A Site of Action of Phenethylbiguanide, a Hypoglycemic Compound*

ARNE N. WICK, ELLEN R. LARSON, AND GEORGE S. SERIF

From the Scripps Clinic and Research Foundation, La Jolla, California

(Received for publication, March 6, 1958)

Ungar, Freedman, and Shapiro (1) recently reported on their pharmacological studies, a new oral (nonsulfonylurea) hypoglycemic drug of the biguanide class (N'-β-phenethylformanidinylaminouracil hydrochloride), hereafter referred to as DBI.† The formula is as follows:

\[
\text{CH}_2\text{CH}_3\text{N} \equiv \text{C} \equiv \text{N} \equiv \text{C} \equiv \text{NH}_2\cdot\text{HCl}
\]

Pomeranz, Fujiy, and Mouratoff (2) presented data showing that this drug can produce a significant decrease in blood sugar concentration in certain diabetic patients. The clinical effectiveness of the compound has been confirmed in a preliminary evaluation study by Krall and Camerini-Davalos (3), although it is realized by all concerned that extended study is necessary to prove the real value of this new drug for human diabetics.

In order to investigate the mechanism by which DBI causes hypoglycemia, we have tested the effect of DBI on the oxidation of glucose, acetate, citrate, succinate, and fumarate, in vitro using rat epididymal adipose tissue. This tissue was chosen because it consistently responds to insulin (4). Since the results from the above experiments suggested a metabolic block in the citric acid cycle and in the area involving the oxidation of succinate, we examined the effect of DBI on the succinic oxidase and cytochrome oxidase enzyme systems. The results of these experiments are reported here.

METHODS

Procedure Used with Adipose Tissue—Nonfasted male rats (Sprague-Dawley) weighing approximately 200 gm. were decapitated and exsanguinated. The pair of epididymal adipose tissues were quickly removed, trimmed of blood vessels, weighed, and deposited in flasks containing the incubation medium. Tissue weight ranged from 200 to 400 mg. after trimming. Paired tissues of equal weight from a given rat were used as a set, one the control tissue for the other. Incubation flasks (stoppered 50-ml. Erlenmeyer flasks with a center well) contained 3 ml. of 0.01 M CYabeled substrate made up in a Krebs-Ringer-phosphate buffer (Ca++ omitted). The labeled substrates used and a comparison of the specific activities are presented in Table I.

* This investigation was supported by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

† This compound has been designated as DBI by U. S. Vitamin Corporation. We are indebted to them for supplies of this drug.

The flasks were incubated at 37° in a Dubnoff metabolic shaking incubator for 3 hours with air as the gas phase. Respired CO₂, trapped in 5 N NaOH contained in the center well, was precipitated as BaCO₃. Sufficient carrier Na₂CO₃ was added to give a final BaCO₃ weight of approximately 120 mg. The radioactivity was determined at infinite thickness of BaCO₃ in the proportional range in a gas flow counter.

Preparation of Homogenate—10 gm. of fresh rat liver was minced with 50 ml. of ice water in a micro Waring Blender, and the resulting liquid filtered through cheese cloth and centrifuged at 3° and 30,000 x g for 30 minutes. The supernatant was discarded and the solids resuspended in 50 ml. of ice water in the micro Waring Blender and centrifuged at 3° and 17,000 x g for 40 minutes. The supernatant was discarded, the solids resuspended in ice water and the volume adjusted with water to provide a 10 per cent homogenate based on original wet tissue weight.

Succinic Oxidase Assay—Succinic oxidase activities were determined using the procedures described by Umbret et al. (5) with minor modifications. A 0.5 ml. volume of the 10 per cent homogenate was employed, and cytochrome c and aluminum ion were omitted except where their inclusion is noted. Calcium ion was omitted in our studies. Succinate was placed in a side arm and tipped in after temperature equilibration. No endogenous O₂ uptake by the homogenate was detectable in the absence of added substrate.

Cytochrome Oxidase Assay—Cytochrome oxidase activities were determined by the method of Umbret et al. (5) with the exception that 0.5 ml. of 10 per cent homogenate was used and aluminum ion was omitted except where specifically noted.

