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Oxidative and nitrosative stress induces peroxiredoxins in pancreatic beta cells

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Abstract

Aims/hypothesis. Insulin-producing beta cells are destroyed by oxidative and nitrosative stress during the pathogenesis of Type I (insulin-dependent) diabetes mellitus. These cells are more sensitive than others due to their deficiency of well known antioxidant enzymes like superoxide dismutase, glutathione peroxidase and catalase. However the peroxiredoxins discovered in the past decade form a large family of highly conserved thioredoxin-dependent peroxide reductases, which are present in most tissues. We investigated whether peroxiredoxins I and II are present in pancreatic beta cells and if they are inducible by oxidative and nitrosative stress.
Methods. To detect these enzymes in insulin-producing beta cells we used semiquantitative RT-PCR, western blots and immunohistochemistry. The expression of peroxiredoxins I and II was analysed after treatment with cytokines, hydrogen peroxide, alloxan or streptozotocin in the rat insulinoma cells INS-1 using RT-PCR and western blots.
Results. We show that peroxiredoxins I and II are present in the cytoplasm of pancreatic islet cells as well as in insulinoma cell lines βTC6-F7 and INS-1. Peroxiredoxins I and II were up-regulated by all stress agents used.
Conclusion/interpretation. Beta cells, undersupplied with well characterized antioxidant enzymes, possess an additional antioxidant system which is inducible by oxidative as well as nitrosative stress. [Diabetologia (2002) 45:867–876]

Keywords Peroxiredoxin, stress, pancreatic beta cells, diabetes, cytokines, alloxan, streptozotocin.

During the pathogenesis of Type I (insulin-dependent) diabetes mellitus, oxidative and nitrosative stress contribute to the destruction of insulin-producing beta cells [1, 2, 3, 4, 5]. Beta cells are very sensitive to cytotoxic stress because they express very little of the well known antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) [6, 7, 8]. Moreover these antioxidant enzymes are able to reduce oxygen radicals but they can not protect against nitrosative stress [9].

Peroxiredoxins (Prxs) represent a recently discovered family of antioxidant proteins without any sequence homology to the antioxidant enzymes mentioned above. Six members of the Prx family have been identified to date (Prx I–VI). They are detected in a wide variety of organisms ranging from prokaryotes to mammals and are expressed in most cell types, though different Prx enzymes have distinct intracellular distributions [10, 11, 12, 13, 14, 15, 16]. Peroxiredoxins act as homodimers or heterodimers [17, 18,

19] and catalyse reduction of both hydrogen peroxide and alkyl peroxides to water or to the corresponding alcohol using thioredoxin (Tx) as their physiological hydrogen donor [20, 21, 22, 23, 24]. Additionally it has been shown that the bacterial peroxiredoxin AhpC (alkylhydroperoxide reductase subunit C) is able to protect against nitrogen radicals [25]. Prx enzymes have been identified in association with various cellular functions apparently unrelated to peroxidase activity. Several lines of evidence support the concept that peroxiredoxins can influence cell proliferation and differentiation, immunological defence, receptor signalling and apoptosis [10, 23, 26, 27, 28, 29]. This is reflected by the numerous synonyms used. Thus Prx I is further known as MSP23 (mouse macrophage stress protein M_r 23 K) [30], OSF3 (mouse osteoblast specific factor 3) [31], HBP23 (heme-binding protein M_r 23 K) [32], PAG (proliferation-associated gene) [10] and NKEF-A (human natural killer cell-enhancing factor-A) [33]. Prx I is a physiological inhibitor of c-Abl kinase activity [34]. It is induced by oxidative stress in peritoneal macrophages exposed to heavy metals, oxidized low-density lipoproteins or heme [30, 35, 36, 37].

Prx II was originally isolated from yeast. It is also named thiol-specific antioxidant (TSA) [38, 39], protector protein (PRP) [11, 26], human natural killer cell-enhancing factor-B (NKEF-B) [33, 41, 40], calpromotin [19, 42, 43] and torin [44]. Prx II is important in erythroid cell differentiation [11, 26] and protects red blood cell membranes from peroxidation [20, 45]. It also regulates signal transduction pathways that directly relate to apoptotic cell death [23, 27, 28, 29, 46]. Prx II is thought to protect neuronal cells against destruction by hypoxia and ischaemia [11, 47] and tumour cells against X-ray treatment and chemotherapy [48].

We investigated the occurrence of peroxiredoxins I and II in pancreatic beta cells and determined whether expression of these enzymes is up-regulated by cytokines, hydrogen peroxide, alloxan and streptozotocin.

