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Liquid chromatography–tandem mass spectrometry method for determination of panel of neurotransmitters in cerebrospinal fluid from the rat model for tauopathy

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ABSTRACT

Alzheimer's disease (AD) is still being recognized today as an unmet medical need. Currently, there is no cure and early preclinical diagnostic assay available for AD. Therefore much attention is now being directed at the development of novel methods for quantitative determination of AD biomarkers in the cerebrospinal fluid (CSF). Here, we describe the liquid chromatography–tandem mass spectrometry method for determination of 5-hydroxytryptamine (SER), 5-hydroxyindoleacetic acid (5-HIAA), homovanilic acid (HVA), noradrenaline (NADR), adrenaline (ADR), dopamine (DA), glutamic acid (Glu), γ-aminobutyric acid (GABA), 3,4-dihydroxyphenylacetic acid (DOPAC) and histamine (HIS) in cerebrospinal fluid (CSF) from the rat model for human tauopathy. The benzoyl chloride was used as pre-column derivatization reagents. Neurotransmitters and metabolites were analysed on ultra performance liquid chromatography (UPLC) on C18 column in combination with tandem mass spectrometry. The method is simple, highly sensitive and showed excellent linearity with regression coefficients higher than 0.99. The accuracy was in a range of 93–113% for all analytes. The inter-day precision ($n=5$ days), expressed as % RSD, was in a range 2–10% for all analytes. Using this method we detected significant changes of CSF levels of two important neurotransmitters/metabolites, ADR and 5-HIAA, which correlates with progression of neurodegeneration in our animal model.

1. Introduction

Alzheimer's disease (AD) is a chronic, irreversible neurodegenerative disease that affects higher structures of the brain [1]. Prominent neuropathologic features of AD are senile plaques, neurofibrillary tangles, neuroinflammation, synaptic and cell loss [2–4]. AD is a leading cause of dementia worldwide. Currently 20–30 million individuals suffer from dementia today, with 4.6 million new cases of dementia every year [5]. In spite of intensive research there is no disease modifying drug for AD treatment available on the market. Currently, most prevalent is symptomatic therapy, which is not able to stop progression of the disease [6].

It has been proposed that therapeutic intervention is most effective when administered early in the disease's progression, before neurodegeneration is too severe and widespread. There is unmet need for novel diagnostic tools that accurately identify Alzheimer's disease in the very early phase of the disease. An ideal diagnostic tool must be sensitive to earliest changes in AD and should differentiate among preclinical AD, normal aging and other brain disorders that cause memory loss [7]. It has been shown that valid clinical biomarkers could be obtained from detail metabolomic studies of body fluids of transgenic animal models of the disease [8]. Major advantage of metabolomics over other 'omics' is that metabolism is conserved during evolution and metabolic networks are essentially very similar in animals and human beings. Therefore the findings from animal models are directly transferable to humans [9].

One group of metabolites that is of particular interest in neuroscience research is a group of neurotransmitters (NT) together with their bioprecursors and metabolites. Several methods have been developed and used for analysis of NT and metabolites in cerebrospinal fluid (CSF) from rat. The most common method is HPLC with electrochemical detection [10–13]. Although very sensitive, this

Abbreviations: AD, Alzheimer's disease; CSF, cerebrospinal fluid; NT, neurotransmitters; UPLC, ultra-performance liquid chromatography; HPLC, high-performance liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; IS, internal standard; Glu, glutamic acid; GABA, γ-aminobutyric acid; HIS, histamine; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanilic acid; SER, 5-hydroxytryptamine; DOPAC, 3,4-dihydroxyphenylacetic acid; NADR, noradrenaline; ADR, adrenaline; DA, dopamine

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method is not applicable for analysis of NT with low intrinsic electroactivity (e.g. histamine) [14]. Radioenzymatic methods have been used for analysis of adrenaline or noradrenaline in rat CSF [15,16]. The radienzymatic methods are selective and very sensitive, however their major disadvantage is a use of the radioactive chemicals. Combination of HPLC with UV [17] or fluorimetric [18] detection was also used for NT/metabolites in rat CSF. Pre-column derivatization with N-hydroxysuccinimide esters followed by HPLC with electrochemical detection is another method that was used previously. However this method is very laborious and requires separate HPLC runs for non-electroactive and electroactive compounds [19].

