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Regional distribution of biogenic amines, amino acids and cholinergic markers in the CNS of the C57BL/6 strain

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Summary. A reliable extrapolation of neurochemical alterations from a mouse model to human metabolic brain disease requires knowledge of neurotransmitter levels and related compounds in control mouse brain. C57BL/6 is a widely used background strain for knockout and transgenic mouse models. A prerequisite for reliable extrapolation from mouse brain to the human condition is the existence of analogous distribution patterns of neurotransmitters and related compounds in control mouse and human brain. We analysed regional distribution patterns of biogenic amines, neurotransmitter and non-neurotransmitter amino acids, and cholinergic markers. Distribution patterns were compared with known neurotransmitter pathways in human brain. The present study provides a reference work for future analyses of neurotransmitters and related compounds in mouse models bred in a C57BL/6 background strain.

Keywords: Amino acids – Biogenic amines – Cholinergic markers – C57BL/6 – Brain – Regional distribution

Introduction

The development of genetically manipulated mouse models has significantly increased our knowledge of the complex domain of human metabolic brain disease. Validation of such models often comprises analyses of multiple neurotransmitter systems. To allow reliable extrapolation from mouse to man, a requisite question has to be answered: are distribution patterns of neurotransmitters and related compounds in control mice comparable with normal human brain? Interspecies differences in (brain) metabolism indeed have to be taken into account when comparing man and mouse (Samorajski and Rolsten, 1973; De Deyn et al., 2000). The rather poorly available information concerning this issue is scattered over literature from the last five decades. Often papers deal with only a limited number of brain regions (e.g., McBride et al., 1976; Shanks et al., 1991; Boatell et al., 1995; Alvarez et al., 1999) or little informative whole brain analyses (e.g., Agrawal et al., 1968; Cheney et al., 1975; Messiha, 1989; Messiha et al., 1990). Differences in tissue sampling, handling and preparation, and analytical methods also impede comparison of different studies.

The present manuscript aims at providing a reference work for future neurochemical analyses of biogenic amines (BA), amino acids (AA) and cholinergic markers in distinct cerebral regions and in spinal cord of control mice. Not only interspecies, but also interstrain differences in neurotransmitter systems have been described (AA: Simler et al., 1977; Waller et al., 1983; BA: Ciaranello et al., 1972; Waller et al., 1983; Messiha, 1989; Puglisi-Allegra and Cabib, 1997; cholinergic markers: Durkin et al., 1977; Waller et al., 1983; Schwab et al., 1990). We performed an elaborate neurochemical assessment of the inbred C57BL/6 strain, as it is widely used for congenic backcrossing of transgenic and knockout lines. It is a commercially available inbred strain that breeds relatively well and that has been characterised in a large number of behavioural paradigms (Crawley et al., 1997).

The regional distribution of BA (noradrenaline: NA, dopamine: DA, serotonin: 5-HT) and certain metabolites (3,4-dihydroxyphenylacetic acid: DOPAC; homovanillic acid: HVA; 5-hydroxy indoleacetic acid: 5-HIAA), neurotransmitter AA (glutamate, aspartate, glycine, and γ-aminobutyric acid: GABA) and 15 other AA was analysed in the CNS and spinal cord of male C57BL/6 control mice aged 7–8 months. In addition, the activity levels of choline O-acetyltransferase (EC 2.3.1.6; ChAT) and acetylcholinesterase (EC 3.1.1.7; AChE) were determined in a restricted number of relevant brain regions. Distribution patterns in C57BL/6 mice were compared with known neurotransmitter pathways in human brain. An additional statistical approach consisted of a correlation analysis studying the relationship between neurotransmitter or marker levels in nuclei and projection areas.

Materials and methods

Animals

Male C57BL/6 mice aged 7–8 months (B&K Universal Ltd., Grimston, UK), were group-housed under conventional laboratory conditions: food and water *ad libitum*, constant room temperature and humidity, 12/12 h light-dark cycle. All experiments were carried out in compliance to the European Communities Council Directive (86/609/EEC) and the Animal Ethics Committee of the University of Antwerp approved all protocols.

Chemicals

5-Hydroxy-N_{ω}-methyltryptamine oxalate (5HNMT), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), tetraisopropyl pyrophosphoramide (iso-OMPA), 1,5-bis(4-allyldimethylammoniumphenyl) pentan-3-one dibromide (BW284c51), ethopropasine-HCl, Coomassie Brilliant Blue G-250 reagent, bovine serum albumin, [^{14}C]acetyl-coenzyme A, choline-HCl, and physostigmine sulfate were purchased from Sigma-Aldrich (Bornem, Belgium). 3,4-dihydroxybenzylamine hydrobromide (DHBA), (−)-epinephrine bitartrate, dopamine hydrochloride, serotonin hydrochloride, L(−)-norepinephrine bitartrate, 3,4-dihydroxyphenylacetic acid, 5-hydroxyindolacetic acid, and 4-hydroxy-3-methoxyphenylacetic acid were obtained from RBI (Research Biochemicals International, Natick, MA, USA). Acetylthiocholine iodide, ninhydrin, and octane-1-sulfonic acid sodium salt monohydrate (OSA) were purchased from Merck (Darmstadt, Germany). Other chemicals were at least of analytical grade.

