The Inhibition of Islet Superoxide Dismutase by Diabetogenic Drugs

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SUMMARY

The enzyme superoxide dismutase (SOD) is a scavenger of superoxide radicals and protects the integrity of cell membranes. We previously reported that streptozotocin inhibits the SOD activity of erythrocytes and retinae in vivo and in vitro. We now report that the three major diabetogenic drugs, i.e., alloxan, streptozotocin, and Vacor (in order of increasing potency), interact with erythrocyte Cu-Zn SOD in vitro. Maximum inhibition of erythrocyte SOD of man, dog, rat, and cow is 40% and is achieved within 10 min. At submaximal doses the effect of the three drugs is additive. Inhibition induced by streptozotocin, but not by the other two agents, is reversible by dialysis. Alloxan (80 mg/kg), like streptozotocin, also inhibits erythrocyte SOD activity when injected i.v. into rats. Glucose or 3-o-methylglucose does not prevent SOD inhibition by alloxan or streptozotocin in vitro. Injection of glucose before alloxan prevents the development of diabetes, but does not prevent alloxan-induced inhibition of erythrocyte SOD.

SOD is present in the islets of Langerhans of rats and dogs, as demonstrated by biochemical assay of isolated islets and by immunofluorescent staining of frozen pancreases. The specific activity of SOD in the islets exceeds that of the exocrine pancreas more than 100-fold. The islet SOD is of the Cu-Zn type, since it is inhibited by KCN, and not by chloroform-ethanol. The mobility of the islet enzyme on polyacrylamide disc gel electrophoresis is different from that of erythrocyte SOD. Streptozotocin, alloxan, and Vacor inhibit the activity of islet SOD in vitro; the rate and magnitude of inhibition is the same as that observed with erythrocyte SOD. The Mn SOD of liver mitochondria is not affected by the diabetogenic drugs. It is suggested that the inhibitory effect of the diabetogenic drugs on islet cell SOD may contribute to their cytotoxicity, and that changes in the amount or activity of this protective enzyme in β-cells may play a role in determining their vulnerability to noxious agents.


Streptozotocin and alloxan are widely used to produce diabetes mellitus in experimental animals. The mode of action of these drugs is incompletely understood; however, they destroy pancreatic β-cells with relative selectivity. Recently, another agent, Vacor, has been reported to produce diabetes in factory workers who contacted and/or accidentally ingested this commercial rodenticide. 1

The enzyme superoxide dismutase (Superoxide oxidoreductase, EC1.15.1.1) is a scavenger of the superoxide radical, a toxic species that has been implicated in lipid oxidation, DNA damage, and protein sulfhydryl oxidation (for review, see ref. 2). Eukaryotic cells contain two types of superoxide dismutase (SOD), one containing manganese (Mn SOD) located in the mitochondria and a copper-zinc protein (Cu-Zn SOD) located in the cytoplasm.

We reported that the SOD activity of rat retinae and erythrocytes is decreased by 30–40% from control levels within 3 h after i.v. injection of a diabetogenic dose of streptozotocin. 3 The action of streptozotocin on this enzyme appears to be somewhat tissue specific, as no change in SOD activity was observed in homogenates of lung, liver, brain, aorta, kidney, and lens from the same animals. In vitro, incubation of retinae, erythrocytes, or pure erythrocyte SOD with streptozotocin resulted in inhibition of the enzyme activity. 3 In this paper we report the extension of this research to alloxan-SOD and Vacor-SOD interactions, the localization of SOD in the islets of Langerhans, and inhibition of islet SOD by diabetogenic drugs in vitro.

MATERIALS AND METHODS

Reagents. Purified bovine erythrocyte SOD was obtained from Truette Labs (Houston, Texas); canine erythrocyte...
SOD, alloxan monohydrate, nitroblue tetrazolium, and collagene (type V) was obtained from Sigma (St. Louis, Missouri). Streptozotocin was kindly supplied by Dr. William Dulin (Upjohn Research Laboratories, Kalamazoo, Michigan). Vacor (N-3-pyridylmethyl-N′-p-nitrophenyl urea) was the generous gift of Rohm and Haas Company (Philadelphia, Pennsylvania). Alloxan, Vacor, and streptozotocin were solubilized immediately before use.

