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Nucleoside Map of the Human Central Nervous System

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Abstract Nucleosides are neuromodulators that have a wide range of biological roles in the brain. In order to better understand the function of nucleosides in the human central nervous system (CNS), we constructed a nucleoside map showing the concentration of various nucleosides and their metabolites using post mortem samples from 61 human brain areas and 4 spinal cord areas. We evaluated in vivo tissue levels of four nucleosides (uridine, inosine, guanosine, and adenosine) and three of their metabolites (uracil, hypoxanthine, and xanthine). The concentrations of nucleosides were unevenly distributed across different brain regions, where the highest levels were found in the cerebral cortex and basal ganglia, whereas the lowest concentrations were located in the locus coeruleus, the zona incerta, the substantia nigra, and the inferior colliculus. The regional differences in nucleoside levels in the CNS may reflect the distinct physiological functions adopted by these compounds in different brain areas.

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Abbreviations

5′NT	5′-Nucleotidase
A_1 receptor	Adenosine A_1 receptor
A_{2A} receptor	Adenosine A_{2A} receptor
ADA	Adenosine deaminase
Ade	Adenine
Ado	Adenosine
AK	Adenosine kinase
AMP	Adenosine monophosphate
CNS	Central nervous system
GMP	Guanosine monophosphate
Gn	Guanine
Guo	Guanosine
Hyp	Hypoxanthine
IMP	Inosine monophosphate
Ino	Inosine
PNP	Purine nucleoside phosphorylase
UP	Uridine phosphorylase
Ura	Uracil
Urd	Uridine
Xn	Xanthine
XO	Xanthine oxidase

Introduction

Some of the purine and pyrimidine nucleosides and nucleotides are both neuromodulators and co-transmitters [1–6]. On binding to its receptors, endogenous adenosine (Ado) participates in the control of sleep, neuronal protection and

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inflammation [3, 7, 8]. Nucleosides, such as Ado, guanosine (Guo), inosine (Ino) and uridine (Urd), may play a role in the mechanisms underlying both physiological processes, such as cognition, memory, and pain, and various diseases, such as epilepsy, depression, schizophrenia, Alzheimer's disease, Huntington's disease and Parkinson's disease [3, 7, 9–11]. As a result, increasing attention is now being given to the role of nucleosides in both physiological and pathological processes in the CNS.

The clinical applications of Ado derivatives for the treatment of epileptic seizures, ischemia, traumatic injuries, and inflammatory diseases [3, 10, 12, 13] are limited by their side effects (e.g., bradycardia, hypotension, and hypothermia) [14, 15]. In addition, nucleoside receptors are widely distributed in several organs other than the human brain [16–18]. For this reason, there is an increasing need to determine the concentrations of nucleosides that are present under both normal and pathological conditions in the CNS.

As purinergic receptors [3, 19, 20], transporters [20, 21] and nucleoside-metabolizing enzymes [22–24] are unevenly distributed across the human brain, it is conceivable that the physiological functions of nucleosides may be different in different brain areas. As we have described previously, the distribution of nucleosides are also uneven in human cortical and white matter areas [25]; however, systematic studies on nucleoside levels in the human CNS are still lacking. Therefore, in the present study, we measured nucleoside concentrations in a number of different regions of the human brain and provided an extensive map of nucleoside levels in the human CNS.

The nucleoside map revealed a region-specific distribution pattern of nucleosides in the CNS. To gain an insight into the role of nucleosides in pathological processes, the pattern and concentrations of these compounds in tissue samples obtained from diseased brains and spinal cords can, in the future, be compared to their distribution under physiological conditions using the map constructed in this study.

Experimental Procedures

Materials

Standards for peak identification (uracil (Ura), hypoxanthine (Hyp), xanthine (Xn), Urd, Ino, Guo and Ado) and other chemicals, reagents, standards, and the like were obtained from Sigma Chemical Co. (St. Louis, Missouri, USA) and Merck Co. (Darmstadt, Germany) in 99% purity (puriss.), analytical or HPLC grade.

Human Brain Tissue Samples

Human brain tissue samples were collected in agreement with the Ethical Rules for Using Human Tissues for Medical Research in Hungary (HM 34/1999) and according to the framework of the Human Brain Tissue Bank, Budapest.

Brains and spinal cords from sudden death and traffic accident victims were investigated. Brains and spinal cords were removed within 2, 4 or 6 h after death in the Department of Forensic Medicine, Faculty of Medicine of the Semmelweis University. The brains and spinal cords were subsequently frozen, before being sliced into 1–1.5 mm thick coronal sections and microdissected using the punch technique [26]. A total of 61 areas were selected from the brain and 4 from the spinal cord of 69 subjects (age: 45.2 $\pm$ 1.03 years). In total, 915 brain and 60 spinal cord samples were analyzed.

Our sample preparation method was previously used to measure nucleoside concentrations in brain tissue samples [27]. Briefly, in average 1.5 mg (1.54 $\pm$ 0.1 mg) from each microdissected tissue pellet was homogenized with a motor-driven Teflon potter for 10 s. The homogenated samples were treated with a 1000W microwave beam for 10 s. The samples were then centrifuged at 13,000 rpm for 20 s by a Beckman airfuge. A 10-µl volume from the 60-µl supernatants was injected into the chromatograph.

HPLC Technique

Our HPLC method for the analysis of nucleosides from brain samples [28] has been proven to be sensitive and selective enough for the measurement of nucleosides and their metabolites in brain tissue at the milligram level. Briefly, nucleosides were measured by an HP 1100 series gradient chromatograph with a diode array detector. The separation was performed on an HP Hypersil ODS C18, 2.1 $\times$ 200 mm analytical column and a 2.1 $\times$ 20 mm guard column. The flow rate was 300 µl/min. Eluent A was 0.02 M formiate buffer containing 0.55% (vol/vol) acetonitrile, pH 4.45, and eluent B was 0.02 M formiate buffer containing 40% (vol/vol) acetonitrile, pH 4.45. The gradient profile was as follows: 0% B at 0–10 min, 10% B at 22 min, and 100% B at 30 min. The column temperature was 10°C. The injection volume was 10 µl. The diode array detector was adjusted so that it measured at 254 nm (reference wave 360 nm) and 280 nm (reference wave 450 nm). Chromatograms were evaluated using the automatic integrator function of HP ChemStation software.

