TEMPORAL RELATIONSHIP OF THE GROWTH HORMONE EFFECTS
ON AMINO ACID TRANSPORT AND PROTEIN SYNTHESIS
IN ISOLATED RAT DIAPHRAGM

By

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ABSTRACT

Experiments were performed to study whether the influence of bovine growth hormone (GH) on the membrane transport of labelled leucine and glycine in the isolated rat diaphragm was similar to that previously found for α-aminoisobutyric acid (Hjalmarson & Ahrén 1967a, b). The relationship between the effects of GH on amino acid transport and protein synthesis was also studied.

Addition of GH in vitro (25 μg/ml) to intact hemidiaphragms from hypophysectomized rats increased the accumulation of glycine in the intracellular water after 2 hours of incubation, while that of leucine was reduced. GH in vitro increased the incorporation rate into muscle protein of both glycine and leucine.

An Intravenous (i. v.) injection of GH (10 μg) to hypophysectomized rats 60 min. before incubation increased the distribution ratio of leucine, while no significant effect was found on the incorporation into protein of this amino acid. On the other hand, an injection of GH (10 μg) 180 min. before incubation increased the in vitro incorporation of both leucine and glycine. This injection did not change the distribution ratio of glycine and that of leucine was significantly decreased.

Repeated injections of GH (50 μg × 4 days) increased the incorporation of both glycine and leucine. This treatment also increased the accumulation of glycine after 2 hours of incubation, while no such effect was seen on the accumulation of leucine.

In vitro addition of GH (25 μg/ml) did not significantly change the distribution ratio of glycine and leucine in diaphragms from hypophysectomized rats previously treated with GH. However, addition of GH in vitro to the diaphragms from these rats further increased the incorporation of glycine into protein. In addition, GH in vitro increased the accumulation of glycine also when the incorporation of this amino acid into protein was completely blocked by puromycin (500 μg/ml).
The present results show that GH, at least in certain doses, may have a biphasic action on the membrane transport of normal amino acids. The results also indicate that GH may have separate effects on the membrane transport and the incorporation into protein of amino acids.

*In vivo* administration of growth hormone (GH) to hypophysectomized rats was recently found to produce two different effects on the membrane transport of the model amino acid α-aminoisobutyric acid (AIB) in the isolated diaphragm (Hjalmarson & Ahrén 1967a, b). A stimulatory effect was obtained when the hormone was injected intravenously less than 3 hours before the start of incubation, while no such effect was seen when GH was injected 3 to 38 hours before the *in vitro* experiment or when repeated injections of the hormone were given for several days. This treatment, however, made the diaphragm refractory to GH subsequently added *in vitro*.

The present experiments were undertaken to study whether the influence of GH on the membrane transport of normal amino acids in the isolated rat diaphragm was similar to that previously found for AIB. Thus, the stimulatory effect of GH on the transport of glycine and leucine was studied and compared to that of AIB with respect to time of onset and duration. It was studied if pretreatment of the hypophysectomized rats with GH could also produce at late inhibitory effect on the transport of glycine and leucine with refractoriness of the diaphragm to further addition of the hormone *in vitro*. Furthermore, it was investigated whether the effects of GH on the amino acid transport and incorporation into muscle protein could be separated temporally. The dependency of the amino acid transport upon the protein synthesis was also studied in experiments where protein synthesis was blocked by puromycin.

**Methods**

*Animals*

Female rats of the Sprague-Dawley strain were maintained on a semisynthetic diet (Gustavsson 1959; Ahrén 1959) and *water ad libitum*. Hypophysectomy was performed by the standard parapharyngeal approach at the age of 4—5 weeks (58—73 g). The completeness of hypophysectomy was checked as described previously (Hjalmarson & Ahrén 1967a). None of the hypophysectomized rats gained more than 5 g in weight during the period between the hypophysectomy and the *in vitro* experiments (14—15 days). The body weights of the rats at autopsy were 60—76 g.

