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Inactivation of cerebral glutamine synthetase by DL-methionine-DL-sulfoximine

Previously, it has been reported that glutamine synthetase (L-glutamate:ammonia ligase (ADP), EC 6.3.1.2) of rat cerebral cortex is readily inhibited by the convulsant DL-methionine-DL-sulfoximine (MSO)[1,2] and that a high degree of structural specificity characterizes this inhibition[3]. Since the administration to rats of convulsant doses of MSO fails to affect the levels of 13 out of 14 other cerebral enzymes tested[4,5], it is likely that glutamine synthetase is the preferred molecular target for the drug. The previously described MSO-elicited effects on cerebral ultrastructure[4,6], function[5,7,8] and metabolism[9–12] may derive as a result. In the present communication treatment of glutamine synthetase by MSO *in vitro* ensuing in the permanent loss of synthetase activity is described. The novel feature of this drug–enzyme interaction is that it takes place in the absence of substrate.

Soluble preparations of glutamine synthetase were obtained as described previously[13,14]. Briefly, postmitochondrial supernatants of cerebral cortex homogenates prepared in water[13] were centrifuged for 1 h at 100 000 × *g* and the resulting pellet was rehomogenized in ice-cold 0.15 M NaCl and recentrifuged as above. The resulting supernatant (Fraction S_{15}) was used as the source of the enzyme. Specific activity was 50–60 units/mg of protein[15], a unit catalyzing the formation of 0.1 μmole ($A_{500\ m\mu}$: 0.085) of γ-glutamohydroxamate in 30 min at 37°. For preincubation of the enzyme with MSO, 1 ml of Fraction S_{15} was mixed with 0.5 ml of solution containing the desired components. For incubation, 0.1 ml of preincubation medium was pipetted into 0.21 ml of assay medium[16]. Inhibition of the enzyme by MSO carried over from the preincubation medium was suitably corrected. Synthetase from deer and sheep brain, respectively, was purified according to published methods[17,18].

The results indicate that the synthetase is effectively inactivated by MSO only when glutamate is absent and Mg^{2+} and ATP are both present. The inactivation by 3.3 mM MSO was 90% complete in 20 min at 37°; conversely, at 4°, MSO was totally ineffective. Preincubation of the enzyme with equimolar amounts of the *S*-isobutyl analog of MSO[3] at 37° led to about 20% inactivation in 20 min. The addition of glutamate resulted in virtually complete protection when a ratio of glutamate:MSO of 5 was reached. As indicated above, the common presence of Mg^{2+} and ATP was found to be essential for effective inactivation. With the concentration of Mg^{2+} maintained at 20 mM and that of ATP exceeding $7 \cdot 10^{-5}$ M, total inactivation ensued; conversely, with ATP below $2 \cdot 10^{-6}$ M, inactivation failed. Half-inactivation occurred with $0.9 \cdot 10^{-5}$ M ATP. Table I shows that dATP caused half-inactivation at $3 \cdot 10^{-5}$ M and that considerably higher concentrations of the other nucleotides were required to achieve this. The inactivating effect of 3.3 mM MSO could be alleviated by suitably adjusting the Mg^{2+} concentration. Thus, with $6.7 \cdot 10^{-5}$ M ATP, 50% inactivation was achieved at 0.3 mM Mg^{2+}; to achieve this when ATP was $1.33 \cdot 10^{-5}$ M, 4 mM Mg^{2+} was required.

It could be established that MSO inactivates cerebral glutamine synthetase equally well regardless of enzyme purity or species. Thus, 50% inactivation by 0.66

Abbreviation: MSO, DL-methionine-DL-sulfoximine.

mM MSO was obtained (species, specific activity and time (min) required) as follows: deer, 38, 9.5; deer, 109, 8; deer, 567, 11; pig, 60, 11 and sheep, 186, 10.

In attempts to reactivate inactivated enzyme MSO-treated Fraction S_{15} was passed through a Sephadex G-100 column equilibrated with 0.02 M potassium phosphate (pH 7.3). The eluted protein had no catalytic activity. When the preincubation medium, in which inactivation of the enzyme had just been carried out was made 20 mM in glutamate and the resulting solution allowed to incubate in the presence

TABLE I

INACTIVATION OF GLUTAMINE SYNTHETASE BY MSO: EFFECT OF NUCLEOTIDES

The pH of the nucleotide solutions was adjusted to 7 before addition to the preincubation medium containing the following substances: MSO: 3.3 mM; Mg^{2+}: 20 mM; 80 mM Tris–HCl, pH 7.2. Final vol.: 1.5 ml. At the end of the preincubation period (30 min, 37°), 0.1-ml aliquots were transferred to prewarmed tubes containing 0.21 ml of the glutamine synthetase assay medium[16] and the incubation was continued for 30 min. Control tubes contained nucleotide, but no MSO.

