Human beta cells exhibit increased resistance against nitric oxide (NO) radicals as compared with rodent islet cells. Here we tested whether endogenous heat shock protein 70 (hsp70) accounts for the resistance of human cells. Stable transfection of the human beta cell line CM in the presence of an antisense hsp70 mRNA-expressing plasmid (ashsp70) caused selective suppression (>95%) of spontaneously expressed hsp70 but not of hsp70 or GRP75 protein. ashsp70 transfection abolished the resistance of CM cells to the NO donors (Z)-1-(2-(2-aminoethyl)-N-(2-ammonioethyl)amino)diazen-1-ium-1,2-diolate and so-
polate (Z)-1-(2-(2-aminoethyl)-N-(2-ammonioethyl)amino)diazen-1-ium-1,2-diolate and so-

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Heat shock proteins (hspS) are a large group of evolutionarily strongly conserved proteins with multiple tasks in trafficking, chaperoning, and stabilizing biomolecules such as mRNAs and proteins with enzymatic and structural functions (1, 2). With these functions the hsps essentially contribute to the protection of vital cell components against injuries induced under conditions of physical (3) or metabolic stress (4) and by a variety of cytotoxic inflammatory mediators like cytokines (5) and radicals (6, 7).

Among the hsps a special protective potential is attributed to the heat shock protein 70 (hsp70), which obviously plays a crucial role in the cellular defense against radical-induced injury. In insulin-producing pancreatic beta cells of rats up-regulation of hsp70 is associated with an improved resistance against NO, reactive oxygen species and the beta cell toxin streptozocin (6). Further evidence for the importance of hsp70 in beta cell defense comes from studies in which the selective overexpression of the hsp70 protein resulted in an improved resistance of rat insulinoma cells against NO (8), which has been identified as an important mediator of beta cell destruction in experimental systems of type 1 diabetes (9, 10).

However, recent findings indicate strong species-specific differences in the sensitivity of beta cells toward NO-induced toxicity. Whereas human beta cells are largely resistant, beta cells from rodents exhibit an increased susceptibility toward the damaging effects of NO (11). The parallel finding of a considerably increased spontaneous expression of hsp70 in human beta cells compared with rodent beta cells (12) led to the suggestion that hsp70 contributes to the strong natural resistance of human beta cells against NO-induced damage (13).

To prove this we established a human beta cell line in which the expression of hsp70 is selectively suppressed by transfection with a plasmid designed for the constitutive expression of antisense-hsp70 mRNA. Our experiments show that the selective inhibition of hsp70 expression results in an increased susceptibility of human beta cells toward NO-induced toxicity. The preservation of the respiratory activity in NO-exposed islet cells identified the mitochondria as the primary targets of hsp70-mediated protection.

**EXPERIMENTAL PROCEDURES**

**Cells**—The study was performed with cells of the rat insulinoma line RINm5F (14), the mouse beta cell line MIN-6 (kindly provided by Drs. J. Miyazaki, Y. Oka, H. Ishihara, Tokyo, Japan (15), and the human beta cell line CM, which was originally isolated from tumor cells present in the ascitic fluid of a patient suffering from insulinoma (16). The cells were cultivated (37 °C, 5% CO₂) in RPMI 1640 (Life Technologies, Inc.) supplemented with 125 mg/liter ampicillin, 75 mg/liter penicillin, 50 mg/liter streptomycin (Serva GmbH, Heidelberg, Germany), 2 mmol/liter L-glutamine, 10 ml/liter 100x nonessential amino acids (Life Technologies, Inc.), 3.4 g/liter NaHCO₃, 2.38 g/liter HEPES (pH 7.3, Serva), and 5% fetal calf serum (Life Technologies, Inc.) (culture medium). Cell cultures were regularly tested for the absence of mycoplasma contamination.

