SULPHHYDRYL PROTECTION AGAINST DEHYDROASCORBIC ACID DIABETES

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Dehydroascorbic acid has been shown to produce diabetes (1, 2). In this respect it is similar to alloxan (3). Whereas alloxan has not yet been shown to occur physiologically, dehydroascorbic acid is accepted as occurring under physiological conditions in the cells of animals (4). Therefore, it is important to study the factors that are known to affect alloxan diabetes in order to determine their effect in dehydroascorbic acid diabetes.

Naturally occurring sulphhydryl compounds, such as glutathione, are believed to exert a protective effect physiologically against diabetogenic compounds (5). Compounds such as cysteine (6), glutathione (6), and 2,3-dimercaptopropanol (BAL) (7) given before an injection of alloxan will protect against the diabetogenic effects of alloxan. When the same substances are injected after alloxan, there is no protection against diabetes. Therefore, the effects of sulphhydryl compounds on the diabetogenic action of dehydroascorbic acid were studied.

EXPERIMENTAL

Dehydroascorbic acid was prepared as previously described (2). The sulphhydryl compounds were administered as aqueous solutions of the following concentrations: cysteine (free base) 10 per cent; neutralized glutathione (GSH) 10 per cent; and 2,3-dimercaptopropanol (BAL) 0.67 per cent.

All substances were administered intravenously to male Sprague-Dawley rats weighing between 100 and 200 gm. A preliminary desensitizing dose (2) of dehydroascorbic acid (0.2 gm. per kilo) was given to all rats on the 1st day of injection only.

A control group (A) was given dehydroascorbic acid (0.7 gm. per kilo) on 3 consecutive days. Diabetes, as defined by a hyperglycemia of at least 200 mg. per cent 1 week after the last injection, was found in 87 per cent of the rats.

A second group (B) was given sulphhydryl compounds 2 minutes before

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each main injection of dehydroascorbic acid. Although the daily dose of various sulphydryl compounds varied from 1.0 to 8.2 mM per kilo, there was complete protection against diabetes in thirty-two animals. Those animals receiving the smallest dose of sulphydryl (as BAL) showed a slight temporary hyperglycemia about 2 to 3 days after the last injection.

A third group (C) was given sulphydryl compounds 10 minutes after each of the three main injections of dehydroascorbic acid. In 75 per cent of the animals diabetes was produced.

**TABLE I**

*Effect of Sulphydryl Compounds on Production of Diabetes*

Dehydroascorbic acid (0.7 gm. per kilo*) was injected intravenously on 3 successive days.

<table>
<thead>
<tr>
<th>Sulphydryl compound</th>
<th>Average blood sugar, mg. per cent</th>
<th>1 wk.</th>
<th>Non-diabetic</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-SH, mm per kilo per day</td>
<td>No. of rats</td>
<td>Initial</td>
<td>2-3 days</td>
</tr>
<tr>
<td>Group A; control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>15</td>
<td>100</td>
<td>364</td>
</tr>
<tr>
<td>Group B; sulphydryl 2 min. before dehydroascorbic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>8.2</td>
<td>13</td>
<td>111</td>
<td>122</td>
</tr>
<tr>
<td>GSH</td>
<td>3.2</td>
<td>8</td>
<td>123</td>
<td>134</td>
</tr>
<tr>
<td>BAL</td>
<td>1.0†</td>
<td>11</td>
<td>102</td>
<td>210</td>
</tr>
<tr>
<td>Group C; sulphydryl 10 min. after dehydroascorbic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cysteine</td>
<td>8.2</td>
<td>12</td>
<td>112</td>
<td>403</td>
</tr>
<tr>
<td>GSH</td>
<td>3.2</td>
<td>5</td>
<td>122</td>
<td>404</td>
</tr>
<tr>
<td>BAL</td>
<td>1.0†</td>
<td>3</td>
<td>102</td>
<td>495</td>
</tr>
</tbody>
</table>

* On the 1st day a desensitizing dose of 0.2 gm. per kilo was given prior to all other injections.
† 0.5 mM of BAL.

