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# Long-term effects of exposure of pancreatic islets to nicotinamide in vitro on DNA synthesis, metabolism and B-cell function

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**Summary.** Using $^3$H-thymidine labeling techniques, we found that rates of DNA synthesis in islet cells doubled when mouse pancreatic islets were cultured for 1 week with 10 mmol/l nicotinamide, a potent poly(ADP-ribose) synthetase inhibitor. Culture with nicotinamide partially inhibited glucose-stimulated insulin release, whereas the islet insulin content and rate of (pro)insulin biosynthesis remained unchanged. Long-term exposure to nicotinamide decreased glucose oxidation and ATP content in the islets. The findings support the view that poly(ADP-ribose)synthetase inhibitors stimulate islet cell replication, but may be accompanied by significant inhibitory effects on islet cell function.

**Key words:** Autoradiography, insulin release, DNA synthesis, nicotinamide, pancreatic islets, poly(ADP-ribose), synthetase.

Poly(ADP-ribose)synthetase is a chromatin-associated enzyme which is involved in cellular DNA repair [1] and differentiation [2-4]. In pancreatic islets the activity of this enzyme increases upon exposure to streptozotocin, leading to cytotoxic cellular NAD depletion [5, 6] coupled to increased DNA repair synthesis [7, 8]. The B-cytotoxic effects of streptozotocin can be counteracted by inhibitors of poly(ADP-ribose) synthetase such as nicotinamide and 3-aminobenzamide [5, 6, 9, 10]. It was recently demonstrated that such compounds may also induce B-cell regeneration in diabetic animals [11]. Okamoto [12] postulated that the poly(ADP-ribose) synthetase inhibitors may counteract a suppression of B-cell DNA replication, thus inducing B-cell regeneration. An increased knowledge on how the B-cell growth is regulated is warranted. Such information may point towards new approaches in the treatment of Type 1 (insulin-dependent) diabetes mellitus, i.e. administration of drugs which induce B-cell regeneration by affecting the nuclear control of DNA replication.

The aim of the present investigation was to study the long-term effects of a potent poly(ADP-ribose) synthetase inhibitor, nicotinamide, on islet cells in vitro. The effects of nicotinamide on islet cell DNA synthesis were determined by $^3$H-thymidine labeling, and those on islet metabolism by measuring glucose oxidation and the contents of adenine and pyridine nucleotides. Specific B-cell functions were monitored by assaying glucose-stimulated insulin release and (pro)insulin biosynthesis.

## Materials and methods

### *Islet preparation and culture*

Pancreatic islets were isolated from adult NMRI mice by a collagenase isolation technique [13]. The islets were cultured free-floating for 7 days at 37 °C in medium RPMI 1640 (11.1 mmol/l glucose) supplemented with 10% calf serum, in an atmosphere of humidified air + 5% $CO_2$ [14], with or without the addition of 10 mmol/l nicotinamide. The culture medium was changed every second day.

### *DNA synthesis and content*

For estimation of islet DNA synthesis, 1 μCi/ml of [methyl-$^3$H]thymidine (Amersham International, Amersham, UK) was added to the culture medium on day 6. After 18 h, duplicate groups of 50 islets were washed in Hanks' solution containing 10 mmol/l thymidine and disrupted ultrasonically in redistilled water. Duplicate aliquots of the homogenates were precipitated with 5% (w/v) trichloroacetic acid, and the labeled DNA was separated from unbound $^3$H-thymidine by filtering through glass microfibre filters (GF/A 2.5 cm; Whatman, UK). After drying, the radioactivity on the filters was determined by liquid scintillation. In another sample of the homogenate, the islet DNA content was determined [15, 16]. From the dishes of islets exposed to $^3$H-thymidine, a group of about 50 islets was harvested and washed as above; after fixation in Bouin's solution, these islets were processed for autoradiography for calculation of the islet cell labeling index [17]. Labeled islet cells were identified, and at least 800 cells were scored to calculate the labeling index in each group of cultured islets.

### *(Pro)insulin and total protein biosynthesis*

Groups of 10 islets were incubated at 37 °C for 2 h (air + 5% $CO_2$) in a bicarbonate buffer [18] containing 10 mmol/l Hepes and 2 mg/ml al-

**Table 1.** DNA content, DNA synthesis and autoradiographic labeling index of mouse pancreatic islets cultured for 1 week in medium RPMI 1640 with or without addition of 10 mmol/l nicotinamide

| | Controls | +10 mmol/l nicotinamide |
|---|---|---|
| DNA content (μg/10 islets) | 0.32 ± 0.043 (14) | 0.34 ± 0.034 (14) |
| DNA synthesis (dpm × $10^{-2}$/10 islets) | 3.6 ± 0.34 (9) | 5.7 ± 0.34 (9)[a] |
| Autoradiographic islet cell labeling index (%) | 2.3 ± 0.44 (9) | 4.6 ± 0.26 (9)[a] |

Values are given as means ± SEM with numbers of culture experiments within parentheses.
[a] $p < 0.001$, unpaired Student's t-test.

