POSSIBLE GROWTH HORMONE REGULATION OF RAT LIVER GLUTAMINE SYNTHETASE ACTIVITY

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SUMMARY: Hypophysectomy results in a marked decrease in glutamine synthetase activity of rat liver homogenates. The enzyme is affected to a lesser extent in the kidneys and is not influenced in the brain. Bovine growth hormone treatment of hypophysectomized rats elevates the diminished glutamine synthetase activity in liver and kidneys but has no effect on the brain enzyme. Adrenalectomy also results in decreased liver glutamine synthetase activity although less than the decline seen with hypophysectomy. Cortisol treatment has no effect on glutamine synthetase activity in hypophysectomized animals. Our results suggest that growth hormone is involved in the regulation of liver glutamine synthetase activity. This regulation may be important in the utilization of α-amino nitrogen from glucogenic amino acids associated with growth hormone enhanced glucose production.

Growth hormone is a potent regulator of cellular metabolism and is a physiological antagonist of insulin. Its absence following hypophysectomy results in altered glucose tolerance (1), increased insulin sensitivity (2), lowered glucose levels and decreased hepatic glucose production (3). The administration of growth hormone to normal and hypophysectomized animals elevates the levels of blood glucose and insulin (3,4), increases hepatic glucose production (5), inhibits the disposal of glucose loads, and in large doses can induce diabetes (6). The increase in glucose production along with the depressed glucose uptake may be an important factor in the diabetogenic action of the hormone. The source of carbon for the hormone stimulated glucose synthesis is unknown, although among the most important precursors are probably the amino acids, whose uptake into cells is stimulated by growth hormone (7). An important step in the net incorporation of amino acid carbon into glucose is the removal of the α-amino nitrogen, via transamination, leading to its eventual elimination as urea. The oft repeated experiments

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of Long and his co-workers (8) show, however, that growth hormone administration results in decreased urea excretion, suggesting that amino acids are not the glucogenic precursors unless an alternate metabolic fate for the α-amino nitrogen can be found. We propose that the synthesis of glutamine by the enzyme glutamine synthetase may be important in such an alternate path. Glutamine plays a central role in nitrogen metabolism; its amide group is a preferred source of nitrogen in the biosynthesis of purines, pyrimidines, glucosamine and amino acids all of which are important precursors for growth hormone enhanced synthesis of RNA, DNA, proteoglycans and proteins. Growth hormone enhanced glutamine synthetase activity could thus divert the α-amino nitrogen of glucogenic amino acids away from urea production.

The experiments described in this paper investigate the effects of short (3-30 days) and long (4-10 months) term hypophysectomy, on glutamine synthetase activities of liver, kidney, and brain. Growth hormone, cortisol, and growth hormone plus cortisol were administered to hypophysectomized rats to determine the influence of these hormones on enzyme activities. We propose that this enzyme may be important in directing the utilization of α-amino nitrogen from gluconeogenic amino acids to the synthesis of compounds needed for cell growth.

MATERIALS AND METHODS

Normal and hypophysectomized male Spraque Dawley rats (140-280g) were obtained from Simonsen Laboratories (Gilroy, CA). Adrenalectomies were performed in our own laboratory. All animals were maintained on Purina Lab Chow ad libitum. Adrenalectomized rats were given 0.9% saline to drink. The completeness of hypophysectomy and adrenalectomy was assessed by autopsy; in the case of hypophysectomized animals the absence of weight gain was also used as an index.

Hypophysectomized rats were injected twice daily for seven days, with a single injection on the eighth day, 1-2 hours before sacrifice. Weight gain confirmed the effectiveness of hormone action. NIAMDD Bovine growth hormone (lot number NIH-GH-B-18) (dose=1mg/100gm/half day) was reconstituted in 0.9% saline, adjusted to pH 9.5 using 0.05N NaOH. Cortisol (hydrocortisone acetate (Towne)) (dose=0.1mg/100gm/half day) was suspended in 0.9% saline, pH 9.5. Control animals were injected with an equivalent volume of pH 9.5 saline.

