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Analysis of Biogenic Amines Exchange in Dystonia

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ABSTRACT

Dystonia – is the debilitating movement disorder of central nervous system often inherited, appearing as involuntary movements, which occur due to deficiency or excess of neurotransmitters, mainly biogenic amines, such as dopamine, noradrenalin, and serotonin. The exact cause has been unveiled only in few forms of dystonia, such as dopa – responsive dystonia, where dopamine deficiency has been established, while the cause of most others forms of dystonia remaining obscure. Numerous studies of these forms have detected neurotransmitters disturbance, but those reports are very contradictory. In present study we explore the latent factors unifying some biogenic amines together into clusters bearing common certain functional mission in norm and in pathologic states such as dystonia. We compare the results obtained in dystonia group and in control of group of patients observed for suspicion for neuroglial tumors and we established that latent factors, unifying groups of biogenic amines, differ between the D group and the control group. We also have compared these two groups by means of discriminative analysis and As a result of discriminate analysis we derived equations of canonical linear discriminate (classification) functions (LCF) that probably can detect or exclude dystonia at examination of latent forms or "formes frustes" of this disorder. We expect to get even much more evident result by recruiting healthy control and by widening the spectre of biogenic amines measured.

Keywords

Biogenic amines, Discriminate analysis, Dystonia, Factor analysis, Neuroglial tumors.

Abstract

Dystonia – is the debilitating movement disorder of central nervous system often inherited, appearing as involuntary movements, that occur due to deficiency or excess of neurotransmitters, mainly biogenic amines, such as dopamine, noradrenalin, serotonin. The exact cause has been unveiled only in few forms of dystonia, such as dopa – responsive dystonia, where dopamine deficiency has been established, while the cause of most others forms of dystonia remaining obscure. Numerous studies of these forms have detected neurotransmitters disturbance, but those reports are very contradictory. In present study we explore the latent factors unifying some biogenic amines together into clusters bearing common certain functional mission in norm and in pathologic states such as dystonia. We compare the results obtained in dystonia group and in control of group of patients observed for suspicion for neuroglial tumors and we established, that latent factors, unifying groups of biogenic amines, differ between the D group and the control group. We also have compared these two groups by means of discriminative analysis and As a result of discriminate analysis we derived equations of canonical linear discriminate (classification) functions (LCF), that probably can detect or exclude dystonia at examination of latent forms or "formes frustes" of this disorder. We expect to get even much more evident result by recruiting healthy control and by widening the spectre of biogenic amines measured.

Introduction

Dystonia (D) – is an involuntary movement disorder, often

inherited, that occurs due to neuromediators disturbances. Different researchers reported changes in metabolism of biogenic amines, most important mediators, and those reports are very contradictory [1-13]. In our first personal studies we revealed tendency to enhancement of the level of Noradrenaline, Hidroxyindolacetic acid and Serotonin in D [14-16]. The penetrance of D. is 30% that means that inherited D is manifesting only in 30% of sibs carrying the mutating gene, while the rest suffer from latent forms, or «forms frustes» of this disorder. Until now only few mutations, responsible for D, have been unveiled, but we expect to exist up to 100 such mutations. Until we uncover all mutations, responsible for D., we require reliable test for diagnosis of latent forms of D. In present study we explore discrimination of dystonia on the base of biogenic amines exchange peculiarities, and the latent factors unifying some biogenic amines together into clasters bearing common certain functional mission in norm and in pathologic states such as dystonia.

Materials and Methods

We considered metabolites of catecholamines and serotonin in two groups of patients as in learning samples for discriminant analysis. The first group consisted of 12 dystonic patients, and the second, control group, comprised 20 patients without dystonia, checked for such tentative neuroglial and neuroendocrine tumors as carcinoid, neuroblastoma, ganglioneuroma. The characteristic of dystonic patients is presented in the Table 1. We explored six independent variables – tryptophane (TRYPT_P), 5 – hydroxytryptophane, (OH5_TR1), serotonin (SER), 5 hydroxyindolacetic acid (HIAA), homovanillic acid (HVA) and tyrosin (TYR_IN_P). Evaluation of tryptophane metabolites – TRYPT_P, OH5_TR1, SER, HIAA, in blood plasma was measured using microbore column high power liquid chromatography (HPLC) with fluometric detection on chromotograph ChZ – 1311 (NPO analitichaskogo priborostroenia, Russia) in the regime of line – gradient elution (ftoroplastic column, 0,5 x 250 mm, packed with the sorbent Nucleosil – 5 – C18, speed of elution 12 mkl/min, acetonnitril gradient in 0,01 H Na – formiatic buffer, ph 3,5, detection on native fluorescence, excitement wave length 285 nm, extinction area – 320 – 480 nm, volume of probe introduced – 70 mkl, time of chromatographic analysis – 30 min).

