Role of Glucagon on the Control of Hepatic Protein Synthesis and Degradation in the Rat in Vivo*

MATILDE S. AYUSO-PARRILLA, ANGELES MARTÍN-REQUERO, JULIO PEREZ-DÍAZ, AND ROBERTO PARRILLA
From the Department of Metabolism, Institute G. Marañón, Consejo Superior de Investigaciones Científicas, Madrid, Spain

The effect of glucagon on hepatic protein synthesis and proteolysis has been investigated. The intraperitoneal administration of 200 μg of glucagon produced an increase of the polypeptide chains completion time which was maximal 5 min after its administration and approached control values at 20 min. The increase of the polypeptide chains completion time observed at 5 min after the hormone administration represents a 38% inhibition of the hepatic protein synthetic rate. When glucagon was continuously supplied by intravascular infusion, maximal inhibition was attained throughout the experiment. This inhibition of protein synthesis brought about by glucagon was accompanied by an increase in the polyribosomal state of aggregation, indicating that the hormone acts mainly if not exclusively, on the elongation or termination step, or both.

The administration of glucagon produced also a progressive increase in the hepatic valine concentration. This increase could not be accounted for by the decrease in plasma valine levels, suggesting that the rise in hepatic valine concentration is an expression of hepatic proteolysis rather than the result of an accelerated transport of amino acids across the hepatocyte plasma membrane. The different time sequence in the glucagon-induced effects on protein synthesis and proteolysis suggests that both effects are independent and probably mediated by different mechanisms.

The administration of glucagon to animals (1, 2) and man (3–5) is followed by an increased nitrogen excretion and a concomitant rise in plasma urea levels. The latter finding made many authors focus their attention on the liver as a main site of the hormone action. Miller (6), using a preparation of isolated perfused rat liver, demonstrated that in addition to the increase in glucose output, glucagon produced an increased urea production. It was then postulated that this action of the hormone on amino acids catabolism might serve to supply the necessary substrates for gluconeogenesis. A more direct proof of the protein catabolic action of glucagon was shown later by several authors by determining the effect of the hormone on the rate of valine output by the isolated perfused rat liver (7, 8).

The effect of glucagon increasing protein catabolism may be exercised on protein synthesis, degradation, or both processes simultaneously. The question of which of these processes is predominantly affected is still open to debate. Pryor and Berthet (9), using rat liver slices, described an inhibitory effect of glucagon on the amino acids incorporation into protein. However, Ayuso-Parrilla and Parrilla (7) reported a predominant, if not exclusive, effect of glucagon on proteolysis according to their findings in the perfused rat liver as well as in vivo. This conflict between the findings of different authors may be at least in part a consequence of the glucagon effect varying the intracellular amino acids pools. Glucagon, through its proteolytic effect, would raise the intracellular concentration of amino acids, resulting in an underestimation of the rate of amino acids incorporation into protein. The problem seems to be complicated further since the hepatic intracellular amino acid pool does not seem to be homogeneous (10–12), and being rather difficult to be certain about the specific radioactivity of those amino acids used for protein synthesis at any given time.

This work aims to study the effect of glucagon on the control of hepatic protein synthesis in vivo, obviating the above mentioned effects of the hormone perturbing the amino acids pools through its proteolytic effect. The objective has been fulfilled by determining the effects of the hormone on the polypeptide chains completion time. Such approach originally described by Haschemeyer (13) and Mathews et al. (14) and modified by Scornik (15) is based on the determination of the average transit time of a radioactive precursor along the messenger RNA.

The results reported herein seem to demonstrate that the administration of glucagon in vivo, under our experimental conditions, is able to produce an inhibitory effect on general hepatic protein synthesis acting mainly, if not exclusively, on the peptide chain elongation or termination step, or both.

EXPERIMENTAL PROCEDURES

Animals—Male albino rats of the Wistar strain, 200 to 220 g body weight, were used in all the experiments. The animals were pur-
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Chased when they had a body weight of 80 to 100 g and were kept under controlled conditions of food intake, light, and temperature until the date of the experiment.

Chemicals—Uniformly labeled L-[U-14C]valine (10 to 20 Ci/mmol) was purchased from C.I.S. (France). All the reagents, of the highest purity available, were obtained from Sigma Chemical Co. (St. Louis, Mo.) or from Merck (Darmstadt, Germany). Glucagon was a gift from Lilly Indiana de España, S.A. and from Novo (Novofarma S.A., Spain).

