Research report

Human brain thioltransferase: constitutive expression and localization by fluorescence in situ hybridization

Sadguna Balijepalli[a], Michael R. Boyd[c], Vijayalakshmi Ravindranath[a,b,*]

[a]*Department of Neurochemistry, National Institute of Mental Health and Neurosciences, Hosur Road, Bangalore 560 029, India*
[b]*National Brain Research Centre, ICGEB Campus, Aruna Asaf Ali Marg, New Delhi 110 067, India*
[c]*Laboratory of Drug Discovery Research and Development, Developmental Therapeutics Program, National Cancer Institute, FCRDC, Frederick, MD 21702-1201, USA*

Abstract

Thioltransferase (glutaredoxin) is a member of the family of thiol-disulfide oxido-reductases that maintain the sulfhydryl homeostasis in cells by catalyzing thiol-disulfide interchange reactions. One of the major consequences of oxidative stress in brain is formation of protein-glutathione mixed disulfide (through oxidation of protein thiols) which can be reversed by thioltransferase during recovery of brain from oxidative stress. Here we have visualized the location of thioltransferase in brain regions from seven human tissues obtained at autopsy. Constitutively expressed thioltransferase activity was detectable in all human brains examined although inter-individual variations were seen. The enzyme activity was significantly higher in hippocampus and cerebellum as compared to other regions. Constitutive expression of thioltransferase mRNA was detectable by Northern blot analysis. Localization of thioltransferase mRNA by fluorescence in situ hybridization revealed its presence predominantly in neurons in the cerebral cortex, Purkinje and granule cell layers of the cerebellum, granule cell layer of the dentate gyrus and in the pyramidal neurons of CA1, CA2 and CA3 subfields of hippocampus. These discrete neuronal concentrations of thioltransferase would be consistent with an essential role in modulating recovery of protein thiols from mixed disulfides formed during oxidative stress. © 2000 Elsevier Science B.V. All rights reserved.

1. Introduction

Brain is highly vulnerable to oxidative injury due to its high oxygen tension and elevated levels of polyunsaturated fatty acids. The more recent discoveries showing association of oxidative injury in the pathogenesis of neurodegenerative diseases such as Parkinson's disease, Huntington's disease and Alzheimer's disease [3,6,7] have further reinforced the need for understanding antioxidant potential of brain, particularly with respect to the ability of brain to recover from oxidative stress.

One of the major consequences of oxidative injury in brain, such as those occurring during reperfusion following cerebral ischemia or in vitro treatment of isolated brain mitochondria with t-butyl hydroperoxide, is the loss of the cellular antioxidant thiol, glutathione (GSH). The reduced GSH is consumed through reaction with protein thiols to form protein-glutathione mixed disulfides (PrSSG; [20,21,25]). The concomitant loss of protein thiols is reflected in the inhibition of activities of enzymes that are known to have thiol groups in their active sites, such as sodium/potassium-ATPase [25]. During mild oxidative stress when the brain recovers from oxidative stress, GSH levels are restored and protein thiols are regenerated, thus re-establishing thiol homeostasis in cells. This response of brain is very different from that seen in other organs wherein the GSH lost during oxidative stress is recovered essentially as GSSG (oxidized glutathione), which is often effluxed out to prevent oxidative modification of proteins [20].

*Corresponding author. Tel.: +91-11-617-7558; fax: +91-11-616-8072.

E-mail address: vijir@vsnl.com (V. Ravindranath).

Thiol-disulfide oxidoreductases are a class of enzymes that are primarily involved in the catalysis of thiol-disulfide interchange reactions. The enzyme systems include thioltransferase (glutaredoxin, EC 1.8.4.2; [16,27]), thioredoxin and protein disulfide isomerase [13]. While thioredoxin and protein disulfide isomerase have broad substrate specificity [18], thioltransferase specifically reduces mixed disulfides that contain glutathione, with greater efficiency than does thioredoxin [11].

Thioltransferase is a low molecular weight protein (about 12 kDa) essentially localized in the cytosol. The hepatic enzymes from rat, rabbit and pig have been characterized [9,10,12]. Thioltransferase has been purified from human red blood cells [17,19]. Studies using the purified enzyme have helped to characterize the substrate specificity of thioltransferase and its ability to reduce certain protein glutathione mixed disulfides more efficiently than thioredoxin [11].

There is a paucity of information on brain thioltransferase. Although the enzyme has been cloned from a human brain cDNA library [5,8], the actual presence of the functional enzyme in human brain, its regional distribution and localization had not been heretofore been demonstrated. In the present study, we have examined the presence of a functional thioltransferase system in brain regions obtained from human autopsy tissue and localized the transcript using fluorescence in situ hybridization.

2. Materials and methods

2.1. Animals

All animal experiments were carried out as per the National Institutes of Health guide for the use and care of animals. All efforts were made to minimize animal suffering, to reduce the number of animals used and to utilize alternatives to in vivo techniques if available. Male Wistar rats (3 to 4 months, 225–250 g) were obtained from the Central Animal Research Facility of the Institute. Animals had access to pelleted diet and water ad libitum.

