論　文

Correlation Analysis between Thyroid-related Hormones and the Plasma Concentration of Amino Acids

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甲状腺関連ホルモンと血漿アミノ酸濃度との相関解析

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【要　　旨】

甲状腺関連ホルモン（TRHs）は甲状腺の内分泌機能調節や代謝プロセスに関与し、その機能亢進により振戦、体重減少、多汗などをきたすことが知られている。そこでTRHsの血漿アミノ酸代謝への影響を調べるためにTRHsと血漿アミノ酸濃度との相関を７人の健常者ボランティアの検体を用いて分析した。TRHsは、甲状腺刺激ホルモン（TSH）、遊離トリヨードサイロニン（FT3）、遊離チロキシン（FT4）の３種類を測定した。さらに、タンパク質を構成する20種類のアミノ酸を含む合計39種類のアミノ酸も測定した。その結果、タウリンとFT4（$p=0.018$）、バリンとFT4（$p=0.043$）、シトルリンとTSH（$p=0.011$）、ヒドロキシプロリンとTSH（$p=0.049$）との間に有意な相関が認められた。また、２種類のアミノ酸間の相関解析も実施したところ、シトルリンとヒドロキシプロリン（$p=0.0079$）で有意な相関が認められた。これらよりTRHsの血漿アミノ酸代謝にシトルリンおよびヒドロキシプロリンの相互作用が関与している可能性が示唆された。

【キーワード】

甲状腺ホルモン　甲状腺刺激ホルモン　アミノ酸　トリヨードサイロニン　チロキシン

【Abstract】

Thyroid-related hormones（TRHs）are involved in the regulation of endocrine function and metabolic processes of the thyroid gland, and hyperthyroidism is known to cause tremors, weight loss, and excessive sweating. As a part of examining the relationship between TRHs and the metabolism of amino acid in the plasma, we analyzed the correlation between TRHs and plasma levels of amino acids using samples from seven healthy volunteers. We measured three types of TRHs: thyroid-stimulating hormone（TSH）, free triiodothyronine（FT3）, and free thyroxine（FT4）in addition to 39 different amino acids

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including the 20 proteinogenic amino acids in the plasma collected from the healthy volunteers. We observed significant correlations between levels of taurine and FT4 (p = 0.018), valine and FT4 (p = 0.043), citrulline and TSH (p = 0.011), and hydroxyproline and TSH (p = 0.049). We also observed a correlation between the levels of citrulline and hydroxyproline (p = 0.0079). These results suggest that the interaction between citrulline and hydroxyproline may be involved in the plasma amino acid metabolism of TRHs.

【Keywords】
Thyroid hormone, thyroid-stimulating hormone, amino acids, triiodothyronine, thyroxine

Introduction

Thyroid-related hormones (TRHs) including triiodothyronine (T3) and thyroxine (T4) are deeply involved in metabolic regulation, growth, and development of various tissues in adults and juveniles.[1)2)] T3 and T4 are key molecules regulating thyroid hormone (TH) metabolism.[1)] The thyroid gland is controlled by thyroid-stimulating hormone (TSH) and is regulated by TH feedback.[1)] Hyperthyroidism (Graves'disease) is an autoimmune disorder, symptoms of which are tremor, excessive sweating, palpitation, agitation, fatigue, increased appetite, weight loss, heat intolerance, and menstrual disorder.[3)4)] Autoimmune damages to the thyroid grand are known to be a major cause of hyperthyroidism and thyrotoxicosis. T3 also affects functions of liver, pancreas, and muscle.[1)] Another review reports the effects of thyroid hormones (THs) on central nervous system differentiation and fetal brain development in animals as well as in humans.[5)] Furthermore, THs are known to produce significant neurological effects by acting as neuroprotective agents and are considered to be future diagnostic and therapeutic targets for Alzheimer's disease.[6)] Recently, the incidence of neurological disorders such as congenital microcephaly, mental disability, spastic paraplegia and polyneuropathy that are known to affect amino acid synthesis has increased.[7)] Therefore, we hypothesized that TH concentrations may be correlated with plasma amino acid concentrations. In this paper, we examined plasma concentrations of THs and amino acids, and discussed the possible relationship between thyroid disorders and amino acid synthesis disorders.

Materials and Methods

Peripheral blood was collected from seven healthy volunteers living in Oita prefecture, Japan (Table 1). Blood sampling was conducted in the morning without dietary restriction. The plasma concentration of amino acids was measured using LC (Liquid chromatography)/MS (Mass spectrometry). Plasma levels of 39 different amino acids were compared to the normal values (Table 2). TRHs (TSH, FT3, and FT4) levels weremeasured at Bungoono City Hospital in Oita prefecture (Table 3).

Table 1. Summary of healthy volunteers.

