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Reduced glutamate decarboxylase activity in rat islet β cells which survived streptozotocin-induced cytotoxicity

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Rat pancreatic β cells exhibit a 16-fold higher glutamate decarboxylase (GAD) activity than islet non-β cells, but a similar glutamate dehydrogenase (GDH) activity. β Cells which survive exposure to 2 mM streptozotocin only contain 10 percent of the GAD activity of control cells, but their GDH activity remains unaltered. Culture of streptozotocin-treated β cell preparations with 2 mM nicotinamide reduces the number of dead cells and prevents in part the decline in GAD activity of surviving β cells. These data indicate that loss in activity of the β cell specific enzyme GAD can serve as marker for β cells which survived a destructive process. It is furthermore demonstrated that nicotinamide increases the percent surviving cells and decreases their loss in GAD activity.

β Cell; Diabetes; Autoantibody; Streptozotocin; Islet, Glutamate decarbocylase

1. INTRODUCTION

In man, development of insulin-dependent diabetes is often associated with the appearance of circulating antibodies that recognize antigens in pancreatic β cells [1–3]. Glutamate decarboxylase (GAD) has been identified as one of the β cell antigens to which autoantibodies are produced during the preclinical phase of the disease [4,5]. It is unknown whether and, if so, how the formation of GAD-antibodies is related to the destruction of β cells which occurs during this period. Autoantibodies often disappear during the clinical course of the disease, which has been interpreted as a sign of a depleted β cell mass and vanishing autoantigens [6]. They may also be present in genetically susceptible individuals who are unlikely to develop the disease [7]. In the present study, we used the cellular GAD-activity to examine the course of the enzyme following in vitro damage to rat islet β cells.

2. MATERIALS AND METHODS

2.1. *Preparation of rat β cells*

Islet β cells were prepared from adult male Wistar rats (150–250 g body weight) according to previously described techniques [8]. Briefly, islets were isolated after collagenase digestion of the pancreas and then dissociated into a cell suspension by treatment with trypsin in a calcium-free medium [8]. Single β cells were purified by autofluorescence-activated sorting of the dispersed cells [8]. In 3 experiments, the population of islet endocrine non-β cells was also isolated [8] with the purpose of comparing enzyme activities in freshly isolated islet β and islet non-β cells.

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2.2. *Culture conditions and exposure to streptozotocin*

Purified β cells were suspended in Ham's F10 medium at 10 mM glucose, supplemented with 2 mM L-glutamine, 50 μM 3'-isobutyl-1-methylxantin, penicillin (0.075 mg/ml) and streptomycin (0.1 ng/ml), and then reaggregated during a 3 h rotary shaking incubation at 37°C [9] The aggregates were subsequently cultured under static conditions for a total period of 9 to 10 days. Every other day, half of the medium volume was replaced by fresh medium. After 7 or 8 days of culture, the cells were distributed over Lux dishes containing 3 ml of the same culture medium ($6 \cdot 10^5$ cells at start per dish). After 2 h of culture in the Lux dishes, streptozotocin (final conc. 2 mM) or only its solvent (1 mM HCl) was added. Streptozotocin exposure lasted 30 min. after which drug-treated and control cells were extensively washed and further cultured for 48 hours in the above culture medium with or without 2 mM nicotinamide. At the end of this period the medium was recovered for insulin assay and the cells extensively washed before sampling for assays.

2 3 *Analysis of cells*

Before and after culture, cells were analysed for their insulin and DNA content [8] as well as for their glutamate decarboxylase and glutamate dehydrogenase activities [10,11]. Hormone content and enzymatic activities are expressed per ng DNA that was measured in the same cell preparation. Results are presented as mean values ± S.E.M. and significance of differences calculated by Student's *t*-test

3. RESULTS

3.1. *Glutamate decarboxylase activity in rat islet β cells*

Freshly purified rat β cells contain a glutamate decarboxylase (GAD) activity (50±6 fmol/min/ng DNA) which is 16-fold higher than that of freshly isolated islet non-β cells (3 fmol/min/ng DNA). A 9 to 10 day culture period at 10 mM glucose does not result in significant alterations of GAD-activity in β cells (37 ± 9 fmol/min/

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ng DNA, p > 0.05). On the other hand, glutamate dehydrogenase (GDH) exhibits comparable activities in islet β cells (4.0 ± 0.2 pmol/min/ng DNA) and islet non-β cells (4.4 pmol/min/ng DNA). As for GAD, GDH-activity is comparable in freshly isolated and cultured β cells (3.8 ± 0.6 pmol/min/ng DNA, $P > 0.05$).

3.2. *Effect of streptozotocin on β cell survival*

Comparison of the DNA content in the control preparation before and after culture indicated that the 9 to 10 day culture period resulted in a loss of 25 percent of the intial number of β cells (5.7 ± 0.4 ng DNA per 1000 cells on day 1 was reduced on day 9 or 10, to 4.2 ± 0.4 ng DNA per 1000 cells at start $P < 0.05$). The effect of streptozotocin on β cell survival was evaluated by comparing the DNA content of streptozotocin-treated preparations with that of paired cultured control cells. After 30 min exposure to 2 mM streptozotocin and a subsequent 48 h culture without the drug, cell recovery was 53 percent lower than in the control condition as derived from the respective DNA contents. The presence of 2 mM nicotinamide in the post-streptozotocin culture period improved cell recovery (only 36 percent reduction as compared to controls). When control preparations were cultured with 2 mM nicotinamide during the final 48 hours, DNA recovery tended to be higher (5.2 ± 0.1 ng DNA per 1000 cells at start) than without this agent (4.2 ± 0.4 ng DNA recovered per 1000 cells at start) but this difference was not statistically significant.

