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ORIGINAL ARTICLE

Differential topochemistry of three cationic amino acid transporter proteins, hCAT1, hCAT2 and hCAT3, in the adult human brain

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Abstract The cellular uptake of L-arginine and other cationic amino acids (such as L-lysine and L-ornithine) is mainly mediated by cationic amino acid transporter (CAT) proteins. Despite the important roles of cationic amino acid transporters for normal brain functioning and various brain diseases there is currently only fragmentary knowledge about their cellular and regional distribution patterns in the human brain. We mapped the immunohistochemical localization of human cationic amino acid transporters 1, 2 and 3 (hCAT1, 2, and 3) throughout five adult human brains and found a wide but uneven distribution of these transporters. All three hCAT1s were mainly localized in neurons, but were also found in numerous astrocytes, oligodendrocytes, plexus choroideus epithelial cells, and small blood vessels. The highest density of hCAT expressing neurons was observed in the hypothalamus, in some areas of the cerebral cortex, the thalamic reticular nucleus and the caudate nucleus, whereas weak to moderate expression was detected in the hippocampus, the prefrontal cortex (hCAT1 only), pons, brain stem and cerebellum. In contrast to what has been found in rodent brain, we detected hCAT2 and hCAT3 also in astrocytes. Overall, each hCAT has its characteristic, individual cerebral expression patterns, which, however, overlap with the others.

Keywords hCAT1 · hCAT2 · hCAT3 · Human brain · Distribution · Immunohistochemistry

Abbreviations

ADMA	Asymmetric dimethylarginine
CA	Cornu ammonis
CAT	Cationic amino acid transporter
hCAT	Human CAT
NO	Nitric oxide
Nnos	Neuronal nitric oxide synthase
iNOS	Inducible nitric oxide synthase
SLC	Solute carrier family

Introduction

The semi-essential amino acid L-arginine is of eminent functional importance as a substrate for protein synthesis and as the precursor of a wide range of biologically active intermediates, such as nitric oxide (NO), urea, creatine, ornithine, and agmatine. There are four principal sources of L-arginine for the central nervous system. L-arginine may (1) originate from the blood, being transported to brain tissue through the blood–brain barrier (O'Kane et al. 2006), (2) come from the cerebral spinal fluid transported through the choroid plexus ependymal cells, (3) derive from protein degradation, or (4) be enzymatically recycled from citrulline via argininosuccinate (Braissant et al. 2001; Wiesinger 2001; Gensert and Ratan 2006). Within the brain and most other tissues the cellular uptake of L-arginine (but also of other cationic amino acids, such as

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L-lysine and L-ornithine) is mainly mediated by sodium-independent transport system y+ carrier proteins (White et al. 1982), the so-called CAT proteins (CAT for cationic amino acid transporters; Kakuda and MacLeod 1994; Closs et al. 1997; Hosokawa et al. 1997; Ito and Groudine 1997; Closs 2002; Wiesinger 2001 and others). The CAT proteins constitute a subfamily of the solute carrier family 7 (SLC7) consisting of four transport proteins: CAT1 (SLC7A1), CAT2A (SLC7A2A), CAT2B (SLC7A2B), and CAT3 (SLC7A3) (Closs et al. 2006). Although closely related, individual members of the CAT protein family have distinct transport properties concerning substrate affinity and sensitivity. Studies of the expression of the individual members of the CATs in brain cells revealed a wide but uneven distribution. While CAT1 can be found in neurons, astrocytes, oligodendrocytes and endothelial cells, CAT2B is expressed in neurons and oligodendrocytes but not in astrocytes, and CAT3 is only detectable in neurons (reviewed in Braissant et al. 2001; Manner et al. 2003). However, CATs may also be expressed in microglial cells upon functional challenge (Kawahara et al. 2001; Czapiga and Colton 2003). Of note, our knowledge about the regional distribution and cellular localization of CATs mainly stems from experiments with rodents. Data on the cellular and regional topochemistry of CATs in human brain (named human CATs, hCATs) are rare, and the reported distribution patterns are incomplete (Vékony et al. 2001). This is surprising when considering that L-arginine and its metabolites (nitric oxide, creatine, and the polyamines) play pivotal roles in normal brain functioning as well as in a wide variety of human brain diseases, ranging from neurotoxicity and/or neuroprotection after cerebral ischemia to their putative implications in Alzheimer′s disease and mental disorders (for recent reviews, see Malinski 2007; Fiori and Turecki 2008; Harston et al. 2010; Mohlake and Whiteley 2010; Baker et al. 2011; Bernstein et al. 2011a, 2012; Cui et al. 2012; Laube and Bernstein 2012; Molderings and Hänisch 2012; Uzbay 2012 and many others). Moreover, hCATs have been assigned to chromosome loci, which have been linked with neurodegenerative and/or neuropsychiatric diseases (hCAT1: 13q12.3—schizophrenia (Hong et al. 2009); hCAT2: 8p22—schizophrenia (Need et al. 2009), major depression (Holmans et al. 2007), and late onset Alzheimer's disease (Baron et al. 2012); hCAT3: Xq13.1—autism (Scherer and Dawson 2011). Since revealing the precise neuroanatomical location of hCATs may potentially contribute to a better understanding of certain aspects of normal human brain function and its pathology, and their modulation might be a possible therapeutic option for the future, we decided to investigate by immunohistochemical means the differential distribution of hCATs 1, 2 and 3 in adult human brain.

