Pharmacology Biochemistry & Behavior, Vol 4, pp. 689–694

Amino Acid Transport Inhibition: Brain and Behavioral Correlates[1]

CLARK T RANDT[2], STANLEY SAMUELS AND IRVING FISH

Milbank Research Laboratories, Department of Neurology, School of Medicine
550 First Avenue, New York NY 10016 U.S.A.

(Received 24 October 1975)

RANDT, C. T., S SAMUELS AND I. FISH. *Amino acid transport inhibition Brain and behavioral correlates* PHARMAC. BIOCHEM. BEHAV. 4(6) 689–694, 1976. – In vivo inhibition of uptake ^{14}C-L-valine by brain following subcutaneous administration of either of two γ-glutamyl cycle enzyme inhibitors, 2-imidazolidone-4-carboxylic acid (ICA), or, L-methionine-S-sulfoximine (MSO) is documented in C57BL/6J mice Dose related decrease in exploratory activity, impairment of memory for foot shock, and reduced operant responding for food reinforcement parallels the time course for interference with uptake of a large neutral amino acid by these two compounds previously shown to inhibit different enzymes in the γ-glutamyl cycle subserving active amino acid transport.

Amino acid transport Behavior Glutamyl cycle Enzyme inhibition

ACTIVE amino acid exchange between blood and brain is supported by a number of transport systems [5,18] Several investigators in the early 1950's discussed the possibility that the reaction catalyzed by γ-glutamyl transpeptidase might be involved in amino acid transport [1, 3, 9, 35]. Orlowski and Meister [20] described the γ-glutamyl cycle for intracellular degradation and synthesis of glutathione which was postulated to act as a γ-glutamyl donor for combination with certain amino acids at brain and other cell membranes in an active transport process. Six enzymes have been shown to participate in this regenerative cycle [16, 17, 20] The conversion of 5-oxoproline to glutamate which is catalyzed by 5-oxoprolinase links reactions involved in the utilization of glutathione to those required for its resynthesis [36]. Inhibition of 5-oxoprolinase may be accomplished by administration of 2-imidazolidone 4-carboxylic acid (ICA) [37,38]. The well known convulsant and persistent glutamine synthetase inhibitor [29,30], L-methionine-S-sulfoximine (MSO), has been demonstrated to inhibit another step in the cycle by acting upon the enzyme γ-glutamylcysteine synthetase [13, 15, 21, 28] thus impairing formation of glutathione Both inhibitors have been shown to disrupt the γ-glutamyl cycle of the brain *in vivo* [22,23].

Three human diseases with inborn errors of metabolism generally associated with mental retardation have been related to possible enzyme defects in the γ-glutamyl cycle. Erythrocyte γ-glutamyl cysteine synthetase deficiency has been identified [11]. Glutathionuria with a deficiency of γ glutamyl transpeptidase has been reported [33]. In 5-oxoprolinuria [7, 8, 10] the enzyme gluthathione synthetase is impaired [40].

Low glutamine levels in blood and behavioral alterations, both clinically in phenylketonuria and maple syrup urine disease [6, 14, 24, 25] as well as in early life undernourished animals [27,31] may be related to defective competitive amino acid transport between blood and brain. Direct evidence of alteration of ^{14}C-L-valine uptake by inhibitors of 2 enzymes in the γ-glutamyl cycle *in vivo* and a survey of behavioral alterations in C57BL/6J mice are the subject of this investigation.

GENERAL METHOD

Animals

Experiments were performed on male C57BL/6J mice 100–150 days old which were obtained from The Jackson Laboratory at 40–50 days of age. They were housed in 17 × 23 × 35 cm wire covered opaque plastic cages in groups of 8–10 in a temperature controlled (21.0 ± 0.6°C) room with lights on 15 hr and off 9 hr.

AMINO ACID UPTAKE

Animals and Procedure

Male mice weighing 23–33 g, fasted overnight, were injected subcutaneously (SC) with (U) ^{14}C-L-valine exactly 30 min before decapitation. ICA (Aldrich Chemical Co.) 22.7 mmoles/kg (2.96 g/kg) was injected (SC) 45 min, 90 min, 3 hr or 24 hr prior to killing. MSO (the L-/R-S isomer, Schwartz-Mann) at a subconvulsant dose of 277.5 μmoles/kg (50 mg/kg) was injected (SC) 2 hr, 4 hr, 24 hr, 48 hr, or 72 hr before killing. Each brain was rapidly removed, divided in the sagittal plane for duplicate analysis, and frozen with dry ice. Brain halves were weighed, minced, dissolved in 12 volumes of tissue solubilizer (NCS,

[1] Supported by The National Foundation – March of Dimes.
[2] Reprint requests should be sent to this author.

