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THE NATURE OF THE INHIBITION *IN VITRO* OF CEREBRAL GLUTAMINE SYNTHETASE BY THE CONVULSANT, METHIONINE SULFOXIMINE*

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Abstract—The nature of the inhibition of glutamine synthetase of rat cerebral cortex by the convulsant agent, DL-methionine-DL-sulfoximine (MSO) has been examined. In view of the high degree of structural latency of the enzyme in brain, the inhibition of the enzyme in particulate and soluble form was studied and found to be the same. Competition between L-glutamate and MSO for the enzyme site(s) could be demonstrated as one of the mechanisms of inhibition. Preincubation of the enzyme with ATP, but not with L-glutamate, afforded some protection toward inhibition by MSO; preincubation with MSO, however, led to no enhancement of the inhibition. L-Methionine, and other compounds structurally related to L-glutamate, failed to alleviate the inhibition.

It is suggested that, as shown here *in vitro*, MSO acts also *in vivo*, by inhibiting the glutamine synthesizing system. It does so by causing depletion of ATP and, possibly, glutamate in the intracellular compartment within the boundaries of which the synthesis of glutamine occurs. It is further visualized that the differences between the effects *in vitro* and *in vivo* of methionine may be due to the necessity, fulfilled only *in vivo*, of replenishing discrete subcellular compartments with substrates (ATP and glutamate).

IN RECENT years, methionine sulfoximine has frequently been used as an experimental convulsant agent.[1–5] Several studies have revealed that, in a number of animal species, its administration results in seizure phenomena that are in many respects similar to those encountered clinically.[2, 4, 6] Since several biochemical correlates to the convulsant properties of MSO have also been noted,[1, 2, 5] MSO has proven to be a useful drug in the study of biochemical events associated with or, perhaps, underlying the epileptogenic process as initiated by this particular agent.

Indications as to the mode of action of MSO at the cellular level have been obtained by Gershoff[7] and by Kolousek and Lodin[8] who noted an inhibition of the incorporation *in vivo* of methionine-^{35}S into the cerebral proteins of MSO-treated vs. normal animals. Of particular interest, however, because not confirmatory of the results *in vivo*, is the recent report of the failure of MSO to inhibit the incorporation *in vitro* of methionine-^{35}S into the proteins of brain slices from normal animals.[9]

Although the inhibition of the reaction:

$$\text{glutamate} + NH_4^+ + \text{ATP} + Mg^{2+} \rightleftharpoons \text{glutamine} + \text{ADP} + PO_4^{3-}$$

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by MSO and by the related methionine sulfoxide had been reported previously,[10, 15] a detailed study of the inhibition of cerebral glutamine synthesis by MSO was first carried out by Peters and Tower,[16] using brain slices. As a result of these studies and on the basis of evidence of a virtual absence of glutamine in brain slices of MSO-treated cats and of markedly subnormal rates of glutamine production noted upon incubation of such slices in a medium containing glucose and 27 mM K^+, MSO-elicited convulsions in cats and the consequent impairment of the cerebral synthesis of glutamine have been causally related. Furthermore, the recent studies of Kolousek and his associates,[17, 18] in which the effect of MSO on the nitrogen metabolism of rat brain after either acoustic epileptogenic stimulation or MSO administration was examined, provide further evidence for the existence of an interrelationship of cellular mechanisms governing the amidation of glutamic acid in brain and seizure phenomena.

Some of the properties as well as the subcellular localization of cerebral glutamine synthetase have recently been described.[19] On the basis of the pronounced structural latency characteristic of this enzyme in brain, it has been suggested[19] that the enzyme is loosely bound to, or associated with, the surface of the subcellular elements which correspond morphologically to the membranous network of the endoplasmic reticulum. It has also been suggested[19] that such a structural arrangement is compatible and may, perhaps, correspond to the metabolic compartment harboring the synthesis of cerebral glutamine and which has been shown to exist in brain.[20–22]

The possibility thus became apparent that the sensitivity of brain *in vivo* toward MSO, in particular as reflected by those cerebral structures associated with the synthesis of glutamine, may be due in part to an MSO-elicited alteration of the structural association of glutamine synthetase with the endoplasmic reticulum which, in turn, would manifest itself in a reduction of measurable glutamine synthetase activity. Analogously, recent studies of other systems[23–28] have shown that enzymes may vary in activity according to the structural state of the microsomes with which they are associated.

