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Short Communication

Regional Distribution of Homocysteine in the Mammalian Brain

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Abstract: The regional distribution of L-homocysteine (Hcy) was determined in brains from mouse, rat, guinea pig, and rabbit, using a sensitive radioenzymatic assay. Large interspecies variations in the Hcy content in various parts of the brain were observed, but cerebellum contained the highest amount in all species investigated. In the rat the amount of Hcy in cerebellum (6.4 nmol/g) was about sixfold higher than in most other parts of the brain, whereas in the mouse and guinea pig the amount in cerebellum (about 1 nmol/g) was only twofold higher than in the other brain regions. There was a remarkably high level of Hcy in all regions of the rabbit brain (4–10 nmol/g); the highest concentration was found in the cerebellar white matter. In this species the amount of Hcy in all brain regions examined exceeded that in the liver.
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Homocysteine (Hcy) is an amino acid that is not supplied by food, but formed from the endogenous transmethylase inhibitor, *S*-adenosylhomocysteine (AdoHcy), through the action of the enzyme AdoHcy hydrolase (EC 3.3.1.1.). Hcy is further metabolized to methionine, or condensed with serine to form cystathionine (Mudd and Levy, 1983). Defects in these metabolic pathways are associated with the genetic disease homocystinuria (Perry, 1974).

Interest in the possible biological functions and effects of Hcy has been stimulated by the observation that exogenously supplied Hcy induces the formation of large amounts of AdoHcy in several cells and tissues (Ueland, 1982), including the mouse brain (Gharib et al., 1983). Further, some transformed cells, in contrast to normal cells, are unable to grow in a methionine-deficient medium containing Hcy, suggesting that altered Hcy metabolism may be associated with malignant transformation (Hoffman, 1982).

The regional distribution of the metabolic precursors of Hcy, *S*-adenosylmethionine (AdoMet) and AdoHcy in the brain, have been described by Gharib et al. (1982). AdoHcy induces sleep in the rat. This effect, however, disappears when the dose is increased (Sarda et al., 1982). AdoHcy may have a depressant effect (Ueland, 1982) whereas AdoMet has an excitatory effect (Phillis, 1981) on nervous function. Hcy itself may be responsible for the convulsive seizures in patients with cystathionine β-synthase deficiency (Dewhurst et al., 1983).

There are no data in the literature on the occurrence of Hcy in brain. This may partly be explained by lack of sensitive methods for the determination of the small amounts of Hcy present in tissues under physiological conditions. The present paper reports on the distribution of Hcy in the CNS of several mammals. The data are based on a sensitive radioenzymatic assay for determination of Hcy, recently developed in our laboratory (Ueland et al., 1984).

MATERIALS AND METHODS

Chemicals

DL-Homocysteine, L-homocysteine thiolactone, and DL-dithioerythritol were purchased from Sigma Chemical, St. Louis, MO. [8-^{14}C]Adenosine (0.59 Ci/mmol) was obtained from the Radiochemical Centre, Amersham. 2′-Deoxycoformycin was a gift from Parke-Davis Research Laboratories, Ann Arbor, MI. Hypersil, 3-μm ODS col-

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Abbreviations used: AdoHcy, *S*-Adenosylhomocysteine; AdoMet, *S*-Adenosylmethionine; Hcy, L-Homocysteine.

umns (10 × 0.5 cm) equipped with a guard column (0.5 × 2.5 cm) were prepared as described (Ueland and Solheim, 1983). AdoHcy hydrolase was purified to apparent homogeneity from mouse liver according to a published procedure (Ueland and Døskeland, 1977).

Animals

Male rats (Mol: Wistar), male mice (NMRI), male guinea pigs (Olac: Dunkin Hartley) and male rabbits (Chbb: CHBB) were used. They were fed a rabbit maintenance feed (rabbit and guinea pigs) or a R3 brood stock feed (rats and mice), both from EWOS AB. The diets contained 2.1 g methionine per 16 g protein N (rabbit 14% and R3 20% protein). Vitamin B_6 was added in an amount of 3 mg/kg (rabbit) or 5 mg/kg (R3).

Preparation of tissue samples

The rats and guinea pigs were killed by a blow in the head and the mice by cervical dislocation. The rabbits were given an intravenous bolus injection of 50 mg/kg pentobarbital. The brains were removed immediately and then placed on an ice-cold glass plate. The regions were dissected out, frozen in liquid nitrogen, and then stored at −80°C for 0–4 days until assay for Hcy. A total time of about 2 min was used on the small animals samples and 5 min on the rabbit samples.

The tissue samples were homogenized in ice-cold 0.8 *M* perchloric acid (1/10, wt/vol) containing 10 m*M* EDTA. The precipitated proteins were removed by centrifugation, and the supernatant neutralized to pH 7.0 by addition of 1.44 *M* KOH/1.2 *M* $KHCO_3$. The potassium perchlorate was removed by centrifugation.

Determination of Hcy in brain extract

Hcy was assayed by a radioenzymatic method described in detail elsewhere (Ueland et al., 1984). In brief, after addition of dithioerythritol to the neutralized brain extract, adenosine and AdoHcy were removed from the extract by adsorption to dextran-coated charcoal, while leaving Hcy in solution. Hcy was then condensed with radioactive adenosine in the presence of AdoHcy hydrolase and the radioactive AdoHcy formed was isolated by gradient elution on a 3-μm ODS Hypersil column. The samples were injected into the column using a HPLC autosampler, which was interfaced with a programmable fraction collector equipped with a peak separator (from ISCO). In this way, radioactive AdoHcy was collected during unattended analysis. The radioactivity was determined by liquid scintillation counting.

