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Plasma and Tissue Concentrations of Free Taurine and Amino Acids in Fasted, and Hyper- or Hypothyroid Diet Fed Rats[1]

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There are many reports concerning with taurine levels in mammalian organs. Especially, the data for the rat have been considerably accumulated by a number of investigators. Some authors show that taurine is preferably present in the intracellular space of tissues rather than in the extracellular one,[3] and others indicate that tissue level of taurine is intimately related to cellular concentrations of several amino acids.[4]

Jacobsen and Smith Jr.[5] described a detailed review for taurine and factors influencing taurine levels in rat tissues. However, unexpectedly few are the works to study plasma and tissue levels of taurine and their changes along with those free amino acids.

In the present study, free taurine and amino acids are determined for the plasma, heart and liver of normal, *ad libitum* fed rats, using an amino acid autoanalyser, and their changes in concentration in fasted, and hyper- or hypothyroid states are investigated.

Experimental method

Male Wistar rats weighing approximately 220—250 g were used in this study. They were housed 3 numbers in a plastic cage in a thermostatically controlled room at 24—25° and given a commercial diet (CLEA, CE-2) and tap water *ad libitum*. These animals were served as normal, *ad libitum* fed controls. In fasting experiment, rats were individually placed in a metal wire cage, withheld the diet and given only water for 72 hr before sacrifice. Hyperthyroidism was induced in rats by adding 2% thyroid powder (J.P. grade) to the diet for 14 days. Body weight and food intake were measured daily and only the animals showing a sufficient decrease of body weight during the period were used for the experiment. Hypothyroidism was produced in rats by feeding the diet containing 1% thiouracil (NB Co.) for 30 days. At sacrifice, the rats showing apparently enlarged thyroid were chosen for use. In every experiment, one or two normal diet fed animals were killed as control. All the animals were intravenously injected heparin under ether anesthesia a few minutes before sacrifice and then immediately the midline-incision was made on the abdomen. Blood was withdrawn from the abdominal arota by means of a dry, sterile syringe and plasma was obtained by centrifugation at low speed for 5 min. After bleeding, the whole body was perfused with 100 ml of warmed Krebs Ringer bicarbonate buffer through the portal vein. Liver and heart were then taken out, soaked in cold buffer, brotted and weighed.

For the determination of free amino acids, samples were prepared according to the method of Stein and Moore.[6] Plasma (2 ml) was deproteinized and extracted by adding five volumes of a 1% picric acid solution. Tissue (600—800 mg) was chopped and extracted under heating for 5 min with ten volumes of the same solution. The supernatant fluid of each sample was applied on an ion exchange resin column (Dowex 2×8, Cl^--type), and eluted with 0.02N HCl for removal of excess picric acid. For the determination of free

1) E. Fujihira, H. Takahashi and M. Nakazawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 1636 (1970).
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3) *a*) P. Campbell and T. Work, *Biochem. J.*, **50**, 449 (1952); *b*) J. Awapara, *Federation Proc.*, **14**, 175 (1955); *c*) A. Minato, S. Hirose, T. Ogiso, K. Uda, Y. Takigawa and E. Fujihira, *Chem. Pharm. Bull.* (Tokyo), **17**, 1498 (1969).
4) *a*) J. Gilbert, Y. Ku, L. Rogers and R. Williams, *J. Biol. Chem.*, **235**, 1055 (1960); *b*) H. Kromphradt, *Biochem. Z.*, **339**, 233 (1963); *c*) H. Christensen, *J. Biol. Chem.*, **239**, 3584 (1964).
5) J. Jacobsen and L. Smith Jr., *Physiol. Rev.*, **48**, 424 (1968).
6) W. Stein and S. Moore, *J. Biol. Chem.*, **211**, 915 (1954).

taurine, the effluent was hydrolized with 6N HCl, according to the method of Garvin.[7] After neutralized, allowed to stand for 4 hr at room temperature, re-acidified and adjusted the final volume, an aliquot of the test-sample was charged on a Hitachi amino acid autoanalyser (LKA-3B) and ninhydrin reactive substances were determined by the method of Speckman, *et al.*[8]

Result and Discussion

Plasma

As shown in Table I, average plasma concentration of taurine was determined to be 23.0 μ moles per 100 ml in normal, *ad libitum* fed rats. This value is in good agreement with those reported by the other workers.[9] Of plasma amino acids, alanine gave the highest concentration, and aspartic acid the lowest.