In vivo studies. Male Wistar rats weighing 125–160 g were fasted for 24 h before injection and then fed ad libitum until killing. The animals were anesthetized with ether and injected via the femoral vein. Alloxan (80 mg/kg body wt) was administered as a solution (50 mg/ml) in 0.3 M sodium phosphate buffer, pH 7.4, with a 0.5-ml saline push. In some studies, D-glucose (2 g/kg body wt, 250 ml/kg) was injected immediately before the alloxan injection to protect the β-cells. Control animals were injected with the same volume of diluent/kg body wt. The animals were killed 24–48 h after injection by decapitation.

Blood samples (0.2 ml) were obtained by cardiac puncture before injection and at various intervals after injection. Heparinized (0.1 ml) aliquots of blood were diluted 1:10 with distilled water, centrifuged (500 × g at 4°C), and the supernatants assayed for SOD activity (see below). Plasma glucose was measured by the glucose-oxidase-O2 analyzer method (Beckman, Palo Alto, California).

Isolation of pancreatic islets of Langerhans. The collagenase digestion procedure of Lacy and Kostianovsky was applied to pancreases obtained from freshly killed dogs or rats. The pancreatic tissues were suspended in buffered Hanks' solution, dissected free of extraneous fat or lymph nodes, and chopped into very fine pieces with scissors. The suspension was drawn into a graduated, stubborned, transfer pipette in which it was allowed to sediment to determine the tissue volume. The tissue was incubated in 5 vol of Hanks' solution containing 1 mg/ml collagenase at 37°C, with vigorous shaking for 15–20 min. The end point of the digestion was assessed visually by the "creamed coffee" appearance of the suspension. After centrifugation (500 × g; 15 min) the pellet was washed twice with Hanks' solution, resuspended in 0.5 ml Hanks' solution in a Petri dish, and the islets were transferred with a Pasteur pipet under a dissecting microscope at 7–10×. The identity of the islets was established by histologic examination of tissues fixed with Bouin's fixative and stained with chromium hematoxylin-phloxine.

Liver mitochondria. Mitochondria were isolated from rat liver homogenates by differential centrifugation according to the method of Schnaitman and Greenwald.

In vitro studies. Heparinized blood samples (0.1 ml) were diluted with 0.3 M sodium phosphate buffer (1 ml, pH 7.4) and incubated with and without alloxan and streptozotocin (0.2–10 mg) for 5–60 min at 37°C. In some studies, lysed blood (diluted with distilled water, 1:5) was used, which was further diluted with sodium phosphate buffer to 0.3 M, pH 7.4.

Isolated islets and small pieces of pancreas or liver mitochondria were suspended in ice-cold 0.3 M sodium phosphate buffer, pH 7.4, sonicated at 4°C for 10 s, and centrifuged (4°C at 500 × g) for 10 min. The supernatant was lyophilized and reconstituted (extract of 10 mg tissue/ml) before incubation, as above, with and without alloxan or streptozotocin (8 mg/ml). In some experiments, commercially obtained purified bovine or canine erythrocyte SOD (20 µg/ml) was incubated with the diabeticogenic drugs.

In some studies, blood samples were incubated with D-glucose (0.1–10 g/dl) for 30 min before the addition of alloxan, or with 3-O-methylglucose (0.1–10 g/dl) before the addition of streptozotocin.

Blood samples, liver mitochondria, and lyophilized extracts of pancreas and islets were stored frozen (−70°C) until assay of SOD activity. In some studies of purified erythrocyte SOD, after exposure to the diabeticogenic drugs, the samples (2-ml aliquots) were dialyzed against 2 L of sodium phosphate buffer with two buffer changes at 4°C for 24 h.