RESULTS

The regional distributions of Hcy in brains of rats, mice, guinea pigs, and rabbits are shown in Table 1. The amount of Hcy in a particular region of the brain varied markedly from one species to another, but cerebellum contained the highest concentration of Hcy in all species investigated (Table 1).

In the mouse and guinea pig, the amount of Hcy was relatively low in all regions of the brain, but the highest levels were observed in cerebellum (about 1 nmol/g tissue). A much higher concentration of Hcy (about 6 nmol/g) was observed in the cerebellum of the rat. Intermediary levels were found in pons/medulla oblongata of this species (about 2 nmol/g) and small amounts in the other regions. For comparison the concentration in the liver was about 4 nmol/g both in rats and mice (Ueland et al., 1984). All parts of the rabbit brain contained remarkably high levels of Hcy (2–10 nmol/g) which exceeded the amount detected in the liver (1 nmol/g). The highest level was again found in the cerebellum, and the white matter contained slightly higher amounts than the cortex.

TABLE 1. *The regional distribution of homocysteine in the brain from various species*

Species	Region	nmol/g wet wt ± SEM (mean ± SEM)
Mouse (6 experiments)	Cerebrum	0.34 ± 0.012
	Pons/medulla oblongata	0.72 ± 0.059
	Cerebellum	0.91 ± 0.099
Rat (6 experiments)	Hypothalamus	1.09 ± 0.11
	Tuberculum olfactorium	0.91 ± 0.17
	Bulbus olfactorius	1.14 ± 0.19
	Thalamus	0.67 ± 0.10
	Corpora quadrigemina	0.76 ± 0.12
	Hippocampus	0.90 ± 0.18
	Corpus striatum	1.12 ± 0.13
	Cortex, occipital	0.54 ± 0.11
	Cortex, frontal	0.49 ± 0.08
	Capsula interna	0.73 ± 0.11
	Mesencephalon	1.04 ± 0.06
	Pons/medulla oblongata	1.83 ± 0.27
	Cerebellum	6.40 ± 0.65
Guinea pig (4 experiments)	Hypothalamus	0.48 ± 0.14
	Thalamus	0.25 ± 0.06
	Hippocampus	0.25 ± 0.10
	Corpus striatum	0.54 ± 0.17
	Pons	0.62 ± 0.13
	Cerebellum	1.07 ± 0.25
Rabbit (3 experiments)	Hypothalamus	5.17 ± 0.56
	Hippocampus	3.83 ± 0.13
	Corpus striatum	6.61 ± 1.17
	Cortex (frontal)	2.11 ± 0.33
	Pons, rostral	6.02 ± 1.59
	Pons, caudal	5.32 ± 0.32
	Cerebellar white matter	10.74 ± 0.60
	Cerebellar cortex	8.23 ± 1.49
	Liver	1.36 ± 0.16

Mice were killed by cervical dislocation, rats and guinea pigs by a blow in the head, and rabbits with a bolus injection of pentobarbital. The tissues were frozen within 2 min for the small animals and 5 min for the rabbits.

DISCUSSION

Hcy was not included in previous studies on the amino acid content in the mammalian brain (Carver, 1965; LaBella et al., 1968; Perry et al., 1971). The existence of a metabolic product of Hcy, cystathionine, has been demonstrated in the brain, and this compound is especially abundant in pineal body (LaBella et al., 1968). High concentrations of cystathionine have been found in the white matter of the human cerebellum obtained at autopsy (Shimizu et al., 1966). No function has hitherto been assigned to this sulfur-containing compound in the CNS.

We recently demonstrated the presence of Hcy in the brain and other tissues of the mouse and rat. The liver contained the highest amount of Hcy in these two species (Ueland et al., 1984). The present paper describes the distribution of Hcy in various regions of the brain of several laboratory animals, i.e., the mouse, rat, guinea pig, and rabbit. There was a remarkable abundance of Hcy in cerebellum of all species investigated, but a large interspecies variation in the concentration of Hcy in the various regions of the brain was observed. Further, all re-

gions of the rabbit brain were rich in Hcy, and the amount of Hcy in rabbit brain was in fact higher than in rabbit liver.

The dissection did not allow freezing of the brain *in vivo*. Therefore, postmortem metabolic alterations might interfere with the determination of Hcy in brain. It should be noted that longer time was required to isolate the brain from rabbits than from small animals such as rats and mice. We found no change in the amount of Hcy in mouse tissues within the first minute after death of the animal (Ueland et al., 1984). In five rabbits given 0.1 mg/kg Hypnorm (containing 10 mg fluanisone and 0.32 mg fentanyl/ml) with subsequent outbleeding for 20 min the Hcy levels were 20% higher. Pentobarbital 50 mg/kg given 20 min before death had no effect on the Hcy level in rat brain (data not shown).

The radioenzymatic method used for the determination of Hcy do not discriminate between this compound and several disulfides containing the homocysteinyl residue. Therefore, part of the amount of Hcy detected in various regions of the brain may exist as homocystine and/or a mixed disulfide *in vivo*.

The abundance of Hcy in cerebellum (Table 1) contrasts with the regional distribution of AdoHcy hydrolase (Broch and Ueland, 1980) and the metabolic precursors of Hcy, namely AdoMet and AdoHcy (Gharib et al., 1982), which are evenly distributed in the brain. This may indicate a role of Hcy in nervous function. In addition, the existence of pathways leading to Hcy other than the AdoHcy hydrolase reaction should be considered.

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