LOW ANTIOXIDANT ENZYME GENE EXPRESSIO IN PANCREATIC ISLETS COMPARED WITH VARIOUS OTHER MOUSE TISSUES

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Abstract—Using a sensitive Northern blot hybridization technique, gene expression of superoxide dismutase (SOD), catalase, and glutathione peroxidase was studied in pancreatic islets and for comparison in various other mouse tissues (liver, kidney, brain, lung, skeletal muscle, heart muscle, adrenal gland, and pituitary gland). Gene expression of the antioxidant enzymes was usually in the range of ±50% of that in the liver. Only in pancreatic islets gene expression was substantially lower. The levels of the cytoplasmic Cu/Zn SOD and the mitochondrial Mn SOD gene expression were in the range of 30–40% of those in the liver. Glutathione peroxidase gene expression was 15%, and catalase gene expression was not at all detectable in pancreatic islets. These low levels of antioxidant enzyme gene expression may provide an explanation for the extraordinary sensitivity of pancreatic beta cells towards cytotoxic damage by diabetogenic compounds and during the development of human and animal diabetes.

Keywords—SOD, Catalase, Glutathione peroxidase, Gene expression, Mouse tissues, Pancreatic islets, Free radicals

INTRODUCTION

Environmental and chemical agents can cause cell damage through generation of reactive oxygen species. Free oxygen radicals may participate in the pathogenesis of many diseases including inflammatory and autoimmune diseases, reperfusion injury, cancer, and diabetes mellitus.1,2 Superoxide dismutase in cytosol (Cu/Zn containing isoform) and mitochondria (Mn containing isoform) converts the superoxide anion into hydrogen peroxide, which is removed by catalase and glutathione peroxidase.1,2 Damage through reactive oxygen species is determined not only by the generation of free oxygen radicals but also by the antioxidant defense status of the cell. This is why different organs can exhibit considerable differences in their susceptibility towards cytotoxic damage. However, enzyme activity measurements may give rise to misinterpretations because small compounds with enzyme-like activity may disturb the measurements, and erythrocytes in the tissues can contain large amounts of antioxidant enzymes.3 As erythrocytes do not contain ribonucleic acids, this is not a problem in gene expression studies. Because cDNAs coding for superoxide dismutase, catalase, and glutathione peroxidase are now available, we have studied their gene expression in various mouse tissues.

MATERIALS AND METHODS

Materials

Restriction enzymes, the SP6/T7 Transcription Kit, and the DIG Nucleic Acid Detection Kit were obtained from Boehringer (Mannheim, Germany). Hybond N hybridization transfer membranes were from Amersham (Braunschweig, Germany). Guanidine thiocyanate was purchased from Fluka (Neu-Ulm, Germany). All other reagents of analytical grade were from Merck (Darmstadt, Germany). The cDNAs coding for rat cytoplasmic Cu/Zn superoxide dismutase, rat mitochondrial Mn superoxide dismutase and rat glutathione per-
Table 1. Cu/Zn and Mn Superoxide Dismutase (SOD) Gene Expression in Various Albino Mouse Tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cu/Zn SOD (% of liver)</th>
<th>Mn SOD (% of liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>100 ± 7</td>
<td>100 ± 17</td>
</tr>
<tr>
<td>Kidney</td>
<td>99 ± 7</td>
<td>125 ± 19</td>
</tr>
<tr>
<td>Brain</td>
<td>77 ± 8</td>
<td>67 ± 16</td>
</tr>
<tr>
<td>Lung</td>
<td>80 ± 12</td>
<td>66 ± 17</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>59 ± 7</td>
<td>95 ± 14</td>
</tr>
<tr>
<td>Heart muscle</td>
<td>70 ± 10</td>
<td>142 ± 9</td>
</tr>
<tr>
<td>Pituitary gland</td>
<td>79 ± 19</td>
<td>47 ± 11</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>175 ± 16</td>
<td>239 ± 25</td>
</tr>
<tr>
<td>Pancreatic islets</td>
<td>38 ± 9</td>
<td>30 ± 5</td>
</tr>
</tbody>
</table>

RNA (5 µg) from each tissue was hybridized with antisense cRNA probes coding for rat cytoplasmic Cu/Zn superoxide dismutase and rat mitochondrial Mn superoxide dismutase. Values are presented as means ± SEM and expressed as % of the liver gene expression. The number of experiments was four to seven for each tissue.

oxidase were generously provided by Dr. Ye-Shih Ho (Detroit, MI). The human catalase cDNA was obtained from the American Tissue Culture Collection (ATCC) (Rockville, MD).

