methionine sulfoximine. It is difficult to determine at present whether the slow elimination of the inhibitor is due to slow enzyme turnover or whether the inhibitor is successively transferred from enzyme molecule to molecule as degradation and resynthesis occur. Accurate data in this regard would necessitate antibody precipitation of labeled enzyme–inhibitor complex. Nevertheless, the latter possibility is more compatible with information gained from experiments on glutamine synthetase repression in rat liver,* which indicates an enzyme half-life of approximately 3 hr.

Since 4 hr after the administration of the drug the animals develop a hyperexcitable state which lasts for only an hour, the data presented imply a lack of a causative relationship between the inhibition of glutamine synthetase by MSO and the seizure state. Other workers have come to similar conclusions. Folbergrova[11] noted that in rats given 200 mg/kg of MSO, glutamine levels in the brain

Despite the fact that of 13 enzymes† investigated,[13] MSO has been reported to show significant inhibition of only alanine aminotransferase and glutamine synthetase, the present evidence indicates that methionine sulfoximine does not produce seizures by its inhibition of glutamine synthetase. Nevertheless, a definitive decision on this point must await studies on the distribution of glutamine in various regions of the brain and in the various subcellular components as well.

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* C. Lamar and H. C. Pitot, unpublished observations.

† The author has noted negative effects of up to 0·1 M concentration of MSO on these enzyme systems: glutamic acid decarboxylase, methionine-activating enzyme, ornithine transaminase, and glutaminase I.

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The effect of various agents on the levels of homocarsonine in rat brain

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THE DISCOVERY of homocarnosine in brain tissue[1, 2] has stimulated investigation of this compound. Recently Hayashi[3] presented evidence that homocarnosine was helpful in the treatment of epilepsy and suggested that it may function as an inhibitory substance in the central nervous system. A similar function has been suggested for γ-aminobutyric acid (GABA).[4] Since certain hydrazines and hydrazides will alter brain GABA levels,[5] hydroxylamine raises[6] and reserpine decreases[7] the levels, it was

deemed desirable to determine if these compounds might affect the levels of brain homocarnosine. It was also convenient to measure the amounts of histidine and carnosine in brain tissue, and the results of these experiments are reported here.

MATERIALS AND METHODS

Albino rats (Holtzman) weighing 350–400 g were used in these experiments. Drugs and chemicals were neutralized if necessary and injected i.p. in the following amounts: hydrazine (Hy) (Eastman), 75 mg/kg; 1,1-dimethylhydrazine (UDMH) (Eastman), 100 mg/kg; 1,2-dimethylhydrazine (SDMH) (Aldrich Chemical Co.), 500 mg/kg; phenylisopropyl hydrazine (PIH) (donated by Lakeside Labs, J.B. 516), 20 mg/kg; hydroxylamine (HA) (Eastman), 30 mg/kg; reserpine (Res) (Sandrill, Lilly), 5·0 mg/kg. The amounts of hydrazines injected were those suggested by Uchida and O'Brien.[5] The general procedure for the isolation and determination of homocarnosine, histidine, and carnosine was that reported by Abraham, Pisano, and Udenfriend.[8]

1½ Hr after injection, the animals were killed by decapitation and their brains removed immediately. In the case of reserpine the animals were sacrificed 18 hr after injection. The brains were immediately homogenized in 8 vol. of cold (0–4°) 80% ethyl alcohol, and the homogenate was then centrifuged in the cold. The supernatant fluid was measured and an aliquot taken for GABA determination. The remaining supernatant fluid was extracted with an equal volume of a 1:1 mixture of isoamyl alcohol–hexane to dissolve lipoidal material. A volume of water was added which equaled one-fifth the combined preceding volumes, and the lower aqueous phase was recovered after repeated mixing.

The extracted sample was then placed on a Dowex 50-X-4 (200–400 mesh) column which had been saturated with 2,6-lutidine.[9] The column was approximately 75 cm × 1 cm. The column was eluted with 0·1 M 2,6-lutidine and collected in 5-ml fractions. An aliquot of each fraction was assayed by the ninhydrin method or by reaction[8] with Fast Red Salt TRN (donated by General Analine and Film Corp), or by both methods. In a typical experiment, the histidine would appear in tubes 30–35, carnosine in tubes 65–75, and homocarnosine in tubes 97–109. The respective fractions were combined and evaporated to dryness under reduced pressure. The sample was taken up in 2·0 ml water and the amount determined colorimetrically at 500 mμ after coupling with Fast Red Salt TRN.

