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EFFECT OF THE CONVULSIVE AGENT 3-MERCAPTOPROPIONIC ACID ON THE LEVELS OF GABA, OTHER AMINO ACIDS AND GLUTAMATE DECARBOXYLASE IN DIFFERENT REGIONS OF THE RAT BRAIN

ARVE KARLSSON, FRODE FONNUM, DIDRIK MALTHE-SØRENSSEN and JON STORM-MATHISEN

Norwegian Defence Research Establishment, Division for Toxicology, PO Box 25-N-2007 Kjeller, Norway

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Abstract—Intraperitoneal injection of 3-mercaptopropionic acid into rats caused severe convulsions which started after about 7 min. Of the amino acids examined only the level of GABA changed after 4 min and immediately before (6·5–7 min) the convulsions started. The decrease in GABA concentration detected immediately before the onset of convulsions was about 35 per cent in the cerebral cortex, corpus striatum and cerebellum, 30 per cent in pons-medulla and 20% in hippocampus. Concomitant with the fall in GABA there was a large, reversible inhibition of glutamate decarboxylase activity in the brain. The uptake of GABA into synaptosomes isolated after injection of the convulsive agent was not reduced, and the uptake of GABA into synaptosomes was not inhibited by high concentrations of 3-mercaptopropionic acid added *in vitro*. During convulsions levels of aspartate and taurine decreased significantly in all the brain regions investigated. A small increase in glutamine was detected in pons-medulla and in cerebellum. Major changes in the concentrations of other amino acids such as glutamate, alanine, serine and glycine were found only in corpus striatum.

SEVERAL convulsive agents alter the levels of amino acids in the brain. In particular attention has been focused on changes in GABA and glutamine.[1–4] Also changes in taurine concentrations in the brain are considered to be of importance in epileptic seizures.[5,6] Injection of high doses of 3-mercaptopropionic acid (MP) leads to running and jumping fits of very sudden onset.[7] Lamar[8] showed that this agent inhibits both glutamate decarboxylase (L-glutamate 1-carboxylyase EC 4.1.1.15, GAD) and bacterial GABA aminotransferase (4-aminobutyrate:2-oxoglutarate aminotransferase EC 2.6.1.19) *in vitro*. Rodríguez de Lores Arnaiz *et al.*[9,10] reported that the levels of several amino acids and several enzymes connected with glutamate metabolism in cerebellum and cerebral cortex change during convulsions induced by MP. In addition morphological changes of Purkinje cells and some terminals were described.

In the present investigation we have studied the effect of MP on the concentrations of amino acids and GAD in different regions of the brain, paying particular attention to the period prior to the convulsions. In this way we hoped to demonstrate the biochemical changes leading to the convulsion rather than the changes caused by the convulsions. In addition we investigated the effect of MP on the uptake of GABA into synaptosomes.

MATERIALS AND METHODS

Adult male Wistar rats (150–200 g) were injected intraperitoneally under light ether anesthesia with a freshly prepared solution of MP (Koch-Light Laboratories Inc.) in 0·9% NaCl. Control animals were injected with 0·9% NaCl only. The rats were decapitated after 4,7,15,30 or 60 min and the brains were immediately taken out and placed on ice (maximum one min). The pons-medulla, cerebellum, hippocampus, corpus striatum and cortex were rapidly dissected out. For amino acid determination the different parts were homogenized in 10% (w/v) trichloroacetic acid (TCA) (maximum 10 min after decapitation). For the determination of GAD and choline acetyltransferase (acetyl-CoA: choline *O*-acetyltransferase EC 2.3.1.6) the tissue was homogenized in 25 mM sodium phosphate buffer, pH 7·4. In some experiments aminooxyacetic acid 25 mg/kg was injected intraperitoneally into rats 1 hour prior to the administration of MP or saline.