Materials and methods

Subjects

All the brains were obtained from the New Magdeburg brain collection. The case recruitment, acquisition of personal data, performance of autopsy, and handling of autoptic material were conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Committee of Magdeburg. In addition, written consent was obtained from the next-of-kin. The brains of five human subjects (3 males, 2 females; mean age: 51.9 ± 4.1 years) without a history of neuropsychiatric disorder were investigated. None of the subjects had a history of substance abuse or alcoholism. An experienced neuropathologist ruled out changes due to neurodegenerative or traumatic processes.

Tissue processing

The subjects' brains were removed within 10–25 h after death and fixed in toto in 8 % phosphate-buffered formaldehyde for at least 2 months (pH 7.0, $T = 15$–20 °C).

The frontal and occipital poles were separated by coronal sectioning anterior to the genu and posterior to the splenium of the corpus callosum. After embedding all of the brain sections in paraffin, serial coronal sections of the middle block were cut (20 µm) and mounted. The distance between the sections was 1 mm. Every 50th section was Nissl and myelin stained (Heidenhain/Woelke).

Immunohistochemistry

Antisera

To immunolocalize hCAT1, hCAT2, and hCAT3, we used monospecific polyclonal antisera. hCAT1 was detected by employing of two different antisera. One was manufactured by immunizing rabbits with the designed appropriate synthetic peptide (anti-human CAT1-IgG; DPC Biermann GmbH, Bad Nauheim, Germany). This antiserum was tested before treatment for their specificity. For this purpose, the hCAT1-peptide [C] QMLRRKVVDCSREETRSLR-30 (NP003036.1, XP 007137.1, P30825) was diluted into different concentrations with the incubation solution and tested with the hCAT-1 antisera. It was demonstrated that a specific 70 kDa protein band reduction was equivalent to the increase in hCAT-1 peptide concentration (Jäger et al. 2008). Additionally, the specific immunostaining disappeared after preincubation of the hCAT-1 antiserum with the respective blocking peptide (Jäger et al. 2008). The second anti-hCAT1-antibody was a commercially available, affinity purified IgG antiserum raised in rabbits

(SLC7A1, Biozol Eching, Germany). Both anti-hCAT1 antisera yielded equally good and specific staining results. hCAT2 was localized using a rabbit polyclonal antiserum generated against the C-terminal domain of the human origin peptide (sc 87036, Santa Cruz Biotechnology, USA). Immunodetection of hCAT3 was carried out using a rabbit polyclonal antiserum produced against the synthetic human peptide (SLC7A3, Biozol Eching, Germany).

Immunostaining protocols

Whole brain frontal sections were collected at intervals of about 1.8 cm from the level 2 cm rostral to the splenium to the posterior splenium and from the central portion of the raphe nuclei to the central portion of the olivary nuclei. After dewaxing, the sections were boiled in 10 mM citrate buffer (pH 6.0) and then pre-incubated with methanol/H_2O_2 to suppress endogenous peroxidases. After repeated washing with phosphate-buffered saline (PBS), the respective antibodies (anti-hCAT 1–3) were applied at dilutions of 1:200 in PBS. For visualization, the avidin–biotin method (Vectastain-peroxidase kit) with 3,3′-diaminobenzidine as chromogen was used. The color reaction was enhanced by adding 2 ml of 0.5 % nickel ammonium sulfate solution to the diaminobenzidine as described previously (Bernstein et al. 1999). The procedure yielded a dark purplish-blue to dark-blue reaction product. For negative controls, the primary antibody was replaced with buffer or normal serum. No immunostaining was found in control sections (Fig. 1h).

Cell countings

To get an impression of between subject differences in staining intensities and numerical densities of immunostained cells, we counted hCAT1, hCAT2 and hCAT3 expressing neurons in two brain areas (layers III and V of the left dorsolateral prefrontal cortex and left hypothalamic paraventricular nucleus). Cell counting method and statistical treatment were as described earlier in detail (Bernstein et al. 2009). The section thickness after the histologic procedures was 18.9 ± 1.0 µm (mean ± SD). A counting grid was used to define a three-dimensional box within the thickness of the section allowing at least 4-µm guard zones at the top and bottom of the section, and to apply a direct, three-dimensional counting method. To estimate the number of immunopositive paraventricular neurons we counted at higher magnification the number of cell profiles per slice using the optical disector method as previously described for nitric oxide synthase-containing hypothalamic neurons (Bernstein et al. 1998).

Results

Regional and cellular distribution patterns of hCAT1, hCAT2 and hCAT3 immunoreactivities

Cerebral cortex

The following cortical gyri were studied: parahippocampal, entorhinal, orbitofrontal, inferior temporal, medial temporal, insular (longus and breves), medial frontal, superior frontal, anterior cingulate, and dorsolateral prefrontal cortex. hCAT1 and hCAT3 immunopositive neurons were found in all cortical areas under investigation. However, the number of hCAT1 immunopositive nerve cells was rather low in prefrontal cortical areas such as anterior cingulate or dorsolateral prefrontal cortex, where immunostaining was restricted to large layer V pyramidal cells (Fig. 1a). In contrast, in other cortical areas, such as medial and inferior temporal, parahippocampal or entorhinal cortex, hCAT1 was found to be present in multiple neurons situated in all cortical layers (Fig. 1b). HCAT3 was widely distributed in all areas of the cerebral cortex (Fig. 1c, d). Intracellularly, the immunoreaction was located in the perikarya and the dendrites of the nerve cells. Typically, cell nuclei were free of the reaction product. The only exception was a subpopulation of parahippocampal interneurons, where some nuclear hCAT1 immunostaining was evident (Fig. 1b). Sometimes, the neuropil showed a weak to moderate immunoreactivity to hCAT1. hCAT2 immunoreactive neurons were rarely found in cortical regions (Fig. 1f). Interestingly, numerous astrocytes located at the border between prefrontal gray matter layer VI and white matter were intensely immunostained for hCAT1 and hCAT3 (Fig. 1e, g). A portion of gray and white matter astrocytes was found to express hCAT2 (Fig. 1f). Their topographic distribution differed from those of hCAT1 and hCAT3, however.