Amersham/Searle) and heated overnight at 40–50°C. The samples were cooled and 0.36 volumes of glacial acetic acid were added followed by 15 ml of toluene based counting fluid. The vials were chilled and total brain radioactivity was measured in a Searle Isocap 300 liquid scintillation counter with external quench correction. All samples were counted to the 2% level of confidence (10,000 counts) and rechecked to verify that chemilumescence was not contributing to the final counts.

The identity of the radioactive tracer was verified by a modification of a thin-layer chromatographic procedure that has been shown to be particularly effective for resolving valine from other amino acids [4] and from acetic acid, glucose, lactic acid and 2-oxoisovaleric acid. Plasma (60.8 μl) was obtained from adult male mice killed exactly 30 min after receiving a subcutaneous injection of (U) ^{14}C-L-valine (152 nCi/g, 655 p moles/g body weight) and was vigorously mixed with 4 volumes of 95% ethanol After centrifugation (710 × g for 10 min), the supernatant was evaporated to dryness with nitrogen and redissolved in 25 μl 80% (aq) ethanol This material was totally applied, as a 1 cm band, to a 20 × 20 cm sheet of Schleicher and Schuell (Keene, NH) microcrystalline cellulose (F1440) for monodimensional thin-layer chromatography (TLC). In adjacent columns, "cold" valine (35 nmoles) and an amino acid mixture (Stuart Amino Acids, Atlas Chemical Co., Pasadena, CA, 175 μg) with added valine (35 nmoles) were applied The TLC sheet was first chromatographed in 80% (aq.) ethanol to a height just above the origin line and air dried. This procedure was repeated in order to sharpen the origin and enhance resolution. After thorough drying, the chromatography was continued in 70% (aq.) n-propanol [4] to about 1 5 cm from the top (7 hr). The chromatogram was air dried overnight and the chromatography in 70% (aq.) n-propanol was repeated After final drying, the side strips were cut off and developed with ninhydrin [32]. The center strip containing the plasma extract was cut into 10 mm or 5 mm (in the valine region) portions and placed in vials for liquid scintillation counting with 15ml toluene containing 0.6% (w/v) 2,5 – diphenyloxazole (PPO). Radioactivity was measured in an Isocap 300 (Searle Analytical) for 100 min or 10,000 counts (2% reliability). Quench was corrected for by the channels ratio method

Results

The effects of ICA and MSO over time on the uptake of (U) ^{14} C-L-valine by the total brain in vivo was documented (Table 1). The brains from ICA treated mice showed an uptake of ^{14} C-L-valine, 45 min after SC administration of 2.96 g/kg, that was 60.6% of that found in control animals (t = 4 51, $p<0.001$). Significant but less marked depression of uptake of ^{14}C-L-valine was recorded 90 min and 3 hr after injection. No difference was found between the controls and other mice killed 24 hr after receiving ICA. Four hr after the SC administration of MSO 50 mg/kg, the uptake of ^{14}C-L-valine in the brain was 74.1% of that found in the controls (t = 5.63, $p<0.001$). Recovery to control values was incomplete 48 hr after administration of this dose of MSO, however, the effect on brain ^{14}C-L-valine injected 30 min before killing was not apparent 2 or 72 hr after MSO injection. Chromatographic evaluation of the soluble radioactivity in the plasma 30 min after administration of (U) ^{14}C-L-valine revealed that the label was predominantly in valine with a small portion (less than 15%) found on the chromatogram in the area characteristic of 2-oxoisovaleric acid and lactic acid About 7% of the soluble radioactivity was found in the chromatographic region occupied by acetate, glucose and the nonessential amino acids Therefore, 78% of the soluble radioactive label available for transport from blood to the brain, was still in L-valine 30 min after its injection (Fig. 1).