SOD activity assay. The blood samples were diluted 1:5 with sodium phosphate buffer (0.3 M, pH 7.4). The lyophilized, soluble proteins from whole pancreas or islet extracts were dissolved, as above, in the same buffer. Protein concentration was determined by the method of Bradford. The SOD activity measurement routinely employed in this study is based on the ability of the enzyme to inhibit the reduction of nitroblue tetrazolium by superoxide radicals, which are generated with xanthine oxidase as described by Beaufay and Fridovich. The assay was performed in 0.5 M sodium carbonate, pH 10.2, with 0.1 M EDTA. The reduction of tetrazolium was measured spectrophotometrically at 560 nm. Unless otherwise stated, the results reported herein were obtained using this assay procedure. Two other assays of SOD activity were used to confirm the observed inhibitory effects of diabeticogenic drugs. One is based on the ability of the enzyme to inhibit the reduction of cytochrome c by superoxide radicals generated by the xanthine–xanthine-oxidase system, as described by McCord and Fridovich, the other assay is based on the ability of the enzyme to inhibit the auto-oxidation of pyrogallol as described by Marklund and Marklund. Cyanide inhibition was measured by the addition of KCN (10 mM) to the assay mixture. Stability to chloroform–ethanol was determined by mixing equal volumes of the homogenate supernatant with chloroform–ethanol (5:3) for 5 min, followed by assay of the aqueous layer.

Gels. Disc electrophoresis was performed in polyacrylamide gels (7.5 g/dl) using the system described by Maurer, except that stacking gels were not employed. Samples (25–50 µl) were mixed with a crystal of bromphenol blue and sucrose (10 mg/ml). The gels were stained for SOD at 37°C for 1 h as previously reported. Absence of uptake indicated reaction of the stain with the enzyme. The gels were stained for protein with Coomassie brilliant blue according to the method of Weber and Osborn.
ined for fluorescence using a fluorescence microscope attached to a silicon intensification target camera, as described by Willingham and Pastan.14

The RITC conjugate was obtained from Cappel Laboratories (Cochranville, Pennsylvania). The specificity of this antiserum was confirmed by immunodiffusion and immunoelectrophoresis against rabbit serum. Antiserum to canine SOD was raised in rabbits by repeated injection of 0.5 mg purified antigen in normal saline (Canine erythrocyte SOD, Sigma Chemical, St. Louis, Missouri). Three intramuscular injections of antigen emulsified with Freund's complete adjuvant were given at 15-day intervals, after which three further injections of SOD without adjuvant were administered at weekly intervals. The animals were exsanguinated 1 wk after the sixth injection. The purity of the antigenic preparation was confirmed by 7.5% acrylamide gel electrophoresis, and specificity of the antiserum obtained was established immunochromically, as described above. Experimental controls for unwanted fluorescence included substitution of PBS (conjugate controls) and normal nonimmune rabbit serum (serum controls) for the first antiserum layer.

Conjugate controls were negative throughout. Serum controls initially showed unacceptable unwanted fluorescence of the entire tissue. This was effectively abolished by prior adsorption of the nonimmune rabbit serum with normal dog serum in solid phase.15 The rabbit and dog antiserum were, therefore, similarly adsorbed for use in subsequent experiments.

RESULTS
In vitro incubation of whole blood or lysed blood (rat, dog, or human) with each of the three agents (alloxan, streptozotocin, or Vacor) resulted in a 40% decline in SOD activity. Pure bovine erythrocyte SOD (Cu-Zn SOD) was affected to the same degree, as determined by three different assays of SOD activity (Table 1). The Mn SOD of liver mitochondria was not affected by incubation with any of the three drugs (Table 1). The relative effectiveness of the three drugs as in vitro inhibitors of erythrocyte SOD was: Vacor > streptozotocin > alloxan (Figure 1). The molar ratio required for half-maximal inhibition was 1:4:10. When submaximal doses of any drug were used, addition of other drug(s) resulted in an additive response, which, however, did not exceed 40% inhibition of enzyme activity either with increased quantities of a single drug or with combinations of drugs (Table 2). The time course of inhibition of pure erythrocyte SOD by each agent was similar and was maximal within 10 min at high drug concentrations (Figure 2).

