FEBS Letters 394 (1996) 300–306 FEBS 17583

Sensitivity of human pancreatic islets to peroxynitrite-induced cell dysfunction and death

Carol A. Delaney[a,*], Björn Tyrberg[a], Luc Bouwens[c], Hamid Vaghef[b], Björn Hellman[b], Décio L. Eizirik[a,d]

[a]*Department of Medical Cell Biology, Uppsala University, Biomedicum, Box 571, S-751 23 Uppsala, Sweden*
[b]*Department of Occupational and Environmental Medicine and Department of Toxicology, Uppsala University, Uppsala, Sweden*
[c]*Laboratory of Experimental Pathology, Vrije Universiteit Brussel, Brussels, Belgium*
[d]*Department of Metabolism and Endocrinology, Vrije Universiteit Brussel, Brussels, Belgium*

Received 22 August 1996

Abstract Nitric oxide and peroxynitrite (generated by the reaction of nitric oxide with the superoxide anion) may both be mediators of β-cell damage in early insulin-dependent diabetes mellitus. We observed that acute exposure of primary cultured human pancreatic islets to peroxynitrite results in a significant decrease in glucose oxidation and islet retrieval. DNA strand breaks in single human and rat islet cells are detectable after acute peroxynitrite exposure, followed by a decrease in islet cell survival after 1 h and 24 h. Cell death appeared to occur via a toxic cell death mechanism (necrosis) rather than apoptosis, as suggested by vital staining and ultrastructural evidence of early membrane and organelle degradation, mitochondrial swelling and loss of matrix. This study demonstrates for the first time that cultured human pancreatic islets are susceptible to the noxious effects of peroxynitrite.

Key words: Peroxynitrite; Nitric oxide; Islets of Langerhans; Apoptosis; DNA damage; Comet assay

1. Introduction

Destruction of the pancreatic beta cells in insulin-dependent diabetes mellitus (IDDM) occurs as part of an autoimmune mediated assault [1]. During an immune response or an inflammatory reaction, activated macrophages are present within the vicinity of the β-cell [2]. It is well documented that activated macrophages can produce both nitric oxide and a mixture of oxygen radicals, including the superoxide anion [3]. The superoxide anion is also produced as a result of normal cellular metabolism and from the oxidation of endogenous biomolecules. Nitric oxide can be formed within the pancreatic β-cells following cytokine induction of nitric oxide synthase (reviewed in [4]). Several studies into the mechanisms involved in cytokine-induced β-cell functional inhibition and cytotoxicity have revealed a fundamental role for nitric oxide and oxygen radicals (for reviews see [5,6]). In addition to the individual toxic effects of these radicals, nitric oxide reacts with a very high rate constant with superoxide to form the peroxynitrite anion ($ONOO^-$), that will be protonated to peroxynitrite, a potent oxidant, during physiological conditions [7]. Recently, several potential sites for peroxynitrite-mediated cytotoxicity in cells have been revealed, many of which were previously thought to be sites for nitric oxide-mediated cytotoxicity (for review see [8]).

We have previously identified a major species difference in the susceptibility to pancreatic β-cell injury, with human islets being less susceptible to several different assaults than islets from rat or mouse [9–11]. More recently, we observed that human islets are more resistant than rodent islets to nitric oxide-induced DNA damage [12], although nitric oxide itself can decrease human islet survival [11]. The aim of the present study was to examine whether primary cultured human islets are susceptible to the deleterious effects of peroxynitrite or its decomposition products, and to compare the susceptibility of human and rodent islets to peroxynitrite exposure.

2. Materials and methods

2.1. Materials

Chemicals were purchased from the following sources: culture medium RPMI-1640, foetal calf serum, penicillin and streptomycin, Hank's balanced salt solution, trypsin, BSA and agarose from Sigma Chemical Co. (St. Louis, MO, USA); D-[U-^{14}C]glucose (55 μCi/mmol/l) from Amersham International (Amersham, UK); collagenase from *Clostridium histolyticum* from Boehringer-Mannheim (Mannheim, Germany). All other chemicals of analytical grade were obtained from E. Merck (Darmstadt, Germany) or Sigma Chemicals.

Peroxynitrite used in the experiments was purchased from Alexis Corp. (San Diego, CA, USA) and stored in alkaline solution at −70°C for several weeks. When using this preparation, the peroxynitrite concentration was checked spectrophotometrically at 302 nm before each experimental procedure [13]. In the experiments a small volume (17 μl) of concentrated peroxynitrite (10 mM in 1 M NaOH), was added to islets placed in 0.8 ml Krebs Ringer bicarbonate buffer (KRBH; pH 7.4) for 10 min and the culture dish was swirled to distribute the peroxynitrite. The amount of peroxynitrite added did not affect the pH of the KRBH solution (data not shown). A negative control containing decomposed peroxynitrite was prepared from the same stock as the active form, and it was used throughout the experiments at the same concentration as peroxynitrite, to exclude the possibility that other reactants were toxic to the islets. The negative control contained the same concentrations of hydrogen peroxide, nitrite and sodium chloride as the peroxynitrite preparation, but did not have any absorbance at 302 nm under alkaline conditions.

2.2. Islet isolation, culture and test agent treatment

Pancreatic islets were isolated by collagenase digestion from adult male Sprague-Dawley rats bred in a local colony (Biomedical Centre, Uppsala, Sweden). The islets were maintained free-floating in culture medium for 2–4 days at 37°C [14] before treatment with the test agents. The culture medium, which was changed every 48 h, was RPMI 1640 containing 10% foetal calf serum, benzylpenicillin (60 mg/ml), streptomycin (0.1 mg) and 11.1 mM glucose.

