Increased mRNA Levels of Mn-SOD and Catalase in Embryos of Diabetic Rats From a Malformation-Resistant Strain

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Previous studies have suggested that reactive oxygen species (ROS) are mediators in the teratogenic process of diabetic pregnancy. In an animal model for diabetic pregnancy, offspring of the H rat strain show minor dysmorphogenesis when the mother is diabetic, whereas the offspring of diabetic rats of a sister strain, U, display major morphologic malformations. Earlier studies have shown that embryonic catalase activity is higher in the H than in the U strain, and maternal diabetes increases this difference in activity. The aim of this study was to characterize the influence of genetic predisposition on diabetic embryopathy by comparing the mRNA levels of ROS-metabolizing enzymes in the two strains. We determined the mRNA levels of catalase, glutathione peroxidase, γ-glutamylcystein-synthetase, glutathione reductase, and superoxide dismutase (CuZn-SOD and Mn-SOD) in day 11 embryos of normal and diabetic H and U rats using semiquantitative reverse transcription–polymerase chain reaction. The mRNA levels of catalase and Mn-SOD were increased in H embryos as a response to maternal diabetes, and no differences were found for the other genes. Sequence analysis of the catalase promoter indicated that the difference in mRNA levels may result from different regulation of transcription. Sequence analysis of the catalase cDNA revealed no differences between the two strains in the translated region, suggesting that the previously observed difference in the electrophoretic mobility in zymograms is due to posttranslational modifications. An impaired expression of scavenging enzymes in response to ROS excess can thus be an integral part of a genetic predisposition to embryonic dysmorphogenesis. *Diabetes* 49:101–107, 2000

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CuZn-SOD, cytoplasmatic form of SOD; γ-GCS, γ-glutamylcystein-synthetase; GR, glutathione reductase; GSH, glutathione; GSHpx, glutathione peroxidase; GSSG, oxidized glutathione; HD, embryos of diabetic H rats; HN, embryos of normal H rats; Mn-SOD, mitochondrial form of SOD; PCR, polymerase chain reaction; ROS, reactive oxygen species; SOD, superoxide dismutase; UD, embryos of diabetic U rats; UN, embryos of normal U rats.

Maternal type 1 diabetes during pregnancy has been known for many years to be associated with an increased risk for congenital malformations in the offspring (1–4). Clinical studies have estimated the risk for a malformed fetus in a type 1 diabetic pregnancy to be in the range of 5–10% (5–9). The malformations are induced before the 7th postconceptional week in human diabetic pregnancy (10). In rats, the teratogenic process is believed to occur during organogenesis (11). Recent reports, however, indicate that a diabetic environment may decrease the inner cell mass (12) and that a high glucose concentration can lead to increased apoptosis already in the preimplantation embryo (13). The teratogenic process and its predisposing factors are not known in detail. In pregnancies with poorly controlled diabetes, however, there is a correlation between the level of HbA_{1c} in maternal blood and the risk for having a malformed child (5,14,15).

The hypothesis has been put forward that an excess of reactive oxygen species (ROS) mediates the teratogenicity of diabetic pregnancy (16–19). The oxygen radicals may be harmful to the cell and its functions by reacting with unsaturated fatty acids in membranes, yielding lipid peroxides and causing decreased membrane fluidity (20) and the formation of reactive aldehydes, which may in turn diffuse to other parts of the cell and there react with macromolecules (21,22). The oxygen radicals can also react directly with proteins, resulting in, for instance, cross-linking of collagen with DNA, causing damage to both bases and sugars (23,24).

There are three major types of findings indicating a role for an excess of free oxygen radicals in diabetic embryopathy. First, diabetes in vivo (25) and hyperglycemia in vitro (26,27) cause an increase in lipid peroxides and free radicals in the offspring (28). Second, developmental defects similar to those seen in diabetic rat pregnancies in vivo and in high-glucose embryo cultures in vitro can be induced by enzymatic production of superoxide ions in embryo culture systems (29). Third, several different scavengers of free oxygen radicals added to the diet (18,30–32) decrease the malformation rate in diabetic rat pregnancy. In vitro, the increased malformation rates caused by both high glucose (16,17) and enzymatic radical production (33) in the culture medium can be diminished by radical scavengers. In addition, radical scavengers normalize glucose-induced overproduction of the isoprostane 8-iso-prostaglandin $F_{2\alpha}$ in cultured rat embryos (28). The ROS theory is also strengthened by the suggestion

that ROS excess is a teratologic mediator of ethanol (34), phenytoin (35), and thalidomide (36).