TABLE 1

THE EFFECT OF 2-IMIDAZOLIDONE-4-CARBOXYLIC ACID (ICA) AND METHIONINE SULFOXIMINE (MSO) ON THE UPTAKE OF L-14C-VALINE BY MOUSE BRAIN *IN VIVO*

Experiment	Drug Injection	Normalized Counts*	N	t	p†
ICA	Control	100 ± 2 59	17		
	45 min	60 6 ± 3 30	6	7 925	0 001
	90 min	84 3 ± 3 08	6	3 193	0 01
	3 hr	89 2 ± 3 46	6	2 154	0 05
	24 hr	95 6 ± 4 34	6	0 835	NS
MSO	Control	100 ± 2 35	19		
	2 hr	94 9 ± 3 14	6	1 078	NS
	4 hr	74 1 ± 1 68	8	6 592	0 001
	24 hr	73 8 ± 2 20	7	6 148	0 001
	48 hr	88 3 ± 4 35	5	2 199	0 05
	72 hr	107 ± 2 02	5	1 427	NS

*Mean ± S E M dpm/mg fresh weight with mean control values adjusted to 100

†Probability, two tailed t-test

BEHAVIOR

Experiment 1

Exploratory activity was measured in a circular open field 45 cm in dia. with a solid white painted floor divided into 9 segments by 2 cm wide black lines under a fluorescent light of 30 lumens intensity. Male adult mice were placed in milkshake cartons, half filled with cotton, with 4 ventilating holes in the tops, after being injected intraperitoneally (IP) with ICA 2.96 g/kg, 1.48 g/kg, or saline 0.3 ml, 45 min before testing. Mice given MSO 50 mg/kg, 25 mg/kg or saline 0.3 ml (IP) were placed in similar cartons 4 hr prior to testing. One week later, after similar intervals in the cartons for each group, the test was rerun without prior ICA, MSO, or saline injections. After being placed in the open field, the frequency with which animals intersected 4 infrared light beams located 1 cm above the floor, thus activating photocells, was recorded automatically for 3 consecutive 5 min intervals.

Results

Analysis of variance indicated that ICA significantly reduced activity 45 min after injection, $F(2,27) = 80.50$, $p<0.001$. MSO injected 4 hr before test likewise decreased activity, $F(2,27) = 28\ 97$, $p<0.001$. Both enzyme inhibitors acted in a dose dependent manner (Fig 2, a and c) Activity decreased as a function of time in the open field (ICA, $F(2,27) = 54.94$, $p<0.001$; MSO, $F(2,27) = 32.67$, $p<0.001$). The interaction between drug and time was significant only for MSO treated mice, $F(2,27) = 13.7$, $p<0.001$. The nature of this interaction is shown in Fig. 2c. Repeat testing 7 days later without further administration of the drugs or saline, showed a return to previously

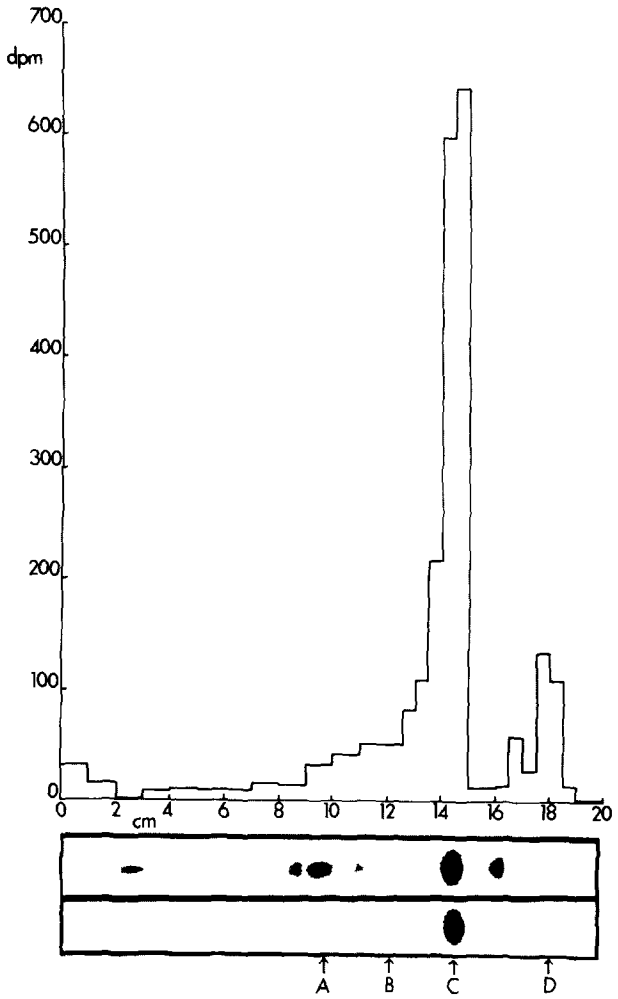

FIG. 1. Thin-layer chromatographic distribution of the soluble radioactivity in the plasma 30 min after administration of (U) ^{14}C-L-valine The origin was at 1.5 cm and the solvent front at 18.5 cm. (A) indicates the area of alanine and glutamate The approximate region of glucose and acetate is indicated by (B) Valine is marked by (C) The chromatographic area characteristic of 2-oxoisovalerate and lactate is indicated by (D)