*Chemicals*

The radioactive substances were obtained from the Radiochemical Centre, Amersham, England and from the New England Nuclear Corp., Boston, USA. The substances were used with the following specific activities: glycine-2-14C, 5.0 μc/μmole; L-leucine-U-14C, 25 μc/μmole; glycine-2-3H, 885 μc/μmole; L-leucine-G-3H, 200 μc/μmole. The iso-
topes were added to the medium to make a molarity of 0.1 mM for glycine-2-\textsuperscript{14}C, 0.025 mM for L-leucine-G-\textsuperscript{3}H, and 0.01 mM for glycine-2-\textsuperscript{3}H and L-leucine-U\textsuperscript{14}C.

Bovine growth hormone (NIH-GH-B-6) was supplied by the Endocrinology Study Section of the National Institutes of Health, USA. The hormones was dissolved in 0.9\% NaCl at pH 10 (1.0—2.5 mg/ml) and diluted to the final concentration with neutral 0.9\% NaCl for the \textit{in vitro} administrations and with Krebs bicarbonate buffer for the \textit{in vivo} experiments. When given in vivo, GH was injected intravenously (i. v.) under light ether anesthesia into a tail vein in a volume of 0.2 ml or intramuscularly (i. m.) in a volume of 0.1 ml.

Puromycin was used as puromycin dihydrochloride obtained from Nutritional Biochem. Corp., Cleveland, USA and was added to the incubation medium in a concentration of 500 µg/ml.

\textbf{Incubation procedure}

The rats were killed by cervical fracture and the intact hemidiaphragm was dissected out and incubated as described in a previous paper (Hjalmarson \& Ahrén 1967a). Incubation was carried out in Krebs bicarbonate buffer, pH 7.4, containing glucose (2.5 mg/ml) and equilibrated with 95\% O\textsubscript{2}—5\% CO\textsubscript{2}. The muscles were preincubated for 10 min. in 10 ml medium in 25 ml flasks. The final incubation was carried out for 2 hours in 4 ml glucose buffer containing the labelled substances with and without GH (25 µg/ml) and puromycin (500 µg/ml). Before the incubations all flasks (25 ml) were gassed with 95\% O\textsubscript{2}—5\% CO\textsubscript{2}, sealed and placed in a gyratory shaking bath at 37°C. After the final incubation, each diaphragm was dissected free from its rib-cage, rinsed rapidly in buffer, blotted, weighed and homogenized in 1 ml of 10\% trichloroacetic acid (TCA).

\textbf{Determination of the intracellular accumulation of the labelled glycine and leucine}

The muscles and incubation media were processed and counted to measure intracellular accumulation of the labelled amino acids by methods described previously (Hjalmarson \& Ahrén 1967a, b). The intracellular accumulation of the substances is expressed as the distribution ratio of radioactivity between the intracellular and extracellular compartments (cpm/ml intracellular water : cpm/ml medium). In all calculations the extracellular space was considered to be 12\% and the total tissue water 77\% of the wet tissue weight (Hjalmarson \& Ahrén 1967a, b; Hjalmarson 1968).

Since normal amino acids can be metabolized to some extent in muscle tissue it is possible that some of the radioactivity in the intracellular water did not represent the original amino acids but their various metabolites. However, after incubation of the diaphragm with labelled glycine and leucine, several authors have reported that with chromatographic analysis at least 90—95\% of the radioactivity in the intracellular water still belongs to the amino acid originally added (Manchester \& Young 1960; Kipnis et al. 1961; Akedo \& Christensen 1962).