There are three main intracellular ROS scavenging enzymes: superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSHpx). The three differ in subcellular distribution and type of catalyzed reaction. SOD catalyzes the conversion of superoxide ions into oxygen and hydrogen peroxide and exists in a cytoplasmatic form (CuZn-SOD) and a mitochondrial form (Mn-SOD). Catalase, on the other hand, is distributed mainly to the peroxisomes and catalyzes the decomposition of hydrogen peroxide into water and oxygen. GSHpx reduces hydrogen peroxide to water using glutathione (GSH), which in turn is oxidized to oxidized glutathione (GSSG). The embryonic expression of these three enzymes seemed relevant to investigate against the background of a possible role for oxygen radicals in diabetic teratogenicity. Hyperglycemia in vitro has been found to decrease embryonic GSH content (27), as has also been demonstrated in vivo in embryos of diabetic rats (37). The enzyme γ-glutamylcystein-synthetase (γ-GCS) catalyzes the rate-limiting step in synthesis of GSH, while glutathione reductase (GR) reduces GSSG back to GSH in a NADPH-dependent reaction. To get a more comprehensive view of glutathione metabolism in the embryo, therefore, we investigated the mRNA levels of the enzymes γ-GCS and GR.

We have used two substrains of the Sprague-Dawley rat, denoted U and H. The U rat developed spontaneously out of the H strain and has been shown in several studies to have a high frequency of congenital malformations in maternal diabetes (38,39). Both strains are now kept under outbreeding conditions. The catalase activity in embryos of the U strain was found to be decreased compared with that of H embryos. This difference increases further when the mother is diabetic, because of a decrease in the activity in U embryos and unchanged activity in H embryos (39). It has also been demonstrated that the catalase proteins purified from these two strains are different with respect to charge, size, or both (40). Taken together, the previous observations indicate that differences in the embryonic capacity to metabolize ROS may determine susceptibility to the teratogenic impact of ROS excess. Such a difference in teratologic susceptibility on a genetic level—that is, in the expression patterns of enzymes metabolizing ROS and GSH—has not been elucidated before.

The aim of this study, therefore, was to investigate if the embryonic mRNA levels of ROS-scavenging and GSH-metabolizing enzymes were different between the H and U substrains. We also studied whether the mRNA levels would change in a state of maternal diabetes. Furthermore, we determined and compared the H and U nucleotide sequences of catalase cDNA and catalase promoter region with the aim of finding discrepancies that may explain the strain-related differences in catalase activity and protein structure.

RESEARCH DESIGN AND METHODS

Animals. We used two outbred substrains of the Sprague-Dawley rat, denoted H and U, with different teratologic susceptibility as discussed above (38,39). The U strain developed out of the H strain during a 20-year period (1962–1982) when it was kept in a commercial breeding facility in Sweden. Since 1982, the U strain has been kept under outbreeding conditions in a colony at the Laboratory Animal Resources of the BioMedical Center in Uppsala. The H strain has been outbred in a colony at a commercial breeder in Sweden (B&K Universal AB, Sollentuna, Sweden) since 1982. In the present study, all rats were subjected to a 12-h light and 12-h dark cycle. They had free access to tap water and commercial food pellets (R36; Lactamin AB, Stockholm, Sweden). Female rats weighing at least 230 g were made diabetic with injection into the tail vein of 40 mg/kg streptozotocin (a gift from Pharmacia & Upjohn, Kalamazoo, MI). Diabetes was defined as a blood glucose level >20 mmol/l 1 week after injection of streptozotocin (measured with a Glucose Analyzer 2; Beckman Instruments, Fullerton, CA). The diabetic rats were not given insulin or any other antidiabetic treatment. Control embryos from both substrains were obtained from normal H and U females not previously injected with streptozotocin. Embryos from normal H rats were denoted HN and embryos of diabetic H rats were denoted HD; the same terminology was applied to embryos of normal and diabetic U rats (UN and UD). The female rats were mated overnight with males of the same substrain. Gestational day 0 was defined as the day when sperm was found in vaginal smear. At day 11 of pregnancy, blood glucose concentration was measured, and the rats were killed by cervical dislocation. Embryos were dissected out of the uterus and transferred to petri dishes containing saline, where they were freed from connective tissue and yolk sac using watchmaker's forceps under a stereo microscope (Wild M3Z, Heerbrugg, Switzerland). Crown-to-rump length was measured using a millimeter ruler under the petri dish, and the number of somites was counted. The embryos were deposited in 1.5-ml plastic tubes, quickly frozen in liquid nitrogen, and stored at –135°C until further preparation. Ten embryos were collected from each of the HN, HD, UN, and UD groups; no more than two embryos from the same litter were used in the determinations of mRNA levels. In the experiments, RNA samples from 8–10 embryos from each group were investigated. The animal experimental procedures were approved by the Animal Ethical Committee of the Medical Faculty of Uppsala University.