The levels of catecholamines - HVA and TYR_IN_P in the blood plasma were measured using microbore column high power liquid chromatography (HPLC) with fluometric detection in regime of isocratic elution. We used an Orlant elution system (Medicant, Russia) and a Kratos fluometric detector (United States). Catecholamines were separated from the physiological liquids using the solid phase extraction method.

The components were separated using a 2 x 150 µm column filled with a Nucleosil – 5 –C 18 sorbent; the elution rate was 100 µl/min, the eluent was 3% acetonnitrile in a 0,01 N sodium formate buffer (pH 3,5). The substances studied were detected using standarts in the 320 – 480 nm range at the excitation of 285 nm. The volume of the probe was 10 µl and the time of chromatography was 10 minutes. The data obtained were analyzed using STATISTICA for Windows (version 5.5).

For testing of distributions of indicators for presence of significant differences from the norm and for relevance of application of parametric methods we used one – sample Kolmogorov – Smirnov test with significance level p = 0,05. Also, taking into account short amount of observations in the sample, we prefer graphic analysis of cumulative probability and analysis of asymmetry and of an excess. All six variables were distributed normally. We also used Wald's criterion, the Mann–Whitney criterion, median chi_ square criterion, the Kruskal–Wallis criterion, and ANOVA for the comparison of biogenic amines levels in the groups studied. We used correlation analysis by means of Pearson's correlations for normally distributed indicators, and monotonous correlations of Spearman for other indicators, and established that correlations within dystonia group differ from those within control group. Then we used factor analysis with principal component method and varimax normalizing rotation and scree plot analysing. Discriminate analysis was held by means of forward stepwise method.

Name	Sex	Age	Severity	Age of onset	Results of DYT – 1 test	MRI, CT	Brain PET
Пр	M	72	Sporadic, polysegmental	Late	-	-	-
Ос	F	26	Familial, cervical	Early	-	-	-
Лв	F	50	Sporadic, Cervical	Late	n/a	Angioma of the left frontal cortex	-
Кз	M	52	Sporadic, Segmental	Late	n/a	-	-
Тм	M	29	Sporadic, polysegmental	Late	_	Protrusions in cervical spine	-
Кр	M	38	Sporadic, Cervical	Late	-	Norm	+
Сн	F	70	Sporadic, Segmental	Late	-	-	+
Ал	M	38	Familial, generalized	Early	-	-	-
И	F	52	Sporadic, polysegmental	Early	-	Norm	+
Ф	M	57	Familial, writer's cramp	Early	n/a	Norm	+
Кч	F	59	Familial, writer's cramp	Late	n/a	Norm	+
Лб	F	37	Sporadic, Cervical	Late	n/a	-	+

Table 1: Characteristic of patients studied.

Results of the research

The data obtained by means of measuring biogenic amines in plasma are presented in the table 2 for D. group, and in the table 3 for control group.

Patient	TYR mcg/Ml	TRYPT_P mcg/ml	OH5_TR1 ng/ml	SER ng/ml	HIAA ng/ml	HVA ng/ml
Norm	8,0-15	5,1 - 14,9	66 – 94	36 - 82	<60	116-264
Пр	7,3		175	110	27,6	240
Ос	6,5		90,6	75		
Лв	25,5	12,6	127,7	3,3	57,6	10
Кз	24	3,27	166,4	249,7	90	30
Тм	12,8	5,1	92,2	28,9		
Кр	9,2	5,1	115,5	186,7	18	110
Сн	19,1	4,9	134,4	192	28	146
Ал	24	3,8	297,6	24,3	32	57,5
И	16,3	4,9	151,2	44,4	192	90
Ф	11	10,9	148	72	12	124
Кч	11	8,6	165	116,3	300	168
Лб	8	9,2	110	138	120	122

Table 2: Independent variables in the group of D.

