Regulation of Glucose Uptake in Muscle

V. THE EFFECT OF GROWTH HORMONE ON GLUCOSE TRANSPORT IN THE ISOLATED, PERFUSED RAT HEART*

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The effects of growth hormone in the whole animal have been recently reviewed (1, 2). The following points are pertinent to the present work. The first injection of growth hormone into the hypophysectomized animal induces a hypoglycemic reaction similar to that which follows a small dose of insulin. This reaction is much less marked in a normal animal. After repeated injections of the hormone over several days in either the hypophysectomized or normal animal, the hypoglycemic reaction no longer occurs and a sustained hyperglycemia develops which varies greatly in intensity depending on the dosage and the species of animal involved. This "diabetic" phase of growth hormone activity is increased in severity by the concurrent administration of gluconeogenic adrenal steroids or by insulin deficiency. It is also characterized by an insensitivity to insulin.

These effects of growth hormone on blood glucose can be accounted for in part by changes in peripheral glucose uptake. Earlier work (3) with the isolated rat diaphragm as an indicator of hormone effects in muscle gave the following findings. A few minutes after the first injection of growth hormone into hypophysectomized rats, glucose uptake by the diaphragm was stimulated. Addition of the hormone to the muscle in vitro had the same effect. This stimulation was not observed in diaphragms taken from hypophysectomized animals treated chronically with growth hormone or in the muscle from normal rats. In fact, repeated injection of growth hormone into either hypophysectomized or normal animals depressed glucose uptake by the excised diaphragm and this depression was augmented by the concurrent administration of adrenal cortical extracts. The muscle also showed an insensitivity to insulin.

Although the above observations in vivo and in vitro seemed to fit well together, recent work suggested a reexamination of the findings. First, the studies of Altszuler et al. (4) have shown that the peripheral utilization of glucose by the hypophysectomized dog in the postabsorptive state is below normal and that utilization rises after repeated growth hormone injections. Second, the "cut" diaphragm is probably quite unreliable as an indicator of hormone effects on muscle. The transport step, which is usually rate-limiting for uptake, is bypassed to a large extent because of cut edges in the preparation. The recent studies of Kipnis (5), who measured penetration of the glucose analogue, 2-deoxyglucose, in the "cut" diaphragm from hypophysectomized rats, suggest that the high rate observed earlier (6–8) was an artifact because the rate in the "intact" diaphragm, which has no cut edges, is low. In accord with these observations, Henderson et al. (9) found that the uptake of glucose itself by the perfused heart from hypophysectomized rats is below normal. These observations in vitro are in line with Altszuler's studies cited above. Third, newly developed techniques (10) for examining separately the transport and phosphorylation steps make it possible to give a more detailed description of growth hormone effects on glucose uptake.

With the above considerations in mind, the effect of growth hormone on glucose uptake by isolated muscle has been reinvestigated. The present study is limited to effects on transport through the cell membrane. Effects on phosphorylation of glucose are presented separately (11, 22). The perfused rat heart has been used as the test object.

EXPERIMENTAL PROCEDURE

Methods and Materials

These experiments were made with rat hearts from normal rats. Hydrocortisone sodium succinate was obtained from The Upjohn Company and bovine growth hormone (a highly purified preparation, lot No. R50109) was kindly supplied by the Endocrinology Study Section of the National Institutes of Health.

RESULTS

Effect of Growth Hormone in Vitro—Glucose uptake by the heart from normal rats was not affected when growth hormone was added directly to the perfusion medium (Fig. 1). These experiments were carried out at three different perfusate (extracellular) glucose concentrations. When the same experiments were performed, however, with hearts from hypophysectomized rats, uptake was stimulated to rates 60 to 100% above the mean control values (Fig. 1). These results confirm those obtained previously with the isolated rat diaphragm. With the heart technique, however, it was possible to characterize the stimulatory effect in more detail as follows.