Islets from 9 human heart-beating organ donors were isolated at the Central Unit of the β-Cell Transplant (Vrije Universiteit Brussel, Brussels, Belgium). The mean age of the donors was 43.5 ± 4.7 years. Aliquots of the islet-enriched fraction were examined routinely by electron microscopy, which indicated less than 6% dead cells or exocrine cells in all preparations. Light microscopical examination of immunocytochemically stained islets was carried out routinely on all

*Corresponding author. Fax: (46) (18) 556401.
E-mail: Carol Delaney@medcellbiol.uu.se

PII S0014-5793(96)00977-5

Table 1
Effect of peroxynitrite on human and rat islet glucose oxidation immediately (acute) or 24 h after exposure, and on islet retrieval after 24 h in culture

Species	Treatment		Glucose oxidation (pmol/20 islets per 1.5 h)			Islet retrieval (%)
Human	Acute					
	Control		127.1 ± 15.8 (6)			
	Peroxynitrite	0.2 mM	88.7 ± 17.8 (6)			
	Peroxynitrite	1.0 mM	57.6 ± 11.0 (6) [a]			
	Neg control	0.2 mM	129.9 ± 14.8 (5)			
	24 h					
	Control		91.6 ± 13.1 (5)	Control		96.9 ± 1.8 (4)
	Peroxynitrite	0.2 mM	36.4 ± 11.5 (5)[a]	Peroxynitrite	0.2 mM	83.8 ± 4.0 (4)[a]
	Peroxynitrite	1.0 mM	2.2 ± 1.6 (5)[b]	Peroxynitrite	1.0 mM	70.8 ± 5.5 (4)[b]
	Neg control	0.2 mM	93.8 ± 13.9 (5)	Neg control	0.2 mM	96.3 ± 2.0 (3)
			(pmol/10 islets per 1.5 h)			
Rat	Acute					
	Control		846.4 ± 85.1 (5)			
	Peroxynitrite	0.2 mM	504.5 ± 87.5 (5)[b]			
	Neg control	0.2 mM	750.5 ± 167.3 (5)			
	24 h					
	Control		716.3 ± 137.2 (5)	Control		99.3 ± 0.7 (7)
	Peroxynitrite	0.2 mM	400.1 ± 104.6 (5)[b]	Peroxynitrite	0.2 mM	87.6 ± 2.9 (7)[b]
	Neg control	0.2 mM	708.8 ± 192.7 (5)	Neg control	0.2 mM	95.4 ± 2.7 (7)

Groups of islets were exposed for 10 min to peroxynitrite and either studied immediately (Acute) or following a subsequent 24 h in culture. Control islets were exposed to vehicle only while the negative (Neg) control contained decomposed peroxynitrite solution. For the glucose oxidation studies the islets were incubated in triplicate groups of 20 (human) or 10 (rat) for 90 min in KRBH supplemented with D-[U-^{14}C]glucose and 16.7 mM glucose. After 24 h the number of islets remaining in culture was counted again (islet retrieval, expressed as a % of the original number of islets placed in culture). The results are means ± S.E.M. of the number of separate experiments indicated in parentheses. [a]$p < 0.05$ and [b]$p < 0.01$ when compared to control islets; paired t-test.

preparations and indicated the prevalence of insulin- and glucagon-positive cells to be 51.2 ± 3.5 and 13.6 ± 1.6%, respectively, $n = 9$. The isolation and culture conditions for human pancreatic islets have been described previously [15]. The islets were subsequently sent by air to Uppsala, where they were cultured in RPMI 1640 medium containing 10% FCS and 5.6 mM glucose. We have previously shown that functional preservation in RPMI 1640 medium is optimal at 5.6 mM glucose for human islets [15] and at 11 mM for rodent islets [10,14]. After 5–6 days in culture, groups of human islets were acutely exposed to peroxynitrite (0.2 or 1.0 mM) for 10 min and then functional and survival studies were performed either immediately after (acute) or following a subsequent period of 24 h in culture without the radical (see below).

2.3. Islet glucose oxidation and retrieval studies

Following exposure to peroxynitrite, the islets were rinsed with KRBH and islet glucose oxidation rates were determined either acutely or after a subsequent 24 h in culture. For this purpose, triplicate groups of 20 human or 10 rat islets were used. This higher number of human islets was selected based on our previous observations that glucose oxidation is much lower in human than in rodent islets [10]. The islets were transferred to glass vials containing KRBH supplemented with D-[U-^{14}C]glucose and non-radioactive glucose to a final concentration of 16.7 mM glucose for 90 min. Islet glucose oxidation was measured as previously described [16]. Islet retrieval was examined in islets treated with vehicle (control), peroxynitrite or negative control for 10 min and then cultured for 24 h. Retrieval was expressed as a % of the original number of islets placed in culture.