Activity was completely restored to the streptozotocin-inhibited enzyme after dialysis. Furthermore, the same degree of inhibition was again observed when any of the three drugs was added to the dialyzed enzyme. However, extensive dialysis failed to restore activity to the SOD enzyme after inhibition by either alloxan or Vacor (Table 3). Administration of 3-O-methylglucose has been reported to prevent the diabetogenic effect of streptozotocin. Preincubation of the pure enzyme with 3-O-methylglucosac had no effect on inhibition of the enzyme activity by

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Species</th>
<th>Streptozotocin</th>
<th>Alloxan</th>
<th>Vacor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>Man</td>
<td>33 ± 3</td>
<td>38 ± 5</td>
<td>37 ± 3</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>34 ± 4</td>
<td>35 ± 4</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>Lysed blood</td>
<td>Man</td>
<td>31 ± 5</td>
<td>32 ± 4</td>
<td>34 ± 5</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>35 ± 3</td>
<td>35 ± 3</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>Purified erythrocyte enzyme</td>
<td>Cow</td>
<td>38 ± 2</td>
<td>36 ± 2</td>
<td>35 ± 3</td>
</tr>
<tr>
<td></td>
<td>Cow*</td>
<td>37 ± 4</td>
<td>32 ± 3</td>
<td>31 ± 4</td>
</tr>
<tr>
<td></td>
<td>Cow†</td>
<td>41 ± 5</td>
<td>40 ± 6</td>
<td>42 ± 3</td>
</tr>
<tr>
<td>Islet</td>
<td>Dog</td>
<td>37 ± 8</td>
<td>39 ± 5</td>
<td>37 ± 4</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>40 ± 10</td>
<td>32 ± 3</td>
<td>35 ± 4</td>
</tr>
<tr>
<td>Liver mitochondria</td>
<td>Rat</td>
<td>2 ± 2</td>
<td>2 ± 2</td>
<td>2 ± 2</td>
</tr>
</tbody>
</table>

Tissues (5 mg islets or mitochondria, 0.1 ml blood, or 20 μg purified enzyme) were incubated with drug (0.04 M) in 1 ml sodium phosphate buffer (0.3 M, pH 7.4) at 37°C for 1 h. Mitochondria and islets were sonicated in 1 ml buffer, centrifuged, and the supernatant used for incubation. N = 3–5; means ± SEM are shown. The NBT reduction method was used for the determination of SOD activity except for *, in which the cytochrome c assay and †, in which the pyrogallol assay was used.
INHIBITION OF ISLET SOD

FIGURE 2. Time course of inhibition of erythrocyte SOD activity during incubation of canine blood (0.1 ml) with 0.4 M streptozotocin (■—■), alloxan (○—○), or Vacor (□—□), and without drug (X—X) at 37°C in 15 ml sodium phosphate buffer (0.3 M, pH 7.4); aliquots were removed at various time intervals for assay. Each point represents the mean of 3 observations ± SEM.

streptozotocin (Table 3). Similar results were obtained with intact red blood cells or lysed blood (data not shown).

Hyperglycemia prevents the diabetogenic action of alloxan in vivo. Preincubation of pure erythrocyte SOD with glucose (3 g/dl) did not affect the alloxan induced inhibition of SOD activity (Table 3). Lower or higher doses of glucose (0.1–10 g/dl) were also ineffective (data not shown).

As previously reported for the in vivo effects of streptozotocin in rats, the injection of a diabetogenic dose of alloxan (80 mg/kg, i.v.) resulted in a 40% inhibition of erythrocyte SOD, which was first observed 3 h after the injection and persisted for 72 h (Figure 3).