**Eukaryotic Expression Vectors**—The antisense (as) hsp70pcDNA3 plasmid was constructed by insertion of a 500-base pair fragment of the
human inducible hsp70cDNA in antisense orientation (974–475) at the multiple cloning site downstream the cytomegalovirus promoter in the pcDNA3 vector (17). The eukaryotic expression vector pZEM-neo was used as control. This vector, with a size (6.3 kilobases) comparable to that of pcDNA3, also contains the neomycin resistance gene but lacks any sequence interfering with hsp70 transcripts.

Transfection and Selection of Stably Transfected Clones—Transfection of the CM cells was performed by electroporation as described previously (8). CM cells (2 × 10^7) were resuspended in 800 μl of phosphate-buffered saline (PBS) containing 40 μg of plasmid DNA and exposed to a double pulse (pulse 1: 330 V/cm, +1, 1500 microfarads; pulse 2: 300 V/cm, 192.0, 900 microfarads) in a Cellject eletroporation device (Eurogentec, Sart Tilman, Belgium). After electroporation the cells were seeded in cloning plates (Greiner, Solingen, Germany) in the presence of 1600 μg/ml G418 (Geneticin, Molecular Biochemicals). From the pool of the surviving G418-resistant cells single cell-derived clones were selected and expanded to a cell number sufficient for analysis by Western blot and for the in vitro experiments.

Western Blot Analysis—Proteins from lysates of CM cells cultivated under standard conditions or exposed to heat shock (42.5 °C, 60 min) (6) were separated on 10% SDS-polyacrylamide gel electrophoresis and blotted onto nitrocellulose membranes. The membranes were incubated for 1 h with 1:1000 dilutions of a mouse monoclonal antibody directed against the inducible form of the human hsp70 (BIOMOL, Hamburg, Germany), a rat monoclonal antibody raised against a synthetic peptide of Chlamydomonas heat shock protein 70 (hsc70, BIOMOL) or a mouse monoclonal antibody specific for the human mitochondrial heat shock protein 70 (GRP75, BIOMOL). The detection was performed with sheep peroxidase-labeled anti-mouse or anti-rat antibodies (1:10,000, Amersham Pharmacia Biotech) (8, 18).

Flow Cytometry—For flow cytometric analysis single cell suspensions of ashp70-transfected cells and CM cells were fixed in methanol/acetic acid (30 min, 4 °C). The cells were washed with PBS and incubated for 18 h (4 °C) with a 1:150 dilution of the mouse anti-human hsp70 antibody (see Western blot analysis). After washing in PBS with 2% fetal calf serum the sample were counted in triplicates.

Determination of Cell Lysis—The proportion of dead cells was determined by the trypan blue exclusion assay (19) in 96-well flat bottom plates ( Falcon/Becton-Dickinson). After incubation under various conditions, 150 μl of the culture supernatant were removed from each well and 15 μl of a trypan blue solution (0.4% in PBS) were added, and the cells were counted for an additional 15 min (37 °C, 5% CO2). Then 200 cells were counted in adjacent microscopic fields in each well, and the percentage of dead cells from the total cell number was calculated.

Determination of Insulin Contents—To investigate the reactivity of CM cells toward glucose stimulation the cells were cultivated in medium containing 16.7 mM glucose, and after 0, 8, and 24 h the cells were lysed, and the released DNA was bound to a mineral carrier material. The fixed DNA was washed over a column (12,000 × g, 2 min) and eluted by incubation in elution buffer (2 min), followed by centrifugation (12,000 × g, 1 min). The DNA fragments were separated by electrophoresis on an agarose gel (2%, Metaphor, FMC Bioproducts, Rockland, ME) containing ethidium bromide and visualized by UV exposure. DNA isolated from apoptotic murine thymocytes included in the InViSorb kit was used as a positive control for a ladder-like DNA fragmentation pattern.

