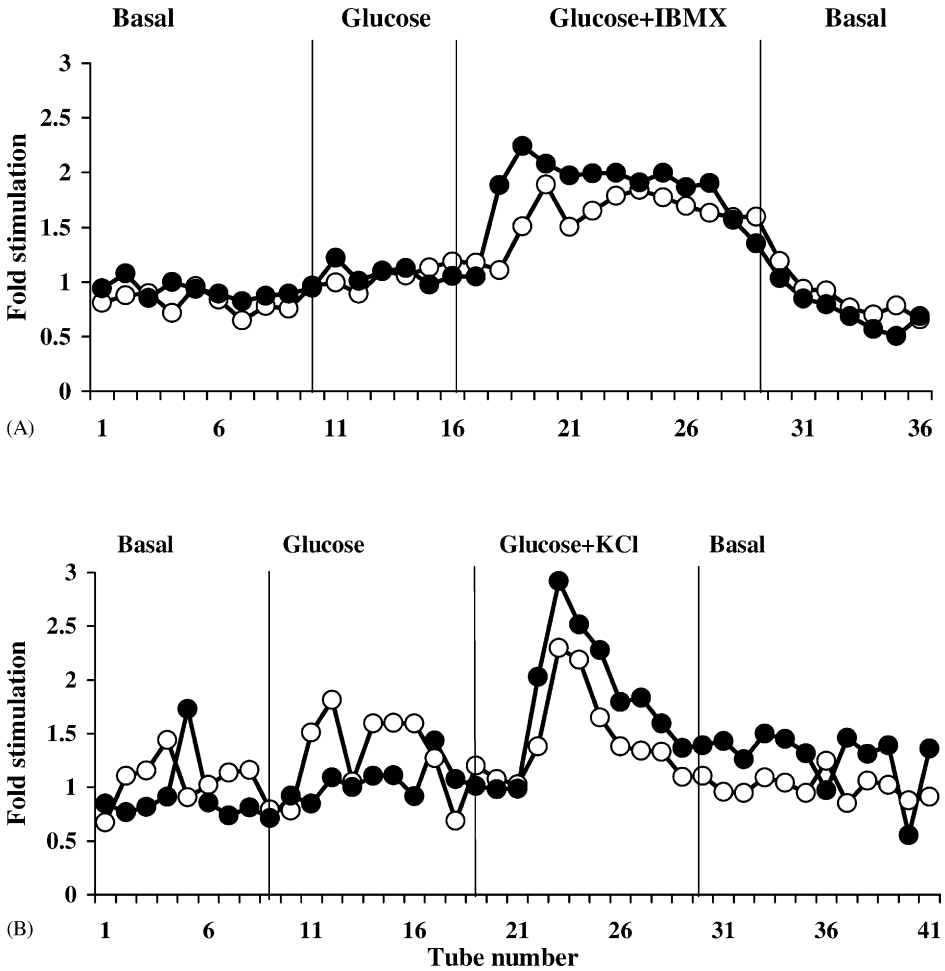

Fig. 5. Effect of IBMX (A) and KCl (B) on glucose-induced insulin secretion by RINm (white circles) and RINmHP (black circles) cells in perifusion assays. Cells were perifused in the absence of secretagogues (basal) and then switched to medium supplemented with glucose (5 mM) alone; glucose (5 mM) with IBMX (0.1 mM) or KCl (30 mM) and then once again switched to basal secretion. Perifusion flow was 0.3 mL/min. Samples were taken every 3 min. Results are expressed as fold stimulation compared to 5 mM glucose alone. Data are representative profiles of two independent perifusion experiments.

the improved resistance to HP and other oxidative stressors was accompanied by a 2-fold higher capacity to degrade HP, and a higher CAT activity, when compared to parental naive cells. Previous attempts to induce overexpression of CAT by stressful treatments, such as high glucose concentration and oxygen tension, as well as by elevated temperature, were not successful [9]. In the present study, only a small portion of HP-resistant cells survived, and maintained their characteristics following *de novo* cell propagation. Probably, HP-based selection, may occur not due to upregulation of CAT expression *via* induction but rather through selection of pre-existing cell populations with higher expression levels. This assumption is in agreement with the fact that parental RINm cells are composed of highly heterogeneous populations containing both insulin- and somatostatin-secreting cells [18].

Using a gene transfection technique, it was shown that a greater than 100-fold overexpression of CAT in RINm5F cells induced a 10-fold greater resistance to HP [9]. Moreover, an approximately 2-fold higher level of CAT activity was found in human than in rat pancreatic islets, a finding that can explain the higher resistance of human islets to HP and other β-cell toxins [31]. These results correspond to our data indicating that a 2.5-fold higher level of CAT activity in selected RINmHP cells induces a 3- and 6-fold greater resistance to 200 and 300 μM HP, respectively, in the MTT-assay. Although these findings indicate a possible involvement of CAT in the resistance of RINmHP cells to HP, additional factors can be implicated in the process of cellular defense, such as reparative enzymes and various intracellular free radical scavengers. Recently, it was found that overexpression of antioxidant enzymes in RINm5F cells confer protection against cytokine-mediated toxicity [11]. The higher resistance of the selected RINmHP cells to a mixture of cytokines generated by activated splenocytes thus could result from the overexpression of CAT, which inactivates cytokine-induced ROS more effectively. One may speculate that the mechanisms by which insulin-producing cells are able to survive the selection procedure, depends on the cell types and the conditions of stress. For example, incubation of RINm INS-1 cells with increasing concentrations of cytokines for 2 months induced a

selection of cells resistant to IL-1β- and γ-interferon. This was due to a reduction in expression of the IL-1 receptor type I, and the impaired production of nitric oxide by the surviving cells [8]. However, a 1-day incubation of isolated rat pancreatic β-cells with IL-1β induced an upregulation of various antioxidant enzymes and the downregulation of β-cell-specific proteins [13].

Our previous reports [6,7], the results of this study, and the observations made by Chen *et al*. [8] indicate that established selected cell populations exhibit not only improved defense properties, but also conserve their regulated insulin secretion. HP treatment of RINm cells did not change intracellular insulin content nor did it alter the capacity of these cells to secrete insulin in response to a combination of glucose and IBMX or KCl, compounds that enhance cAMP levels and potentiate β-cell membrane depolarization, respectively. These results indicate that at least cAMP-dependent, and K^+-ATP based pathways of insulin secretion in selected cells were not impaired.

In summary, our data suggest that a cell selection strategy, based on the repeated exposure of insulin-producing cell lines to β-cell injurious compounds, offers cell subpopulations an enhanced defense system against oxidative stress, probably through the selection of cells that overexpress CAT. Such a procedure does not impair cellular function as reflected by an unchanged intracellular insulin content and unaltered secretion in response to secretagogues. The possibility of engineering cells with a higher resistance to oxidants might be of importance in pancreatic β-cell transplantation, in particular, when using an immunoisolation technique that prevents the penetration of cells and large molecules (such as immunoglobulins), but does not offer protection against cytokine attack and oxidative stress. Thus, toxin-based selection of resistant insulin-producing cells may be beneficial in prolonging cell survival and function in the post-transplantation period. In addition, these cells may be useful for studying the mechanisms of cell sensitivity to pharmacological agents and various toxins.

Acknowledgments

This work was partially supported by Grant 6774195 from the Israeli Ministry of Science and Art and from INTAS Grant 00-057 of EU.

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