GLYCOGENOLYTIC ACTION OF GLUCAGON AS INFLUENCED BY INSULIN AND OTHER COMPOUNDS*

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The exact metabolic relationship between insulin and glucagon is still a matter of controversy. Some authors (2, 3) have proposed that glucagon, like insulin, enhances the peripheral utilization of glucose. However, much evidence has been obtained which suggests that glucagon antagonizes the action of insulin. The results obtained by Thorogood and Zimmerman (4) suggested that, since alloxan-diabetic dogs required less insulin after pancreatectomy, the pancreas contained a factor which could act as an insulin antagonist. Later, de Duve et al. (5) were able to decrease considerably the amount of glucose necessary to maintain a normal blood sugar level in rabbits injected with insulin by adding insulin which contained small amounts of glucagon to the infusion. Those experiments have been confirmed and extended by Tyberghein (6), who maintained unchanged blood sugar and liver and glycogen levels in insulinized rabbits by an intravenous infusion of glucagon. Vuylsteke and de Duve (7) also demonstrated that a subcutaneous injection of glucagon decreased the action of insulin by 30 per cent. With his experiments on cross-circulation, Foa (8) came to the conclusion that glucagon is the anti-insulin hormone of the pancreas. The work of Candela (9), Drury et al. (10), Snedecor et al. (11), and Pincus et al. (12) suggests that glucagon antagonizes the action of insulin in extrahepatic tissues.

In order to investigate further the metabolic relationship between insulin and glucagon, the present study dealing with their action on liver slices has been carried out. Although insulin has no apparent action on carbohydrates in liver tissue in vitro, it is well known that glucagon stimulates glycogenolysis in liver slices (13). It was therefore of interest to investigate whether insulin can modify the glycogenolytic action of glucagon, and if such an action is specific for insulin.

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**Materials and Methods**

Slices were made from the livers of well fed rabbits according to the method described by Sutherland and Cori (13). In each experiment three sets of slices were prepared, each set being cut from the same piece of liver. The weight of the slices, each about 100 mg., did not differ more than 10 per cent from one another. The slices were placed in 20 ml. beakers containing 2 ml. of a solution of 4 parts of 0.9 per cent sodium chloride and 1 part of a 0.1 M potassium phosphate buffer, pH 7.5. The mixtures were incubated at 37° for 45 minutes in a Dubnoff incubator which was shaken at 110 oscillations per minute. Aliquots were then removed for glucose determinations according to the method of Nelson (14). In each set, two slices incubated in buffer alone served as control; another slice served to indicate the glucose output as influenced by 7.5 γ of glucagon, an amount which gives maximal stimulation of glycogenolysis; the remaining slices were used to study the effect of the other compounds on the glycogenolytic action of glucagon.

Two glucagon preparations were used. Lot No. 208-158B-214A contained 50 per cent glucagon and 50 per cent inert protein, and lot No. 208-158B-131-2 was crystalline glucagon, which is considered to be pure. The Novo insulin2 was a crystalline glucagon-free preparation and the Lilly insulin1 an amorphous preparation. Other purified substances used in this study were crystalline bovine plasma albumin, α-casein, α-corticotropin, ox growth hormone, α-lactalbumin, and crystalline ribonuclease.3

**RESULTS AND DISCUSSION**

In a preliminary study, the glucose output from liver slices was measured under the influence of 30 γ of glucagon-free insulin (Novo insulin) per ml. and of the same amount of insulin containing approximately 1 per cent of glucagon (Lilly insulin). The results are given in Table I. It is evident that in every experiment glucagon-free insulin had no effect. However, the insulin preparation containing 1 per cent of glucagon caused a marked increase in glucose output (p < 0.001) and had approximately the same glycogenolytic effect as an excess amount of glucagon. This suggests that 0.3 γ of glucagon, when acting in the presence of insulin, might have the same effect as 7.5 γ of glucagon alone. These findings have been veri-

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1 Glucagon preparations and amorphous insulin were kindly supplied by Dr. O. K. Behrens and Dr. C. W. Pettinga of Eli Lilly and Company.

2 Crystalline glucagon-free insulin was a gift from Dr. K. Halls-Møller of the Novo Terapeutisk Laboratorium.

3 α-Casein was kindly supplied by Dr. T. L. McMeekin, α-corticotropin and growth hormone by Dr. C. H. Li and Dr. I. I. Geschwind, and α-lactalbumin by Dr. P. E. Wilcox. Crystalline bovine plasma albumin was obtained from the Armour Laboratories and crystalline ribonuclease from the Worthington Biochemical Corporation.
fied by comparing the glycogenolytic action of 0.075 γ of glucagon, the action of 30 γ of glucagon-free insulin (Novo insulin), and the action of both when added together. The results (Table II) indicate that neither 0.075 γ of glucagon nor 30 γ of insulin influence glycogenolysis significantly, but that together they greatly increase the glucose output from liver slices.

TABLE I

<table>
<thead>
<tr>
<th>System</th>
<th>Glucose output in mg. per 100 mg. of liver tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>1.10</td>
</tr>
<tr>
<td>Glucagon, 7.5 γ per ml.</td>
<td>1.60</td>
</tr>
<tr>
<td>Insulin (Novo), 30 γ per ml.</td>
<td>1.21</td>
</tr>
<tr>
<td>&quot; (Lilly), 30 γ per ml.</td>
<td>1.60</td>
</tr>
</tbody>
</table>

Each value is the mean of three different slices.