LC/MS methods are becoming popular in neuroscience research due to their sensitivity, specificity and applicability for complex matrices such as tissues and body fluids. Ultra performance liquid chromatography (UPLC) is a modern method that runs chromatographic separations using columns packed with small size particles. The small size particles allow for increased speed with superior resolution and sensitivity. In combination with mass spectrometry, UPLC has been previously used for analysis of few NT/metabolites in various biological matrixes [20–22].

Chemical derivatization is often used to enhance the detection sensitivity in ESI/MS. Many derivatization reagents previously used for UV or fluorescence detection were found to be useful also for MS [23]. Recently, the derivatization with dansyl chloride was used for analysis of panel of NT in human plasma [24]. However, derivatization with dansyl chloride has several disadvantages such as need for increased temperature during the derivatization reaction, longer time for derivatization and photosensitivity of derivates. Furthermore, there is no stable isotope labelled dansyl chloride available commercially and must be synthetically prepared for analysis. Derivatization with benzoyl chloride was hence used as a better alternative for neurochemical monitoring of NT and metabolites in brain microdyalisate [25] or brain tissue and plasma samples [26].

Here we developed rapid and sensitive method for analysis of panel of neurotransmitters and metabolites in rat CSF. Benzoyl chloride was used as pre-column derivatization reagent. All selected analytes were separated and quantified within the single chromatographic run. The use of UPLC/MS offered an advantage of short analysis time and therefore very good sample throughput, applicability for all analytes of interest and selectivity. To demonstrate its suitability in neuroscience research, the method was subsequently used for analysis of CSF samples from the transgenic rat model for tauopathy.

2. Experimental

2.1. Instrumentation

A Waters (Waters, Praha, CZ) Quattro Premier XE mass spectrometer coupled to a Waters ACQUITY UPLC system was used. Mass spectra were acquired using positive electrospray ionization and SRM. The capillary voltage was 3 kV and the source temperature and desolvation temperature were 120 and 450 °C, respectively. The cone gas and desolvation gas flowed at 50 and 750 L/h, respectively. Argon was used as collision gas at a manifold pressure of 2.8×10^{-3} mbar. The collision energies and source cone voltages were manually optimized for each SRM transition. Data were acquired with MassLynx 4.0 and calibrated and quantified by QuanLynx software.

2.2. Chemicals and materials

Glutamic acid, γ-aminobutyric acid, 5-hydroxytryptamine, 5-hydroxyindoleacetic acid, homovanilic acid, noradrenaline, adrenaline, dopamine, 3,4-dihydroxyphenylacetic acid, histamine, ^{12}C-benzoyl chloride, $^{13}C_6$-benzoyl chloride, ammonium formate and LC/MS grade acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO, USA). D6 4-aminobutyric acid was from C/D/N isotopes (Quebec, Canada). Water was purified using a Millipore system (Bedford, MA, USA). All other reagents used in the study were of analytical grade.

2.3. Preparation of standard solutions

Individual stock solutions of 5-hydroxytryptamine (SER), 5-hydroxyindoleacetic acid (5-HIAA), homovanilic acid (HVA), noradrenaline (NADR), adrenaline (ADR), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), histamine (HIS) were freshly prepared in methanol/water mixed solution (1:1, v/v). Glutamic acid (Glu) and γ-aminobutyric acid (GABA) stock were prepared in water. For NADR, ADR and DA, 250 μM ascorbic acid was added. For every sample set, working solutions were prepared from the freshly prepared stock solution every day. The benzoyl chloride (^{12}C and $^{13}C_6$) solution (2% in acetonitrile, v/v) was prepared fresh before each analysis. Calibration curves of the analytes were prepared by diluting the stock solutions with 50% methanol/water (v/v) to the concentration ranging from 0.25 to 4000 ng/ml and spiking into matrix. Internal standard was prepared by derivatization of standard mixture with $^{13}C_6$ benzoyl chloride using the same procedure as ^{12}C reagent, then diluted in DMSO.