2.4. Measurement of DNA damage

Islets were treated with vehicle (control), peroxynitrite or negative control and then rinsed in KRBH before retrieval for DNA damage. DNA strand breakage was quantified using the alkaline version of the comet assay (single cell gel electrophoresis) (for recent review on this technique see [17]), performed as previously described for islet tissue [18], with the modifications described below. After the appropriate treatments, groups of 100 islets were rinsed in Hanks buffered saline solution (HBSS) and then gently dispersed into single cells with trypsin. Islet single cells (approx. 5×10^4 per slide) were embedded in duplicate, on top of a 0.6% low melting point agar base layer (on slides precoated with 0.6% agar) and placed in an incubator at 37°C for 1 h, to allow repair of putative trypsin-induced damage. Slides were then placed in lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Na sarcosinate, 10% DMSO and 1% Triton) for 1 h to lyse away non-nuclear cell components and then placed in electrophoresis buffer (0.3 M NaOH, 1 mM EDTA) for 40 min. This treatment allows the DNA containing strand breaks to unwind, and the DNA fragments to move towards the anode, in a comet tail, during electrophoresis carried out at 20 V and 300 mA for 24 min. The slides were then neutralised and stained with ethidium bromide (20 μg/ml). The length and the intensity of the fragmented DNA in the tail region is proportional to the extent or severity of DNA damage [17]. DNA damage was evaluated using two parameters: the tail length, which can be defined as the distance fragmented DNA has migrated following electrophoresis, and the tail moment, which additionally takes into account the proportion of DNA in the fragmented or damaged tail/comet region. DNA damage was directly quantified using a fluorescent image analysis system [19]. Briefly, the slides were examined at 500× magnification in a fluorescence microscope (excitation filter, 515–560 nm; barrier filter, 590 nm) attached to a black and white CCD video camera (Model ICD-42E, type F/L. Ikegami Tsushinki Co., Tokyo, Japan), connected to a computer-based image analysis system. The image analysis program Aequitas (IA version 1.3. DDL Ltd., Cambridge, UK), with its special application for the 'comet assay' AutoCell (version 8A, Reppalon AB, Hägersten, Sweden), was used when evaluating the degree of DNA damage in individual cells. Details on the program and performance of the image analysis system were described previously [19].

2.5. Assessment of islet cell survival

After peroxynitrite treatment intact islets were rinsed in HBSS and dispersed with trypsin into single cells. The cells were stained with either trypan blue solution (0.4%) or with the DNA binding fluorophore propidium iodide (50 μg/ml). Trypan blue (TB) staining was used to evaluate loss of membrane integrity, associated with cell necrosis [20] and propidium iodide (PI) to indicate DNA fragmentation

and chromatin condensation associated with late apoptosis [21] or necrosis. Cell survival was quantified by counting the total number of cells and the percentage TB or PI positive cells examined at the appropriate time points.

2.6. Electron microscopic examination of peroxynitrite exposed human islets

Following exposure to peroxynitrite for 10 min, 40–60 islets were rinsed and placed in culture in RPMI-1640 medium containing 10% FCS and 5.6 mM glucose for 6 or 24 h. After this the islets were rinsed in KRBH and fixed with 2.5% glutardialdehyde in 0.1 M cacodylate buffer, pH 7.4. The pellets were postfixed in 1% aqueous osmium tetroxide, stained 'en bloc' with 1% uranyl acetate, dehydrated in increasing ethanol concentrations and embedded in Spurr resin. Ultrathin sections were prepared and examined with a Zeiss EM109 transmission electron microscope. At least 50 cells were counted (by an observer unaware of the identity of the samples) to determine the percentage of cells with either necrotic or apoptotic degradative changes.

2.7. Statistical analysis

Data are presented as means ± S.E.M., and statistical differences between groups were determined using Student's paired or unpaired *t*-test, as appropriate. In all experiments, each islet preparation (islets obtained from one donor) was considered as one individual observation, even when experiments were performed in duplicate or triplicate.

3. Results

3.1. Effect of peroxynitrite on islet glucose oxidation and islet retrieval

Treatment of human islets with peroxynitrite at 0.2 mM did not significantly affect glucose oxidation acutely, but after 24 h in culture glucose oxidation was decreased by 60% (Table 1). When human islets were treated with 1.0 mM peroxynitrite glucose oxidation was markedly decreased (60–95% decrease) both acutely and after a 24 h culture period (Table 1). In rat islets treatment with 0.2 mM peroxynitrite was sufficient to inhibit glucose oxidation both acutely and after a 24 h culture period. Exposure to 1.0 mM peroxynitrite induced widespread destruction of rat islets, preventing further functional studies at that concentration (data not shown). Islet retrieval data revealed that peroxynitrite exposure decreased the number of both human and rat islets remaining in culture after 24 h. The negative control when applied at 0.2 mM did not adversely affect either glucose oxidation or islet retrieval, although a higher dose (1.0 mM) was found to partly inhibit glucose oxidation in rat islets (data not shown).

3.2. Peroxynitrite induced DNA strand breakage

A 10 min exposure of human islets to peroxynitrite caused extensive DNA strand breakage in individual islet cells, as indicated both by an increase in the overall mean comet length (Fig. 1) and by an increase in the tail moment from 164.0 ± 18.6 (control) to 397.5 ± 80.8 (0.2 mM peroxynitrite; $p < 0.05$ versus control using Student's unpaired *t*-test). Mean comet length (Fig. 1) and tail moment 188.1 ± 25.5 ($n = 5$–6) in the negative control were similar to values observed in the control. In rat islets there was also an increase in the overall mean comet length (Fig. 1) and an increase in the tail moment with peroxynitrite treatment (data not shown).