Material and Methods

Materials. Female BalbC mice (6–8 weeks) were purchased from the Department of Pathophysiology and the Department of Zoology, NMRI mice were from the Department of Neurology, Greifswald University. We confirm that the 'Principles of laboratory animal care' (NIH publication no. 85-23, revised 1985) were followed as well as any specific national laws applicable in Germany. Collagenase type IV and antibody against glucagon were obtained from Sigma (Deisenhofen, Germany). Taq polymerase was from Amersham Pharmacia Biotech (Freiburg, Germany). Cytokines and antibody against insulin were obtained from Roche Diagnostics (Mannheim, Germany). Rotiblock was from Carl Roth (Karlsruhe, Germany). Streptozotocin was obtained from Calbiochem Novabiochem (Schwalbach, Germany) and alloxan was from ICN (Eschwege, Germany). Preparation of antiserum against Prx I and II and

preimmunsera has been described in [49]. The secondary antibodies anti mouse-Cy2 and anti rabbit-Cy3 were obtained from Dianova (Hamburg, Germany). All other chemicals were from commercially available sources.

Cell culture. Mouse insulinoma cells βTC6-F7 (passages 15–20) were propagated in DMEM medium containing 5.5 mmol/l glucose, supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin and 100 µg/ml streptomycin. Rat insulinoma cells INS-1 (passages 13–20) were propagated in RPMI-1640 medium containing 11 mmol/l glucose, supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, 10 mmol/l HEPES-buffer, 2 mmol/l L-glutamine, 1 mmol/l sodium pyruvate, 50 µmol/l β-mercaptoethanol. All cells were cultured at 37°C in a humidified atmosphere containing 95% air and 5% CO_2.

Isolation of pancreatic islets. Islets were isolated from female NMRI mice (8 weeks old). Pancreas were dissected, rinsed with ice-cold HBSS solution, minced and digested with 1 mg/ml Typ IV collagenase for 12 min at 30°C. Digestion was stopped by adding ice-cold HBSS solution containing 5% FCS. Pancreatic islets were selected under a stereomicroscope, collected into cell culture dishes and cultured under the same conditions as INS-1 cells.

For western blot analyses islets were collected by centrifugation, resuspended in 500 µl lysis buffer (480 mg/ml urea, 152 mg/ml thiourea, 40 mg/ml CHAPS, 20 mg/ml DTT and 9.6 mg/ml Tris) and sonicated. The lysate was ultracentrifugated for 1 h at 8°C and 103000×g.

Exposure of INS-1 cells and pancreatic islets to stress agents. Monolayers of confluent INS-1 cells or isolated pancreatic islets were incubated for the indicated periods with or without stress agents in culture medium. After exposure to alloxan or streptozotocin, cells were incubated for an additional 8 h in medium without stress agents. INS-1 cells and pancreatic islets were harvested for RNA or protein isolation. Preparation of cytosolic and nuclear extracts for western blot analysis were done as described [50].

Semiquantitative RT-PCR. RT-PCR quantitated *Prx I* and *Prx II* mRNA in oxidant-treated cells. Total INS-1 RNA was isolated from 60 mm dishes using a commercial kit (Qiagen). cDNA was prepared by reverse transcription of 2 µg of total RNA using 0.5 µg BamTT-primer (3′-CGC GGA TCC TTT TTT TTT TTT TTT TTT-5′) and superscript II reverse transcriptase. *Prx I* and *Prx II* were amplified with Taq DNA polymerase in a DNA thermal cycler (Eppendorf) using the following gene-specific primer pairs: *Prx I*: 5′-GTG GAT TCT CAC TTC TGT CAT CT-3′ and 5′-GGC TTA TCT GGA ATC ACA CCA CG-3′ with an expected product of 470 bp; *Prx II*: 5′-GAG GGA AGT ACG TGG TCC TCT-3′ and 5′-GGT AGG TCA TTG ACT GTG ATC TG-3′ with an expected product of 339 bp. The conditions for each cycle were 30 s at 94°C, 40 s at 63°C or 57°C and 60 s at 72°C (25 cycles). In parallel, RT-PCR was carried out with primers specific for *GAPDH* (5′-GTC GTG GAG TCT ACT GGC GTC TTC-3′ and 5′-GTT GTC ATT GAG AGC AAT GCC AGC-3′ with an expected product of 635 bp) as a control for equal amounts of RNA used in the RT-reaction.

Western blot. Proteins (20 µg) were separated by 12.5% SDS-PAGE and electroblotted onto nitrocellulose membranes using a semi-dry blotter. The blot was blocked with "Rotiblock" for 1 h at room temperature and subsequently incubated in a 1:2000 dilution of a polyclonal rabbit anti-Prx I or anti-Prx II

antiserum. After washing the blot with PBS-Tween 0.05%, an alkaline phosphatase-conjugated goat anti-(rabbit) IgG (1:10000) was used as a secondary antibody. Bound antibody was detected with BCIP and NBT as substrate.

Cryostat slices. Pancreas from BalbC mice was incubated for 4 to 6 h at 4°C in paraformaldehyde (4% in 5× phosphate buffer) and then 12 to 16 h in sucrose (5% in 5× phosphate buffer). After shock-freezing in isopentane the tissue was embedded in Tissue Tec and stored at –80°C. Cryostat sections of 8 to 10 μm were used for immunohistochemistry.