Determination of amino acids. Amino acids were analyzed by a modification of the method of Briel and Neuhoff.[11] The method is based on reaction of the amino acids with (dimethyl-^{14}C)-1-dimethylamino-napthalene-5-sulfonyl chloride ((^{14}C)DNS Cl, Commissariat à l'Energie Atomique, Departement des Radioélément, Gif-sur-Yvette, France). The (^{14}C)DNS-amino acids were separated by two dimensional t.l.c., and the amounts of (^{14}C)DNS-amino acids were determined by liquid scintillation counting. The yields of DNS-amino acids are governed by pH, temperature, reaction time and the ratio between the concentration of DNS Cl and amino acids,[11] and care was taken to standardize the reaction conditions. The TCA homogenates were centrifuged for 30 min at 20,000 *g*, the residues washed with 10% (w/v) TCA and recentrifuged for 10 min at 20,000 *g*. The combined supernatants were shaken with ether to remove TCA. The solutions were then evaporated to dryness and the residues dissolved in 0·2 M $NaHCO_3$, pH 9·0, giving a final concentration of 0·5 mg wet wt of brain tissue/20 μl, i.e. about 25 nmoles of amino acids/20 μl of $NaHCO_3$ solution. To 20 μl of this solution was added 20 μl 12·5 mM solution of (^{14}C)DNS Cl in acetone (DNS Cl: amino acid ratio about 10:1). The dansylation was allowed to proceed at room temperature for 2 hr. After evaporation to dryness the DNS-amino acids were solubilized in acetone–1N HCL (19:1). The DNS-amino acids were separated by two-dimensional chromatography on silica gel.[12] The thin layer chromatograms were developed with toluene–pyridine–acetic acid (100:50:3·5, by vol.) as the primary solvent and toluene–2-chloroethanol–25% NH_3 (100:80:6·7, by vol.) as the secondary solvent; DNS-taurine was separated from DNS-OH after removing all the other DNS-amino acid spots and rechromatographing DNS-taurine in the secondary solvent (Fig. 1). To the fluorescent spots of (^{14}C)DNS-amino acids was added a solution of 2·5% (w/v) collodion in ether/alcohol (7:1, v/v). After drying, the spots could be removed with a pincette and transferred to counting vials containing 10 ml toluene–Triton (2:1, v/v) scintillation mixture. The recoveries of the amino acids were determined in separate experiments adding tracer amounts of (^{3}H)amino acids to the homogenates. The recoveries were: glutamate 60%, GABA 90%, aspartate 95%, glycine 75%, serine 65%, alanine 100%, glutamine 80%, taurine 80%. The recoveries were taken into account in the tables and figures.

Determination of choline acetyltransferase activity. Choline acetyltransferase was assayed as described by Fonnum.[13] To 2 μl of a 40% (w/v) brain homogenate was added 2 μl of an incubation medium to give (final concn): 200 mM NaCl, 4 mM

EDTA, 17 mM sodium phosphate buffer pH 7·4, 0·06 mM physostigmine, 6 mM choline and 0·10 mM (1-^{14}C)acetyl-CoA (New England Nuclear Corp., Boston Mass), and 0·05% Triton X-100. After prewarming of both enzyme and substrate at 37°, the incubation was carried out for 1 min.