3.3. Cell survival following peroxynitrite exposure

A decrease in cell viability, as demonstrated by the inability to exclude trypan blue, was observed in human islet cells already after a 10 min exposure to either 0.2 or 1.0 mM peroxynitrite (Fig. 2A). At this time point there was no effect of peroxynitrite on propidium iodide staining. After a subsequent period of 24 h (Fig. 2B) a decrease in cell viability was observed after both 0.2 and 1.0 mM peroxynitrite treatment, as judged by both trypan blue and propidium iodide staining. The survival of rodent islets was affected both acutely and after a 24 h recovery period according to trypan blue staining (Fig. 2C,D), but there was no increase in propidium iodide staining at either time points in rat islets. In both the human and rat experiments the negative control did not decrease cell survival. As a whole, these results suggest that peroxynitrite induced early cell necrosis.

3.4. Effect of peroxynitrite on ultrastructure of human islets

Electron microscopic analysis of human islets revealed well preserved control islets, while peroxynitrite-treated islets had a high percentage of necrotic cells (Fig. 3). Necrosis did not appear to be restricted to a particular type of islet cell, as it was observed in all islet cell types and also in the small fraction of contaminating non-endocrine cells. In islets studied 6 h after exposure to 0.2 mM peroxynitrite, the percentage of necrotic cells ranged between 5 and 50% (average 35%), whereas with 1 mM peroxynitrite an average of 95% of cells were necrotic (data based on 3 separate human islet preparations). Necrotic characteristics included membrane degradation, 'extraction' of cytoplasmic and nucleoplasmic components, and degradation of organelles. With 0.2 mM peroxynitrite treatment, where relatively less severe degradation occurred, we noted the frequent occurrence of mitochondrial swelling and loss of mitochondrial matrix. Cells with characteristics of apoptotic cell death, such as nuclear condensations and fragmentation, were very rarely observed in peroxynitrite treated cells (similar to controls and negative control). The percentage of dead cells in control cultures (no peroxynitrite) and in cultures treated with the negative control was < 1%. In islets retrieved 24 h after peroxynitrite exposure, the mean percentage of necrotic cells was 77% (50–100%) at 1 mM and 10% at 0.2 mM (control and negative control, < 1%). This apparently reduced fraction of necrotic cells at 24 h, compared to 6 h, may be explained by a selective loss of completely degraded cells from whole islets during the culture period. This is confirmed by first the 'cloudy' appearance (macroscopically) after the 24 h culture of peroxynitrite-treated islets, with tissue debris in the medium; and second, by a clear reduction in the total number of cells in the pellets. Again, there were very few cells showing signals of apoptosis after 24 h in all studied groups.

4. Discussion

The main question addressed by this paper is whether human pancreatic islets are susceptible to peroxynitrite-induced cell dysfunction and death. Peroxynitrite caused a progressive decrease in glucose oxidation, which was accompanied by an early induction of DNA strand breaks. Peroxynitrite toxicity to pancreatic islets was confirmed both by ultrastructural signs of membrane and organelle damage, leading to necrotic cell death, and by vital staining techniques (trypan blue and propidium iodide), indicating death of more than 50% of islet cells 24 h after an acute exposure to 0.2–1.0 mM peroxynitrite. The fact that a negative control containing decomposed peroxynitrite solution i.e. hydrogen peroxide, nitrite, nitrate