The present report describes results of experiments in which the effect of MSO on particulate and soluble preparations of glutamine synthetase of rat cerebral cortex was examined in an effort to determine differences in response to MSO as a result of differences in structural association of the enzyme. In both states the enzyme is inhibited by MSO, and the nature of the inhibition is shown to be competitive with respect to one of its substrates, L-glutamate. Some other features of the MSO inhibition of glutamine synthetase are also described.

MATERIALS AND METHODS

Chemicals

D- and L-glutamic acid, D-, L-, and DL-methionine, L-, and DL-ethionine, N-acetyl-L-glutamic acid, γ-aminobutyric acid, DL-methionine-DL-sulfoximine, and 2,3-dimercaptopropanol (BAL) were products of the Mann Research Laboratories, New York, and were of the highest purity available. ATP and L-glutamohydroxamic acid were purchased from the Sigma Co., St. Louis, Mo.

Preparation of microsomal fraction from rat cerebral cortex

Male Holtzman rats (150 to 200 g) were used in all experiments. After decapitation, the brain (one or two per experiment) was exposed and the cortical layers quickly removed and placed in a tared beaker containing 1 ml ice-cold distilled water. After weighing, the cortices were transferred to a homogenizer tube fitted with a Teflon pestle (clearance: 0·005 in.)[29] and homogenization was accomplished mechanically in a volume 4 times the tissue weight. The resulting homogenate was centrifuged at 25,000 × *g* for 10 min (Spinco model L centrifuge, rotor no. 40), and the sedimented material was resuspended mechanically (one up-and-down stroke with the pestle) and recentrifuged at the same speed for 10 min. The combined supernatant fluid and washings were then centrifuged at 145,000 × *g* for 60 min. The pellet (P) represents the microsomal fraction.* It was suspended in water and the suspension diluted so as to contain approximately 60 mg of tissue equivalent/ml. The supernatant fluid (S), representing the soluble cell material, contained approximately 50 mg of tissue equivalent/ml.

Chemical determinations

Protein was determined according to Lowry *et al.*[30] with human serum albumin as standard.

Glutamine synthetase assay

Except as otherwise noted the previously described[19] incubation mixture (3 ml) was used. Incubation time (37°) was 130 min. The enzymic reaction was terminated by the addition of 2 ml 0·5 N HCl solution, 10% (w/v) in $FeCl_3$ and 8% (w/v) in trichloroacetic acid. The tube contents were filtered (Whatman No. 42, 9 cm), and the optical density of the clear filtrate was determined at 500 mμ. A standard curve was prepared with L-glutamohydroxamic acid.

RESULTS

Particulate vs. soluble glutamine synthetase activity

The production of glutamohydroxamate by varying amounts of particulate and soluble glutamine synthetase is shown in Fig. 1. The activity of glutamine synthetase in particulate form always exceeded that of the enzyme in soluble form. This was further evidenced by the specific activity of the particulate enzyme which exceeded that of glutamine synthetase in soluble form by approximately 8- to 10-fold. In all incubations, amounts of tissue equivalent ranging between 10 and 20 mg were used. It was established in separate experiments that at these levels of tissue equivalent the production of glutamohydroxamate proceeded linearly with time, up to about 3 hr.