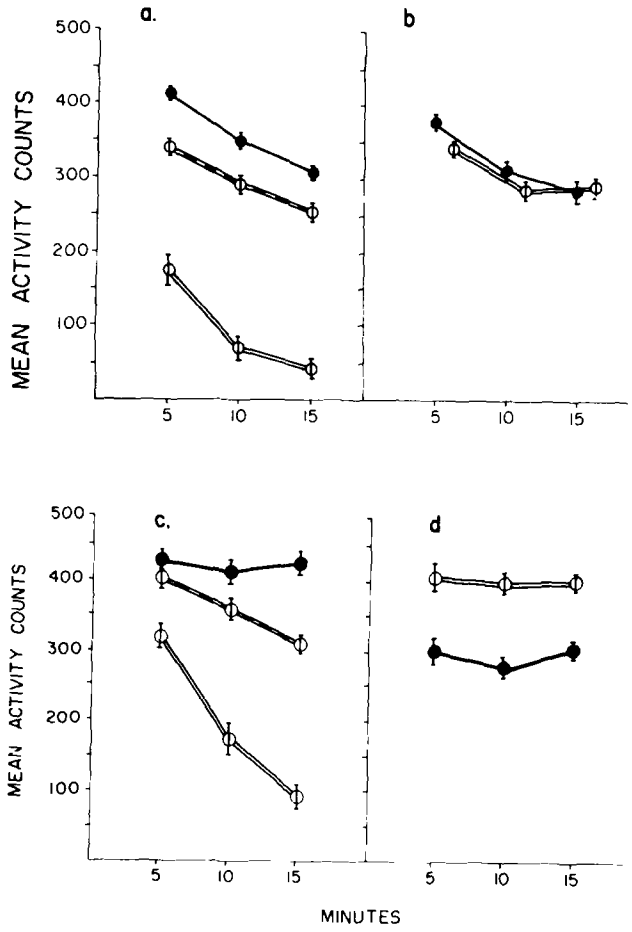

FIG 2 ICA and MSO effects on open-field activity for 6 groups of mice, N = 10 each, mean activity counts with SEM for 3 consecutive 5 min intervals. (a) ICA (1.48 g/kg) ▭, ICA (2.96 g/kg) ▭ saline ●—● (0.3 ml) given IP 45 min before test. (b) ICA and saline mean activity counts 7 days after ICA (c) MSO (25 mg/kg) ▭ MSO (50 mg/kg) ▭ and saline (0 3 ml) ●—● given IP 4 hr before test (d) MSO and saline mean activity counts 7 days after MSO.

established saline injected control levels of activity in the MSO (50 mg/kg) treated mice suggesting possible lack of retention of memory for the first exposure, or, alternatively, increased activity 7 days after drug administration (Fig. 2d). The mice given ICA (2.96 g/kg) 7 days prior showed only the expected slight decrease in activity similar to saline controls presumably due to adaptation to the test situation (Fig. 2b).

Experiment 2

Retention of a 1 trial inhibitory avoidance response was tested in a 2 compartment black Plexiglas apparatus 7 x 14 cm and 10 x 28 cm both 12 cm high, with covers, separated by a sliding door. Male C57BL/6J mice single caged for 24 hr prior to training were placed in the start compartment for 15 sec following which the door was elevated activating a timer. The latency to step through to the large chamber was measured by lowering the door after entry which simultaneously stopped the clock and initiated scrambled foot shock (0.16 mA, 2 sec) through the grid floor. The details of injection, training, and test are given in Table 2.