2.2. Cytosol preparation for post-mortem studies

Rats were sacrificed by cervical dislocation and the animals were kept at 20°C for different time-periods (12 h, 18 h and 24 h). At the end of each predetermined time-period, animals were decapitated and brains were removed rapidly. Control animals were sacrificed by cervical dislocation and brains were removed immediately for preparation of cytosol. Each brain was homogenised in 20 ml of ice-cold potassium phosphate buffer (0.137 M, pH 7.6). The homogenate was centrifuged at 100,000 g for 1 h and the supernatant was used for the assay of thioltransferase.

2.3. Studies with human brain

Human brain from male and female traffic accident victims were obtained from the Human Brain Tissue Repository of the Institute. The age of the deceased, post-mortem delay and clinical details are given in Table 1. After autopsy, brains were washed in ice-cold saline and based on standard anatomical markings and were dissected into different regions including cortex, hippocampus, striatum, midbrain, cerebellum and thalamus. All regions were frozen and stored at −70°C immediately. Prior to use in experiment, the tissue was thawed briefly on ice. Approximately 2 g of tissue from each region was homogenized in 10 ml of potassium phosphate buffer (0.137 M, pH 7.6). The homogenate was centrifuged at 100,000 g for 1 h. The supernatant (cytosol) was aliquoted, stored at −70°C and used for assay of thioltransferase.

Table 1
Details of human brain autopsy tissue used in determining thiol transferase activity

Case number	Gender	Age (years)	Postmortem delay (h)	Cause of death	Medications administered
I	Male	22	5 h	Subarachnoid hemorrhage	Mannitol, Ampicillin, Epsolin
II	Male	32	5.5 h	Head injury (diffuse axonal injury)	Mannitol, Adrenaline
III	Male	26	5.5 h	Severe head injury, cardio-respiratory arrest	No treatment
IV	Male	65	5.45 h	Diffuse cerebral edema	Mannitol
V	Male	47	6 h	Severe degree of diffuse cerebral edema with traumatic sub-arachnoid hemorrhage	Mannitol
VI	Female	19	6.5 h	Severe head injury	Mannitol, Adrenaline
VII	Female	19	2.5 h	Diffuse cerebral edema, cerebral contusion with subdural hematoma and left epidural hematoma	Mannitol, Epsolin, chloramphenicol

2.4. Assay of thioltransferase

Activity of thioltransferase was estimated spectrophotometrically at 340 nm using cysteine-*S*-sulfate as substrate [10]. Cysteine-*S*-sulfate was synthesized according to the method of Segel and Johnson [22] by aerating stoichiometric amounts of cysteine and sodium sulfite in aqueous ammonia containing trace amounts of cupric chloride. The assay was carried out at room temperature. The assay mixture (0.5 ml) consisted of 100–300 μg of cytosolic protein, 50 μl of 0.5 mM GSH, 50 μl of 2.5 mM cysteine-*S*-sulfate, 50 μl of GSH reductase solution (24 units/ml) and the volume was made up to 450 μl with potassium phosphate buffer (0.137 M, pH 7.6). The reaction was initiated by adding 50 μl of 3.5 mM solution of NADPH. The decrease in absorbance at 340 nm was recorded for 1 min. Blanks were run simultaneously without addition of cytosol to measure non-enzymatic reaction. The net enzymatic rate was obtained by subtraction of non-enzymatic rate from total rate. The activity was calculated using the molar extinction coefficient of NADPH, which is 6220 $M^{-1}.cm^{-1}$. Protein was estimated in the cytosol by dye-binding method [2].

Thioltransferase activity was also measured using cysteine glutathione disulfide as substrate. The assay was carried out essentially as described above for cysteine-*S*-sulfate, except that the final substrate concentration was 0.1 mM.

2.5. Estimation of K_m and V_{max} for human brain thioltransferase

The effect of protein concentration on the activity of thioltransferase in human brain cytosol was determined by adding various concentrations of cytosolic protein to the reaction mixture (100 μg to 500 μg of protein) and measuring enzyme activity. The activity of thioltransferase in human brain cytosol was also measured for varying periods of time ranging from 10 to 60 s. Blank reactions were run simultaneously for each time-point without addition of cytosol. The activity of thioltransferase in human brain regions (hippocampus and striatum) was estimated using various concentrations of the substrate, S-sulfocysteine (0.1 mM to 3 mM). The protein concentration of 300–420 μg and a time period of 30 s was chosen such that they were in the linear range of enzyme activity. The K_m and V_{max} values were calculated from Eadie–Hofstee plot.

2.6. Northern analysis

The cDNA to human brain thioltransferase which was cloned from a human brain cortex cDNA library [5] was used for the Northern and in situ hybridization experiments. Total RNA from human brain cortex and rat brain was extracted as described by Chomczynski [4]. RNA was separated electrophoretically and transferred onto positively charged nylon membranes by capillary transfer [14]. After UV crosslinking, the membranes were hybridized overnight at 55°C with digoxigenin-labelled antisense and sense riboprobes prepared using the cDNA to human brain thioltransferase. The membranes were washed and incubated with antibody to digoxigenin fab fragments conjugated with alkaline phosphatase. Bands were visualized using chromogenic substrates for alkaline phosphatase, namely, nitroblue tetrazolium salt and 5-bromo-4-chloro-3-indolyl phosphate.