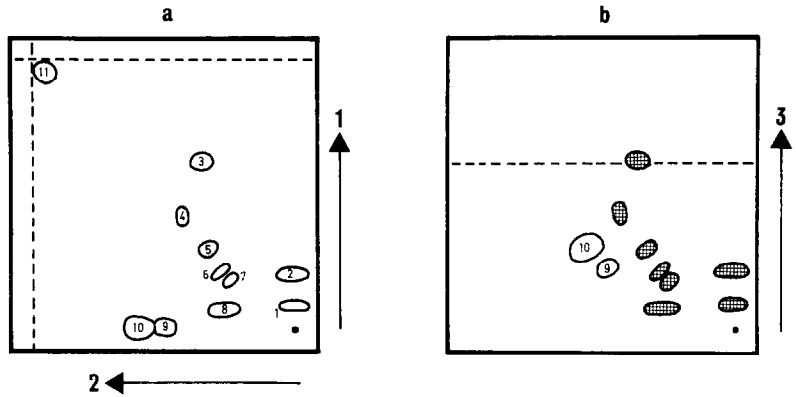

FIG. 1. Separation of DNS-amino acids from brain tissue by t.l.c. on silica gel. The directions of chromatography are indicated by arrows. (a) Two-dimensional separation of DNS-amino acids. (b) Separation of DNS-taurine and DNS-OH, by a third run in the direction of the first run after the other DNS-amino acid spots have been removed. The numbers on the chromatograms refer to: 1. DNS-aspartate; 2. DNS-glutamate; 3. DNS-GABA; 4. DNS-alanine; 5. DNS-glycine; 6. DNS-threonine; 7. DNS-serine; 8. DNS-glutamine + DNS-asparagine; 9. DNS-taurine; 10. DNS-OH; 11. DNS-NH_2.

Determination of GAD activity. GAD was assayed as previously described.[14] To 2 μl of a brain homogenate was added 2 μl of an incubation mixture to give (final concn): 0·97 mM L-(-^{14}C)glutamate (The Radiochemical Centre, Amersham, 20 mCi/mmol), 3·85 mM L-glutamate, 0·2 mM pyridoxal phosphate, 2 mM dithiothreitol, 38 mM sodium phosphate buffer, pH 6·5, 1 mM KCl and 0·2% Triton X-100. In addition about 2 mM L-glutamate (final concn) were suuplied by the 40% brain homogenate. The incubation was carried out for 10 min at 41°C with a 40%(w/v) homogenate and for 30 min with more dilute homogenates.

GABA uptake. GABA uptake activity was measured *in vitro* largely as described by Iversen and Johnston.[15] Twenty μl of homogenate prepared in 0·32 M sucrose was added to 2 ml of Krebs phosphate solution and after 5 min preincubation at 25° 200 μl of (2,3-^{3}H)GABA (10 Ci/m-mole, New England Nuclear Corp., Boston, Mass.) was added to give a final concentration of 5×10^{-8} M (*in vivo* injection of MP) or 10^{-7} M (MP added *in vitro*) of labelled GABA. The incubation was continued for 10 min, the samples were then cooled on ice, and centrifuged at 27,000 *g* for 10 min. The pellets were rinsed with 5 ml of cold 0·9% NaCl, resuspended in 1·2 ml of 10% Triton X-100 and counted in Triton–toluene (1 : 2) scintillation mixture. Passive binding of GABA was corrected for as described.[15] When MP was present in the incubation medium it was added 5 min before the labelled GABA.

RESULTS

Intraperitoneal injections of MP into rat caused after about 7 min a very sudden onset of convulsions. The time for the onset of the convulsion was almost independent of the doses used by us. With low doses (35 mg/kg) the animals apparently recovered from convulsions after 15 min, with higher doses (90 mg/kg) they recovered

after 30 min, and with even higher doses (150 mg/kg and 180 mg/kg) the animals died within 30 min. Rats injected with 25 mg/kg of aminocoxyacetic acid 1 hour prior to the administration of MP (50 mg/kg) showed no convulsions. The onset of convulsions was delayed to about 15 min in animals receiving higher doses of MP (90 mg/kg). Rats treated with aminooxyacetic acid appeared very sedate.