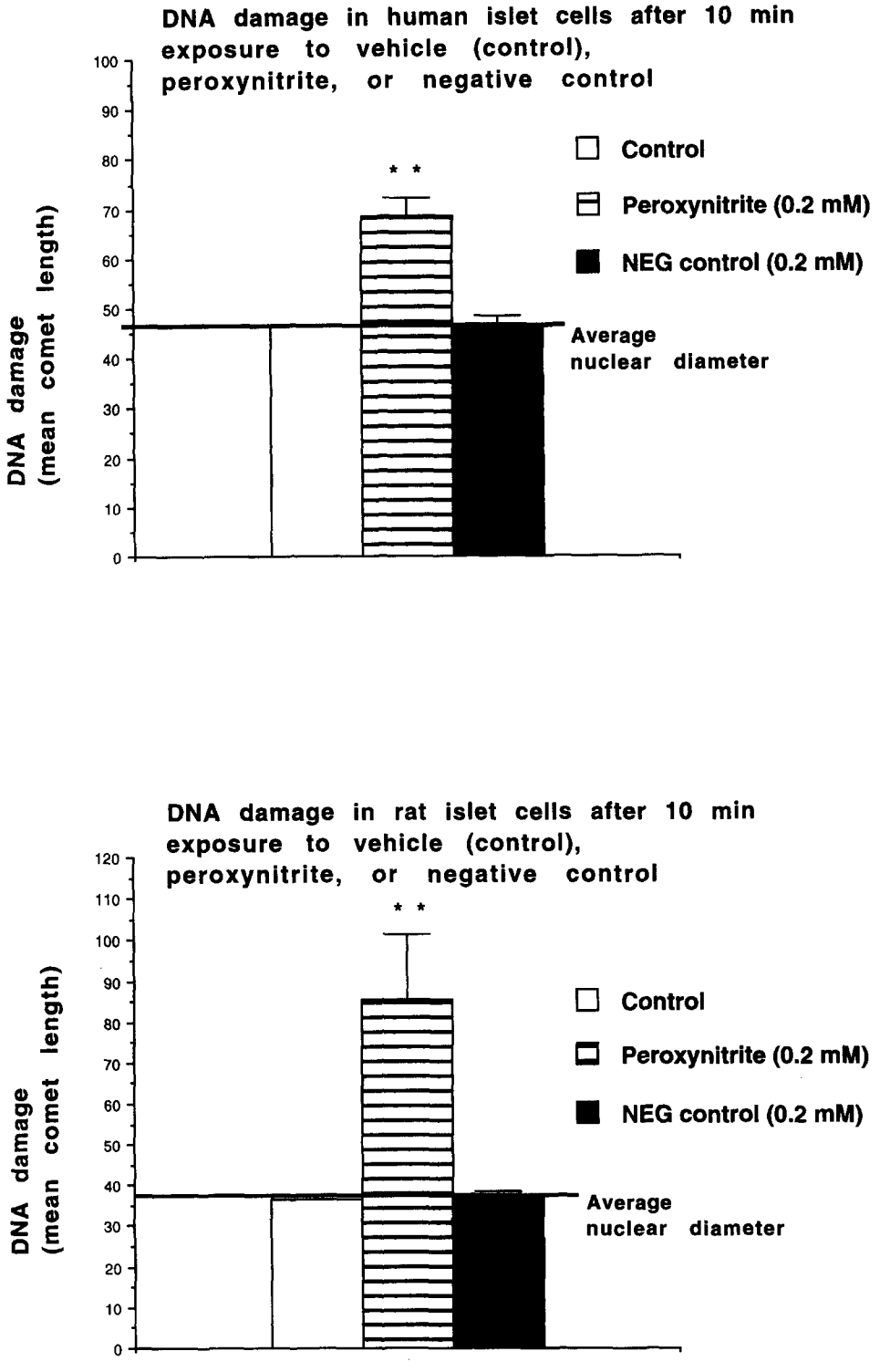

Fig. 1. DNA damage following treatment of human (top graph) and rat (lower graph) islets with vehicle only (control), peroxynitrite (0.2 mM) or negative control (0.2 mM) for 10 min. Results represent the overall mean comet length (intact head diameter and tail length) ± S.E.M. from 5–6 human (total of 320 nucleoids analysed) and 3 rat (total of 146 nucleoids analysed) separate experiments. The line represents the average nuclear diameter of the head/intact DNA region, whereas the area above the line represents the tail region or damaged/fragmented DNA. $^{**}p < 0.001$ versus control, by Students unpaired t-test.

and sodium chloride, did not affect human islet function or survival, suggests that peroxynitrite (or its reactive decomposition intermediates) is the main toxic effector molecule.

We have previously observed that human pancreatic islets are less susceptible than rat or mouse pancreatic islets to the damaging effects of cytokines [9], nitric oxide donors [10,11], streptozotocin [10], alloxan [10], cycasin [22] and hydrogen peroxide [23]. Thus, it is noteworthy that in the present series of experiments peroxynitrite induced similar damage to human and rat pancreatic islets, as judged by a similar decrease