2.7. Fluorescence in situ hybridization

Human brain regions obtained at autopsy were rinsed in sterile saline (0.9%, w/v), fixed in buffered formalin for 2–4 h and transferred to 70% ethanol in water. Tissue was processed for paraffin embedding, and serial sections (8–10-μm-thick) were cut under RNase-free conditions. Sections were dewaxed, hydrated in graded ethanol, acetylated and treated with proteinase K. The sections were then rinsed in PBS and dehydrated using graded ethanol. Digoxigenin-labelled sense (for control sections) and antisense cRNA probes were synthesized from cDNA human brain thioltransferase in Bluescript vector using T3 and T7 RNA polymerases, respectively. Sections were hybridized overnight at 45°C with sense or antisense probes. After hybridization, the sections were washed, blocked with 0.5% bovine serum albumin (w/v, NEN Life Sciences products, USA), and incubated with antibody to digoxigenin fab fragments conjugated to horseradish peroxidase. After washing, the sections were incubated with biotinylated tyramide (NEN Life Sciences Products, USA) followed by FITC-labelled streptavidin. Finally the sections were washed, dried and mounted prior to examination under a fluorescence microscope.

3. Results

3.1. Studies on effect of post-mortem delay on the activity of thioltransferase of rat brain

The activity of thioltransferase was estimated in cytosol prepared from brain tissue of rats subjected to various time-periods of post-mortem delay. As shown in Fig. 1, no change in the activity of the enzyme was observed even after 12 h of post-mortem delay. There was only a small loss (32%) in the activity of thioltransferase in the brain cytosol after 24 h. These studies indicated the considerable stability of the brain thioltransferase to autolytic changes that are known to occur during post-mortem period. The studies on human brain thioltransferase were hence carried out using human autopsy tissue that was obtained within 12 h of post-mortem delay.
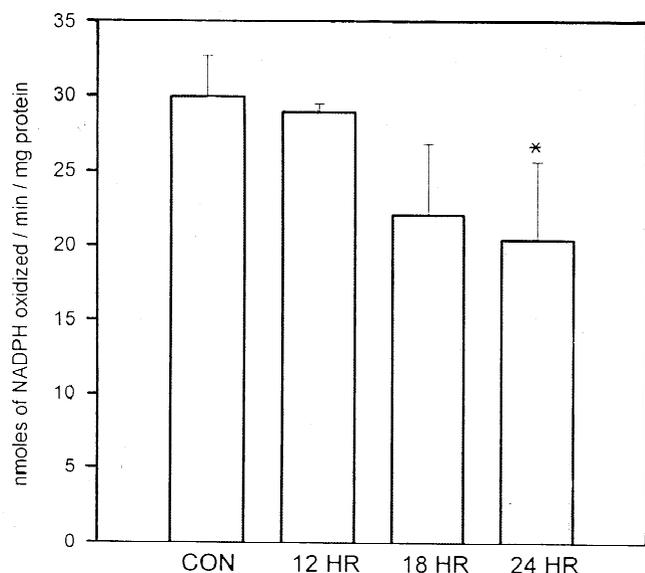
Fig. 1. Effect of post-mortem delay on the activity of rat brain thioltransferase. Activity of thioltransferase was estimated in cytosol prepared from whole brain of rats sacrificed after various periods of post-mortem delay (12, 18 and 24 h). Enzyme activity is expressed as nmoles of NADPH oxidized/min/mg protein and values are mean±S.E.M. (n=4 animals). Asterisks indicate values that are significantly different from controls ($P<0.05$).

3.2. Activity of thioltransferase in various regions of human brain

Activity of thioltransferase was estimated in the brain regions of autopsy tissue obtained from seven human subjects. The age, time interval between death and autopsy and the cause of death are given in Table 1. Activity of the enzyme was estimated in the cytosol prepared from various regions of human brain including cortex, hippocampus, striatum, midbrain, cerebellum and thalamus, using cysteine-S-sulfate as substrate. Variations in enzyme activity was seen among the individuals (Fig. 2A). For example, in hippocampus, the activity of the enzyme varied from 20 to 40 nmoles of NADPH oxidized/min/mg protein. The activity varied within the regions also (Fig. 2B). The activity of thioltransferase in hippocampus and cerebellum was significantly higher than other regions studied (cortex, striatum, midbrain and thalamus), while striatum and midbrain showed the lowest activity (Fig. 2B).

3.3. Kinetic studies on human brain thioltransferase

Kinetic studies on thioltransferase of human brain were carried out and K_m and V_{max} values were determined in hippocampus and striatum regions from each of the seven subjects using cysteine-S-sulfate as substrate. An increase in rate of enzyme activity was observed with increasing protein concentration (200–600 μg) and time-period of reaction in all the human brains samples (data not shown). The mean of the apparent K_m and V_{max} values obtained

from Eadie–Hofstee plots in hippocampus and striatum regions of six subjects (subjects II to VII) are given in Table 2. Interindividual variations were seen in both affinity and maximum velocity. Thioltransferase in hippocampus of two female brains had higher affinity for thioltransferase as compared to any of the five brains from male subjects. However, the gender-related differences need to be further examined in a larger sample size.