Following decapitation, rat tissues were excised and homogenized (1gm of tissue to 5ml of 0.25M sucrose) at 0°C with a Polytron homogenizer at full speed for 30 seconds. The homogenate was diluted with an equal

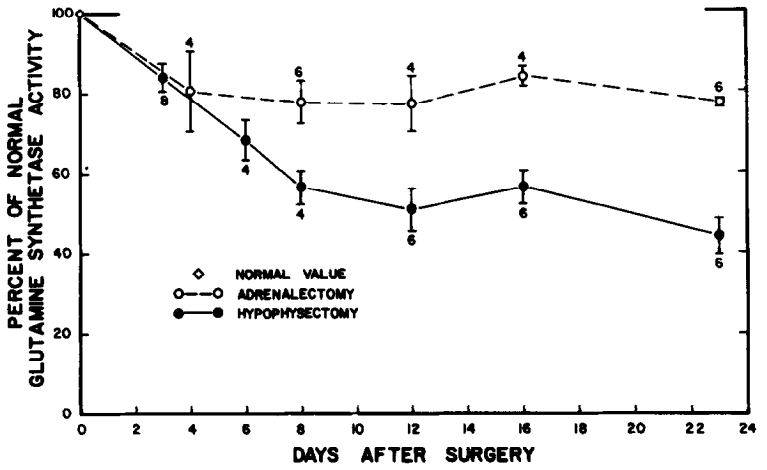

Figure 1. Glutamine synthetase activity of rat liver homogenates at different time intervals following hypophysectomy and adrenalectomy. Numbers above and below the standard error of the mean bars indicate the number of experiments.

volume of 40mM Tris-HCl, pH 7.2 and centrifuged for 20 minutes at 20,000 x g. The activity of glutamine synthetase was assayed by the method of Vorhaben et al (9) modified to contain 0.5ml of the tissue supernatant and 5mM ATP in the reaction mixture. For the glutamate dehydrogenase assay, rat liver was excised, frozen, thawed and homogenized (1g of tissue to 10ml of 0.1M Tris-acetate, pH 8) at 0°C with a Polytron homogenizer at full speed for 30 seconds. The homogenate was centrifuged for 20 minutes at 20,000 x g and 0.025ml of the supernatant was used as the enzyme source. The 1.25ml assay mixture contained the enzyme; 56mM Tris-acetate, pH 8, with 5.6 x 10^{-6} M EDTA; 48mM L-glutamate, 0.2mg NAD; 3% glycerol and 0.1mM ADP. The reaction was started by the addition of the L-glutamate and the reduction of NAD was monitored at 22°C. Protein was determined by the method of Lowry et al (10).

RESULTS

Hypophysectomy (Figure 1) results in a decrease in liver glutamine synthetase activity as early as 3 days after surgery. Enzyme activity continues to fall and reaches a plateau value of 51% of normal activity between 8 and 23 days. Liver glutamine synthetase is also decreased between 8 and 23 days after adrenalectomy, although not to the same extent as hypophysectomy (79% vs 51% of normal values). Long term hypophysectomy (Table 1) depresses enzyme activity still further to 0.244 ± 0.018μmoles/30min/mg protein. Short and long term hypophysectomy both result in a substantial diminution in kidney

Table 1 - Glutamine Synthetase Activity of Liver, Kidney and Brain following Hypophysectomy and Adrenalectomy

Rat State	Time After Gland Ablation	Liver	Kidney	Brain
		μmoles γ-glutamyl hydroxamate/30min/mg protein		
Normal		0.679 ± 0.015 (60)	0.755 ± 0.030 (28)	1.057 ± 0.019 (38)
Hypox	8 to 23 days	0.313 ± 0.024 (22)	0.462 ± 0.046 (12)	0.947 + 0.026 (22)
Adrex	8 to 23 days	0.534 ± 0.014 (20)	0.850 ± 0.043 (16)	0.897 ± 0.021 (17)
Hypox	4 to 9 months	0.244 ± 0.018 (10)	0.514 ± 0.075 (4)	0.940 ± 0.030 (6)

The values represent the mean ± the standard error of the mean.

