Effects of methionine sulfoximine on levels of free amino acids and related substances in brain*

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METHIONINE SULFOXIMINE (MSO) is a toxic substance which induces convulsive seizures, episodic behavioral changes, and other symptoms. Its effects in many species were reviewed recently by Proler and Kellaway.[1] A disturbance in the cerebral nitrogenous metabolism of animals treated with this agent is indicated by a number of observations. MSO inhibits the activity of brain glutamine synthetase *in vitro*[2, 3] by a mechanism in which MSO is competitive with respect to L-glutamate.[4] A glutamyl transferase system also is inhibited by MSO *in vitro*.[2] In rats, brain analyses after decapitation and freezing of the head indicate a decrease in glutamine, slight decreases in protein nitrogen and ammonia, and an increase in nonprotein nitrogen induced by large doses of MSO.[5] Treatment of cats with MSO decreases the initial levels and rates of production of glutamine, glutamic acid, and γ-aminobutyric acid in incubated brain slices[6] and also decreases the rate of formation of protein-bound glutamine.[7] Large doses of glutamine or asparagine protect mice against MSO toxicity.[8] MSO is known to be a methionine antagonist,[1] and its toxic effects are overcome by large doses of methionine. MSO retards the incorporation of methionine into brain proteins *in vivo*,[8, 9] but does not itself enter the protein fraction.[10]

We wish to report experiments indicating that the influence of MSO on the nitrogenous metabolism of the brain is more extensive than has been apparent heretofore.

Adult male dogs were given DL-methionine-DL-sulfoximine i.p., and food was withheld thereafter. Two animals given 6 mg/kg and two given 10 mg/kg had severe generalized seizures after 16–18 hr. In another instance 6 mg/kg induced pronounced symptoms of toxicity, but probably no seizures occurred (the dog was not observed continuously). Two animals given 6 mg/kg showed only minimal symptoms 15–24 hr after the injection and were not studied further.

When seizures had been observed, or at 21·5 hr in the animal not convulsing, morphine was given (5 mg/kg s.c.) and after 30–40 min, transient anesthesia was induced with thiopental sodium (about 12 mg/kg i.v., with subsequent small doses as needed). The recurrent seizures were not suppressed by morphine but were eliminated by thiopental. The cranium was exposed and opened, and most of the calvarium was removed, the dura mater remaining intact. Blood pressure (recorded from a cannulated femoral artery) was in the normal range in all experiments. When the surgical procedures had been completed, a period of 40 min or longer was allowed for disappearance of thiopental effects. Seizures did not recur during this time. The brain was frozen with liquid nitrogen 2·5–3 hr after the morphine was given, and the exposed parts of the cortex were quickly removed to a depth of about 1 cm. The tissue was analyzed for the following constituents: N-acetylaspartate, alanine, γ-aminobutyrate, ammonia, arginine, aspartate, citrate, glutamate, glutamine, glutathione, glycerophosphoethanolamine, glycine, histidine, lactate, leucine, lysine, the sum of methionine and cystathionine, phenylalanine, phosphoethanolamine, serine, taurine, threonine, tyrosine, urea, and valine. The amino acids were separated by ion-exchange chromatography. Further details of the experimental procedures and analytical methods are given elsewhere.[11] Arterial blood oxygenation was judged to be adequate at the time of freezing. In control experiments, food was withheld from the animals for 18–20 hr prior to injection of morphine.

The results (Table 1) confirm the reports that MSO induces a great decrease in glutamine[5, 6] and a significant decrease in glutamic acid.[6] The mean decrease in γ-aminobutyric acid is not statistically significant, but if the value from the animal not convulsing (0·838 μmoles/g) is omitted, the mean and standard deviation become 0·720 $\pm$ 0·015 μmoles/g, and the decrease is significant at the 5% level. This is in agreement with the observations of Peters and Tower.[6] The decrease in the peak representing methionine and cystathionine supports other indications[1] of a disturbance in methionine metabolism.

The marked increase in the ammonia level is in contrast to the slight decrease found by Koloušek and Jiráček[5] in rats. The rise in cerebral ammonia is not due to accumulation of this substance in the blood. In the two animals given 10 mg MSO/kg, analysis of blood taken from the arterial cannula

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just before the brain was frozen gave ammonia values of 0·15 and 0·20 μmole/ml; control values ranged from 0·1 to 0·2 μmole/ml.

Other observed changes include increases in cerebral alanine, lysine, phosphoethanolamine, and serine, and decreases in aspartic acid, leucine, and valine. Lactic acid showed a small but significant increase. The levels of the other constituents measured were not significantly different from control values reported previously.[11] It has also been found that dogs fed a ration containing 'agenized, flour and subject to convulsions show normal cerebral levels of phosphocreatine, acid-soluble nucleotides, inorganic phosphate, glucose, and glycogen.[12]