Values were corrected for recovery by adding a known amount of homocarnosine, histidine, and carnosine to the homogenates and carrying the samples through the procedure. Recoveries were found to be 78 per cent for homocarnosine, 86 per cent for histidine, and 83 per cent for carnosine. Further evidence for the identity of the materials eluted from the columns was accomplished by thin-layer chromatography (Eastman chromatogram sheet 6061, silica gel) with a solvent system of $CHCl_3$–methanol–17% NH_3 (2:2:1). GABA was determined on an aliquot of the original supernatant by separation using descending paper chromatography on Whatman No. 1 paper and a solvent system of *n*-butanol–acetic acid–H_2O (120:30:50). After drying, the paper was sprayed with 0·5% ninhydrin in 95% ethanol and GABA was determined by the procedure of Maynert, Klingman and Kaji.[10] Each sample was run in triplicate. Recovery of GABA was found to be 80 per cent.

RESULTS AND DISCUSSION

The amount of homocarnosine found in brains of control rats agrees well with that reported by Abraham *et al.*[8] and by Kanazawa and Sano.[11] Homocarnosine was significantly lowered in every case except with the administration of SDMH, which is considered to be a non-toxic hydrazine (Table 1). It was also interesting to find that reserpine caused the greatest lowering of homocarnosine in brain of the compounds tested. It was noted that all the agents tested raised histidine levels and reserpine raised histidine nearly equimolar with homocarnosine lowering. The equimolar ratio was close for Hy and UDMH. A wide variation in histidine content has been previously reported.[12] However, the values here agree well with those reported recently by Levi, Kandera and Lajtha.[13] None of the agents tested significantly altered carnosine levels. The GABA levels were in general agreement with previous work. Since GABA levels are so much higher than homocarnosine, it would probably be difficult to demonstrate any relation in changes of concentration if it existed.

Whether the lowering of homocarnosine is related to the toxic action of the hydrazines or not will have to await further experiments. Since reserpine also lowered the homocarnosine in brain, the decrease may be taking place by two different mechanisms. It has been suggested that reserpine may

block the storage of amines in intracellular particles.[14] In a similar manner, reserpine may be interfering with the storage of homocarnosine. On the other hand, the hydrazines may be interfering with the synthesis of homocarnosine by a loss in energy metabolism.[15] The results of the experiments reported here do indicate a relation between levels of homocarnosine and histidine in rat brain.

TABLE 1. RAT BRAIN CONSTITUENTS*

Treatment	Histidine	Carnosine	Homocarnosine	GABA
Control	3·72 ± 0·24 (8)	1·51 ± 0·21 (9)	4·71 ± 0·26 (9)	220 ± 13 (6)
Hydrazine	6·62 ± 0·70† (7)	0·51 ± 0·70 (8)	2·23 ± 0·31† (7)	477 ± 56† (6)
1,1-Dimethylhydrazine	6·89 ± 0·52† (6)	1·12 ± 0·21 (6)	2·29 ± 0·26† (6)	201 ± 14 (6)
1,2-Dimethylhydrazine	5·66 ± 0·41† (6)	1·04 ± 0·08 (6)	3·82 ± 0·50 (6)	201 (2)
Phenylisopropyl hydrazine	8·02 ± 0·67 (5)	1·41 ± 0·06 (5)	2·72 ± 0·17† (5)	267 ± 10‡ (6)
Hydroxylamine	9·75 ± 0·87† (6)	1·94 ± 0·41 (6)	2·19 ± 0·24 (6)	201 ± 23 (6)
Reserpine	7·07 ± 0·35† (6)	1·90 ± 0·22 (6)	1·73 ± 0·06† (6)	137 ± 7† (6)

* Results are expressed as μmole/100 g ± S.E.M. Numbers in parentheses are numbers of samples.
† $P < 0·01$ by *t*-test.
‡ $P < 0·02$ by *t*-test.

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