	Age	Sex
Case 1	37	Male
Case 2	39	Male
Case 3	47	Male
Case 4	24	Female
Case 5	47	Female
Case 6	55	Male
Case 7	64	Female

Table 2. Plasma concentrations of amino acids (nmol/ml).

Amino acids	Volunteers							Normal range*
	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	
Taurine	75.6	116.4	74.2	62.5	64.2	63.4	41.8	39.5 – 93.2
Aspartic acid	4.7	3.7	3.1	2.6	TR	2.1	TR	≤2.4
Hydroxyproline	TR	14.6	11.2	11.4	TR	17.1	TR	≤21.6
Threonine	100.1	123.3	122.9	163.7	122.9	126.4	139.6	66.5 – 188.9
Serine	96.7	127.1	118.2	160.8	107.5	114.5	102.7	72.4 – 164.5
Asparagine	40.8	41.5	43.9	46	48.7	37.3	44.7	44.7 – 96.8
Glutamic acid	47.1	28.4	33.5	27.6	17.5	35.7	20	12.6 – 62.5
Glutamine	488.7	605.3	504.5	581.3	581.9	481.2	574.6	422.1 – 703.8
Sarcosine	ND	TR	ND	ND	ND	ND	ND	TR
α-Aminoadipic acid	ND	ND	ND	ND	ND	ND	ND	ND
Proline	120.6	183.9	124.4	175.8	147.6	155	104.5	77.8 – 272.7
Glycine	205.9	184.2	260.4	243.3	211.6	167.7	187.3	151.0 – 351.0
Alanine	293.7	249.6	336.1	369.4	255.7	380.1	314.8	208.7 – 522.7
Citrulline	20.6	20.3	25.7	23.4	21.6	28.9	21.8	17.1 – 42.6
α-Aminobutyric acid	27.3	13.7	19.6	16.9	18.4	19.4	18.6	7.9 – 26.6
Valine	256	173.7	214.8	177.7	209.3	196.5	183.6	147.8 – 307.0
Cystine	20.3	13.5	18.1	10.6	5.7	15.6	18.3	13.7 – 28.3
Cystathionine	ND	ND	ND	ND	4.8	ND	ND	TR
Methionine	23.4	25.1	24	20.5	16.9	16.2	23.6	18.9 – 40.5
Isoleucine	63.3	49	60.3	48.1	59.1	48	49.3	43.0 – 112.8
Leucine	128.9	89.9	115.4	87	111.1	101	100.4	76.6 – 171.3
Tyrosine	65.5	52.6	44.9	63.2	53.4	51.7	66.9	40.4 – 90.3
Phenylalanine	64.9	60.5	52	47.1	53.6	46.6	58.1	42.6 – 75.7
γ-Amino β-hydroxybutyric acid	ND	ND	ND	ND	ND	ND	ND	ND
β-Alanine	3.8	3.5	4.2	TR	3.4	TR	TR	TR
β-Amino-iso-butyric acid	ND	ND	ND	ND	TR	3	3.4	TR
γ-Aminobutyric acid	ND	ND	ND	ND	ND	ND	ND	ND
Monoethanolamine	7.8	7.3	6.5	6.8	5.8	5.8	6.8	≤10.4
Homocystine	ND	ND	ND	ND	ND	ND	ND	ND
Histidine	73.4	75.9	63.4	71.2	78.1	73	75.5	59.0 – 92.0
3-Methylhistidine	4.3	TR	4.6	TR	TR	TR	TR	≤5.0
1-Methylhistidine	6.6	ND	ND	ND	7.9	5.2	TR	≤18.5
Carnosine	ND	ND	ND	ND	ND	ND	ND	ND
Anserine	ND	ND	ND	ND	ND	ND	ND	ND
Tryptophan	53.9	42.1	55.9	46.5	39.3	47.5	48.9	37.0 – 74.9
Hydroxylysine	ND	ND	ND	ND	ND	ND	ND	ND
Ornithine	49.1	52.4	49.3	49.9	57.3	52.1	47.4	31.3 – 104.7
Lysine	199.2	175.3	184.4	150.6	124.9	163.8	177.3	108.7 – 242.2
Arginine	87	101.2	86.9	75	44.6	95.4	103.4	53.6 – 133.6
Total AA	2590.3	2594.9	2620.9	2703.8	2440.4	2504.9	2504.5	2068.2 – 3510.3
NEAA	1627.2	1780.1	1727.8	1891.4	1625.2	1685.9	1648.2	1381.6 – 2379.4
EAA	963.1	814.8	893.1	812.4	815.2	819	856.3	660.0 – 1222.3
BCAA	448.2	312.6	390.5	312.8	379.5	345.5	333.3	265.8 – 579.1
EAA/NEAA	0.59	0.46	0.52	0.43	0.5	0.49	0.52	0.40 – 0.63
BCAA/Total AA	0.17	0.12	0.15	0.12	0.16	0.14	0.13	0.11 – 0.18
Fischer ratio	3.44	2.76	4.03	2.84	3.55	3.51	2.67	2.43 – 4.40

ND: not detected.
TR: trace.
*Normal ranges were obtained from SRL. Inc.
Table 2 references the preceding reports[16].