TABLE 1. EFFECTS OF METHIONINE SULFOXIMINE ON LEVELS OF CEREBRAL CONSTITUENTS

Constituent	Control (Mean ± S.D.)	MSO* (Mean ± S.D.)
Alanine	0·14 ± 0·04 (9)	0·58 ± 0·19†
γ-Aminobutyric acid	0·829 ± 0·081 (9)	0·744 ± 0·054
Ammonia	0·26 ± 0·07 (13)	0·77 ± 0·13†
Aspartic acid	2·45 ± 0·22 (19)	1·30 ± 0·26†
Glutamic acid	7·81 ± 0·65 (19)	6·56 ± 0·62†
Glutamine	5·6 ± 1·7 (13)	1·6 ± 0·6†
Lactic acid	1·04 ± 0·26 (17)	1·53 ± 0·37†
Leucine	0·089 ± 0·011 (9)	0·041 ± 0·006†
Lysine	0·15 ± 0·03 (9)	0·28 ± 0·04†
Methionine + cystathionine	0·31 ± 0·08 (9)	0·14 ± 0·04†
Phosphoethanolamine	1·28 ± 0·20 (8)	1·83 ± 0·18†
Serine	0·39 ± 0·05 (9)	0·86 ± 0·14†
Valine	0·13 ± 0·03 (7)	0·09 ± 0·02‡

Values are in μmoles/g. Numbers of control experiments in parentheses.
* Five animals with symptoms of toxicity; four of these had seizures.
† Significant difference at the 1% level.
‡ Significant difference at the 5% level.

Okumura *et al.*[13] have reported amino acid analyses of cortical gray matter, cerebral white matter, and other parts of the dog brain (data from two females killed by decapitation). For the most part our control data are in conformity with their results on cortical gray matter, if allowance is made for the large post-mortem increases in alanine and γ-aminobutyrate known to occur in unfrozen tissue.[11, 14] It appears that the inclusion of small amounts of white matter in our frozen specimens could have contributed to the higher value that we found for lysine and to the slightly lower values for glutamate and phosphoethanolamine, but more data would be required for a statistically valid evaluation. Under post-mortem conditions the γ-aminobutyrate level also is much lower in cerebral white matter than in cortical gray matter; assuming a similar difference in the frozen brain, our values for this constituent are slightly lower than the true levels in gray matter of the cortex. It is unlikely that incomplete extraction of γ-aminobutyrate is a factor in our analyses, since our use of picric acid for extraction gives results on frozen mouse brain (mean value 2·26 μmoles/g) which are almost identical with those of Lovell and Elliott[14] based on extraction with 90% ethanol. The true levels of γ-aminobutyrate and of alanine in the dog cortex are evidently much lower than those reported for brain tissue of other species.

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The protective effects of cysteamine against ionizing radiation in mouse fibroblasts growing *in vitro**

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THE radioprotective action of cysteamine (β-mercaptoethylamine, MEA) has been widely confirmed, primarily in intact animals,[1] bacteria,[2] and mammalian cells *in vitro*.[3, 4] Therkelsen,[5] however, reported little or no protection of 1·5 mM MEA against X-ray damage of L-strain fibroblasts in tissue culture. Vos and associates,[3, 4] on the other hand, have reported significant degrees of protection (dose-reduction factors of up to approximately 4) for human kidney cells, grown *in vitro*, irradiated in the presence of MEA, and tested by the ability of treated cells to form clones. The present experiments, using the parameter of population growth of L-strain mouse fibroblasts after irradiation in the presence of different concentrations of the agent, were undertaken in an attempt to determine whether MEA exerts a radioprotective effect. Some additional studies were performed, dealing with the radioprotective effect of MEA at reduced oxygen tension.

METHODS

Mouse fibroblasts (Earle's 'L' cells) were grown at 37° as monolayer cultures in 8-oz prescription bottles containing 20 ml. of Eagle's culture medium.[6] The gas phase was 5% CO_2 in air. The use of 5% CO_2 in all gas mixtures served to fix the pH of the bicarbonate medium at a constant value of 7·15. Replicate cultures of about 10^5 cells per bottle were dispensed by an automatic, sterile pipet machine. On the following day, the bottles were removed from the incubator and the medium replaced with fresh medium or with the appropriate experimental medium containing MEA.† The cells remained in the experimental medium for 15 min prior to irradiation, and the period of irradiation ranged from 2–14 min depending on the dose of radiation to be administered. The culture medium was drained off after a total time of 30 min in the experimental medium (regardless of radiation dosage); the cells were washed twice with 10 ml of fresh medium; 20 ml of fresh medium was then added, and the bottles were returned to the incubator.

Cell counts were obtained in an electronic cell counter. Representative bottles were counted on day 1, after the manipulations described above, and final counts obtained 4 days later. Each count reported in this paper is the mean count of 3 replicate bottles.

Anoxic conditions were achieved by the following procedure: 1 hr before irradiation, the gas phase in the culture bottles was replaced with a mixture of 5% CO_2–95% N_2. Analysis of this gas mixture revealed an oxygen content of less than 50 ppm. One hour later, the medium was withdrawn through hypodermic needles and replaced with experimental media (with or without MEA); these media were previously freed of oxygen by bubbling 5% CO_2–95% N_2 through them for 90 min. The bottles were flushed once again with 5% CO_2–95% N_2, and the gas flow was continued throughout the irradiation (or sham-irradiation) period. Irradiation commenced 15 min after addition of the experimental media.

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† Cysteamine hydrochloride (Fluka AG) was supplied in vacuum-sealed ampoules. It was dissolved in water, sterilized by filtration, and added to culture medium immediately before use.