(1·25 to 2·5 mg/kg), the brain levels of amphetamine 6 hr after its administration were 5- to 9-fold greater than those of the control animals. At these low doses the adrenergic blocking action of chlorpromazine does not appear sufficient to prevent the action of the norepinephrine which is released in the presence of increased amounts of amphetamine. In contrast, higher doses of chlorpromazine antagonize the action of emphetamine even in the presence of strikingly elevated levels of amphetamine in brain (Table 1). Since the action of amphetamine appears to depend on the release of newly synthesized norepinephrine, the data obtained with high doses of chlorpromazine furnish additional evidence for the central adrenergic blocking properties of this phenothiazine derivative. This interpretation is consistent with recently obtained data by Bradley *et al.*,[14] whose studies demonstrated that chlorpromazine specifically blocked the excitatory effects elicited by norepinephrine on single neurons of the brain stem reticular formation. Moreover, the results of the present study explain the desipramine-like potentiation of the action of amphetamine by low doses of chlorpromazine.

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The duration of the inhibition of glutamine synthetase by methionine sulfoximine*

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METHIONINE sulfoximine (MSO) is a powerful convulsant whose mode of action remains obscure. Sellinger and Weiler[1] showed that this compound competitively inhibits rat cerebral glutamine synthetase *in vitro*. Lamar and Sellinger[2] demonstrated that glutamine synthetase isolated from brains of rats treated with MSO, when compated to untreated controls, was affected in a manner described for noncompetitive inhibition.[3] These and other studies involving the incubation *in vitro* of glutamine synthetase, ATP, magnesium, 2, 3 dimercaptopropanol, and 1-^{14}C-MSO in the presence and absence of glutamate led to the hypothesis that MSO inhibited this enzyme either by causing a permanent

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structural change in the molecule[2] or by binding irreversibly with the enzyme.[4] Sellinger[5] has demonstrated that pre-incubation of cerebral glutamine synthetase with MSO at 37° effectively inactivated the enzyme only in the presence of Mg^{2+} and ATP and in the absence of glutamate. Interestingly, this effect was not seen if the pre-incubation was carried out at 4°. Ronzio and Meister[6] recently presented proof that MSO was bound to the active site of glutamine synthetase.

In view of the evidence for the apparent irreversible binding of MSO to glutamine synthetase, it seems pertinent to study the duration of the glutamine synthetase inhibition in various tissues of the rat and to follow the removal of 1-^{14}C-MSO from the body.

MATERIALS AND METHODS

1-^{14}C-DL-methionine sulfoximine (sp. act. 0·2 μc/mg) was added to a carrier solution of ^{12}C-DL-methionine sulfoximine (20 mg/ml) with a resulting sp. act. of 0·05 μc/mg. Male Holtzman rats (Holtzman Farms, Madison, Wis.) weighing 130–160 g were injected i.p. at a dose of 200 mg/kg. The animals were maintained on laboratory chow throughout the experiment and were sacrificed by decapitation at various intervals after the administration of the drug. The liver, brain, and kidneys were removed quickly and homogenized (10%, w/v) in 0·01 M, Tris buffer, pH 7·2, by means of a homogenizing tube and a tight-fitting Teflon pestle.

Glutamine synthetase activity was measured on 0·2-ml fractions of the homogenate by the method described by Sellinger and De Balbian Verster.[7] Glutamine transferase was assayed in a medium in which the proportions of manganese and arsenate had been shown to be at an optimal ratio (a fine