Results

Mean latency to step-through into the large compartment was significantly shorter on testing 24 hr later following ICA (2.96 g/kg) given IP 45 min before training as compared with saline injected mice (t = 2.29, $p<0.05$). No significant difference in latency was apparent between MSO (50 mg/kg) and saline given 4 hr before training on testing 24 hr later. MSO and ICA given 24 hr before training with testing 24 hr later showed significantly decreased latencies to enter only for MSO as compared with saline (t = 2.19, $p<0.05$). Decreased activity in the open field test noted in Experiment 1 was not reflected in 24 hr test latencies which were, in fact, shorter as compared with saline controls in the 2 instances described above. The initial step out latencies for training 45 min after ICA

TABLE 2

STEP-THROUGH LATENCIES INTO FOOT SHOCK CHAMBER ON TRAINING AND ON TEST, 24 HR LATER TEST LATENCIES NORMALIZED FOR PARAMETRIC ANALYSIS BY SQUARE ROOT TRANSFORMATION

Group	N	Time of Injection (IP) Prior to Training	Mean Training Latency ± SEM (second)	t	p	Mean Test Latency 24 Hours After Training ± SEM (seconds)	t	p
Saline 0 3ml	15	45 min	14 ± 1 1			239 ± 24 7		
ICA 2 96g/Kg	14	45 min	30 ± 4 1	4 27	< 001	148 ± 35 2	2 29	<0 05
Saline	15	24 hr	14 ± 1 9			260 ± 19 2		
ICA 2 96g/Kg	15	24 hr	9 ± 1 3		NS	208 ± 22 3		NS
Saline 0 3ml	25	4 hr	15 ± 2 1			153 ± 20 9		
MSO 50mg/Kg	22	4 hr	14 ± 2 2		NS	94 ± 15 6		NS
Saline 0 3ml	29	24 hr	13 ± 1 9			142 ± 19 8		
MSO 50mg/Kg	29	24 hr	9 ± 1 1		NS	86 ± 15 5	2 19	<0 05

injections were significantly longer (t = 4.27, $p<0.001$) than saline injected controls, however, no difference in latency to step out prior to training shock was seen in the MSO treated groups.

Experiment 3

Performance on a fixed interval (FI) 2 min schedule of food reinforcement was used to assess the effects of ICA and MSO on operant responding. Three enclosed black painted wooden chambers 11 × 14 × 15 cm with stainless steel rod floors were equipped with single levers extending 2 cm from the wall, 2 3 cm above the floor, requiring 2.0 g to close a microswitch which delivered a 20 mg Noyes pellet from a feeder into a dispenser located adjacent to the lever. Male C57BL/6J mice were reduced by food restriction to 80% of their initial body weights and given training to lever press for food until a stable rate was achieved on the FI 2 min schedule. Constant rates were established after 30 min sessions given daily for 24 days. This training was followed by 4 daily sessions before which saline injections (0.3 ml) were given subcutaneously (SC) between the scapulae 45 min or 4 hr prior to the 30 min test period for adaptation to the injection. On the following day ICA (2 96 g/kg) SC 45 min, or, MSO (50 mg/kg) SC 4 hr before testing was administered to mice in each of the 2 groups.

Results

Fixed interval performance for 2 representative mice, one given ICA and the other MSO is illustrated (Fig. 3a). Correlated t tests were used for the following intragroup comparisons (Fig. 3b). The total presses during a 30 min period on the FI 2 min schedule showed 70% (t = 5.0, $p<0.01$) mean reduction following ICA and 88% (t = 5.45, $p<0.01$) decrease of mean bar presses after MSO. Bar pressing returned to predrug rates 24 hr later in the ICA group. There was persistent marked reduction of bar pressing of the MSO treated mice for at least 24 hr (t = 4.8, $p<0$ 01) with recovery to predrug levels at 48 hr (Fig. 3 a and b). Despite access to food in their home cages comparable to that previously observed, all animals lost weight during the 24 hr period following drug administration. The ICA group showed mean body weight reduction of 4.5% (t = 5.69, $p<0.01$) and, in the MSO treated mice there was 7.5% mean weight loss (t = 7.77, $p<0.001$).

GENERAL DISCUSSION

Inhibition of uptake of ^{14}C-L-valine by whole brain in vivo results from parenteral administration of ICA and MSO. Valine was chosen for the initial phase of this study because it is a large neutral amino acid with a moderately high rate of entry into the brain [19] and relatively slow incorporation into proteins [12]. Uptake of valine by the brain has been shown to be accompanied by an increase of tissue glutamine [2]. Administration of valine to mice treated with ICA has been demonstrated previously to cause a greater accumulation of 5-oxoproline in the kidney than did the enzyme inhibitor alone [38]. Our data directly confirm Meister's postulated action of 2 γ-glutamyl cycle inhibitors on transport of at least 1 amino acid. These preliminary observations should be extended to encompass other amino acids to assess the generality of the findings as regards active amino acid transport between blood and brain. More convincing behavioral correlations would make observations on 2 other large neutral amino acids, tyrosine and tryptophan which are aminergic neutrotransmitter precursors, of particular interest.