2.4. Ultra-performance liquid chromatography

Acquity UPLC BEH C18 column (2.1 mm × 150 mm, 1.7 μm particle size) with VanGuard pre-column was used for analysis. Column temperature was set for 30 °C. Mobile phase A consisted of 10 mM ammonium formate/0.15% formic acid in water, and mobile phase B was acetonitrile. Mobile phase gradient program was as follows: 10% B for 2 min; increased to 90% B from 2 min to 10 min; 90% B from 10 min to 11 min, and then 10% B from 11 min to 13.3 min. The flow rate was 0.3 mL/min and the injection volume was 5 μL.

2.5. Method validation [27]

The inter-day precision and accuracy of the method was determined by analysing the 3 different concentrations over 5 days. Intra-day accuracy and precision was calculated from 6 repeat injections. The limit of detection (LOD, S/N=3:1) and the limit of quantification (LOQ, S/N=10:1) were calculated from standard chromatograms. Stability of derivatized standards was tested by analysing the sample stored at RT, 4 °C and −80 °C for up to 24 h after derivatization.

2.6. Animals

The generation and characterization of a transgenic rat model for tauopathy expressing human truncated tau151–391 was described in details elsewhere [28,29]. For this study, heterozygous transgenic rats (6–7 month old) and non-transgenic SHR age-matched controls were used. All animals were housed under standard laboratory conditions with free access to water and food and were kept under diurnal lighting conditions (12 h light/dark cycles with light starting at 7:00 am). All experiments on animals were carried out according to the institutional animal care guidelines conforming to international standards and were approved by the State Veterinary and Food Committee of Slovak Republic and by Ethics Committee of Institute of Neuroimmunology. Efforts were made to minimise the number of animals utilised and to limit discomfort, pain or any other suffering of the experimental animals used in this study.

2.7. Collection of CSF

Cerebrospinal fluid was collected from the cisterna magna. Animals were anesthetized with tyletamin/zolazepam/xylazine mixture, fixed in a head holder and a midline incision in the skin was made up to the head area to permit easy access to the cisterna magna. Approximately 80 μl of CSF was collected from each animal. After a short centrifugation step (3 min at 5000 g, 4 °C) all CSF samples were immediately flash frozen in liquid nitrogen and stored at −70 °C till used.

2.8. Sample preparation

Fifty μl of standards in aCSF (145 mM NaCl, 2.68 mM KCl, 1.1 mM $MgSO_4$, 1.22 mM $CaCl_2$, 0.5 mM NaH_2PO_4, 1.55 mM Na_2HPO_4 and 0.5 mg/ml BSA; pH was adjusted to 7.4) or CSF sample was mixed with 50 μl of acetonitrile. Subsequently 25 μl of borate buffer (sodium tetraborate, 100 mM) and 25 μl of benzoyl chloride (2% in acetonitrile, v/v) was added for derivatization. Then 10 μl of internal standard was added, the mixture was vortexed, centrifuged (30000xg/10 min) and supernatant was transferred to sample vials and analysed with UPLC/MS.

2.9. Data statistic

The data from animal samples were analyzed by one-way ANOVA followed by Tukey's multiple comparison test (Prism 5.0 software, GraphPad, inc, San Diego, CA). Values are expressed as mean ± S.E.M. Differences at $p < 0.05$ were accepted as statistically significant.