We also examined the activity of thioltransferase in the cytosolic preparation from hippocampus using cysteinyl glutathione as substrate. In general, the detected activity of the enzyme was higher using this substrate. The activity increased with increasing protein concentration (100–500 μg/ml), increasing time-period of reaction (0.5–3 min) and increasing substrate concentration (50–100 μM; data not shown). A comparison of the activities using the two substrates (cysteine-S-sulfate and cysteinyl glutathione) is given in Table 3.

3.4. Northern analysis

Northern blot analysis of total RNA from human brain cortex using the cDNA to human brain thioltransferase demonstrated the constitutive expression of thioltransferase mRNA. The molecular mass of the transcript was approximately 0.3 Kb which was similar to that seen in rat brain (Fig. 3A). No signal was detected in the blots hybridized with the sense cRNA probe to thioltransferase (Fig. 3B). Northern analysis of total RNA extracted from the brains of rats subjected to various periods of post-mortem delay revealed a band of 0.3 Kb with intensity similar to that seen in normal rat brain indicating that mRNA to thioltransferase was relatively resistant up to 12 h to autolytic changes that occur during post-mortem (data not shown).

3.5. Localization of thioltransferase mRNA in human brain by fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) studies demonstrated the presence of thioltransferase mRNA predominantly in neuronal cells in human brain regions. High levels of thioltransferase mRNA was seen in the cerebral cortex (Fig. 4A, C and D). Neuronal cells in cerebral cortex showed intense cytosolic staining, indicating presence of thioltransferase mRNA (Fig. 4D), while sections hybridized with the sense probe showed no fluorescence (Fig. 4B). In the cerebellum, the Purkinje cells and granule cell layer showed intense fluorescence (Fig. 4G). Intense fluorescence was seen in hippocampus, in the pyramidal neurons of CA1, CA2, CA3 subfields and in the granule cell layer in the dentate gyrus (Fig. 5A,C,E,F,G), while similar sections hybridized with the sense digoxigenin labelled probe showed no fluorescence (Fig. 5B and D). In the midbrain, the reticular neurons were selectively labelled, indicating the predominant presence of thioltransferase mRNA in these cell populations (Fig. 4E), although
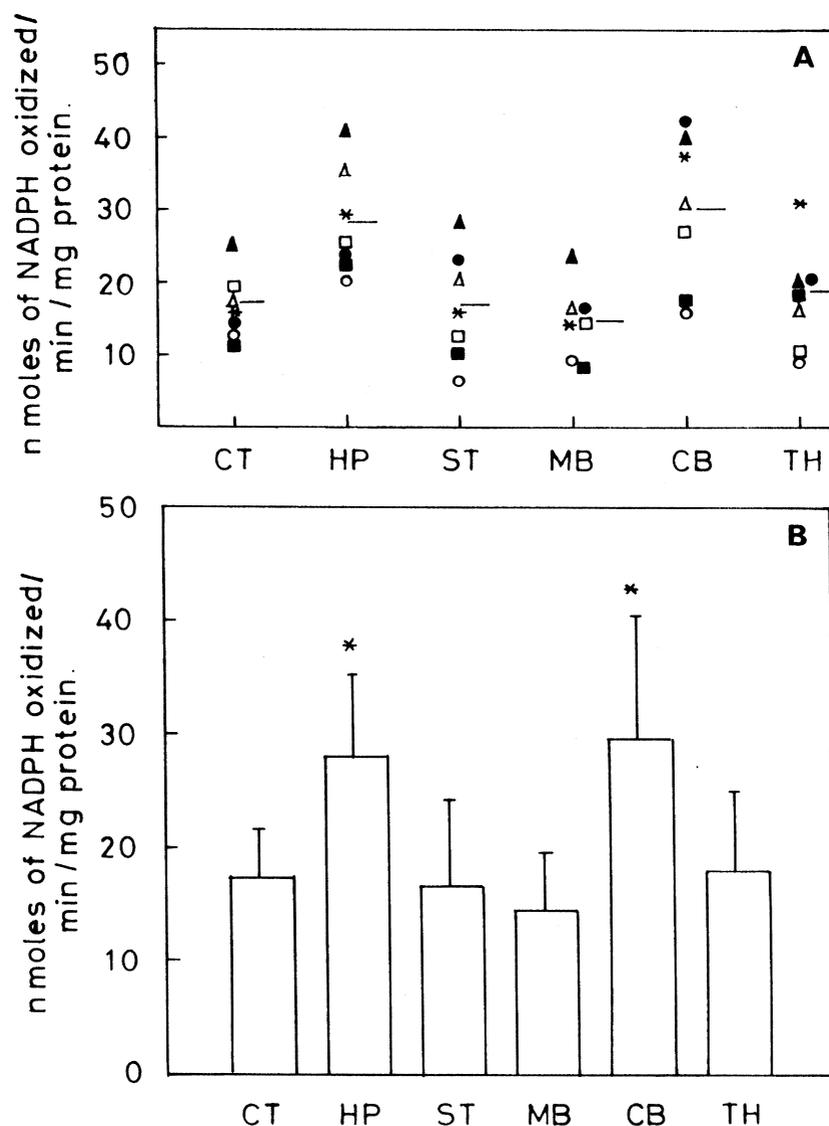
Fig. 2. Thioltransferase activity in various regions of human brain. (A) Distribution of thioltransferase activity in various regions of human brain. The ages of the subjects are as follows: Subject I, 22 yrs (△); subject II, 32 yrs (□); subject III, 26 yrs (○); subject IV, 65 yrs (★); subject V, 47 yrs (▲); subject VI, 19 yrs (■); subject VII, 19 yrs (●). Subjects I to V were male and VI and VII were female. (B)Activity of thioltransferase was estimated in cytosol prepared from brain regions of seven human subjects (subjects I to VII) obtained at autopsy. Values are expressed as mean±S.E.M. ($n=7$ subjects). The values significantly different from cortex are indicated by asterisks ($P<0.05$). CT, cortex; HP, hippocampus; ST, striatum; MB, midbrain; CB, cerebellum; TH, thalamus. The enzyme activity is expressed as nmoles of NADPH oxidized/min/mg protein.