Immunohistochemistry. Slices were successively incubated for 5 min in ice-cold methanol, acetone and IF-buffer (0.2% BSA, 0.05% saponine, 0.1% sodium azide in PBS pH 7.4) followed by an overnight incubation at 4°C with polyclonal rabbit anti-Prx I or anti-Prx II antiserum and monoclonal mouse anti-glucagon or anti-insulin antibody diluted 600-fold in IF-buffer. After washing with IF-buffer, the immunoreacted primary antibodies were visualised with red fluorescent Cy3-conjugated goat anti-rabbit IgG (1:400) and green fluorescent Cy2-conjugated goat anti-mouse IgG (1:400), by incubation for 2 h at room temperature in the dark. After washing with IF-buffer again, slices were covered with glycerol gelatin and observed by fluorescent microscopy (400x magnification) with an IX-70 microscope (Olympus). Control staining with preimmune sera gave no immunoreactive signals.

Results

Detection of peroxiredoxins I and II in pancreatic beta cells. To establish the expression of *Prx I* and *Prx II* in pancreatic beta cells, insulinoma cells were cultured for 24 h. The amount of mRNA of both Prx enzymes was examined by RT-PCR using total RNA from mouse βTC6-F7 cells (data not shown) and rat INS-1 cells (Fig. 1A). We detected *Prx I* as well as *Prx II* mRNA in both insulinoma cell lines. Each primer set yielded a single PCR product of the size predicted (Fig. 1A).

To determine the subcellular distribution of both peroxiredoxins, nuclear and cytosolic fractions of INS-1 cells were subjected to immunoblot analysis using polyclonal antisera. Prx I and II were detected, at different expressions, almost exclusively in the cytosolic fraction of INS-1 cells. The western blot showed a major band at M_r 23 K, representing the monomer, and a fainter band at M_r 46 K, representing the dimer. There were only weak multiple protein bands in the nuclear fraction (Fig. 1B).

Furthermore we stained cryostat slices of pancreas from BalbC mice to analyse the presence of peroxiredoxins in endocrine and exocrine tissue. Figure 2 shows the green fluorescent, centrally situated insulin-producing beta cells (a, g) and the surrounding glucagon-producing alpha cells (d, j). The red fluorescent signals represent Prx I (b, e) or Prx II (h, k). Immunostaining signals were not visible when the corresponding preimmune sera were used. The colocalisation of Prx I (c, f) or Prx II (i, l) with insulin or glucagon results in yellow immunostaining. Peroxiredoxins are
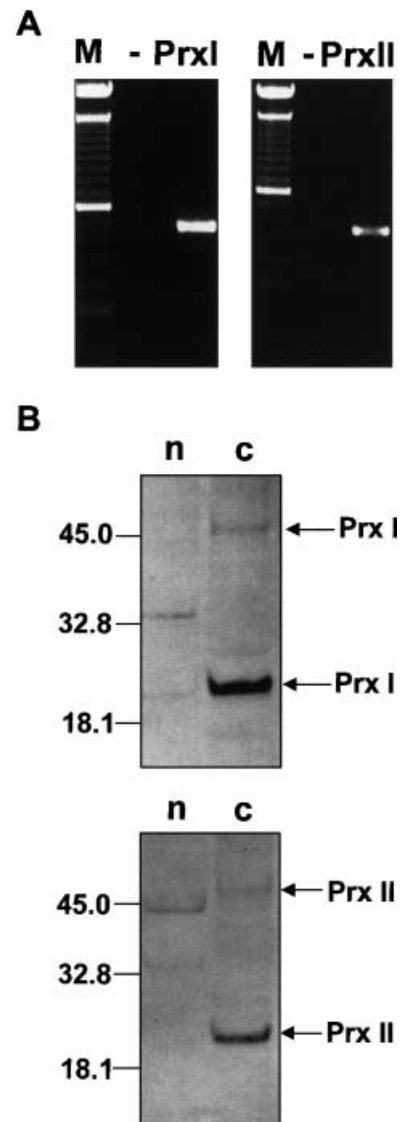
Fig. 1A, B. Expression of Prx I and Prx II in pancreatic beta cells. **A** RT-PCR. Total RNA from INS-1 cells was analysed. A negative control (–) without RNA was carried out. The major bands of the marker (M) represent DNA size of 0.6; 1.5 and 2 kb, respectively. **B** Western blot analysis. 20 μg nuclear (n) or cytosolic (c) proteins were separated on SDS-12.5% polyacrylamide gels. Molecular size standards (in K) are indicated on the left

predominantly expressed in pancreatic islets, only low signals are observed in exocrine cells of the pancreas. The micrographs confirmed cytosolic localisation of both peroxiredoxins shown (Fig. 1B).