**Table 2.** (Pro)insulin and total protein biosynthesis, insulin content and insulin release, and glucose oxidation in mouse pancreatic islets cultured for 1 week in medium RPMI 1640 with or without addition of 10 mmol/l nicotinamide

| | Controls | +10 mmol/l nicotinamide |
|---|---|---|
| (Pro)insulin biosynthesis (dpm × $10^{-3}$/10 islets × 2 h) | 34.2 ± 5.5 (10) | 35.9 ± 5.5 (10) |
| Total protein biosynthesis (dpm × $10^{-3}$/10 islets × 2 h) | 130 ± 17.7 (10) | 147 ± 18.2 (10) |
| Fraction (pro)insulin of total protein biosynthesis (%) | 25.7 ± 1.0 (10) | 24.0 ± 0.65 (10) |
| Insulin content (ng/10 islets) | 427 ± 45.6 (22) | 352 ± 45.6 (22) |
| Insulin release (ng/10 islets × 1 h) | | |
| 1.67 mmol/l glucose | 4.1 ± 0.67 (22) | 4.2 ± 0.76 (22) |
| 16.7 mmol/l glucose | 44 ± 6.7 (22) | 24 ± 3.3 (22)[a] |
| Glucose oxidation (pmol glucose/10 islets × 90 min) | 525 ± 25.7 (11) | 446 ± 37.4 (11)[b] |

Values are given as means ± SEM with numbers of culture experiments within parentheses.
[a] $p < 0.02$, Student's unpaired t-test; [b] $p < 0.05$, Student's paired t-test

**Table 3.** Adenine nucleotide (AMP + ADP + ATP) and pyridine nucleotide (NADH + NAD) contents of mouse pancreatic islets cultured for one week in medium RPMI 1640 with or without addition of 10 mmol/l nicotinamide

| | Controls | +10 mmol/l nicotinamide |
|---|---|---|
| AMP + ADP + ATP content (pmol/islet) | 7.4 ± 0.44 (15) | 6.2 ± 0.44 (16) |
| ATP content (pmol/islet) | 4.2 ± 0.18 (15) | 3.3 ± 0.24 (16)[a] |
| NADH + NAD content (pmol/10 islets) | 5.0 ± 0.30 (16) | 6.0 ± 0.34 (16)[a] |
| NADH/NAD | 0.106 ± 0.013 (16) | 0.112 ± 0.021 (16) |

Values are given as means ± SEM with numbers of culture experiments within parentheses.
[a] and [b] denote $p < 0.05$, Student's unpaired and paired t-test respectively

bumin, hereafter referred to as KRBH, 16.7 mmol/l glucose and 50 μCi/ml L-[4.5-$^3$H]leucine (Amersham). After the incubation the islets were washed and homogenised, and the biosynthesis of total protein and (pro)insulin was measured [19].

### *Insulin release and content*

Triplicate groups of 10 islets were incubated for 1 h in 250 μl of KRBH containing 1.67 mmol/l glucose (37 °C; $CO_2/O_2$: 5/95). The medium was then replaced by KRBH supplemented with 16.7 mmol/l glucose for another 1-h period. Insulin released to the media was determined by radioimmunoassay [20]. Insulin content was measured after extraction of the islets overnight at +4 °C in acid ethanol (0.18 M HCl in 70% (v/v) ethanol).

### *Glucose oxidation*

The procedure has been described previously [21]. Groups of 10 islets in triplicate were incubated for 90 min in 100 μl of KRBH containing D-(U-$^{14}$C) glucose (Amersham) and non-radioactive glucose to give a final concentration of 16.7 mmol/l and a specific radioactivity of 0.5 mCi/mmol.

### *Adenine and pyridine nucleotide contents*

Cultured islets in groups of 10 or 30 were transferred to plastic tubes containing 0.1 ml of KRBH plus 11.1 mmol/l glucose with or without 10 mmol/l nicotinamide. The tubes were incubated for 60 min at 37 °C (air + 5% $Co_2$), and the incubation media were then rapidly withdrawn and exchanged for 40 μl of 0.02 mol/l NaOH (adenine nucleotides) or 30 μl of 0.04 mol/l NaOH (pyridine nucleotides) and instantly frozen in liquid nitrogen. The islet contents of ATP + ADP + AMP and NADH + NAD were measured by bioluminescence methods [22] as described previously [9].