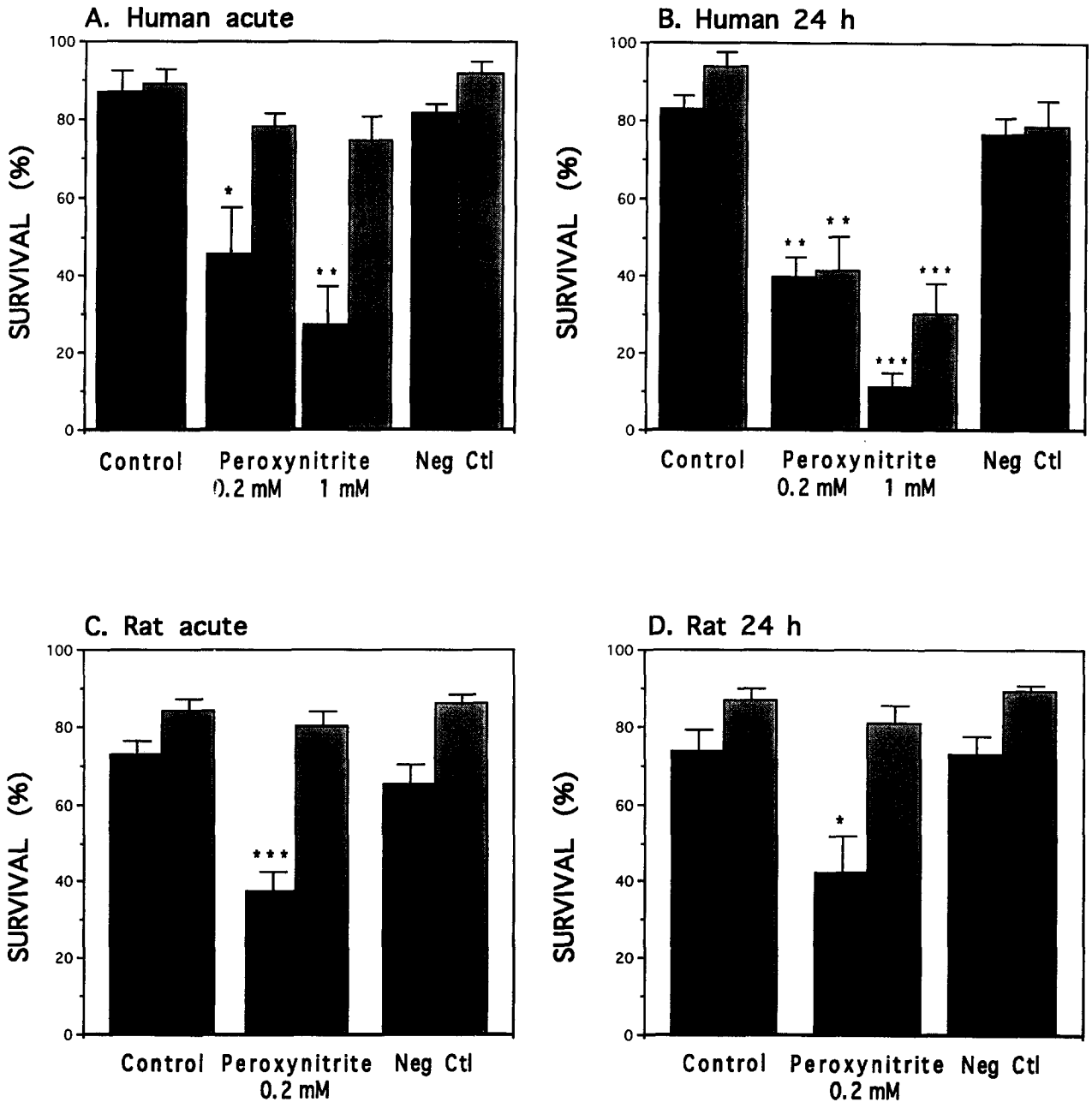

Fig. 2. Cell survival in human and rat islets following exposure to vehicle only, peroxynitrite (0.2 mM) or negative control (0.2 mM). Black panels represent data obtained using the trypan blue exclusion test and grey panels represent results obtained with propidium iodide staining. The top two graphs show data from 5 separate human islet cell preparations: (A) acute data; (B) data obtained after 24 h in culture. The lower two graphs show rat islet cell data from 6 individual experiments: (C) acute data; (D) 24 h data. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ versus control, using Students unpaired t-test.

in islet glucose oxidation and islet retrieval, and similar levels of DNA strand breakage. Indeed, to our knowledge, peroxynitrite is the first toxic agent to which human islets are as sensitive as rat islets. This, and the fact that both primary cultured human and rat islets seem to be more sensitive to peroxynitrite-induced cell dysfunction and death than other primary cultured cells (such as human peripheral blood mononuclear cells and human umbilical vein endothelial cells [24]), reinforce a possible role for this potent oxidant as an inflammatory mediator of human β-cell destruction.

Human islets express larger amounts of the heat shock protein 70 and possess higher activities of the free radical scavenger enzymes manganese superoxide dismutase (SOD) and catalase than rodents islets [23]. This may at least in part explain the decreased susceptibility of human islets to the different toxic agents listed above (reviewed in [25]). However, the present data showing similar susceptibility of human and rodent islets to peroxynitrite suggest that higher amounts of these 'defence' proteins are not sufficient to protect human islets against this reactive species. Peroxynitrite is formed by the interaction of nitric oxide with superoxide [7], and there is data suggesting that human pancreatic islets exposed in vitro to cytokines generate both these radicals [26,9,27]. Considering the high activity of SOD in human islets [23], it can be

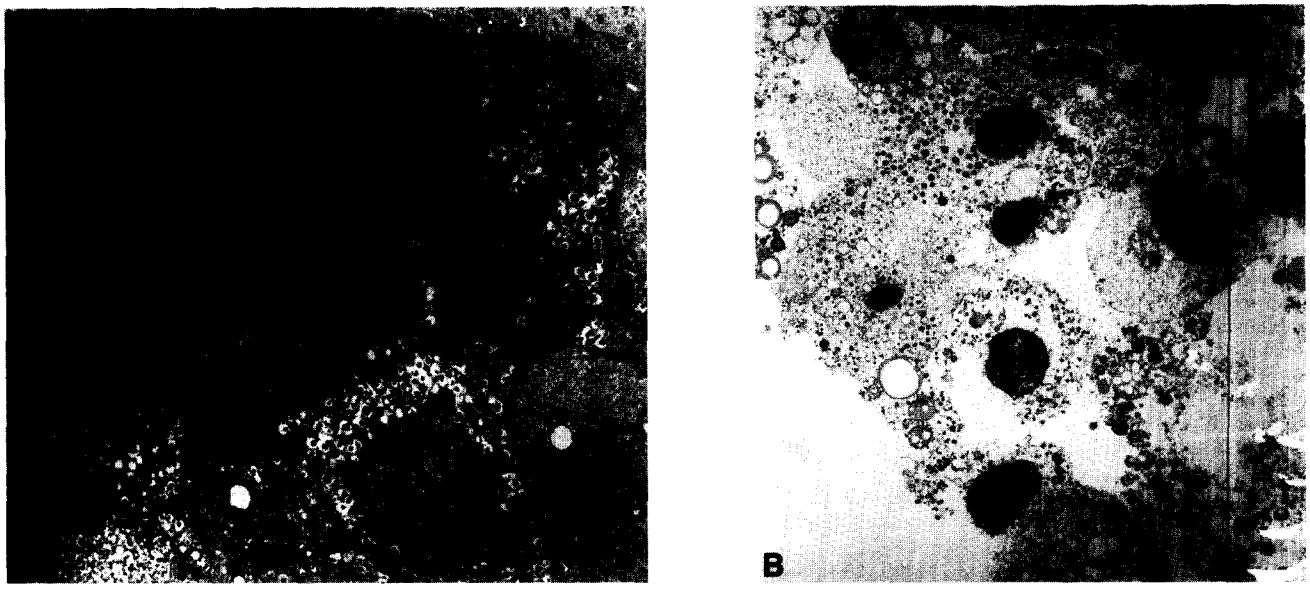

Fig. 3. Electron microscopy of human islets examined 24 h after an initial 10 min exposure to vehicle (control) or peroxynitrite (0.2 mM). (A) Islets exposed to vehicle only (control), showing well preserved and normal appearing organelles and insulin secretory granules. Magnification 4000×; (B) islets exposed to 0.2 mM peroxynitrite for 10 min, showing generalised signals of β-cell necrosis (degradation of membrane and organelles). Note also a necrotic α-cell in the centre of the picture. Magnification 2800×.