Sample collection and preparation

Mice were killed by submersion in liquid nitrogen – an ethically approved method for small rodents weighing less than 40 g (van Zuthpen et al., 1998) – and immediately stored at −75°C to prevent compound degradation. Upon dissection, the brain and medulla spinalis (at the cervical level) were quickly removed and washed in ice-cold saline solution. Cerebellum and olfactory bulb were isolated and, like the medulla spinalis sample, immediately frozen on dry ice and stored at −75°C. The remainder of the cerebrum was cut into 2-mm-thick coronal slices employing a chilled rodent brain matrix (type RMB-2000C, adult mouse, ASI Instruments, Warren, MI, USA). These brain slices were placed on a petridish filled with a mixture of ice and dry ice. Eleven different brain regions (frontal, parietal, and occipital cortex, striatum, hippocampus, thalamus, hypothalamus, tectum + colliculi, mesencephalon, pons, and medulla oblongata) were microdissected under a binocular microscope illuminated by a fiber optic cold light source. Tissue fragments were immediately frozen on dry ice and stored at −75°C. For the analysis of cholinergic markers, fewer brain regions were sampled, based on their importance in the cholinergic system (orbitofrontal cortex, amygdala, hippocampus, basal forebrain nuclei, anterior part temporal lobe).

Tissue fragments were weighed and subsequently homogenised using a rotor/stator-type tissue homogeniser (Tissue-tearor®, model 985-370 type 2, 4500–30000 rmp, Biospec Products, Bartlesville, OK, USA). All sample handling was carried out in an ice bath.

For BA analyses, 7–10 mg of tissue was homogenized (5000 rpm, 30 s) in 340 μL homogenisation solution (0.1 M HCl + antioxidant mixture 0.1% $Na_2S_2O_5$, 0.01% Na_2EDTA) to which 10 μL of an internal standard mixture containing 1024 ng/mL DHBA and 4096 ng/mL 5HNMT was added. The homogenisation probe was washed in 350 μL homogenisation solution, which was mixed with the first tube. After centrifugation (20800 g, 30 min, 4°C), 300 μL of the supernatant was mixed with 20 μL homogenisation solution. After further 1/4 dilution, the sample was filtrated over a 0.2-μm-membrane (Ultrafree®-MC filter unit, Millipore Corporation, Bedford, MA, USA) and stored at −75°C until HPLC-ED analysis.

For AA analysis, 5–8 mg of tissue was homogenized (4500 rpm, 1 min) in 350 μL of 0.5 N perchloric acid containing norvaline (19.2 μmol/L) as internal standard. To prevent the formation of foam, 1 μL of octanol was added. After centrifugation (20800 g, 15 min, 4°C), 250 μL of the clear supernatant was mixed with 70 μL of 2 M $KHCO_3$. After 15 min on ice, samples were vortexed and centrifuged (20800 g, 15 min, 4°C). Finally, 375 μL of sample dilution buffer (66.67 mM tri-lithiumcitrate tetrahydrate, 0.091% phenol, 0.5% thio-di-ethanol, pH 1.3) was added to 250 μL of the clear supernatant.

Samples for analysis of cholinergic markers were homogenised in 50 mM sodium phosphate buffer (pH 7.4), thereby yielding a 5× diluted stock solution.

Biogenic amine determination

Analysis was accomplished using ion-pair reversed phase chromatography. A BAS 200B HPLC system (Bioanalytical Systems, West Lafayette, USA) was equipped with a Ag/AgCl reference electrode and a dual glassy carbon working electrode for electrochemical detection (adapted from Cheng et al., 1993). The mobile phase, constantly kept at 35°C, consisted of a sodium phosphate-citrate buffer (pH 3.1) to which OSA was added to form ion pairs with BA. Methanol was added as organic phase. Isocratic separation of compounds was performed on a microbore reversed phase column (Hypersil, C_{18} BDS, 3 μm, 150 mm × 1.0 mm I.D., LC Packings, Zurich, Switzerland) preceded by a guard column. A calibrated splitter system (Acurate™, LC Packings) allowed only 10% of total pump flow-rate (400 μl/min) to pass over the analytical column, while the remaining 90% was recycled. ChromGraph® Report software (Bioanalytical systems, West Lafayette, IN, USA) was used for analysis of chromatographs and calculation of compound concentrations by comparing ratios of each endogenous substance to the internal standard with linear calibration graphs. Compared with conventional analytical columns of 3.0–4.6 mm I.D., a microbore column leads to an increase in detection sensitivity, significantly reduces detection limits, and allows analysis of small sample volumes. Detection limits (2× peak-to-peak noise of the baseline) were 57 ng/g wet weight for NA, 117 ng/g wet weight for DA, 79 ng/g wet weight for DOPAC, 148 ng/g wet weight for HVA, 273 ng/g wet weight for 5-HT and 172 ng/g wet weight for 5-HIAA. Reproducibility, as obtained from day-to-day analyses of standard mixtures and calculated from peak height, ranged from 1.0 to 2.6%.

Amino acid determination

The concentration of neurotransmitter AA and other AA (taurine, threonine, serine, glutamine, alanine, valine, cystine, methionine, isoleucine, leucine, tyrosine, ornithine, lysine, histidine, and arginine) was determined with a Biotronik LC 6000E AA analyser (Biotronik, Maintal, Germany).

Compounds were separated over a cation exchange column using lithium citrate buffers in a step-wise gradient elution with increasing lithium concentration, pH and temperature. Detection was accomplished with the ninhydrin colorimetric method as previously described in detail (Pei, 1994). The detection limit (2× peak-to-peak noise of the baseline) ranged from 0.009 μmol/g wet weight for valine to 0.038 μmol/g wet weight for glutamine. Reproducibility, as obtained from day-to-day analyses of standard mixtures and calculated from peak height, ranged from 0.64 to 2.8%.