Purification of RNA and cDNA synthesis. Total RNA was purified from the frozen embryos using 1 ml Ultraspec total RNA isolation reagent (Biotecx Laboratories, Houston, TX) and 200 µl chloroform for each sample. After centrifugation, the RNA was precipitated with isopropanol (Merck, Darmstadt, Germany). The RNA sample was then washed twice with 75% ethanol, dried to a pellet, and dissolved in 100 µl diethylenepyrocarbonate (DEPC)-treated water. The RNA concentration was measured as absorbance at 260 nm, and 1 µl RNase inhibitor was added to the sample (RNAguard; Pharmacia Biotech, Uppsala, Sweden). cDNA was produced with 1 µg RNA as template. We used Ready-To-Go first-strand beads (Pharmacia Biotech) containing buffer, nucleotides, and Moloney murine leukemia virus (M-Mulv) reverse transcriptase and followed the instructions provided by the manufacturer. Oligo d(T) primers were used with this kit to reverse-transcribe the mRNA.

Analysis of mRNA levels. Parts of the cDNAs of interest, each 150–550 bp, were amplified by polymerase chain reaction (PCR) (41) using gene-specific primers. Each reaction mix comprised 50 µl and contained 5 µl 10× PCR Buffer II (500 mmol/l KCl and 100 mmol/l Tris-HCl, pH 8.3) (Perkin Elmer AB, Stockholm, Sweden), 2.5 mmol/l $MgCl_2$, 0.8 mmol/l dNTP, 0.25 µg of each primer, 1 µl of template cDNA, and 0.25 µl AmpliTaq Gold polymerase. The gene-specific primers (Table 1) were designed using available gene sequences. The oligomers were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). Other PCR chemicals were purchased from Perkin Elmer AB. The PCRs were run on a Touch Down Thermal Cycler (Hybaid, Teddington, U.K.). The programs (Table1) were optimized for each primer pair to get a linear proportion between the amount of template used and the amount of product after the PCR reaction (data not shown). All programs started with a 10-min period at 94°C and ended with a 5-min extra elongation period at 72°C. The PCR products were run on 1.5% agarose gels (SeaKem GTG agarose; FMC BioProducts, Rockland, ME). All four groups were represented on each gel. Gels were stained with ethidium bromide, visualized with ultraviolet light, and photographed with a digital camera. The bands were identified and quantified by comparing with a lane of 125 ng of DNA-standard VIII (Boehringer Mannheim, Mannheim, Germany) on each gel, using the Kodak Digital Science Electrophoresis, Documentation, and Analysis System for Macintosh computers (Kodak AB, Stockholm, Sweden).

DNA sequence analysis of the catalase gene. A 2,202-bp cDNA fragment was amplified by PCR. A 2,015-bp portion of this fragment, comprising the complete coding part of the catalase gene as well as 13 and 418 bp of the 5′ and 3′ untranslated regions, respectively, was sequence-analyzed. For PCR amplification, we used the Expand Long Template System (Boehringer Mannheim) following their protocol using 5 µl of cDNA as template and the catalase-specific primers 5′-ATTGC CTACCCCGGGTGGAG and 5′-CCAGAAGATCATTTTATTGTAAAAATG. The program used for PCR amplification was denaturation at 94°C for 2 min, followed by 30 cycles consisting of 94°C for 10 s, 60°C for 30 s, 68°C for 2 min with a 20-s increase per cycle at 68°C for the last 20 cycles, and a final elongation step at 68°C for 7 min.