Animals and tissue isolation

Fed female albino (NMRI) mice (30–35 g b.wt.) and fed ob/ob mice of either sex (40–50 g b.wt.) were sacrificed by decapitation. After isolation tissues were washed in ice-cold phosphate-buffered saline and homogenized in a precooled buffered 4 M guanidine thiocyanate solution.

Northern blot experiments

Two hundred pancreatic islets isolated by collagenase digestion were homogenized in 300 µl precooled buffered 4 M guanidine thiocyanate solution. The other tissues were homogenized in the same solution. Total RNA was isolated by a combined water saturated phenol-chloroform-isoamyl alcohol extraction according to Chomczynski and Sacchi with an addition of ultrapure glycogen to achieve full precipitation of islet RNA. Five micrograms total RNA per lane were subjected to electrophoresis on denaturing formamide/formaldehyde 1% agarose gels and transferred to nylon membranes. The cDNAs coding for rat cytoplasmic Cu/Zn superoxide dismutase, rat mitochondrial Mn superoxide dismutase, rat glutathione peroxidase, and human catalase were subcloned in the pBluescript SK(+) vector (Stratagene, La Jolla, CA) for the generation of cRNA probes. Hybridization was performed at 68°C overnight in a solution containing 50% deionized formamide, 5 × SSPE (SSPE = 180 mmol/l sodium chloride, 1 mmol/l EDTA, and 10 mmol/l NaH2PO4, pH 7.4), 10 × Denhardt’s solution, 0.5% SDS, 100 µg/ml sonicated nonhomologous DNA from herring sperm and 11-DIG-UTP-labeled antisense cRNA probes. The DIG-labeled hybrids were detected by an enzyme-linked immunossay using an anti DIG-alkaline-phosphatase antibody conjugate. The subsequent enzyme-catalyzed chemiluminescence detection with the substrate AMPPD visualized the hybrids on a lightsensitive film for quantification by densitometry with the NIH Image 1.52 program (Bethesda, MD). Ribosomal bands were used as control markers for gel loading.

Statistical analyses

The experimental data are expressed as mean values ± SEM. Statistical analyses were performed using Student’s t-test.

RESULTS AND DISCUSSION

The gene expression of the antioxidant enzymes superoxide dismutase (SOD), catalase, and glutathione peroxidase was studied in liver, kidney, brain, lung, skeletal muscle, heart muscle, adrenal gland, pituitary gland, and pancreatic islets from albino mice. Only in pancreatic islets very low levels of gene expression of all antioxidant enzymes were observed. In all other tissues the level of gene expression of the cytoplasmic Cu/Zn SOD isoenzyme and the mitochondrial Mn SOD

Table 2. Catalase and Glutathione Peroxidase Gene Expression in Various Albino Mouse Tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Catalase (% of liver)</th>
<th>Glutathione Peroxidase (% of liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>100 ± 10</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>Kidney</td>
<td>78 ± 8</td>
<td>91 ± 9</td>
</tr>
<tr>
<td>Brain</td>
<td>36 ± 10</td>
<td>39 ± 8</td>
</tr>
<tr>
<td>Lung</td>
<td>50 ± 10</td>
<td>58 ± 9</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>41 ± 12</td>
<td>40 ± 10</td>
</tr>
<tr>
<td>Heart muscle</td>
<td>72 ± 11</td>
<td>39 ± 7</td>
</tr>
<tr>
<td>Pituitary gland</td>
<td>23 ± 2</td>
<td>66 ± 11</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>45 ± 7</td>
<td>77 ± 12</td>
</tr>
<tr>
<td>Pancreatic islets</td>
<td>n.d.</td>
<td>15 ± 6</td>
</tr>
</tbody>
</table>

RNA (5 µg) from each tissue was hybridized with antisense cRNA probes coding for human catalase and rat glutathione peroxidase. Values are presented as means ± SEM and expressed as % of the liver gene expression. The number of experiments was four to seven for each tissue.

n.d. = not detectable.
isoenzyme (Table 1) as well as of catalase and glutathione peroxidase (Table 2) was in the range of ± 50% compared with liver values. In all tissues the Cu/Zn SOD isoenzyme gene was expressed as a single 0.7 kb mRNA transcript, while the Mn SOD isoenzyme gene was expressed in two major forms, a 1.2 and a 4.2 kb mRNA transcript (Fig. 1). The level of gene expression in albino mouse pancreatic islets of the Cu/Zn SOD isoenzyme was 38%, and of the Mn SOD isoenzyme 30% of that in the liver (Table 1). The catalase gene was expressed as a 2.4 kb mRNA transcript in all albino mouse tissues studied with the exception of the pancreatic islets, where catalase gene expression was not detectable (Fig. 2 and Table 2).