\textbf{Determination of incorporated radioactivity in muscle protein}

The method for determining the incorporation of \textsuperscript{14}C-labelled amino acids into the muscle protein was a slight modification of the method described by Manchester \& Young (1958), which has been described in detail in a previous paper from our laboratory (Arvill \& Ahrén 1967). The samples were counted in the scintillation spectrometer with and without internal standard for correction of quenching. The amount of radioactivity incorporated into protein was calculated and expressed as µmoles of the amino acid, from which it originated, per gram protein.
The following method was used for determining the incorporation of $^3$H-labelled amino acids into protein. The muscle TCA precipitate containing protein was washed with cold TCA in excess of non-labelled amino acids. The precipitate was then kept in TCA at 90°C for 10 min. and after centrifugation the precipitate was washed 3 times with ethanol-ether-chloroform (2:2:1). After the final wash the precipitate was dissolved in 1 ml of 1 N NaOH and boiled for 5 min. The concentration of protein in the NaOH solution was measured according to Lowry et al. (1951). The radioactivity of the NaOH solution was determined by the Schöniger combustion technique (Schöniger 1955) in a modified form (Hamberger et al., to be published) and liquid scintillation counting. The samples were corrected for quenching by internal standardization. The incorporated radioactivity was then calculated and expressed as disintegrations per minute (DPM)/100 µg protein.

Statistical analysis
Mean values are given ± the standard error of the mean (S.E.). Differences between mean values were analysed according to Student's t-test and by the method of paired analyses (Snedecor 1956). A p-value of 0.05 or less is considered significant in this study.

RESULTS

Effects of GH on the accumulation of glycine and leucine
Table 1 shows that addition of GH (25 µg/ml) to the isolated diaphragms from hypophysectomized rats significantly increased the accumulation of glycine-$^{14}$C in the intracellular water after 2 hours of incubation. The distribution ratio of leucine-$^{14}$C showed a significant reduction when GH was added in vitro.

When a single i. v. injection of GH in a dose of 10 µg was given to the hypophysectomized rats 60 min. before incubation, the accumulation of leucine-$^3$H by the diaphragm after 2 hours of incubation was increased (Fig. 1), while no effect was seen on the distribution ratio of glycine-$^3$H (Table 3). However, a similar injection of GH in a higher dose (50 µg) also significantly increased the accumulation of glycine-$^3$H (Table 3). An i. v. injection of the hormone (10 µg) 180 min. before incubation significantly reduced the accumulation of leucine-$^3$H after 2 hours of incubation (Fig. 1), while no change in the distribution ratio of glycine-$^3$H was seen (Table 3).

Injections of GH (50 µg/day) for 4 days to hypophysectomized rats increased the in vitro accumulation of glycine-$^{14}$C in the diaphragm after 2 hours of incubation, while no changes was seen in the distribution ratio of leucine-$^{14}$C (Table 1).

Tables 1 and 3 show that addition of GH in vitro did not change the distribution ratios of labelled glycine and leucine in diaphragms from the rats given repeated daily injections of GH or a single i. v. injection of GH 180 min. before incubation.
Effects of GH on the incorporation into protein of glycine and leucine

The incorporation rates of radioactivity from the labelled amino acids are calculated and expressed as μmoles of the labelled amino acid incorporated per gram protein after 2 hours of incubation (Table 2) or as disintegrations per minute (DPM)/100 μg protein (Table 3 and Fig. 1).

Addition of GH (25 μg/ml) to the isolated diaphragms from hypophysectomized rats significantly increased the incorporation rate into protein of both glycine-14C and leucine-14C. The injections of GH (50 μg/day) for 4 days to hypophysectomized rats also increased the in vitro incorporation of the amino acids into muscle protein.

A single i. v. injection of GH in a dose of 10 μg given 180 min. before incubation stimulated the in vitro incorporation rates of both glycine-3H (Table 3) and leucine-3H (Fig. 1), while no such effects were seen when the same dose of GH was given 60 min. before incubation. However, a higher dose of GH (50 μg) injected 60 min. before the in vitro experiment, increased the incorporation of glycine-3H into protein.

Addition of GH in vitro (25 μg/ml) to diaphragms from the hypophysectomized

Table 1

Effects of GH in vivo and in vitro on the uptake of glycine-14C and leucine-14C by the diaphragm from hypophysectomized rats with and without puromycin1).