The number of experiments are indicated in parentheses.

glutamine synthetase activity. Adrenalectomy, however, produces a small but significant ($p<0.05$) increase. Neither hypophysectomy nor adrenalectomy affects brain enzyme activity.

Bovine growth hormone administration (Table 2) to long term (7-10 months) hypophysectomized rats results in a significant increase in liver glutamine synthetase activity. The hormone replacement therapy does not, however, completely restore enzyme activity to normal levels. Growth hormone administration also increases the mean enzyme activity of the kidney but not to the extent produced in the liver. Brain enzyme activity is not affected by growth hormone. Short term (2-4 weeks) hypophysectomized animals exhibit a pattern of liver, kidney and brain response to growth hormone similar to that seen in the long term animals. The enzyme activities measured in this group of hypophysectomized animals is lower than previously determined; the liver glutamine synthetase activities of the normal controls, measured during this time, however, were also lower. (62.2 ± 8.8% of the value in Table 1). The

Table 2 - Effect of Bovine Growth Hormone and/or Cortisol Treatment on Glutamine Synthetase Activity in Short Term (2-4 weeks) and Long Term (7-10 months) Hypophysectomized Rats.

Rat State	Weight Change		Liver	Kidney	Brain
Treatment	%	grams	μmoles of γ-glutamyl hydroxamate/30min/mg protein		
Long Term Hypox					
Saline	- 0.9 ± 0.7	- 1.0 ± 0.9	0.171 ± 0.038 (5)	0.451 ± 0.085 (5)	0.922 ± 0.037 (4)
Growth Hormone	+19.0 ± 1.1	+27.8 ± 1.9	0.353 ± 0.044 (5)	0.614 ± 0.079 (5)	0.941 ± 0.063 (4)
Short Term Hypox					
Saline	+ 0.8 ± 1.0	+ 1.1 ± 1.5	0.178 ± 0.026 (7)	0.335 ± 0.034 (7)	0.657 ± 0.021 (4)
Growth Hormone	+30.9 ± 2.2	+48.3 ± 2.7	0.302 ± 0.018 (7)	0.391 ± 0.056 (7)	0.687 ± 0.019 (4)
Cortisol	- 1.3 ± 1.2	- 2.0 ± 1.8	0.187 ± 0.024 (4)	0.327 ± 0.025 (4)	0.873 ± 0.059 (4)
Growth Hormone plus Cortisol	+28.7 ± 2.7	+43.8 ± 3.8	0.285 ± 0.010 (4)	0.393 ± 0.018 (4)	0.910 ± 0.021 (4)

The values represent the mean ± the standard error of the mean. The number of experiments are indicated in parentheses.

Table 3 - Effect of Long Term (4-9 months) Hypophysectomy and Bovine Growth Hormone Treatment on Rat Liver Glutamate Dehydrogenase Activity

Rat State	Treatment	nmoles NAD reduced/min/mg protein
Normal (5)		45.3 ± 4.2
Hypophysectomized (6)	Saline	34.5 ± 2.7
Hypophysectomized (4)	Growth Hormone	38.7 ± 5.7

The values represent the mean ± the standard error of the mean. The number of experiments are indicated in parentheses.

dose of cortisol (0.2mg/100gm/day) administered has no influence on hypophysectomized liver and kidney enzyme activities. Furthermore, cortisol does not enhance the growth hormone stimulated glutamine synthetase activity in liver and kidney. Growth hormone does not influence brain enzyme activity. Cortisol administration does not result in an increase in brain glutamine synthetase activity per unit wet weight of tissue (data not shown). The Lowry assay results do, however, show a reduction in total brain protein with cortisol administration thus resulting in the apparent increase in enzyme activity per mg protein shown in Table 2.