### *Statistical analysis*

Data were computed as means ± SEM, and compared using the Student's t-test for unpaired samples. Levels of significance are as shown in Tables 1-3.

## Results

Tissue culture of isolated islets in the presence of 10 mmol/l nicotinamide increased $^3$H-thymidine incorporation by about 60% (Table 1). The possibility that this increase was due to enhancement of islet cell DNA synthesis was supported by the finding that the autoradiographic islet cell labeling index was doubled. Furthermore, there was a significant correlation between the rates of DNA synthesis in the islets and their labeling index ($r = 0.69$, $p < 0.01$, $n = 18$). The increased DNA replication in the islets cultured with nicotinamide was not, however, accompanied by a net increase in the islet content of DNA (Table 1).

Culture in the presence of 10 mmol/l nicotinamide did not affect either the rates of (pro)insulin and total protein biosynthesis or the islet insulin content (Table 2). Glucose-stimulated insulin release was reduced, however, by about 50% in islets cultured in the nicotinamide-supplemented medium. Likewise, the rate of glucose oxidation in the islets was slightly inhibited

(Table 2). In acute experiments with freshly isolated islets, the presence of 10 mmol/l nicotinamide did not influence the islet glucose oxidation rate (data not shown). There was no difference in the total content of adenine nucleotides between islets cultured in the presence and absence of nicotinamide, but the content of ATP was reduced in the former islets (Table 3). It was also found that the total islet content of NADH + NAD was increased by about 20% in the experimental group, but the redox state as reflected by the NADH/NAD ratio was unaltered.

## Discussion

The present study shows that long-term exposure of isolated pancreatic islets to nicotinamide in vitro at a concentration which inhibits the poly(ADP-ribose) synthetase activity by about 90% [10] has a number of effects on the islets. The observation of a nicotinamide-induced increase of the islet DNA synthesis as measured by incorporation of $^3$H-thymidine cannot be explained by an effect on the intracellular thymidine pool. Thus, the number of grains covering the labelled cells in the autoradiographs was very high in both experimental groups and well above the number defined to constitute a labelled cell (> 15 grains/cell). The increased DNA replication in the islet cells found in this study supports the in vivo findings by Yonemura et al. [11]. However, these authors also observed enlargement of the islets in animals treated with poly(ADP-ribose) synthetase inhibitors, whereas in the present experiments we failed to demonstrate an elevated DNA content of the mouse islets cultured with nicotinamide. Enhanced DNA synthesis in the islet cells without an accompanying increase in their total DNA content has been reported after islet culture with 3-isobutyl-1-methylxanthine [23]. This phenomenon may suggest a loss of cells in vitro of sufficient magnitude to prevent a net growth of the islets. It should also be noted that in the study by Yonemura et al. (1984) the animals were hyperglycaemic and a growth of the islet mass could well be supported by a high glucose concentration.

The impairment of glucose-stimulated insulin release from the islets cultured with nicotinamide is obscure. A reduction of ATP in the B cells, which is required for the energy-dependent process of insulin secretion, may contribute to this impairment. However, it was not evaluated in this investigation if the islets cultured with nicotinamide are able to increase their generation of ATP in response to a high glucose concentration. On the other hand, the biosynthesis of both total protein and (pro)insulin was intact, despite the disturbance in oxidative metabolism. The most probable reason for net increase in islet NADH + NAD content after culture with nicotinamide is that nicotinamide had been metabolized as a precursor of NAD synthesis. The observed reduction in islet ATP is in accordance with the finding by Hoshino et al. [24] that ATP was decreased in liver cells cultured with nicotinamide. To what extent the long-term effect of nicotinamide on islet glucose oxidation reflects an inhibition of poly(ADP-ribose) synthetase is unclear, and obviously this impairment has to be induced over a prolonged period. Thus, we observed no acute effects of nicotinamide on islet glucose oxidation and previously demonstrated a lack of effect on (pro)insulin biosynthesis, oxygen uptake, and adenine and pyridine contents in islet cells [9]. Similarly, Zawalich et al. [25] found that nicotinamide did not affect islet glucose utilization. In their study, however, nicotinamide potentiated glucose-stimulated insulin release, in contrast to the long-term inhibitory effect shown in this study. In conclusion, the present investigation supports the view that poly(ADP-ribose) synthetase inhibitors can stimulate the generation of new islet cells. It is clear that nicotinamide exerts several effects which may well be separated from an inhibition of poly(ADP-ribose) synthesis. As pointed out by Milam and Cleaver [26], such other effects on metabolic processes emphasize the risks of attributing data obtained by using poly(ADP-ribose) synthetase inhibitors to reductions of poly(ADP-ribose) concentrations alone.

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