argued that the enzyme will remove superoxide and prevent peroxynitrite formation. However, the rate constant for the reaction of the superoxide anion with nitric oxide is considerably higher than that for superoxide removal by SOD [28], increasing the possibility for generation of peroxynitrite in vivo. Indeed, to prevent effectively peroxynitrite formation SOD must be present in sufficiently greater amounts than nitric oxide and must be close to the site of interaction between nitric oxide and superoxide.

Nitric oxide reacts with superoxide to form the peroxynitrite anion, which is stable in alkaline solution but rapidly decays to form the protonated peroxynitrous acid which subsequently decomposes to form several intermediate reaction products such as the nitronium ion, nitrogen dioxide and either the hydroxyl radical or a 'hydroxyl radical-like intermediate'. The exact nature of the cytotoxic peroxynitrite reactive species is a matter for debate but the early decomposition products may each have varying potencies as toxic intermediates [29]. The production of peroxynitrite has been implicated in many pathological conditions, such as atherosclerosis, acute endotoxemia and neurological disorders (reviewed in [8]). Peroxynitrite causes hydroxylation and nitration of aromatic amino acids, such as tyrosine, leading to the in vivo formation of nitrotyrosine. Increased nitrotyrosine levels have been found both in lung sections of patients with lung injury and in the lungs of animals exposed to hypoxia [30]. The formation of peroxynitrite may also be involved in autoimmune diseases, as suggested by the observations that blood serum and synovial fluid from patients with rheumatoid arthritis contain 3-nitrotryosine [31] and that nitrotyrosine is increased in kidney proteins in a mouse model of arthritis and glomerulonephritis [32]. Furthermore, a role for peroxynitrite produced from activated macrophages has been suggested in active inflammatory processes such as chronic human renal allograft rejection [33].

The ultimate mechanism(s) by which peroxynitrite leads to β-cell dysfunction and death remains to be clarified. However, our data suggest that the radical induces an early impairment in glucose oxidation, which is paralleled by DNA damage. It has previously been reported that peroxynitrite decreases the activity of the Krebs cycle enzyme aconitase [34,35] which may contribute to the observed decrease in glucose metabolism. Moreover, peroxynitrite reacts with guanine to form 8-nitroguanine [36] and causes DNA strand breaks in islet cells (present data) and in the macrophage cell line RAW264.7, where it was found that peroxynitrite causes oxidative DNA damage [37]. It is therefore conceivable that peroxynitrite-induced DNA strand breakage may lead to poly ADP-ribose synthetase (PARP) activation and NAD consumption. A reduction in NAD may contribute to a further decrease in islet glucose metabolism and ATP generation and ultimately insulin biosynthesis and release. This, together with other potential direct effects of the oxidant, may induce overwhelming cell damage, leading to the presently observed early β-cell necrosis. However, it cannot be excluded that prolonged in vivo exposure of β-cells to low concentrations of peroxynitrite and/or other radicals may lead to apoptosis. Indeed, data obtained from different cell types [24,38] suggest that both nitric oxide and peroxynitrite may lead to either necrosis or apoptosis, depending on the intensity and duration of exposure to these agents.

Acknowledgements: We thank E. Törnelius, I.-B Hallgren, A. Nordin and M. Engkvist for excellent technical assistance and A. Nuyts for her expert technical help in electron microscopy. This work was supported by grants from the Juvenile Diabetes Foundation International, the Swedish Medical Research Council (12X-9886; 12X-109; 12X-9237), the Swedish Centrala Försöksdjursnämnden, the Swedish Work Environment Fund (Grant No. 92-1454) and Lions Cancer Research Fund. Human islets were prepared by the Central Unit of the β-Cell Transplant, with financial support of a Shared Costs Action in Medical and Health Research of the European Community. We are grateful to Professor Danny Pipeleers, Coordinator of the β-Cell Transplant project, for providing human islet preparations and infor-

mation on human islet cell composition. C.A.D. holds a Juvenile Diabetes Foundation International Postdoctoral Fellowship. D.L.E is presently at the Department of Metabolism and Endocrinology, Vrije Universiteit Brussel, Brussels, Belgium.