The results obtained during the 1st week following injection are summarized in Table I. The rats were followed for a 2nd week with no change in results except in the case of three diabetic rats that had received the large dose of cysteine 10 minutes after dehydroascorbic acid. At the end of 2 weeks the blood sugars of these rats had dropped below 200 mg. per cent. At the end of 2 weeks, therefore, diabetes was found in only 50 per cent of the animals in the third group, whereas the other groups were unchanged. Table I shows the results on all rats that survived the in-
jection period. The mortality during the injection period was as follows: the control group (A), 42 per cent; the group (B) receiving sulfhydryl before dehydroascorbic acid, 26 per cent; and the group (C) receiving sulfhydryl after dehydroascorbic acid, 57 per cent.

**DISCUSSION**

When dehydroascorbic acid is injected intravenously following an injection of sulfhydryl compound, three reactions which tend to remove it from the blood stream can occur. First, the compound can decompose spontaneously to diketogulonic acid at a rate such that it is half destroyed in 2 minutes (8); second, it can be reduced by sulfhydryl to ascorbic acid (9); and third, it can combine with a sulfhydryl compound to give an addition product (10). These reactions are similar to those that occur with alloxan (11, 12). Diketogulonic acid (13) and ascorbic acid (14) are not diabetogenic. A combination of dehydroascorbic acid with sulfhydryl removes an active group of dehydroascorbic acid, and so the resultant combination is probably not diabetogenic.

These three mechanisms, therefore, may combine to remove dehydroascorbic acid from the blood and thus prevent an adequate concentration from reaching the B cells of the islets of Langerhans in the pancreas. It is assumed that these same mechanisms would also be effective within the cell. Similar observations with alloxan led to the hypothesis that the diabetogenic effect of alloxan was due to the inactivation of essential enzymes through combination with a sulfhydryl group (7, 15). This same hypothesis is applicable to the mechanism of action of dehydroascorbic acid.

Sulfhydryl compounds given 10 minutes after dehydroascorbic acid fail to protect the animals from diabetes. Therefore, dehydroascorbic acid must bring about the necessary alterations for the production of diabetes within a few minutes after injection. The short time required for this diabetogenic action suggests that dehydroascorbic acid acts either by rapidly destroying or combining with an essential component of cellular metabolism. If this block involves the sulfhydryl of an enzyme, as has been suggested in the case of alloxan (7), the process must involve the irreversible addition of dehydroascorbic acid to the sulfhydryl and not an oxidation of the sulfhydryl to a disulfide linkage. For one would expect a disulfide linkage to be reduced by the sulfhydryl compounds given after the dehydroascorbic acid and diabetes would thus be prevented. This is not the case. Therefore, if an enzymatic sulfhydryl group is blocked, it is probably the result of an irreversible combination with dehydroascorbic acid.

Dialuric acid and ascorbic acid are the respective reduction products of
alloxan and dehydroascorbic acid. Dialuric acid is relatively insoluble and readily oxidized; hence it is difficult to test it for diabetogenic properties. Ascorbic acid is readily soluble. Repeated large daily doses of ascorbic acid (5.0 to 6.0 gm. per kilo) given to rats in this laboratory did not produce hyperglycemia. This analogy lends further support to earlier work indicating the inability of dialuric acid to produce diabetes (15).

Sulfhydryl compounds have a similar effect in dehydroascorbic acid and in alloxan diabetes. These two types of diabetes are also similar in that there is a triphasic blood sugar response following injection (1), and in that they both respond to small doses of insulin (2). Preliminary work indicates that they produce similar histological lesions in the islets of Langerhans and liver. However, the necrosis in the former is not as marked after dehydroascorbic acid administration.

SUMMARY

Cysteine (8.2 mM of sulfhydryl per kilo), glutathione (3.2 mM of sulfhydryl per kilo), or 2,3-dimercaptopropanol (1.0 mM of sulfhydryl per kilo) given intravenously 2 minutes before the intravenous administration of 0.7 gm. per kilo of dehydroascorbic acid on 3 successive days completely prevented the development of diabetes in thirty-two rats. Of fifteen rats not receiving sulfhydryl compounds, thirteen developed diabetes. The above dose of cysteine, glutathione, or 2,3-dimercaptopropanol injected 10 minutes after dehydroascorbic acid did not prevent the development of diabetes in fifteen out of a total of twenty rats.

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