Inhibition of glutamine synthetase by MSO

Particulate and soluble preparations of glutamine synthetase were incubated under standard conditions (glutamate: 40 mM, BAL: 0·5 mM) in the presence of increasing

* Electron microscopic examination reveals a homogeneous picture of rounded vesicles and concentric aggregates of membranes. Free or attached ribosomes may also be seen, as well as occasional myelin fragments. No double-membrane structures are seen, presumably as a result of the use of water as the homogenizing fluid. A survey of contaminating, extramicrosomal enzymes revealed acid phosphatase (lysosomal) and traces of aspartic transaminase (mitochondrial) and lactic dehydrogenase (soluble phase) activities (O. Z. Sellinger, F. de Balbian Verster, J. C. Harkin; unpublished observations).

concentrations of MSO (Table 1). At the existing glutamate concentration, the particulate and the soluble glutamine synthetase activity was inhibited to an approximately equal extent both by 10^{-4} M and 10^{-3} M MSO.

Preincubation of glutamine synthetase with MSO

Preincubation of glutamine synthetase with MSO (37°, 10 to 40 min) was carried out in an effort to determine whether such treatment, followed by subsequent incubation in the routine assay system, would modify the inhibition of glutamine synthetase seen upon direct incubation with MSO. Whether preincubation was carried out in

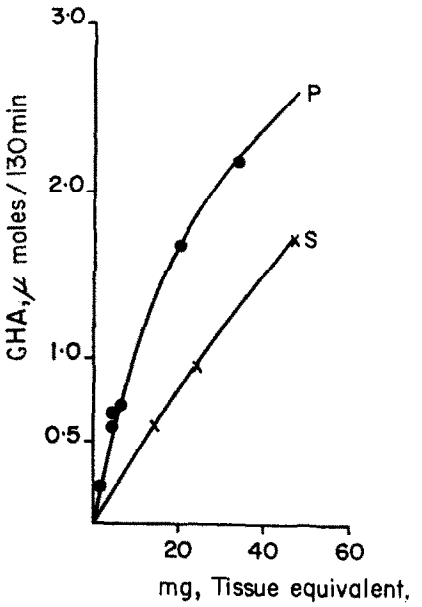

FIG. 1. Activity of cerebral glutamine synthetase in particulate (P) and soluble (S) form; GHA: L-glutamohydroxamate.

TABLE 1. INHIBITION OF PARTICULATE AND SOLUBLE PREPARATIONS OF CEREBRAL GLUTAMINE SYNTHETASE BY METHIONINE SULFOXIMINE

	Fraction	Methionine sulfoximine (M)	Activity (%)
Expt. 1			
	P	0	100
	P	10^{-5}	97·5
	P	10^{-4}	89·5
	P	10^{-3}	56·5
Expt. 2			
	P	0	100
	P	10^{-3}	58·5
	S	10^{-4}	96·5
	S	10^{-3}	47·5

Microsomal suspension, P; soluble fraction, S; 20·4 mg of tissue equivalent/tube; incubation, 130 min, 37°.

the presence of MSO (2×10^{-3} M) for 10 (Table 2, Expt. 1) or 40 min (Table 2, Expt. 2), the inhibition of glutamine synthetase, as measured at the end of the subsequent incubation period, was the same as that seen after preincubation of the enzyme alone. Identical results were obtained if preincubation was altogether omitted (Table 2, Expt. 2). Preincubation with MSO failed to affect the extent of inhibition whether 4 mM or 20 mM glutamate was used during incubation (Table 2, Expt. 3).