Genomic DNA was purified by incubation of embryos overnight in lysis buffer containing 10 ml of 1 mol/l Tris-HCl, 1 ml of 0.5 mol/l EDTA, 2 ml of 10% SDS, and 4 ml of 5 mol/l SDS. The sample was then centrifuged at 13,000 rpm for 10 min, and the DNA in the supernatant was precipitated with 2-propanol. The DNA pellet was

TABLE 1
PCR primers and programs for mRNA quantitation

cDNA	Forward primer	Reverse primer	PCR program
β-Actin	5′-CTGACCGAGCTGGCTAC	5′-CCTGCTTGCTGATCCACA	94/30/55/30/72/45/22
Catalase	5′-GGCAGCTATGTGAGAGCC	5′-CTGACGTCCACCCTGACT	94/30/55/30/72/15/30
GSHpx	5′-CTCTCCGCGGTGGCACAGT	5′-CCACCACCGGGTCGGACATAC	94/30/60/60/72/30/32
γ-GCS	5′-ACACGGAGATCTACGAGCAGC	5′-CCACAAATACCACATAGGCAG	94/30/57/30/72/20/32
GR	5′-CTCAACACCGCCAGCGTTCTCC	5′-TCACTGCTCCGCACATCC	94/30/57/30/72/20/32
CuZn-SOD	5′-GTTCCGAGGCCGCCGCGCGT	5′-GTCCCCATATTGATGGAC	94/30/55/30/72/20/28
Mn-SOD	5′-CTGAGGAGAGCAGCGGTCGT	5′-CTTGGCCAGCGCCTCGTGGT	94/30/55/30/72/30/32

Primers were designed using the known sequences for the respective genes. Programs are given as denaturation temperature (°C)/denaturation time (s)/annealing temperature (°C)/annealing time (s)/elongation temperature (°C)/elongation time (s)/number of cycles. Additionally, all programs started with a period of 94°C for 10 min and finished with 5 extra min at 72°C. For example, the program for β-actin was 94°C for 10 min followed by 22 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 45 s, and ending with 5 min at 72°C.

washed twice in 75% ethanol, dried, and dissolved in distilled water. A 1.8-kb DNA fragment, reaching 1,680 bp 5′ of the start codon of the catalase gene, was PCR-amplified using the specific primers 5′-CTCGCCGCCTCCCAAGTTCTCG and 5′-CTCCACCACGTGGACCTGGGTA. The PCR program was denaturation at 94°C for 10 min, followed by 40 cycles consisting of 94°C for 30 s, 65°C for 30 s, 72°C for 60 s with a 5-s increase per cycle from cycle 15 to 40, and a final 5 min at 72°C.

The DNA sequences of the PCR products were determined using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit and catalase-specific primers in the ABI PRISM 377 DNA semiautomatic sequencer (PE Biosystems, Foster City, CA).

Statistics. Tests for significant differences were made by analysis of variance with Fisher's protected least significant difference (42) using the program Statview for Macintosh. A difference was considered significant when $P < 0.05$.

RESULTS

The mean crown-to-rump lengths were equal in HN and UN groups, whereas embryos of diabetic mothers of both substrains were smaller than embryos from controls (Table 2). The decrease in size induced by maternal diabetes was 14% in the H strain and 18% in the U strain. The mean somite number was ~25 and similar in all four groups (Table 2).

The catalase-to-actin ratio, reflecting the catalase mRNA levels, was two times as high in the HD group as in the HN group (Fig. 1). There was no difference in mRNA levels of catalase between the UN and UD groups (Fig. 1). The catalase mRNA level in the HD group was higher than in all other groups.

No differences between any of the groups were detected in the mRNA levels of GSHpx, γ-GCS, or GR (Fig. 2). The GSHpx-to-actin ratio in the HD group appeared to be numerically higher than in any of the other embryo groups, but the variations in all groups were considerable, which precluded any statistically significant difference. The pattern (Fig. 2), however, was remarkably similar to that seen for catalase expression (Fig. 1).

The CuZn-SOD mRNA levels did not display any variation in response to diabetes or between the substrains (Fig. 3). In the H strain, embryos from diabetic mothers displayed increased Mn-SOD mRNA levels compared with embryos from normal mothers; the ratios were 3.4 and 0.9, respectively. Mn-SOD expression was unchanged by maternal diabetes in embryos of the U substrain (Fig. 3).