To determine if glucose could prevent the effect of alloxan on SOD in vivo, rats were injected i.v. with glucose (2 g/kg) before administration of alloxan (80 mg/kg). Although diabetes was prevented, erythrocyte SOD activity decreased to the same extent in alloxan-treated rats, regardless of treatment with glucose (Figure 4).

TABLE 3
Effect of dialysis and hexoses on drug inhibition of erythrocyte SOD

<table>
<thead>
<tr>
<th>Drug</th>
<th>% Inhibition of SOD activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 M Streptozotocin, followed by dialysis</td>
<td>40 ± 2</td>
</tr>
<tr>
<td>0.01 M Streptozotocin dialyzed, then dialysis</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>0.04 M Alloxan</td>
<td>36 ± 5</td>
</tr>
<tr>
<td>0.04 M Alloxan, followed by dialysis</td>
<td>36 ± 5</td>
</tr>
<tr>
<td>0.04 M Vacor</td>
<td>35 ± 3</td>
</tr>
<tr>
<td>0.04 M Vacor, followed by dialysis</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>30 mg/ml 3-o-Methylglucose + 0.01 M Streptozotocin</td>
<td>37 ± 1</td>
</tr>
<tr>
<td>30 mg/ml Glucose + 0.04 M Alloxan</td>
<td>39 ± 4</td>
</tr>
</tbody>
</table>

Purified bovine erythrocyte SOD (Sigma, 20 µg) in 1 ml sodium phosphate buffer (0.3 M, pH 7.4) was incubated with drugs for 1 h at 37°C. Hexoses, when present, were added 30 min before the incubation with the drugs. Dialysis was for 24 h at 4°C against 1000 vol phosphate buffer; N = 4; means ± SEM are shown.

Since these drugs are selectively cytotoxic to β-cells, it was important to establish the presence or absence of the Cu-Zn SOD enzyme in islets of Langerhans. The islets from both dog and rat were found to contain SOD with a specific activity exceeding that of the exocrine pancreas by more than 100-fold (Table 4). Islet extracts contained several soluble proteins but only one with SOD activity as determined by the use of a stain specific for SOD on polyacrylamide gels (Figure 5). This protein had a different mobility on 7.5% polyacrylamide gel than did erythrocyte SOD. The enzyme was completely inhibited by cyanide and stable to chloroform-ethanol (Table 4).

The SOD activity of dog and rat islet extracts was inhibited 40% in vitro by each of the three diabetogenic drugs (Table 1). The rates of inhibition were comparable with those observed with pure erythrocyte SOD (within 10 min).

FIGURE 3. Changes in erythrocyte SOD activity in control rats (○—○) and rats injected i.v. with 80 mg/kg alloxan (■—■); N = 4; brackets represent SEM. Plasma glucose levels were greater than 300 mg/dl in the alloxan treated animals after 48 h.

FIGURE 4. Changes in erythrocyte SOD activity in rats after i.v. injection of alloxan (80 mg/kg) with or without preinjection of D-glucose (2 g/kg). White bars represent untreated; cross-hatched, alloxan; and black, glucose- and alloxan-treated rats; N = 4; brackets represent SEM. *P < 0.05 as compared with control. Mean plasma glucose levels were not significantly different in the three groups 3 h after injection; at 24 and 48 h, rats treated with alloxan alone were hyperglycemic (mean plasma glucose > 400 mg/dl). These values were different (P < 0.05) from controls and from rats treated with glucose and alloxan (mean plasma glucose 130–160 mg/dl in both groups).
TABLE 4
Islet and pancreatic SOD activity

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Species</th>
<th>Treatment</th>
<th>Activity (µg SOD/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole pancreas</td>
<td>Rat</td>
<td>—</td>
<td>&lt;0.02 ± 2</td>
</tr>
<tr>
<td>Islets</td>
<td>Rat</td>
<td>0.1 M KCN</td>
<td>2.7 ± 0.5</td>
</tr>
<tr>
<td>Whole pancreas</td>
<td>Dog</td>
<td>—</td>
<td>&lt;0.03 ± 3</td>
</tr>
<tr>
<td>Islets</td>
<td>Dog</td>
<td>0.1 M KCN</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>Islets</td>
<td>Dog</td>
<td>CH₃Cl-ETOH</td>
<td>3.5 ± 0.3</td>
</tr>
</tbody>
</table>