RESULTS

A summary of the effect of DBI on the oxidation of the C¹⁴ labeled substrates by adipose tissue is presented in Table II. It is apparent that this hypoglycemic agent, when tested at 0.5 mg. per ml. (0.002 M), substantially depresses the oxidation of glucose, acetate, and succinate but has a much less intense inhibitory effect on the oxidation of citrate and fumarate. In Fig. 1 are data found which show that the inhibition of acetate oxidation is related to the concentration of DBI in the incubating fluid. For example, DBI at 6.75 x 10⁻⁵ molar concentration produced a 30 per cent inhibition of acetate oxidation, whereas 85 to 95 per cent inhibition was obtained at 8 x 10⁻⁵ molar concentration.

It is apparent from the data presented in Table III that DBI at 0.5 mg. per ml. significantly inhibits the uptake of oxygen by the rat liver succinic oxidase system when succinate...
**TABLE I**

*Specific activity of substrates*

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Specific Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (uniformly labeled)</td>
<td>22,600 c.p.m./ml, 12,560 c.p.m./mg, 2.26 million c.p.m./mmole</td>
</tr>
<tr>
<td>Acetate-1-C¹⁴</td>
<td>15,800 c.p.m./mg, 1.58 million c.p.m./mmole</td>
</tr>
<tr>
<td>Fumarate-1-C¹⁴</td>
<td>74,000 c.p.m./mg, 7.40 million c.p.m./mmole</td>
</tr>
<tr>
<td>Citrate-1,5-C¹⁴</td>
<td>63,000 c.p.m./mg, 6.3 million c.p.m./mmole</td>
</tr>
<tr>
<td>Succinate-2-C¹⁴</td>
<td>67,000 c.p.m./mg, 6.67 million c.p.m./mmole</td>
</tr>
</tbody>
</table>

**TABLE II**

*Effect of DBI on oxidation of C¹⁴-labeled substrates by rat adipose tissue*

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Number of experiments</th>
<th>Control</th>
<th>DBI 0.5 mg/ml</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (uniformly labeled)</td>
<td>9</td>
<td>0.063</td>
<td>0.02</td>
<td>68</td>
</tr>
<tr>
<td>mg. oxidized</td>
<td></td>
<td>3.5 × 10⁻⁴</td>
<td>1.1 × 10⁻⁴</td>
<td>77</td>
</tr>
<tr>
<td>mmoles oxidized</td>
<td></td>
<td>0.056</td>
<td>0.013</td>
<td>16</td>
</tr>
<tr>
<td>Acetate-1-C¹⁴</td>
<td>4</td>
<td>0.056</td>
<td>0.3 × 10⁻⁴</td>
<td>16</td>
</tr>
<tr>
<td>mg. oxidized</td>
<td></td>
<td>2.1 × 10⁻⁴</td>
<td>1.1 × 10⁻⁴</td>
<td>16</td>
</tr>
<tr>
<td>mmoles oxidized</td>
<td></td>
<td>0.016</td>
<td>0.013</td>
<td>16</td>
</tr>
<tr>
<td>Fumarate-1-C¹⁴</td>
<td>4</td>
<td>0.016</td>
<td>1.3 × 10⁻⁴</td>
<td>16</td>
</tr>
<tr>
<td>mg. oxidized</td>
<td></td>
<td>1.3 × 10⁻⁴</td>
<td>1.1 × 10⁻⁴</td>
<td>77</td>
</tr>
<tr>
<td>mmoles oxidized</td>
<td></td>
<td>0.027</td>
<td>0.026</td>
<td>3</td>
</tr>
<tr>
<td>Citrate-1,5-C¹⁴</td>
<td>7</td>
<td>0.027</td>
<td>1.39 × 10⁻⁴</td>
<td>46</td>
</tr>
<tr>
<td>mg. oxidized</td>
<td></td>
<td>1.34 × 10⁻⁴</td>
<td>1.14 × 10⁻⁴</td>
<td>77</td>
</tr>
<tr>
<td>mmoles oxidized</td>
<td></td>
<td>0.032</td>
<td>0.017</td>
<td>46</td>
</tr>
<tr>
<td>Succinate-2-C¹⁴</td>
<td>3</td>
<td>0.032</td>
<td>2.7 × 10⁻⁴</td>
<td>77</td>
</tr>
<tr>
<td>mg. oxidized</td>
<td></td>
<td>1.4 × 10⁻⁴</td>
<td>1.04 × 10⁻⁴</td>
<td>77</td>
</tr>
<tr>
<td>mmoles oxidized</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Results expressed as total oxidation per gm. of tissue per hour. It is assumed that the release of each C¹⁴ atom as CO₂ represents complete oxidation of the substrate molecule.