TABLE 2. PREINCUBATION OF GLUTAMINE SYNTHETASE WITH METHIONINE SULFOXIMINE*

Preincubation	Incubation L-Glutamate 4 mM −MSO GHA (μmoles)	4 mM +MSO GHA (μmoles)	20 mM −MSO GHA (μmoles)	20 mM +MSO GHA (μmoles)
Expt. 1				
Enzyme, 10 min	0·65	0·35		
Enzyme + MSO, 10 min		0·34		
Expt. 2				
None	0·91	0·55		
Enzyme, 20 min	0·97	0·58		
Enzyme, 40 min	0·90	0·58		
Enzyme + MSO, 20 min		0·66		
Enzyme + MSO, 40 min		0·65		
Expt. 3				
None	1·28		1·57	
Enzyme, 20 min	1·21	0·55	1·48	1·32
Enzyme + MSO, 20 min		0·46		1·26

* Concentrations of MSO: preincubation, 2×10^{-3} M; incubation, 2×10^{-4} M. GHA, L-glutamohydroxamate; preincubation, 37°.
Enzyme: particulate glutamine synthetase was used. Tissue equivalent: Expt. 1, 16·4 mg; Expts. 2 and 3, 19·3 mg.

TABLE 3. EFFECT OF CONCENTRATION OF GLUTAMATE ON THE INHIBITION OF GLUTAMINE SYNTHETASE BY METHIONINE SULFOXIMINE*

L-Glutamate mM	L-Glutamohydroxamate (μmoles/130 min) −MSO	+MSO†	Inhibition (%)
0·4	0·29	0	100
2	0·76	0·07	91
8	1·18	0·38	68
40	1·65	1·00	40
80	1·78	1·20	33

* Particulate glutamine synthetase: 18·3 mg tissue equivalent/tube was used.
† 5×10^{-4} M.

Effect of varying the concentration of L-*glutamate and MSO on the inhibition of glutamine synthetase by MSO*

The dependence of the inhibition of particulate preparations of glutamine synthetase by MSO (5×10^{-4} M) on the prevailing concentrations of glutamate is illustrated in Table 3. Better to define some of the modalities of the substrate–inhibitor interplay

necessary for inhibition, these two parameters were varied and the extent of inhibition determined. Thus, inhibition was examined: (a) at 2×10^{-4} M MSO over a narrow range of L-glutamate concentrations (2 to 8 mM); and (b) at 8 and 20 mM glutamate with MSO concentrations ranging between $0{\cdot}1 \times 10^{-3}$ and 5×10^{-3} M. The results of these experiments (Figs. 2 and 3) show that the inhibition by MSO is of a competi-

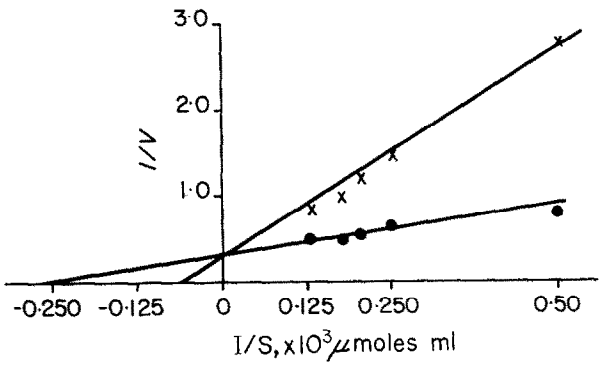

FIG. 2. Inhibition of cerebral glutamine synthetase by DL-methionine-DL-sulfoximine (MSO); S = concentration of L-glutamate; V = μmoles glutamohydroxamate/130 min. Particulate enzyme: 19 mg of tissue equivalent/tube; ●——●: no inhibitor; ×——×: MSO, 2×10^{-4} M.

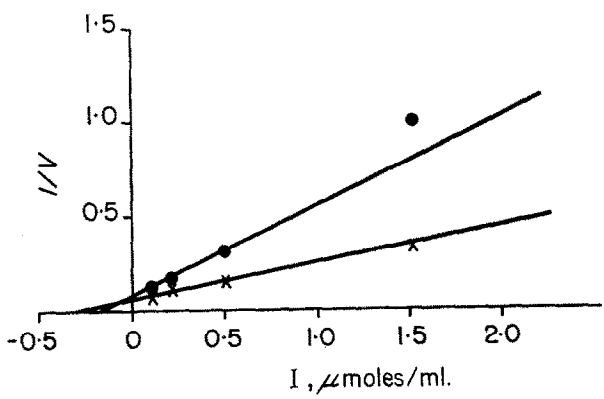

FIG. 3. Inhibition of cerebral glutamine synthetase by DL-methionine-DL-sulfoximine (I), as a function of L-glutamate concentration; V as in legend to Fig. 2; ●——●: L-glutamate, 8 mM; ×——×: L-glutamate, 20 mM.