References

[1] Eisenbarth, G.S. (1986) N. Engl. J. Med. 314, 1360–1368.
[2] Kolb, H., Kolb-Bachofen, V. and Roep, B.O. (1995) Immunol. Today 170, 170–172.
[3] Nathan, C. (1992) FASEB J. 6, 3051–3064.
[4] Eizirik, D.L., Flodström, M., Karlsen, A.E. and Welsh, N. (1996) Diabetologia 39, 875–890.
[5] Rabinovitch, A. (1994) Diabetes 43, 613–621.
[6] Delaney, C.A. and Eizirik, D.L. (1996) Braz. J. Med. Biol. Res. 29, 569–579.
[7] Beckman, J.S., Beckman, T.W., Chen, J., Marshall, P.A. and Freeman, B.A. (1990) Proc. Natl. Acad. Sci. USA 87, 1620–1624.
[8] Darley-Usmar, V., Wiseman, H. and Halliwell, B. (1995) FEBS Lett. 369, 131–135.
[9] Eizirik, D.L., Sandler, S., Welsh, N., Cetkovic-Cvrlje, M., Nieman, A., Geller, D.A., Pipeleers, D.G., Bendtzen, K. and Hellerström, C. (1994) J. Clin. Invest. 93, 1968–1974.
[10] Eizirik, D.L., Pipeleers, D.G., Ling, Z., Welsh, N., Hellerström, C. and Andersson, A. (1994) Proc. Natl. Acad. Sci. USA 91, 9253–9256.
[11] Eizirik, D.L., Delaney, C.A., Green, M.H.L., Cunningham, J.M., Thorpe, J.R., Pipeleers, D.G., Hellerström, C. and Green, I.C. (1996) Mol. Cell Endocrinol. 118, 71–83.
[12] Delaney, C.A., Eizirik, D.L., Lowe, J.E., Green, M.H.L., Thorpe, J.R., Pipeleers, D.G., Hellerström, C. and Green, I.C. (1995) Diabetologia 38, A17.
[13] Hughes, M.N. and Nicklin, H.G. (1968) J. Chem. Soc. A, 450–452.
[14] Andersson, A. (1978) Diabetologia 14, 397–404.
[15] Eizirik, D.L., Korbutt, G.S. and Hellerström, C. (1992) J. Clin. Invest. 90, 1263–1268.
[16] Andersson, A. and Sandler, S. (1983) Cryobiology 20, 161–168.
[17] Fairbairn, D.W., Olive, P.L. and O'Neill, K.L. (1995) Mutat. Res. 339, 37–59.
[18] Delaney, C.A., Green, M.H.L., Lowe, J.E. and Green, I.C. (1993) FEBS Lett. 333, 291–295.
[19] Hellman, B., Vaghef, H. and Boström, B. (1995) Mutat. Res. 336, 123–131.
[20] Tennant, J.R. (1964) Transplantation 2, 685–694.
[21] Crompton, T., Peitsch, M.C., MacDonald, H.R. and Tschopp, J. (1992) Biochem. Biophys. Res. Commun. 183, 532–537.
[22] Eizirik, D.L. and Kisby, G.E. (1995) Biochem. Pharmacol. 50, 355–365.
[23] Welsh, N., Margulis, B., Borg, L.A.H., Wiklund, H., Saldeen, J., Flodström, M., Mello, M.A., Andersson, A., Pipeleers, D.G., Hellerström, C. and Eizirik, D.L. (1995) Mol. Med. 1, 806–820.
[24] Lin, K.-T., Xue, J.-Y., Nomen, M., Spur, B. and Wong, P.Y.-R. (1995) J. Biol. Chem. 270, 16487–16490.
[25] Eizirik, D.L. (1996) Horm. Metab. Res. 28, 302–305.
[26] Corbett, J.A., Sweetland, M.A., Wang, J.L., Lancaster, J.R. and McDaniel, M.L. (1993) Proc. Natl. Acad. Sci. USA 90, 1731–1735.
[27] Rabinovitch, A., Suarez-Pinzon, W.L., Strynadka, K., Schulz, R., Lakey, J.R.T., Warnock, G.L. and Rajotte, R.V. (1994) J. Clin. Endocrinol. Metab. 79, 1058–1062.
[28] Huie, R.E. and Padmaja, S. (1993) Free Rad. Res. Commun. 18, 195–201.
[29] Koppenol, W.H., Moreno, J.J., Pryor, W.A., Ischiropoulos, H. and Beckman, J.S. (1992) Chem. Res. Toxicol. 5, 834–842.
[30] Haddad, I.Y., Pataki, G., Hu, P., Galliani, C., Beckman, J.S. and Matalon, S. (1994) J. Clin. Invest. 94, 2407–2413.
[31] Kaur, H. and Halliwell, B. (1994) FEBS Lett. 350, 9–12.
[32] Keng, T., Privalle, C.T. and Weinberg, J.B. (1996) Annals of the Second International Conference on the Biochemistry and Molecular Biology of Nitric Oxide, Los Angeles, CA, p. 118 (Abstr.).
[33] MacMillan-Crow, L.A., Crow, J.P., Beckman, J.S. and Thompson, J.A. (1996) Annals of the Second International Conference on the Biochemistry and Molecular Biology of Nitric Oxide, Los Angeles, CA, USA, p. 119 (Abstr.).
[34] Hausladen, A. and Fridovich, I. (1994) J. Biol. Chem. 269, 29405–29408.
[35] Castro, L., Rodriquez, M. and Radi, R. (1994) J. Biol. Chem. 269, 29409–29415.
[36] Yermilov, V., Rubio, J., Becchi, M., Friesen, M.D., Pignatelli, B. and Ohshima, H. (1995) Carcinogenesis 16, 2045–2050.
[37] DeRojas-Walker, T., Tamir, S., Ji, H., Wishnok, J.S. and Tannenbaum, S.R. (1995) Chem. Res. Toxicol. 8, 473–477.
[38] Bonfoco, E., Krainc, D., Ankarcrona, M., Nicotera, P. and Lipton, S.A. (1995) Proc. Natl. Acad. Sci. USA 92, 7162–7166.