the intensity was considerably less as compared to cortex, hippocampus or cerebellum.

4. Discussion

Thioltransferase, which belongs to the class of thiol-disulfide oxido-reductases, is an enzyme that is specifically involved in the reduction of protein glutathione mixed disulfides to protein thiols in the presence of GSH. Since formation of protein glutathione mixed disulfides is one of the major consequences oxidative stress in brain, thioltransferase would potentially play an important role in re-

covery of brain from oxidative stress when protein thiol homeostasis in brain is being restored. The present study is the first attempt to examine the presence of the functional enzyme in human brain and study its regional distribution. The enzyme is known to be present in rat brain [1] and the activity is comparable to that seen in liver.

Studies on effect of post-mortem delay on thioltransferase activity and mRNA to thioltransferase revealed that brain thioltransferase and its transcript were relatively stable to changes that normally occur during post-mortem delay. The enzyme activity was stable up to 12 h after death and significant activity was lost only after 24 h post-mortem (Fig. 1). No observable degradation of thiol-

Table 2

The K_m and V_{max} of thioltransferase in hippocampus and striatum regions of various human brains as obtained from Eadie–Hofstee plots[a]

Subjects	Hippocampus		Striatum	
	K_m	V_{max}	K_m	V_{max}
I	0.147	41.2	–	–
II	0.707	50.6	0.143	25.1
III	0.670	40.3	0.159	21.2
IV	0.164	50.0	0.070	22.3
V	0.110	66.0	0.072	29.6
VI	0.063	30.9	0.170	28.4
VII	0.086	24.3	0.170	26.9
Mean±S.D.	0.28± 0.29	43.3±13.8	0.13±0.47	25.6± 3.3

[a] Apparent K_m and V_{max} are expressed as mM and nmoles of NADPH oxidized/min/mg protein, respectively.

transferase mRNA was seen up to 12 h of postmortem delay and only minimal degradation was seen after 24 h of post-mortem delay (data not shown). Thus, human brain autopsy tissue obtained within 12 h post-mortem provided suitable source material for these studies.

Northern blot analysis of human brain mRNA using the cDNA to human brain thioltransferase revealed the presence of a transcript of 300 bp. Higher molecular weight bands were also seen in the Northern blot (Fig. 3). This is in concurrence with the observation of Fernando et al. [8] who also observed a transcript of 700 bp when screening a human brain library due to the presence of the untranslated region in the mRNA. Immunoblot analysis [15,26] using antibody to human red blood cell thiotransferase indicated constitutive expression of the enzyme in human brain (data not shown).

Significant thioltransferase activity was detected in human brain regions obtained at autopsy. The activity of the enzyme was assayed using two kinds of substrates, namely, cysteine-*S*-sulfate (an artificial substrate) and cysteine glutathione disulfide (which is structurally related to the endogenous substrates of thioltransferase). The presence of this enzyme in high amounts in brain indicates a probable role in the reduction of protein glutathione mixed disulfides, specially those that are formed in high

Table 3

Comparison of thioltransferase activities in human brain hippocampus using two different substrates[a]

Subjects	Thioltransferase activity using	
	Cysteine-*S*-sulfate	Cysteinyl glutathione
III	20.5	33.14
IV	28.11	43.40
V	40.72	73.13
VI	23.37	27.33
VII	24.3	27.75
Mean±S.D.	27.4±7.93	40.95±19.12