Measurement of Incorporation of Amino Acids into Liver Protein—The animals were anesthetized with Nembutal (40 mg/kg of body weight) Ten minutes thereafter the portal vein was exposed by midline abdominal incision and 20 μCi of L-[U-14C]valine, dissolved in 0.2 ml of saline (0.9% NaCl) solution, were administered by an injection, the vein which took approximately 30 s. Glucagon-treated animals were given 200 μg of glucagon intraperitoneally as a single dose. Small liver biopsies were taken 0.5, 1, 1.5, and 2 min after the completion of the amino acid administration, in such a manner that only two biopsies were taken from each liver (at 0.5 and 1.5 min or at 1 and 2 min, respectively). In order to prevent hemorrhage liver lobules were ligated immediately before the biopsies were taken. Liver samples were suddenly frozen by means of aluminum clamps (16), precooled in liquid nitrogen or in a solid CO2/methanol mixture, and stored in liquid nitrogen until they were homogenized with 5 volumes of 0.3 M sucrose. The procedure followed for the determination of radioactivity in total and nascent proteins was similar to the one described by Sornik (15) and was as follows. Three milliliters of 10% perchloric acid were added to 0.2 ml of homogenate. After sedimenting at 8000 rpm for 30 min in a Sorvall refrigerated centrifuge, the pellet was washed twice with 2 ml of 10% perchloric acid. The pellet after the last wash was resuspended in 3 ml of 10% perchloric acid and heated at 90° C. After centrifugation, the pellet was washed with 50 mM magnesium acetate and ether and kept at room temperature. The dry protein residue was solubilized in 1 ml of 5% trichloroacetic acid containing 10% of toluene-based scintillation mixture. The radioactivity was determined using a Tri-Carb scintillation spectrometer.

The incorporation of radioactive amino acids into nascent chains was determined by using 1 ml of homogenate dilute in 1 ml of 0.1 M-Tris-HCl, 2 mM magnesium acetate, pH 7.8, at 0° C. After centrifugation at 5000 rpm for 15 min at 4°, sodium deoxycholate (2% final concentration) was added to the supernatant, placed on the top of a tube containing 6 ml of 1 M sucrose, 1 mM magnesium acetate and centrifuged at 150,000 × g for 3 h in a Beckman ultracentrifuge at 0° C. The supernatant was discarded, 1 ml of distilled water at 0° C, 1 ml of 0.2 M-Tris-hydroxymethylaminomethane (pH 7.3), 5 mM MgCl2, 25 mM KCl, and 7 mM mercaptoethanol. In this set of experiments, glucagon, when administered, was injected directly into the portal vein immediately after a zero time liver biopsy was taken. Following addition of sodium deoxycholate (1.3% final concentration), the polyribosomes were purified through a discontinuous sucrose gradient made up as described before (17). The discontinuous gradients were centrifuged for 16 h at 85,000 rpm in a Beckman 65 fixed angle head. The yield of the polyribosomes isolation procedure was as originally described (18). The pelleted was resuspended in 0.1 ml of buffer containing 50 mM triethanolamine (pH 7.3), 5 mM MgCl2, 25 mM KCl, and 7 mM mercaptoethanol and layered on 5 ml of a 20 to 40% linear sucrose gradient made up in the same buffer. The gradient was then centrifuged in the SW 50.1 Beckman rotor for 35 min at 45,000 rpm and pumped from the bottom of the tube through a flow cell of an ice ultra-violet monitoring system. The absorbance at 254 nm was continuously recorded.

Determination of Hepatic Valine Concentration and Specific Radioactivity—One-milliliter aliquots of homogenates from six livers were pooled and deproteinized with 6 ml of 12% perchloric acid, and the precipitate was removed by centrifugation. After neutralization of the supernatant with 3 ml of K2CO3, the samples were freeze-dried. Valine concentration was measured either way, chromatographically in a Jeol automatic amino acids analyzer or following an isotope dilution involving the determination of radioactivity incorporated into nascent peptides (n) and the radioactivity incorporated into total peptides (t) is assumed that following its injection, the radioactive amino acid will meet the ribosomes in the middle of the translation of a message of average size. When a full cycle is completed, the whole peptide on the ribosomes will be labold, while the chains that have been terminated and released will be only half-labeled. Thus, the ratio between the radioactivity incorporated into nascent peptides in the polyribosomes (n) and the radioactivity incorporated into total peptides (t) will be reduced by 50% and the time required to reach this 50% value is taken as the average polypeptide chains completion time (15). This seems to be true to the extent that the specific radioactivity of the precursor does not change over the 2-min period. A similar observation was previously described by Mathews et al. (14) under similar experimental conditions. In the experiments reported herein the total acid-soluble valine specific activity was determined at each experimental time in five different pools made up of aliquots from the perchloric acid supernatants of six livers. The results were: 40 ± 2, 32 ± 2, 35 ± 2, and 37 ± 16 pmol/mmol at 0.5, 1, 1.5, and 2 min, respectively. These small variations were not statistically significant as determined by the t test.