Previous work (9) had established that membrane transport is the principal limiting step1 for glucose uptake by the heart of the

1 Transport is predominantly limiting for uptake when the intracellular free glucose remains very low. Under these conditions, glucose uptake provides a measure of transport into the cell. With excess insulin, transport becomes so fast that intracellular, free glucose accumulates and uptake is significantly limited by phosphorylation. A full exposition of these relationships has been given earlier (10, 13).
hormone caused a marked acceleration of transport in hearts from hypophysectomized-diabetic rats, as seen by the pronounced rise in glucose uptake (Table II). Transport did not become fast enough, however, to cause a detectable accumulation of free, intracellular glucose. No significant acceleration was seen in the muscle from diabetic animals (Table II). It was concluded from these experiments that the growth hormone effect did not require the presence of insulin in the tissues, and that it was suppressed by endogenous pituitary secretion.

The failure of hypophysectomy of the diabetic animal to improve the rate of glucose uptake, as seen in Table II, has been discussed elsewhere (11, 22).

Early Effect of Growth Hormone in Vivo—An acceleration of glucose transport also occurred in vivo as shown by the rates obtained in hearts removed 1 hour after growth hormone injection into the animal. Table I shows faster accumulation of L-arabinose and Table III shows accelerated transport as reflected by the higher rates of glucose uptake in muscle from hypophysectomized and normal rats. Table II shows a similar result with hearts from diabetic-hypophysectomized animals. The magnitude of the effects in the studies with glucose also suggest that sensitivity to this action of the hormone is greater in the muscle from animals lacking endogenous pituitary secretion.

TABLE I

Effect of growth hormone in vitro and in vivo on transport of L-arabinose in isolated, perfused heart of hypophysectomized and normal rats

<table>
<thead>
<tr>
<th>Heart donor rats</th>
<th>Treatment</th>
<th>L-Arabinose space (µg)</th>
<th>% equilibrium concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypophysectomized</td>
<td>None</td>
<td>500 ± 10* (14)</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>+ GH in vitro</td>
<td>568 ± 20 (14)</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>60 µg/ml</td>
<td>646 ± 21 (8)</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>+ GH in vivo</td>
<td>580 ± 90 (12)</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>1 hr</td>
<td>428 ± 22 (8)</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>24 hrs</td>
<td>494 ± 13 (8)</td>
<td>44</td>
</tr>
<tr>
<td>Normal</td>
<td>None</td>
<td>453 ± 14 (8)</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>+ GH in vitro</td>
<td>500 ± 15 (7)</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>60 µg/ml</td>
<td>488 ± 17 (8)</td>
<td>40</td>
</tr>
</tbody>
</table>

*Standard error of the mean.

** p < 0.01 versus the corresponding control.

2 Whereas glucose uptake was reduced below the normal rate in the heart from the hypophysectomized rat with transport as the limiting step (Fig. 1), L-arabinose transport was not depressed (Table I). Earlier studies (9), however, with larger series, have shown a reduction of L-arabinose transport after hypophysectomy. The present discrepancy presumably reflects the tendency of transport to rise in the hypophysectomized muscle when no metabolizable substrate is present in the medium. This point has been discussed earlier in detail (9).
Effect of growth hormone on uptake and intracellular accumulation of glucose in isolated, perfused heart of hypophysectomized-diabetic and diabetic rats

The experiments were performed as described in Fig. 1 except that the initial perfusate concentration was 100 mg per ml in all cases. The extracellular space was $360 \pm 10 \mu l$ per g of heart in the diabetic and $351 \pm 9 \mu l$ per g in the hypophysectomized-diabetic rats (10). The number of hearts tested is given by the figure in parentheses.

Diabetes was induced by injection of 60 mg per kg of alloxan intravenously 40 to 72 hours before killing. Growth hormone was added in vitro in a concentration of 6 pg per ml of perfusate, and was injected in vivo in a dose of 0.1 mg per 100 g of rat 1 hour before removal of the heart for testing.

