Plasma Amino Acids in Hypothyroid and Hypertyroid Rats

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Summary

Plasma amino acid concentrations, together with other metabolic parameters were determined in thyroidectomized rats treated with daily injections of saline (hypothyroid), and 250 μg/kg L-T$_4$ (hypothyroid). Data were compared with sham-operated controls. There is a general increase in plasma amino acid concentrations in hyperthyroidism, a limited increase only in several amino acid concentrations in hypothyroid rats as compared with controls, and a considerable difference between the plasma aminograms of both groups. Amino acid homeostasis seems to be subject to greater modification in hyperthyroidism than in hypothyroidism.

Key-Words: Amino Acids — Hyperthyroidism — Hypothyroidism

Introduction

Thyroid hormones increase protein synthesis in key organs of experimental animals (*Michels, Cason* and *Sokoloff* 1963; *Carter, Faas* and *Wynn* 1971) through stimulation of ribosomal and nuclear RNA synthesis (*Tata* and *Widnell* 1966; *Hulbert* 1978). The incorporation of labeled amino acids into proteins (*Michels, Cason* and *Sokoloff* 1963; *Carter, Faas* and *Wynn* 1971) is related to the increase of target tissue oxygen utilization induced by thyroid hormones (*Gordon* and *Hening* 1944; *Weiss* and *Sokoloff* 1963). The action of thyroid hormones on protein synthesis would be, then, partly due to increased availability of energy produced in the oxidation of substrates by mitochondria (*Hanson, Lindsay* and *Barker* 1963; *Primack, ꝓley* and *Buchanan* 1972) and specifically due to specific mRNA synthesis and translation (*Garren, Richardson* and *Crocco* 1967; *Herd, Kaplay* and *Sanadi* 1974; *Kurtz, Sippel* and *Feigelson* 1976; *Seo, Vassart, Brocas* and *Refetoff* 1977; *Hulbert* 1978); actions mediated by thyroid hormones binding to nuclear chromatin (*Sterling* 1979).

The administration of pharmacological doses of thyroid hormones to experimental animals nevertheless provokes a marked decrease in protein (*Rupp, Paschkis* and *Cantarow* 1949; *Sokoloff* and *Kaufman* 1959). This same effect has been observed in animals chronically treated with overdoses of thyroid hormones (*Klye, Ball* and *Doolan* 1966). Chronic hypothyroidism also seems to provoke an increase in proteolysis (*Menahan* and *Wieland* 1969); the amino acids thus produced are not utilized for protein synthesis (*Hoberman* and *Graaf* 1950).

We have studied here the changes in plasma amino acid homeostasis under chronic hyperthyroidism and hypothyroidism in the rat, for further characterization of the metabolic changes induced by hypo- and hyperthyroidism.

Materials and Methods

Wistar male rats weighing approximately 45 g were used. The animals were separated into three groups: the animals of the first and second groups were thyroidectomized under ether anesthesia (*Zarrow, Yochin* and *McCarthy* 1964), the third group (controls) was sham-operated also under ether anesthesia. All animals were kept in collective cages in a temperature (22 ± 1 °C) and light (12 hours light/12 hours darkness) controlled animal room. They were fed a low iodine diet (*Remington* and *Levine* 1935) supplemented in the case of controls with 1 ppm iodine in the form of potassium iodate.

Hyperthyroid rats were injected daily i.p. with L-Thyroxine (Sigma) (250 μg/kg of body weight). Hypothyroid rats were injected i.p. with 0.9 % NaCl; the volume of the injections was of 2.5 ml/kg of body weight. The treatment was continued for 40 days, when the animals were killed by decapitation with a guillotine, with as low stress as possible, at the beginning of a light cycle, and blood was collected into dry heparinized beakers and capillary tubes.

The hematocrit value was obtained by centrifugation. Plasma total proteins were determined with the Folin phenol reagent procedure (*Lowry, Rosebrough, Farr* and *Randall* 1951; *Wang* and *Smith* 1975), using defatted bovine serum albumin (Sigma) as standard. After deproteinization with perchloric acid and KHCO$_3$ neutralization, plasma glucose (*Hugget* and *Nixon* 1957), acetoacetate (*Mellanby* and *Williamson* 1974), beta-hydroxybutyrate (*Williamson* and *Mellanby* 1974), lactate (*Hohorst* 1974) and glycerol (*Eggstein* and *Kühlman* 1974) were estimated enzymatically. Urea was determined colorimetrically with the indophenol reaction (*Fewcett* and *Scott* 1960).