Determination of cholinergic markers

Total protein content was determined with the spectrophotometric method (Hitachi UV-VIS 2001, 595 nm) according to Bradford (1976), using Coomassie Brilliant Blue G-250 reagent and bovine serum albumin as a standard. A protocol adapted from Ellman et al. (1961), with acetylthiocholine iodide as a substrate, was employed for measuring AChE activity (Harkany et al., 1995). Ethopropasine-HCl (10^{-4} M) was added to the

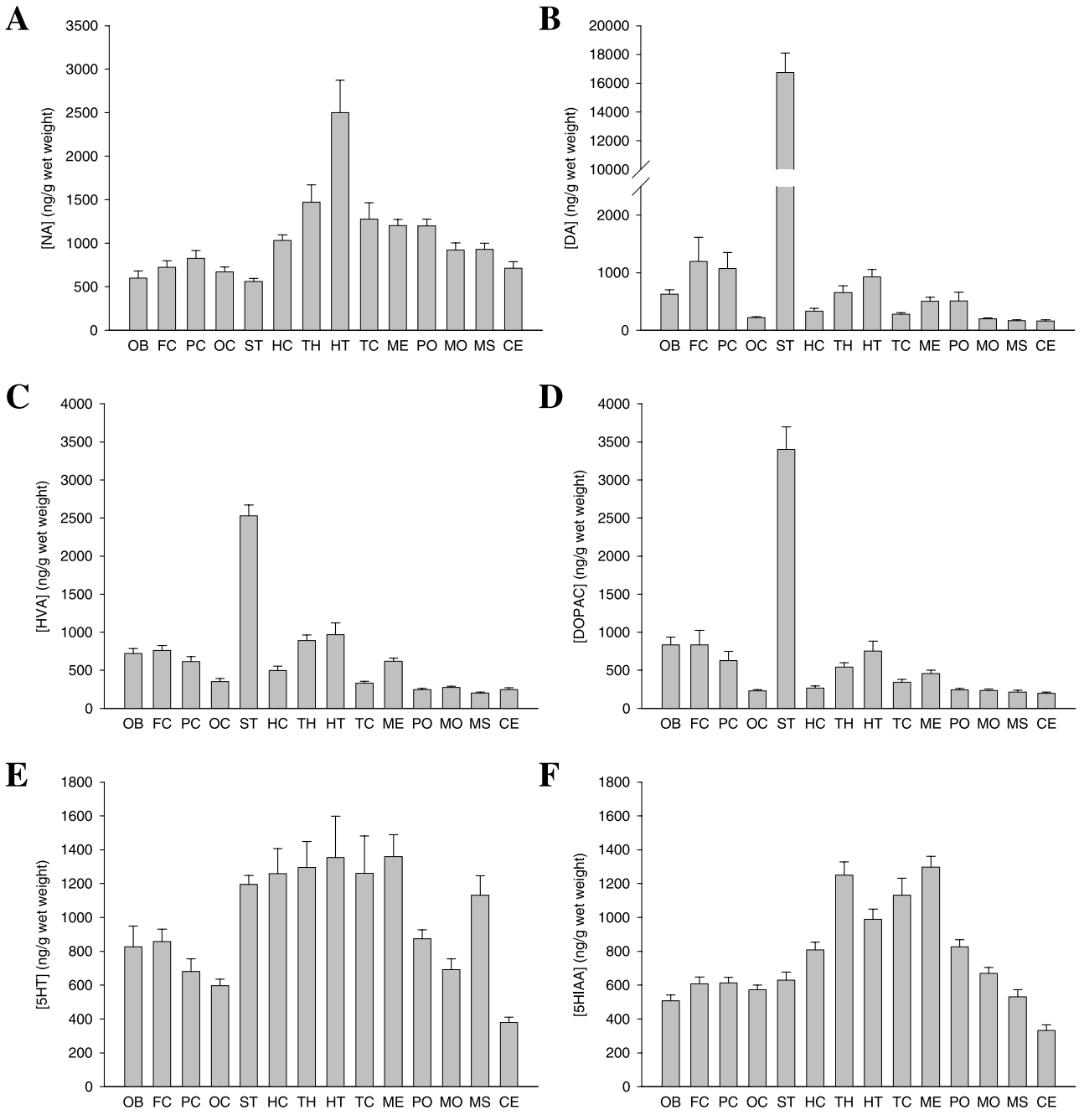

Fig. 1. Biogenic amine content in different brain areas of C57BL/6 mice. Each data point represents the mean (±SEM) of between 9 and 16 animals. *OB*, olfactory bulb; *FC*, frontal cortex; *PC*, parietal cortex; *OC*, occipital cortex; *ST*, striatum; *HC*, hippocampus; *TH*, thalamus; *HT*, hypothalamus; *TC*, tectum + colliculi; *ME*, mesencephalon; *PO*, pons; *MO*, medulla oblongata; *MS*, medulla spinalis; *CE*, cerebellum. Distribution patterns of neurotransmitters (**A**, **B** and **E**) and certain metabolites (**C**, **D** and **F**) are depicted: (**A**) noradrenaline, *NA*; (**B**) dopamine, *DA*; (**C**) homovanillic acid, *HVA*; (**D**) 3,4-dihydroxyphenylacetic acid, *DOPAC*; (**E**) serotonin, 5-HT; and (**F**) 5-hydroxy indoleacetic acid, 5-HIAA. Bars represent mean (±SEM) compound levels expressed as ng/g wet weight

incubation mixture to inhibit nonspecific cholinesterase activity. AChE activity was measured spectrophotometrically at 412 nm (Hitachi UV-VIS 2001), and expressed as μmol acetylthiocholine iodide hydrolysed per min/mg protein. ChAT activity was determined by the radiochemical method of Fonnum (1975), adapted by Harkany et al. (1995). The final concentrations in the incubation mixture were: 50 mM sodium phosphate