Tissue (5 mg) was homogenized in 1 ml sodium phosphate buffer (0.3 M, pH 7.4) at 4°C, centrifuged, and the supernatant frozen at −20°C until assay. Treatment with CH₃Cl-ETOH consisted of mixing equal volumes of the homogenate supernatant with chloroform-ethanol for 5 min followed by assay of the aqueous layer. Potassium cyanide, when used, was added immediately before assay. N > 3; means ± SEM are shown.

The selective localization of SOD in dog islets was confirmed by immunofluorescence staining of frozen slices of dog pancreas with rabbit anti-dog SOD. In initial studies of frozen tissue, no fluorescence was observed. Since the low molecular weight cytosolic SOD antigen may diffuse out of the tissue during washing, further studies were performed on acetone-fixed tissues, which revealed fluorescence localized to the pancreatic islets (Figure 6). The majority of cells in each islet and all islets in each section appeared positive. While in Figure 6 staining appears to be heavier in the center of the islet, this was not a consistent observation and the opposite was also observed in other islets. Studies are in progress to refine the technique and attempt to localize the enzyme to specific islet cells, by counterstaining with antibodies to their hormone products.

DISCUSSION
The Cu-Zn SOD is significantly inhibited (40%) by three agents that manifest selective toxicity toward β-cells. The inhibition of activity was observed using three different assay methods and was therefore not artifactual. The remarkable consistency of inhibition, which in vivo and in vitro never exceeded 40%, suggests that the drugs may be acting at a specific site of the enzyme, modifying the protein and converting it to a less active form. Alternatively, the enzyme may have two or more active sites, not all of which are accessible to the diabetogenic drugs; or possibly two isoenzymes are present, but only one is susceptible to drug inhibition.

No inhibition of the Mn SOD was observed, as extracts of liver mitochondria showed no decrease in SOD activity after direct incubation with the three drugs. The Mn SOD is readily differentiated from the Cu-Zn SOD, since the former is not inhibited by cyanide but is inhibited by chloroform-ethanol; the reverse applies to the Cu-Zn enzyme. By these criteria, the SOD activity of the rat liver mitochondria preparation was confirmed as Mn SOD while the SOD activity of the islet extracts was identified as Cu-Zn SOD.

We previously reported that the inhibition of SOD by streptozotocin represents interaction of the drug with the enzyme. The inhibition of the enzyme after incubation with alloxan suggests that the in vivo SOD inhibition observed after alloxan injection is likewise due to drug-enzyme interaction. The relative rapidity of the in vitro inhibition, which was detectable within 5 min with all three agents, and the similar time course observed with the pure enzyme and with tissue
extraction of the i.v. dose with the extracellular space, but within the range of concentration expected in plasma immediately the drugs were rapidly bound to components of the cell membrane. Binding at or near the glucose receptors of β-cells has been proposed for alloxan and for streptozotocin. Rapid selective uptake of alloxan by cells, particularly the islets, has been shown by autoradiography after injection of [14C-2]-alloxan. Submaximal inhibition of erythrocyte SOD was observed in the present study in vitro with 5 mM streptozotocin or 10 mM alloxan (Figure 1), which is 3–5 times higher than expected plasma levels after equilibration of the i.v. dose with the extracellular space, but within the range of concentration expected in plasma immediately after i.v. injection. We have no information concerning the possible in vitro inhibitory effects of smaller drug doses upon prolonged incubation with the enzyme. Such studies would be difficult to interpret, in view of the known instability of the drugs in aqueous solution at neutral pH. The inhibitory effect on erythrocyte SOD is pH and temperature dependent, and is maximal in the physiologic range. The discrepancy between in vivo and in vitro doses required for maximal erythrocyte SOD inhibition could be explained if the drugs were rapidly bound to components of the cell membrane. Binding at or near the glucose receptors of β-cells has been proposed for alloxan and for streptozotocin. A direct drug-enzyme interaction would require internalization of the drug into the cytosol. There appears to be no consensus concerning the site (e.g., extracellular and/or intracellular) of the cytotoxic effects of alloxan. We cannot, however, rule out the possibility that the in vivo inhibition of SOD by the diabetogenic drugs may be at least, in part, an indirect effect.