Up-regulation of Prx I and II expression by cytokines. To mimic the conditions during autoimmune destruction in the pancreas, insulinoma cells INS-1 were exposed to IL-1β, IFN-γ and TNF-α for 48 h. The effects on mRNA and protein level were examined by semiquantitative RT-PCR and western blot analysis. Treating with IL-1β (50 U/ml) increased the *Prx I* mRNA amount. The combination of IL1β
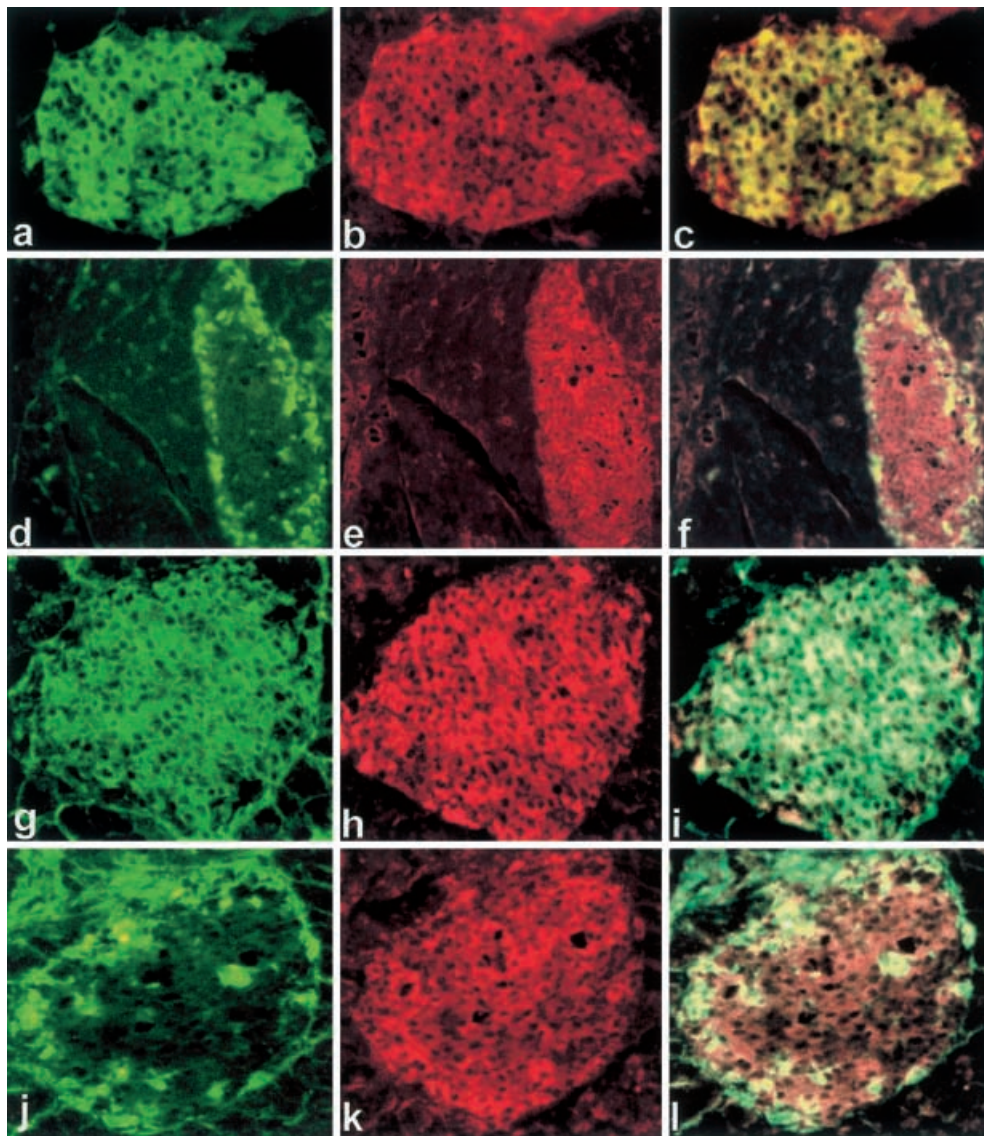
Fig. 2a–l. Expression of Prx I and Prx II in pancreatic beta cells. Immunohistochemistry. Cryostat slices of BalbC mouse pancreas were stained with antisera against Prx I (b, c, e, f) or Prx II (h, i, k, l) in parallel with antibodies against insulin (a, c, g, i) or glucagon (d, f, j, l). Immunopositive staining is indicated by green fluorescence for insulin or glucagon and by red fluorescence for Prx I or Prx II. Colocalisation results in a yellow fluorescence. (magnification ×400)