[a] Thioltransferase activity was estimated in the cytosol from hippocampal region of human brains using either cysteine-*S*-sulfate or cysteinyl glutathione as substrate. The enzyme activity is expressed as nmoles of NADPH oxidized/min/mg protein.
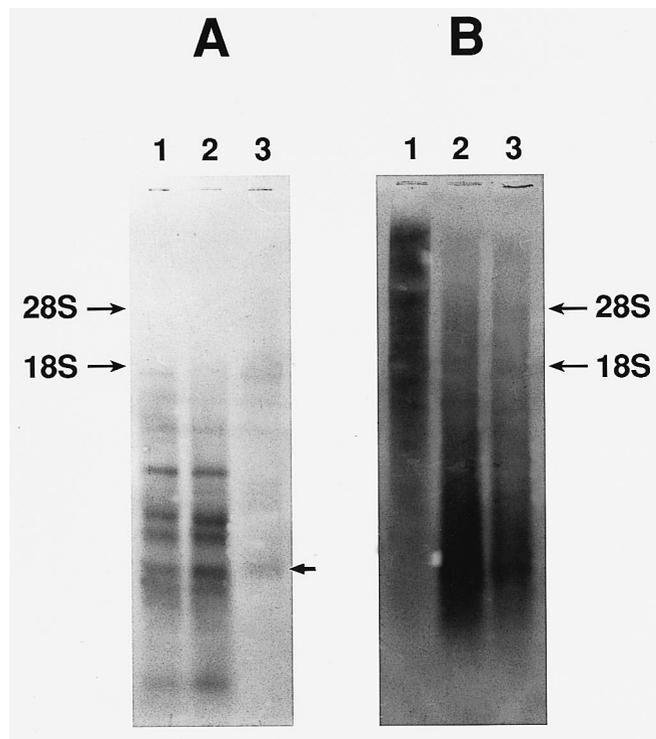

Fig. 3. Northern blot analysis of human brain RNA using cDNA to thioltransferase. Total RNA was isolated from human brain cortex (lanes 1 and 2 — 15 and 30 μg, respectively) and electrophoresed under denaturing conditions along with total RNA from rat brain cortex (15 μg). Lane 3 contained total RNA from rat brain (15 μg). After transfer to nylon membrane, the blots were hybridized with antisense (A) and sense (B) riboprobe prepared using human brain thioltransferase cDNA. The arrow indicates the thioltransferase mRNA of 300 bp. The mobility of the 18S and 28S ribosomal RNA is indicated.

amounts during oxidative stress in brain [23–25]. The enzyme is not homogeneously distributed in brain as demonstrated by the variable activity seen in different regions. In the human brain, highest activities were seen in hippocampus and cerebellum. Interindividual differences were also seen in thiotransferase activity. However, these may also be derived in part from ante-mortem effects since most of the patients were subjects of head injury. The localization of thioltransferase mRNA clearly revealed its predominant presence in the hippocampus (Fig. 5), wherein the transcript could be detected in pyramidal neurons of CA1, CA2 and CA3 sub-fields in addition to the granule cell layer of dentate gyrus. In the cerebellum, mRNA to thiotransferase could be visualized in Purkinje and granule cell layers. Significant amounts of the transcript were detected in the cortical neurons, but in the midbrain comparatively low levels of the transcript were detected, predominantly in the reticular neurons.