Hippocampus and amygdala

A majority of CA_1, CA_2 and CA_3, polymorphic and subicular pyramidal neurons showed moderate hCAT1 immunostaining (Fig. 2a). Dentate gyrus neurons were only weakly stained for hCAT1. A few hippocampal interneurons highly expressed the hCAT1 protein (Fig. 2b). Some hCAT2 immunoreactivity was seen in a few pyramidal neurons, which appeared weakly stained. Many polymorphic neurons moderately expressed hCAT2 and hCAT3 (Fig. 2c, d). hCAT3 immunoreactivity was restricted to single interneurons and a small subpopulation of CA_1 and CA_2 pyramidal cells. Alveus astrocytes were heavily immunolabeled for hCAT1 (Fig. 2e), hCAT2 and hCAT3 (Fig. 2f). Numerous amygdaloid neurons were

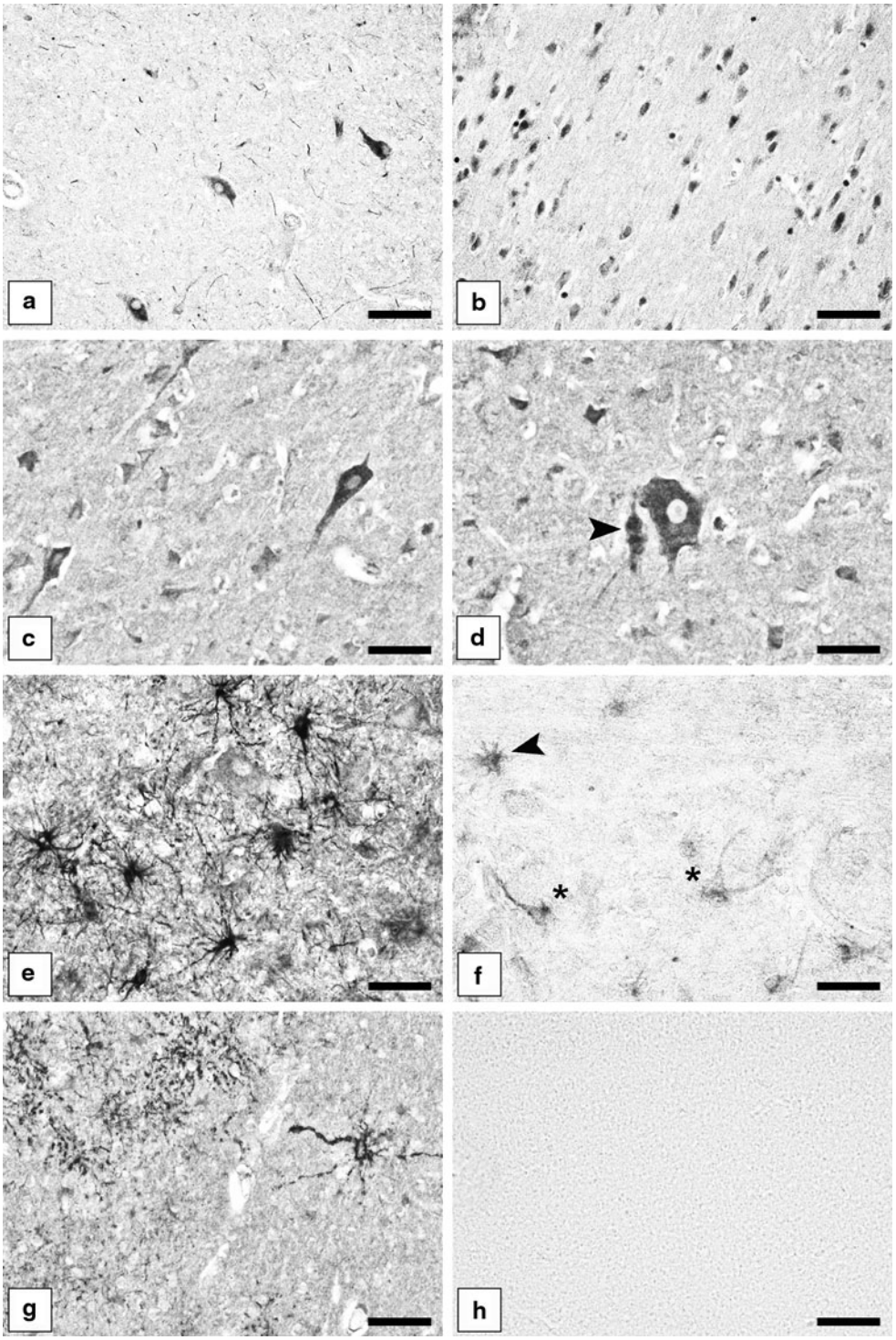

Fig. 1 Expression of hCAT1, 2 and 3 in human cerebral cortex. **a** Neurons immunoreactive for hCAT1 in the dorsolateral prefrontal cortex. Immunoreactivity is restricted to some pyramidal cells. Bar = 80 μm. **b** Multiple hCAT1 immunopositive neurons in the parahippocampal gyrus. Bar = 100 μm. **c** Neurons immunoreactive for hCAT3 in the dorsolateral prefrontal cortex. Large pyramidal cells and some interneurons are immunostained. Bar = 40 μm. **d** HCAT3 expressing neurons in the anterior cingulate cortex. Besides numerous small interneurons two large-sized neurons are shown (a pyramidal cell to the *right* and a spindle shaped von Economo neuron to the *left*, *arrowhead*). Bar = 40 μm. **e** Astrocytes expressing hCAT1 at the border between gray and white matter. Bar = 30 μm. **f** Small hCAT2 immunoreactive neurons (*asterisks*) and astrocytes (*arrowhead*) in the orbitofrontal cortex. Bar = 40 μm. **g** Astrocytes expressing hCAT3 at the border between gray and white matter. Bar = 40 μm. **h** Control section. After replacement of the primary anti-hCAT1 antiserum by normal serum no specific immunostaining is visible. Bar = 80 μm