TABLE II

<table>
<thead>
<tr>
<th>System</th>
<th>Glucose output in mg. per 100 mg. of liver tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>1.43</td>
</tr>
<tr>
<td>Glucagon, 7.5 γ per ml.</td>
<td>2.08</td>
</tr>
<tr>
<td>&quot; 0.075 γ per ml.</td>
<td>1.51</td>
</tr>
<tr>
<td>Insulin (Novo), 30 γ per ml.</td>
<td>1.42</td>
</tr>
<tr>
<td>&quot; &quot; + glucagon, 0.075 γ per ml.</td>
<td>2.11</td>
</tr>
</tbody>
</table>

Each value is the mean of three different slices.

(p < 0.001). According to these observations, minute amounts of glucagon, in the presence of insulin, may produce the same glycogenolytic effect as much larger amounts when used alone. This increased glycogenolytic effect is obtained only if insulin and glucagon are both added at the same time to the incubation medium. Even if insulin is added only 10 minutes after the addition of glucagon, the glucose output is not increased.

4 This observation was made independently by Dr. C. de Duve, who reported this finding at the Lilly Insulin Symposium in May, 1955.
This interaction of insulin and glucagon may be explained by recent observations in our laboratories (15), which suggest that the "proteolytic" system of the liver, which degrades insulin, is probably not an absolutely specific one. Certain other hormonal and non-hormonal proteins, among them glucagon, are probably also degraded by the same system in vitro. Excess amounts of these substances are thought to be able to decrease the rate of degradation of insulin in vitro by serving as substrate competitors for this enzyme system. Other studies suggest that insulin also can be degraded by enzymatic reduction of its disulfide linkages. However, it is unlikely that this system is the one involved, since glucagon probably does not contain any disulfide linkages (16). This hypothesis of a common "proteolytic" system for insulin and glucagon may explain the present findings. Minute amounts of glucagon do not produce any significant increase in glucose output probably because small amounts of glucagon are completely degraded rapidly. However, if relatively large amounts of insulin are added to the incubation medium, insulin may serve as a substrate competitor and decrease the extent of destruction of glucagon.

To investigate this hypothesis, other compounds which, according to the insulin degradation studies, might serve as substrate competitors for this enzyme system of the liver have been added to 0.075 \( \gamma \) of glucagon, and the mixture has been tested for glycogenolytic activity. The quantities of other compounds added to glucagon were equimolar to 30 \( \gamma \) of insulin (15). Each compound has been tested in three different experiments. The results given in Fig. 1 represent the mean value of the percentages of maximal stimulation by 0.075 \( \gamma \) of glucagon in the presence of each compound, calculated according to the following formula: \( (X - C_2/M - C_1) \times 100 \). Each letter indicates the glucose output in mg. per 100 mg. of liver tissue, \( C_1 \) being the glucose output in buffer alone, \( C_2 \) the glucose output

\[ \text{Fig. 1. Effect of various purified proteins on the glycogenolytic action of glucagon in liver slices.} \]

\( ^6 \) H. T. Narahara, unpublished results.
in buffer plus possible substrate competitor, \( M \) the glucose output in buffer plus an excess amount of glucagon, and \( X \) the glucose output in buffer plus 0.075 \( \gamma \) of glucagon and possible substrate competitor. It is obvious from the data in Fig. 1 that the addition of insulin or other substances, such as \( \alpha \)-corticotropin or growth hormone, to 0.075 \( \gamma \) of glucagon greatly increases the glucose output from liver slices. Other compounds such as cysteine-inactivated insulin and \( \alpha \)-casein have the same effect. However, not all proteins serve as substrate competitor for the enzyme system of the liver in vitro. Indeed, ribonuclease, \( \alpha \)-lactalbumin, and bovine plasma albumin have little or no effect. The results of these experiments are strikingly similar to those of the earlier experiments concerning degradation of insulin (15). The degrees of effectiveness of successful competitors in the insulin degradation study and in the present study are quite comparable. In addition, compounds that had little effect in the insulin experiments are similarly ineffective in the present study. It is still possible that substances effecting the sparing action are inhibitors rather than substrates for the glucagon-degrading system. However, the similarity in the results of this and the previous study strengthens the hypothesis that the "proteolytic" system in the liver which degrades insulin in vitro is also capable of degrading glucagon, \( \alpha \)-corticotropin, and growth hormone.

The fact that small amounts of glucagon may produce a significant increase in the glucose output from liver slices, when acting in the presence of insulin or certain other substances, makes this technique a very sensitive method for assaying glucagon.

**SUMMARY**

Evidence has been presented that insulin does not antagonize the glycogenolytic action of glucagon in liver slices. Moreover, a minute amount of glucagon, too small to influence glycogenolysis significantly when acting alone, may produce a marked glycogenolytic effect when acting in the presence of excess insulin or proteins such as \( \alpha \)-corticotropin or growth hormone. This phenomenon is best explained as being the result of competition of these substrates for the same degradative enzyme system of liver.

**BIBLIOGRAPHY**

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