Aliquots of plasma were deproteinized with cold acetone (*Arola, Herrera* and *Alemany* 1977) and the supernatants were used for determination of individual amino acids (*Arola, Palou, Herrera* and *Alemany* 1976) by means of their derivatization with Dns-Cl (^{14}C), thin layer chromatography separation and evaluation of radioactivity present in individual spots (*Arola* et al. 1976). Tests for significant differences between the means were done using the Student's t test.

Results

In Table 1 the body weights attained at the end of the chronic treatment with 0.9 % NaCl (hypothyroid rats) and L-T$_4$ (hyperthyroid rats) can be seen. Hypothyroid animals suffered a significant decrease in their growth rate; the differences are also significant versus the hyperthyroid animals. This last group showed a significant hepatomegaly with respect to controls and hypothyroid rats. The hematocrit value was significantly lower in hypothyroid versus controls, and normal in hyperthyroid rats.

Table 1   Body and liver weight and hematocrit of hypothyroid and hyperthyroid rats compared with controls

	control	hypothyroid	hyperthyroid
Body weight (g)	179 ± 10	110 ± 10 *†	169 ± 11
Liver weight (% of body weight)	4.47 ± 0.12	4.69 ± 0.33*	6.46 ± 0.24†
Hematocrit	47.0 ± 0.9	41.6 ± 1.2 †	47.2 ± 2.7

All data are the mean ± S.E.M. of 5–6 different animals.
Significance of the differences versus controls: † = p < 0.05
Significance of the differences between hypothyroid and hyperthyroid groups: *= p < 0.05

Table 2   Glucose, lactate, glycerol, urea, total proteins and ketone bodies concentrations in the plasma of hypothyroid and hyperthyroid rats compared with controls

	control	hypothyroid	hyperthyroid
Glucose (mM)	8.13±0.25	6.80±0.39†	7.48±0.81
Lactate (mM)	2.93±0.12	2.53±0.27	3.11±0.81
Glycerol (μM)	178±22	106±21	146±20
Urea (mM)	12.91±0.85	15.10±0.84	12.71±1.05
Total proteins (g/l)	66.10±1.10	66.50±1.50*	60.50±1.50†
β-hydroxybutyrate (μM)	38.21±5.20	69.21±3.29†	49.79±6.71
Acetoacetate (μM)	67.28±6.21	95.60±7.12†	89.43±13.0

All data are the mean ± S.E.M. of 5–6 different animals.
The symbols used are the same as in Table 1

Table 3   Plasma amino acid concentrations in hypothyroid and hyperthyroid rats compared with controls

amino acid	control	hypothyroid	hyperthyroid
Gluconeogenic total	2967±103	2955±139	3118±262
Alanine	897± 41	808±105	867±132
Glutamate + glutamine	1180± 71	1116± 37	1118±101
Aspartate + asparagine	121± 4	120± 6*	103± 3†
Glycine	148± 9	247± 34†	226± 30†
Serine	248± 7	334± 18†	399± 33†
Threonine	352± 14	330± 28	362± 59
Imino acids total	698± 18	779± 68*	1017± 26†
Proline	609± 51	733± 97	968± 33†
Hydroxyproline	54± 2	46± 5	49± 6
Branched chain total	748± 9	1013± 60†	1126± 32†
Leucine + isoleucine	430± 38	592± 53*	752± 32†
Valine	333± 33	421± 49	374± 19
Basic total	662± 48	639± 33*	1023± 11†
Lysine	166± 33	115± 16*	298± 9†
Arginine	340± 26	347± 25*	463± 14†
Histidine	94± 13	90± 11	146± 10†
Citrulline	87± 15	77± 17	115± 10
Sulphur total	569± 24	855± 67†*	582± 11
Taurine	455± 27	664±108*	388± 14
Cysteine + cysteate	32± 4	46± 10	44± 2
Methionine (+ ornithine)	82± 4	75± 10*	151± 11†
Aromatic total	388± 31	389± 46	383± 23
Tyrosine	195± 22	159± 16	165± 24
Phenylalanine	84± 4	109± 12	107± 7†
Tryptophan	110± 6	121± 20	113± 5
Non essential total	4359±175	4785±104	4945±213
Essential total	1652± 57	1852± 50†*	2303± 97†
Total	6010±226	6628±151	7248±300†

All values are expressed in μmoles/l and are the mean ± S.E.M. of 5–6 different animals.
The symbols used are the same as in Table 1.