tive nature with respect to L-glutamate. A KM value of $3{\cdot}8 \times 10^{-3}$ M was calculated from the data plotted in Fig. 2. K_i values of $6{\cdot}4 \times 10^{-5}$ M (Fig. 2) and $\equiv 5 \times 10^{-5}$ M (Fig. 3) were calculated. When the data shown in Fig. 2 were replotted according to Hunter and Downs,[31] a straight line with slope > 0 was obtained, in further confirmation of the competitive nature of the MSO inhibition.

Interdependence of glutamine synthetase (GS) activation by BAL and inhibition by MSO

The possibility was tested that some of the inhibitory effects of MSO might be due to an interaction of MSO with the glutamine synthetase activator, BAL, resulting in enzyme "deactivation" by virtue of effective removal of the activator by the inhibitor.

Particulate preparations of GS were incubated with MSO (5×10^{-4} M) in the presence and absence of BAL (5×10^{-4} M) and at two different concentrations of L-glutamate. It was found that considerable inhibition of GS had already ensued in the absence of BAL, ruling out an MSO effect by virtue of enzyme "deactivation" alone. The inhibition in the absence of BAL was particularly evident at low concentrations of glutamate. Significantly, raising the latter from 4 to 40 mM led to lowering the inhibition of GS, both in the absence and presence of BAL. These results point out some common features between GS activation by BAL and inhibition by MSO inasmuch as, at both concentrations of L-glutamate, greater inhibition by MSO was noted in the presence of BAL than in its absence. Conversely, when the effect of MSO on the activation of GS by BAL was examined, it was noted that, in each of four consecutive experiments, activation was greater in the absence of MSO. However, with MSO present, activation by BAL was significantly higher at 40 mM than at 4 mM glutamate, in agreement with the concept that activation by BAL and inhibition by MSO constitute separate, not exclusive effects with a common dependence on the prevailing concentration of glutamate.

Attempts to relieve the inhibition of glutamine synthetase by MSO

(*a*) *Preincubation with glutamate.* Preincubation of particulate glutamine synthetase (10 to 20 min, 37°) with varying concentrations of L-glutamate (4 to 40 mM) followed by incubation (glutamate: 13·3 mM) afforded no protection toward inhibition by MSO.

TABLE 4. EFFECT OF PREINCUBATION WITH ATP AND Mg^{2+} ON GLUTAMINE SYNTHETASE ACTIVITY*

Addition to preincubation	L-Glutamohydroxamate (μmoles/65 min)	Inhibition (%)
None	1·30	30
L-Glutamate	1·36	27
L-Glutamate + ATP	1·45	22
ATP	1·60	14
ATP + methionine sulfoximine†	1·28	31·5
ATP + Mg^{2+} (1:1·5)	1·32	29
ATP + Mg^{2+} (1:1·5) + glutamate	1·48	21·5
ATP + Mg^{2+} (1:1·0) + glutamate	1·54	17
ATP + Mg^{2+} (1:0·5) + glutamate	1·32	29

* Controls consisted of identical preincubation tubes, incubated in the absence of methionine sulfoximine. L-Glutamohydroxamate (average) formed in the controls: 1·86 μmoles. Inhibition refers to per cent of control value. Preincubation conditions: L-glutamate, 7·2 mM; ATP, 25 mM; Mg^{2+} as indicated; 10 min, 37°. Incubation conditions: L-glutamate, 4 mM; ATP, 13·3 mM; methionine sulfoximine, 0·2 mM; 65 min, 37°.
† 2 mM.