<table>
<thead>
<tr>
<th>Labelled</th>
<th>In vivo² treatment</th>
<th>Distribution ratio³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>aminoacid</td>
<td>Control in vitro</td>
<td>Control GH 25 μg/ml</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.19 ± 0.08 (9)</td>
<td>2.76 ± 0.12 (9)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.005</td>
<td>p&lt;0.005</td>
</tr>
<tr>
<td></td>
<td>GH 50 μg × 4 days</td>
<td>N. S.</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.24 ± 0.05 (8)</td>
<td>1.01 ± 0.10 (8)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GH 50 μg × 4 days</td>
<td>N. S.</td>
</tr>
</tbody>
</table>

¹) Intact hemidiaphragms from hypophysectomized rats were incubated for 2 hours with glycine-14C (0.1 mM) and leucine-14C (0.01 mM) with and without bovine GH (25 μg/ml) and puromycin dihydrochloride (500 μg/ml).

²) Bovine GH was injected i. m. for 4 days before the in vitro experiment. The last injection was given approximately 3 hours before start of incubation.

³) Mean value ± S. E. Number of muscles in parenthesis. The injections of GH significantly increased the uptake of glycine-14C in the muscles incubated without GH (p<0.01).
rats given repeated injections of GH, further increased the incorporation of glycine-14C, while no such effect was seen on the incorporation of leucine-3H. No further increase in the incorporation rate of glycine-3H was seen by addition of GH in vitro to diaphragms from hypophysectomized rats injected with 50 µg of GH 60 min. before incubation or 10 µg of the hormone 180 min. before incubation.

Effects of puromycin in vitro
Puromycin was added to the medium to a concentration of 500 µg/ml, which in each experiment was found to completely block the incorporation of the labelled amino acids into the muscle protein. Negligible amounts of radioactivity were, thus, found in the protein fractions after the addition of puromycin.

Table 1 shows that addition of GH in vitro (25 µg/ml) also in the presence of puromycin increased the distribution ratio of glycine-14C in the diaphragm from hypophysectomized rats not pretreated with GH. No such effect of GH was seen on the accumulation of leucine-14C after 2 hours of incubation. Neither did GH in vitro in presence of puromycin increase the distribution ratios of glycine-14C and leucine-14C in diaphragms from the hypophysectomized rats repeatedly

<table>
<thead>
<tr>
<th>Table 2</th>
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</table>

Effects of GH in vivo and in vitro on the incorporation of glycine-14C and leucine-14C into the protein of the diaphragm from hypophysectomized rats.

<table>
<thead>
<tr>
<th>Labelled amino acid</th>
<th>In vivo treatment²)</th>
<th>Incorporation rate³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>GH 25 µg/ml</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.16±0.01 (9)</td>
<td>0.24±0.01 (9)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.21±0.01 (4)</td>
<td>0.31±0.03 (4)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>0.10±0.01 (5)</td>
<td>0.18±0.01 (5)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.15±0.01 (5)</td>
<td>0.15±0.01 (5)</td>
</tr>
<tr>
<td></td>
<td>N.S.</td>
<td></td>
</tr>
</tbody>
</table>

¹) Intact hemidiaphragms from hypophysectomized rats were incubated for 2 hours with glycine-14C (0.1 mM) or leucine-14C (0.01 mM). Bovine GH was added in a concentration of 25 µg/ml.

²) Bovine GH was injected i. m. for 4 days before the in vitro experiment. The last injection was given approximately 3 hours before start of incubation.

³) The incorporation of radioactivity is calculated and expressed as µmoles of the labelled amino acid per gram protein. Mean value ± S. E. Number of muscles in parenthesis. The injections of GH significantly increased the incorporation rate of the muscles incubated without GH (p<0.01).
injected with GH. The distribution ratio of leucine-\(^{14}\)C was significantly increased in all groups when puromycin was added to the medium.