Calculation of Nucleoside Content and Data Analysis

To determine the concentrations of nucleosides in the living human brain, we applied a previously elaborated reverse

Table 1 Concentration of nucleosides and their metabolites (pmol/mg w.w., mean ± SEM) in the human brain and spinal cord

	Concentration of nucleosides and their metabolites						
	Ura	Hyp	Xn	Urd	Ino	Guo	Ado
Brain	7.0 ± 0.4	58.2 ± 1.7	35.2 ± 1.2	35.1 ± 1.3	75.0 ± 3.0	13.6 ± 0.8	8.3 ± 0.6
Spin. C.	9.0 ± 1.3*	57.6 ± 8.7	35.0 ± 4.8	37.2 ± 5.8	80.6 ± 9.9	10.6 ± 1.1**	9.1 ± 1.0
Ratio *B/S*	0.78	1.01	1.01	0.94	0.93	1.28	0.91

Ura uracil, *Hyp* hypoxanthine, *Xn* xanthine, *Urd* uridine, *Ino* inosine, *Guo* guanosine, *Ado* adenosine, *SPIN.C.* spinal cord, *ratio B/S* ratio of nucleoside and metabolite levels in the brain versus spinal cord

* $P < 0.05$; ** $P < 0.005$

extrapolation method using neurosurgical brain samples with a 30 s to 24 h post mortem delay [27]. Briefly, human brain tissue samples were removed in the surgical room within less than 30 s and divided to five fractions. One of the fractions was frozen to −70°C after immediately (called as 30 s sample). Four fractions were kept on room temperature (+25°C) for 2, 4, 6 and 24 h respectively before freezing to −70°C. Nucleoside concentrations were measured from each samples and normalized to the 30 s sample values. So we calculated the post mortem deterioration function in time for each particular compound separately. Estimating that degradation of nucleosides runs fluently after death, a degradation curve was fitted to the measured data points by polynomial curve fitting. The ratio of concentration at 30 s and at each degradation curve points provided time dependent multiplying factor curve (back-extrapolation coefficients). Reading the multiplying factor at a particular post mortem time point the in vivo concentration of a nucleoside can be calculated from any post mortem data.

Data were calculated for 1 mg wet weight (w.w.). The levels of nucleosides and their metabolites in brain and spinal cord areas and in specific macrostructures (cerebral cortex and white matter, limbic cortex and limbic areas, basal ganglia, thalamus, hypothalamus, midbrain, pons, medulla oblongata, cerebellum) were compared to the grand average concentration values of the total 61 brain and 4 spinal cord areas (Table 1). The statistical significance of these differences was assessed using the Student's *t*-test. Changes were regarded as significant at $P < 0.05$.

Results

Concentrations of Nucleosides in the Human Brain and Spinal Cord

Four nucleosides (Urd, Ino, Guo and Ado) and three nucleobases (Ura, Hyp and Xn) were measured in all tissue samples (Tables 1–3). Initially, the grand average nucleoside concentrations of all brain and spinal cord areas (Table 1) were calculated (mean ± SEM). To determine the in vivo levels of nucleosides and their metabolites, back-extrapolation coefficients were used as described previously [27]. With the exception of Ura and Guo, the average in vivo levels of nucleosides and their metabolites were similar in the brain and spinal cord. Levels of Ura were significantly higher in the spinal cord than in the brain, whereas Guo was present at a significantly lower concentration in the spinal cord than in the brain (Table 1).

Nucleoside Levels in Regions of the Brain and Spinal Cord

Uracil

The highest Ura levels in the brain were found in the lateral hypothalamic area, the cochlear nuclei, the motor facial nucleus, and the spinal trigeminal nucleus. The lowest levels were located in the parahippocampal cortex, the ventral lateral nucleus, the habenula and the hypothalamic paraventricular nucleus (Tables 1, 2). The lowest levels of Ura were found in the cerebral cortex, whereas the highest concentration was located in the cerebral white matter (Fig. 1).

Hypoxanthine

Of all the compounds studied, Hyp was the most uniformly distributed, and it was also present at the high overall concentration in the human brain (Tables 2, 3). Relatively low Hyp concentrations were found in thalamic areas, especially in the ventral anterior nucleus and the spinal cord white matter. We measured high Hyp levels in the nucleus accumbens, the cochlear nuclei, and in the central gray of the spinal cord. The highest level was found in the reticular formation of the midbrain and the lowest level was detected in the preoptic area. A significantly high Hyp concentration was measured in the cerebellum (Fig. 1).

Table 2 Concentration of nucleosides and their metabolites in different brain macrostructures: cerebral cortex and white matter, limbic cortex and limbic areas, basal ganglia and thalamus (pmol/mg w.w., mean ± SEM)