Fig. 1 shows the rate of decay of the hepatic n/t value at different times after glucagon administration. As it can be seen, 5 min after glucagon administration the slope of the decay was less steep than in the control livers, while at 20 min the slope was similar to the starting values. These changes in slope 5 min after glucagon administration indicate a slower ribosomal transit time along the messengers. The extrapolation of the n/t value to zero time, in agreement with previously reported transit time determinations (14, 15), does not result in a ratio of 1. This observation can be easily explained taking into account the approximately 30-s period that it took the injection of the precursor. In fact, extrapolation of the line gives a ratio of 1 somewhere between ~20 and ~40 s, which is the usual time taken for the isotope administration. Fig. 2 shows the values of the polypeptide chains completion times calculated using the data from experiments reported in Fig. 1. The polypeptide chains completion time in livers from control rats was 90 s. Five minutes after glucagon treatment this value raised to 146 s and at 20 min after the hormone administration the completion time returned to the control values. This transient effect of glucagon is probably related to its rapid destruction by the organism. In order to support this point of view and also to confirm that the changes in n/t ratios were directly related to the presence of the hormone, experiments were designed to determine variations in n/t ratios in liver of animals which had been continuously supplied with glucagon. Fig. 3 shows how 30 min after continuous infusion of glucagon the slope of the decay in the hepatic n/t ratios remained lower than in control livers. Fig. 4 demonstrates clearly how the continuous infusion of glucagon resulted in a sustained effect on the ribosomal transit time which was 130 s.
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Fig. 1. Effect of glucagon on the rate of decay of the ratio between the radioactivity incorporated into hepatic nascent peptides and the radioactivity incorporated into total peptides (n/t). The experimental procedure was as described under "Experimental Procedures." Each point represents the average value of 10 to 14 experiments and the vertical bars represent the standard error of the mean.

Fig. 2. Effect of the intraperitoneal glucagon administration on the polypeptide chains completion time. The polypeptide chain completion time was determined from the data on Fig. 1. It was taken to be the time required to reduce the n/t ratio by 50%.

Fig. 3. Effect of the continuous glucagon infusion on the rate of decay of the ratio between the radioactivity incorporated into hepatic nascent peptides and the radioactivity incorporated into total peptides (n/t). Glucagon was continuously infused into the inferior vena cava of anesthetized rats at a rate of 26 μg/min for the first 5 min and 10 μg/min thereafter. The determination of the amino acids incorporation into liver proteins was performed as described under "Experimental Procedures." a, 20 min of saline infusion; b, 20 min of glucagon infusion. Each point represents the mean value of at least eight experiments and the vertical bars represent the standard error of the mean.

Fig. 4. Effect of the continuous glucagon infusion on the polypeptide chains completion time. The polypeptide chains completion time was determined from the data on Fig. 3 as described in the legend of Fig. 2.
treated rats. As shown in Fig. 5 glucagon treatment increased the ribosomal state of aggregation. A quantitative determination of this increase is displayed in Table I. Since a block of initiation equal or higher than the inhibition of elongation should result in either one unaltered or broken polyribosomal profile, respectively (21, 22), these results indicate that glucagon acts preferentially on the elongation or termination step, or both.

This conclusion is further supported by the fact that 5 min after glucagon administration, the radioactive valine incorporation into hepatic polyribosomes reached a maximum at an earlier time than in control livers (Fig. 6). This earlier accumulation of radioactive precursor in glucagon-treated rats can be interpreted as the consequence of elongation being limiting relative to initiation. In other words, this implies that the rate of entry of the precursor is higher than its rate of movement along the mRNA (23-25). Fig. 6 also shows that 20 min after the hormone administration the kinetics of the incorporation of radioactivity was similar to the one in livers of control rats.

Effect of Glucagon on Hepatic Protein Catabolism. - The variations in plasma urea levels after glucagon administration are shown in Fig. 7. In contrast to the transient effect of glucagon on the polypeptide chains completion time, urea levels increased progressively along the 20 min of experimental time. This increase in plasma levels reflects a larger availability of amino acids for the process of urea synthesis as is clearly shown in Fig. 8 in which it can be appreciated how the hepatic valine concentration increases after glucagon administration in a similar manner than urea. Valine is an amino acid poorly oxidized and transaminated by the liver (26) so that variations in its concentration can be accepted as a net index of proteolytic activity (7, 27). Glucagon has been described to accelerate the transport of amino acids across the hepatocyte plasma membrane (28) and to decrease the plasma amino acids content (29). However, as it can be shown in Table II, the decrease in plasma valine concentration cannot possibly account for the observed rise in its hepatic concentration, even if it is assumed that valine is evenly distributed in the extracellular water and that the decrease in plasma valine reflects its decrease in the total extracellular volume. These findings clearly indicate that a dissociation exists between the proteolytic and protein synthetic effects of glucagon in the hepatocyte, suggesting that both effects are probably mediated by different mechanisms.