Table 3. Thyroid-related hormones levels.

	FT3(pg/mL)	FT4 (ng/dL)	TSH(μlU/mL)
Case 1	3.39	1.42	0.623
Case 2	2.81	0.95	0.470
Case 3	3.24	1.13	1.196
Case 4	3.56	1.07	1.153
Case 5	3.08	1.23	0.607
Case 6	3.04	1.08	3.422
Case 7	2.97	0.76	0.934

Pearson's product-moment correlations between plasma levels of TRHs and the 39 amino acids were calculated. The correlation analysis was done as described previously.[8)9)] Statistical analyses were conducted using the R statistical software package (http://www.r-project.org). We calculated the correlation coefficients and p-values using the following R command prompt. cor.test(x[,i],x[,j]), where "i" and "j" are plasma levels of amino acids and TRHs, respectively.

Results

We examined correlations between plasma levels of each TRH and each of the measured amino acids (Table 4). We observed significant positive correlations in four combinations, taurine vs FT4 ($r = 0.889$, $p = 0.018$), valine vs FT4 ($r = 0.827$, $p = 0.043$), hydroxyproline vs TSH ($r = 0.814$, $p = 0.049$) and citrulline vs TSH ($r = 0.915$, $p = 0.011$). In addition, we calculated the correlation for all the possible pairs of the measured amino acids shown in Table 2. We observed significant correlations in 26 of these combinations (Table 5). Out of the four amino acids (taurine, valine, citrulline, and hydroxyproline) that were correlated with either FT4 or TSH, a significant correlation was found between citrulline and hydroxyproline ($r = 0.926$, $p = 0.0079$). Interaction of citrulline and hydroxyproline may affect the regulation of plasma amino acid metabolism by the TRHs. It is also notable that among the 26 significantly correlated pairs of amino acids, 16 pairs involved essential amino acids (Table 5). Seven essential amino acids that were involved in the 16 pairs were leucine, isoleucine, lysine, phenylalanine, threonine, tryptophan, and valine. Some essential amino acids showed significant correlations with other amino acids. Leucine was correlated with valine, threonine, isoleucine, β-alanine, and α-aminobutyric acid. Valine was correlated with leucine, α-aminobutyric acid, threonine, and isoleucine. Threonine was correlated with leucine, valine, serine, and α-aminobutyric acid (Table 5).

Discussion

In this study, we showed significant positive correlations between the plasma levels of FT4 and each of valine and taurine. A mutation substituting valine with glycine in the thyroid peroxidase gene has been reported to be associated with congenital hypothyroidism and multinodular goiter.[10)] In addition, a mutation substituting alanine with valine in the thyrotropin receptor gene has been reported to be associated with autosomal dominant non-autoimmune hyperthyroidism.[11)] These suggested that the coordination between valine and FT4 is a key factor for normal thyroid function. Also, taurine levels in platelets have been reported to be associated with thyroid function.[12)] The interaction of FT4 with taurine observed in this study suggests that FT4 may also be associated with thyroid function. There are few reports on the association of TSH with citrulline and hydroxyproline. It has been reported that plasma levels of citrulline and hydroxyproline are significantly increased in patients with Alzheimer's disease.[13)] In contrast, plasma levels of citrulline and hydroxyproline have been reported to be signifi-

Table 4. Correlation analysis between thyroid-related hormones and the plasma concentration of amino acids.

