THE EFFECT OF GROWTH HORMONE AND CORTISONE ON THE ACTION OF BOUND INSULIN

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(Received for publication, March 13, 1952)

There is abundant evidence that pituitary and adrenal cortical hormones influence insulin so as to produce marked alterations in its customary action on tissue metabolic patterns. There is little doubt that this reciprocal influence is manifest in many types of tissue and is particularly well established in the cases of liver and muscle.

The injection of pituitary or adrenal hormones into normal hypophysectomized, or adrenalectomized animals is also known to influence the action of insulin on the metabolism of various organs. Evidence is accumulating which strongly suggests that growth hormone is the pituitary principle which in the main is responsible for effects on carbohydrate metabolism. The possibility, however, that other pituitary principles may so act cannot be excluded. A variety of adrenocortical hormones are reported to influence different aspects of carbohydrate metabolism, but cortisone appears to be the most potent one.

The rat diaphragm, first shown by Gemmill (1) to synthesize extra glycogen from glucose in the presence of insulin, has been extensively used to demonstrate interhormonal effects on glucose uptake or glycogen synthesis. Nelson (2) first showed that prior injection of pituitary extract diminished the subsequent synthesis of glycogen by rat diaphragm in vitro. Park and Krahl (3) reported that injection of certain pituitary fractions depressed the glucose uptake in vitro by diaphragm from normal, hypophysectomized, and hypophysectomized-adrenalectomized rats, but that this depression was counteracted by insulin. Krahl, in a comprehensive but brief review of the recent literature (4), discussed extensive studies on the effects of pituitary and adrenal hormones on the action of insulin on glucose uptake by the rat diaphragm in vitro.

Attempts to demonstrate hypersensitivity of the isolated diaphragm of the hypophysectomized rats to insulin have been reported. Early reports have been negative (Perlmutter and Greep (5), Krahl and Park (6), Stadie, Haugaard, Hills, and Marsh (7)). Bornstein and Nelson (8), using rats 14...
days after hypophysectomy, reported an insulin effect on glycogen synthesis about double that in normal animals.

Bornstein and Trehwella (9) studied glycogen synthesis with diaphragms from normal, diabetic, diabetic-hypophysectomized, diabetic-adrenalectomized, and diabetic-hypophysectomized-adrenalectomized rats. They found marked alterations in glycogen synthesis in all cases, which were counteracted by insulin. In no case, however, was the action of insulin enhanced above normal. They also concluded that pituitary and adrenal factors acted synergistically in opposing insulin action.

In a previous communication (10) Stadie, Haugaard, and Marsh described a new phenomenon: a rat hemidiaphragm momentarily dipped in an insulin solution subsequently responded by an increased synthesis of glycogen from glucose. Since this effect persisted after prolonged washing of the diaphragm and, as shown by controls, could not be due to insulin in solution, it was concluded that insulin during the brief preliminary period was bound to the diaphragm by forces not yet understood and in this bound form exhibited its customary effect on metabolic reactions. More recent work (11) with insulin labeled with S35 and I131 has confirmed this concept. By this method it was shown that the action of the bound insulin was greatly increased in hypophysectomized rats.

In this paper we report more detailed studies on the action of insulin on the rat diaphragm, extending our studies to include adrenalectomized as well as hypophysectomized rats, and also the effect of injected growth hormone or cortisone alone or in combination. The experiments illustrate the usefulness of this new method for studying interhormonal effects on carbohydrate metabolism and bring out in a striking way the hypersensitivity of the hypophysectomized rat diaphragm to insulin and also the synergy of growth hormone and cortisone in opposing insulin action, a problem about which there is still much controversy.

**Methods**

The techniques used in the study of the effect of bound insulin on the synthesis of glycogen by the rat diaphragm follow closely those described in previous communications (10).