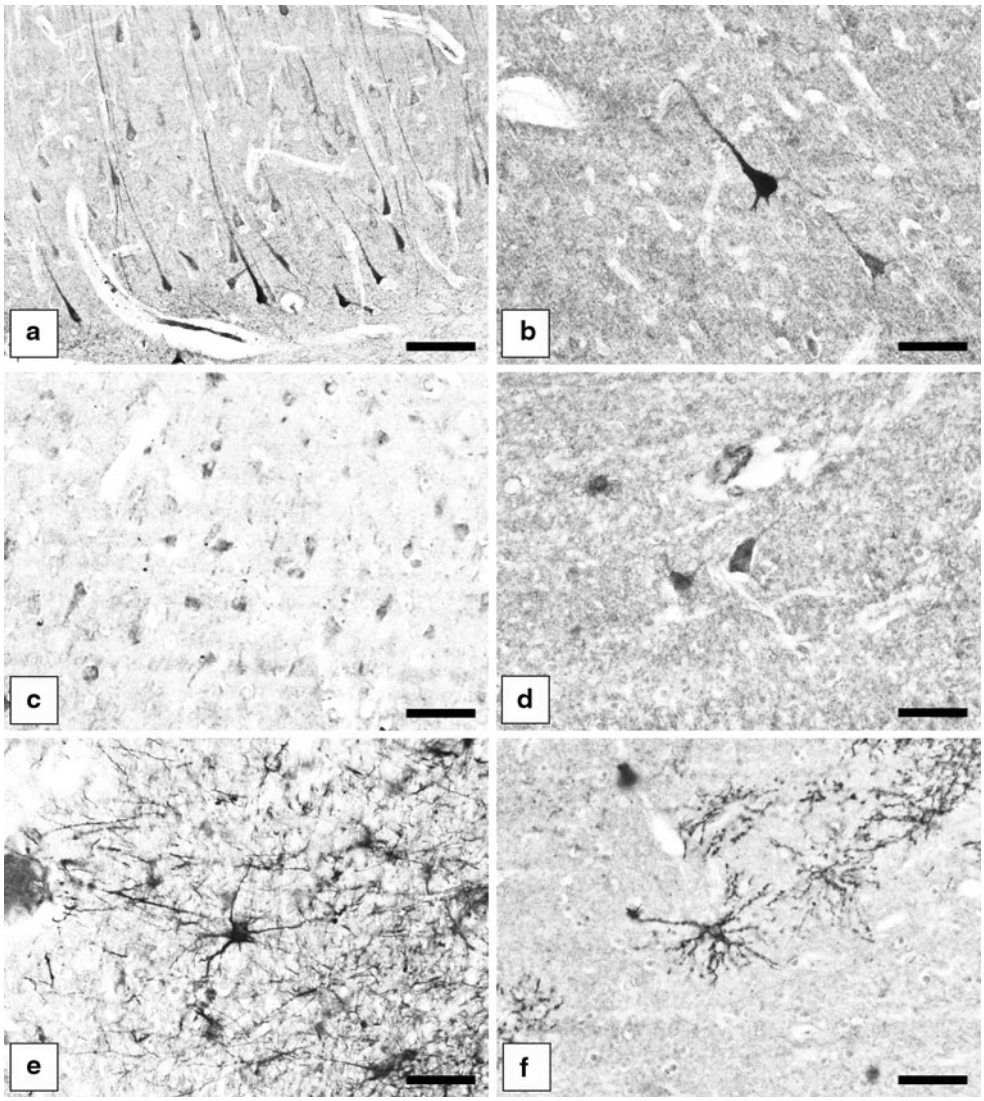

Fig. 2 Expression of hCAT1, 2 and 3 in human hippocampus. **a** Multiple hCAT1 immunoreactive hippocampal pyramidal cells situated in the CA_2 region. Bar = 100 μm. **b** HCAT1 immunopositive neurons in the subiculum. Bar = 40 μm. **c** Polymorphic layer neurons immunoreactive for hCAT2. Bar = 100 μm. **d** Polymorphic layer neurons immunoreactive for hCAT3. Bar = 60 μm. **e** Alveus astrocytes expressing hCAT1. Bar = 40 μm. **f** Alveus astrocytes expressing hCAT3. Bar = 40 μm

immunoreactive for hCAT1 and hCAT3 but not for hCAT2. The immunostaining was fairly weak, however.

Basal ganglia, thalamus and pineal gland

A very strong hCAT1 immunolabeling was found in caudate nucleus neurons and the surrounding neuropil (Fig. 3a). The same holds true for the thalamic reticular nucleus (Fig. 3b). The majority of Nucleus basalis of Meynert and medial habenular neurons were revealed to express both hCAT1 and hCAT3. Neurons belonging to the lateral geniculate nucleus were intensely immunostained for both hCAT1 and hCAT3. Moderately hCAT1, hCAT2 (Fig. 2c) and hCAT3 (Fig. 3d) immunopositive nerve cells were scattered over the entire striatum. Numerous hCAT1 and hCAT2 expressing astrocytes were found in thalamic nuclei and the internal capsule. The pineal gland was found to abundantly express hCAT1 s. Intense hCAT1 (Fig. 3e) and hCAT3 immunostained cells were observed in the subventricular zone.