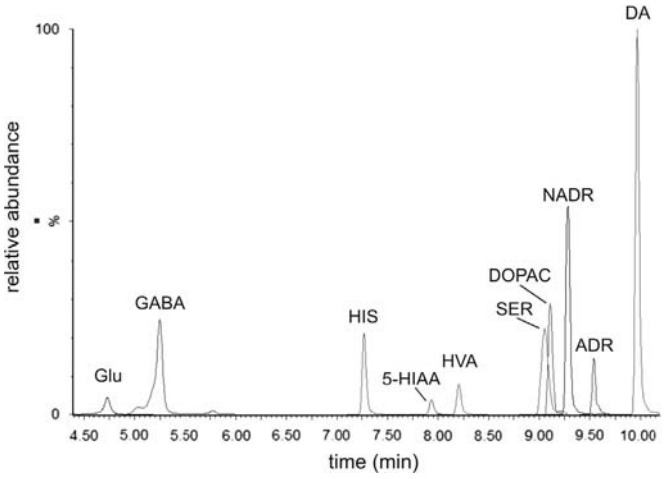

Fig. 1. Extracted ion chromatogram of 10 targeted analytes. Selected reaction monitoring (SRM) was used to detect targeted analytes. Chromatographic conditions: column, Acquity UPLC BEH C18 column (2.1 mm × 150 mm, 1.7 μm particle size) with VanGuard pre-column; flow rate=0.3 mL/min; column temperature 30 °C; gradient elution conditions are described in Section 2.4; peaks: Glu – glutamic acid, GABA – γ-aminobutyric acid, HIS – histamine, 5-HIAA – 5-hydroxyindoleacetic acid, HVA – homovanilic acid, SER – 5-hydroxytryptamine, DOPAC – 3,4-dihydroxyphenylacetic acid, NADR – noradrenaline, ADR – adrenaline, DA – dopamine.

Table 1
SRM conditions of benzoylated neurotransmitters, metabolites and their internal standards.

	Precursor ion (m/z)	Product ion (m/z)	Dwell time (s)	Cone voltage (V)	Collision energy	
Benzoyl-DA	**466.1**	**105.1**	**0.05**	**30**	**25**	**Used for quantification**
	466.1	**241.2**	**0.05**	**30**	**14**	
$^{13}C_6$ Benzoyl-DA	**484.1**	**111.1**	**0.05**	**30**	**25**	
Benzoyl-GABA	**208.1**	**105.1**	**0.1**	**20**	**15**	**Used for quantification**
	208.1	**190.1**	**0.1**	**20**	**8**	
Benzoyl-d_6-GABA	**214.2**	**105.1**	**0.1**	**20**	**15**	
Benzoyl-Glu	**252.05**	**105.1**	**0.1**	**20**	**20**	**Used for quantification**
	252.05	**129.6**	**0.1**	**20**	**15**	
$^{13}C_6$ Benzoyl-Glu	**258.05**	**111.1**	**0.1**	**20**	**20**	
Benzoyl-SER	**385.1**	**105.1**	**0.1**	**30**	**20**	**Used for quantification**
	385.1	**263.9**	**0.1**	**30**	**20**	
$^{13}C_6$ Benzoyl-SER	**397.1**	**111.1**	**0.1**	**30**	**20**	
Benzoyl-ADR	**496.2**	**105.1**	**0.05**	**25**	**25**	**Used for quantification**
	496.2	**478.2**	**0.05**	**25**	**14**	
$^{13}C_6$ Benzoyl-ADR	**514.2**	**111.1**	**0.05**	**25**	**25**	
Benzoyl-NADR	**482.1**	**105.1**	**0.05**	**30**	**25**	**Used for quantification**
	482.1	**464.2**	**0.05**	**30**	**13**	
$^{13}C_6$ Benzoyl-NADR	**500.1**	**111.1**	**0.05**	**30**	**25**	
Benzoyl-DOPAC	**394.1**	**105.1**	**0.05**	**25**	**25**	**Used for quantification**
	394.1	**132.9**	**0.05**	**25**	**25**	
$^{13}C_6$ Benzoyl-DOPAC	**406.2**	**111.1**	**0.05**	**25**	**25**	
Benzoyl-HVA	**304.1**	**105.1**	**0.1**	**17**	**16**	**Used for quantification**
	304.1	**136.9**	**0.1**	**17**	**16**	
$^{13}C_6$ Benzoyl-HVA	**310.3**	**111.1**	**0.1**	**17**	**16**	
Benzoyl-5HIAA	**313.1**	**105.1**	**0.1**	**17**	**25**	**Used for quantification**
	313.1	**250**	**0.1**	**17**	**25**	
$^{13}C_6$ Benzoyl-5HIAA	**319.2**	**110.5**	**0.1**	**17**	**25**	
Benzoyl-HIS	**320.1**	**105.1**	**0.05**	**30**	**20**	**Used for quantification**
	320.1	**76.9**	**0.05**	**30**	**40**	
$^{13}C_6$ Benzoyl-HIS	**332.3**	**111.1**	**0.05**	**30**	**20**	

Glu – glutamic acid, GABA – γ-aminobutyric acid, HIS – histamine, 5-HIAA – 5-hydroxyindoleacetic acid, HVA – homovanilic acid, SER – 5-hydroxytryptamine, DOPAC – 3,4-dihydroxyphenylacetic acid, NADR – noradrenaline, ADR – adrenaline, DA – dopamine.