**DISCUSSION**

Accurate information regarding the possible action of glucagon controlling protein synthesis, particularly in liver, was hindered by its potent proteolytic effect. In the present work this obstacle has been obviated by determining the polypeptide chains completion time. Since it has been shown that the specific activity of the total acid-soluble valine does not change significantly along the experimental time, the use of ratios of radioactivity incorporated into nascent and total polypeptide chains offers the advantage of being independent of the precursor pool size since any possible variations would be cancelled out. An important limitation of this procedure is that it only detects variations in the elongation or termination step, or both, of protein synthesis. In other words, by using this procedure we are only measuring the rate of movement of a ribosome along the messenger RNA, independently of the...
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The step of the protein synthetic process affected by the hormone seems to be mainly the elongation or termination step, or both. One of the arguments against an effect on the initiation step comes from the fact that the ribosomal state of aggregation increased after glucagon administration (Fig. 5 and Table I). On the other hand, the kinetics of labeling of polyribosomes (Fig. 6) does not support the idea of an effect on initiation. If the initiation step was inhibited as to become rate-limiting one should expect that the maximum labeling would be reached later than in the control (23-25). In our case, in livers from 5-min glucagon-treated rats the maximum labeling was reached earlier than in the control giving support to the idea that there is an effect on the elongation or termination step, or both.

The polypeptide chains completion time as determined in control livers was found to be 1.5 min, which yields a rate of protein synthesis of 120 nmol of protein/g of liver/h or 6 mg of protein/g of liver/h. These calculations have been made taking the ribosomes concentration as 3 nmol/g and the average polypeptide chain size, 50,000 daltons (14). This value is within the same order of magnitude of those previously reported (14, 15, 30). However, it is probably an under estimation since the synthesis of plasmatic proteins has not been measured and it can account for about 20% of the overall synthetic capacity (31).

The intraperitoneal administration of glucagon produced an increase of the polypeptide chains completion time (Fig. 2), suggesting an interaction of the hormone at the level of the elongation or termination step, or both. At 5 min after glucagon administration, when the effect was maximal, the completion time was 2.4 min; that is, 75 nmol, or 3.75 mg, of protein/g of liver/h. This represents an inhibition of about 38% of the protein synthetic capacity. This finding is in line with results reported by Woolside et al. (18) who found that glucagon inhibited by 37% total protein synthesis in the isolated rat liver perfused with a mixture of amino acids at a concentration similar to those found in plasma. The fact that the effect of glucagon lasts for less than 20 min seems to be related to the rapid degradation of the hormone by the organism. This point of view seems to be substantiated by the observation that the continuous supply of the hormone by intravascular infusion maintained maximal effects all along the infusion period (Fig. 4). Glucagon stimulates insulin secretion in vivo as well as in vitro (32-34). Even though it is not yet known whether insulin prevents the glucagon effects on protein synthesis, as it has been described with some other metabolic events (35-37), the possibility cannot be excluded that the transient effect of glucagon was the result of a rise in insulin levels, shifting the glucagon:insulin ratios to the control values.

The finding of the effect of glucagon inhibiting the bulk protein synthesis seems to be in conflict with the known effects of the hormone inducing several hepatic enzymes (38-41). However, both processes are qualitatively and quantitatively...
different. The enzyme induction is a very slow process compared to the acute effects herein reported and can take place in the absence of substantial variations in the overall rate of protein synthesis.

A very interesting finding was the different time course in the glucagon effects on protein synthesis (Fig. 2) and on proteolysis (Fig. 8). This dissociation between both effects lends support to the interpretation that both metabolic events are mediated by different mechanisms. As far as we know this is the only report in which both effects appear so clearly independent from each other, demonstrating that the increased availability of amino acids does not play a primary role in the hormonal effects on protein synthesis. Previous work on this subject (7) failed to demonstrate any effect of glucagon on hepatic protein synthesis in vivo. The most plausible explanation in view of the above reported findings is that by the time (20 min) the liver samples were taken, the glucagon effects had vanished (Fig. 2).

From a physiological point of view the effect of glucagon on hepatic protein synthesis in fed animals is quite relevant for the understanding of the metabolic transition from normal fed to starvation. The latter condition is characterized by a decrease in plasma glucagon levels (48). Glucagon, thus, may play a key role in vivo.

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