Patient	TRYPT_P mcg/ml	OH5_TR1 ng/ml	SER ng/ml	HIAA ng/ml	TYR mcg/ml	HVA ng/ml
Ш	12.9	36.5	166	13.2	9.8	80.1
А	11.3	35.2	128.3	42.5	14.9	227.7
Аг	14.357	29.228	135.578	281.115	10.676	95.661
Б	11.6	35.3	69.6	13	8.8	140
Бес	13.1	48.9	317.1	44.2	18.5	167.8
Бл	11.9	33.5	209.2	147.8	11.1	370.9
Бр	10.5	56.2	74.75	12.25	9.6	87.1
В	12.2	35.3	97	9.5	12	28.3
Вас	9.7	39.3	70.4	24.5	5.07	162.4
Ви	5.3	19.3	51.4	29.8	6.4	126
Г	12.3	38.737	35.046	50.7	9.11	31.7
Го	10.7	35.9	261.1	37.1	12.7	97.5
Гу	11.7	28.2	57.6	5.6	9.3	120.8
Гус	11.3	42.5	218.4	18.4	11.9	79.1
Ег	12.8	42.2	141.5	12.4	16.7	76.8
Ес	8.1	33.8	96.7	284.3	10.2	197.8
Еф	12.667	19.338	164.715	21.364	14	338.149
Ж	8.7	23.6	37.2	5.53	6.4	22.7
З	12.44	27	206	9.2	10.06	51.8
За	14	43.1	237.3	44.8	21.5	134.1

Table 3: Independent variables in the control group.

Results of the factor analysis

Two latent factors were extracted in the group of D. by means of factor analysis with principal component method with rotation by means of normalized varimax (Figure 1).

The graphs of rotation are demonstrated in the figures 2.

In the control group the principal component method has extracted also two latent factors after normalyzed varimax rotation (Figure 3).

Results of discriminate analysis

The independent variable were tryptophane (TRYPT_P), 5 – hydroxytryptophane, (OH5_TR1), serotonin (SER), 5 hydroxyindolacetic acid (HIA in plasma A), homovanillic acid (HVA) and tyrosin (TYR_IN_P). From those the most valuable differential indicators were picked, namely the level of HIAA in plasma and 5 OH T in plasma (Figure 5).

Continue... Extraction: Principal components (Marked loadings are > .700000)

Variable	Factor 1	Factor 2
TRYPT_P	.309959	-.901627
OH5_TR1	-.631310	.290196
SER	.300419	.755760
HIAA	.629559	.104152
TYR_IN_P	-.900181	.115893
HVA	.833391	.186995
Expl.Var	2.486088	1.527564
Prp.Totl	.414348	.254594

Figure 1: Factor loading in D. group after varimax rotation.

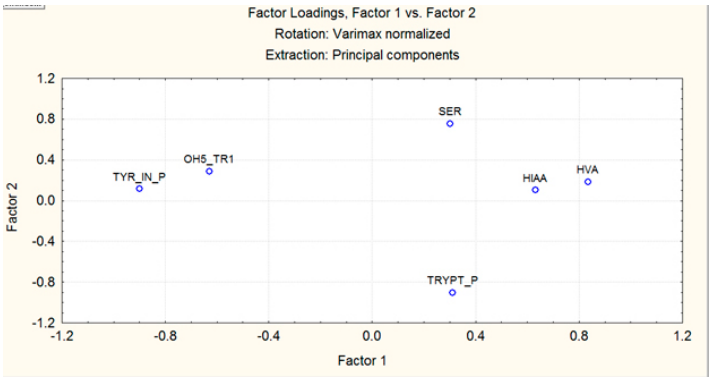


Figure 2: The graph of factor analysis after varimax normalized rotation.

Continue... Extraction: Principal components (Marked loadings are > .700000)

Variable	Factor 1	Factor 2
TRYPT_P	.766693	-.028692
OH5_TR1	.467671	-.108367
SER	.798162	.234861
HIAA	-.123689	.806380
TYR_IN_P	.912574	.104499
HVA	.169656	.778818
Expl.Var	2.320470	1.335453
Prp.Totl	.386745	.222575

Figure 3: Factor loading in the control group after varimax rotation.