(50 U/ml), IFN-γ (100 U/ml) and TNF-α (10 ng/ml) had an additive effect on the content of *Prx I* mRNA. However, mRNA amount of the *Prx II* gene was not altered (Fig. 3A). The western blot (Fig. 3B) showed a low basal expression of Prx I and II markedly enhanced by cytokines. The amount of the monomers as well as the dimers was increased. These results were confirmed with pancreatic islets isolated from NMRI mice and treated with cytokines (Fig. 3C).
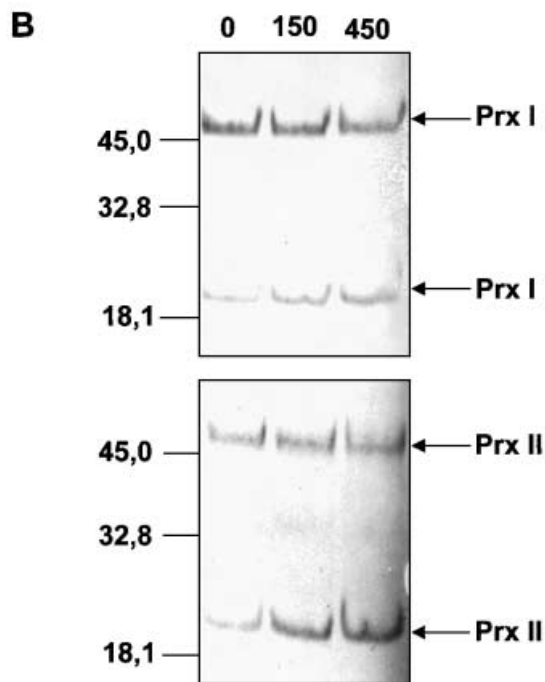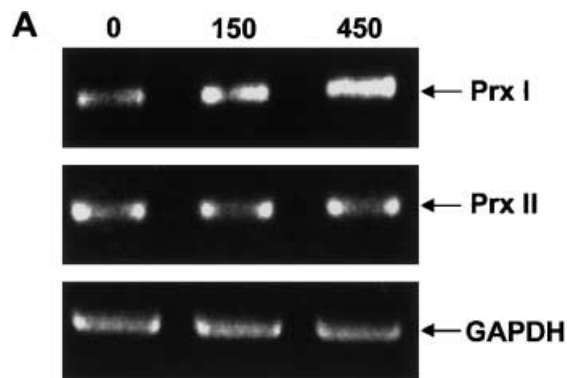
Up-regulation of Prx I and II expression by hydrogen peroxide. To analyse the effect of oxidative stress,

INS-1 cells were treated for 2 h with hydrogen peroxide at a concentration of 150 μmol/l or 450 μmol/l. Semiquantitative RT-PCR (Fig. 4A) showed a dose-dependent up-regulation of *Prx I* mRNA. The amount of *Prx II* mRNA however, was not changed. Protein contents were examined by immunoblotting. The synthesis of the monomers of Prx I as well as Prx II was increased (Fig. 4B).