In animal models, the hippocampus responds to oxidative stress by substantial decease in GSH levels and formation of PrSSG. Further, it is in this region that the maximum rebound of GSH synthesis is observed and the protein thiol homeostasis is restored following moderate
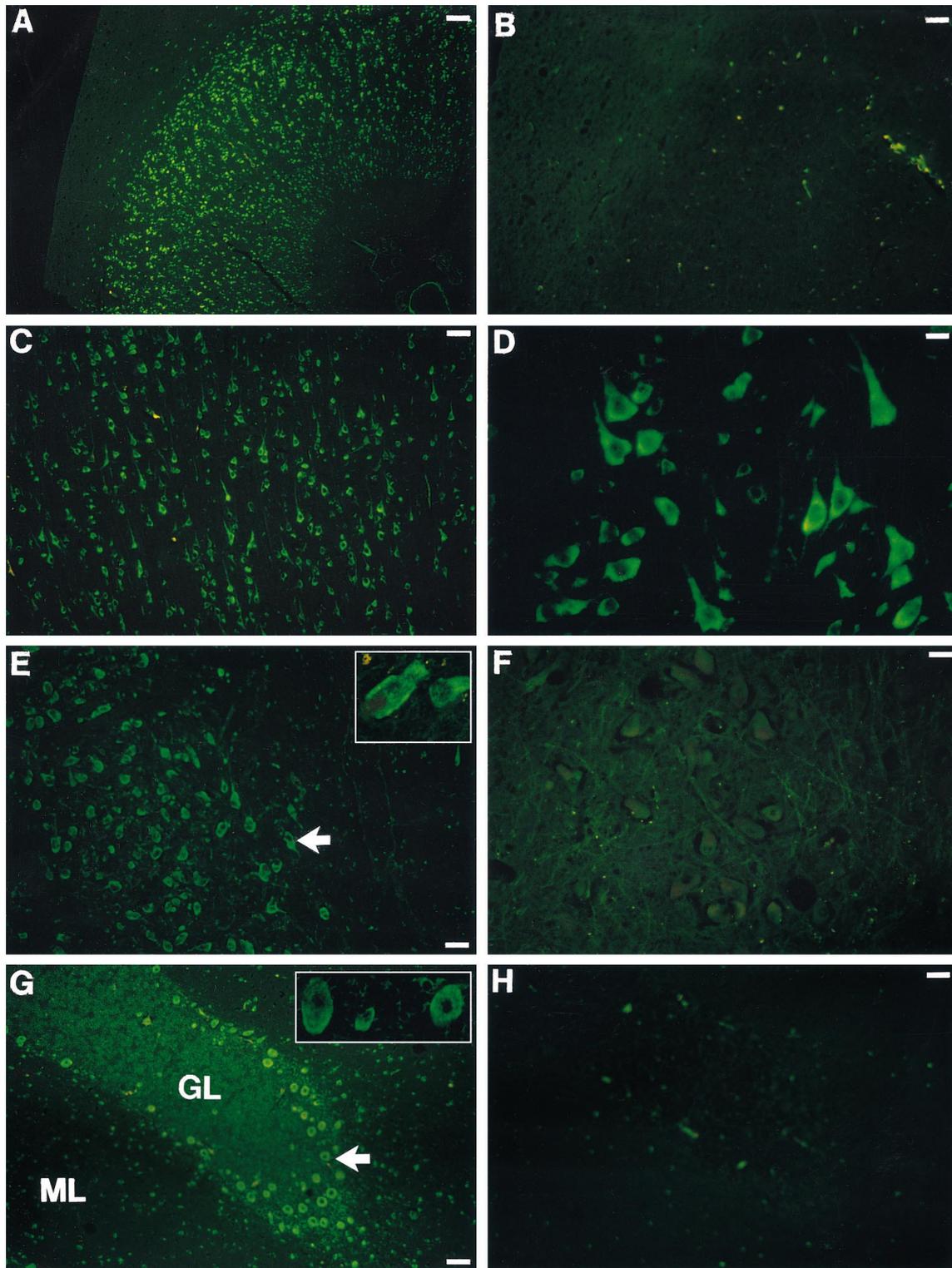
Fig. 4. Localization of thioltransferase mRNA in human brain by fluorescent in situ hybridization. (A) Intense fluorescence was seen in the neuronal cell layers of the human brain temporal cortex. Differential fluorescence was seen delineating the laminar architecture of the cortex. Scale bar=200 μm. (B) Control section hybridized with the sense probe. Scale bar=100 μm. (C) An enlarged view of the cortical neurons with the apical dendrites. Scale bar=100 μm. (D) Higher magnification of the cortical neurons with intense cytosolic staining indicating the presence of thioltransferase mRNA. Scale bar=25 μm. (E) The reticular neurons (arrow) in the midbrain expressed thioltransferase mRNA. Scale bar=100 μm. Inset: Higher magnification of giant reticular neuron. (F) Control section of midbrain hybridized with the sense probe. Scale bar=50 μm. (G) Fluorescent labelling of the Purkinje cells (arrow) and the granule cell layer (GL) in the human cerebellum. Sparse staining of the cells in the molecular layer (ML) was also noted. Scale bar=100 μm. Inset: Higher magnification of the Purkinje neurons containing thioltransferase mRNA. Control section of human cerebellum hybridized with the sense probe is depicted in (H). Scale bar=100 μm.
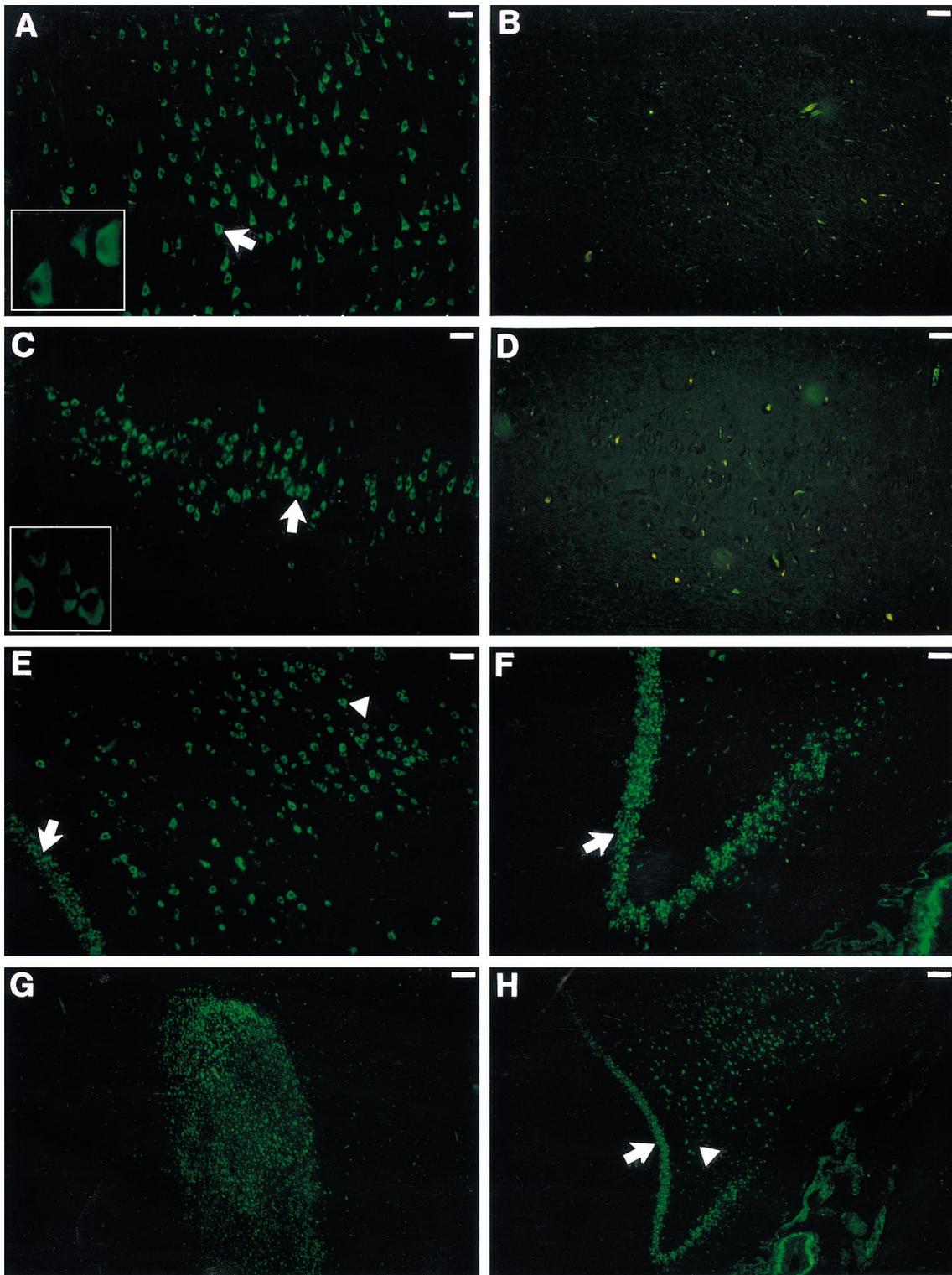
Fig. 5. Localization of thioltransferase mRNA in human brain using fluorescent in situ hybridization. (A) The presence of thioltransferase mRNA in the pyramidal neurons of CA1 subfield in the hippocampus (arrow). Inset: A higher magnification of CA1 neuron. (B) Similar section of hippocampus hybridized with the sense probe. Scale bar=100 μm. (C) Intense fluorescence was seen in the CA2 pyramidal cell layer of the human brain (arrow). Inset: Higher magnification of the CA2 neuron. (D) Control section hybridized with the sense probe. Scale bar=100 μm. (E) The presence of thioltransferase mRNA was observed in the CA3 neurons (arrowhead). Granule cell layer of the dentate gyrus (arrow) is also seen. (F) A closer view of the dentate gyrus (arrow). Scale bar=100 μm. (G) Intense staining was seen in the neurons of pes hippocampus. (H) Another view of the dentate gyrus (arrow) is depicted. Staining of the interneurons of the hilus (arrowhead) was also observed. Scale bar=200 μm.