The amino acid values, as obtained with the dansylation technique, correspond with those previously obtained by other methods.[16] In two-dimensional thin layer chromatograms (Fig. 1) DNS-glutamine and DNS-asparagine were not separated and the values were given as the sum of these two. Difficulties were encountered in separating DNS-taurine from DNS-OH, and made the determination of taurine more laborious and consequently less accurate, than that of the other amino acids. Instantaneous freezing of the tissue was deemed unnecessary for the present study since amino acids such as glutamate and GABA change relatively slowly *post mortem*.[17]

GABA was the only amino acid that was significantly changed prior to the convulsions. A decrease of 20–30 per cent was found in all regions of the brain examined 4 min after the injection (Table 1). Just before the onset of the convulsion, about 7 min after injection, the concentration of GABA was decreased 30–35 per cent in medulla-pons, cerebellum, cerebral cortex and corpus striatum, and 20 per cent in the hippocampus (Table 2). Animals which showed signs of convulsions at this time were not used. The other amino acids showed only small and hardly significant changes.

During convulsions (Table 3), at 15 min after injection, there were significant changes in the levels of several amino acids in all regions of the brain. The largest changes took place in corpus striatum and the smallest changes in hippocampus. The levels of GABA, aspartate and taurine decreased in all parts of the brain examined. All the amino acids examined except glutamine-asparagine decreased significantly in corpus striatum. There was a small increase of glutamine-asparagine in pons-medulla and cerebellum and a less pronounced, but significant, increase of glutamate in pons-medulla. In one animal which received 150 rather than 90 mg/kg the results were similar to those in Table 3, but the changes in amino acids were slightly more pronounced. After 30 min the convulsions subsided and the amino acid levels approached normal values (2 animals). The GABA levels at this time were 10 per cent above those obtained at 15 min, the aspartate levels were still below normal in pons, hippocampus and striatum; glycine was low only in hippocampus.

The effect of MP on GAD was also investigated. The assay conditions were selected to be suboptimal with respect to glutamate concentration, because *in vitro* experiments have shown that the convulsant is a competitive inhibitor of the enzyme.[8] This was confirmed in experiments where the animals were killed 15 min after injection of MP (90 mg/kg), the degree of enzyme inhibition being very dependent on the final concentration of tissue. Thus with 20 per cent homogenates (final concentration) of hippocampus and cortex in the incubation medium 43 and 54 per cent of the enzyme activity was inhibited, respectively. In 2·5 per cent homogenates (final concentration) the corresponding inhibition was 10 and 0 per cent, and in 1 per cent homogenates no inhibition could be detected. In the subsequent experiments we therefore selected 20% homogenates for enzyme assays. There was a pronounced and significant decrease of GAD already 4 min after injection of MP (50 mg/kg). The

TABLE 1. LEVELS OF FREE AMINO ACIDS IN DIFFERENT REGIONS OF RAT BRAIN 4 min AFTER INTRAPERITONEAL INJECTION OF 3-MERCAPTOPROPIONIC ACID (90 mg/kg)

	Pons + medulla		Cerebellum		Hippocampus		Corpus striatum		Cerebral cortex	
Amino acid	C	MP	C	MP	C	MP	C	MP	C	MP
Asp	1·98 ± 0·05	94 ± 5	2·14 ± 0·11	90 ± 6	2·05 ± 0·08	95 ± 6	2·15 ± 0·10	93 ± 5	2·88 ± 0·11	95 ± 6
Glu	5·53 ± 0·14	98 ± 3	11·24 ± 0·63	98 ± 6	11·30 ± 0·45	94 ± 8	9·94 ± 0·47	100 ± 5	11·67 ± 0·49	97 ± 7
GABA	1·62 ± 0·04	77 ± 3*	1·79 ± 0·05	69 ± 3*	1·84 ± 0·07	81 ± 4†	2·06 ± 0·06	72 ± 3*	1·75 ± 0·09	71 ± 5†
Gly	3·52 ± 0·24	106 ± 8	1·22 ± 0·03	99 ± 5	1·33 ± 0·11	99 ± 11	1·18 ± 0·05	97 ± 6	1·16 ± 0·04	106 ± 6
Ala	0·64 ± 0·03	98 ± 7	0·76 ± 0·05	96 ± 8	0·77 ± 0·06	99 ± 9	0·75 ± 0·03	93 ± 9	0·67 ± 0·01	94 ± 4
Ser	0·84 ± 0·07	90 ± 10	0·91 ± 0·16	104 ± 19	1·18 ± 0·05	114 ± 6	1·12 ± 0·05	104 ± 7	1·19 ± 0·05	104 ± 5
Gln + Asn	3·20 ± 0·20	107 ± 8	4·41 ± 0·16	105 ± 5	3·94 ± 0·29	108 ± 9	4·29 ± 0·15	106 ± 6	4·99 ± 0·31	101 ± 7
Tau	1·84 ± 0·15	102 ± 10	4·21 ± 0·16	99 ± 7	2·76 ± 0·12	97 ± 8	4·21 ± 0·15	94 ± 4	5·07 ± 0·25	97 ± 5