Table 2
Linearity parameters, limit of quantification and limit of detection.

Analyte	Regression equation[a]	r	LOQ[b]	LOD[c]	Linear range[d]
DA	0.0842x−0.006	0.998	0.15	0.02	0.15–100
ADR	0.0583x+0.002	0.998	0.07	0.04	0.07–50
NADR	0.0681x+0.003	0.998	0.2	0.05	0.2–50
DOPAC	0.0495x+0.282	0.997	1	0.5	1–200
HVA	0.1442x+0.133	0.998	5	1	5–200
SER	0.0183x−0.0001	0.998	0.15	0.03	0.15–50
5-HIAA	0.1539x+1.305	0.997	5	1	5–200
GABA	0.0702x+0.039	0.999	4	2	4–800
Glu	0.0976x+0.121	0.985	100	20	100–4000
HIS	0.1435x+0.070	0.989	0.12	0.03	0.12–50

DA – dopamine, ADR – adrenaline, NADR – noradrenaline, SER – 5-hydroxytryptamine, 5-HIAA – 5-hydroxyindoleacetic acid, HVA – homovanilic acid, DOPAC – 3,4-dihydroxyphenylacetic acid, HIS – histamine, GABA – γ-aminobutyric acid, Glu – glutamic acid.

[a] The calibration curves were constructed by plotting the IS/analyte ratio versus the concentration of each analyte. Each calibration curve was derived from seven data points ($n=7$).

[b] LOQ referred to the limits of quantification in ng/ml.

[c] LOD referred to the limits of detection in ng/ml.

[d] linear range in ng/ml.

Table 3
Inter-day accuracy and precision statistics.

	Nominal (ng/ml)	Mean (ng/ml)[a]	Accuracy (%)	%RSD
Glu	200	205.91	103.0	4.5
	1000	1058.56	105.9	5.0
	4000	3987.67	99.7	4.5
GABA	40	39.21	98.0	3.0
	200	196.10	98.1	1.3
	400	393.70	98.4	2.5
5-HIAA	10	9.31	93.1	10.4
	50	48.58	97.2	7.4
	100	98.87	98.9	3.7
HVA	5	5.17	103.4	4.8
	10	9.87	98.7	4.4
	50	49.02	98.0	2.8
DA	0.5	0.57	113.9	8.5
	1	1.02	102.1	5.3
	5	4.71	94.2	7.4
ADR	0.5	0.52	104.3	7.1
	1	1.03	102.4	8.3
	5	4.75	95.0	6.8
NADR	0.5	0.53	105.9	5.9
	1	0.99	99.2	5.1
	5	4.83	96.6	3.4
SER	0.5	0.51	103.3	4.8
	1	0.98	97.9	2.4
	5	4.91	98.2	3.4
DOPAC	5	4.88	97.6	9.7
	10	9.96	99.6	8.6
	50	50.41	100.8	3.3
HIS	1	1.08	111.9	5.7
	5	5.11	106.0	10.5
	10	9.72	102.2	12.6

The inter-day precision (%RSD) and accuracy assays were carried out using the 3 different concentrations over the 5 different days.
DA – dopamine, ADR – adrenaline, NADR – noradrenaline, SER – 5-hydroxytryptamine, 5-HIAA – 5-hydroxyindoleacetic acid, HVA – homovanilic acid, DOPAC – 3,4-dihydroxyphenylacetic acid, HIS – histamine, GABA – γ-aminobutyric acid, Glu – glutamic acid.

[a] Calculated concentration – average for five measurements at each concentration level ($n=5$).