Table 1. Significant correlations (Pearson product moment correlation) between biogenic amine content (NA, DA, and 5-HT) in different brain regions of C57BL/6J mice putatively representing known neurotransmitter pathways

	Origin		Projection region	Pathway	Pearson product moment correlation
NA	PO	→	HC	Pathways originating from Pons:	
		→	TH	locus coeruleus + lateral tegmental neurons	
		→	HT		$r = 0.670$; $n = 9$
		→	FC		$P = 0.0484$
		→	PC		
		→	OC		
		→	MS		
		→	CE		
DA	ME	→	ST	Nigrostriatal pathway	$r = 0.704$; $n = 15$
		→	HC	Mesolimbocortical pathway	$P = 0.00338$
			TH	Mesothalamic pathway	
		→	PO	Mesopontine pathway	
5-HT	PO	→	MS	Caudal pathway	$r = 0.714$; $n = 14$
					$P = 0.00412$
	MO	→	MS		$r = 0.624$; $n = 14$
					$P = 0.017$
	ME	→	CE	Cerebellar rostral pathway	
	PO	→	CE		
	ME	→	TC	Dorsal ascending pathway	$r = 0.631$; $n = 13$
					$P = 0.0208$
	PO	→	TC		$r = 0.653$; $n = 13$
					$P = 0.0156$
	ME	→	HC	Ventral ascending pathway	$r = 0.589$; $n = 13$
					$P = 0.0342$
		→	TH		$r = 0.582$; $n = 15$
					$P = 0.023$
		→	HT		$r = 0.738$; $n = 10$
					$P = 0.0148$
		→	ST		$r = 0.795$; $n = 15$
					$P = 0.000399$
		→	FC		
		→	PC		$r = 0.539$; $n = 16$
					$P = 0.0313$
		→	OC		
		→	OB		$r = 0.697$; $n = 14$
					$P = 0.00557$
	PO	→	HC	Ventral ascending pathway	$r = 0.648$; $n = 13$
					$P = 0.0167$
		→	TH		
		→	HT		$r = 0.664$; $n = 10$
					$P = 0.0363$
		→	ST		$r = 0.536$; $n = 15$
					$P = 0.0394$
		→	FC		
		→	PC		$r = 0.527$; $n = 16$
					$P = 0.036$
		→	OC		
		→	OB		

CE, cerebellum; *FC*, frontal cortex; *HC*, hippocampus; *HT*, hypothalamus; *ME*, mesencephalon; *MO*, medulla oblongata; *MS*, medulla spinalis; *OB*, olfactory bulb; *OC*, occipital cortex; PC, parietal cortex; *PO*, pons; *ST*, striatum; *TC*, tectum + colliculi; *TH*, thalamus
If no *r* and *P*-values are shown, correlations failed to reach the desired level of significance

buffer (pH 7.4), 0.6 mM [^{14}C]acetyl-coenzyme A, 10 mM choline-HCl, 300 mM NaCl, 20 mM EDTA and 0.1 mM physostigmine sulfate. The radioactivity of the samples was analysed in a liquid scintillation counter, resulting in ChAT activity expressed as nmol acetylcholine synthesized per hour/mg protein. For easier comparison, results for AChE are expressed as μmol acetylthiocholine iodide hydrolysed per hour/mg protein in Fig. 3A.