The experiments were performed as described in Fig. 1 except in hearts from hypophysectomized rats (10). The number of hearts tested is given by the figure in parentheses. Growth hormone was given as a single intraperitoneal injection of 0.1 mg per 100 g of rat at the time indicated except in the 4% hour experiments in which a second injection was given at 24 hours. Hydrocortisone was injected subcutaneously at the same times in a dosage of 2.5 mg per 100 g. The extracellular space was $351 \pm 9 \mu l$ per g and $360 \pm 10 \mu l$ per g in the hearts from hypophysectomized and diabetic rats, respectively (10).

Late Effect of Growth Hormone in Vivo—When the heart was tested 24 or 48 hours after growth hormone injection into the animal, no consistent effect on transport could be shown. L-Arabinose accumulation seemed to be reduced at 24 hours in hearts from hypophysectomized rats (Table I), but this effect could not be demonstrated by measurement of glucose uptake at the same time interval (Table III) or in hearts from normal animals (Table III). The concomitant administration of cortisone had no effect (Tables III and IV). After 48 hours, glucose uptake was marginally reduced in hearts from hypophysectomized rats, but no such depression was seen in the normal hearts. These results differ from earlier observations (3) that growth hormone induced a consistent, late inhibition of glucose uptake by the isolated diaphragm.

Effect of Growth Hormone on Insulin Sensitivity—It was shown previously (9) that glucose transport in hearts from hypophysectomized rats is hypersensitive to insulin. It was, therefore, of interest to observe if sensitivity would be reduced by growth hormone, cortisone administration, or both. When hypophysectomized rats were treated with growth hormone, hydrocortisone, or both together for 4 days, no effect on transport could be detected in the absence of added insulin as measured by L-arabinose accumulation (Table IV), in agreement with the conclusions reached in the preceding paragraph. When transport was determined, however, in the presence of a low level of insulin, either growth hormone or cortisone individually diminished the sensitivity to insulin, and, when administered together, the insulin effect was reduced to that in the normal tissue.

<table>
<thead>
<tr>
<th>Heart donor rats</th>
<th>Treatment</th>
<th>Glucose uptake</th>
<th>Glucose space</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg g$^{-1}$ hr$^{-1}$</td>
<td>µl/g</td>
</tr>
<tr>
<td>Hypophysectomized-diabetic</td>
<td>None</td>
<td>2.6 ± 0.5 (12)</td>
<td>266 ± 6 (8)</td>
</tr>
<tr>
<td></td>
<td>+ GH in vitro</td>
<td>7.5 ± 0.3 (8)</td>
<td>322 ± 19</td>
</tr>
<tr>
<td></td>
<td>+ GH in vivo, 1 hr</td>
<td>7.2 ± 0.6 (4)</td>
<td>271 ± 7</td>
</tr>
<tr>
<td>Diabetic</td>
<td>None</td>
<td>2.0 ± 0.3 (8)</td>
<td>286 ± 27</td>
</tr>
<tr>
<td></td>
<td>+ GH in vitro</td>
<td>2.7 ± 0.3 (9)</td>
<td>383 ± 22</td>
</tr>
</tbody>
</table>

$^a$ Standard error.

$^b$ p < 0.01 versus corresponding control.

Effect of growth hormone in vivo on glucose uptake by perfused heart from hypophysectomized and normal rats

The experiments were carried out as described in Fig. 1. The number of hearts tested is given by the figure in parentheses. Growth hormone was given as a single intraperitoneal injection of 0.1 mg per 100 g of rat at the time indicated except in the 48-hour experiments in which a second injection was given at 24 hours. Hydrocortisone was injected subcutaneously at the same times in a dosage of 2.5 mg per 100 g. The extracellular space was $351 \pm 9 \mu l$ per g and $360 \pm 10 \mu l$ per g in the hearts from hypophysectomized and normal rats, respectively (10).
Delayed effect of growth hormone and cortisone in vivo on sensitivity of transport to insulin as measured by intracellular accumulation of L-arabinose in isolated, perfused heart

The experiments were performed as described in Table I. Growth hormone and cortisone were injected daily in a dosage of 0.1 mg per 100 g, and 2.5 mg per 100 g, respectively, over a 4-day period before the animal was killed. The number of hearts tested is given by the figure in parentheses.