**Lipid Peroxidation**—Lipid peroxidation was assessed by measurement of malondialdehyde (MDA). The cells (2 × 10^6 in 10 ml) were seeded in Petri dishes and incubated for 24 h in the presence of NP (0.6 mM). To induce lipid peroxidation in the cells FeCl3 (0.1 mM) and ascorbate (0.25 mM) were used as a positive control. Then the cells were scraped from the Petri dishes in the presence of butyldihydroxytoluid (Sigma), disrupted by sonication and frozen at −20 °C until use. To 1 ml of the cell lysate 0.35 ml of 20% trichloroacetic acid (Sigma) and 0.5 ml of 1.4% (w/v) thioarsorbic acid (Merck) were added. After heating (15 min, 95 °C) the samples were centrifuged (5 min, 250 × g), and 2 ml of the supernatant were mixed with 2 ml of 1-butanol. The resulting thiobarbituric acid-MDA complex was separated on reversed-phase HPLC and quantitated with a fluorescence detector (excitation 515 nm, emission 553 nm) (23).

**Statistics**—Statistical differences were calculated using the Student’s t test with a significance level of p < 0.05.

### RESULTS

**Enhanced Resistance of Human Compared with Rodent Insulinoma Cells toward Radicals**—The study was performed to investigate the role of hsp70 in the natural resistance of human beta cells against damage induced by reactive radicals. For the experiments, cells of the human insulinoma line CM were used, which was maintained under tissue culture conditions since its isolation from an insulinoma patient. To confirm the beta cell characteristics of this cell line, the insulin contents of the cells were determined before and after stimulation by an increased glucose level in the culture medium. As shown in Table I the CM cells contained high amounts of insulin (8.5 microunits/10^6 cells) and were able to release the stored hormone in a time-dependent manner in response to stimulation with an elevated glucose concentration.

To compare the sensitivity of human and rodent beta cells to radical-induced injury, CM cells and cells of the rat line RINm5F and of the mouse line MIN-6 were exposed to NO and

| Duration of stimulation (h) | Insulin contents (μg/ml) |
|-----------------------------|-------------------------|
| 0                           | 8.5 ± 2.7               |
| 8                           | 0.4 ± 0.2               |
| 24                          | 0.05 ± 0.04             |
reactive oxygen species generating agents for 18 h. Both agents dose dependently damaged cell functions as determined by the MTT test. The human insulinoma cell line showed a significantly improved metabolic activity in the presence of the NO donor NP (Fig. 1A) as well as after exposure to the O$_2$/H$_2$O$_2$ generating system hypoxanthine/xanthine oxidase (Fig. 1B) compared with the insulinoma cells from mouse and rat (p < 0.05 and p < 0.005, respectively). Next we analyzed for spontaneous expression of hsp70 in untreated cells. Western blot analysis revealed spontaneous expression of hsp70 in human CM cells, whereas the rodent cells contained almost undetectable amounts of the protein (inset in Fig. 1B). Both rodent cell lines expressed hsp70 in response to heat stress (not shown).

Suppression of Spontaneous hsp70 Expression in Human Insulinoma Cells—To investigate the role of hsp70 in the enhanced natural resistance of the human insulinoma cells against NO radicals, the expression of hsp70 protein was suppressed in CM cells by transfection with a plasmid expressing an antisense hsp70 mRNA. The efficiency and the selectivity of the antisense strategy was proven by Western blot analysis. Transfection of the CM cells with the ashsp70 construct resulted in a 15-fold reduction of hsp70 expression in cells cultivated under standard conditions (37 °C, 5% CO$_2$), whereas cells transfected with the control plasmid pZEM showed an hsp70 protein expression comparable to the level in the untransfected controls (Table II). Expression of the antisense construct did not influence the morphology and growth rate of the cells. Exposure of the cells to heat shock (42.5 °C, 60 min) 4 h prior to protein extraction resulted in a significant increase of the hsp70 signal in all three cell lines tested. In the control lines (CM and pZEM-transfected CM) the hsp70 signal increased about 1.7-fold. Interestingly, heat shock induced a more than 30-fold increase of the hsp70 signal also in ashsp70-transfected cells thereby reaching a level of expression comparable to the control cells (Table II). The deficient expression of hsp70 in the antisense line was also confirmed by cytofluorometry. As shown in Fig. 2, there was a reduction of the fluorescence activity in ashsp70-transfected cells yielding a single peak, which indicated homogeneously reduced hsp70 expression in antisense plasmid-transfected cells.