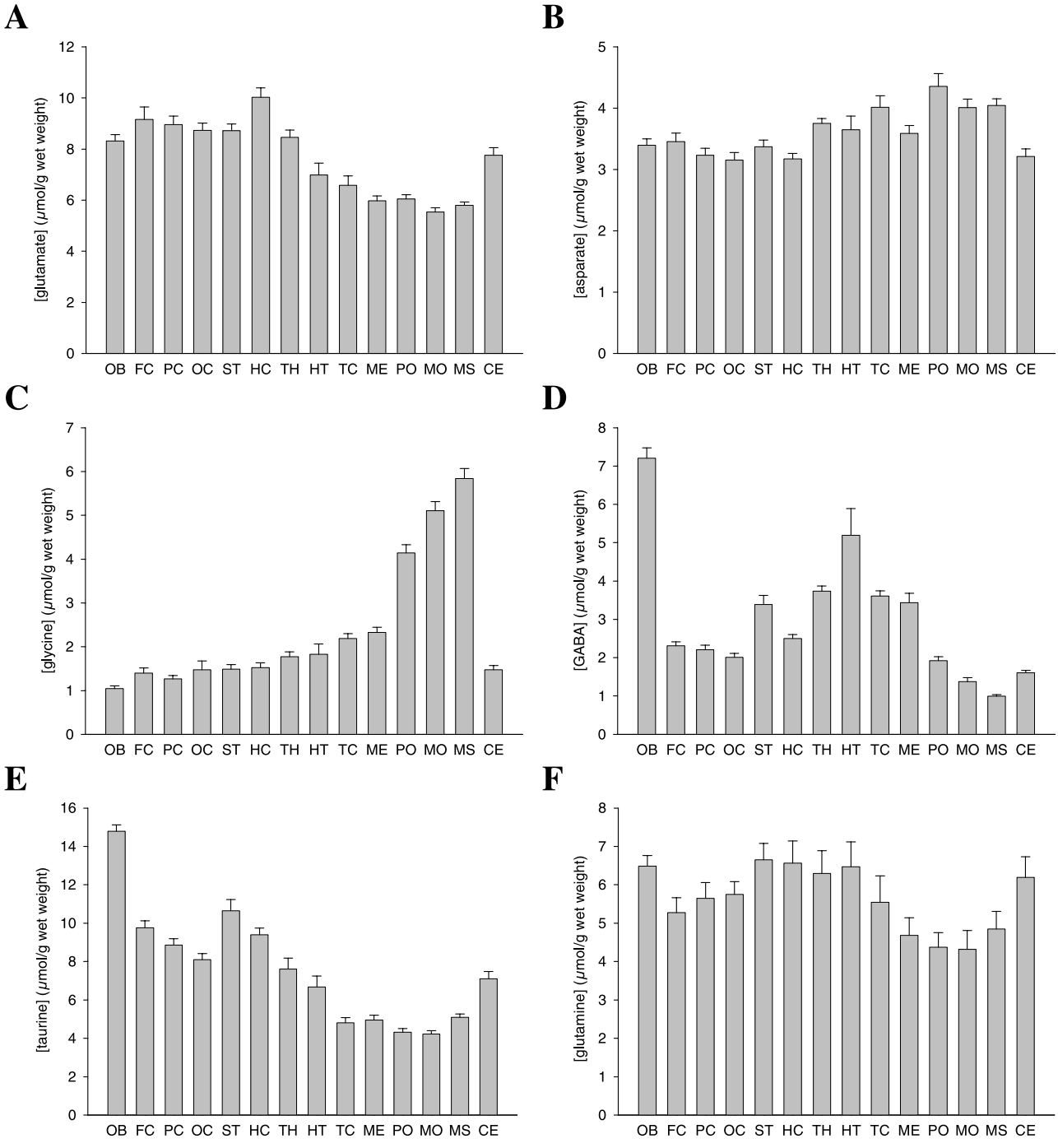

Fig. 2. Amino acidergic neurotransmitter content in different brain areas of C57BL/6 mice. Each data point represents the mean (±SEM) of between 10 and 18 animals. *OB*, olfactory bulb; *FC*, frontal cortex; *PC*, parietal cortex; *OC*, occipital cortex; *ST*, striatum; *HC*, hippocampus; *TH*, thalamus; *HT*, hypothalamus; *TC*, tectum + colliculi; *ME*, mesencephalon; *PO*, pons; *MO*, medulla oblongata; *MS*, medulla spinalis; *CE*, cerebellum. Excitatory (**A** and **B**) and inhibitory (**C** and **D**) amino acid neurotransmitter concentrations are depicted: (**A**) glutamate, (**B**) aspartate, (**C**) glycine, and (**D**) g-aminobutyric acid, GABA, as well as (**E**) taurine and (**F**) glutamine distribution patterns. Bars represent mean (±SEM) compound levels expressed as μmol/g wet weight

Statistical analysis

All data are presented as mean ± SEM. Statistics were performed with SigmaStat software (SPSS Inc., Erkrath, Germany) with the level of probability set at 95%. One-way analysis of variance with correction for repeated measures (1-way RM-ANOVA) was employed to investigate whether a compound displayed a region-specific distribution pattern. Post hoc comparison between pairs of means was performed using Turkey multiple comparisons analysis. Pearson product moment correlation was employed for correlation analysis of acknowledged neurotransmitter pathways.

Table 2. Significant correlations (Pearson Product Moment correlation) between amino acid content in different brain regions of C57BL/6J mice putatively representing known neurotransmitter pathways

	Origin		Projection region	Pathway	Pearson product moment correlation
GLU	FC	→	PC	Corticocortical pathways	$r = 0.800$; $n = 16$ $P = 0.000199$
		→	OC		$r = 0.515$; $n = 15$ $P = 0.0497$
	PC	→	OC		
	FC	→	ST	Corticostriatal pathways	
	PC	→	ST		
	OC	→	ST		
	FC	→	TH	Corticothalamic pathways	
	PC				
	OC				
	FC	→	PO	Corticopontine pathways	
	PC				
	OC				$r = 0.593$; $n = 15$ $P = 0.0198$
	OC	→	TC	Corticotectal pathway (visual cortex)	
	FC	→	ME	Prefrontal cortex → substantia nigra	$r = 0.536$; $n = 16$ $P = 0.0322$
	MO	→	CE	Olivocerebellar tract	
ASP	FC	→	PC	Corticocortical pathways	$r = 0.570$; $n = 16$ $P = 0.0212$
		→	OC		
	PC	→	OC		
	FC	→	ST	Corticostriatal pathways	$r = 0.552$; $n = 15$ $P = 0.0329$
	PC				
	OC				
	ME	→	CE	Olivocerebellar pathway	$r = 0.503$; $n = 16$ $P = 0.0471$
GABA	ST	→	ME	Strionigral pathway	
	CE	→	PO	Purkinje cells → lat. vestibular nucleus	$r = 0.610$; $n = 17$ $P = 0.00934$
	ME	→	TC	Nigrotectal + nigrocollicular pathway	
	ME	→	TH	Nigrothalamic pathway	
GLY	FC	→	HT	Corticohypothalamic pathway	
	PC				
	OC				
	MO	→	ME	Brainstem → substantia nigra	$r = 0.595$; $n = 16$ $P = 0.015$
	PO				$r = 0.595$; $n = 17$ $P = 0.0118$
	PO	→	MS	Raphe magnus nucleus → MS	$r = 0.509$; $n = 17$ $P = 0.0371$