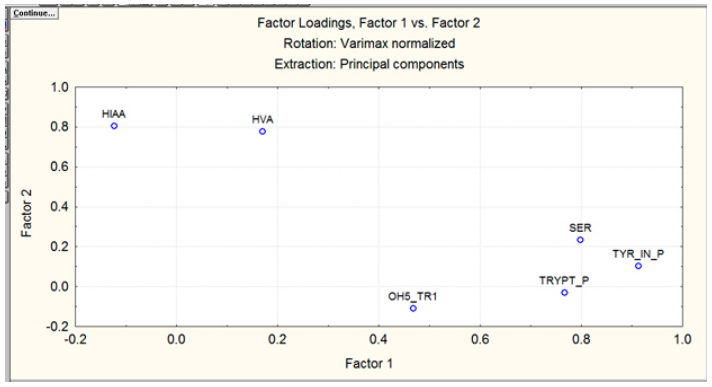
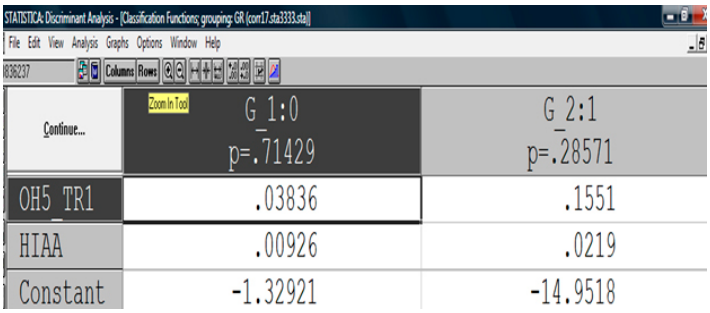


Figure 4: The graph of factor analysis with rotation in the control group.



Continue...	G_1:0 p=.71429	G_2:1 p=.28571
OH5_TR1	.03836	.1551
HIAA	.00926	.0219
Constant	-1.32921	-14.9518

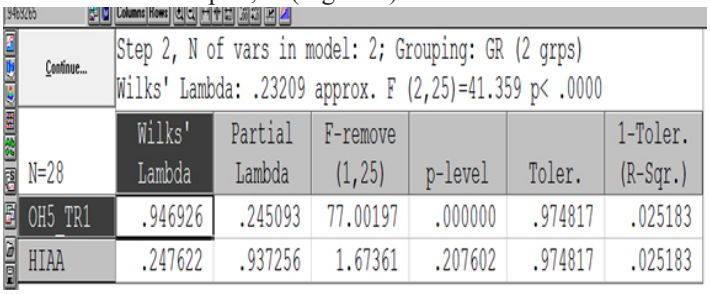
Figure 5: Two most valuable variables derived from stepwise procedure and the meanings of coefficients of linear classification functions.

As a result of discriminate analysis we derived equations of canonical linear discriminate (classificational) functions (LCF) that probably can detect or exclude dystonia at examination of latent forms or "formes frustes" of this disorder. These are the equations formulas –

LCF 1 = -1,329 + 0,038 x 5 OH Tr p + 0,009 x HIAA p equation for group without D. (G0)

LCFЛКФ 2 = -14,95 + 0,155 x 5 OH Tr p + 0,02 X HIAA p equation for group with D. (G1)

The significance of derived discriminative functions p<0,0000 turned to be below p<0,05 (Figure 6).



Continue... Step 2, N of vars in model: 2; Grouping: GR (2 grps)
Wilks' Lambda: .23209 approx. F (2,25)=41.359 p< .0000

N=28	Wilks' Lambda	Partial Lambda	F-remove (1,25)	p-level	Toler.	1-Toler. (R-Sqr.)
OH5_TR1	.946926	.245093	77.00197	.000000	.974817	.025183
HIAA	.247622	.937256	1.67361	.207602	.974817	.025183

Figure 6: Significance of discriminate functions derived. Abbreviations – HIAA – hydroxyindolacetic acid in plasma, OH5 TR1 – 5 OH – tryptophan in plasma.

When we check the patient for probable form fustes of dystonia we just measure the level of 5 OH Tr и HIAA in plasma and obtained data substitute into these two equations and then compare results of LCF 1 and LCF 2. If LCF 2 exceeds the LCF 1, our patient relates to the group G 1, suffering from D. And if LCF 1 exceeds LCF 2, the patient is related to the group G 0, free from D.

Discussion

Factor analysis

We guess that it is better to start discussion of factor analysis results rather from the question than from the answer. What results we expected from factor analysis? We undertook it to test the following two hypothesizes. The first one – that there should be the unique set of factors in metabolism of amines special for healthy state. Because we studied two pathways of metabolism – the pathway of catecholamines and the pathway of serotonin, we should expect to detect two factors pertinent to each of these pathways, but we can find relations of components inside of each pathway as well. And the second hypothesis is that this pattern should change in its own unique way in pathologic state whether in D., the movement disorder, or in tumors of nervous and glial tissue, and the pattern of the change could give a clue to differential diagnosis.