Hypothalamus

The hypothalamus was among the brain regions with the most prominent immunolocalization of hCATs 1 and 3. The most pronounced immunostaining appeared in parvocellular neurons of the paraventricular nucleus as as well as in neurons situated in the supraoptic, the lateral and the

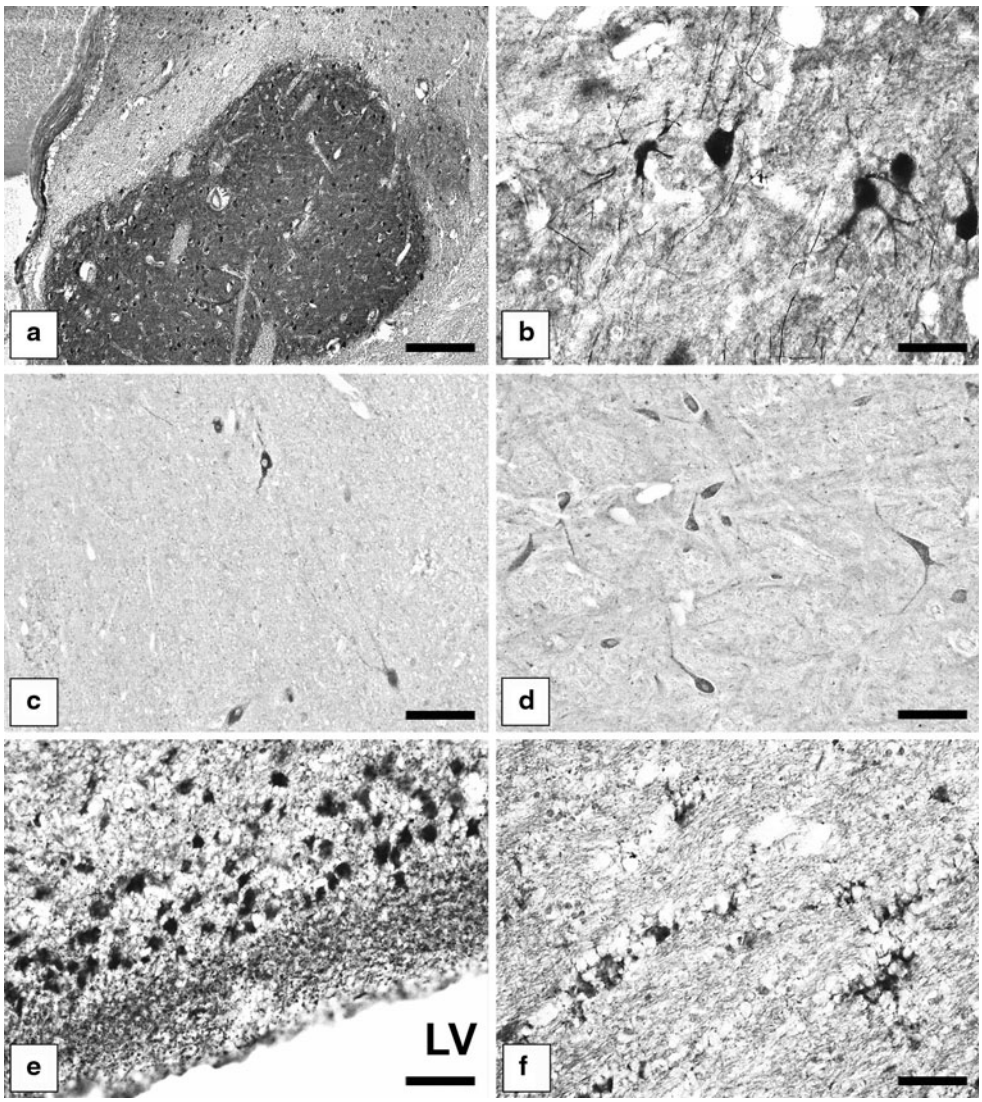

Fig. 3 Expression of hCAT1, 2 and 3 in basal ganglia, thalamus, the striatum and pineal gland. **a** Low power microphotograph demonstrating very strong expression of hCAT1 in caudate nucleus neurons and the surrounding neuropil. Bar = 250 μm. **b** Neurons highly expressing hCAT1 protein in the reticular thalamic nucleus. Bar = 40 μm. **c** HCAT2 immunoreactive striatal neurons. Bar = 100 μm. **d** HCAT3 immunoreactive striatal neurons. Bar = 100 μm. **e** Strong expression of hCAT1 in cells of layer II of the subventricular zone. *LV* lateral ventricle. Bar = 40 μm. **f** Corpus callosum parafascicular oligodendrocytes immunopositive for HCAT1. Bar = 30 μm

arcuate nuclei. Besides, numerous hCAT1 and hCAT3 immunoreactive astrocytes were observed (Fig. 4a–f). Nerve fibers in the pituitary stalk were also strongly immunostained for hCAT1 and hCAT3 (data not shown). A faint to moderate neuronal immunostaining for hCAT2 was seen in some neurons located in the paraventricular nucleus and in some astrocytes.

Cerebellum

Somata of some Purkinje neurons were weakly immunostained for hCAT1 and hCAT3. Sometimes, the reaction product was also observable in Purkinje cell dendrites. Besides, a few granule cells and Golgi cells strongly expressed hCAT1, but not hCAT3 immunoreactivity (Fig. 5a). HCAT2 was not found in these cells. hCAT1 was detected in certain neurons of deep cerebellar nuclei (i.e. dentate nucleus, emboliform, fastigial, and globose nuclei).