3. Results and discussion

3.1. UPLC/MS method development and optimization

NT and metabolites were singly benzoylated on amino/hydroxyl group (Glu, GABA, 5-HIAA, HVA) or doubly or triply benzoylated if they contained also additional amino/hydroxyl groups or heterocyclic nitrogen (2xBzCl – HIS, DOPAC, SER; 3xBzCl NADR, ADR, DA). For HVA, DOPAC and 5-HIAA ammonium adducts were the most intensive peaks in spectra and were therefore used as parent ions for selected reaction monitoring (SRM) method development. Strong sodium adducts were also identified within the spectra of several compounds probably due to the use of sodium borate buffer for derivatization. All analytes were detected by MS/MS under collision activated dissociation (CAD) conditions. Similarly to previous report [25], the benzoyl fragment (m/z 105.1) or corresponding ^{13}C benzoyl fragment (m/z 111.1) were the most abundant product ions and were therefore used for SRM. Table 1 demonstrates the mass spectrometry parameters used for analysis of NT and metabolites. In addition to improved ionization in ESI, the benzoylation is also effective for enhancing the retention on reverse phase columns. The UPLC BEH C18 column with UPLC BEH C18 VanGuard pre-column was used for analysis. For optimal separation the gradient elution program was established by use of ammonium formate with formic acid as mobile phase A and acetonitrile as mobile phase B. The retention time for targeted analytes was 4.75 min for Glu, 5.26 min for GABA, 7.27 min for HIS, 7.94 min for 5-HIAA, 8.21 min for HVA, 9.06 min for SER, 9.11 min for DOPAC, 9.30 min for NADR, 9.55 min for ADR, 9.97 min for DA. Fig. 1 and Fig. S1 (Supporting information) displays

Table 4
Comparison of mean NT/metabolites concentrations in rat CSF to the literature.

Compound	Concentration (ng/ml)	Reference
Glu	3864 ± 1540	[30]
	10,000 ± 4930	[39]
	706 ± 44	[40]
	614 ± 317	Current study
GABA	89 ± 6	[41]
	31.9	[42]
	344 ± 183	[30]
	2400 ± 670	[39]
	35 ± 18	Current study
5-HIAA	104 ± 8.8	[43]
	90–100	[10]
	101.3 ± 15.4	Current study
HVA	25 ± 2.9	[43]
	10–20	[10]
	33.5 ± 3.9	[11]
	16.4 ± 2.2	Current study
DOPAC	5–9	[10]
	24.7 ± 1.4	[11]
	10.1 ± 4.1	Current study
SER	0.46 ± 0.08	[18]
	2.64–4.4	[12]
	0.45 ± 0.2	Current study
HIS	1.5–2	[44]
	0.77 ± 0.27	[22]
	1.7 ± 0.74	Current study
NADR	0.49 ± 0.18	[15]
	0.85 ± 0.15	[12]
	0.8 ± 0.26	Current study

NADR – noradrenaline, SER – 5-hydroxytryptamine, 5-HIAA – 5-hydroxyindoleacetic acid, HVA – homovanilic acid, DOPAC – 3,4-dihydroxyphenylacetic acid, HIS – histamine, GABA – γ-aminobutyric acid, Glu – glutamic acid.

a typical chromatograms of targeted analytes in standard solution or rat CSF.

3.2. Linearity, limit of detection (LOD) and limit of quantification (LOQ)

All quantitative analyses were performed by internal standard procedure using corresponding 13C-benzoylated or deuterated compounds as internal standards (IS). Linear calibration curves (correlation coefficient > 0.99) were obtained for all analytes within the concentration ranges studied. The method proves to be enough sensitive to detect physiological concentrations of NT and metabolites in CSF and potential changes due to the pathological processes. The linear regression equations, correlation coefficients, limit of detection (LOD) and the limit of quantification (LOQ) are summarized in a Table 2.