To further investigate a possible role of the catalase gene in the process of malformation in the U strain, we determined the DNA sequence of the catalase cDNA as well as 1.3 kb of the promoter region in both H and U rats. No differences were found between H and U in the coding region. In the 3′ untranslated region 178 bp downstream of the translation stop codon, however, an A-to-G substitution was found in the U strain (Fig. 4). The DNA sequence upstream of the catalase transcription start site revealed no differences between H and U, except for a heterozygosity in the H strain. In one of the H alleles, a deletion of two nucleotides (AA) was observed in positions –910 and –911 in relation to the start codon (Fig. 4).

DISCUSSION

The main finding of this study was that maternal diabetes causes an increase in the mRNA levels of the radical scavenging enzymes catalase and Mn-SOD in embryos from the malformation resistant rat strain (H), while there is no such increased expression in embryos of a malformation-prone strain (U). The catalase result for the U embryos is in line with earlier studies showing no differences in mRNA levels between embryos of normal and diabetic U rats (43). Interestingly, in the previous study, Mn-SOD tended to be increased in embryos of diabetic rats (43), similar to the result in the present investigation. It may be speculated, therefore, that maternal diabetes could induce a small increase in U-strain mRNA levels of Mn-SOD. Such an increase would escape detection due to small magnitude and methodologic imperfection, and, at any rate, be of less functional importance than the clear-cut response to maternal diabetes in the mRNA levels of Mn-SOD in H embryos. In addition, we did not detect any differences between the H and U strains in the mRNA levels of either CuZn-SOD or the three GSH-metabolizing enzymes.

TABLE 2
Crown-to-rump lengths and somite numbers

Group	*n*	Crown-to-rump length (mm)	Somite number
HN	10	3.94 ± 0.21	25.4 ± 0.58
HD	18	3.38 ± 0.14*	26.2 ± 0.79
UN	16	3.83 ± 0.10†	24.9 ± 0.49
UD	12	3.14 ± 0.11*‡	24.3 ± 0.73

Data are means ± SE. Significant difference versus *HN, †HD, and ‡UN.

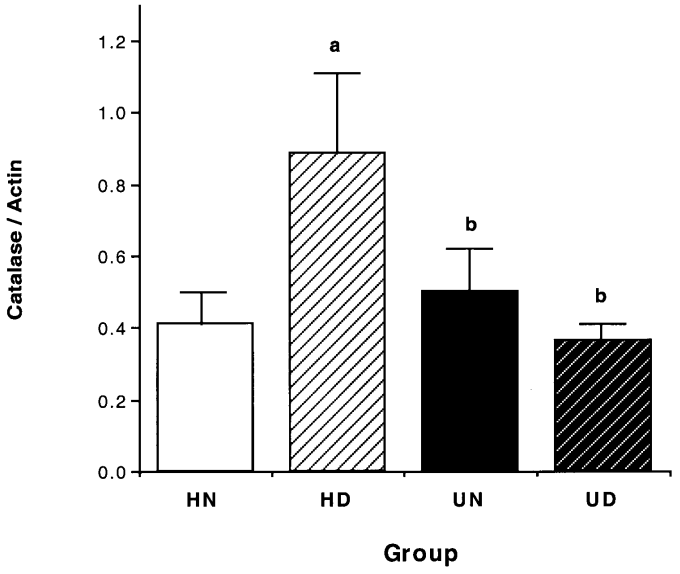

FIG. 1. The catalase expression in all groups expressed as mean ratios of catalase/β-actin mRNA (± SE); n = 9–10. a, significant difference versus HN; b, significant difference versus HD; c, significant difference versus UN.

Another finding was that a diabetic environment hampers embryonic growth, measured as crown-to-rump length. This observation is in line with earlier studies showing decreased inner cell mass in blastocysts from diabetic rats (44). The crown-to-rump length is likely a reflection of a general environmental stress factor during embryonic growth, whereas the fetal malformation difference between H and U strains relates to specific genetic susceptibilities expressed under diabetic conditions. The somite number is approximately the same in all the groups. Because this parameter is a measure of the developmental stage of the embryo, we can conclude that our observations were performed at the same developmental stage in all four groups—an important consideration, since the pattern of gene expression varies between different developmental stages. The findings suggest that embryonic growth is hampered in maternal diabetes by a process different from that causing malformations, and that the developmental stage of the embryos in this study is largely unaffected by maternal diabetes.