Nucleoside level of the human central nervous system areas							
Area	Ura	Hyp	Xn	Urd	Ino	Guo	Ado
Cerebral cortex and white matter							
Prefrontal cortex	4.2 ± 0.8*	49.8 ± 6.6	30.0 ± 4.1	38.2 ± 4.4	81.4 ± 9.7	14.5 ± 1.8	11.4 ± 2.0
Frontal cortex	4.8 ± 0.6**	54.9 ± 3.6	38.1 ± 2.9	39.4 ± 3.1	100.6 ± 8.3*	14.1 ± 1.4	5.2 ± 0.7**
Insular cortex	4.8 ± 0.6**	63.2 ± 4.3	39.4 ± 3.6	41.2 ± 2.7*	84.2 ± 5.6	15.9 ± 1.3*	7.5 ± 1.1
Somatomotor cortex	6.9 ± 0.6	70.4 ± 4.8*	43.7 ± 3.0*	39.5 ± 3.1	77.7 ± 5.5	14.5 ± 1.5	7.8 ± 0.8
Somatosensory cortex	5.8 ± 0.6*	62.6 ± 3.6	40.5 ± 3.2	39.6 ± 3.7	84.4 ± 6.7	14.1 ± 1.4	5.8 ± 0.9*
Temporal cortex	5.5 ± 0.8*	67.5 ± 4.7	42.2 ± 5.6	49.3 ± 5.0*	99.8 ± 7.6*	19.0 ± 2.1*	11.1 ± 1.9
Occipital cortex	5.2 ± 0.6**	59.4 ± 4.7	41.4 ± 2.8*	45.7 ± 3.3*	93.0 ± 5.4**	17.9 ± 1.7*	8.0 ± 1.2
Cerebral white matter	9.1 ± 0.8*	59.1 ± 3.2	36.5 ± 2.5	29.1 ± 2.0**	78.8 ± 5.1	10.8 ± 1.1*	10.3 ± 1.3
Limbic cortex and limbic areas							
Cingulate cortex	5.5 ± 1.5	60.3 ± 8.2	41.2 ± 5.7	41.7 ± 5.4	71.2 ± 10.9	15.3 ± 3.2	3.8 ± 0.9*
Entorhinal cortex	4.0 ± 0.5*	43.7 ± 4.9	27.6 ± 3.9	25.5 ± 2.4*	50.1 ± 5.8*	10.9 ± 1.5	2.4 ± 0.5**
Parahippocampal cortex	3.6 ± 0.4**	40.8 ± 5.3*	27.8 ± 3.9	27.3 ± 2.7	50.7 ± 6.7*	11.1 ± 1.9	7.4 ± 0.9
Hippocampus	4.2 ± 0.4*	57.6 ± 2.3	38.7 ± 2.1	38.3 ± 3.4	53.7 ± 4.8*	12.7 ± 2.1	6.9 ± 0.4*
Nuclei of diagonal band	8.1 ± 1.4	56.6 ± 7.3	36.9 ± 5.7	30.9 ± 5.4	49.3 ± 7.6*	8.9 ± 1.6*	5.2 ± 0.9*
Septum	8.7 ± 0.7	90.7 ± 11.5	49.7 ± 5.9	50.9 ± 8.6	58.6 ± 10.6	8.0 ± 1.3*	2.8 ± 0.4**
Amygdala	5.1 ± 0.4*	75.6 ± 6.5	63.3 ± 5.8*	66.2 ± 11.2	101.3 ± 13.8	26.4 ± 4.2	8.7 ± 1.4
Basal ganglia							
Caudate nucleus	6.2 ± 0.6	61.3 ± 3.9	43.2 ± 3.0*	43.9 ± 3.1*	101.5 ± 9.9*	19.5 ± 2.4*	9.6 ± 1.2
Putamen	6.5 ± 0.7	63.4 ± 3.9	42.9 ± 3.3*	37.9 ± 2.5	81.4 ± 5.8	16.3 ± 1.6	7.3 ± 1.0
Nucleus accumbens	6.8 ± 0.6	76.2 ± 5.6*	45.8 ± 2.3**	34.5 ± 2.6	78.9 ± 6.8	16.1 ± 1.6	7.3 ± 0.9
Globus pallidus (externa)	7.3 ± 0.6	52.5 ± 2.6*	37.5 ± 2.3	29.0 ± 1.8**	76.7 ± 7.1	14.0 ± 1.3	6.4 ± 0.8*
Globus pallidus (interna)	9.7 ± 0.9*	66.4 ± 2.9*	46.3 ± 2.4**	31.6 ± 2.9	72.6 ± 7.0	14.4 ± 1.5	7.2 ± 0.9
Substantia innominata	5.2 ± 0.4**	50.4 ± 2.7*	36.4 ± 1.9	37.7 ± 2.6	105.0 ± 5.6**	17.1 ± 1.5*	11.0 ± 1.3
Nucleus basalis	8.7 ± 0.6*	61.0 ± 3.4	42.7 ± 2.1**	39.6 ± 2.2	106.4 ± 5.9**	22.6 ± 1.8**	8.3 ± 0.8
Thalamus							
Anterior nuclei	5.1 ± 0.3**	45.0 ± 6.1	29.2 ± 3.7	26.3 ± 2.7*	73.5 ± 6.6	14.3 ± 1.5	9.9 ± 1.4
Dorsomedial nucleus	9.9 ± 1.0	58.7 ± 7.0	33.6 ± 4.1	30.9 ± 2.8	94.5 ± 10.1	13.6 ± 2.0	10.1 ± 1.4
Ventral anterior nucleus	4.4 ± 0.7*	36.1 ± 2.8**	20.5 ± 1.7**	16.7 ± 1.3**	61.2 ± 7.0	11.7 ± 1.4	15.4 ± 3.1
Ventral lateral nucleus	3.1 ± 0.4**	40.3 ± 6.9	25.5 ± 3.5	26.0 ± 3.2	67.1 ± 9.4	14.2 ± 1.8	4.7 ± 0.8*
Ventral posterior nucleus	8.9 ± 1.6	47.7 ± 6.2	34.3 ± 4.9	29.9 ± 3.7	69.9 ± 5.7	11.2 ± 1.1	10.1 ± 1.9
Habenula	3.1 ± 0.3**	45.6 ± 3.4*	25.1 ± 3.5	24.9 ± 3.8	35.1 ± 6.0*	4.9 ± 0.9**	2.2 ± 0.4**
Lateral geniculate body	5.2 ± 0.7	60.6 ± 8.7	45.0 ± 4.3	34.9 ± 2.9	66.5 ± 9.6	9.7 ± 1.0*	6.5 ± 0.8
Pulvinar	4.9 ± 0.7	49.1 ± 4.8	33.1 ± 4.1	31.5 ± 3.0	48.5 ± 5.7*	7.3 ± 0.9*	4.3 ± 0.5**
Medial geniculate body	7.8 ± 1.2	77.2 ± 6.3	54.2 ± 8.2	43.0 ± 5.5	90.2 ± 10.8	23.5 ± 3.2*	12.9 ± 1.5
Zona incerta	3.9 ± 0.5*	39.9 ± 5.0*	18.7 ± 2.2**	15.7 ± 2.5**	35.9 ± 5.3*	5.1 ± 0.8**	2.2 ± 0.3**

Ura uracil, *Hyp* hypoxanthine, *Xn* xanthine, *Urd* uridine, *Ino* inosine, *Guo* guanosine, *Ado* adenosine

* $P < 0.05$; ** $P < 0.005$

Xanthine

Xn and Hyp displayed similar distributions, and their highest and lowest values overlapped (for example, see the somatomotor cortex, nucleus accumbens, ventral anterior nucleus in Tables 2, 3). In addition, high Xn levels were found in the amygdala, the globus pallidus (interna), the reticular formation of the midbrain, and the central gray of the spinal cord. The lowest levels were found in the inferior olive, the ventral anterior nucleus, the zona incerta, the preoptic area, and the hypothalamic paraventricular nucleus. Relatively high, significantly different Xn concentrations were also found in the basal ganglia (Fig. 1).