**Temporal relationship between GH effects on the accumulation and incorporation into protein of glycine and leucine**

Table 3 shows that the injection of GH in a dose of 50 \(\mu\)g given 60 min. before incubation significantly increased the distribution ratio and incorporation rate of glycine-\(^{3}\)H after 2 hours of incubation. A corresponding injection of GH in a dose of 10 \(\mu\)g did not give these effects. However, when 10 \(\mu\)g of GH was injected 3 hours before incubation, the hormone significantly increased the incorporation rate of the amino acid, while no change was seen on the accumulation of glycine-\(^{3}\)H in the cell water.

From Fig. 1 it can be seen that an i. v. injection of GH in a dose of 10 \(\mu\)g to the hypophysectomized rats 60 min. before start of incubation increased the distribution ratio of leucine-\(^{3}\)H in the diaphragms, while no significant effect was found on the incorporation into protein of this amino acid. However, when the

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**Table 3**

Effects of GH, injected i. v. 60 or 180 min. before incubation and added *in vitro*, on the accumulation and incorporation into protein of glycine-\(^{3}\)H in diaphragms from hypophysectomized rats\(^1\).

<table>
<thead>
<tr>
<th>In vitro treatment(^2)</th>
<th>Distribution ratio(^3)</th>
<th>Incorporation rate(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>In vitro</em></td>
<td>GH 25 (\mu)g/ml</td>
</tr>
<tr>
<td>Control</td>
<td>1.17 ± 0.02</td>
<td>1.35 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.005</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>GH 10 (\mu)g i. v. 60 min. before</td>
<td>1.14 ± 0.06</td>
<td>1.39 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.005</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>GH 50 (\mu)g i. v. 60 min. before</td>
<td>1.72 ± 0.10(^a)</td>
<td>1.92 ± 0.15(^a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GH 10 (\mu)g i. v. 180 min. before</td>
<td>1.23 ± 0.05</td>
<td>1.25 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

\(^1\) Intact hemidiaphragms from hypophysectomized rats were incubated for 2 hours in glucose buffer (2.5 \(\text{mg/ml}\)) containing glycine-\(^{3}\)H (0.01 \(\text{mM}\)). Bovine GH was added in a concentration of 25 \(\mu\)g/ml.

\(^2\) Bovine GH (10 or 50 \(\mu\)g) was injected i. v. 60 or 180 min. before start of incubation. The control rats were injected with 0.2 ml 0.9 \(/\text{NaCl}\) i. v. 60 min. before incubation.

\(^3\) Mean value ± S. E. There are 5 muscles in each group. The incorporation of radioactivity into proteins is expressed as DPM/100 \(\mu\)g protein.

\(^a\) Significantly different from the value of the corresponding muscles from the rats not injected with GH (p<0.001).
Effects of an i. v. injection of GH (10 μg) given to hypophysectomized rats 60 and 180 min. before incubation on the accumulation in cell water and incorporation into protein of leucine-$^3$H in the diaphragm. The control rats were injected with 0.2 ml 0.9 % NaCl i. v. 60 min. before incubation. The incubation of the intact hemidiaphragms was carried out for 120 min. There are 5 muscles in each group and the standard error is indicated at the top of each bar. The injection of GH 60 min. before incubation increased the accumulation of leucine-$^3$H (p<0.001). The injection of GH 180 min. before incubation decreased this accumulation rate (p<0.05), while the incorporation rate was increased (p<0.05). The incorporation rate of leucine-$^3$H into protein is expressed as DPM/100 μg protein.

The same dose was injected 180 min. before incubation the incorporation rate of leucine-$^3$H was increased and the distribution ratio was significantly decreased.

Thus, after a single i. v. injection of GH in a dose of 10 μg, the effects of the hormone on the membrane transport and on the incorporation into protein of the amino acids in the diaphragm could be separated temporally.