It has been shown that catalase activities from H and U rats behave differently in zymograms (40). We wanted to find out if this difference was due to a structural difference in the catalase gene. We discovered that there is a base pair exchange in the 3′ untranslated region of the catalase cDNA in the malformation-prone U strain compared with the H strain and the sequence shown by Furuta et al. (45). The relevance of this finding is not clear, but the change could affect mRNA stability or processing. In addition, embryos of the H and U strains also respond differently to maternal diabetes with respect to catalase activity (39). We therefore sequenced the catalase promoter region, since the differences in embryonic catalase activity and effect of maternal diabetes could be due to promoter sequence differences. The rat catalase promoter is a TATA-less promoter containing three CCAAT boxes and contains at least eight transcription starting points (46). The 5′-flanking region of catalase has different regulatory effects on reporter genes in dif-

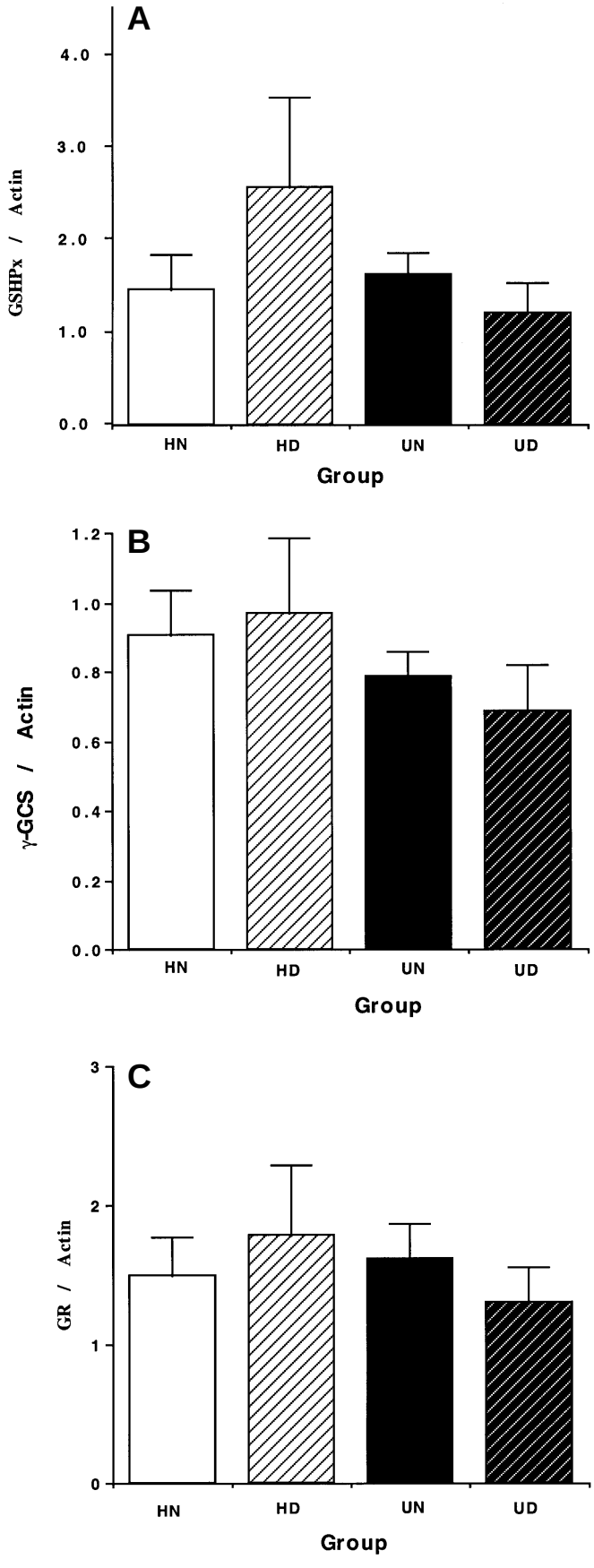

FIG. 2. The mean ratios (± SE) of GSHPx mRNA (A), γ-GCS (B), and GR (C) to β-actin; n = 8–10. No significant differences were found.