Up-regulation of Prx I and II expression by alloxan. The beta cell toxin alloxan is used to generate animal models of Type I diabetes mellitus. The substance is able to destroy insulin-producing beta cells by generating oxygen radicals [51]. We investigated the effect of alloxan on Prxs expression in INS-1 cells using semiquantitative RT-PCR and western blot analysis. After exposing cells to different concentrations of alloxan for 30 min followed by an 8-h recovery period, the amount of *Prx I* mRNA, but not *Prx II* mRNA, increased (Fig. 5A). The immunoblot (Fig. 5B) shows a dose-dependent enhancement of both, Prx I and Prx II,
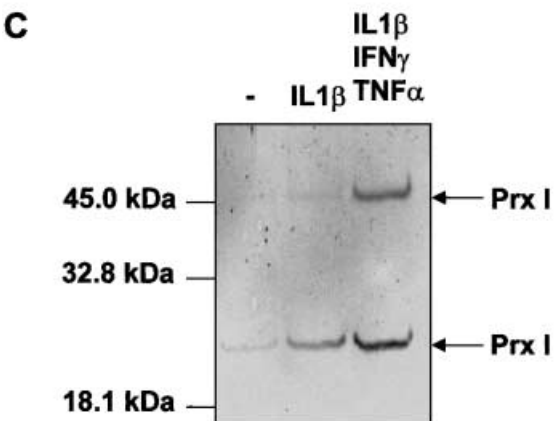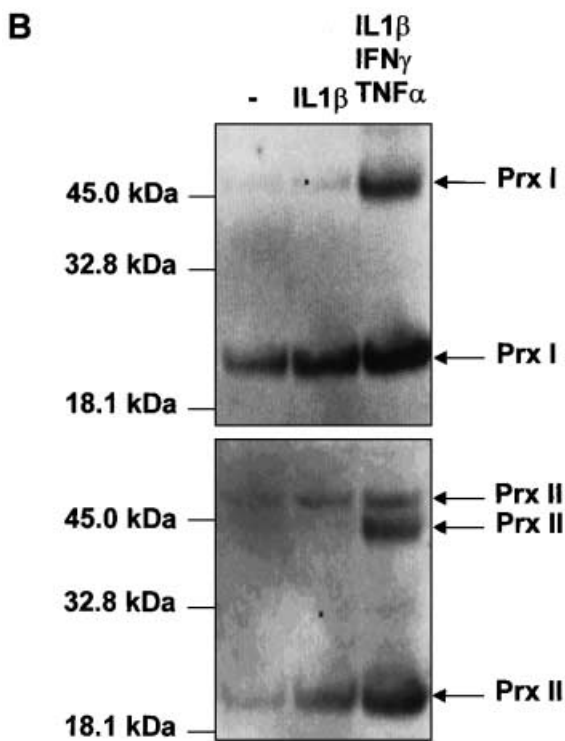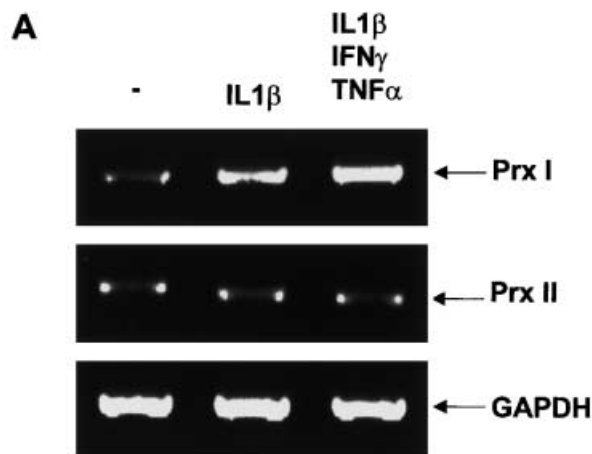
Fig. 3A–C. Up-regulation of Prx I and Prx II expression in INS-1 cells and islets by cytokines. INS-1 cells and isolated islets were exposed for 48 h to 50 U/ml IL-1β or a combination of 50 U/ml IL-1β, 100 U/ml IFN-γ and 10 ng/ml TNF-α. **A** RT-PCR. Total RNA from INS-1 cells was analysed. As control, cDNA was amplified with GAPDH-specific primers, confirming similar loading in all lanes. Western blot analysis. 20 μg cytosolic proteins from INS-1 cells (**B**) and isolated islets (**C**) were separated on SDS-12.5% polyacrylamide gels. Molecular size standards are indicated on the left in K
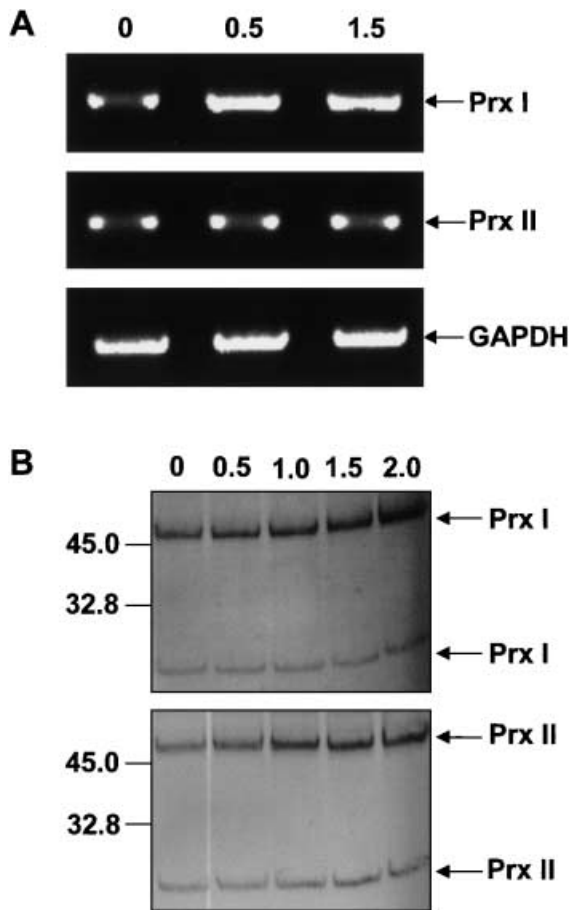

Fig. 4A, B. Up-regulation of Prx I and Prx II expression in INS-1 cells by hydrogen peroxide. **A** RT-PCR. RNA was isolated from INS-1 cells treated for 2 h in the absence (0) or the presence of 150 μM or 450 μM of hydrogen peroxide. As control, cDNA was amplified with GAPDH-specific primers. **B** Western blot analysis. 20 μg cytoplasmic proteins of INS-1 cells exposed for 2 h to 0, 150 or 450 μmol/l hydrogen peroxide were separated by SDS-PAGE (12.5%). Molecular size standards are indicated on the left in K

protein amount. In contrast to hydrogen peroxide, treatment with alloxan led to an increased synthesis of only the dimers.

Up-regulation of Prx I and II expression by streptozotocin. In contrast to alloxan, the diabetogenic substance streptozotocin induces nitrosative stress in pan-