cerebral ischemia-reperfusion [25]. The variability in the regional distribution of the enzymes is of great significance as the brain is heterogeneous anatomically, functionally and biochemically. Brain regions like cerebellum and hippocampus which have high activity of thioltransferase could potentially recover more competently from the damage caused by oxidative stress. Recent reports suggest that the thioltransferase levels are lowered in a variety of chemically transformed cells when compared to normal cells suggesting that variations in thioltransferase levels may have important cellular consequences [28]. Interestingly, while significantly higher levels of thioltransferase activity was detected in human cerebellum, this region had very low activity in rat brain [1]. The activity of the enzyme in human brain varied between individuals (Fig. 2) and this could not be attributed to the age of the subject.

The studies on the pH dependence of the enzyme revealed that human brain thioltransferase had maximum activity at pH 8.5 (data not shown) which is similar to rat liver and brain thioltransferase [1,9]. The human brain thioltransferase was thermostable in presence of disulfide reductant, dithiothreitol and protease inhibitor (data not shown), which was also similar to the observations made using rat brain [1] but different from the liver wherein the enzyme was thermostable even in the absence of protease inhibitor and disulfide reductant [9].

Thioltransferase enzyme plays a major role in the maintenance of thiol/disulfide ratio in the cell. Most of the studies that have been carried out to date have mainly focused on characterization and purification of the enzyme from various sources. The regulation and function of thioltransferase under normal conditions as well as during oxidative stress has not been elucidated in any system so far [18]. While the present study has demonstrated for the first time the presence of a functional enzyme in discrete neuronal cell populations in human brain, it will be important to elucidate further the physiological role of thioltransferase in brain. In view of the increased formation of protein mixed disulfides in brain during oxidative stress, an understanding of the role played by thioltransferase in brain during oxidative stress assumes greater importance.

Acknowledgements

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