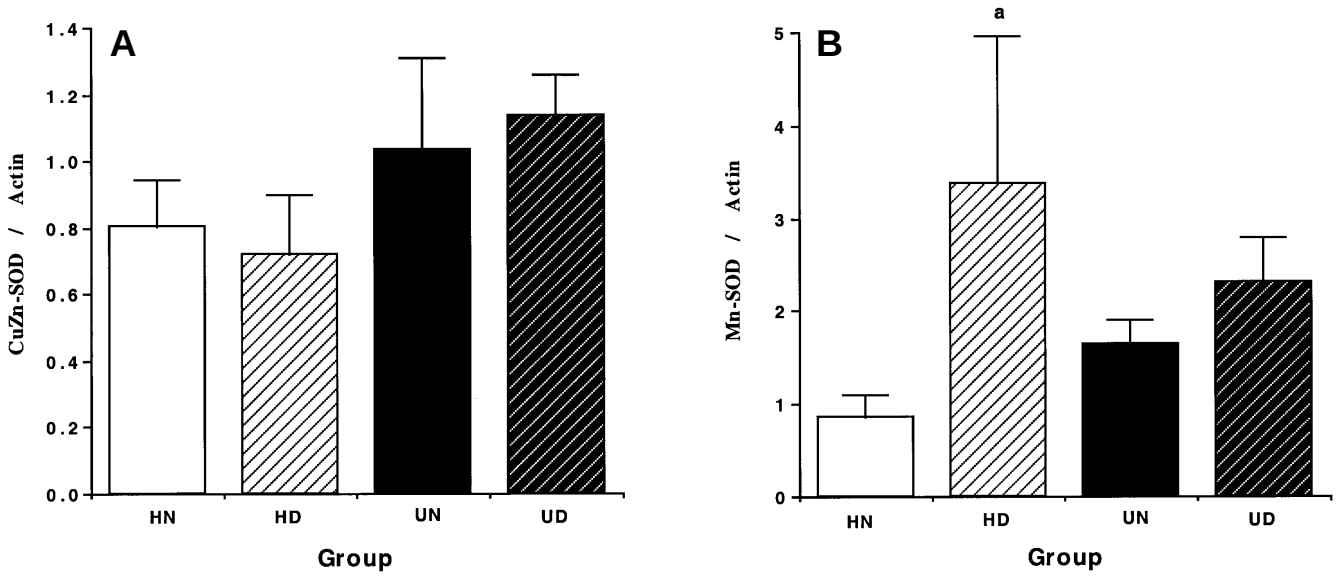

FIG. 3. The ratio (± SE) of CuZn (*A*) and Mn-SOD (*B*) to β-actin mRNA; *n* = 8–10. a, significant difference versus HN; b, significant difference versus HD; c, significant difference versus UN.

ferent cell types in cell-line experiments (47). We did not find any difference in DNA sequence of the promoter region between H and U strains, apart from a heterozygosity in the H strain that is not likely to affect expression. This finding may indicate that the differences in catalase activity and mRNA levels between H and U embryos are due to differences in the transcriptional regulation rather than the genes and promoters per se. This conjecture supports the notion that the difference in catalase activity is not the primary lesion leading to malformations; rather, it is a reflection of differences between the H and U strains in regulation of gene expression.

Diabetes is known to induce the production of free radicals in rats (48). It is also known that the addition of free radical scavengers in vitro (16) and in vivo (25,31) can reduce maldevelopment in rat embryos cultured in high glucose concentrations and in embryos from diabetic mothers. The mechanism and location of radical production are still disputed; however, a probable site of production is the mitochondrion (49). It can be speculated that radicals are formed within the embryonic mitochondria, since they are subjected to substrate overload in the diabetic environment. In favor of this hypothesis are the facts that diabetes induces increased uterine blood flow in early rat pregnancy (50), GLUT-1 is not