TABLE I. Plasma Concentration of Taurine and Amino Acids[a]

Compds.	*Ad libitum* fed	72 hr fasted	Thyroid powder fed	Thiouracil fed
Taurine	23.0±3.0	71.4±6.7	87.7±2.3	65.8±4.8
Aspartic	2.0±0.1	1.3±0.1	5.0±0.6	1.3±0.3
Glutamine[b]	29.6±4.6	25.0±1.2	54.7±7.4	28.2±3.2
Serine	24.8±2.0	25.1±1.3	42.6±3.9	20.7±1.5
Glutamic	12.4±1.3	13.1±2.4	25.0±2.0	9.4±1.1
Glycine	35.3±2.8	40.4±4.0	36.3±1.7	36.3±2.4
Alanine	52.2±3.6	30.9±2.3	94.0±2.1	23.2±0.6
Lysine	34.3±1.9	32.6±3.0	12.1±0.9	26.4±2.1
Histidine	4.7±0.6	3.8±0.5	10.6±1.3	3.2±0.2
Valine	24.4±2.8	15.0±1.3	40.7±5.3	16.9±2.3
Methionine	4.9±0.4	4.4±0.6	8.7±0.9	3.8±0.3
Isoleucine	9.8±0.8	8.1±1.8	20.6±2.1	8.1±0.8
Leucine	16.3±0.9	13.1±1.4	33.2±2.0	15.0±1.3
Tyrosine	8.1±0.9	5.0±0.6	11.9±1.2	7.5±1.1
Phenylalanine	6.0±0.7	5.0±0.5	10.0±0.9	6.6±0.7
Tryptophan	7.5±0.5	5.0±0.5	5.0±0.2	trace
Arginine	6.8±0.6	5.6±0.6	5.7±0.6	trace

a) μmoles per 100 ml (S.E.); five determinations
b) contaminated with a small amount of threonine.
Experimental conditions are shown in the text.

The three experimental conditions, 72 hr fast, and hyper- and hypothyroidisms, caused a significant increase of taurine level in rat plasma from the control level. Previously, Wu[9a)] and Awapara[10] described the markedly increased level of taurine in the plasma of long-fasted rats. A generalized increase in plasma concentration of amino acids was found only in hyperthyroidism. The concentrations of aspartic and glutamic acids, histidine, isoleucine and leucine were 2.0—2.5 times higher than those found in euthyroid state. As an exception, plasma lysine was significantly low in hyperthyroidism, as compared to the control level. On the other hand, 72 hr fast and hypothyroidism caused a slight fall of plasma levels of several amino acids, or did not change the normal levels of the other acids. From the well-known phenomenon of nitrogen inbalance in hyperthyroidism, and the Wu's description on the increased urinary taurine associated with the decreased amino acids in fast, the plasma levels

7) J. Garvin, *Arch. Biochem. Biophys.*, **91**, 291 (1960).
8) D. Speckman, W. Stein and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).
9) *a*) C.Wu, *J. Biol. Chem.*, **207**, 775 (1954); *b*) T. Riggs and L. Walker, *J. Biol. Chem.*, **238**, 2663 (1963).
10) J. Awapara, *J. Biol. Chem.*, **218**, 571 (1956).

of taurine and amino acids can be explained to reflect the urinary excretion of these compounds in each state.

