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SHORT COMMUNICATION

Brain glutamine transferase: different rates of reappearance of the particulate and soluble components after inhibition by the convulsant methionine sulphoximine[1]

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RECENTLY, LAMAR (1968) has reported on the long duration of the inhibition of glutamine transferase (EC 6.3.1.2.) (GT) in rat liver, kidney and brain cortex after a single, subconvulsive dose of the convulsant methionine sulphoximine (MSO) (LAMAR and SELLINGER, 1965). We have independently observed a similar inhibition of this enzyme in the rat thalamus, hypothalamus and cerebellum (SELLINGER, AZCURRA and OHLSSON, 1968), noting, in addition, that the inhibition may be partially prevented by methionine and selected derivatives. We have also observed differences in the time required for methionine to achieve protection of the particulate as compared with the soluble component of cerebral GT activity (SELLINGER, DE BALBIAN VERSTER, SULLIVAN and LAMAR, 1966; DE ROBERTIS, SELLINGER, ALBERICI and RODRIGUEZ DE LORES ARNAIZ, 1967; SELLINGER and AZCURRA, 1968). More recently, we examined the time-course of the reappearance of activity of MSO-inhibited GT at its different subcellular sites in the hope that such a probe might reveal whether the activity of the enzyme returns to normal levels uniformly at all of its subcellular locations (SELLINGER and BORENS, 1969). BARONDES (1966) has examined, in a somewhat similar fashion, the reappearance of activity of isopropylhydrazine-inhibited monoamine oxidase of brain. In the experiments reported in this communication, the reappearance of activity of GT was determined in the homogenate and in three subcellular fractions which were isolated in parallel from the cerebral cortex and the cerebellum.

Pairs of male rats (Sprague–Dawley, 200–250 g) were injected intraperitoneally with 125 mg/kg of L-methionine-DL-sulphoximine (Pierce Chemical Co., Rockford, Illinois). After decapitation, the tissue was homogenized in 20 mM-Tris–HCl (pH 7·2), containing 10 mM-Mg-acetate (SELLINGER and AZCURRA, 1968), at about 1300 rev./min for 30 sec and the resulting suspension was centrifuged at 25,000 *g* for 10 min. The sediment was washed once (fraction *NEML*) and the final supernatant was centrifuged at 104,000 *g* for 60 min yielding a pellet (fraction *Mic*) and soluble fraction *S*. The activity of GT in the soluble and particulate fractions (SELLINGER *et al.*, 1968; SELLINGER and AZCURRA, 1968) was assayed according to SELLINGER *et al.* (1968). Table 1 provides a comparison of the activity and the intracellular distribution of the control and the inhibited GT, determined at 350 hr post-MSO. The distribution of the enzyme changed noticeably during this period: 29·3 per cent of the cortical activity was found in control fraction *NEML* compared to 40·3 per cent for the 350 hr post-MSO samples; the corresponding cerebellar component shifted similarly: from 28·6 per cent to 37·4 per cent. The activity of GT in the soluble fraction from both regions (Fraction *S*, Table 1) shifted in the opposite direction, from control values of 59·8 and 62·4 per cent for cortex and cerebellum respectively, to 47·5 and 53·3 per cent for the 350 hr post-MSO samples.

When the reappearance of GT was determined at seven times (Fig. 1) during the 308-hr experimental period, the rates of reappearance of the soluble and the particulate components of activity of GT differed significantly in both regions. The reappearance of activity of the former proceeded at a rate which was appreciably slower than the rate of reappearance of GT in fraction *NEML*. Reappearance of activity in the homogenate was at a rate which was ostensibly an average of the particulate and soluble rates.

It was shown previously (LAMAR, 1968; SELLINGER *et al.*, 1968) that the activity of GT decreases to about 10 per cent of control levels at all of its subcellular sites (*NEML*, *Mic*, *S*) within 2 hr after a single, subconvulsive dose of MSO. The present results, which show that activity in the microsomal component initially reappears the least rapidly (Fig. 1), complement previous findings which

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Abbreviations used: GT, glutamine transferase; MSO, L-methionine-DL-sulphoximine.