Fig. 5A, B. Up-regulation of Prx I and II expression by alloxan. After overnight culture cells were incubated for 30 min with alloxan for the indicated concentrations (mmol/l), followed by an 8 h recovery period. **A** RT-PCR. Total RNA was analysed. As control, cDNA was amplified with GAPDH-specific primers, confirming similar loading in all lanes. **B** Western blot analysis. 20 μg cytosolic proteins were separated on SDS-12.5% polyacrylamide gels. Molecular size standards are indicated on the left in K
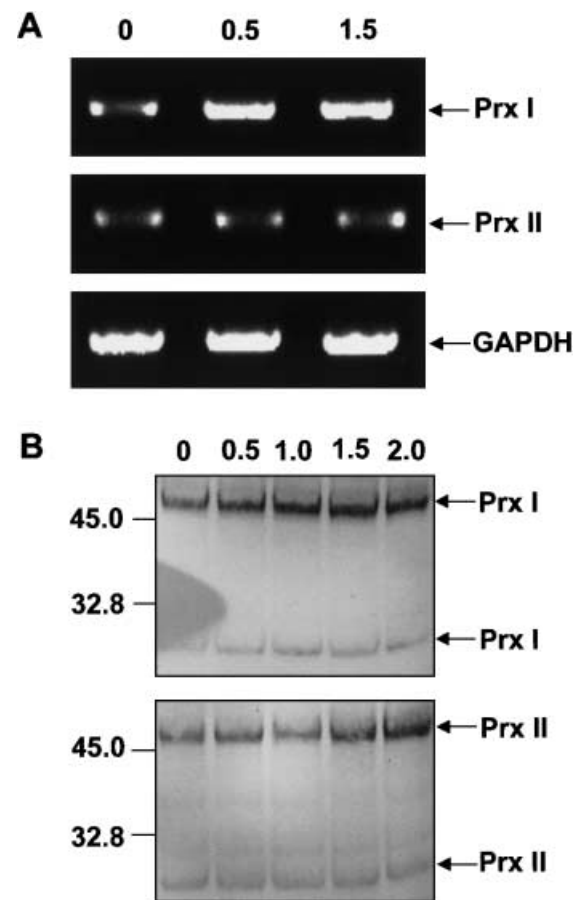
Fig. 6A, B. Up-regulation of Prx I and Prx II expression by streptozotocin. **A** RT-PCR. Total RNA was isolated from INS-1 cells exposed to 0–1.5 mmol/l streptozotocin for 30 min. As control, cDNA was amplified with GAPDH-specific primers. **B** Western blot analysis. 20 μg cytosolic proteins were separated on SDS-12.5% polyacrylamide gels. Molecular size standards are indicated on the left in K

creatic beta cells by inducing overproduction of free radical nitric oxide [52]. INS-1 cells were cultured for 30 min in the absence or the presence of increasing concentrations of streptozotocin. RNA and cytosolic proteins were isolated for semiquantitative RT-PCR and western blot analysis. The mRNA contents of *Prx I* were up-regulated. However, mRNA amount of the *Prx II* gene was not altered (Fig. 6A). Exposure of INS-1 cells to streptozotocin increased Prx I and Prx II protein content dose-dependently (Fig. 6B).

Discussion

In aerobic organisms, many metabolic processes generate reactive oxygen species, which cause cellular damage by oxidising lipids, proteins, and nucleic acids. These processes have been implicated in apoptosis, ageing and in the aetiology of various diseases, in-

cluding diabetes. As protection against these cytotoxic oxygen species, all aerobic organisms have evolved a number of antioxidant proteins that can scavenge reactive oxygen intermediates as well as repair or remove the damaged cellular components.

Peroxiredoxins, a large antioxidant gene family, have a wide tissue distribution but the highest expression of *Prx I* and *Prx II* occur in bone marrow, liver, testis, ovary and heart, followed by spleen and brain [13, 15]. Using immunohistochemical staining of mouse pancreas we show that Prx I and Prx II expression is mainly localized to pancreatic islets, with almost no signals in exocrine pancreas. Islets represent only 2 to 3% of the pancreatic tissue. This could be the reason why Prx I as well as Prx II have not been detected in the total pancreas by northern blot analysis [15]. A similar expression pattern within the pancreas was found for Prx VI, but in contrast to our findings for Prx I and Prx II immunohistochemical analyses

showed an extensive staining of nuclei in islet cells [53]. Using semiquantitative RT-PCR and western blot analyses we were able to detect Prx I and Prx II mRNA and protein of both peroxiredoxins in rat insulinoma cells INS1 and mouse insulinoma cells βTC6-F7.

Peroxiredoxins are in different subcellular compartments. While Prx III is localized to mitochondria [12, 22, 54, 55], Prx I and Prx II are found in the cytoplasm [15, 23, 32, 43]. Furthermore Prx I is thought to be translocated into the nucleus by association with other proteins such as tyrosine kinase c-Abl, though given its small size it could enter passively [34]. Our findings support that Prx I and Prx II are localized to the cytoplasm (Fig. 1B, Fig. 2).

With monomer masses of about M_r 23 K peroxiredoxins are small peroxide reductases. Peroxiredoxins form both homodimers and heterodimers, which are linked through head-to-tail disulfide bonds between the conserved amino-terminal and carboxy-terminal cysteines. Although both cysteines are required to form dimers, only the amino-terminal cysteine is absolutely required for the antioxidation function. By coupling with the redox cycle of thioredoxin, peroxiredoxins can transfer the reducing equivalents to scavenge reactive oxygen species. In western blot analyses we found both monomers and dimers. However, formation of heterodimers was difficult to distinguish from homodimers based on electrophoretic migration in polyacrylamide gel electrophoresis, because peroxiredoxins have very similar molecular sizes. Furthermore we detected an additional dimer signal of Prx II after exposure of INS-1 cells to a combination of three cytokines, probably caused by the interaction with cytoplasmic proteins. Several lines of evidence support the view that cyclophilins with molecular masses around 20 K are able to bind to peroxiredoxins and could enhance their antioxidant activity [56, 57].