Now we can proceed to answers to these two hypothesises. It seems that in the group of D we detected rather the healthy pattern of factors than disturbed one. The first factor contributes mainly from TYR_IN_P and HVA, and both of these components are pertinent to catecholamines metabolism pathway. And the second factor in D. group contributes mainly from TRYPT_P and SER, both of which are pertinent to serotonin metabolic pathway. But since earlier we revealed disturbance of both pathways in D., namely enhancement of serotonin and noradrenaline, we still cannot exclude pathologic nature of the components derived. In the present study we report only small part of our results of observation of biogenic amines exchange. We explore 22 indicators of biogenic amines exchange in blood plasma and 24 – hours urine and we performed factor analysis for those 22 indicators, but we report here only 6 of them, those that correspond to available in control group. We managed to gather control group with only 6 indicators and since calculate here only 6 indicators from D. group. In our unpublished factor analysis of the rest of biogenic amines we found evidence for disturbed pattern of factors pertinent to dystonic disorders, factor comprises set of components of Noradrenaline in urine and HIAA in plasma, which is much more likely to reflect pathologic changes.

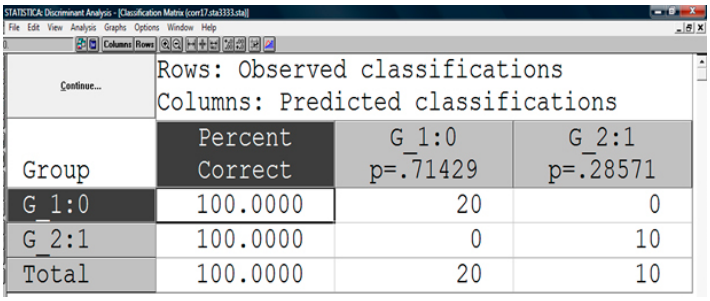
In contrast, in control group, we revealed factors reflecting peculiarities of neuronal and glial tumors. Thus, the first factor with TRYPT_P, SER, TYR_IN_P is pertinent to neuronal and glial tumors, while the second with HIAA and HVA is pertinent to carcinoid tumors.

We postulate that recruiting appropriate healthy control group for factor analysis of widen set of biogenic amines in blood plasma and urine should yield a much more valuable result of such study.

Discriminate analysis

Discriminate analysis of neuromediators exchange in D. resulted in creation of equations of linear classifications. For evaluation of effectiveness of these linear classifications derived, we have estimated the relative frequency of attribution of objects from our learning sample to one from two groups – one with dystonia disorder, and another without D. If our control group had been represented by healthy subjects, similar exploration of diagnostic test would have meant estimation of its sensitivity and specificity, i. e. estimation of ability of the suggested test to detect disorder and ability of the new test to exclude it. But our research as yet does not meet all strict criteria of new diagnostic test study because we do not know percentage of patients from control group turned to be healthy, meanwhile the rest of this group appeared to suffer from neuroglial and neroendocrine tumors. We just believe that if we substituted the control group for healthy individuals, the parameters of this new elaborated test would not change, but we cannot prove such statement so far. Until we recruit healthy subjects for control group, our study must be considered just as elaboration only of the model of diagnostic test, but sensitivity and specificity of our model proved to be very high – both constitute

100%, as shown in the matrix of classifications results (Figure 7). As we can see from the matrix, estimated LCF 1 and LCF 2 did not make any mistake – all suffered from dystonia were attributed to the main group, and all those, free of dystonia, were attributed to control group. Though the size of the main group sample in our study is limited, the excellent clinical parameters of this model of the new diagnostic test predict its usability also in comparison with healthy subjects.



Group	Percent Correct	G_1:0 p=.71429	G_2:1 p=.28571
G_1:0	100.0000	20	0
G_2:1	100.0000	0	10
Total	100.0000	20	10

Figure 7: The matrix of results of classification of total of 30 subjects studied to one from two groups – the group of dystonia patients (10 patients) and the control group of 20 patients.

Thus, obscure scientific medical issues could be clarified by use of such methods of statistical and mathematical investigations as factor and discriminate analysis. [17-19].

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