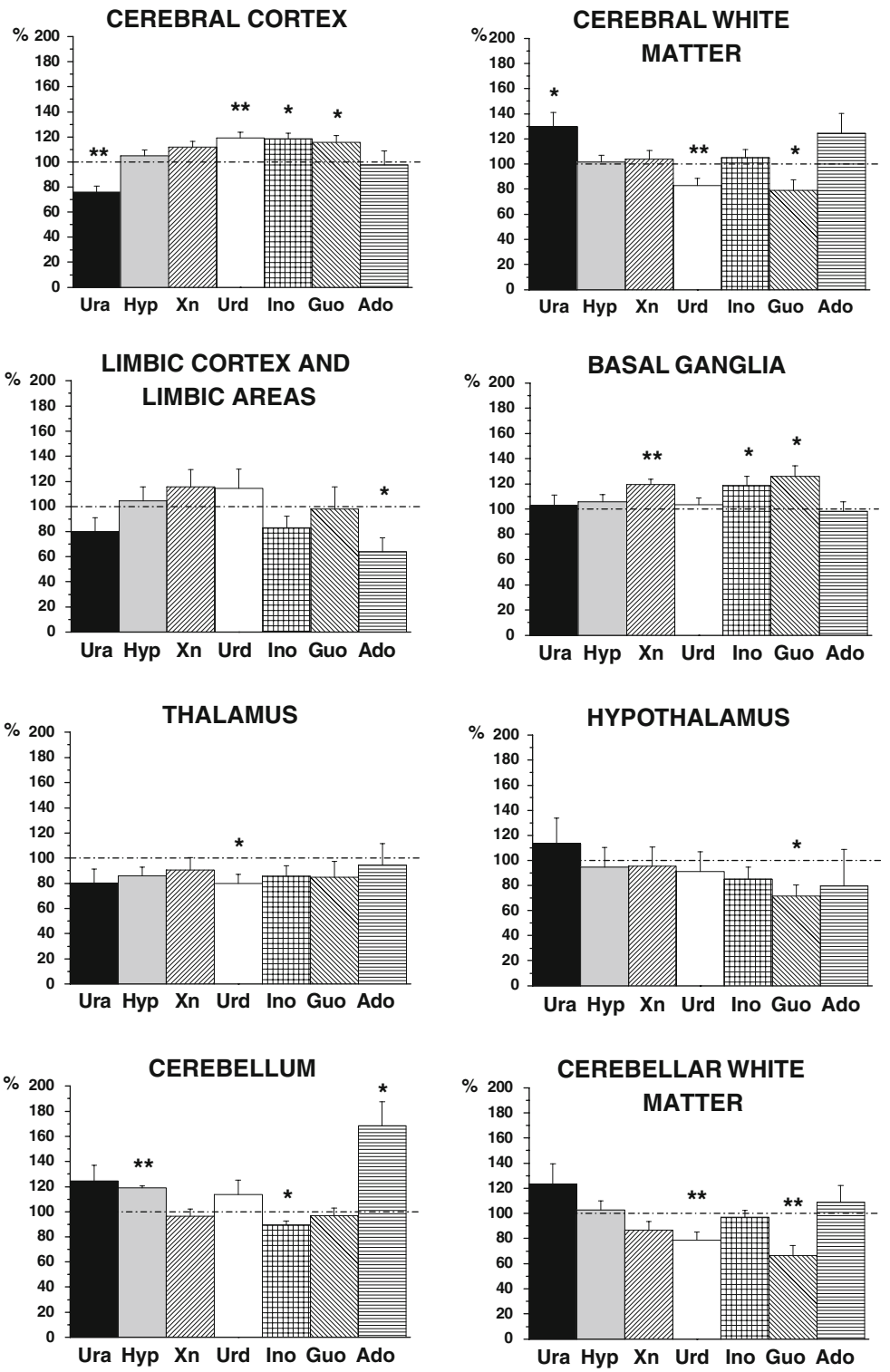

Fig. 1 Level of nucleosides and their metabolites in brain macrostructures. When a comparison was made between the nucleoside levels in the midbrain, pons, and medulla oblongata with the concentrations of these substances in 61 brain areas, no significant difference was found. *Abbreviations*: *Ura* uracil, *Hyp* hypoxanthine, *Xn* xanthine, *Urd* uridine, *Ino* inosine, *Guo* guanosine, *Ado* adenosine; * $P < 0.05$; ** $P < 0.005$

Uridine

Urd was relatively evenly distributed throughout the CNS. High concentrations were detected in the forebrain (temporal, occipital and insular cortex), the caudate nucleus, the cerebellar cortex and the central gray of the spinal cord (Tables 2, 3) while the highest significantly different concentration was measured in the cochlear nuclei. Some of the thalamic and hypothalamic nuclei contained low concentrations of nucleoside; the lowest Urd levels were

Table 3 Concentration of nucleosides and their metabolites in different brain macrostructures: hypothalamus, midbrain, pons, medulla oblongata, cerebellum and spinal cord (pmol/mg w.w., mean ± SEM)