		FT3		FT4		TSH	
Type	Amino acids	Correlation coefficient	*p*-value	Correlation coefficient	*p*-value	Correlation coefficient	*p*-value
Essential amino acids	Histidine	−0.367	0.474	−0.044	0.933	−0.127	0.810
	Isoleucine	0.160	0.762	0.749	0.087	−0.562	0.245
	Leucine	−0.045	0.933	0.683	0.135	−0.339	0.511
	Lysine	0.137	0.796	0.085	0.873	−0.018	0.973
	Methionine	0.270	0.605	−0.112	0.832	−0.546	0.262
	Phenylalanine	−0.023	0.966	0.313	0.546	−0.626	0.184
	Threonine	0.226	0.666	−0.647	0.165	0.088	0.868
	Tryptophan	0.263	0.615	0.107	0.840	−0.001	0.998
	Valine	0.184	0.727	0.827	0.043*	−0.301	0.563
Non-essential amino acids	Alanine	0.231	0.660	−0.346	0.501	0.733	0.097
	Anserine	NA	NA	NA	NA	NA	NA
	Arginine	−0.168	0.750	−0.466	0.352	0.399	0.433
	Asparagine	0.107	0.840	−0.124	0.815	−0.743	0.091
	Aspartic acid	0.695	0.126	0.647	0.165	0.030	0.956
	Carnosine	NA	NA	NA	NA	NA	NA
	Citrulline	−0.213	0.685	−0.192	0.716	0.915	0.011*
	Cystathionine	−0.287	0.581	0.259	0.620	−0.331	0.522
	Cystine	−0.027	0.959	−0.066	0.901	0.111	0.834
	Glutamic acid	0.433	0.391	0.613	0.195	0.221	0.674
	Glutamine	0.009	0.987	−0.443	0.379	−0.517	0.293
	Glycine	0.633	0.178	0.207	0.694	−0.495	0.318
	Homocystine	NA	NA	NA	NA	NA	NA
	Hydroxylysine	NA	NA	NA	NA	NA	NA
	Hydroxyproline	0.150	0.777	−0.115	0.828	0.814	0.049*
	Monoethanolamine	0.557	0.251	0.271	0.604	−0.516	0.295
	Ornithine	−0.230	0.661	0.370	0.470	0.050	0.925
	Proline	0.457	0.363	0.183	0.729	0.356	0.488
	Sarcosine	NA	NA	NA	NA	NA	NA
	Serine	0.644	0.168	−0.163	0.757	0.113	0.831
	Taurine	0.528	0.281	0.889	0.018*	−0.030	0.955
	Tyrosine	0.229	0.662	−0.167	0.752	−0.380	0.457
	α-Aminoadipic acid	NA	NA	NA	NA	NA	NA
	α-Aminobutyric acid	0.206	0.696	0.673	0.143	−0.210	0.689
	β-Alanine	0.138	0.794	0.709	0.115	−0.507	0.305
	β-Amino-iso-butyric acid	−0.714	0.111	−0.729	0.100	0.567	0.240
	γ-Amino β-hydroxybutyric acid	NA	NA	NA	NA	NA	NA
	γ-Aminobutyric acid	NA	NA	NA	NA	NA	NA
	1-Methylhistidine	−0.187	0.722	0.683	0.135	0.033	0.950
	3-Methylhistidine	0.337	0.514	0.553	0.255	−0.295	0.571

*$p < 0.05$

Table 5. Correlation analysis result between two amino acids with significant difference.

	Two amino acids		Correlation coefficient	p-value
Essential amino acids involved	Lysine*	Cystine	0.980	0.0006
	Isoleucine*	β-Alanine	0.978	0.0007
	Leucine*	Valine*	0.957	0.0027
	Leucine*	Threonine*	−0.954	0.0031
	Lysine*	Tryptophan*	0.938	0.0057
	Valine*	α-Aminobutyric acid	0.925	0.0083
	Leucine*	Isoleucine*	0.916	0.0102
	Valine*	Threonine*	−0.910	0.0117
	Tryptophan*	Cystine	0.897	0.0154
	Isoleucine*	Valine*	0.881	0.0204
	Leucine*	β-Alanine	0.851	0.0317
	Leucine*	α-Aminobutyric acid	0.843	0.0350
	Threonine*	Serine	0.830	0.0408
	Phenylalanine*	Hydroxyproline	−0.824	0.0436
	Threonine*	α-Aminobutyric acid	−0.821	0.0453
	Lysine*	Ornithine	−0.817	0.0470
Essential amino acids not involved	Aspartic acid	Glutamic acid	0.937	0.0059
	Citrulline	Hydroxyproline	0.926	0.0079
	Cystathionine	Ornithine	0.901	0.0141
	Arginine	Cystathionine	−0.888	0.0181
	Alanine	Hydroxyproline	0.888	0.0181
	Asparagine	Glutamine	0.872	0.0235
	Arginine	Cystine	0.869	0.0246
	Glutamine	Glutamic acid	−0.862	0.0273
	Arginine	Ornithine	−0.851	0.0318
	Cystine	Ornithine	−0.827	0.0425

*Essential amino acids

cantly decreased in patients with traumatic brain injury.[14] In addition, some types of neurological disorders have been known to be caused by thyroid diseases.[15] We propose that plasma amino acids, such as taurine, valine, citrulline, and hydroxyproline, may be potential targets for diagnosis and treatment of thyroid and neurological diseases. Furthermore, considering the correlation of TRHs with plasma essential amino acids, for e.g. leucine, valine, and threonine, it may be useful to treat patients of thyroid or neurological diseases with dietary control. To further clarify this, it is necessary to obtain data on TRH levels and plasma amino acids concentrations from patients with thyroid and neurological diseases, and to perform a similar correlation analysis on it.

Compliance with ethical standards

This study was approved by the Ethics Committees of Beppu University.

Conflicts of interest/disclosures

We declare that we have no financial or other conflicts of interest in relation to this re-

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