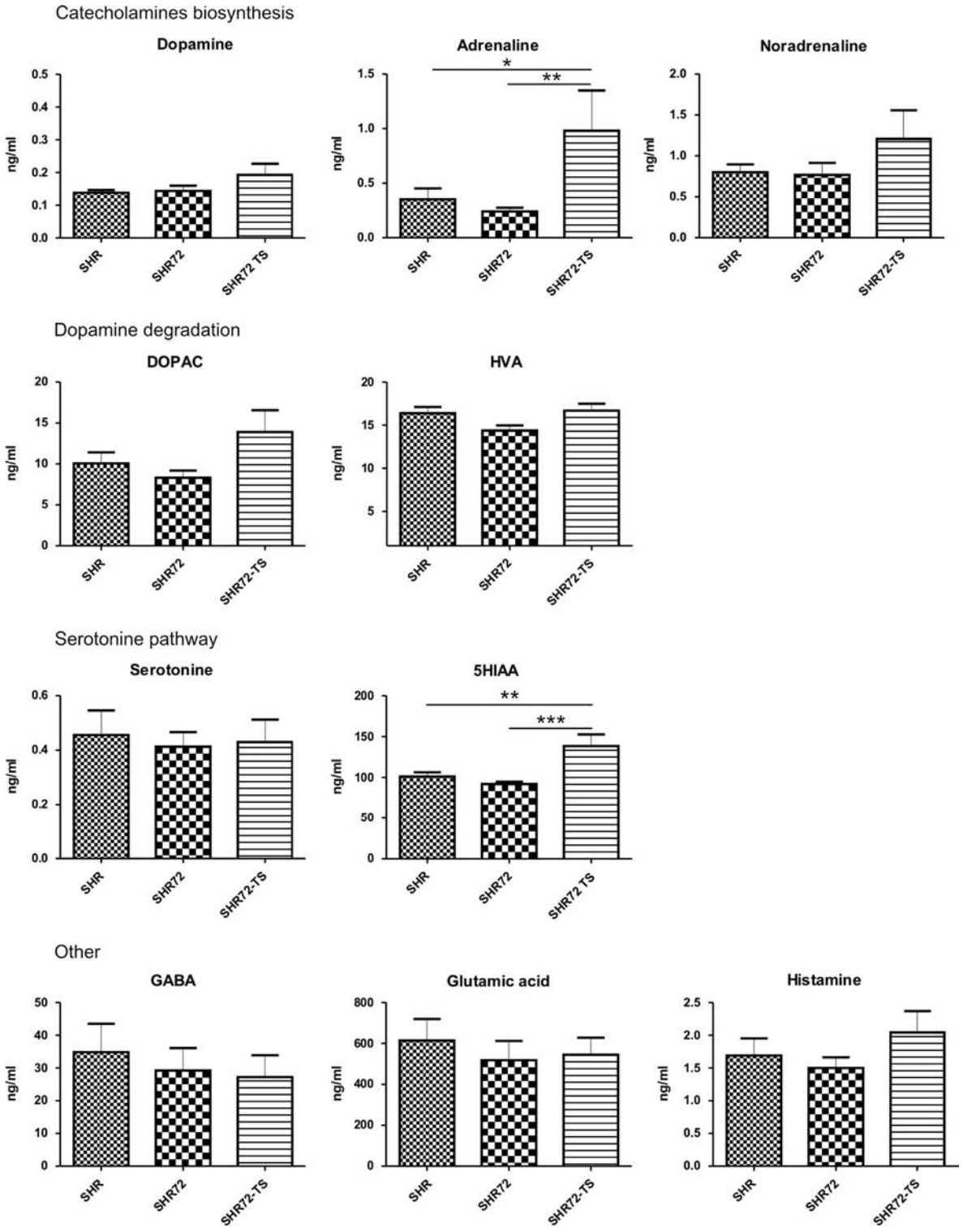

Fig. 2. Concentrations of 10 NT and metabolites in CSF from control rats (SHR; $n=9$), transgenic rats in early stage (SHR72; $n=13$) and in the late terminal stage (SHR72 TS; $n=6$). Data are shown as mean ± standard error of measurement $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

3.3. Precision,accuracy and stability

The intra-day reproducibility for 6 repeat injections was 12.1% (R.S.D.) for Glu, 5.4% (R.S.D.) for GABA, 3.7% (R.S.D.) for HIS, 4.4% (R.S.D.) for 5-HIAA, 5.8% (R.S.D.) for HVA, 3.2% (R.S.D.) for DOPAC, 2.4% (R.S.D.) for SER, 5.8% (R.S.D.) for NADR, 6.3% (R.S.D.) for ADR, and 2.2% (R.S.D.) for DA. The inter-day precision and accuracy assays were carried out using the 3 different concentrations over the 5 different days. The results, expressed as CV ranged from 1.3 to 12.6% (Table 3). Stability of derivatized analytes was analysed by comparison of initial responses with those after storage at RT, 4 °C or −80 °C. There were no significant differences in response for Glu, GABA, HVA, DOPAC, SER, NADR, ADR and DA stored for 24 h at RT (peak area 94.6–118.7%). Derivates of HIS and 5-HIAA were stable for up to 4 h at RT (peak area 112.5–116.1%). All derivates were stable for at least 24 h when stored at 4 °C (autosampler condition) or at −80 °C (peak area 96.3–111.5%).