Nucleoside level of the human central nervous system areas							
Area	Ura	Hyp	Xn	Urd	Ino	Guo	Ado
Hypothalamus							
Preoptic area	7.4 ± 1.3	30.5 ± 5.4*	20.2 ± 2.6*	16.0 ± 0.9**	61.7 ± 15.1	11.9 ± 2.1	4.2 ± 0.8*
Supraoptic nucleus	8.4 ± 1.1	73.1 ± 9.5	39.1 ± 7.0	46.9 ± 5.9	83.1 ± 7.5	10.2 ± 1.4	16.1 ± 2.5*
Paraventricular nucleus	3.1 ± 0.3**	40.4 ± 6.0	20.9 ± 2.4*	25.7 ± 3.1	38.5 ± 6.8*	5.9 ± 1.0*	5.5 ± 0.7*
Dorsomedial nucleus	8.8 ± 1.0	77.1 ± 7.1	44.3 ± 7.4	41.5 ± 5.5	67.4 ± 11.4	12.5 ± 2.0	2.9 ± 0.5**
Lateral hypothalamic area	12.0 ± 1.2*	54.4 ± 7.1	43.6 ± 3.5	29.7 ± 3.2	68.1 ± 6.5	8.1 ± 1.0*	4.4 ± 0.6*
Midbrain							
Reticular formation	10.8 ± 1.1**	85.4 ± 7.8**	47.9 ± 3.5**	42.0 ± 4.4	81.7 ± 6.6	15.5 ± 1.7	8.8 ± 1.2
Substantia nigra	5.3 ± 0.5*	50.5 ± 4.4	31.6 ± 2.4	26.0 ± 2.1*	48.9 ± 3.6**	6.9 ± 1.0**	3.2 ± 0.4**
Red nucleus	4.6 ± 0.5*	54.3 ± 9.3	31.7 ± 4.2	31.9 ± 5.5	87.1 ± 11.0	12.7 ± 1.5	10.3 ± 1.7
Superior colliculus	5.0 ± 0.2**	65.0 ± 6.1	38.2 ± 3.3	38.2 ± 3.9	59.2 ± 8.9	7.1 ± 1.3*	5.7 ± 1.0
Inferior colliculus	4.2 ± 0.5**	51.9 ± 4.5	28.9 ± 2.4	28.9 ± 1.9*	39.5 ± 5.4**	4.1 ± 0.7**	3.5 ± 0.4**
Pons							
Pontine tegmentum	7.5 ± 0.7	62.1 ± 4.0	34.2 ± 1.9	36.6 ± 3.4	76.3 ± 6.7	13.0 ± 1.2	9.4 ± 1.4
Locus coeruleus	6.4 ± 0.6	57.9 ± 7.3	24.9 ± 2.8*	25.6 ± 2.9*	29.8 ± 3.9**	4.7 ± 0.8**	1.4 ± 0.2**
Reticular formation	9.6 ± 1.0*	62.1 ± 4.2	32.1 ± 2.0	38.5 ± 3.8	78.5 ± 8.4	14.8 ± 1.5	9.1 ± 1.0
Pontine nuclei	8.0 ± 0.8	58.3 ± 4.3	32.6 ± 2.7	39.8 ± 3.3	92.3 ± 8.5	15.7 ± 1.4	8.5 ± 1.1
Vestibular nuclei	6.7 ± 1.0	50.4 ± 9.6	25.8 ± 3.1	52.0 ± 8.7	101.9 ± 14.6	38.9 ± 16.3	22.0 ± 3.7*
Medulla oblongata							
Cochlear nuclei	12.3 ± 1.6*	77.1 ± 2.2**	47.4 ± 5.9	55.1 ± 4.8*	161.5 ± 19.6*	26.1 ± 3.8*	23.9 ± 2.1*
Motor facial nucleus	14.1 ± 1.9*	44.4 ± 5.7	21.7 ± 3.7*	15.9 ± 2.3**	75.8 ± 10.6	13.5 ± 1.1	11.0 ± 1.6
Spinal trigeminal nucleus	17.2 ± 2.7*	61.2 ± 8.9	32.8 ± 5.6	36.6 ± 6.4	115.7 ± 12.9*	20.3 ± 3.1	8.1 ± 1.4
Inferior olive	5.4 ± 0.6	39.4 ± 4.2*	17.3 ± 2.4**	24.6 ± 1.8*	75.1 ± 8.2	14.3 ± 1.6	7.1 ± 1.0
Reticular formation	7.9 ± 0.6	53.5 ± 2.8	28.8 ± 1.5**	31.2 ± 1.5*	90.4 ± 4.2**	12.7 ± 0.9	9.7 ± 1.3
Dorsal vagal nuclei	8.5 ± 1.0	44.9 ± 6.9	22.7 ± 4.1	27.6 ± 6.3	78.9 ± 11.1	9.9 ± 1.7	4.8 ± 0.7*
Cerebellum							
Vermis	8.3 ± 0.9	71.4 ± 4.2*	34.9 ± 3.2	42.3 ± 4.1	70.4 ± 8.3	13.8 ± 1.2	12.2 ± 1.8
Cortex (hemisphere)	7.3 ± 0.8	69.6 ± 3.9*	38.0 ± 3.2	46.6 ± 3.3**	66.5 ± 4.8	13.1 ± 1.2	17.2 ± 1.9**
Flocculo-nodular lobe	7.9 ± 1.0	66.8 ± 5.4	34.5 ± 3.3	42.3 ± 3.7	70.9 ± 6.1	14.9 ± 2.0	16.0 ± 2.2*
Cerebellar nuclei	11.3 ± 1.0**	68.9 ± 4.9*	28.5 ± 3.0*	28.3 ± 2.4*	60.3 ± 5.0*	10.8 ± 1.2*	10.5 ± 1.4
Cerebellar white matter	8.6 ± 1.1	59.9 ± 4.1	30.5 ± 2.4	27.6 ± 2.3**	72.7 ± 4.2	9.0 ± 1.1**	9.0 ± 1.1
Spinal cord							
Dorsal horn	8.9 ± 0.7	55.1 ± 4.1	32.9 ± 2.5	32.9 ± 2.2	69.7 ± 5.7	10.9 ± 0.9	9.3 ± 1.4
Spinal central gray	11.4 ± 1.2	77.7 ± 7.4*	45.2 ± 3.7*	48.3 ± 4.5*	90.8 ± 9.0	9.9 ± 1.0	6.4 ± 1.2*
Ventral horn	10.3 ± 1.5	61.7 ± 6.3	39.2 ± 3.6	44.7 ± 3.0*	102.9 ± 7.0**	13.5 ± 1.0*	9.9 ± 1.1
Spinal white matter	5.4 ± 0.4**	35.7 ± 2.3**	22.6 ± 1.4**	22.8 ± 1.5**	59.1 ± 4.6**	8.1 ± 0.7**	10.9 ± 1.3

Ura uracil, *Hyp* hypoxanthine, *Xn* xanthine, *Urd* uridine, *Ino* inosine, *Guo* guanosine, *Ado* adenosine

* $P < 0.05$; ** $P < 0.005$

detected in the ventral anterior nucleus, the zona incerta, the preoptic area, and the motor facial nucleus. We found significantly higher Urd levels in the cerebral cortex but lower amounts in the thalamus, cerebral white matter, and cerebellar white matter (Fig. 1).

Inosine

Of all the nucleosides and their metabolites, Ino had the highest overall concentration in the brain. This nucleoside was particularly well represented in the cochlear nuclei, the

spinal trigeminal nucleus and in the basal ganglia (caudate nucleus, substantia innominata, nucleus basalis; Tables 2, 3). Relatively high concentrations were also detected in the frontal cortex and the ventral horn of the spinal cord. The lowest levels were found in the habenula, the zona incerta and the locus coeruleus. Significantly higher than average Ino levels were measured in the cerebral cortex and basal ganglia but lower levels were detected in the cerebellum (Fig. 1).

Guanosine

The distribution of Guo in the CNS is relatively uneven. The highest values were found in the cochlear nuclei, the medial geniculate body, the caudate nucleus, and the nucleus basalis, whereas the habenula, the zona incerta, the inferior colliculus and the locus coeruleus had the lowest values (Tables 2, 3). The cerebral cortex and basal ganglia contained significantly higher levels of Guo, whereas the lowest concentrations of Guo were found in the hypothalamus, in the cerebral white matter and in the cerebellar white matter (Fig. 1).