Because the constitutively expressed hsc70 and the mitochondrial GRP75 have a high amino acid sequence homology with hsp70, we investigated whether the expression of these proteins was impaired in cells transfected with ashsp70. As shown in Fig. 3 (lane 1) the CM cells spontaneously express hsp70, hsc70, and GRP75. Transfection with the ashsp70 construct strongly reduced the expression of hsp70, whereas the signal strengths of hsc70 and GRP75 remained unchanged (lane 2). CM cells transfected with the control plasmid pZEM expressed the same levels of hsp70, hsc70, and GRP75 as the wild type CM cells (lane 3). After heat shock treatment a strong increase mainly of the hsp70 protein expression was observed in all three cell lines (lanes 4–6).

**Table II**

| Condition                  | CM cells untransfected | CM cells ashsp70 transfected | CM cells pZEM transfected |
|----------------------------|------------------------|------------------------------|---------------------------|
| Untreated                  | 354.9 ± 20.2           | 20.4 ± 10.2$^b$             | 319.8 ± 23.5              |
| Heat shock treated         | 570.9 ± 27.1$^b$       | 682.1 ± 18.7$^b$            | 571.1 ± 24.7$^b$          |

$p < 0.01$ compared to the signal of untransfected CM cells or the pZEM-transfected cells.

$p < 0.01$ versus the signal of the corresponding samples without heat shock treatment.

Suppression of Spontaneous hsp70 Expression Abolishes Resistance to NO Radicals—To investigate whether the suppression of spontaneous hsp70 protein expression by the antisense plasmid would increase the sensitivity of the CM cells toward NO radical-induced injury, the cell lines were exposed to the NO donors NP (Fig. 4A) and DETA/NO (Fig. 4B). As an end point of necrotic cell death, the irreversible loss of membrane integrity was determined by the inability of the cells to exclude trypan blue. Within the first 24 h of NO exposure the rates of cell death slightly increased up to 5–10% (Fig. 4A and B). No significant difference in sensitivity could be observed between the ashsp70-transfected cells and the cells transfected with the control plasmid. Prolongation of the exposure time resulted in a steady increase of the death rate in the control cells up to a maximum of 10% after 48 h. In contrast, ashsp70-transfected cells showed a strongly accelerated death rate reaching 50% in the NP-(0.6 mM) exposed cells and more than 50% in the DETA/NO- (0.2 mM) exposed cells ($p < 0.05$ compared with the specific
These findings clearly show an increased susceptibility toward NO-induced necrosis in cells with suppressed spontaneous hsp expression.

To further prove the role of hsp70 we tested the hypothesis that the (re-)induction of hsp70 protein in ashsp70-transfected CM cells by heat shock exposure (Fig. 3) will re-establish the resistance of the cells toward NO-induced damage. In fact, heat shock treatment resulted in a significant reduction of DETA-NO-induced lysis of ashsp70-transfected cells from 38.4 to 12.0% for 0.1 mM DETA-NO and from 55.6 to 17.3% for 0.2 mM DETA-NO (p < 0.05, Fig. 5). Heat shock did not improve the resistance of untransfected and pZEM-transfected CM cells that constitutively express hsp70.

**Analysis of DNA Fragmentation**—In parallel samples the effect of hsp70 expression on the NO-induced apoptotic pathway of cell death was examined by analyzing nuclear chromatin condensation and DNA fragmentation. As shown in Fig. 6 exposure to NO resulted in an increased proportion of cells showing apoptotic alterations. Acridin orange staining revealed condensation of nuclear chromatin and formation of apoptotic bodies in a significantly higher percentage of ashsp70-transfected CM cells (24.0 ± 0.5%) (Fig. 6A) when compared with identically treated cells transfected with the control plasmid pZEM (13.8 ± 2.2%) (p < 0.05). In the untreated samples, about 4% of the cells formed apoptotic bodies. To investigate the mode of DNA degradation, DNA was isolated from ashsp70-transfected CM cells and controls after 72 h of NO exposure. After separation of the DNA by electrophoresis a ladder-like fragmentation pattern was clearly visible in ashsp70-transfected cells (lane 3), whereas only faint signals of DNA degradation were detectable in untransfected CM cells (lane 1) and in CM cells transfected with the control plasmid pZEM (lane 4) (Fig. 6B). These observations indicate that suppression of spontaneous hsp70 expression increased the susceptibility of the CM cells toward NO-induced apoptosis.

