Biochemical Pharmacology, Vol. 37, No. 11, pp. 2287–2288, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

Regional distribution of malonaldehyde in mouse brain

(*Received* 28 *July* 1987; *accepted* 10 *November* 1987)

Aging in the CNS is characterized by neuronal depletion that affects with a different degree of severity various anatomical structures [1]. Certain theories of aging ascribe a primary role to free radical formation within the cell [2, 3]. Release of free radicals and lipid peroxidation would trigger the chain of events leading to membrane disruption first and cellular damage and death later [4], overcoming the natural protecting systems.

It has been suggested that malonaldehyde (MDA) is a primary culprit in disrupting membrane integrity by promoting cross-linkage between proteins and phospholipids through a reaction involving the beta amino group of lysine [5, 6]. Therefore, we assessed the content of MDA in various regions of the mouse brain to establish whether its distribution is uniform or differentially concentrated and whether differential concentration would match with the rate of aging and neuronal death.

C57 Black mice weighing 18–22 g were used for the experiments. The mice were fasted from the evening before and guillotined the following morning. The brain was removed and dissected under 2.5 times magnification on crushed ice according to a protocol that allows a maximum of 50-sec time variability for each sample across animals. The substantia nigra (SN) was dissected from the rostral surface of a coronal cut started ventrally from the deepest part of the interpeduncular fossa. The dissected regions were frozen in liquid nitrogen and analyzed within 4 hr from dissection. The samples were transferred to 0.6 ml of an ice-cold 0.05 M Tris–HCl buffer (pH 7.4). The tissue was kept in the buffer at 0° for 10 min and then homogenized. Thiobarbituric acid reactive material (MDA) was measured using a micromethod modified from Slater and Sawyer [7]: 0.5-ml aliquots of the brain homogenate were extracted with 0.5 ml of 20% (w/v) trichloroacetic acid; after centrifugation, 0.9 ml of the supernatant fraction was added to 1 ml of 0.67% thiobarbituric acid (Sigma, St. Louis, MO) dissolved in 0.026 M Tris–HCl buffer (pH 7.0). The samples were heated in boiling water for 10 min. After cooling, the absorbance was determined at 432 nm on a Beckman spectrophotometer. Extraction blanks were prepared and treated in the same way as the experimental samples but an equal volume of buffer was substituted for homogenate. MDA was quantitated using MDA (Aldrich, Milwaukee, WI) and expressed in nanomoles per mg of protein. Proteins were measured according to the method of Smith *et al.* [8], using bicinchoninic acid (BCA, Pearce, Rockford, IL) as reagent.

To assess whether time after death was a variable affecting MDA levels, experiments were performed in which varous cerebral regions and the liver were dissected at fixed times after decapitation. Figure 1 shows that, although a tendency to higher values after death was seen, this was negligible within the time variability of the experiment. Table 1 reports the levels of MDA in the cerebral regions that were examined, compared to the values found in the liver and kidney. The SN had the highest level found, with an MDA content 8.4 times greater than the cortex, and 4 times higher than the septal area, the next highest structure. Statistical evaluation of the data was performed with the ANOVA test which gave a $P \leq 0.001$. Intergroup variability was assessed with the two-tailed Wilcoxon rank sum test. The difference in the MDA concentration between the SN and other areas was highly significant (Table 1). The septal area was also significantly different from hippocampus, cerebellum and cortex ($P < 0.01$, as calculated by the Wilcoxon rank sum test). Since the SN sampling is subject to variation in size and contect of the underlying penduncular white matter, we also measured the content of MDA in the corpus callosum, a white matter structure. MDA content in the white matter was found to be 0.420 ± 0.07 nmol/mg protein (Table 1). This was significantly higher than the level found in cortex, hippocampus or cerebellum but much lower than the SN value ($P < 0.001$). Thus, contamination of the white matter cannot explain the high levels found in this mesencephalic nucleus.