Adenosine

Not only was Ado the most unevenly distributed of all the nucleosides and their metabolites in the brain, it was also present at a lower concentration than any of the other compounds (Tables 2, 3). The highest levels were found in the vestibular and cochlear nuclei, while the lowest levels were measured in the entorhinal cortex, the locus coeruleus, the habenula, and the zona incerta. We found Ado levels that were significantly higher in the cerebellum but lower in the limbic cortex and other limbic areas (Fig. 1).

Discussion

Nucleoside Concentrations in Brain and Spinal Cord Areas

In the present study, we determined the concentrations of four nucleosides and three of their metabolites from 975 post mortem human tissue samples that were microdissected from 61 brain and 4 spinal cord areas (Tables 2, 3). Brain is an extremely inhomogeneous tissue structurally changing form place to place. Collecting reproducible brain samples is a difficult problem and one of the best we can do is to collect few mm diameter samples from easily identifiable sites [26]. Using larger brain samples is misleading because the big sample averages out regional changes and cutting a big sample is not so reproducible as punching a small one due to the inhomogeneity of the brain tissue. So as a first step of nucleoside mapping of the human brain we collected about 1.5 mg small but anatomically reproducible and representative samples of the 65 areas studied. Obviously, several other sampling strategies can be applied later to establish the distribution of nucleosides inside a larger brain area as hippocampus or basal ganglia. However, we preferred the reproducibility and homogeneity of the samples in this first mapping study on nucleosides, for which the punching technique was appropriate.

To decrease the variability that may be caused by different factors such as age, activity of brain before death, and density of brain capillaries, we selected homogeneous brain tissue samples that were derived from individuals that were of a similar age at death and in which the cause of death was similar. Our aim was to provide a general map of nucleoside distributions in the human CNS. We collected female and male brain and spinal cord samples in nearly equal number (brain/spinal cord: women 454/29 and man 461/31). Also we collected samples from brains of age 46.4 ± 0.97 from men and 44.3 ± 1.99 from women. Water contents of white and gray matters are different [29]. Since nucleosides are water soluble, it could increase the differences between tissues. We calculated the concentrations to wet tissue weight which partly reflects the tissue water content. Respecting to all different factors increasing the data dispersion of our studies, our first nucleoside map can be a good reference data set for further studies on age, gender and other issues.

Because of nucleoside pools in the intracellular stores [28], the concentrations of nucleosides may be several times higher intracellularly than extracellularly in the brain tissue [28, 30]. Nucleosides can be metabolised both extra- and intracellularly under both normal and pathophysiological conditions [31–34]. The function of nucleoside metabolizing enzymes and transporters results in continuously changing nucleoside concentrations, both intra- and extracellularly [32, 35–37]. The metabolic pathways of nucleosides are relatively well known. Guanosine monophosphate (GMP), inosine monophosphate (IMP) and adenosine monophosphate (AMP) can be degraded to Guo, Ino and Ado respectively by 5′-nucleotidase (5′NT). Purine nucleoside phosphorylase (PNP) can catalyze Guo-guanine (Gn), Ino-Hyp and Ado-adenine (Ade) conversion. Adenosine deaminase (ADA) can metabolize the Ado to Ino and adenosine kinase (AK) catalyzes Ado conversion to AMP. Gn can be catabolized to Xn by guanase while xanthine oxidase (XO) catalyzes Hyp-Xn and Xn-uric acid (UA) conversion. Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) is a purine salvage enzyme, which can catalyze Gn and Hyp conversion to GMP and IMP respectively [32, 33, 37]. Uridine monophosphate (UMP) can be metabolized to Urd by 5′NT while Urd–Ura conversion can

be catalyzed by uridine phosphorylase (UP) [38]. Under ischemic conditions, nucleosides are found at levels that are between 4 and 35 times higher than under normal conditions [32, 35]. As a result of metabolic enzymes that function in the brain for hours after death [22, 23], extracellular and intracellular concentrations of nucleosides also change under post mortem conditions [27, 28]. As we have described previously [27], the formation of Hyp and Xn coincided with a substantial decrease in the levels of Ino, and, to a lesser extent, also Ado and Guo content under post mortem conditions in the human brain. Fluctuation in the levels of Urd may result from the function of UP [38]. Consequently, a back-extrapolation method was used to calculate the in vivo nucleoside levels from post mortem samples [27]. Using this method, the measured physiological concentrations of nucleosides and nucleobases and their ratios are in general agreement with sporadic data about some brain areas reported previously by others [30, 38].

Uneven Distribution of Nucleosides

The major finding of our study is the first map of nucleoside distribution in 65 separate sites of the human CNS. Nucleosides have been implicated in epilepsy, depression, schizophrenia, Alzheimer's, Huntington's and Parkinson's diseases, cognition, memory, sleep, pain, ischemia and drug abuse via adenosine A_1 receptor (A_1 receptor) and adenosine A_{2A} receptor (A_{2A} receptor) [3, 10, 39–44]. Several nucleoside derivatives [13, 15, 45–48], AK inhibitors [49–51] and adenosine uptake inhibitors [52, 53] are used in clinical applications to treat epileptic seizures, ischemia, movement disorders, traumatic brain and spinal cord injuries, and inflammatory diseases, as well as in cancer chemotherapy [13, 46, 51–53]. Ino and Guo have anticonvulsant, neuroprotective and trophic effects [9, 54, 55]. Urd and its derivatives have been shown to decrease seizure activity [56, 57], induce hypothermia [58], modify the firing pattern of neurons [59], and contribute to epilepsy-related neuronal activity changes [60]. They may also act as sleep-promoting substances [61]. The distribution of purinergic receptors [3, 19, 20, 50], transporters [21, 53, 62] and nucleoside-metabolizing enzymes [22, 23, 63] is uneven in the brain. Overall, these data suggest that the roles of nucleosides differ in different brain areas.