**hsp70 Does Not Protect Cells From Lipid Peroxidation**—Exposure to NO radicals may lead to cell injury via the formation of toxic compounds from cellular components. It has been suggested that lipid peroxides mediate radical toxicity in islet cells (24, 25). Therefore, it was analyzed whether hsp70-mediated protection from NO toxicity affects lipid peroxidation. As a measure of lipid peroxidation, we determined MDA. CM cells transfected with the ashsp70 plasmid or the pZEM control plasmid were incubated in the presence of the NO donor NP or the potent reactive oxygen species generating system FeCl₃ and ascorbate. As expected, HPLC analysis of the cell lysates revealed a strong accumulation of MDA (about 8800 nM/10⁶ cells) in cells exposed to FeCl₃/ascorbate for 24 h (Fig. 7). In contrast, NP-exposed cells showed only a slight, but significant (p < 0.05) increase to 0.86 nM MDA/10⁶ cells compared with untreated cells (0.07 nM MDA/10⁶ cells). However, the analysis of the ashsp70-transfected cells and the pZEM transfected CM cells did not reveal any difference in the degree of lipid peroxidation in response to either NO or reactive oxygen species (Fig. 7).

**hsp70 Preserves Mitochondrial Function in the Presence of...**

**FIG. 2.** Decreased hsp70 expression in ashsp70-transfected human insulinoma cells as determined by cytofluorometry. Untreated CM cells (A) and CM cells transfected with ashsp70 (B) were processed and subjected to fluorescence-activated cell sorter analysis for the detection of hsp70 expression as described under “Experimental Procedures.” Shown are data of a single representative experiment.

**FIG. 3.** Spontaneous expression of hsp70 in human insulinoma cells and suppression by the ashsp70 plasmid. The expression of hsp70 (A), hsc70 (B), and GRP75 (C) was analyzed by Western blot in the lysates of 10⁵ cells/lane. Lysates were prepared from untreated cells (lanes 1–3) or from heat shock-treated cells after a 4 h recovery period (lanes 4–6). Lanes 1 and 4 show the signals of CM cells, lanes 2 and 5 show the signals of ashsp70-transfected CM cells, and lanes 3 and 6 show the signals of pZEM-transfected CM cells. Shown are data of a single representative experiment.
Fig. 4. Suppression of spontaneous hsp70 expression abolishes resistance to necrosis induced by NO released from NP (A) or DETA-NO (B). Cells transfected with pZEM (squares) and ashsp70-transfected CM cells (circles) were incubated in the presence of 0.3 (open symbols) and 0.6 mM (solid symbols) of the NO donor NP (A) or in the presence of 0.1 mM (open symbols) and 0.2 mM (solid symbols) of DETA-NO. After 0, 24, and 48 h the specific lysis of the cells was determined by the trypan blue exclusion assay. Spontaneous lysis never exceeded 15%. The data show mean ± S.D. from four experiments. *, p < 0.05 compared with the ashsp70-transfected cells.

Fig. 5. Heat shock reverses the increased sensitivity of ashsp70-transfected insulinoma cells. Untransfected CM cells and CM cells transfected with the control plasmid pZEM or with ashsp70 were cultivated under standard conditions at 37°C (open bars) or exposed to heat shock (42.5°C, 60 min, hatched bars). Thereafter, the cells remained untreated (medium control) or they were incubated in the presence of DETA-NO (0.1 and 0.2 mM). After 48 h cell lysis was determined by the trypan blue exclusion assay. Data show mean ± S.D. from two experiments performed in triplicate. **, p < 0.01 compared with the samples incubated at 37°C.