3.4. Matrix effect

The collection of rat CSF requires special skills and is time consuming. Additionally, the method for CSF collection we used is applicable only for collection of relative small volumes (up to 80 μl from one animal). Initial experiments with several different batches of rat CSF and artificially prepared cerebrospinal fluid (aCSF) were therefore conducted to identify the possible differences between both matrixes. Based on the extraction recoveries for DOPA, NADR, ADR, HVA, 5-HIAA, DOPAC, HIS and SER any matrix effect was excluded for these compounds. Glu and GABA showed ion enhancement effect. To overcome this problem, deuterated IS for Glu and GABA were used for analysis. The application of deuterated IS significantly reduced matrix effect for GABA (extraction recovery 90.2 ± 5%) and was used for further validations and analysis of samples from animals. The use of deuterated IS for Glu did not cause reduction of matrix effects and $^{13}C_6$ benzoylated IS was used for further Glu analysis. The alternative method for analysis of Glu concentrations in rat CSF could be for example ion-pair HPLC/MS [30].

3.5. Comparison of developed method to the literature

According to our knowledge no extensive studies correlating rat strain/age/sex and concentrations of different NT in CSF exist. Therefore we compared values for non-trangenic SHR animals obtained in current study with several previously published studies. For most NT/metabolites the concentrations measured by the developed method are within the ranges published previously (Table 4).

3.6. Analysis of samples from transgenic animals

The developed method was used for analysis of CSF from transgenic and age matched control animals. Many scientific studies demonstrated importance of analysis of metabolites in body fluids of AD patients in relationship to discovery of novel drug targets. Analysis of monoamine neurotransmitters in brain tissues of AD patients revealed decreased concentrations of dopamine, noradrenalin, 3,4-dihydroxyphenyalanine, serotonin and increase of tyrosine [31]. These findings confirm and extend findings of monoaminergic systems disturbances in AD and suggest that pharmacotherapy of AD, should be directed to these systems. The transgenic rat model for tauopathy recapitulates several features of Alzheimer's disease including tau hyperphosporylation, formation of neurofibrillary tangles, insoluble tau complexes [28], white matter damage [32], increase ROS [33], and mitochondria damage [34]. All these pathological changes are accompanied with extensive neuroinflammation [35]. In this study, we did not detect substantial changes of neurotransmitters and metabolites in CSF of transgenic animals (early and late stage of neurodegeneration) (Fig. 2). Only two compounds, ADR (mean 0.35 ng/ml in CTRL vs 0.98 ng/ml in SHR72 TS) and 5-HIAA – serotonine metabolite (mean 101 ng/ml in CTRL vs 139 ng/ml in SHR72 TS) were statistically significantly elevated in transgenic animals.

Previously, it was shown that the concentration of adrenalin was increased in the CSF of AD patients. These findings suggest that adrenergic activity may be increased with disease progression [36]. In previous study, we have found neurofibrillary degeneration in adrenergic brainstem nuclei such as nucleus of the solitary tract (unpublished data). This can suggest direct causative link between degenerating adrenergic neurons and the increased levels of adrenalin in the CSF of transgenic rats.

There is no clear correlation between the CSF levels of 5-HIAA and AD. Several authors reported decreased concentrations of 5-HIAA in CSF from AD. However it is not evident if these changes are related to cerebral or spinal cord dysfunction [37]. There is also no significant correlation between the MMSE, duration of disease, age or APOE 4 and 5-HIAA levels in CSF [38]. Here we detected significant increase in 5-HIAA levels in transgenic rats in late stages of neurodegeneration. This finding correlates with the neurofibrillary pathology in serotonergic brain stem area (unpublished data). However further studies are needed to investigate this hypothesis.

4. Concluding remarks

In this study, we developed and further validated new UPLC/MS method using a benzoyl chloride as pre-column derivatization reagent for the simultaneous determination of 10 NT and metabolites in rat CSF. The developed method is easy, highly sensitive and requires only small volume of CSF sample. The concentrations determined by the current method are in the same order of magnitude as in the literature. Using this method we analysed neurotransmitters and metabolites in the CSF of transgenic rat model for human tauopathy.We believe that this method will be especially useful in the field of transgenic animal models for diseases, where similarly to human you can analyze and compare several important parameters from individual CSF sample from individual animal in different stage of the disease.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2013.10.027.

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