Midbrain

Some immunopositive neurons were seen in the hCAT1 and hCAT3 ventral tegmentum. Numerous astrocytes were observed to express all three hCATs.

Brain stem

Moderate immunostaining for hCAT1 antibody was observed in some brain stem nuclei (especially in the neurons belonging to the trigeminal and vestibular nuclei as well as to the inferior olivar nucleus. hCAT3 was observed in a few cells belonging to the locus coeruleus (Fig. 5b). hCAT2 was not detectable in brain stem cells (data not shown).

White matter

Multiple white matter astrocytes were observed to express hCAT1, hCAT2 and hCAT3 immunoreactive material.

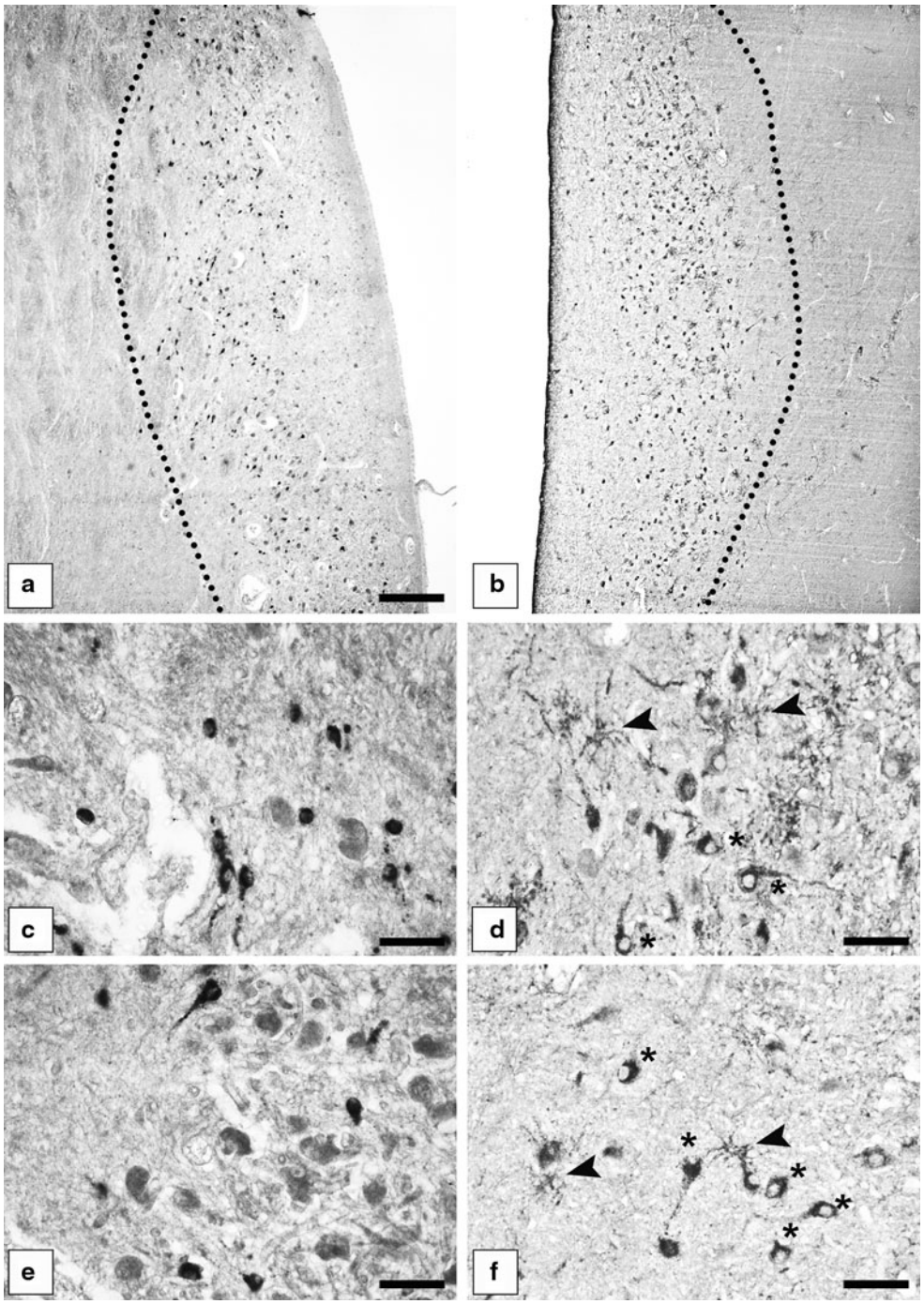

Fig. 4 Expression of the transporters hCAT1, 2 and 3 in the hypothalamus. **a** Low power microphotograph of hCAT1 expressing paraventricular neurons. The *dotted line* delineates the nucleus. Bar = 400 μm. **b** Low power microphotograph of hCAT3 expressing paraventricular neurons. The dotted line delineates the nucleus. Bar = 400 μm. **c** HCAT1 expressing neurons in the paraventricular nucleus shown at higher magnification. Parvocellular neurons are intensely immunostained, while magnocellular neurons show a less intense staining. Bar = 40 μm. **d** HCAT3 immunoreactive neurons (*asterisks*) and astrocytes (*arrowheads*) in the paraventricualr nucleus. Bar = 40 μm. **e** HCAT1 expressing supraoptic neurons. Bar = 40 μm. **f** HCAT3 immunoreactive neurons (*asterisks*) and astrocytes (*arrowheads*) in the supraoptic nucleus. Bar = 40 μm

Besides, hCAT1 was seen in numerous parafascicular oligodendrocytes (especially in the corpus callosum; Fig. 3f). A few interstitial white matter neurons were found to be immunopositive for hCAT1. Interestingly, hCAT1 immunoreactivity was associated with many white matter crossing nerve fibers (Fig. 5c).