Fig. 6. Suppression of spontaneous hsp70 expression abolishes resistance toward NO-induced apoptosis. CM cells transfected with pZEM (open bars) and ashsp70-transfected CM cells (hatched bars) were cultivated on chamber slides and exposed to 0.6 mM NP. After 48 h the percentage of cells showing apoptotic nuclear alterations was determined by acridin orange staining. Data show mean ± S.D. from four experiments. *, p < 0.05 compared with the pZEM-transfected cells. The micrograph inset documents nuclear histology in NP-treated ashsp70-transfected cells. A fraction of NP-treated cells displays nuclear condensation (arrow) (A). Analysis of DNA fragmentation by electrophoretic separation of DNA samples from untransfected (lane 2), ashsp70-transfected (lane 3), and pZEM-transfected CM cells (lane 4) after 72 h of NO exposure (B). DNA from apoptotic thymocytes was used as a positive control for the demonstration of internucleosomal DNA cleavage (lane 5). Lane 1 shows 100-base pair (bp) markers.
Because the mitochondrial respiratory system and energy metabolism in general were found to be highly susceptible to NO radicals, it was determined whether hsp70 might exert its protective action at this level. CM cells transfected with the ashsp70 construct and with the control plasmid pZEM were incubated for 24 h in the presence of increasing doses of NP or DETA/NO, and after different time intervals the residual metabolic activity was assessed by the capacity of the cells to reduce MTT into its formazan product (Fig. 8). In CM cells transfected with pZEM, the metabolic activity was decreased by 40% after exposure to NP, and no significant changes were noted after DETA/NO exposure. In contrast, the ashsp70-transfected CM cells showed a significantly decreased capacity to convert MTT after 24 h of exposure to either NP or DETA/NO ($p < 0.01$). In an additional sample CM cells were used, which derived from the pool of ashsp70-transfected cells after selection for G418 resistance but before selection of clones. After NO exposure (0.6 mM NP) these cells showed a significant reduction of mitochondrial activity to a level comparable to the residual MTT activity of the NO-exposed ashsp70-transfected clone originally selected for the experiments.

**DISCUSSION**

The present study was performed to elucidate the role of hsp70 in the protection of human beta cells from radical-induced damage. As an experimental model, the human cell line CM was selected, which has retained basic beta cell-specific characteristics such as glucose-stimulated insulin release and an expression pattern of beta cell surface markers and autoantigens almost identical to native human beta cells (26).

The CM cells used for the experiments were found to express hsp70 under standard culture conditions, whereas rodent beta cell lines, studied in parallel, expressed only trace amounts of hsp70. After exposure of the cells to chemically generated NO or O$_2$.H$_2$.O$_2$, the human beta cell line exhibited largely preserved metabolic activity, in contrast to substantial impairment of metabolic activity in rodent cells.

These observations confirm previous observations obtained with freshly isolated islets (13, 27, 28) and refute the argument that species differences result from different degrees of stress occurring during the isolation procedure of human versus rodent islets.