**DISCUSSION**

The studies in vitro with succinic oxidase (Table III) show positively that DBI exerts a blocking action at one of the events concerned with the conversion of succinate to fumarate. The following scheme illustrates the electron transfer pathway for this oxidation and indicates the point of entry of ascorbate:

```
Succinate → Cytochrome b → "Factor" → Cytochrome c → Cytochrome a → Ascorbate → Cytochrome oxidase → O₂
```

Since DBI does not significantly inhibit electron transfer from ascorbate to molecular oxygen it would appear from this study that the most sensitive site of inhibition is at a point between succinate and cytochrome c. This conclusion is not in complete agreement with that recently reported by Steiner and Williams (6). The observations of these authors suggest that the most likely site of inhibition is on cytochrome oxidase. Although our data indicate that the most sensitive site of action is before reduced cytochrome c, it does not preclude other sites of action which may be revealed when a higher concentration of the drug is used.

The metabolic block described here in the electron transport scheme associated with the oxidation of succinate, correlates with the data obtained from the study of the effect of DBI on the labeled substrates presented in Table II. An inhibition in oxidation of glucose would result from a block at any step in its metabolism. The strong inhibition of the oxidation of carboxyl-
have to pass the succinate block and be released as CO₂, in part, so that it contributes very little to the production of CO₂.

labeled acetate is expected since the labeled carbon would not be released as CO₂ until the carbon redistribution occurred beyond the succinate stage. On the other hand, DBI should and does exert only a slight inhibiting effect on the oxidation of fumarate-1-C⁰⁰. This labeled carbon is released without passing through the block postulated at the succinate level. The inhibitory effect of DBI on citrate-1,5-C⁰⁰ is somewhat less than first anticipated. No theoretical inhibition should be associated with the release of carbon 5 but the label on carbon 1 would have to pass the succinate block and be released as CO₂, in part, in its second trip through the Krebs cycle. An explanation of this result may be based on the possibility that the major proportion of the CO₂ activity obtained as CO₂ in the citrate study is derived from carbon 5. Beyond the point at which carbon 5 is lost as CO₂, the radioactivity associated with carbon 1 may be diluted by a metabolic pool or diverted via another pathway so that it contributes very little to the production of CO₂.

It is apparent from the studies reported here that with respect to the oxidation of glucose in adipose tissue the action of this hypoglycemic agent is diametrically opposed to that of insulin. Thus, the current problem is one of explaining the hypoglycemic action of DBI in terms of its effect on known metabolic pathways. Williams et al. (7), and Tyberghein and Williams (8) have suggested that the hypoglycemia may be attributed to increased anaerobic glycolysis. They found that DBI caused an increase in glucose uptake, a decrease in glycogen storage, increased lactic acid production, and a decrease in oxygen uptake in their rat diaphragm studies in vitro. They also presented evidence suggesting that there is a decrease in gluconeogenesis in vitro. From the data presented in this paper, a possible correlation may be drawn between studies in vitro and clinical observations. The succinate block discussed here may produce an inhibition of the Pasteur effect. The resulting increase in glycolysis will provide a greater glucose utilization with a concomitant increase in lactate in all tissues to which DBI may gain access. It is suggested that the liver may be such a site of action. The biguanide may not, however, enter muscle cells of the extrahepatic tissues and thus may fail to produce a similar effect in this tissue. The lactate produced in the liver then would be readily oxidized by the muscle cells (9). A mechanism such as this could result in a lowering of the blood sugar in diabetic patients.

**SUMMARY**

1. A hypoglycemic drug (N¹-s-phenethylformamidinyliminourea hydrochloride) has been evaluated in vitro with respect to its inhibitory action on the oxidation of C¹⁴-labeled glucose, acetate, citrate, succinate, and fumarate.

2. A site of action of the drug was shown to exist in the electron transport system associated with the succinic oxidase of rat liver mitochondria. The demonstration that cytochrome oxidase is not inhibited by concentrations of this drug which produce a marked inhibitory effect on the total succinic oxidase system further localized the most sensitive point of action as between succinic dehydrogenase and reduced cytochrome c.

3. A possible correlation was drawn between the effects, in vitro, of N¹-s-phenethylformamidinyliminourea hydrochloride and the clinical observation that the drug is an effective hypoglycemic agent.

**REFERENCES**


A Site of Action of Phenethylbiguanide, a Hypoglycemic Compound

Arne N. Wick, Ellen R. Larson and George S. Serif


Access the most updated version of this article at http://www.jbc.org/content/233/2/296.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/233/2/296.citation.full.html#ref-list-1