(b) *Preincubation with ATP and* Mg^{2+}. Glutamine synthetase was preincubated in the presence of ATP, glutamate + ATP, MSO + ATP, and Mg^{2+} + ATP. Also, the ratio of ATP to Mg^{2+} was varied during preincubation from 0·67 to 2·0. The results are shown in Table 4. Of all conditions tested, only preincubation with ATP partially protected glutamine synthetase from the inhibitory effect of subsequent incubation with MSO. The protective effect of ATP was readily reversed by MSO and could not

be enhanced by inclusion of either glutamate alone or glutamate + Mg^{2+} to the preincubation medium; it could, however, be maintained in the presence of equimolar amounts of ATP and Mg^{2+}.

(c) *Incubation in the presence of* L-*methionine.* In the light of the correction by the combination L-methionine + NH_4^+ of the impaired synthesis of glutamine noted in epileptogenic brain slices,[16] L-methionine (2, 4, 8, 10, 20, and 40 mM final concentration) was added to incubation mixtures containing GS and MSO (2×10^{-4} M). These experiments were carried out at 50 mM and 200 mM hydroxylamine and 4 mM L-glutamate. It was established separately that, with the concentration of glutamate constant at 4 mM, inhibition by MSO was independent of the concentration of hydroxylamine. No protection by L-methionine[10] at either concentration of hydroxylamine was noted.

(d) *Incubation in the presence of* D-*methionine,* L- *and* DL-*ethionine, γ-aminobutyrate, and* N-*acetyl*-L-*glutamate.* Addition of the above amino acids (40 mM, except L-ethionine, 8 mM; DL-ethionine, 13 mM) to incubation mixtures containing GS and MSO resulted in no diminution of inhibition. It was separately shown that none of the amino acids tested acted as substrate or otherwise affected the glutamine synthetase reaction.

(e) *Incubation in the presence of* D-*glutamate.* It has been shown previously that D-glutamate may serve as a substrate for glutamine synthetase[32] and that production of lower amounts of D-glutamohydroxamate observed in comparative studies of the two enantiomorphs is not due to steric preference in binding to the enzyme but, rather, to differential steric selectivities of the rate-limiting step of the glutamine synthetase reaction—viz. the reaction of the enzyme-bound activated glutamate with hydroxylamine.[33–35] The results of incubations of cerebral particulate preparations of GS with D-glutamate are illustrated in Table 5. Of interest is the protection from MSO inhibition achieved by incubating with L- and D-glutamate simultaneously. The observed

TABLE 5. INHIBITION BY MSO WITH D-GLUTAMATE AS GLUTAMINE SYNTHETASE SUBSTRATE

Substrate	Glutamohydroxamate formation*				Inhibition (%)	
	−MSO		+MSO†			
	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
L-Glutamate, 4 mM	1·15	1·05	0·55	0·45	52	57
L-Glutamate, 4 mM + D-glutamate, 40 mM	1·03	0·85	0·67	0·58	35	32
D-Glutamate, 40 mM	0·74	0·64		0·30		53

* μMoles/120 min.
† 2×10^{-4} M.

degree of inhibition may be compared to that seen with saturating concentrations of L-glutamate alone (Table 3). The results of Table 5 are readily interpreted on the basis of the recently proposed mechanism of action for glutamine synthetase,[35] for they indicate equally efficient competition between D- and L-glutamate and the inhibitor for, presumably, identical enzyme site(s).