<table>
<thead>
<tr>
<th>Heart donor rats</th>
<th>Treatment of rats</th>
<th>Insulin</th>
<th>L-Arabinose space</th>
<th>Intracellular L-arabinose</th>
<th>% equilibration concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypophysectomized</td>
<td>None</td>
<td>0</td>
<td>509 ± 26 (7)</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GH</td>
<td>0</td>
<td>478± 21 (7)</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cortisone</td>
<td>0</td>
<td>545± 29 (7)</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GH + cortisone</td>
<td>0.1</td>
<td>542 ± 21 (8)</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>None</td>
<td>0.1</td>
<td>723± 10 (8)</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GH</td>
<td>0.1</td>
<td>659± 19 (7)</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cortisone</td>
<td>0.1</td>
<td>664± 16 (8)</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GH + cortisone</td>
<td>0.1</td>
<td>603± 12 (9)</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>0.1</td>
<td>540 ± 12 (18)</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>0.1</td>
<td>582± 10 (6)</td>
<td>62</td>
<td></td>
</tr>
</tbody>
</table>

* Standard error of the mean.

p > 0.05 versus hypophysectomized, no treatment.

p < 0.01 versus hypophysectomized, no insulin.

p = 0.02 versus hypophysectomized, plus insulin.

p < 0.01 versus hypophysectomized, plus insulin.

p > 0.05 versus hypophysectomized + insulin + GH + cortisone.

Discussion

The effect of growth hormone in vivo on transport is the same as that of a very low concentration of insulin. As a result, glucose uptake is accelerated in the more sensitive muscle from the hypophysectomized animal (8, 9, 16) and is affected slightly, if at all, in muscle from normal or diabetic animals. The effect apparently is directly on the transport process and does not depend on the mobilization of tissue-bound insulin, as has been suggested (17, 18), inasmuch as it occurs in the muscle from diabetic-hypophysectomized rats. It should be noted, however, that concentrations of hormone were used in these studies that were many times greater than equally effective concentrations of insulin. Thus, contamination of growth hormone by a very active insulin-like substance is difficult to exclude. Previous efforts by Park et al. (3) to separate such material from growth hormone preparations were not successful, but the studies of Ottaway and Paul (19) continue to suggest this possibility.

The early stimulatory effect of growth hormone in vivo on glucose uptake also seems to be caused by a direct effect of the protein on the transport system because it occurs in animals lacking the capacity to secrete insulin. This is shown in the present experiments with diabetic-hypophysectomized rats, which confirm earlier studies with depancreatized-hypophysectomized dogs (20) and hypophysectomized-ecinercate rats (3). It may be noted, however, that uptake is also stimulated in the normal muscle after growth hormone in vivo, whereas no response is obtained with addition of the protein in vitro to normal muscle. This difference may be caused simply by the longer exposure time and superior physiological conditions of the experiments in vivo. Normal muscle is not completely refractory to the stimulatory effect of growth hormone in vitro because Ottaway (18) has observed stimulation of glucose uptake under special conditions and with high concentrations of the protein. None of these experiments, however, rules out the possibility that growth hormone injection may also stimulate pancreatic release of insulin. In this connection, growth hormone administration is known to cause hypertrrophy of the beta cells of the rat islets. Whether or not this is a direct effect or is secondary to an elevation of blood glucose is uncertain.

Prolonged administration of growth hormone apparently reduces the sensitivity of transport to insulin, as seen in the present studies, or to the immediate acceleratory effect of growth hormone itself, as suggested in earlier studies (3). These observations fit well with a growth hormone-induced decrease in the insulin sensitivity of the whole animal observed by de Bodo et al. (21), and with the well known insulin insensitivity seen in many cases of acromegaly. Hydrocortisone also diminishes insulin sensitivity and its effect is additive or synergistic with that of growth hormone. Enhancement of the anti-insulin effect of growth hormone by adrenal steroids has been noted earlier (3).