We have no data from regionally different activity of DNA or RNA metabolism in the human CNS but DNA and RNA known to be stable for long post mortem periods [64–67]. Therefore, we hypothesized that regional different nucleoside levels measured in our study may not result from RNA metabolism differences in several CNS areas but could not rule out the possibility that nucleic acid metabolism may differently affect nucleoside levels in several brain and spinal cord areas. However, a possible explanation of spatially regulated nucleoside distribution is the existence of regional variations in the glia/neuron ratio. The metabolism of nucleosides differs markedly in neurons and glial cells due to different expression and activity of purine metabolic enzymes in the two cell types. Activities of XO, PNP and ADA are higher in glial cells than neurons. Consequently, adenosine degradation pathways are more active in glial cells [22, 23, 68]. Glia/neuron ratios have not been extensively investigated in the human brain areas. Therefore, we found data on glia/neuron ratio only for some of the human brain areas, which were investigated in our study. Thus, the glia/neuron ratio is relatively low in the cerebral cortex, moderate in the brain nuclei, and high in the white matter [69–73]. It was revealed that glia/neuron ratio of temporal, occipital, prefrontal, somatosensory and frontal cortex are 4.55, 0.67–1.0, 0.55–1.75, 1.65, and 1.55–2.19, respectively [69–72]. Frontal, insular, temporal and occipital cortex showed high Urd, Guo and/or Ino levels in our study. Modified Urd, Guo and Ino levels were measured in the prefrontal, the somatomotor and in the somatosensory cortex. However, glia/neuron ratio of temporal cortex was about five times higher than this in the occipital cortex but levels of Urd, Ino and Guo were similarly high in both brain areas. The lowest Urd and Guo concentrations were detected in the cerebral white matter in cerebral cortex and white matter areas (Table 2). Similar concentrations of nucleosides were found in the cerebral and cerebellar white matter. The levels of nucleosides in the cerebellar white matter were also different from those in the cerebellar cortex (Tables 2, 3). Furthermore, the nucleoside content of white matter in the spinal cord was different from that of cerebral and cerebellar white matter (Tables 2, 3). Limbic cortex and limbic areas showed lower Urd, Ino, Guo and Ado levels but similar glia/neuron ratio to cerebral corticies (Table 2; Fig. 1). Glia/neuron ratio in cingulate cortex, entorhinal cortex, hippocampus and amygdala were 0.82–1.72, 4.35, 0.29–2.0, and 5.19, respectively [74–77]. Glia/neuron ratio of dorsomedial thalamic nucleus with moderate nucleoside levels was 17.1 [78] but nucleoside levels of this area were similar to brain areas with lower glia/neuron ratio (for example the prefrontal cortex). One of the highest glia/neuron ratios were measured in the basal ganglia. Glia/neuron ratio of globus pallidus externa and interna were 158 and 159 respectively [79]. In these brain areas we measured moderate or low nucleoside levels, which were similar to nucleoside concentrations of white matter areas with very high glia/neuron ratio as well as limbic cortex and limbic areas with relatively low glia/neuron ratio (Table 2). Based on these data, we did not find very strong correlation between nucleoside levels and glia/neuron ratio in different brain areas that could explain the differences in nucleoside

concentrations between distinct brain areas. However, glia/neuron ratio was changed from individual to individual [72] and affected by cortical layers [75]. In addition, differences were detected in glia/neuron ratio within several brain areas such as different type of cerebral corticies and hippocampus [70, 77]. Based on data from the literature, we hypothesized previously that different glia/neuron ratio might result in the regionally distinct distribution of nucleosides in the human cortical and white matter areas [25]. However, the current results suggest that different neuron/glia ratios may only be one of the factors that could lead to the heterogeneous distribution of nucleoside concentrations in the human brain.

It is noteworthy that glia/neuron or astrocyta/neuron ratio was changed by different CNS diseases in affected brain areas. For example, glia/neuron ratio of subgenual prefrontal cortex, somatosensory cortex, entorhinal cortex, temporal cortex and amygdala was decreased in major depressive and bipolar disorder as well as in schizophrenia [69, 71, 76]. Astrocyta/neuron ratio of nucleus accumbens and caudate nucleus were higher in the Huntington's patients than in the control group [80]. Glia/neuron ratio was increased in cornu ammonis of hippocampus by hippocampal sclerosis [77]. In suprachiasmatic nucleus, glia/neuron ratio was increased in Alzheimer's disease and in frontotemporal dementia [81]. These results suggest that changes in glia/neuron ratio of several brain areas may be one of the key features of several types of CNS diseases [75]. We hypothesized that these changes in glia/neuron ratio may cause alterations in nucleoside metabolism and nucleoside levels in affected brain areas because of different purine metabolism of neurons and glial cells. As revealed previously, the levels of nucleosides change extremely in the brain under pathological conditions, which may play a role in the generation of certain diseases including Alzheimer's disease, Parkinson's disease and schizophrenia. This finding warrants an increasing attention to the understanding of the roles of nucleosides in the CNS [3, 7, 13, 82]. Our results will allow the comparison of nucleoside levels from diseased human brains and spinal cords to those in normal patients thereby contributing to the understanding of the role of nucleosides in pathomechmanisms of CNS diseases and the development of more potent drugs to treat them [13, 15, 45–53].

The architecture of iso- and allocortex (for example, the number of cortical layers) is different [83–85]. In spite of the different structures, our map suggests that nucleoside levels in isocortex may be similar to nucleoside concentrations measured in allocortex. For example, we measured moderate or low nucleoside levels in the somatosensory cortex and in the hippocampus (Table 2). In addition, nucleoside levels of isocortical areas with different cortical structures such as somatosensory and somatomotor cortex [86, 87] were not markedly different. These results suggest that no correlation could be found between the cortical structure and nucleoside levels in human cerebral cortical areas.

Nucleoside metabolic pathways form a complex network, in which nucleoside metabolic enzymes, such as 5′NT, ADA, PNP, AK and guanase are functioning simultaneously under physiological conditions [22, 23, 63, 88, 89]. Consequently, measuring of the activity all of these enzymes in parallel is needed to investigate exact concentrations of Ado, Ino and Guo and ratio of different nucleosides and their metabolites in the brain. However, only sporadic data from nucleoside metabolic enzyme activity were found in the literature. Activity of 5′NT, ADA, PNP and AK were revealed in the temporal cortex, the cerebellar cortex, and in the cerebral white matter in the human brain. 5′NT, ADA, PNP and guanase activities were measured in the caudate nucleus, the putamen, the anterior nuclei of thalamus, the substantia nigra, the pontine tegmentum and in the cerebellar cortex. In other human brain areas, the activities of only some of the enzymes were revealed [22, 23, 63, 88, 89]. Activity of PNP, ADA and AK were moderate or high in the temporal cortex and in the cerebral white matter. However, the activity of 5′NT was high and low in the temporal cortex and in the cerebral white matter, respectively, which may be the reason of the high Ino level in the temporal cortex (Table 2). Brain areas, in which high Ino levels were measured, such as the caudate nucleus, 5′NT shows high activity. In addition, low 5′NT activity and moderate Ino level were measured in the cerebellar cortex and in the cerebral white matter. It is noteworthy that we measured high Ado level in relation to low activity of ADA in the cerebellar cortex; this may partly explain why the highest Ado levels were found in the cerebellum (Fig. 1). We measured low Ado level in the frontal cortex, the cingulate cortex, the hippocampus, the globus pallidus etc. with high or moderate ADA level. Based on these results, we hypothesize that high activity of 5′NT and low activity of ADA result in high Ino or Ado level in the brain (Tables 2, 3; Fig. 1). Level of Hyp was moderate in brain structures with moderate PNP activity, for example in the temporal cortex and in the caudate nucleus. The cerebellar cortex contained high concentration of Hyp (Table 3; Fig. 1), which correspond to the low activity of Hyp metabolizing enzyme XO in the cerebellum [90]. 5′NT as well as PNP and guanase activity was high and moderate, respectively, in the caudate nucleus where high Guo level was measured. In other brain areas with low Guo levels, guanase activity was high such as in the some of the thalamic areas. These results suggest that moderate or low guanase, moderate PNP and high or moderate 5′NT activity may cause high Guo level in some of the cerebral cortical areas and basal ganglia (Table 2; Fig. 1). We have