Values from control animals (C) are expressed as μmoles/g wet wt ± S.E.M. and those from experimental animals (MP) as per cent ± S.E.M. of control values. S.E.M. for the quotient MP/C was calculated from those of MP and C[18] (and expressed as per cent of C), to take into account the uncertainty in the control value. Each value represents results from 5 extracts. Values differing significantly from controls are denoted by * for $P < 0.001$ and by † for $0.001 < P < 0.005$ (Student's *t*-test performed on the absolute values for MP and C).

TABLE 2. LEVELS OF FREE AMINO ACIDS IN DIFFERENT PARTS OF THE RAT BRAIN 7 min AFTER INJECTION OF 3-MERCAPTOPROPIONIC ACID (90 mg/kg)

	Pons − medulla		Cerebellum		Hippocampus		Corpus striatum		Cerebral cortex	
Amino acid	C	MP	C	MP	C	MP	C	MP	C	MP
Asp	2·11 ± 0·10	97 ± 7	2·27 ± 0·06	91 ± 3	2·18 ± 0·06	99 ± 5	2·17 ± 0·08	97 ± 6	2·79 ± 0·10	100 ± 7
Glu	5·65 ± 0·30	110 ± 6	11·88 ± 0·75	94 ± 9	11·24 ± 0·53	103 ± 6	9·75 ± 0·45	108 ± 9	12·06 ± 0·48	114 ± 7
GABA	1·60 ± 0·08	69 ± 5†	1·80 ± 0·12	64 ± 4*	1·83 ± 0·09	79 ± 4†	1·92 ± 0·06	67 ± 3*	1·46 ± 0·06	67 ± 4*
Gly	3·63 ± 0·43	112 ± 17	1·08 ± 0·10	101 ± 16	1·29 ± 0·11	103 ± 12	1·07 ± 0·09	109 ± 10	1·14 ± 0·07	97 ± 7
Ala	0·62 ± 0·17	77 ± 24	0·67 ± 0·10	91 ± 16	0·77 ± 0·06	103 ± 9	0·74 ± 0·15	89 ± 19	0·63 ± 0·02	95 ± 4
Ser	0·71 ± 0·03	87 ± 10	1·00 ± 0·15	97 ± 21	1·17 ± 0·06	100 ± 6	1·08 ± 0·07	93 ± 9	1·07 ± 0·06	92 ± 6
Gln + Asn	3·07 ± 0·19	106 ± 13	4·26 ± 0·20	110 ± 7	4·00 ± 0·20	98 ± 6	4·41 ± 0·26	93 ± 8	4·13 ± 0·19	92 ± 9
Tau	1·99 ± 0·12	99 ± 8	4·40 ± 0·21	97 ± 5	2·78 ± 0·18	97 ± 7	4·03 ± 0·23	92 ± 6	5·08 ± 0·13	97 ± 6

The data are presented as in Table 1.