Gross behavioral descriptions of the subconvulsive effects of MSO in cat and rat have been reported [26, 34, 39] We observed similar reactions in C57BL/6J mice. MSO 100 mg/kg (IP) was followed by hyperirritability, generalized seizures, and death within 3 to 6 hr. Doses of 75 mg/kg induced hyperirritability and seizures within 24 hr. Seizures were only rarely seen after MSO 50 mg/kg however,

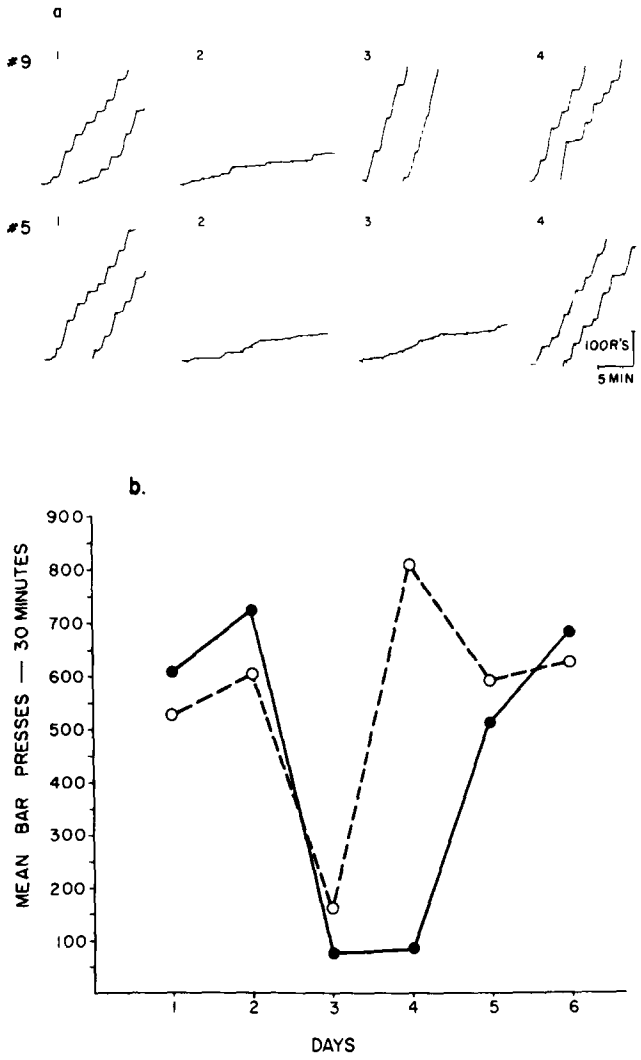

FIG 3. Fixed interval (FI) 2 minute schedule with food reinforcement. (a) Cumulative records depicting rate of bar pressing from 2 mice upper records, No 9, ICA lower, No. 5, MSO (1) saline injection, (2) ICA 2.96 g/kg SC, 45 min before and MSO 50 mg/kg SC 4 hr before test, (3) Twenty-four after ICA and MSO injections, and (4) Forty-eight after ICA and MSO injections. (b) mean rates during 30 min periods on FI 2 minute schedule, ICA o--o, N = 6, given SC 45 min before trial on Day 3, and, MSO •—•, N = 6, given SC 4 hr before trial on Day 3 in same doses as in (a) Mean rates for 3 subsequent days are shown.

a second dose of MSO 50 mg/kg within 24 hr after the first routinely produced seizures.

ICA in doses 100 fold higher (4.92 g/kg) produced lack of righting reflexes within 30 min and progressive immobility to death within 2 to 4 days. No seizures were apparent following ICA administration.

In this report, standard behavioral measurements of exploratory activity, one trial inhibitory avoidance of foot shock, and operant responding for food reinforcement following administration of ICA and MSO were documented. These observations constitute an initial survey providing information regarding dosage, duration of action and similarity in the characteristics of behavioral responses with both γ-glutamyl cycle inhibitors. This first systematic investigation of the effects of γ-glutamyl cycle inhibitors on behavior shows a dose dependent decrease in activity. Significantly shorter latencies on single trial passive avoidance of foot shock in the ICA and MSO treated animals tends to support the presumption of lack of retention unrelated to decrease in activity although the magnitude of effect is small and state dependency has not been ruled out. Reduction of response rate on the FI 2 min schedule may reflect decrease in activity or a reduction in motivation for food due to anorexia. Precise explanations await more extensive studies.