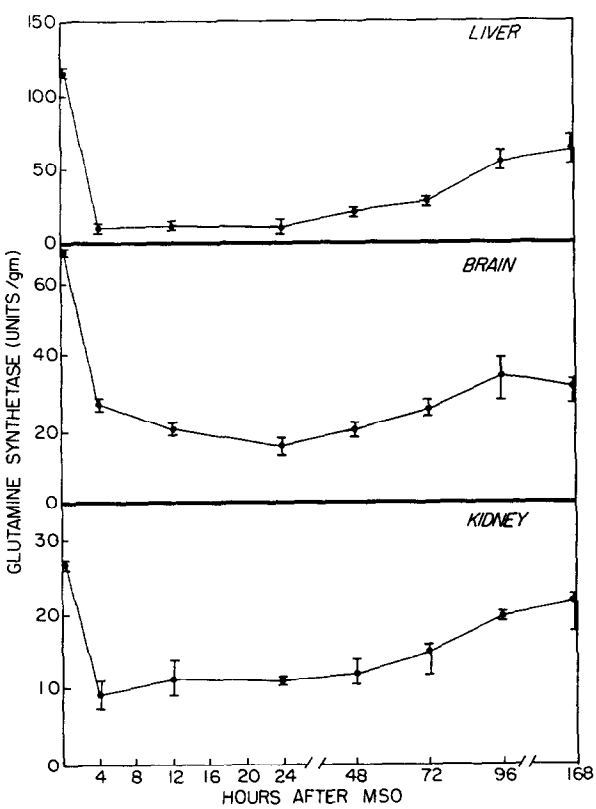

FIG. 1. The inhibition of glutamine synthetase in various tissues of the rat after i.p. administration of methionine sulfoximine (MSO), 200 mg/kg. Each point represents the average of 4 animals, and the brackets encompass all determinations.

precipitate which forms does not interfere with the reaction). The incubation volume of 1·5 ml contained in final concentrations per ml: Tris buffer, pH 7·4, 13·5 μmole, fresh and neutralized hydroxylamine, 50 μmoles; glutamine, 73 μmole, Mn^{++}, 3·3 μmole; $NaHAsO_4$, 53 μmole; and ADP, 0·03 μmole. After a 15-min incubation, the reaction was stopped by the addition of 1 ml ferric chloride solution.[7] A standard curve was prepared with L-glutamylhydroxamic acid (GHA). A unit of enzyme activity in both assays refers to the formation of 1 μmole GHA in 60 min.

Radioactive MSO was synthesized from 1-^{14}C-methionine by the method of Bentley *et al.*[8] The synthesized MSO was shown to be approximately 75 per cent pure by paper chromatography. The descending technique was used and the solvents employed were aqueous phenol (80%, w/v) and a mixture of phenol (80%) 150, ethanol 40, ammonia 1, and water 10 (contaminant, unreacted methionine sulfoxide). The radioactivity in the homogenate was measured, after digestion of 0·3 ml in 1 ml NCS™ solubilizer (Nuclear Chicago) for 2 hr at 50°, by adding 15 ml of Bray's scintillation counting fluid.[9] The samples were counted for 300 min in a Packard Tri-Carb liquid scintillation counter system, model 500D.

The microsomal pellet was obtained by centrifuging the homogenate in a Sorvall refrigerated ultracentrifuge, model RC2-B, 10,000 rpm for 10 min, and then by centrfuging the supernatant at 140,000 *g* for 60 min in a Spinco ultracentrifuge, model L. The radioactivity pattern in the microsomal pellet was similar to that determined in the homogenate.

RESULTS

The degree of inhibition of glutamine synthetase and glutamine transferase activities in liver, brain, and kidney after a single injection of methionine sulfoximine is shown in Figs. 1 and 2. It is apparent that in liver and kidney enzyme inhibition is maximal in 4 hr and in brain at 24 hr. The inhibition remains to a considerable degree for 72 hr and even at the end of 1 week the activity has not reached