GLU, glutamate; *ASP*, aspartate; *GABA*, γ-aminobutyric acid; *GLY*, glycine; *CE*, cerebellum; *FC*, frontal cortex; *HC*, hippocampus; *HT*, hypothalamus; *ME*, mesencephalon; *MO*, medulla oblongata; *MS*, medulla spinalis; *OB*, olfactory bulb; *OC*, occipital cortex; *PC*, parietal cortex; *PO*, pons; *ST*, striatum; *TC*, tectum + colliculi; *TH*, thalamus

If no *r* and *P*-values are shown, correlations failed to reach the desired level of significance

Results

Biogenic amines

Each compound exhibited a brain region-specific distribution pattern ($P<0.001$ in all cases with $F_{13,173}=17.317$ for NA, $F_{13,169}=115.115$ for DA, $F_{13,176}=61.414$ for DOPAC, $F_{13,168}=85.244$ for HVA, $F_{13,171}=13.177$ for 5-HT, and $F_{13,178}=21.129$ for 5-HIAA). Levels of dopaminergic compounds were most prominent in the striatum: 16748 ± 1344 ng/g wet weight for DA (Fig. 1B), 2527 ± 144 ng/g wet weight for HVA (Fig. 1C), and 3402 ± 295 ng/g wet weight for DOPAC (Fig. 1D). The highest NA concentration was observed in hypothalamus (2499 ± 374 ng/g wet weight), being almost twofold higher than the concentration in the other brain regions (Fig. 1A). The mesencephalon contained the highest 5-HT (1359 ± 129 ng/g wet weight) and 5-HIAA (1298 ± 62 ng/g wet weight) levels. Brain region-specific differences, however, were not as pronounced as in the dopaminergic and noradrenergic system (Figs. 1E and F). The results from Pearson Product Moment correlation analysis are summarized in Table 1.

Amino acids

For neurotransmitter AA, one-way RM-ANOVA disclosed a significant effect of brain region on compound level ($P<0.001$ for all compounds with $F_{13,203}=32.203$ for glutamate, $F_{13,203}=10.256$ for aspartate, $F_{13,210}=176.531$ for glycine, and $F_{13,210}=82.569$ for GABA). Highest glutamate levels were located in hippocampus and cortical areas with values ranging from 8.73 ± 0.29 μmol/g wet weight in occipital cortex up to 10.0 ± 0.37 μmol/g wet weight in hippocampus (Fig. 2A). Aspartate levels were highest in pons (4.32 ± 0.20 μmol/g wet weight), and comparable values were noted for medulla spinalis, tectum + colliculi and thalamus (4.04 ± 0.11; 4.02 ± 0.19 and 3.75 ± 0.08 μmol/g wet weight, respectively; Fig. 2B). Glycine levels were highest in medulla spinalis (5.84 ± 0.23 μmol/g wet weight) and brainstem, consisting of medulla oblongata, pons and mesencephalon (5.10 ± 0.21; 4.14 ± 0.19 and 2.33 ± 0.12 μmol/g wet weight, respectively; Fig. 2C). GABA content was maximal in the olfactory bulb (7.20 ± 0.27 μmol/g wet weight), followed by hypothalamus, thalamus and tectum + colliculi (5.20 ± 0.70; 3.74 ± 0.13 and 3.61 ± 0.14 μmol/g wet weight, respectively; Fig. 2D). As can be perceived in Fig. 2, glutamate and aspartate exhibit a rather constant regional distribution pattern, whereas distribution patterns of glycine and GABA display more prominent peaks. The results of Pearson Product Moment correlation are presented in Table 2.

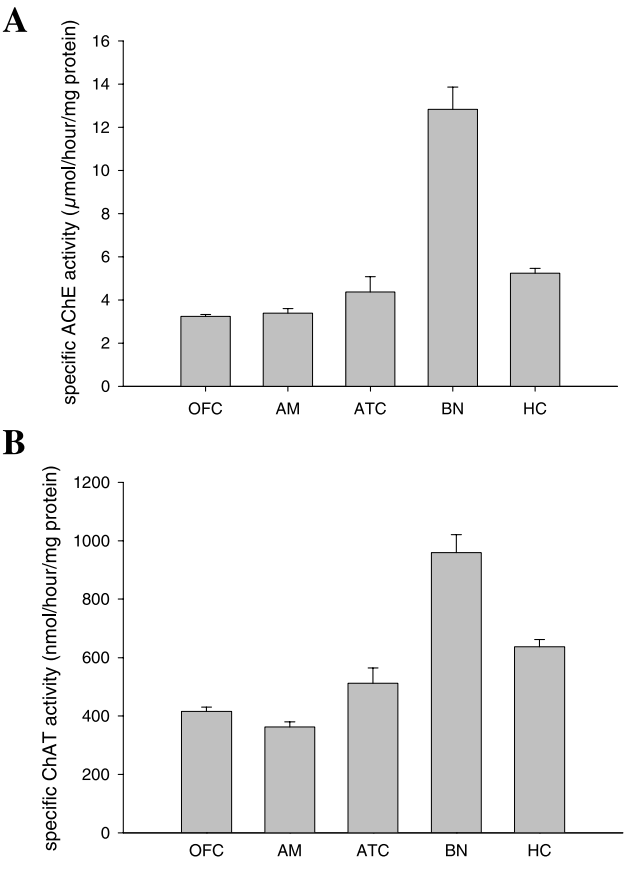

Fig. 3. Enzymatic activity of (**A**) acetylcholinesterase (*AChE*), and (**B**) choline acetyltransferase (*ChAT*) in different brain regions of C57BL/6 animals ($n=8$ for each data point). *OFC*, orbitofrontal cortex; *AM*, amygdala; *ATC*, anterior temporal cortex; *BN*, basal forebrain nuclei; *HC*, hippocampus. Bars represent mean (±SEM) specific activity