**DISCUSSION**

When studying the membrane transport of normal amino acids it is important to bear in mind that the intracellular accumulation of these amino acids is not only dependent upon the rate of inward and outward transport, but also upon the rate
of synthesis and break down of protein and upon the utilization of these substances e.g. for supply of energy. Furthermore, in studies using labelled amino acids the accumulation of radioactivity rather than amino acid is studied and there is no assurance that the ratio of radioactivity between intra- and extracellular water after incubating diaphragm really reflects the concentration ratio for the unlabelled amino acid. As mentioned under “Methods”, however, after incubation of the diaphragm with labelled glycine and leucine, chromatographic analysis of the muscle extracts have shown that at least 90—95% of the radioactivity in the intracellular water still belongs to the amino acid originally added. From these results it was assumed that the amount of radioactivity in the intracellular water and in the protein fraction reflects the amount of labelled amino acid in these fractions. For the interpretation of the present results, it has generally been supposed that an increase in the accumulation of glycine and leucine in the intracellular water, expressed as distribution ratio, is due to a stimulation of the inward transport of the amino acids, while a decrease in these accumulations is supposed to be secondary to an increased rate of incorporation of the amino acids into the muscle protein. An increased intracellular accumulation of the labelled amino acids could, however, also be due to a decreased incorporation rate of these amino acids into protein. In fact, this is the case in the experiments with puromycin. Furthermore, an inhibition of the net inward transport, or changes in the utilization of the amino acids could also influence upon the intracellular concentrations of the labelled amino acids.

From the present investigation it is obvious that a single i. v. injection of GH given 60 min. before incubation of the diaphragm produced other effects on the membrane transport and the incorporation into protein of glycine and leucine than did a similar injection of GH 180 min. before incubation. The injection of 10 µg of GH 60 min. before the in vitro experiment increased the accumulation of leucine in the diaphragm after 2 hours of incubation, while a decrease in this accumulation was seen when the hormone was injected 180 min. before incubation. None of these injections increased the distribution ratio of glycine, although the injection of GH 180 min. before the in vitro experiment abolished the stimulatory effect of GH subsequently added in vitro. The decrease in the distribution ratio of leucine and the failure of GH added in vitro, to stimulate the accumulation of glycine could, however, be due to the fact that the injection of GH 180 min. before incubation markedly increased the incorporation rates of both amino acids into the muscle protein. The injection of GH in a dose of 10 µg 60 min. before incubation did not stimulate the incorporation rate of glycine and leucine, which is notable. Thus, there are several data in the present study indicating that GH, at least in certain doses, has a biphasic action on the membrane transport of naturally occurring amino acids. In this connection it could be mentioned that a biphasic action of GH has been reported on the plasma level of amino acids in both hypophysectomized and diabetic animals (e.g. Li et al. 1949; Lotspeich 1950).
It has been suggested that the stimulatory effect of GH on the incorporation rate of amino acids into muscle protein is not secondary to the effect of the hormone on the intracellular accumulation of amino acids (Kostyo 1964; Knobil 1966). This idea is supported by the present results showing that GH, injected daily for several days or given as a single i.v. injection to hypophysectomized rats or added in vitro to the diaphragm, could increase the incorporation rate of radioactivity from leucine and glycine into muscle protein, when no change or even a decrease in the intracellular concentration of the amino acids was seen. Similar results have also been obtained with insulin in isolated muscle tissue (Manchester & Krabl 1959; Wool 1965; Arvill & Abrén 1967).