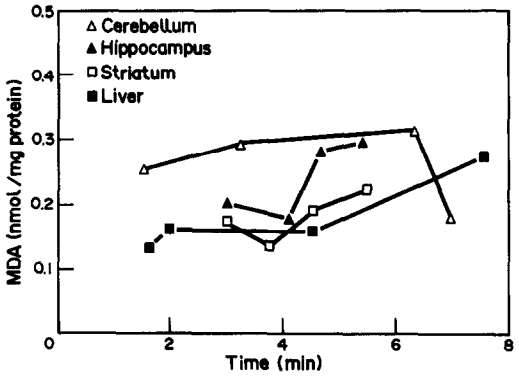

Fig. 1. Malonaldehyde (MDA) levels in brain regions and liver at different times after death. The plots demonstrate overall insignificant variations between 2 and 8 min.

Table 1. Regional distribution of malonaldehyde (MDA) in mouse brain

	N	MDA (nmol/mg protein)
Substantia nigra	8	1.51 ± 0.33*
Septum	6	0.377 ± 0.15
Striatum	8	0.265 ± 0.11
Cerebellum	8	0.242 ± 0.10
Hippocampus	8	0.241 ± 0.10
White matter	3	0.420 ± 0.07
Liver	8	0.183 ± 0.08
Kidney	8	0.200 ± 0.08

Values are means ± SD.
* SN levels were significantly higher than all other values shown at $P < 0.001$.

Furthermore, to ascertain that the small size of the nigral sample would not distort the results of the assay, nigrae were pooled from four mice and processed for the assay. This experiment provided an MDA nigral value of 1.3 nmol/mg protein.

Others have reported regional levels of MDA in the rat brain. Mizuno and Ohta [9] found low MDA levels in the nigra, but their animals were killed under barbiturate anesthesia, which both reduces the level of peroxidation and interferes with the TBA assay. MDA values reported by Noda *et al.* [10] were similar to ours in other regions, but not in the SN. Species differences, long time after sacrifice, technique of sampling, and absence of neuromelamin in rats [11] may all contribute in various degrees to this discrepancy.

Our findings may have implications relevant to the theory of aging and neuronal degeneration. The septal area is a predominantly cholinergic structure and cholinergic neurons are primarily affected in primary degenerative dementia of the Alzheimer's type [12]. SN neurons are depleted during physiological aging [1] and even more so in all neurodegenerative processes associated with Parkinsonian symptoms [13]. The high content of MDA may predipose these areas to damaging effects of the pathogenic noxae.

The SN contains the highest levels of MDA and iron [14], the lowest levels of the scavenger GSH [15] and ubiquinone Q10 with a skewed ratio between the reduced and oxidized form in favour of the oxidized Q10 [16]. All these factors suggest that this mesencephalic nucleus may be particularly vulnerable to oxidative stress and has an intrinsically reduced capability to cope with it.

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Biochemical Pharmacology, Vol. 37, No. 11, pp. 2288–2291, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

Purification and partial characterization of human intestinal glutathione *S*-transferases

(*Received* 8 *October* 1987; *accepted* 31 *December* 1987)

Glutathione *S*-transferases (EC 2.5.1.18) are a family of multifunctional enzymes which play an important role in detoxification, or storage and transport, of xenobiotics and physiologic compounds, in a wide variety of tissues and species [1–4].

In humans, the enzyme system is present in most, if not all epithelial tissues [2, 3], but is most studied so far in the liver [2, 3, 5–11]. However, the intestinal tract is the first route of many toxic compounds, digested with food or drugs. The intestine, in particular the epithelial mucosal cells, are primarily exposed to dietary xenobiotics, and a proper functioning of the detoxificating enzyme system, like the glutathione *S*-transferases, is very important here. Little is known about human intestinal glutathione *S*-transferases [12–14]. In this study adult human intestinal glutathione *S*-transferases are purified and partially characterized. Their properties are compared with those of human liver enzymes.

Materials and methods

Tissue. Human tissue was obtained after autopsy or surgical resections. Liver tissue was obtained from a kidney donor who died by cerebral damage after a traffic accident. Two specimens, one of jejunum and one of ileum, were obtained from another kidney donor, who also died by cerebral damage. Other ileum specimens were obtained