TABLE 1.—THE ACTIVITY AND THE INTRACELLULAR DISTRIBUTION OF GLUTAMINE TRANSFERASE IN THE CEREBRAL CORTEX AND THE CEREBELLUM BEFORE AND 350 hr AFTER A SINGLE DOSE OF L-METHIONINE-DL-SULPHOXIMINE

Region subcellular fraction	Cerebral cortex						Cerebellum					
	Protein		Glutamine transferase $t = 0$*		$t = 350$†		Protein		Glutamine transferase $t = 0$*		$t = 350$†	
	mg/g	%	u/g‡	%	u/g	%	mg/g	%	u/g	%	u/g	%
Homogenate	115·0	100	604	100	250	100	116·7	100	556	100	183	100
NEML	90·7	80·0	177	29·3	111	40·8	92·7	79·4	171	28·6	74·6	37·4
Mic	2·32	2·2	65·9	10·9	31·9	11·7	1·58	1·4	53·7	9·0	18·5	9·3
S	20·3	17·8	361	59·8	128	47·5	22·4	19·2	373	62·4	106	53·3
Recovery	99·4		99·8		108·4		101·8		107·5		108·9	

* Controls, uninjected animals.
† Hr after MSO.
‡ Units/g of wet tissue.

The tissue was obtained from uninjected ($t = 0$) and injected (see text) animals. The fractions consist of: myelin and the membranes of nerve endings, mitochondria and lysosomes (*NEML*); the smooth and rough endoplasmic reticulum (*Mic*) and the cell sap (*S*). A unit of GT is defined as the amount of enzyme forming 0·1 μmole of glutamohydroxamate under the conditions of the assay (SELLINGER *et al.*, 1968).

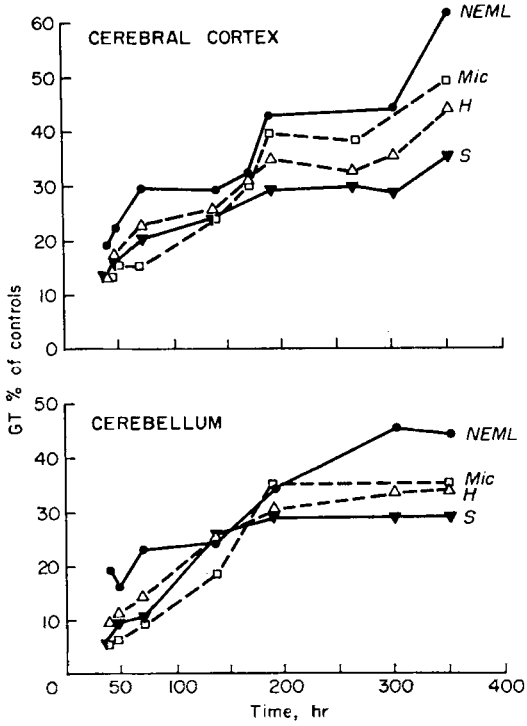

FIG. 1.—The time-course of reappearance of activity of glutamine transferase (GT) in the homogenate (*H*) and three subcellular fractions of rat cerebral cortex and cerebellum. *Abscissa:* Time, hr after administration of MSO; *ordinate*: per cent of activity determined in homogenate or fraction of uninjected controls (for absolute values and for definition of fractions *NEML*, *Mic* and *S*, see Table 1). Three sets of experiments are represented with death occurring as follows (hr post-MSO): set 1 at 41,137 and 305; set 2 at 45 and 188 and set 3 at 72 and 350 hr.

revealed that activity in this component also exhibits the most precipitous drop immediately after administration of MSO (SELLINGER *et al.*, 1968). It seems likely that the unusually slow reappearance of cortical and cerebellar activity of GT after a single dose of MSO reflects synthesis of new GT protein rather than a gradual, catalytic, reactivation of the enzyme. Whether synthesis takes place at more than one intracellular site (BARONDES, 1968; GORDON and DEANIN, 1968: BRAY and AUSTIN, 1968), and whether it proceeds at more than one rate remain to be elucidated.

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