DISCUSSION

When MSO was used to inhibit cerebral glutamine synthetase of either particulate or soluble origin (Table 1), a closely similar response to inhibition was obtained, indicating that susceptibility to inhibition is not affected in this instance by the structural state of the enzyme. Since results of previous studies from this laboratory[19] have provided evidence that cerebral glutamine synthetase exists, *in situ*, in loose association with the surface elements of the endoplasmic reticulum, the present results suggest that detachment of the enzyme from its subcellular structural matrix does not bring about structural alterations of the enzyme such as would be reflected in an altered reactivity toward an inhibitor. Experiments in which the activation of freshly isolated particulate glutamine synthetase by 2,3-dimercaptopropanol was compared to that of the enzyme after the latter was eluted off the particulate elements of the endoplasmic reticulum by means of 0·15 M NaCl,* lend further support to the above suggestion. The mode of the inhibition *in vitro* of glutamine synthetase by MSO was examined in some detail, since it was of interest to ascertain the extent of interaction of the inhibitor with the enzyme. Demonstration of such interaction was attempted by incubating the enzyme with the inhibitor in the absence of glutamate (Table 2). When 2×10^{-3} M MSO was preincubated with the enzyme for periods of up to 40 min, the inhibition noted at the end of the subsequent incubation period was not enhanced over suitable controls. Although by no means ruled out by these experiments, the occurrence of irreversible enzyme MSO interactions, under the conditions employed here, appears unlikely.

Evidence for the competitive nature of the MSO inhibition was obtained when the latter was measured at two levels of MSO in the presence of increasing concentrations of L-glutamate (Fig. 2, Table 3), as well as at increasing concentrations of inhibitor in the presence of two levels of L-glutamate (Fig. 3). Total inhibition of glutamine synthetase was achieved with an MSO to L-glutamate ratio of $\equiv 1$ (Table 3); partial inhibition (5×10^{-4} M MSO) could be readily demonstrated over a 200-fold range of L-glutamate concentrations. A K_m value of $3{\cdot}8 \times 10^{-3}$ M, in good agreement with the value recently reported by Pamiljans *et al.*[34] for the purified sheep brain enzyme, was calculated. The values for K_i, smaller than the corresponding K_m value by two orders of magnitude, point to a marked affinity of the inhibitor for the enzyme. As recently discussed by Koshland,[36] kinetics typical of competitive inhibition may result even if a mechanism of inhibition other than simultaneous competition of substrate and inhibitor for enzyme occupancy is operative. A change in the shape of the protein accompanying the presumed binding of MSO to the enzyme, albeit not revealed in the experiments reported here, may indeed have occurred, thus vitiating the normal interaction of the enzyme with substrate(s). The possibility of an interaction of BAL with MSO that results in effective removal of the activator or alternatively, as discussed by Reiner,[37] in the formation of an inhibitor–activator complex which can combine with the enzyme and inhibit it directly, is considered unlikely in view of enzyme inhibition observed in the total absence of BAL. Since the relationship between the activation by BAL and the concentration of glutamate has not been examined, it is difficult to interpret at present the observed interdependence of the activating and inhibitory processes, other than to point out their mutual dependence on the concentration of L-glutamate.

* O. Z. Sellinger and F. de Balbian Verster, unpublished observations.

Although shown for glutamine synthetase from peas,[38] activation of the rat brain particulate enzyme by preincubation with ATP could not be demonstrated (Table 4, legend). Preincubation with ATP led, however, to greatest protection from inhibition by MSO.* Addition of MSO caused annihilation of protection, indicating competition between the drug and the nucleotide for the glutamate-reacting enzyme site(s). Binding of ATP to the enzyme from peas has previously been demonstrated[39] and, moreover, protection by ATP against inactivation of the pea enzyme by iodoacetate has also been shown.[39] With regard to the brain enzyme studied here, it was found (Table 4) that addition of Mg^{2+} in molar excess (ATP: Mg^{2+} = 1:1·5) resulted in a block of the ATP-afforded protection. Protection similar to that noted when ATP alone was used during preincubation was achieved only with equimolar ATP and Mg^{2+} and in the presence of glutamate.