Although ICA and MSO are dissimilar structurally and, as far as is known, share only their effects on enzymes which involve the function of the γ-glutamyl cycle, the

congruence of behavioral alterations and time courses which parallel the effects of these enzyme inhibitors on the uptake of ^{14}C-L-valine between blood and brain is apparent. These observations may reflect a more general defect in amino acid transport with behavioral consequences. Additional investigation will be required before a causal relationship between amino acid transport interference and behavioral alterations can be established. These observations suggest novel approaches to further investigation of protein metabolic defects as they affect brain and behavior.

REFERENCES

1. Ball, E. G., O. Cooper and E. C Clarke. On the hydrolysis and transpeptidation of glutathione. *Biol Bull.* **105**: 369–370, 1953.
2. Battistin, L. A Grynbaum and A Lajtha. The uptake of various amino acids by the mouse brain *in vivo*. *Brain Res* **29**: 85–99, 1971.
3. Binkley, F. Metabolism of Glutathione. *Nature* **167**: 888–889, 1951.
4. Brenner, M and A Niederwieser. Dunnschicht-Chromatographie von Aminosauren. *Experientia* **16**: 378–383, 1960.
5. Cohen, S. R. and A. Lajtha. In *Handbook of Neurochemistry*, vol. 7, edited by A Lajtha. New York Plenum, 1972, pp. 543–572.
6. Dancis, J and M. Levitz. Abnormalities of branched chain amino acid metabolism (maple syrup urine disease). In *The Metabolic Basis of Inherited Disease, 3rd edition*, edited by J B Stanbury, J B. Wyngaarden, and D S Fredrickson. New York McGraw Hill – Blakiston, 1972, p 426–439.
7. Eldjarn, L., E. Jellum and O. Stokke. Pyroglutamic acidemia. Studies on the enzymic block and on the metabolic origin of pyroglutamic acid. *Clin Chim Acta* **40**: 461–476, 1972.
8. Hagenfeldt, L , A Larsson and R Zetterstrom. Pyroglutamic acidemia. *Acta paediatr scand* **63**: 1–8, 1973.
9. Hird, J F R., Doctoral Dissertation, Cambridge University, England 1950.
10. Jellum, E , T Kluge, H C , Borresen, O. Stokke and L. Eldjarn. Pyroglutamic acidema. A new inborn error of metabolism. *Scand. J clin Lab. Invest.* **26**: 327–335, 1970.
11. Konrad, P. N., F Prichards II, W. N. Valentine and D. E Paglia. γ-glutamyl cysteine synthetase deficiency. *New Engl J Med* **286**: 557–561, 1972.
12. Lajtha, A. and J Marks. Protein turnover. *Handbook of Neurochemistry*, vol 5B, edited by A. Lajtha. New York Plenum, 1971, p 551.
13. Levitow, L., A. Meister, E. L Kuff and G. H. Hogeboom. Studies on the relationship between the enzymatic synthesis of glutamine and glutamyl transfer reaction. *J. Am Chem Soc* **77**: 5304–5308, 1955.
14. McKean, C M and N. A. Peterson. Glutamine in the phenylketonuric central nervous system. *New Engl J Med.* **283**: 1364–1367, 1970.
15. Meister, A. *The Enzymes.* Vol 3, New York Academic Press, pp. 443–468, 1962.
16. Meister, A. On the enzymology of amino acid transport. *Science* **180**: 33–39, 1973.
17. Meister, A. Symposium on Brain Dysfunction in Metabolic Disorders, F Plum ed. Res Publ. A.R.N.M.D., New York Raven Press, 1974, pp. 273–291.
18. Neame, K. D. Applied *Neurochemistry*, edited by A N Davison and J. Dobbing, Philadelphia, Penn Davis, 1968, pp 119–147.
19. Oldendorf, W H. Brain uptake of radiolabelled amino acids, amines, and hexoses after arterial injection. *Am J. Physiol* **221**: 1629–1633, 1971.
20. Orlowski, M and A. Meister. The γ-glutamyl cycle: A possible transport system for amino acids. *Proc. Natn Acad Sci.* **67**: 1248, 1970.