In previous studies we were unable to detect a direct effect of the diabetic state on SOD activity, since insulin treatment did not reverse the streptozotocin-induced inhibition in red cells or in the retina. If the metabolic alterations associated with diabetes affect SOD activity, this may have to be established in a model that has not been exposed to chemical diabetogenic agents.

On a molar basis, the in vitro inhibitory activity of the agents is Vacor, streptozotocin, alloxan (in decreasing order of potency) in an approximate ratio of 10:4:1, although the same level of maximum inhibition was obtained with each drug. The complex formed between streptozotocin and SOD is reversible as the drug is removed on dialysis. Alloxan and Vacor apparently form an irreversible complex(es) with the enzyme or denature the enzyme, since the activity is not restorable with dialysis.

Cu-Zn SOD was found to be present in the islets of Langerhans in two species. The pancreas as a whole contains very little SOD activity, suggesting that the major focus of SOD in the pancreas is in the islets. This was confirmed by immunofluorescent studies, which showed staining throughout the islets. Precise localization to specific cell types will require counterstaining, but the homogeneity of staining suggests that the β-cells almost certainly contain SOD, since they constitute the majority of islet cells. The islet SOD appears to be an isoenzyme of the erythrocyte SOD, as a shorter migration distance was observed on gel electrophoresis. Numerous isoenzymes of the Cu-Zn SOD have been described in mammals with varying tissue distribution.

The superoxide anion has previously been suggested to be involved in the diabetogenic activity of alloxan, as alloxan can be recycled by reducing agents such as ascorbate to dialuric acid which, in turn, reacts spontaneously with oxygen to generate O2-. In vitro studies have shown that the addition of SOD counteracts the toxicity of alloxan against islet cells. The toxic radical in alloxan action has been proposed to be the hydroxyradical, which is generated by a metal catalyzed reaction of O2 and H2O2. SOD catalyzes the removal of O2 and thus prevents the formation of OH- and resultant β-cell damage.

Robbins et al. observed protection against streptozotocin-induced diabetes by prior administration of SOD to rats. Fischer and Hamburger reported that alloxan action is inhibited in isolated pancreatic islets by predialysis of SOD. Both of these results are consistent with the proposal that hydroxyl radicals mediate the deleterious effects of these diabetogenic drugs.

If the diabetogenic drugs indeed exert their effect via generation of O2 or OH it seems likely that concomitant inhibition of SOD (the major protective enzyme system) would potentiate their cytotoxicity. In this study, pretreatment with glucose prevented alloxan-induced diabetes but did not prevent SOD inhibition in erythrocytes. However, it is the SOD activity in the islets after alloxan injection with or without glucose that needs to be measured to strengthen the hypothesis of the role of SOD inhibition in drug-induced diabetes. Such in vivo experiments are technically difficult and have not yet been carried out successfully.

The localization of SOD in the islet of Langerhans opens the question of the possible role of this enzyme in β-cell homeostasis. The etiology of insulin-dependent diabetes mellitus is poorly understood, although genetic, infectious, immunologic, and toxic factors have been implicated. The genetic locus of Cu-Zn SOD has been identified in mouse and in man, the latter on the long arm of chromosome 21. Multiple agents, including dietary components, microbial products, hormones, drugs, and O2 have been shown to induce or inhibit the activity of this enzyme in the lung. It is tempting to speculate that genetic and/or environmental factors may regulate the amount or activity of islet SOD, and that decreased activity of this enzyme may contribute to the vulnerability of the β-cells.

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