To prove the assumed role of hsp70 in the protection of human beta cells against radical-induced injury, we generated a cell line in which the expression of the stress protein was suppressed by more than 95% after transfection with a plasmid for the constitutive transcription of antisense hsp70 mRNA. The suppression induced by the ashsp70 construct was highly specific for the inducible hsp70. As demonstrated by Western blot analysis the expression of hsc70 or of the mitochondrial hsp 70 (GRP75) remained unchanged although these proteins share a high degree of homology of their amino acid sequences with hsp70 (1). Suppression of hsp70 was observed in ashsp70-transfected cells cultivated under standard conditions at 37 °C. After heat shock treatment ashsp70-transfected cells expressed similar high amounts of hsp70 protein as untransfected cells or cells transfected with the control plasmid pZEM. This observation indicates that the capacity of the transfected cells to produce ashsp70 mRNA seems to be limited to an amount sufficient for blocking hsp70 mRNA expression generated under normal culture conditions. It may not suffice to block the large amounts of hsp70 protein as untransfected cells or cells transfected with the control plasmid pZEM. This observation indicates that the capacity of the transfected cells to produce ashsp70 mRNA seems to be limited to an amount sufficient for blocking hsp70 mRNA expression generated under normal culture conditions. It may not suffice to block the large amounts of hsp70 mRNA newly transcribed under heat shock conditions, because the transcription rate of the constitutively expressed antisense mRNA is under the control of the cytomegalovirus.
promoter and therefore it is not up-regulated in response to heat stress. To investigate the protective effect of hsp70 against the beta cell toxic radical NO, the hsp70-deprived CM cells were cultivated under standard conditions (37°C) in the presence of chemical NO donors. However, it cannot be excluded that these substances may exert at least a part of their cytotoxic activity via the release of additional compounds formed during decomposition. Toxic CN⁻ ions, which may be spontaneously released from NP, are effectively scavenged by rhodanese and thiosulfate (19). Recent findings further indicated that NP also releases reactive oxygen species (29). To address this critical issue we used an additional chemically unrelated NO donor, DETA/NO, with a different mode of NO release. Interestingly, both chemicals induced comparable amounts of cell death and metabolic inhibition. These observations strongly indicate that the damaging effects observed after exposure to NP or DETA/NO are attributable to the NO radical released from the two compounds.

The investigations on the role of hsp70-mediated protection focused on the analysis of signs of apoptosis and necrosis as the major pathways of beta cell death (30). After 48 h of exposure to NP or DETA/NO, the antisense-transfected human beta cell line exhibited strongly increased susceptibility to NO-induced cell death. In these cells necrosis was assessed by the trypan blue assay, which detects membrane leakage as an irreversible lethal damage of the cell. Both NO donors dose dependently induced necrotic cell death in the hsp70-deficient beta cell line but little cell lysis in hsp70-expressing control cells. A similar outcome was noted when apoptosis was used as an end point of NO toxicity. A significantly higher proportion of hsp70-deficient cells showed morphological signs of apoptosis compared with the control cell line. In addition, detectable amounts of DNA fragments resulting from apoptotic DNA degradation could only be demonstrated in ashp70-transfected CM cells. A possible regulatory role of hsp70 in apoptosis was recently also found by Robertson et al. (31), who reported that the suppression of hsp70 by antisense oligomers enhanced proteasome inhibitor-induced apoptosis in FL5.12 cells. As a further control, hsp70 expression was re-induced in the antisense line by heat shock. Concomitantly, resistance to NO toxicity was established at a level comparable to that of nontransfected or pZEM control transfected cells. We have reported previously that the induction of hsp70 by heat shock also is associated with improved resistance to NO toxicity in murine islet cells (6, 8).

These findings suggest that the constitutive expression of hsp70 in human beta cells is critical for the resistance against NO-induced necrosis as well as apoptosis. Recent findings suggest that radical-dependent beta cell apoptosis involves lipid peroxidation with subsequent formation of cytotoxic aldehydes (24, 25). Furthermore, hsp70 was found to protect rat hepatocytes from tumor necrosis factor α-mediated cell death. In the latter study hsp70 attenuated lipid peroxidation and subsequent apoptosis, which are the consequence of tumor necrosis factor α-induced radical formation (32). We therefore determined whether lipid peroxidation in the CM cells was affected by the presence or absence of hsp70. However, when using MDA levels as a surrogate, we did not observe an association between degrees of lipid peroxidation and levels of hsp70. Another line of evidence indicates that mitochondria are involved in the initiation of cell death processes (33). Interestingly, hsp70 was found to play an important role in the translocation of polypeptides from the cytoplasm to the mitochondria (34, 35) thereby contributing to mitochondrial biogenesis and to the structural and functional integrity of the organelle (36). In addition, a recent study identified the mitochondrial function as the primary intracellular target of hsp70-mediated cell death. In the latter study hsp70 attenuated lipid peroxidation and subsequent apoptosis, which are the consequence of tumor necrosis factor α-mediated cell death.

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