In Table 2 the plasma concentrations of lactate, glucose, glycerol, urea, proteins, beta-hydroxybutyrate and aceto-acetate are shown. Hypothyroidism provoked a discrete, but significant decrease of plasma glucose levels. The thyroid status did not change the urea, lactate and glycerol concentrations, but lowered the plasma protein concentrations in hyperthyroid animals, both with regard to the controls and hypothyroid rats. Plasma beta-hydroxybutyrate and acetoacetate concentrations in the hypothyroid group were significantly higher than those of controls.

Changes in the plasma aminogram were more marked in the hyperthyroid than in the hypothyroid group when compared with controls. As can be seen in Table 3, the combined total amino acids concentration was significantly higher in hyperthyroid animals than in the controls; and was not significantly different from that of hypothyroid rats. The combined non essential amino acid concentrations were not statistically different in any of the three groups. Both hyperthyroid and hypothyroid animals had significantly higher essential amino acid concentrations than controls, and the hyperthyroid animals showed significantly higher values than did the hypothyroid rats.

Glycine, serine and branched chain amino acid concentrations were higher than controls in both groups. The hypothyroid group also showed a significant increase in sulphur amino acids concentration, both versus controls and hyperthyroid rats.

The hyperthyroid animals showed significant increases versus controls in proline, imino acids, basic amino acids (lysine, arginine, histidine) leucine + isoleucine, methionine + ornithine and phenylalanine, together with a significant decrease (versus controls and hypothyroid) in aspartate + asparagine concentration.

Discussion

The slower growth of hypothyroid rats together with their lower hematocrit value seems to point towards a hampered protein synthesis, in agreement with data found in the literature (*Aranda, Montoya* and *Herrera* 1972; *Castro* and *Herrera* 1973). This can be attributed to the diminished availability of growth hormone provoked by the deficiency in thyroid hormones (*Sterling* 1979; *Seo* et al. 1977). In the hyperthyroid animals this situation is not observed. However, their low plasma proteins, seem to indicate that the excess of thyroid hormones also affects protein turn-over in agreement with data in the literature (*Rupp, Paschkis* and *Cantarow* 1949; *Sokoloff* and *Kaufman* 1959; *Kyle, Ball* and *Doolan* 1966; *Menahan* and *Wieland* 1969).

Plasma glucose concentrations of hyperthyroid animals were similar to those of controls, in disagreement with data in the literature (*Aranda, Montoya* and *Herrera* 1972); these differences can be tentatively attributed to differences in the protocol of drug administration to the animals. The slight hypoglycemia found in the hypothyroid animals seems not to be compensated by an enhanced gluconeogenesis from amino acids, as gluconeogenic and non essential amino acids show a slight increase versus controls because of an increase in both glycine and serine concentrations. Glycerol and lactate — also good gluconeogenic substrates (*Exton* 1972) — showed no differences versus

controls. This apparent lack of ability to compensate for the low glucose concentration in hypothyroid animals is somewhat circumvented by the increased availability of circulating ketone bodies, index of an active fat mobilization. This situation differs from that found in the control and hyperthyroid rats. The lack of variation in the concentrations of urea in both groups agrees with the unaffected amino acid utilization indicated above.

The hypothyroid plasma aminogram shows little variation with respect to that of controls. The general trend is towards increased concentrations of essential amino acids, mainly branched chain and sulphur amino acids; but also serine and glycine show high concentrations. This can in some measure be related to an impairment of gluconeogenic utilization of these amino acids. This lower gluconeogenesis is in agreement with lower glucose concentrations and has been postulated in hypothyroidism (*Menahan* and *Wieland* 1969; *Bargoni, Grillo, Rinaudo, Fossa, Tourn* and *Bozzi* 1966). The glucose steady state can be maintained, however, due to decreased tissue glucose utilization in the hypothyroid state (*Bargoni* et al. 1966).

Hyperthyroidism is characterized by an increase in gluconeogenesis (*Menahan* and *Wieland* 1969; *Murad* and *Freedland* 1967) but our steady state data do not show changes pointing towards an enhanced gluconeogenesis from amino acids, as here also both glycine and serine concentrations are increased with unchanged alanine and glutamate plus glutamine, and only a significant decrease in aspartate plus asparagine. Nevertheless, given the increase in essential amino acid concentrations as a consequence of increased tissue proteolysis (*Hoberman* and *Graaf* 1950), while the non essential ones remain — in a general way — at the same concentrations as controls, it can be safely assumed that they are used for gluconeogenic or other energetic purposes; this is in agreement with the postulated enhanced gluconeogenesis in hyperthyroidism.