Nucleotide	*Nucleotide concn. ($M \times 10^5$)*	*Glutamine synthetase inactivation (%)*
ATP	0.9	49
ATP	6.7	100
dATP	3.2	50
dATP	15	83
CTP	85	91
dCTP	11	0
dCTP	265	76
ITP	34.5	51
UTP	49	31
GTP	58	35

of suitable controls for up to 5 h prior to assay, no enzymic activity could be detected.

The previously observed effectiveness of MSO as an inhibitor of glutamine synthetase is revealed by comparing the $K_{i(MSO)}$ ($6 \cdot 10^{-5}$ M) and the apparent $K_{m(glutamate)}$ ($3.8 \cdot 10^{-3}$ M) values[1,2]. That MSO is somewhat less effective as an inactivating agent is now shown by the fact that $3.3 \cdot 10^{-4}$ M was required to half-inactivate the enzyme for subsequent catalysis (ATP: $0.9 \cdot 10^{-5}$ M). Glutamate appears to modulate inhibition and inactivation differently, since for half-reversal of the inhibition concentrations 40 times higher were required than for half-reversal of inactivation.

A tentative interpretation of these observations assumes the simultaneous presence of two forms of the enzyme, the catalytically operant form with bound ATP and glutamate and a catalytically incomplete form to which only ATP is bound; MSO inactivates by somehow altering the site reserved for the binding of glutamate to the latter form; thereby, the conversion of the protein from inactive to active form is irreversibly impaired. This model is compatible with the hitherto proposed mechanism(s) of action of glutamine synthetase[19,20]. It is here supported by the finding that MSO alone, in the absence of ATP and Mg^{2+}, fails to alter the catalytic potential of the "native" enzyme. In further support is the observation that the amount of ATP

required to sensitize the enzyme to inactivation by MSO is about 5000 times less than that required for catalysis, closely approximating bound levels[20].

In conclusion, the observed modulation of the catalytic potential of glutamine synthetase by the interplay between MSO and its substrates may help toward the understanding of the drug's convulsant effects. If, indeed, MSO effectively prevents the absorption of glutamate by the obligatory enzyme–ATP complex, the concentration of glutamate is likely to rise in the perimembranous pool immediately available to the membrane-associated enzyme[13,14]. It may become sufficiently high as to elicit presynaptic excitatory foci[21] which, eventually, may spread to culminate in a seizure[22].

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1 O. Z. SELLINGER AND P. G. WEILER, JR., *Biochem. Pharmacol.*, 12 (1963) 89.
2 C. LAMAR, JR., AND O. Z. SELLINGER, *Biochem. Pharmacol.*, 14 (1965) 489.
3 O. Z. SELLINGER AND A. GARAZA, *Biochem. Pharmacol.*, 15 (1966) 396.
4 E. DE ROBERTIS, O. Z. SELLINGER, G. RODRIGUEZ DE LORES ARNAIZ, M. ALBERICI AND L. M. ZIEHER, *J. Neurochem.*, 14 (1967) 81.
5 O. Z. SELLINGER AND G. D. RUCKER, *Life Sci.*, 5 (1966) 163.
6 B. HARRIS, *Arch. Neurol.*, 11 (1964) 388.
7 E. PETERS AND D. B. TOWER, *J. Neurochem.*, 5 (1959) 80.
8 M. PROLER AND P. KELLAWAY, *Epilepsia*, 3 (1962) 117.
9 K. S. WARREN AND S. SCHENKER, *J. Lab. Clin. Med.*, 74 (1964) 442.
10 Z. S. GERSHENOVICH, A. A. KRICHEVSKAYA AND J. KOLOUSEK, *J. Neurochem.*, 10 (1963) 79.
11 J. K. TEWS AND W. E. STONE, *Biochem. Pharmacol.*, 13 (1964) 543.
12 J. FOLBERGROVA, *Physiol. Bohemoslov.*, 13 (1964) 21.
13 F. DE BALBIAN VERSTER, O. Z. SELLINGER AND J. C. HARKIN, *J. Cell Biol.*, 25 (1965) 69.
14 O. Z. SELLINGER, F. DE BALBIAN VERSTER, R. J. SULLIVAN AND C. LAMAR, JR., *J. Neurochem.*, 13 (1966) 501.
15 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
16 O. Z. SELLINGER AND F. DE BALBIAN VERSTER, *J. Biol. Chem.*, 237 (1962) 2836.
17 V. PAMILJANS, P. R. KRISHNASWAMY, G. DUMVILLE AND A. MEISTER, *Biochemistry*, 1 (1962) 153.
18 K. SCHNACKERZ AND L. JAENICKE, *Z. Physiol. Chem.*, 347 (1966) 127.
19 T. WIELAND, G. PFLEIDERER AND B. SANDMANN, *Biochem. Z.*, 330 (1958) 198.
20 V. P. WELLNER AND A. MEISTER, *Biochemistry*, 5 (1966) 872.
21 K. KRNJEVIC, *Brit. Med. Bull.*, 21 (1965) 10.
22 P. WIECHERT AND A. HERBST, *J. Neurochem.*, 13 (1966) 59.

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