3.3. *Characteristics of cells surviving after streptozotocin exposure*

Cells surviving streptozotocin-treatment and subsequent culture were compared to those recovered from control cultures by expressing the respective hormone contents and enzyme activities per ng recovered DNA (Table I).

The cells recovered in the streptozotocin condition exhibited a similar hormone content (5.6 ± 1.3 ng insulin/ng DNA) than control cells (3.8 ± 0.7 ng insulin/ng DNA, p > 0.05); their incubation medium contained, however, significantly less hormone suggesting a reduction in their secretory activity during the final 2 days of culture (Table I). Addition of nicotinamide to the culture medium did not increase total hormone content per ng cellular DNA but raised the fraction that was released in the medium both in control cells and in streptozotocin-treated cells (Table I).

Streptozotocin-pretreated cells presented similar GDH activity as control cells, but their GAD-activity was only 7 percent of control values (Table I). The presence of nicotinamide in the culture medium did not alter cellular GDH activity but was found to partially protect against the decline in cellular GAD-activity of cells surviving streptozotocin treatment (Table I).

4. DISCUSSION

This study confirms that islet β cells contain glutamate decarboxylase and that the enzyme is β cell specific in rat islet tissue [12,13]. A 10 day culture period did not alter GAD activity in rat β cells. Cultured β cells were used to examine whether exposure to a damaging agent such as streptozotocin alters GAD activity in β cells which have not been killed by the diabetogenic compound. The cells were incubated with streptozotocin for 30 min, extensively washed to remove the drug and then cultured for 48 h at 10 mM glucose during which irreversibly damaged cells detach from the aggregates and lose their viability; a second wash allows to remove dead cells as indicated by neutral red staining. Under the conditions used in this study, approximately 50 percent of the β cells were killed by the streptozotocin treatment. The remaining cells were neutral red-positive and contained a similar insulin content than the control cells; the hormone content in their culture medium was, however, lower suggesting that their secretory activity is reduced. These data confirm that intercellular differences exist in the β cell sensitivity to cytodestructive agents [14,15] with a proportion of cells dying and with surviving cells that exhibit signs of functional impairment [16].

β Cells which survived streptozotocin treatment pre-

Table I

Hormone and enzyme content of rat islet β cells surviving in vitro exposure to streptozotocin*

Treatment	Control		Streptozotocin	
Post-treatment culture with nicotinamide 2 mM	–	+	–	+
Insulin content (ng/ng DNA)				
Cells	3.8 ± 0.7	1.9 ± 0.1	5.6 ± 1.3	4.7 ± 1.4
Medium	7.0 ± 1.2	5.4 ± 0.4	1.9 ± 0.4*	3.1 ± 0.6
Total	10.8 ± 1.8	7.3 ± 0.4	7.5 ± 1.5	7.8 ± 1.7
GAD activity (fmol/min/ng DNA)	37.4 ± 8.7	35.3 ± 3.8	2.6 ± 0.8**	8.3 ± 1.7*#
GDH activity (pmol/min/ng DNA)	3.8 ± 0.6	3.1 ± 0.3	3.9 ± 0.4	2.9 ± 0.6

*Contents expressed per DNA content in the same sample, containing cells which survived treatment and post-treatment culture. Data are means ± S.E.M., of 4 to 8 independent experiments; statistical significance of differences is calculated by paired Student's *t*-test: *P* vs control: *$P < 0.05$, **$P < 0.02$; *P* vs. STZ without nicotinamide: #$P < 0.02$.

sented a similar GDH activity as control cells but their GAD activity fell to 10 percent of control values. Their signs of functional impairment are thus associated to a decline in a cell specific enzyme. This is not necessarily an indication for a regulatory role of GAD in the process of glucose-induced insulin release. It may as well indicate that surviving cells have lost specific glucose-regulated features of differentiated β cells. Assuming that similar cells can occur in vivo following a β cell toxic process, the question arises as to whether they can induce autoimmune responses through presentation of autoantigens, or, conversely, reduce autoimmune reactivity by a decline in the expression of cellular autoantigens such as GAD. The latter mechanism may result in a decrease of circulating autoantibodies, however, in the presence of surviving β cells.

Culture of streptozotocin-treated β cells in the presence of nicotinamide reduced the proportion of destroyed cells as well as the functional impairment of surviving cells. The cells that were recovered from this condition had maintained a higher rate of hormone release and presented also higher GAD levels than those collected without nicotinamide. These results extend the notion that nicotinamide is partially protectective against β cell damage, even when administered after cellular interaction with the damaging agent [13,14]. This property may be responsible for a beneficial effect of the agent in prediabetic conditions (17).

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