Heart

Table II shows the concentrations of taurine and amino acids in the rat heart under each experimental condition. A remarkably high concentration of taurine was noted in the heart of normal rats. However, this value is approximately intermediate among those reported in the literature (1600—3796 μmoles per 100 g).[7,10,11] Glutamic acid, glutamine, aspartic acid and alanine were predominant amino acids in the rat heart. The tissue to plasma concentration ratio thus indicates an extremely high concentration gradient of heart taurine, aspartic and glutamic acids against their plasma levels.

TABLE II. Heart Concentration of Taurine and Amino Acids[a]

Compds.	*Ad libitum* fed	72 hr fasted	Thyroid powder fed	Thiouracil fed
Taurine	2405±70.5 (105)	4243±187 (59.4)	4446±297 (50.6)	3351±125 (50.9)
Aspartic	373±23.1 (186)	256± 21.3 (197)	371± 29.2 (74.2)	220± 10.0 (169)
Glutamine[b]	493±80.8 (18.3)	568± 57.4 (22.7)	522± 42.5 (9.5)	550± 48.3 (19.5)
Serine	64± 5.2 (2.6)	63± 5.8 (2.4)	152± 10.7 (3.5)	59± 4.3 (2.8)
Glutamic	579±61.5 (46.7)	572± 18.4 (43.6)	1083± 40.1 (43.3)	348± 18.6 (37.0)
Glycine	99± 6.7 (2.8)	105± 10.0 (2.5)	110± 9.8 (3.0)	102± 8.2 (2.8)
Alanine	238±10.7 (4.5)	403± 30.9 (13.0)	386± 17.4 (4.1)	165± 14.0 (7.1)
Lysine	53± 2.2 (1.5)	49± 2.4 (1.5)	55± 5.0 (5.5)	55± 0.5 (2.0)
Histidine	21± 2.5 (4.4)	20± 1.2 (5.7)	28± 1.7 (2.7)	22± 1.0 (6.8)
Valine	24± 0.5 (1.0)	22± 0.3 (1.5)	25± 0.1 (0.6)	21± 0.3 (1.2)
Methionine	8± 0.5 (1.7)	8± 1.0 (2.6)	10± 1.2 (1.1)	9± 0.8 (2.3)
Isoleucine	23± 2.2 (2.4)	23± 1.5 (2.9)	26± 2.3 (1.3)	25± 2.1 (3.0)
Leucine	24± 0.9 (1.5)	22± 1.0 (1.7)	21± 2.0 (0.6)	22± 0.8 (1.5)
Tyrosine	8± 0.7 (1.0)	7± 0.7 (1.4)	8± 0.7 (0.7)	7± 0.5 (0.9)
Phenylalanine	7± 0.7 (1.2)	8± 0.6 (1.6)	9± 1.0 (0.9)	7± 0.7 (1.1)
Tryptophan	4± 0.2 (0.5)	trace	4± 0.2 (0.8)	4± 0.2
Arginine	24± 1.1 (3.5)	24± 2.0 (4.3)	24± 1.6 (4.2)	24± 2.1

a) μmoles per 100 g of wet weight (S.E.); five determinations
b) contaminated with a trace amount of threonine.
Heart to plasma concentration ratio is in parentheses.
Method of sample preparation is shown in the text.

Like the case of the plasma level, the heart content of taurine was significantly increased by fasting and feeding the hyper-and hypothyroid diets. There are no information available in the literature concerning any change of heart taurine in rats under any kind of experimental conditions.[10,12] One of the previous authors noted a significant increase of taurine content in the skeletal muscle of fasted rats, but not in their hearts.[10] Neverthless the increased taurine content in the hearts of thyroid powder fed rats, approximately 1.8 times higher than the control level, is of much interest, if permissible to connect this to other phenomena in hyperthyroidism, such as cardiac muscular excitability. Some workers[13] showed that hyperthyroidism caused a decrease of myocardial response to digitalis, and others[14] also demonstrated that administered taurine suppressed completely digitoxine-induced premature