Normal, adrenalectomized, and hypophysectomized rats were used and the effect of prior injection of growth hormone and cortisone into these animals on the response of the isolated diaphragm to bound insulin was studied. The assay for the activity of the bound insulin was always carried out in the same way; viz., a hemidiaphragm was equilibrated in a phosphate-saline medium containing 0.1 unit per ml. of insulin for 1 minute. It was then washed twice for 30 seconds each in 25 ml. of medium and finally equilibrated at 38° for 90 minutes in a phosphate-saline medium, at
pH 6.8, containing 0.4 per cent glucose (gas phase, oxygen). The final glycogen content was determined. The control, paired hemidiaphragm was carried through the same steps except for the omission of insulin. The difference between the final glycogen content of the experimental and control hemidiaphragms is called the insulin effect. It is given in micromoles of glucose equivalents per gm. of wet weight per 90 minutes.

The normal rats were albino rats of the Wistar strain. Adrenalectomies were carried out under ether anesthesia and the animals were maintained for 5 to 7 days with salt added to the drinking water before being used for the experiments. The hypophysectomized rats were obtained from the Hormone Assay Laboratories of Chicago and were of the Sprague-Dawley strain. We have shown previously that similar results were obtained in our experiments with the two strains of rats. They were used 5 to 8 days after operation.

The preparations of growth hormone (Lot 22KRl) were obtained from the Armour Research Laboratories through the courtesy of Mr. I. Bungling. Cortisone acetate was obtained from the Research Laboratories of Merck and Company. The hormones were administered intraperitoneally in saline solutions or suspensions.

All animals were fasted 24 hours before being used in the experiments, except for the adrenalectomized and hypophysectomized rats. We have not found any significant differences in the insulin effect on glycogen synthesis between fasted and fed rats.

**Results**

The results are summarized in Table I, together with a statistical analysis outlined in the table. The significance of the mean values in the various categories is evaluated by $P$, the probability obtained from Fisher's $t$ test that the differences are due to sampling errors.

**Normal Rats**—Injection of 2 to 7 mg. of growth hormone 14 hours prior to the experiment or 0.2 mg. of cortisone 5 hours previously caused a significant decrease of the insulin effect on glycogen synthesis. This is in conformity with our earlier experiments (10).

**Hypophysectomized Rats**—The response of the diaphragm to combined insulin is greatly increased over that of the normals. However, the injection of growth hormone or cortisone separately has no effect; when injected together, the insulin effect is significantly decreased to the level observed in normals.

**Adrenalectomized Rats**—No change in insulin action resulted from adrenalectomy alone. The injection of growth hormone caused no change in the insulin effect, but, when it was injected with cortisone, there was a significant decrease. Cortisone alone appeared to result in a small but
significant increase rather than decrease, a result which at the moment is difficult to explain.

**TABLE I**

*Effect of Prior Injection of Growth Hormone (14 Hours) and Cortisone (6 Hours) on Action of Bound Insulin in Synthesis of Glycogen from Glucose by Isolated Diaphragms from Normal, Hypophysectomized, or Adrenalectomized Rats*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>N = No. of rats</th>
<th>Insulin effect, mg.</th>
<th>Standard deviation</th>
<th>Difference from &quot;none&quot; in group</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>2-7</td>
<td>96</td>
<td>7.7</td>
<td>4.1</td>
<td>−2.4 &lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Growth hormone</td>
<td>2-7</td>
<td>34</td>
<td>5.3</td>
<td>2.9</td>
<td>−4.4 &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Cortisone</td>
<td>0.2</td>
<td>15</td>
<td>3.3</td>
<td>2.9</td>
<td>+5.9§ &lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Hypophysectomized</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>2-7</td>
<td>25</td>
<td>13.6</td>
<td>4.3</td>
<td>−1.6</td>
<td></td>
</tr>
<tr>
<td>Growth hormone</td>
<td>2-7</td>
<td>15</td>
<td>12.0</td>
<td>5.0</td>
<td>−1.6</td>
<td></td>
</tr>
<tr>
<td>Cortisone</td>
<td>0.2</td>
<td>10</td>
<td>14.1</td>
<td>7.6</td>
<td>+0.5</td>
<td></td>
</tr>
<tr>
<td>Growth hormone +</td>
<td>2-7</td>
<td>6</td>
<td>8.8</td>
<td>1.0</td>
<td>−4.8 &lt;0.02</td>
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<tr>
<td>Adrenalectomized</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>2-7</td>
<td>16</td>
<td>7.6</td>
<td>3.8</td>
<td>−0.1§</td>
<td></td>
</tr>
<tr>
<td>Growth hormone</td>
<td>2-7</td>
<td>13</td>
<td>7.9</td>
<td>3.7</td>
<td>+0.3</td>
<td></td>
</tr>
<tr>
<td>Cortisone</td>
<td>0.2</td>
<td>16</td>
<td>10.9</td>
<td>5.9</td>
<td>+3.3 &lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Growth hormone +</td>
<td>2-7</td>
<td>14</td>
<td>4.2</td>
<td>2.9</td>
<td>−3.4 &lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Pooled standard deviation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.2 §</td>
<td></td>
</tr>
</tbody>
</table>