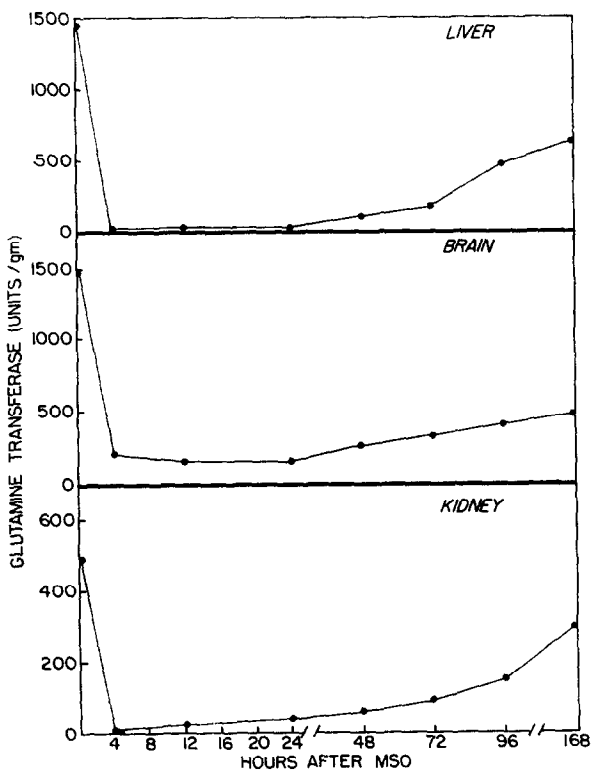

FIG. 2. Identical to Fig. 1, but the inhibition of glutamine transferase is represented.

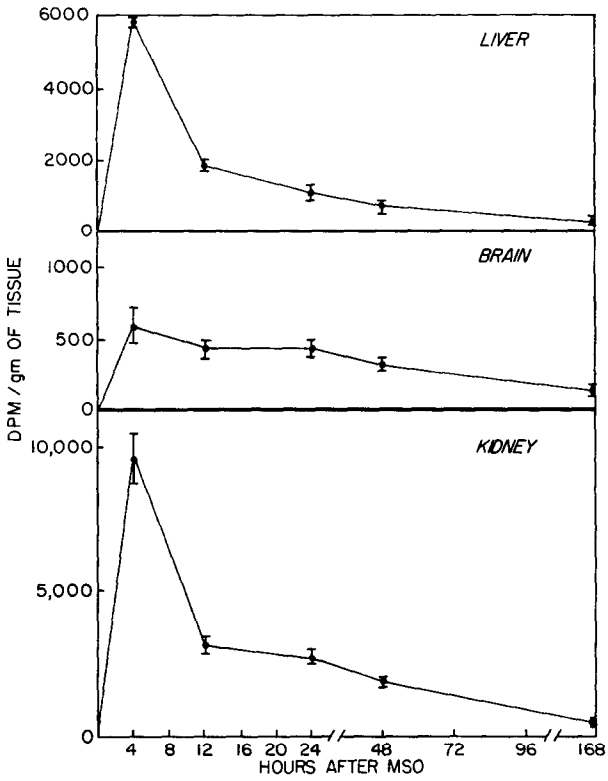

FIG. 3. The level in the homogenate of radioactive 1-^{14}C-methionine sulfoximine (sp. act. of administered compound, 0·05 μc/mg). Conditions identical to Fig. 1. See text for further explanation

pretreatment levels. Glutamine transferase activity shows proportionately more inhibition than glutamine synthetase. Its activity in kidney and liver dropped to 1 per cent of pretreatment levels. The radioactivity present in each tissue is represented in Fig. 3. It can be seen that in kidney and liver there is a marked elevation in radioactivity at 4 hr. The radioactivity pattern in the microsomal pellet of brain and liver failed to show the spike at 4 hr, whereas that from kindey demonstrated a slight initial elevation. At 4 hr the radioactivity in the microsomal pellet when compared to that in the homogenate was approximately one-tenth for brain and one-fiftieth in the case of liver and kidney. For these reasons, it is thought that the initial spike represents pool saturation rather than binding to the enzyme. It is noteworthy that the radioactivity is removed from the various tissues slowly and that its removal corresponds closely to the slow return toward normal in enzyme activity.

DISCUSSION

The present report indicates that in contrast to the duration of the pharmacological effect of most drugs, which is measured in hours, the inhibition of glutamine synthetase by MSO extends over a period of several days. These findings are compatible with the recent evidence for the binding *in vitro* of MSO to the glutamine synthetase molecule.[6] At present, an explanation of the mechanism for the continued to be depressed even after the animals had recovered from the seizures. When lower doses of MSO were given, although no seizures occurred, glutamine concentrations fell to levels seen in convulsed animals. Sellinger and Ohlsson[12] have studied the degree of glutamine synthetase inhibition by MSO in various regions of rat brain and have shown a lack of direct relationship between the convulsive event elicited by MSO and enzyme inhibition.

prolonged inhibition is speculative. It is apparent from the radioactivity data that MSO remains in the tissue during the period of inhibition. Roth *et al.*[10] reported similar findings with ^{35}S-labeled

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