11) *a*) R. Scharff and I. Wool, *Nature*, **202**, 603 (1964); *b*) P. Boquet and P. Fromageot, *Biochim. Biophys. Acta*, **97**, 222 (1965).
12) *a*) V. Kostos and J. Kocsis, *Proc. Soc. Exp. Biol. Med.*, **106**, 659 (1961); *b*) D. Bowden and R. Goyer, *Arch. Pathol.*, **74**, 137 (1962); *c*) R. Goyer, M. Yin and D. Bowden, *Proc. Soc. Exp. Biol. Med.*, **116**, 534 (1964).
13) *a*) R. Frye and E. Braunwald, *Circulation*, **23**, 376 (1961); *b*) D. Morrow, T. Gaffney and E. Braunwald, *J. Pharmacol. Exp. Therap.*, **140**, 324 (1963).
14) *a*) W. Read and J. Welty, *J. Pharmacol. Exp. Therap.*, **139**, 283 (1963); *b*) J. Welty and W. Read, *ibid.*, **144**, 110 (1964).

ventricular contractions, probably due to prevention of potassium loss from the cardiac tissue.

Hyperthyroidism also increased the heart levels of alanine, glutamic acid and serine. 72 hr fast increased only alanine content in the heart. While, hypothyroidism caused a slight decrease in concentration of heart aspartic and glutamic acids, and alanine from their control levels.

In every three states, the heart to plasma concentration ratio of taurine commonly decreased from that in control state. As for free amino acids, alanine showed a markedly high concentration gradient between the heart and plasma in 72 hr fasted rats. In hyperthyroidism, only lysine demonstrated a highly elevated concentration ratio, while aspartic acid, glutamine and histidine were decreased in their concentration ratio from control.

Liver

The data for the liver are shown in Table III. Normal, *ad libitum* fed rats showed the liver content of taurine to be 770 μmoles per 100 g. In the literature, there is a considerable variation among the values for the liver taurine of male rats (60—550 μmoles per 100 g).[7,9a,10,15] The discrepancy is possibly attributable to the differences in procedures of tissue preparation, diet, and age and strain of rats used, from laboratory to laboratory.

The highest concentration of liver amino acids was noted in alanine, which was followed by aspartic acid, glycine and glutamic acid. The tissue to plasma concentration ratio of free amino acids was generally higher for the liver than for the heart, while only the values for liver taurine and glutamic acid were lower.

TABLE III. Liver Concentration of Taurine and Amino Acids[a)]

Compds.	*Ad libitum* fed	72 hr fasted	Thyroid powder fed	Thiouracil fed
Taurine	770±45.0 (33.5)	747±20.5 (10.4)	475±48.0 (5.4)	678±50.8 (10.3)
Aspartic	369±41.4 (184)	550±32.2 (423)	275±31.2 (55.0)	477±49.3 (367)
Glutamine[b)]	87±16.2 (2.9)	96± 8.6 (3.8)	72±11.1 (1.3)	77± 7.4 (2.7)
Serine	110± 2.6 (4.4)	101± 9.3 (4.0)	103±13.2 (2.4)	110± 2.5 (5.3)
Glutamic	192±19.2 (15.4)	385±42.0 (29.3)	198± 5.4 (7.9)	229±18.7 (24.3)
Glycine	271±19.8 (7.6)	596±31.8 (14.7)	264±14.2 (7.2)	339±10.3 (9.3)
Alanine	550±59.4 (10.5)	477±24.8 (15.4)	349±17.4 (3.7)	363±24.3 (15.6)
Lysine	88± 5.7 (2.6)	55± 5.1 (1.7)	72± 7.0 (1.8)	62± 0.5 (2.3)
Histidine	67± 5.8 (14.3)	19± 1.9 (5.5)	49± 7.2 (4.6)	27± 0.9 (8.3)
Valine	68± 5.6 (2.8)	64± 6.4 (4.3)	28± 0.8 (0.7)	58± 3.6 (3.3)
Methionine	25± 2.2 (5.0)	27± 0.6 (8.7)	25± 2.3 (2.9)	trace
Isoleucine	21± 2.5 (2.1)	28± 0.3 (3.4)	20± 0.6 (1.0)	22± 2.0 (2.7)
Leucine	55± 6.0 (3.3)	92± 9.5 (6.7)	28± 2.1 (0.8)	58± 0.5 (3.9)
Tyrosine	24± 2.6 (3.0)	trace	25± 2.3 (2.1)	28± 1.0 (3.7)
Phenylalanine	24± 2.6 (4.0)	28± 0.3 (5.5)	24± 2.4 (2.4)	28± 1.0 (4.2)
Tryptophan	trace	trace	trace	trace
Arginine	trace	trace	trace	trace