In addition, one-way RM-ANOVA indicated that several other AA displayed a brain region-specific pattern as well: taurine ($F_{13,209}=94.364$, $P<0.001$; Fig. 2E), threonine ($F_{13,209}=2.067$, $P=0.017$), serine ($F_{13,209}=3.265$, $P<0.001$), glutamine ($F_{13,203}=11.711$, $P<0.001$; Fig. 2F), alanine ($F_{13,210}=5.147$, $P<0.001$), methionine ($F_{13,209}=4.499$, $P<0.001$), lysine ($F_{13,209}=3.292$, $P<0.001$), and arginine ($F_{13,193}=2.339$, $P=0.007$).

Cholinergic markers

Both AChE and ChAT displayed a region-specific distribution pattern ($F_{4,28}=45.923$, $P<0.001$ for AChE, and $F_{4,28}=46.385$, $P<0.001$ for ChAT; Fig. 3A and B) with highest activity levels in the basal nuclei of the forebrain; 12.83 ± 1.036 μmol/hour/mg protein for AChE (Fig. 3A), and 959 ± 61 nmol/hour/mg protein for ChAT (Fig. 3B).

For neither of the enzymes, significant correlations between activity levels in basal nuclei and their projection areas were observed (data not shown).

Discussion

Regional heterogeneity in the CNS exists not only at the morphological and physiological level, but also in chemical composition and metabolism. Distribution patterns of BA, AA and cholinergic markers in C57BL/6 brain were compared with widely acknowledged neurotransmitter pathways in human brain. A supplementary approach for validation of our data encompassed the calculation of the correlation between neurotransmitter or enzyme activity levels in acknowledged nuclei and projection areas. Possible pitfalls of this approach should be taken into consideration; e.g. compound analysis as carried out in the present study cannot differentiate interstitial from intracellular levels.

The dopaminergic system is highly topographically organized (Feldman et al., 1996; Kuhar et al., 1999), as indeed manifested in the distribution pattern in C57BL/6 brain. The major dopaminergic pathways are the ventral (i.e. mesolimbical) and dorsal (i.e. nigrostriatal) mesostriatal pathways. Latter pathway is indicated by the significant correlation between mesencephalic and striatal DA values. Projections arising from substantia nigra spread throughout the whole of the striatum, thereby giving rise to the striking peak values of DA, HVA and DOPAC in this structure. Human brain levels of these compounds were also reported to be highest in caudate and putamen (Arai et al., 1984). We measured the highest NA concentration in the hypothalamus of C57BL/6 mice, corresponding with reports on human brain content, which noted the highest NA levels in hypothalamus and in locus coeruleus (Arai et al., 1984). Two principal nuclei groupings in the brainstem contain noradrenergic neurons: locus coeruleus and the more scattered lateral tegmental neurons (Feldman et al., 1996; Kuhar et al., 1999). In correspondence, the concentration in the pontine area was also among the highest levels found in C57BL/6 mice. The significant correlation between NA levels in pons and hypothalamus might reflect the ascending noradrenergic pathway from locus coeruleus to the diencephalon (Feldman et al., 1996; Kuhar et al., 1999). The serotonergic system is the most extensive monoaminergic system with nine serotonergic (raphe) nuclei (B1–B9) located in the brainstem (Feldman et al., 1996; Frazer and Hensler, 1999). Projections are diffuse and spread out to many cortical and subcortical areas, as reflected by the more homogenous distribution pattern in C57BL/6 brain. The manifold of significant correlations between 5-HT content in selected brain regions, might reflect the serotonergic caudal pathway (from B1–B4 in brainstem to spinal cord), the dorsal ascending pathway (from B7–B8 in pons and mesencephalon to colliculi), and the ventral ascending pathway (from B6–B8 in pons and mesencephalon to numerous parts of the diencephalon, limbic system, and cortex) (Feldman et al., 1996; Kuhar et al., 1999). Arai et al. (1984) measured the highest 5-HT and 5-HIAA levels in the raphe area (factor 2 to 4 compared to average diencephalon levels). Due to size limitations of mouse brainstem, it was technically impossible to perform a regional dissection of raphe nuclei. The 5-HT and 5-HIAA levels, therefore, can be considered 'diluted' by inclusion of non-serotonergic compounds. Nevertheless, serotonergic levels were among the highest in mesencephalon of C57BL/6. In analogy with human brain (Arai et al., 1984), levels of serotonergic compounds were high in different parts of the diencephalon and the limbic system, while lower levels were measured in cortical areas.