The present experiments with puromycin indicate that GH could produce a stimulatory effect on the membrane transport of glycine independent of an effect of the hormone on the protein synthesis. Puromycin in a concentration of 500 μg/ml medium completely blocked the effect of GH on the incorporation of radioactivity from the amino acid into protein. Similar results showing hormonal effects on the membrane transport of amino acids in muscle tissue independent of the protein synthesis have been reported by several authors for both GH and insulin (Fritz & Knobil 1963; Carlin & Hechter 1964; Scharff & Wool 1965; Knobil 1966). In all these studies, however, puromycin and the hormones were both initially present in the medium and it is possible that the hormones may exert some unmeasurable influence upon protein synthesis before the inhibition with puromycin was complete. Kostyo (1968) found that if the diaphragms were preincubated for one hour in buffer containing puromycin (185 μg/ml) before GH and AIB were added to the medium, the hormone had no effect on the AIB transport. Similar results were obtained from our laboratory after preincubation of the diaphragm with puromycin (200 μg/ml) for 3 hours before the effect of GH was studied on the AIB transport (Hjalmarson 1968). It is, however, possible that GH may stimulate the membrane transport of amino acids into the muscle cells by different mechanisms. An increased transport rate could be the result of hormonal activation of available enzymes involved in the amino acid transport processes, but it could also be due to formation of new protein or peptide molecules required for the transport of amino acids across the cell membrane. Preincubation with puromycin for 1—3 hours may block the continual synthesis of transport proteins so that there will be a deficiency state in the cell membranes of the muscle with refractoriness to subsequent hormonal stimulation. The fact that the normal operation of the amino acid transport processes requires continual synthesis of protein has been reported by several authors (Kostyo & Redmond 1966; Elsas et al. 1967; Arvill & Abrén 1967).

Repeated injections of GH (50 μg × 4 days) to the hypophysectomized rats significantly increased the accumulation of glycine in the isolated diaphragm, while no such effect was found on the accumulation of leucine or, as recently reported, on the uptake of AIB (Hjalmarson & Abrén 1967a). However, addition
of GH in vitro to the diaphragms or an i. v. injection of GH 60 min. before incubation to hypophysectomized rats not previously treated with GH, increased the intracellular accumulation of these three amino acids as well as of several other naturally occurring amino acids (for ref. see Knobil & Hotchkiss 1964; Engel & Kostyo 1964). The fact that under certain conditions GH could increase the accumulation of glycine but not that of leucine and AIB favours the interpretation that there are different transport systems for amino acids in the muscle cell membrane.

Independent of the mode of administration of GH, in vivo to the hypophysectomized rats or in vitro to the diaphragm, the hormone increased the incorporation rate of radioactivity from the labelled glycine and leucine into the muscle protein. These observations are in agreement with previous reports (for ref. see Knobil & Hotchkiss 1964; Engel & Kostyo 1964). It was also found that although the in vitro incorporation rate of glycine into the protein of the diaphragm was increased by pretreatment of the hypophysectomized rats with GH for several days, addition of the same hormone in vitro could further stimulate this incorporation. Contrary to this finding addition of GH in vitro to the diaphragm did not increase the accumulation of leucine and glycine in the intracellular water after in vivo pretreatment with GH.

The present study shows some differences between the effects of GH on the membrane transport and on the incorporation into protein of glycine and leucine with respect to time of onset of the effects. In this connection it could be mentioned that there are various effects of GH which can be arranged in temporal sequence. For example, the early insulin-like effect of GH with increased transport of sugars and amino acids into muscle tissues could be seen within 30 min. (e.g. Henderson et al. 1961; Kostyo 1968), while the lipidmobilizing effect of GH has a lag period of several hours (e.g. Swislocki & Szego 1965; Fain et al. 1965). Furthermore, a significant activation of muscle RNA synthesis does not occur until several hours after the administration of GH (Florini & Breuer 1966), and the effect on the in vitro incorporation of thymidine-3H into costal cartilage from hypophysectomized rats was not seen until 24 hours after injection of GH (Daughaday & Reeder 1966). It does not seem very likely that GH initiates its various effects in a temporal sequence from perhaps a few minutes to 24 hours after the administration of the hormone. It is therefore tempting to suggest that even the most diverse effects of GH may be causally related and could be traced back to an early interaction between GH and susceptible tissue. In view of this, further clarification of the sequence of reactions induced by GH during the latent period from the initial hormone-receptor interaction in the tissues to the onset of its various effects is necessary to explain the mechanism of action of GH.
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