a) μmoles per 100 g of wet weight (S.E.); five determinations
b) contaminated with threonine.
Liver to plasma concentration ratio is in parentheses.
Experimental conditions are shown in the text.

The normal level of taurine in rat liver was not changed significantly by fasting, but generally decreased by feeding the hyper-and hypothyroid diets. In contrast, Wu[9a)] and Awapara[10)] reported previously a considerable increase of taurine content in the livers of long-fasted

15) *a*) J. Awapara, *Proc. Soc. Exp. Biol. Med.*, **90**, 435 (1955); *b*) J. Bremer, *Acta Chem. Scand.*, **9**, 683 (1955); *c*) J. DuRuisseau, J. Greenstein, M. Wintz and S. Birnbaum, *Arch. Biochem. Biophys.*, **68**, 161 (1957); *d*) J. Jacobsen and L. Smith Jr., *Nature*, **200**, 575 (1963).

male but not female rats. An exact explanation for the difference between this and others' results is impossible, but merely possible to point out some differences in experimental procedures, for example, duration of fasting and employment of liver perfusion. On the other hand, the decrease of liver taurine in hyperthyroidism could be explained by the fact that thyroxine inhibits greatly the activities of cysteinesulfinic acid and cysteic acid decarboxylases in rat liver.[16] Liver taurine was not so much decreased in hypothyroidism as seen in hyperthyroidism. Bergeret, *et al.*[17] reported that thyroidectomy had no effect on the activities of such enzymes as the mentioned above.

As compared to control, a trend to increase in liver concentration of aspartic and glutamic acids and glycine, and to decrease in that of alanine, lysine and histidine was observed in both states of 72 hr fast and hypothyroidism. Contrary to them, hyperthyroidism decreased generally liver amino acids from their normal levels. Therefore, a significantly low concentration ratio of free amino acids was established between the liver and plasma of the thyroid powder fed rats.

Although both fast and hyperthyroidism are equally in a catabolic state, changes of amino acid concentration in the liver as well as in the plasma and heart was apparently opposite between them. Ryan, *et al.*[18] reported an initial rise and a subsequent fall of free amino acids in the plasma and muscular tissue of rats following the repeated administrations of hydrocortisone. Thompson, *et al.*[19] found no general pattern of response for all amino acids in the rat liver during long periods of fast. It thus appears that plasma and tissue levels of free amino acids are much variable during the course of catabolic process. Overall parallelism between the changes in concentration of taurine and amino acids was seen only in hyperthyroidism but not in both fast and hypothyroidism. There was no specific amino acid showing the changes in plasma and tissue concentrations closely related to those of taurine through the three experimental conditions.

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17) B. Bergeret, J. Labouesse and F. Chatagner, *Bull. Soc. Chim. Biol.*, **40**, 1923 (1958).
18) W. Ryan and M. Carver, *Proc. Soc. Exp. Biol. Med.*, **114**, 816 (1963).
19) T. Thompson, P. Schurr, L. Henderson and C. Elvehjem, *J. Biol. Chem.*, **182**, 39 (1950).