Glutamate dehydrogenase (Table 3), another important enzyme in liver nitrogen metabolism, functions in one pathway leading to the urea cycle and in a second pathway, along with glutamine synthetase, leading to synthesis of purines, pyrimidines, glucosamine, etc. Long term hypophysectomy results in a decrease in enzyme activity from 45.3 ± 4.2 to 34.5 ± 2.7 nmoles/min/mg protein. This decrease in liver glutamate dehydrogenase activity (-24%) is not as extensive as the fall in glutamine synthetase activity (-64%). Growth hormone administration does not increase the diminished glutamate dehydrogenase activity.

DISCUSSION

These results may shed new light on the apparent paradoxical physiological observation that growth hormone stimulates hepatic glucose production (5) and concurrently decreases urea excretion (8). The growth hormone elevated glucose production is probably via gluconeogenesis from amino acids. Alanine gluconeogenesis is depressed in hypophysectomized animals (11) and at least partially restored by growth hormone administration (unpublished results). If gluconeogenesis is elevated by growth hormone an alternate metabolic fate must be found for the α-amino nitrogen since enhanced gluconeogenesis is commonly associated with increased urea production (8). Our results indicate that liver glutamine synthetase may be important in this alternate metabolic fate and add additional significant evidence for the already accepted central role of this enzyme and glutamine in the regulation of metabolism and as a nitrogen donor for biosynthetic processes and growth.

The possibility that growth hormone is involved in glutamine synthetase activity is suggested by depressed enzyme activity observed in the liver and kidneys of hypophysectomized animals and the partial restoration of activity with growth hormone treatment. Since much evidence exists for the involvement of adrenal corticosteroids in enzyme synthesis and since hypophysectomized animals, because of ACTH absence, have essentially no adrenal cortical function, the glutamine synthetase activity of adrenalectomized rats was investigated. Although adrenalectomy results in decreased glutamine synthetase activity, this depression is significantly less than in hypophysectomized rats. Furthermore, adrenal corticosteroids, in the low doses used, does not increase liver and kidney enzyme activity in hypophysectomized rats nor does it enhance the effectiveness of growth hormone treatment.

In order to assess whether the effects of hypophysectomy on glutamine synthetase activity are specific for this enzyme or are a reflection of the general depression of protein synthesis in hypophysectomized animals, liver glutamate dehydrogenase, another important enzyme in nitrogen metabolism

was investigated. Hypophysectomy results in a small depression of enzyme activity compared to the decrease in glutamine synthetase activity. Furthermore, growth hormone has no effect on glutamate dehydrogenase activity. Perhaps the comparatively small decrease in glutamate dehydrogenase activity may be related to the absence of adrenal corticosteroids rather than growth hormone. Additional studies (not presented) also indicate that the adenosine 3', 5'-cyclic monophosphate phosphodiesterase activity of liver homogenates is not influenced by hypophysectomy.

The plateau of glutamine synthetase activity remaining in the liver 8 days after hypophysectomy suggests either the existence of hormone sensitive and insensitive enzyme forms or a lesser amount of a single form. Preliminary kinetic studies (not shown) indicate that the Michaelis constant for liver enzyme activity does not change with long term hypophysectomy. The existence of alternate enzyme forms has been demonstrated between different mammalian tissues. Tate and Meister (12) have shown that the glutamine synthetase of sheep brain, rat liver and other tissues respond differently to various effectors in the degree of inhibition or activation that is measured. It has been suggested that in the brain, glutamine synthetase may regulate the levels of glutamate which is an excitatory neurotransmitter. Interestingly our results show that unlike the glutamine synthetase of liver and kidneys the brain enzyme does not respond to growth hormone.

In addition to serving as a nitrogen donor, glutamine may play an important role in regulating gluconeogenesis and/or glycogen synthesis. Katz et al (13) showed that glutamine addition enhances total carbohydrate formation from gluconeogenic precursors in isolated hepatocytes. Thus during growth hormone enhanced amino acid gluconeogenesis, stimulation of glutamine synthetase activity would not only provide an alternate path for α-amino nitrogen leading to RNA, DNA, proteoglycans, etc. needed for hormone stimulated growth, but might also provide an activator of carbohydrate synthesis.

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