Presumably, the relative insensitivity of muscle from normal as compared to that from hypophysectomized rats is caused by endogenous growth hormone and adrenal steroid secretions. It should be emphasized, however, that effects on transport constitute only one aspect of insulin insensitivity with respect to peripheral glucose uptake. Morgan et al. (22) have shown a depression of phosphorylation by growth hormone which contributes to insulin insensitivity, as will be described in the paper to follow (11).

The present work has shown no consistent late depression of glucose uptake in distinction to earlier studies with the rat diaphragm (3). We suggest that the diaphragm results, although technically correct, did not reflect the physiological regulation of uptake as reliably as do the present studies with the perfused heart. Legitimate objections have been raised with regard to the diaphragm preparation used at the earlier time, including the presence of cut edges, excessively long diffusion pathways from medium to cells, and transport instability (5, 9, 10).

A general concept of the action of growth hormone on glucose uptake will be discussed in the paper which follows (11).

Summary

The effect of growth hormone on the transport of glucose through the cell membrane has been studied with the isolated, perfused rat heart as the test object. The following conclusions have been reached.

1. Growth hormone in vitro has a weak stimulatory action on transport that causes an acceleration of glucose uptake. This insulin-like effect is readily observed in the hypersensitive muscle from hypophysectomized rats. The effect does not depend on the mobilization of tissue bound insulin.

2. A similar effect is also observed 1 hour after administration of growth hormone in vivo and is probably caused by a direct action on the muscle. An additional stimulation, however, secondary to the release of pancreatic insulin, cannot be excluded.

3. As a late effect, growth hormone in vivo reduces the sensitivity of transport to stimulation by insulin. Hydrocortisone...
has a similar activity and its effect is additive to that of growth hormone.

The previously reported depression of glucose uptake in the isolated rat diaphragm as a late effect of growth hormone in vivo is not thought to be physiologically significant and has not been observed in the heart.

4. The effects of growth hormone on the isolated heart correlate well with recent observations from other laboratories on the effect of growth hormone on the peripheral utilization of glucose in intact animals.

Addendum—It was of interest to determine whether growth hormone and cortisone in vivo would have any late effects on glucose transport in a situation in which insulin secretion could not be stimulated. Hypophysectomized-diabetic rats were, therefore, injected with these hormones and uptake was measured 24 hours later. Growth hormone was injected in a dosage of 0.1 mg per 100 g at 24 hours and again at 12 hours before killing. The dosages of cortisone were 2.5 mg per 100 g at 24 hours and 1.25 mg at 12 hours and 4 hours. Uptake was estimated over a 30-minute period in a medium containing 100 mg per 100 ml of glucose but no insulin.

The following rates were observed (in milligrams per gram per hour): control, 3.6 ± 0.9 (6); GH, 2.2 ± 0.5 (4); cortisone, 2.0 ± 0.7 (7); and GH plus cortisone, 1.9 ± 0.6 (7). These rates reflected transport activity since no intracellular glucose could be found in any instance.

It was obvious that the early stimulation of transport by growth hormone had disappeared during this period of time. The data suggested, in fact, that some depression had been induced, but the differences from the control rate were not statistically significant because of the scatter of individual values and the small number of hearts tested. A depression, if present, could be best explained as follows. A very low level of insulin secretion presumably persists after alloxan treatment and exerts a slight stimulatory effect on transport. Growth hormone or cortisone, or both, in the dosage employed block this stimulation. In the normal or hypophysectomized preparation, on the other hand, transport is usually not depressed because of a compensatory increase in insulin secretion. It would appear unlikely that growth hormone and cortisone inhibit glucose transport directly in view of the results to be presented in the accompanying paper (11).

REFERENCES

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