The glutathione peroxidase gene was expressed in all albino mouse tissues as a 1.2 kb mRNA transcript (Fig. 2 and Table 2), with 15% the lowest level of gene expression was detected in pancreatic islets (Table 2).

Some of the earlier studies on antioxidant enzyme activities have been performed in pancreatic islets from ob/ob mice that contain more than 90% beta cells. Pancreatic islets from albino mice have only 70–80% beta cells, the remainder representing mainly alpha and delta cells. Therefore, we have analyzed the gene expression of these antioxidant enzymes also in pancreatic islets isolated from ob/ob mice. The level of the Cu/Zn SOD isoenzyme gene expression in ob/ob mouse pancreatic islets was 32 ± 11% (n = 4) of the expression in ob/ob mouse liver. That is similar to the level detected in albino mouse islets (Table 2). The Mn SOD isoenzyme gene expression in ob/ob mouse pancreatic islets was 10 ± 2% (n = 4), which is significantly lower (p < 0.05) than in albino mouse islets (Table 2). Catalase gene expression, like in albino mouse islets, was undetectable in ob/ob mouse pancreatic islets. In contrast to albino mouse islets, glutathione peroxidase gene expression was also not detectable by Northern blot hybridization of ob/ob mouse pancreatic islets. Thus, in pancreatic islets from ob/ob mice that contain fewer nonbeta cells the overall level of antioxidant enzyme gene expression was even lower than in albino mouse islets. Thus, pancreatic islets exhibit a far lower level of antioxidant enzyme gene expression than other tissues, including both endocrine and nonendocrine tissues.

This is consistent with well established evidence from several previous studies that the activities of antioxidant enzymes are very low in pancreatic islets. For example, in pancreatic islets SOD isoenzyme activities were around one-third compared with liver, and

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**Fig. 1.** Northern blot analysis of Cu/Zn and Mn superoxide dismutase (SOD) gene expression in liver (L), kidney (K), and pancreatic islets (I) from albino mice. Five micrograms total RNA were loaded per lane. mRNA levels were related to ribosomal bands and visualized in an ethidium bromide stained agarose gel. Blots were probed with antisense cRNAs coding for rat cytoplasmic Cu/Zn superoxide dismutase (left panel) and rat mitochondrial Mn superoxide dismutase (right panel) by nonradioactive hybridization. Shown are representative blots of at least four independent experiments.

**Fig. 2.** Northern blot analysis of glutathione peroxidase and catalase gene expression in liver (L), kidney (K), and pancreatic islets (I) from albino mice. Five micrograms total RNA were loaded per lane. mRNA levels were related to ribosomal bands and visualized in an ethidium bromide-stained agarose gel. Blots were probed with antisense cRNAs coding for rat glutathione peroxidase (left panel) and human catalase (right panel) by nonradioactive hybridization. Shown are representative blots of at least four independent experiments.
catalase and glutathione peroxidase activities were negligible. These reductions of enzyme activities are in the same range as those of gene expression noted in the present study. Although none of the organs other than the pancreatic islets showed very low levels of antioxidant enzyme gene expression in the present study (Tables 1 and 2), a number of other tissues, in particular, skeletal muscle, heart muscle, and brain, exhibited enzyme activities that were as low or even lower than those in pancreatic islets. This may be an indication for differences in posttranscriptional modifications in the various tissues. It may also reflect differences in tissue contamination with antioxidant enzyme activities originating from erythrocytes, and a variable degree of nonspecific enzyme-like activities in the tissues.

The low levels of superoxide dismutase, catalase, and glutathione peroxidase gene expression may be responsible for the extraordinary sensitivity of pancreatic beta cells towards oxidative stress in human and animal diabetes. They can also explain the selective destruction of pancreatic beta cells by alloxan (for review, see refs. 12, 13) as a result of this low antioxidant defense status of the pancreatic beta cell and the protective effect of superoxide dismutase and catalase against the toxic action of alloxan.

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REFERENCES