The general outline of the plasma aminogram in the hyperthyroid animals shows considerable increases in the essential amino acids, with no changes in the non essential. All this seems to suggest a considerable release of amino acids from peripheral tissues, not counteracted by an increased essential amino acids utilization, in spite of their considerable liver hypertrophy; thus, most amino acid concentrations in plasma are increased.

As a general conclusion, it can be advanced that the effect of the thyroid status on the plasma amino acid homeostasis produces a greater modification in hyperthyroidism than in hypothyroidism, and that both situations tend to alter in a similar way the concentration of certain amino acids, while their effect is completely different in some others. These results can be the direct consequence of both an impaired protein synthesis (*Rupp, Paschkis* and *Cantarow* 1949; *Sokoloff* and *Kaufman* 1959; *Kyle, Ball* and *Doolan* 1966) (thus affecting preferentially the concentrations of essential amino acids), combined with a higher proteolysis (*Menahan* and *Wieland* 1969) in the case of hyperthyroid animals, and other factors not yet known.

The aminograms generated by this experimental situation are considerably different in both cases between themselves and also versus controls, but more dynamic information is needed in order to explain the differences, as the single steady state data are not enough.

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Effect of Adrenalectomy on Hepatic, Pulmonary and Intestinal Mixed Function Oxidase in the Rat

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Summary

Aryl hydrocarbon hydroxylase (AHH) and 7-ethoxycoumarin O-deethylase (ECD) activities of microsomes from liver, lung, and intestine of control and adrenalectomized male and female rats were compared. In addition, adrenalectomized-ovariectomized rats were also used. Adrenalectomy decreased hepatic and pulmonary AHH and ECD activities in microsomes of both sexes. However, adrenalectomy increased the AHH activity of intestinal microsomes of both male and female rats, but had no effect on the intestinal ECD activity of either sex. The hepatic cytochrome P-450 content was decreased in male and female animals by adrenalectomy. The effects of adrenalectomy-ovariectomy on AHH and ECD activities were similar to the results obtained for adrenalectomy alone, indicating that physiological levels of estrogens do not regulate these enzymes. Organ specific alterations of microsomal mixed function oxidases occur with adrenalectomy of both male and female rats.

Key-Words: *Aryl Hydrocarbon Hydroxylase (AHH) – 7-Ethoxycoumarin O-Deethylase (ECD) – Cytochrome P-450 – Adrenalectomy – Adrenalectomy-Ovariectomy – Hepatic – Extrahepatic*

Introduction

Remmer (1959) first reported that the hydroxylation of hexobarbital and N-demethylation of monomethyl-4-aminoantipyrine in male rats was impaired by adrenalectomy and that the administration of cortisone or prednisolone to intact rats increased hexobarbital metabolism above the control levels. Similarly, *Kato* and *Gillette* (1965) demonstrated that adrenalectomy of male rats impaired the metabolism of aminopyrine and hexobarbital, but adrenalectomy did not significantly affect the metabolism of aniline and zoxazolamine. Furthermore, adrenalectomy of female rats did not decrease the metabolism of any of these substrates (*Kato* and *Gillette* 1965). However, *Nebert* and *Gelboin* (1969) reported a decrease in microsomal aryl hydrocarbon hydroxylase (AHH) activity of liver, lung, small intestine and kidney from adrenalectomized male rats. More recently, *Marshall* (1971) has reported that adrenalectomy of male rats decreased aminopyrine N-demethylation without affecting aniline hydroxylation.

Castro, Greene, Gigon, Sasame and *Gillette* (1970) observed that treatment with cortisone reversed the decrease in aminopyrine metabolism produced by adrenalectomy in male rats. However, *Furner* and *Stitzel* (1968) could not detect reversal by cortisol treatment of the decreased metabolism of hexobarbital and ethylmorphine in adrenalectomized male rats. The reason for this discrepancy is not known.

Several investigators have shown that the content of cytochrome P-450 is significantly decreased in liver microsomes from adrenalectomized male rats (*Wade, Higoshi, Umemoto* and *Sahamoto* 1964; *Ichii* and *Yago* 1969; *Orrenius, Gnosspelius, Pas* and *Ernster* 1968; *Castro* et al. 1970). *Wade* et al. (1964) reported that the decrease in cytochrome P-450 content in adrenalectomized rats was restored by cortisone administration. However, this was not confirmed