L-Methionine failed to alleviate the MSO inhibition of glutamine synthetase, whether in the presence of 50 mM or 200 mM hydroxylamine. It is unlikely that the lack of methionine effectiveness is to be attributed to the use of hydroxylamine in lieu of ammonia for, with L-glutamate as substrate, glutamine synthetase (from sheep brain) has been shown to exhibit identical K_m values for hydroxylamine and ammonia.[34] The failure of methionine to counteract the effects *in vitro* of MSO on glutamine synthetase may thus be compared to the failure of MSO to inhibit the incorporation *in vitro* of ^{35}S-methionine into proteins of brain slices[9] and to the efficacy of MSO as an inhibitor of glutamine synthetase in a rat liver s-RNA synthesizing system.[40]

L-Methionine, as well as L-phenylalanine, have recently been shown[41] to promote the cerebral synthesis of phosphocreatine by slices of guinea-pig brain respiring in a glucose medium. Also, a good correlation between the synthesis of glutamine from glutamate and ammonia and the disappearance of phosphocreatine could be established,[41] making it plausible to assume that the role of methionine in such a tissue-slice system was to aid in the maintenance of phosphocreatine levels and not to act directly on the enzymic system catalyzing the synthesis of glutamine. It may be suggested, therefore, that success of the combined effect of methionine and ammonia in stimulating the synthesis of glutamine in slices of cerebral cortex of animals that had undergone MSO seizures[16] may be ascribed to a methionine-mediated re-establishment *in situ* of normal high-energy phosphate levels. Indications of antagonistic action between MSO and ATP have, indeed, been noted in the present study (Table 4). In view of the compartmentalization of glutamine synthesis[20, 22] in brain it is, furthermore, possible that exogenous high-energy phosphate donors may not be functionally equivalent to ATP generated within, or in the proximity of, the glutamine-synthesizing compartment, thus explaining the inability of ATP, when added to an intact whole cell system such as a slice system,[16] to correct the impaired synthesis of glutamine. In this respect it would be of interest to establish, and to compare to glutamine synthetase, the subcellular localization and the structural latency of cerebral creatine phosphokinase[42] and of other, if any, microsomal high-energy phosphate-generating systems.[43, 44]† The failure of methionine to counteract the effect of MSO in the system

* Protection by ATP + Mg^{2+} from heat inactivation[34] has also been established (O. Z. Sellinger and C. Lamar, unpublished observations).

† It has been established in this laboratory that the cerebral microsomal ATPase[45, 46] and glutamine synthetase may readily be differentiated in terms of their structural latency—i.e. respective association with the endoplasmic reticulum (Sellinger and Verster, unpublished observations).

used in this study is therefore, in all probability, the result of the presence in this system of levels of ATP much higher than limiting. Studies are currently in progress in which an evaluation of the MSO effect and of the action thereupon of methionine, under conditions of limiting high-energy phosphate supply, is being sought.

To our knowledge it has not been established whether the administration of MSO *in vivo* results in alterations of cerebral high-energy phosphate levels. Minard and Davis[49] have recently shown that electroshock stimulation leads, within 10 sec after application of the stimulus, to appreciable breakdown of cerebral ATP and phosphocreatine, with a return to normal levels soon thereafter. Changes in the cerebral RNA content as a results of pentylenetetrazole (Metrazol) administration[50] or of application of electroshock[51] have also been reported recently. On the other hand, it is known that central nervous system stimulants such as amphetamine and like substances cause increase of cerebral ATP levels, presumably via an effect on ATP-resynthesizing systems.[52]

If the administration of MSO *in vivo* were to result in a protracted cellular or subcellular deficiency of high-energy phosphate donors, *inter alia*, ATP for the synthesis of glutamine, the impairment of the latter process would be but one of many manifestations of such a deficiency. The observed fact that both methionine and glutamine (and asparagine) will control MSO seizures[2] may then be understood if it is assumed that the former amino acid acts by somehow mobilizing, or stimulating the formation of, ATP and the latter by furnishing glutamate—both generating sufficient amounts of substrate to overcome the inhibition and thus prevent the full development of the epileptogenic picture. Some of these ideas are being tested.

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