A small number of blood vessels were immunostained for hCAT1 (Fig. 5d) or hCAT3.

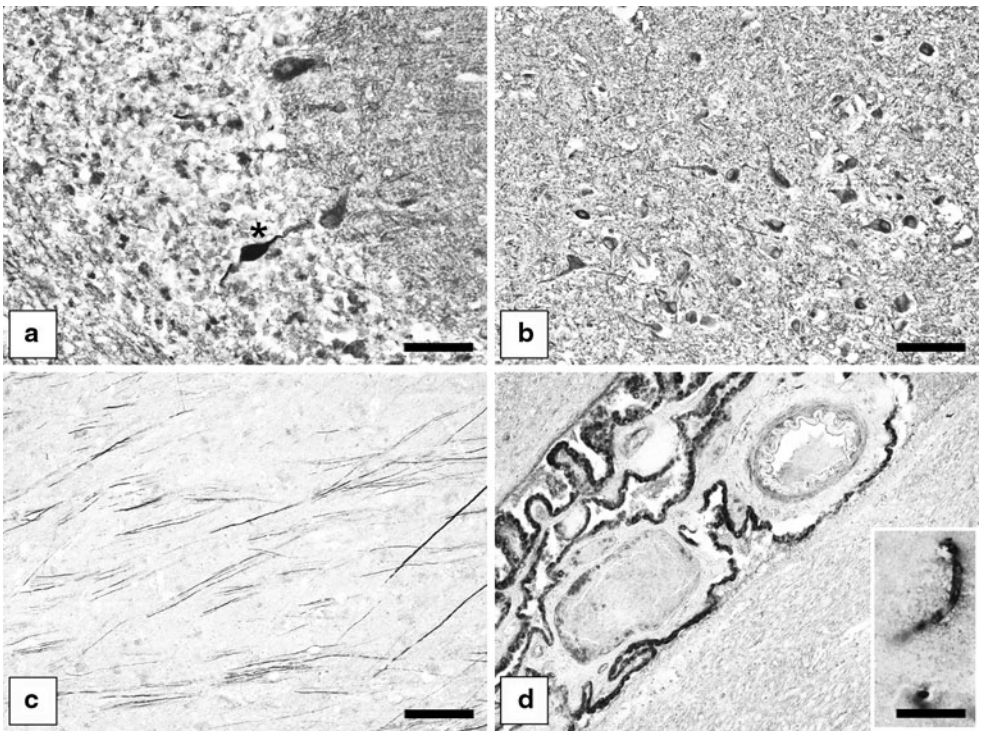

Fig. 5 Expression of hCAT1, 2 and 3 in the cerebellum, brain stem, white matter and choroid plexus. **a** Expression of hCAT1 in cerebellar neurons. Some Purkinje cells, Golgi cells (*asterisk*) and granule cells are immunoreactive. Bar = 60 μm. **b** HCAT3 immunoreactive neurons in the locus coeruleus. Bar = 80 μm. **c** Multiple hCAT1 immunoreactive nerve fibers in the white matter. Bar = 40 μm. **d** HCAT1 immunoreactivity in the choroid plexus. Note the intensely immunostained epithelial cells. Insert: small blood vessel immunoreactive for hCAT1. Bar (valid for plexus) = 100 μm. Bar (valid for inserted blood vessel) = 40 μm

Choroid plexus

HCAT1 (Fig. 5d), hCAT2 and hCAT3 under study were highly expressed in the cuboidal epithelial cells and in numerous capillaries.

Major findings on the distribution of hCATs are summarized in the Table 1.

Quantitative findings of select brain regions

The estimated neuronal densities were for the left dorsolateral prefrontal cortex (layer III: hCAT1: 6,200 ± 1100 cells/mm^3, hCAT2 0; hCAT3 9,730 ± 900 cells/mm^3; layer V: hCAT1: 4,320 ± 870 cells/mm$^{3;}$ hCAT2: 0; hCAT3: 9,900 ± 680 cells/mm^3) and for left paraventricular nucleus: (hCAT1: 32,550 ± 6,300 cell/mm^{3,} hCAT2: 18,225 ± 5,800 cells/mm^3 and hCAT3: 30,440 ± 7,200 cells/mm^3).

Discussion

The indisputable significance of L-arginine and other cationic amino acids for the maintenance of normal brain functioning as well as their emerging roles in brain pathological states has renewed the interest in cationic amino acid transporters, which supply the cells with these important chemical compounds or (as in the case of the arginine derivative, asymmetric dimethylarginine, ADMA Teerlink 2005) export them from the cells. This is, to our knowledge, the first detailed study on the expression patterns of the three hCATs (1, 2 and 3) in the human brain. Herein we show that hCAT proteins are very widely distributed throughout the adult human brain, which is in good accordance with previously reported results on the regional distribution of hCATs as obtained using cDNA dot blot analysis (Vékony et al. 2001). However, a closer look at the cellular distribution of hCATs revealed (besides many similarities) certain peculiarities that have not been observed in the much better studied rat and mouse brains (Hosokawa et al. 1999; Braissant et al. 2001; Manner et al. 2003). In accordance with the data from rodent brain, hCAT1 was found in multiple neurons, numerous astrocytes, white matter oligodendrocytes and blood endothelial cells. This evolutionarily conserved nearly ubiquitous distribution is not surprising, when taking into account that the presence of hCAT1 is indispensable for differentiation and functioning of almost all cells and tissues (except the