Glutamate is the principal excitatory neurotransmitter in the CNS being employed at approximately two thirds of all synapses (Fonnum, 1984; Feldman et al., 1996; Dingledine and McBain, 1999). Overall glutamate levels were on the average 2 to 3 times higher than aspartate levels in C57Bl/6 brain. Glutamate concentrations were highest in hippocampus and the three cortical areas, parallel with glutamate being the main neurotransmitter in neocortical and hippocampal pyramidal neurons (Francis et al., 1993). Glutamate and aspartate are the main neurotransmitters of corticofugal and corticocortical associations (Young, 1987), as reflected by significant correlations of aspartate concentrations in-between the different cortical areas and between cortex and striatum. Other glutamatergic pathways indicated by correlation analysis were the corticopontine pathway and the projection from prefrontal cortex to substantia nigra (Feldman et al., 1996).

Glycine levels in C57BL/6 brain exhibited a rostrocaudal gradient with highest glycine levels in brainstem and spinal cord, the latter corresponding with previous publications in mammals (Curtis and Johnston, 1974; Tossman et al., 1986; Feldman et al., 1996; Olsen and Delorey, 1999). Initially, glycine was almost exclusively established as neurotransmitter in inhibitory spinal cord interneurons, but nevertheless, there is ample evidence of glycine involvement in a number of CNS pathways, e.g. spinal afferents from B3 and reticular formation (Feldman et al., 1996; Olsen and Delorey, 1999),

putatively reflected by the significant correlation between glycine content in pons and medulla spinalis. Correlation analysis was also indicative of the glycinergic projections from brainstem to substantia nigra (Feldman et al., 1996). The reticular formation is a set of interconnected nuclei located throughout the brainstem rich in glycinergic neurons (Banay-Schwartz et al., 1993). Analogously, we observed high glycine levels in pons and medulla oblongata. With as many as 10–40% of the nerve terminals in the cerebral cortex, hippocampus, and substantia nigra using GABA, it is the major inhibitory neurotransmitter in mammalian CNS (Feldman et al., 1996). Tossman et al. (1986) reported highest GABA levels in substantia nigra, globus pallidus, hypothalamus and olfactory tubercle, paralleling our observations with highest GABA levels in, amongst others, the olfactory bulb, (hypo)thalamus, mesencephalon and striatum. The significant correlation between GABA levels in cerebellum and pons might illustrate the GABAergic projection from Purkinje cells to the lateral vestibular nucleus.

Of the eight non-neurotransmitter AA that exhibited a specific regional distribution pattern, taurine and glutamine reached regional concentrations $\geq 4\,\mu$mol/g wet weight, whereas levels of the other AA were on the average $\leq 1.5\,\mu$mol/g wet weight. These prominently higher levels of taurine and glutamine can be linked to their CNS function. Taurine is one of the most abundant free AA in brain where it acts as an organic osmolyte (Pasantes-Morales et al., 2000), and neuromodulator (del Olmo et al., 2000; Mori et al., 2002). Taurine is also involved in late-phase long-term potentiation (del Olmo et al., 2003; 2004). We observed the highest taurine concentration in the olfactory bulb ($14.8 \pm 0.33\,\mu$mol/g wet weight). Similar abundant levels of taurine have been reported in rat olfactory bulb with levels clearly exceeding those of glutamate and GABA (Banay-Schwartz et al., 1989b), as was indeed the case in the C57BL/6 strain as well (glutamate: $8.32 \pm 0.25\,\mu$mol/g wet weight, and GABA: $7.20 \pm 0.27\,\mu$mol/g wet weight). In human brain also, large quantities of taurine can be found in the olfactory epithelium and bulb (Ashihara et al., 1992). Glutamine levels are closely linked to glutamatergic neurotransmission, and the metabolic interconversions of glutamine and glutamate known as the glutamine cycle (Nicklas et al., 1987). When comparing Figs. 2E and F, it is clear that large similarities exist between the distribution patterns of glutamate and glutamine. Glutamate levels were on the average 1.4 times higher than glutamine levels. Similar proportions were previously reported in rat brain (Banay-Schwartz et al., 1989a).

Regional differences in threonine, serine, alanine, methionine, lysine and arginine levels might well be attributable to metabolic differences and are presumably not neurotransmission-related. Regional differences were small and often within the 10% methodological/biological variation, as was the case in previous reports of rat brain levels of these AA (Banay-Schwartz et al., 1990a, b).

Mammalian basal forebrain contains a major cholinergic projection system originating from four overlapping cell groups, numbered Ch1 through Ch4 (Feldman et al., 1996; Selden et al., 1998). In accordance, we measured highest AChE and ChAT activities in the basal forebrain harbouring Ch1–Ch4. Correlation analysis failed to show significant correlations between enzyme activity in basal forebrain and the four other brain regions. It remains to be elucidated whether specific cholinergic pathways can be detected through similar correlation analysis employing acetylcholine levels. The presently used methodology to sacrifice the animals prohibited the determination of acetylcholine levels because of enzymatic catabolism. The use of microwave irradiation to deactivate enzyme activities would overcome this limitation. However, since we wanted to provide a reference work, we opted for a simple and inexpensive technique that is financially and technically feasible for many research groups.

Conclusion

Discrepancies between human and murine distribution patterns can be linked to species-specificity, but also to scaling differences. Size limitations make micro-dissection in mouse brain more vulnerable to contamination with neighbouring regions. Nevertheless, major similarities exist between distribution patterns of neurotransmitters and related compounds in human normal brain and in the brain of C57BL/6 mice. Changes in compound levels in C57BL/6-based models of metabolic brain disease can, therefore, be reliably linked with changes in human pathology. The present study provides a reference work for future analyses of neurotransmitters and related compounds in mouse models bred in a C57BL/6 background.

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