* Difference between final glycogen (micromole glucose equivalents per wet gm.) of insulin-treated and control hemidiaphragms.
† Standard deviation = \( \sqrt{\sum(X - \bar{X})^2/(N - 1)} \).
‡ Probability by Fisher's *t* test that the mean difference is due to sampling error when the pooled standard deviation is used to calculate the standard error of the mean difference; i.e., \( 4.2 \sqrt{(1/N_1) + (1/N_2)} \).
§ Difference from "none" of normals.
|| Not significant.
¶ Pooled standard deviation = \( \sqrt{\sum(X - \bar{X})^2/\Sigma(N - 1)} \).

**DISCUSSION**

The experiments reported here demonstrate that the action of insulin on the metabolism of isolated muscle is influenced by the endocrine state of the experimental animal. The striking increase of insulin action in the hypophysectomized rats is in conformity with the well known hypersensitivity to insulin of this endocrine state. This observation again is evidence
against the hypothesis that the action of insulin is to release an inhibitory action of the pituitary hormone. The finding that in adrenalectomized or hypophysectomized rats it is necessary to administer these substances together in order to decrease the effect of the bound insulin supports the conclusion that these hormones act synergistically in opposing the action of insulin. The synergy of these two hormones is in accordance with other evidence in the literature (4, 12). On the other hand de Bodo et al. (13) have reported experiments in hypophysectomized dogs in which the contra-insulin activity of growth hormone alone was demonstrated. Reconciliation of these two points of view must await further experiments, but it must be pointed out that differences of animal species, tissue studied, and mode of measuring the contra-insulin action of growth hormone are also included in these contradictory experiments.

Whatever the explanation of our experimental results may be, we feel that they support the concept that a pituitary hormone in conjunction with adrenal cortical hormones opposes the action of insulin in the peripheral tissue. Such a concept is in accord with most of the evidence obtained from studies of the intact animal.

The mechanism of this contra-insulin action remains unknown. We have previously pointed out that our experiments showing hormonal effects on bound insulin do not allow us to distinguish between two possibilities; viz., (a) when the endocrine balance is altered, the tissue binds the same amount of insulin as in the normal state, but there is an inhibition of the action of insulin per unit mass bound, or (b) the hormones act competitively so that the binding of insulin by the tissue is inhibited. Accordingly, although the activity of the insulin per unit mass bound is unaltered, the amount is diminished and there is an apparent inhibition of insulin action. The distinction between these two possibilities is of fundamental importance in the problem of interaction, and is being studied here by the use of labeled insulin preparations.

SUMMARY

1. With the action of bound insulin as a criterion it was shown that the isolated rat diaphragm of the hypophysectomized rat synthesizes an extra amount of glycogen. This is in accord with the well known "hypersensitivity" of the hypophysectomized animal to insulin. Adrenalectomy produced no change.

2. In the case of hypophysectomized and adrenalectomized rats, prior injection of both growth hormone preparation and cortisone was required to decrease the accelerating action of bound insulin as measured by the glycogen synthesis by rat diaphragms in vitro.

3. The significance of these findings, particularly the synergy between the two hormones, is discussed.
BOUND INSULIN

BIBLIOGRAPHY

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