

Further Evidence for Amino Acid Transport as a Site of Action of Growth Hormone.* (27403)

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Hypophysectomy decreases and the addition of growth hormone *in vitro* markedly increases the penetration of the nonmetabolizable amino acid α -aminoisobutyric acid (AIB) into the cells of the isolated "intact" rat diaphragm(1,2). These observations have been confirmed using *in vivo* experiments wherein the AIB was injected into rats following hypophysectomy or growth hormone administration(3). Since the intracellular accumulation of AIB under the influence of growth hormone occurs against a concentration gradient it was considered that this action of growth hormone may represent the stimulation of a system which "actively" transports the amino acid into muscle cells. The formulation of a physiologically meaningful hypothesis relating these observations to the site and mode of action of growth hormone in the regulation of protein metabolism requires the demonstration that growth hormone stimulates the transport of naturally occurring amino acids as well as that of the "model amino acid", AIB, heretofore employed. The following study was undertaken to ascertain, using the indirect criterion of competitive inhibition, whether growth hormone can influence the transport of some natural amino acids.

Methods. The methods used in the following experiments for measurement of the distribution of AIB-1-C¹⁴ between the extracellular and intracellular compartments of the isolated, intact rat diaphragm have been described in detail(1,2). Fed, hypophysectomized female rats of the Charles River strain weighing 60-70 g were used 3 weeks following the operation. Their diaphragms were removed with the rib cage attached and in-

cubated with shaking for 2 hours at 37°C in Krebs bicarbonate buffer, pH 7.4, in the presence of .05 mM AIB-1-C¹⁴ (25,000 c.p.m./ml), glucose (.01M) and an atmosphere of 95% O₂ and 5% CO₂. This was preceded by a 10-minute preincubation period in buffer alone. Bovine growth hormone (NIH-BGH-2) when added was present at a concentration of 25 μ g/ml of medium. Competitive inhibition of AIB-1-C¹⁴ transport by the following non-labelled amino acids was studied by their addition to the above systems in a concentration of 5 mM: glycine, L-leucine, L-phenylalanine and L-glutamic acid. Results are expressed as distribution ratios of AIB-1-C¹⁴

$$\left(\frac{\text{c.p.m./ml intracellular H}_2\text{O}}{\text{c.p.m./ml incubation medium}} \right).$$

Results. As previously reported, addition of growth hormone to the intact, isolated diaphragm of the hypophysectomized rat nearly doubles the accumulation of AIB by muscle cells (Fig. 1 and 2). Fig. 1 illustrates the finding that this stimulation of AIB-1-C¹⁴ transport was virtually abolished by addition of non-labelled leucine and glycine to the incubation medium. Addition of these amino acids to the diaphragms in the absence of growth hormone resulted in a small but consistent inhibition of the intracellular accumulation of AIB. In contrast, addition of phenylalanine and glutamic acid did not significantly ($P > .1$) inhibit the increased intracellular accumulation of AIB effected by addition of growth hormone (Fig. 2).

Discussion. It may be inferred, from the observation that glycine and leucine inhibit the entry of AIB into muscle cells, that the transport system for AIB is shared by these 2 natural amino acids. It follows further, that if growth hormone accelerates AIB transport it must, in all probability, also accelerate the transport of glycine and leucine. Similar relationships between other natural

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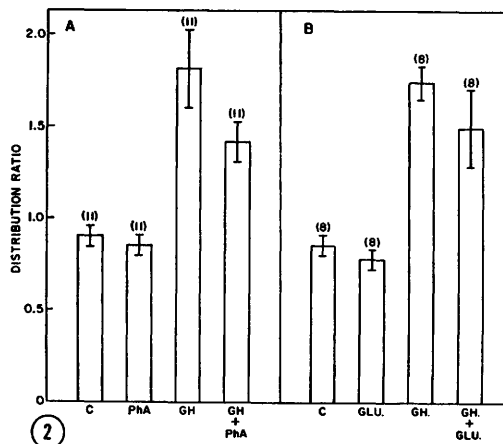
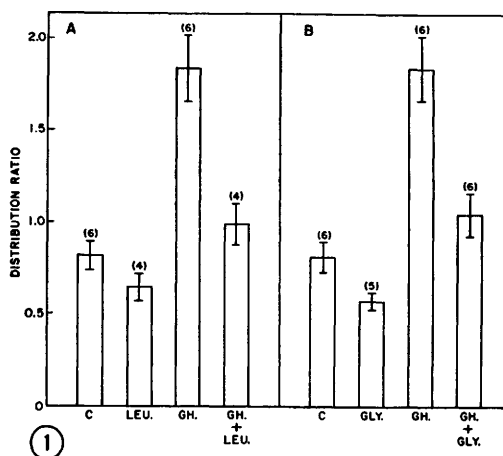


FIG. 1. Distribution ratio of AIB-1-C¹⁴ in isolated "intact" diaphragms of hypophysectomized rats (C) and effect thereon of added bovine growth hormone (GH), leucine (LEU) and glycine (GLY) in presence and absence of growth hormone. Vertical lines represent stand. errors of mean and numbers in parentheses indicate number of observations.

FIG. 2. Distribution ratio of AIB-1-C¹⁴ in isolated "intact" diaphragms of hypophysectomized rats (C) and effect thereon of added bovine growth hormone (GH), phenylalanine (PhA) and glutamic acid (GLU) in presence and absence of growth hormone.

amino acids and AIB transport have recently been observed (Peckham and Knobil, unpublished). Phenylalanine and glutamic acid, on the other hand, did not significantly compete with the transport of AIB into muscle cells under the condition of these experiments, suggesting that these amino acids are transferred into the cells by a system other than that utilized by AIB, glycine and leucine. Of interest in this regard are the ob-

servations that bovine growth hormone added to diaphragms of hypophysectomized rats *in vitro* increases the incorporation of glycine-2-C¹⁴ and leucine-2-C¹⁴ into muscle protein but not that of glutamic acid-2-C¹⁴ (4). This apparent parallelism between the stimulation of the entry of an amino acid into the intracellular pool and its accelerated incorporation into protein by addition of growth hormone, while suggestive of a primary site of action of the hormone on an amino acid transport mechanism, nevertheless raises the possibility that the previously reported effect of growth hormone on incorporation of labelled amino acids into protein *in vitro* (4,5,6) may have been due to an increase in specific activity of the intracellular amino acid pool rather than to a stimulation of protein synthesis. This question is being investigated.

Summary and conclusions. Addition of growth hormone to intact, isolated diaphragms of hypophysectomized rats accelerates the transport of AIB-1-C¹⁴ (α -amino-isobutyric acid) into the intracellular compartment of muscle cells. This is competitively inhibited by leucine and glycine but not by phenylalanine or glutamic acid. These findings suggest that growth hormone stimulates the transport of some, but not all, naturally occurring amino acids as well as that of the nonmetabolizable amino acid AIB. These findings further support the hypothesis that amino acid transport may be a site of action of growth hormone in the regulation of protein metabolism.

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