Table 1 Differential expression of hCAT1, hCAT2 and hCAT3 in adult human brain

	hCAT1	hCAT2	hCAT3
Neurons	++/+++	+/++	++/+++
Astrocytes	+++	++	++
Oligodendrocytes	++	+	+/++
Telencephalon			
Prefrontal cortex	++	−	+++
Other cerebral cortex areas	+++	−/+	+++
Nuc. caudatus and putamen	+++	++	++
Hippocampus	++	+	++
Subfields CA_1-CA_3	+/++	+	++
Dentate gyrus	+	+	+
Polymorphic/molecular layers	++	++	+
Subiculum	++/+++	+	++
Amygdala	+	+	+
Diencephalon			
Thalamus	++	+	++
Nuc. reticularis	+++	++	+++
Cgl	+++	++	+++
Hypothalamus	+++	+/++	+++
Nuc. paraventricularis	+++	++	+++
Nuc. supraopticus	+++	++	+++
Hypophysial stalk	+++	−/+	++
Pineal gland	+++	+++	+++
Mesencephalon	++	+ (++)	+
Ventral tegmentum	++	+	++
Brain stem	++	−	++
Nucc. trigeminus and vestibularis	++	−	++
Nuc. olivaris, Locus coeruleus	++	−	++
Cerebellum	+/++	−	+/++
Purkinje cells	+/++	−	+/++
Granule and Golgi cells	++	−	−
Deep cerebellar nuclei	++	−/+	−/+
Epithelia	++	++	++
Oligodendroglia	++	++	++
Choroid plexus	+++	++	+++
Epithelia	+++	++	+++

Average signal intensities: − Absent; +: Weak expression; ++: Moderate expression; +++: Strong expression

liver, Manner et al. 2003), and that the homozygous deletion of the CAT1 gene in mice is even lethal (Perkins et al. 1997). It should be emphasized, however, that by far not all neurons or glial cells were found to express hCAT1 protein, and it appears difficult to relate these cellular patterns to established cell markers. The observed neuronal expression of CAT1, for example, roughly resembles that of some enzymes involved in NO metabolism, such as human brain nNOS (cerebral cortex, thalamus, cerebellum, brain stem, Egberongbe et al. 1994; hypothalamus, Bernstein et al. 1998; cerebellum, Bernstein et al. 2001; hippocampus, Oliveira et al. 2008; locus coeruleus, Bielau et al. 2012) and murine brain arginase isoenzymes I and II (cerebral cortex, hippocampus, cerebellum, pons, Braissant et al. 1999; Yu et al. 2001), but also shows some overlap with the distribution of human agmatinase (habenula and cerebellum, but not hippocampus, Bernstein et al. 2011b) and the expression of glutamic acid decarboxylase and parvalbumin (reticular thalamic nucleus, unpublished observation). Interestingly, the distribution patterns of the other two hCATs (hCAT2 and hCAT3) markedly differ from what is known from the rodent brain. While hCAT2 is not (or only very barely) detectable in untreated rodent astrocytes (Braissant et al. 2001; Manner et al. 2003), we observed hCAT2 immunopositive astroglial cells in some human brain areas. Interestingly, the transporter CAT2 is of considerable functional importance for these cells, since inducible NOS (iNOS) activity is impaired by 84 % in activated CAT2-deficient mouse astroglial cells (Manner et al. 2003). Moreover, it was shown that CAT2 and iNOS mRNAs are concomitantly and strongly inducible in cultured astrocytes (Stevens et al. 1996). At present it is unclear why hCAT2 immunoreactivity is "constitutively" expressed in some human brain astrocytes. Although the brains studied here did not show any signs of neurologic, neurodegenerative or psychiatric disorders, it cannot fully be ruled out that this expression reflects some kind of local inflammatory processes. Interestingly, these CAT2 immunoreactive astrocytes did not express iNOS, which argues against prominent immune/inflammatory activation of these neuroglial cells (not shown). Moreover, a constitutive expression of hCAT2 was also demonstrated in resident human skin kerationocytes showing that, in principle, hCAT2 may be expressed in non-inflamed, extrahepatic tissues and cells (Schnorr et al. 2003). Lastly, we looked for the distribution of hCAT3. Previously, the distribution of this amino acid transporter within the human brain has been reported by Vékony et al. (2001). These authors found that hCAT3 is, unlike hCAT1 and hCAT2, fairly restricted in its expression, being absent from all cortical areas, cerebellum, substantia nigra, Nuc. accumbens, putamen and pons. Herein we show that hCAT3 immunoreactive neurons and glial cells are present in virtually all regions under study, acknowledging that in some regions, where Vékony et al. (2001) failed to identify hCAT3 using the dot blot technique, only a few hCAT3 immunopositive cells were detectable (i.e. prefrontal cortex, cerebellum, putamen). However, other cortical areas stood out by multiple hCAT3 expressing neurons and glial cells. This discrepancy is difficult to explain and warrants further attention.

One possible reason might be that dot blot analysis is normally performed using small tissue specimens. Taking into account the considerable heterogeneity of human brain tissue with regard to cell composition, it is conceivable that some probes did not contain "hCAT3 immunoreactive cells". The presence of hCAT3 immunoreactive material in subpopulations of white and gray matter astrocytes and some blood vessels has not been reported yet and argues against the assumption that in the brain CAT3 is a neuron-specific transporter protein (Hosokawa et al. 1997; Braissant et al. 1999; Vékony et al. 2001). In sum, our detailed analysis of the regional and cellular distribution of hCATs 1, 2 and 3 in normal human brain revealed transporter-specific (though partly overlapping) expression patterns, knowledge of which could be a good basis for further disease-related studies.

Conflict of interest The authors declare that they have no conflict of interest.

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