Up-regulation of peroxidoredoxins in pancreatic beta cells by cellular stress. Although several functions and activities of peroxiredoxins have already been described in a variety of cells and tissues, the role of these peroxide reductases in the pancreas, especially in islet cells, is not known. We investigated Prxs expression on the level of mRNA and protein in mouse pancreatic islets and rat insulin-producing cells INS-1 after exposure to stress.

Our study shows that expression of peroxiredoxins I and II is enhanced in cultured INS-1 cells exposed to various stress agents, including the diabetogenic substances alloxan and streptozotocin. Furthermore we indicate that Prx I and II are differentially regulated at the transcriptional and translational level during stress conditions. Semiquantitative RT-PCR showed up-regulation of *Prx I* but not *Prx II* mRNA. In contrast, western blot analyses showed enhanced protein contents of both Prx I and Prx II (Figs. 3, 4, 5, 6). The dif-

ferent regulation of peroxiredoxins on mRNA and protein level is thought to be dependent on the stress agent and cell type [29, 58].

Cytokines like IL-1β, IFN-γ and TNF-α have been implicated in the pathogenesis of Type I diabetes and could be a major source of free radical insult in pancreatic islet cells [5, 59, 60]. An effect of cytokines (IFN-γ) on expression of peroxiredoxins has been shown only in rat Kupffer cells [37]. We show that cytokines up-regulate Prxs in INS-1 cells as well as in isolated pancreatic islets. The highest induction was observed with a combination of three cytokines. The cytokine-induced increase of peroxiredoxin expression could correlate with the control of reactive oxygen radical and nitrogen oxide, which are generated by IL1-β, IFN-γ and TNF-α in beta cells.

We found that treatment of INS-1 cells with hydrogen peroxide did have an effect on the accumulation of Prx I mRNA and protein, but that the Prx II protein content was more dramatically increased than that of Prx I. An up-regulation of peroxiredoxins in response to hydrogen peroxide has been observed in the mouse macrophage cell line RAW [58], and in mouse peritoneal macrophages [30], but not in endothelial cell lines ECV304 [61] and HUVEC [62]. This strongly suggests that induction of peroxiredoxins by stress inducing agents is dependent on cell type.

Alloxan and streptozotocin cause a selective destruction of insulin-producing cells by overproduction of reactive oxygen and nitrogen intermediates [51, 52, 63]. Up to now there are no data on their influence on peroxiredoxin expression. We show the induction of both Prx I and Prx II expression in INS-1 cells by both substances. Up-regulation of peroxiredoxins by streptozotocin was more effective than by alloxan. An influence of substances, which directly release nitric oxide on peroxiredoxin expression has already been reported. A dose-dependent increase of peroxiredoxin I mRNA amount in rat Kupffer cells were found after exposure to both the nitric oxide (NO)-donor SNAP and the peroxynitrite donor SIN-1 [37].

Since there are only low concentrations of SOD, CAT and GPX in pancreatic beta cells [6, 7, 8, 9], peroxiredoxins could play an important role as an antioxidant system particularly because, in contrast to these well characterized antioxidant enzymes [8, 9, 64], gene expression of peroxiredoxins is adjustable by oxidative and nitrosative stress agents. The induction of cellular stress by high glucose, high oxygen, and heat shock treatment has been shown not to affect CAT, SOD or GPX expression in rat pancreatic islets or in RINm5F cells [8]. Insulin-producing cells could not adapt the low antioxidant basal enzyme activity to typical situations of cellular stress by an up-regulation of mRNA and protein content [8].

In addition, inactivation of glutathione peroxidase by nitric oxide has been observed [65]. The bacterial peroxiredoxin AhpC (alkylhydroperoxide reductase

subunit C) has been shown to protect cells against reactive nitrogen intermediates, such as nitric oxide [25]. There is evidence that detoxification of peroxynitrite by peroxiredoxin AhpC is seven times faster than by glutathione peroxidase. The reduction of peroxynitrite was shown to be fast enough to forestall the oxidation of bystander molecules such as DNA [66]. Peroxiredoxins are at least twice as effective as catalase in protecting glutamine synthetase from inactivation [33, 41]. The overexpression of both Prx I and Prx II protected fibroblasts and lymphoma cells against oxidant-induced apoptosis [27, 67]. Furthermore, targeted overexpression of thioredoxin, the physiological reducing agent of peroxiredoxins, in pancreatic beta cells of NOD mice remarkably reduced the incidence of diabetes in untreated and in streptozotocin-treated animals. Thus a thioredoxin-dependent antioxidant system is able to prevent beta cell destruction [68].

Our data suggest that the induction of Prx expression is part of the cellular response to oxidative and nitrosative stress as well. For this reason peroxiredoxins are important antioxidant proteins providing protection against toxic agents. However, the mechanisms that control the content of intracellular Prxs are still not known. Therefore, a better understanding of both function and regulation of peroxiredoxins could be of clinical interest and important for the prevention of diabetes.

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