21. Orlowski, M and A Meister. Partial reactions catalyzed by γ-glutamyl cysteine synthetase and evidence for an activated glutamate intermediate. *J biol Chem* **246**: 7095–7105, 1971.
22. Orlowski, M and S Wilk. *In vivo* inhibition of γ-glutamyl cysteine synthetase by L-methionine-RS-sulfoximine, influence on intermediates of the γ-glutamyl cycle. *J Neurochem.* **25**: 601–606, 1975.
23. Palekar, A G., S. S Tate and A Meister. Decrease in glutathione levels of kidney and liver after injection of methionine sulfoximine into rats. *Biochem biophys Res Commun.* **62**: 651–657, 1975.
24. Perry, T L , S Hansen, B Tischler, R Bunting and S. Diamond. Glutamine depletion in phenylketonuria. *New Engl J. Med* **282**: 761–766, 1970.
25. Prensky, A L. and H. W. Moser. Brain lipids, proteolipids, and free amino acids in maple syrup urine disease. *J Neurochem* **13**: 863–867, 1966.
26. Proler, M. and P. Kellaway. The methionine sulfoximine syndrome in the cat. *Epilepsia* **3**: 117–120, 1962.
27. Randt, C T and B. M. Derby. Behavioral and brain correlations in early life nutritional deprivation. *Archs Neurol* **28**: 167–172, 1973.
28. Richman, P G , M. Orlowski and A. Meister. Inhibition of γ-glutamyl cysteine synthetase by L-methionine-S-sulfoximine. *J Biol Chem* **248**: 6684–6690, 1973.
29. Rowe, W B. and A Meister. Identification of L-methionine-S-sulfoximine as the convulsant isomer of methionine sulfoximine. *Proc. Natn Acad. Sci.* **66**: 500, 1970.
30. Ronzio, R. A., W B. Rowe and A Meister. Studies on the mechanism of inhibition of glutamine synthetase by methionine sulfoximine. *Biochem* **8**: 1066–1075, 1969.
31. Samuels, S. Early life nutritional deprivation: persistent alteration of blood and urine amino acids in mice. *Proc. Soc exp. biol. Med.* **143**: 1215–1217, 1973.
32. Samuels, S. High-resolution screening of amino-aciduria. *Archs Neurol* **10**: 322–326, 1964.
33. Schulman, J. D , S I. Goodman, J. W. Mace, A. D Patrick, F. Tietze and E. J.Butler. Glutathionuria: Inborn error of metabolism due to tissue deficiency of γ-glutamyl transpeptidase. *Biochem. biophys Res Commun.* **65**: 68, 1975.
34. Sellinger, O Z , J M Azcurra, W. G Ohlsson, H. H Kohl and R Zand. Neurochemical correlates of drug-induced seizures: selective inhibition of cerebral protein synthesis by methionine sulfoximine. *Fedn Proc* **31**: 160–165, 1972.
35. Springell, P H , Doctoral Dissertaion, Melbourne Univer , Australia, 1953.
36. Van der Werf, P , M Orlowski and A Meister. Enzymatic Conversion of 5-Oxoproline to L-Glutamate Coupled with Cleavage of ATP to ADP. *Proc. Natn. Acad. Sci.* U.S.A **68**: 2982–2985, 1971.
37. Van der Werf, P., R A Stephani, M Orlowski and A Meister. Inhibition of 5-oxoprolinase by 2-Imidazolidone-4-carboxylic acid. *Proc. Natn Acad. Sci.* U.S.A. **70**: 759–761, 1973.
38. Van der Werf, P , R A. Stephani, and A Meister. Accumulation of 5-oxoproline in mouse tissues after inhibition of 5-oxoprolinase and administration of amino acids: evidence for function of the γ-glutamyl cycle. *Proc. Natn Acad. Sci.* U.S.A. **71**: 1026–1029, 1974.
39. Wada, J. A , H. Ikeda, and K Berry. Reversible behavioral and electrographic manifestations induced by methionine sulfoximine. *Neurology* **17**: 854–868, 1967.
40. Wellner, V P , R Sekura, A Meister, and A Larsson. Glutathione synthetase deficiency, an inborn error of metabolism involving the γ-glutamyl cycle in patients with 5-oxoprolinuria (pyroglutamic aciduria). *Proc Natn Acad. Sci* U S A **71**: 2505–2507, 1974.