no data on the activity of Urd metabolizing enzymes in different brain areas, but we hypothesize by analogy that decreased activity of UP and increased activity of 5′NT have a role in producing Urd. Indeed, we measured high Urd level and low or moderate Ura concentration some of the cerebral cortical areas and spinal cord areas as well as caudate nucleus and cerebellar cortex (Tables 2, 3; Fig. 1). In the cochlear nuclei, we measured high Urd and Ura levels in relation to postulated low activity of Ura metabolizing dihydropyrimidine dehydrogenase (DPD). Based on these results, we conclude that regionally different nucleoside metabolic enzyme activity causes regionally different nucleoside levels in the human brain.

High Urd, Ino and Guo levels may be related to specific functions of different cortical areas in the brain involved in higher mental functions, motor control, emotions, receiving of visual and auditory information etc. [91–95]. It was reported that A_1 and A_{2A} receptors may have a role in cognition and memory [3]. We measured low or moderate Ado levels in cerebral cortical areas, which may cause upregulation of A_1 receptor levels in the cortical areas as was demonstrated in the frontal cortex and in the occipital cortex [20]. In addition, low Ado level and high A_1 receptor density was revealed in hippocampus (Table 2), a brain area involved in cognition and memory [3, 20]. A_1 receptor density was similar to the expression pattern of NBTI (S-(4-nitrobenzyl)-6-thioinosine) sensitive type of equilibrative nucleoside transporters in different human brain areas with the exception of the hippocampus [20]. These results support the role of Ado in memory and cognition and suggest that Ado may modulate higher mental functions, motor control and emotions, via adenosine receptors. To support this hypothesis measuring of nucleoside levels in different part of cortical areas, which have established role in different physiological processes (such as Wernicke's and Broca's area) is needed. It is noteworthy that several nucleosides (Urd, Ino, Guo and Ado) showed high levels in the vestibular nuclei, the cochlear nuclei, the medial geniculate body, the flocculonodular lobe and in the spinal trigeminal nucleus (Tables 2, 3). These brain areas receive sensory inputs with different modalities [96, 97] suggesting that Urd, Ino, Guo and Ado may have a role in the modulation of sensory information processing in the human brain. It was revealed that Ado has a modulatory role in homeostatic and signal transduction processes within the retina and inner ear [18]. However, other sensory relay nuclei including the lateral geniculate body, the superior colliculus and the inferior colliculus showed moderate or low nucleoside levels (Tables 2, 3) indicating that a postulated regulatory role of nucleosides is relevant only to some brain structures of sensory systems.

High Urd and/or Ino and Guo levels were also measured in the basal ganglia (caudate nucleus, substantia innominata, and nucleus basalis; Table 2). Caudate nucleus is one of the dopamine rich areas of the basal ganglia involved in learning and memory as well as control of movement. Caudate nucleus is affected by both Huntington's disease and Parkinson's disease [98]. Nucleus basalis (of Meynert) is a nerve cell group in the substantia innominata rich in acetylcholine. Degeneration of these nuclei may generate Alzheimer's disease [99]. It was demonstrated that A_1 and A_{2A} receptor agonists or antagonists might be useful drugs for the treatment of Alzheimer's, Parkinson's and Huntington's disease [3, 10]. Brain areas with low or moderate Ado levels in the basal ganglia (Table 2) show high A_1 and A_{2A} receptor density (for example caudate nucleus) in relation to physiological function of Ado. However, our results suggest that Urd, Ino and Guo and not only Ado may have a role in physiological and pathophysiological functions of the basal ganglia. Low nucleoside and nucleoside metabolite levels were measured in the zona incerta, the substantia nigra as well as in the locus coeruleus (Tables 2, 3), brain structures suggested to be involved in the pathophysiology of Parkinson's disease [100, 101]. Our results suggest that Urd, Ino and Guo are not involved in the regulation of these brain structures.

On the basis of our nucleoside mapping we suggest that regional distribution of nucleoside levels in the CNS may be caused by different factors as neuron/glia ratio, spatially organized metabolism of nucleosides, as well as the distinct activity of the nucleoside metabolic enzymes in different brain areas. Since nucleoside transporters would equilibrate the nucleosides and nucleobases between extracellular and intracellular space [12, 28, 36] we assume that higher nucleoside levels of brain tissues reflect higher extracellular nucleoside levels, which could activate their receptors under physiological conditions. Nucleoside receptors are unevenly distributed in the human brain [3, 19, 20] and the receptor map shows correlations with our nucleoside map. However, to address a correlation between the functional significance of a certain nucleoside receptor system in a certain brain area needs further investigations. The presented map of regional distributions of nucleosides and their metabolites in the CNS, however, will facilitate further functional studies. Brain areas with very high and low concentration of nucleosides can be particularly interesting for functional approaches.

In conclusion, our results are the first demonstration that the distribution of nucleosides and their metabolites in 65 regions of the human CNS is uneven. Glia/neuron ratio of different human brain areas is only one of the factors that affect nucleoside levels in different human brain areas. Furthermore, we propose that regionally distinct nucleoside concentrations result mainly from spatial differences in nucleoside metabolism. The uneven density of nucleoside receptors and transporters as well as heterogeneous

distribution of nucleoside concentrations across the human CNS may contribute to the different functions of nucleosides in different brain areas. We first demonstrated the availability of Urd, Ino, Guo and not only Ado for putative modulatory roles in several human brain structures including sensory systems and basal ganglia. Our nucleoside map will form the basis of additional studies on the role of nucleosides in the CNS.

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