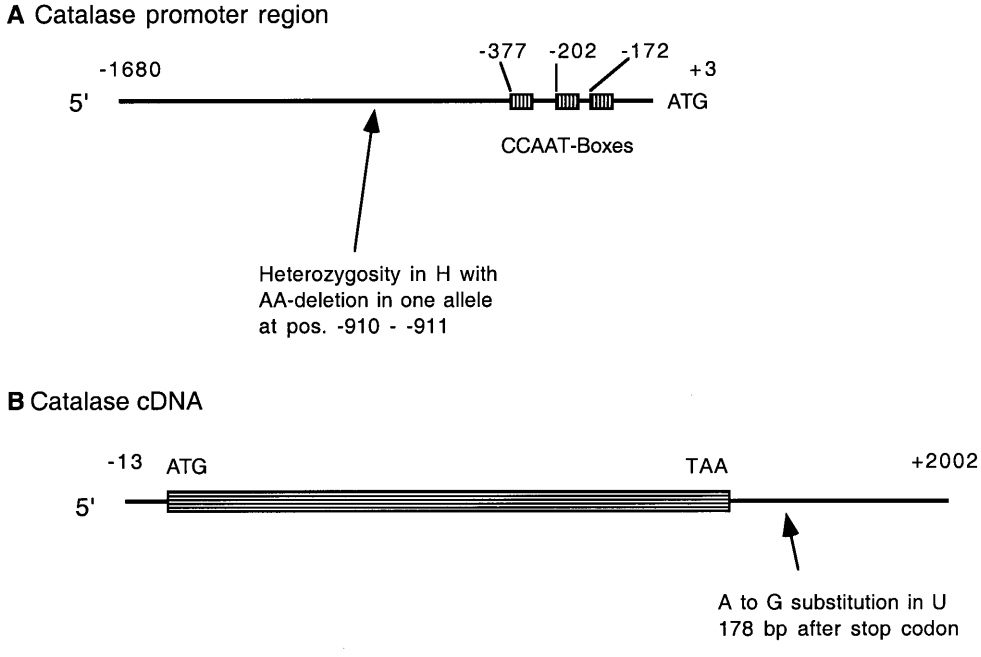

FIG. 4. A schematic figure of the rat catalase promoter region (*A*) and cDNA (*B*), outlining the differences in DNA sequence between the H and U strains.

downregulated in embryonic tissues despite exposure to a diabetic environment (51), and pyruvate uptake inhibitors decrease glucose-induced malformations in vitro (17). Embryonic rat mitochondria also show high-amplitude swelling in diabetic pregnancy, a phenomenon prevented by maternal antioxidant treatment (52). Also, more superoxide is produced from the neuroepithelium of rat embryos when cultured in high glucose than in normal glucose concentrations, a finding paralleled by inhibition of oxygen uptake and increased glucose utilization (26). Earlier studies have shown both increased malformation rate and low catalase activity in our malformation-prone U substrain (39). The catalase mRNA results in the present study display the same pattern as the previous activity data; that is, the U embryos tend to have less protection against hydroxyl radical production when the mother is diabetic, whereas maternal diabetes seems to have the opposite effect on the H embryos. Apart from catalase, Mn-SOD expression is also increased in HD embryos. Hence, these embryos most likely have an increased protection against both superoxide ions and hydrogen peroxide and subsequently lower hydroxyl radical formation. One recent study has shown a correlation between low activities of SOD and catalase and neural tube defects in rat embryos; however, the authors did not find any correlation with diabetes (19). Mn-SOD is the mitochondrial form of SOD, and the mRNA increase is in line with the notion that the mitochondria are the main sites of free radical production in embryos exposed to maternal diabetes. It is known, however, that overexpression of CuZn-SOD in mice protects embryos from malformation in a diabetic environment (53), which may be due to a large surplus of cytosolic SOD. None of the investigated enzymes involved in GSH metabolism were affected by maternal diabetes, which may indicate that the GSH system is mainly reducing basal levels of hydrogen peroxide, whereas catalase would be more inducible by high amounts of oxygen radicals. This idea is in line with indications from RNA and enzyme activity studies that GSHpx is more important than catalase under normal conditions in mouse embryos (54). In embryos exposed to high glucose in vitro, Trocino et al. (27) found decreased expression of γ-GCS, a finding we were unable to repeat in embryos of diabetic rats. The reasons for this discrepancy are not completely clear but may be related to the use of different techniques for measuring mRNA and differences in the experimental conditions, such as the use of different rat strains.

One possible way to interpret the role of free oxygen radicals in diabetic embryopathy is that the radicals could induce apoptosis in the embryos of diabetic mothers, resulting in too few cells to produce a well-formed embryo (13). Apoptosis has recently been shown to be associated with the development of neural tube defects in embryos of diabetic mice (55). It has also been shown that oxidative stress induces apoptosis in cultured neurons from rat fetuses (56). The neural crest cells have been proposed to be a prime target of oxygen radicals in diabetic pregnancy (57), a hypothesis supported by the facts that tissues displaying malformations are those that are neural crest–derived (58) and that the migratory capacity of neural crest cells is reduced by high glucose in vitro (59).

Mn-SOD and catalase enzymes are thus likely to be involved in the protection of embryos against diabetes-induced malformations. An impaired response to high levels of free radicals in the expression of these enzymes may therefore cause increased embryonic vulnerability to maternal diabetes.

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