TABLE 3. LEVELS OF FREE AMINO ACIDS IN DIFFERENT PARTS OF THE RAT BRAIN 15 min AFTER INJECTION OF 3-MERCAPTOPROPIONIC ACID (90 mg/kg)

Amino acid	Pons + medulla		Cerebellum		Hippocampus		Corpus striatum		Cerebral cortex	
	C	MP	C	MP	C	MP	C	MP	C	MP
Asp	2·31 ± 0·05	80 ± 7	2·35 ± 0·10	71 ± 4*	2·45 ± 0·06	64 ± 4*	2·18 ± 0·07	54 ± 3*	3·12 ± 0·04	71 ± 2*
Glu	5·95 ± 0·25	124 ± 8‡	11·20 ± 0·60	102 ± 9	11·21 ± 0·41	94 ± 4	9·55 ± 0·49	70 ± 6†	12·96 ± 0·32	93 ± 9
GABA	1·54 ± 0·06	68 ± 3*	1·70 ± 0·06	60 ± 4*	1·68 ± 0·04	77 ± 2*	1·92 ± 0·04	48 ± 4*	1·60 ± 0·04	68 ± 3*
Gly	3·69 ± 0·26	103 ± 10	1·23 ± 0·06	94 ± 6	1·44 ± 0·06	83 ± 4†	1·14 ± 0·06	60 ± 7*	1·11 ± 0·05	89 ± 5
Ala	0·67 ± 0·07	94 ± 11	0·81 ± 0·02	83 ± 10	0·80 ± 0·02	109 ± 8	0·91 ± 0·09	69 ± 8‡	0·68 ± 0·03	109 ± 10
Ser	0·76 ± 0·07	99 ± 11	0·79 ± 0·03	100 ± 8	1·11 ± 0·07	96 ± 9	1·25 ± 0·05	63 ± 4*	1·13 ± 0·07	101 ± 7
Gln + Asn	3·00 ± 0·27	135 ± 17	5·02 ± 0·18	120 ± 7	3·87 ± 0·15	116 ± 8	4·30 ± 0·23	93 ± 6	4·77 ± 0·18	102 ± 11
Tau	1·99 ± 0·11	68 ± 7†	4·44 ± 0·11	70 ± 3*	2·65 ± 0·11	76 ± 5†	3·98 ± 0·09	60 ± 3*	5·06 ± 0·14	66 ± 3*

The data are presented as in Table 1, but 6 rather than 5 sets of extracts were analyzed in each group. ‡ denotes $0{\cdot}010 < P < 0{\cdot}025$.

TABLE 4. ACTIVITIES OF GLUTAMATE DECARBOXYLASE AND CHOLINE ACETYLTRANSFERASE IN DIFFERENT REGIONS OF RAT BRAIN 15 min AFTER INJECTION OF 3-MERCAPTOPROPIONIC ACID (90 mg/kg)

Enzyme	Pons + medulla		Cerebellum		Hippocampus		Corpus striatum		Cerebral cortex	
	C	MP	C	MP	C	MP	C	MP	C	MP
Glutamate decarboxylase	16·3 ± 0·4	47 ± 1*	18·6 ± 0·1	46 ± 1*	19·1 ± 0·1	57 ± 2*	17·2 ± 0·3	61 ± 1*	20·8 ± 0·1	38 ± 1*
Choline acetyltransferase	16·1 ± 0·4	93 ± 3	1·09 ± 0·07	106 ± 9	10·30 ± 0·4	135 ± 9	26·4 ± 1·4	102 ± 6	8·3 ± 0·3	117 ± 5

The results are presented as in Table 1, except that the units for C are in μmole g^{-1} hr^{-1}. Each value is based on results from four animals. The final concentration of tissue in the assay mixture was 20%.

Values differing significantly from control values are denoted by * for $P < 0{\cdot}001$.

time course for GAD activity (Fig. 2) agrees well with that of GABA concentration (Tables 1–3) prior to and during convulsions. When the convulsions subsided the activity of GAD returned to normal. Table 4 shows that GAD, but not ChAc, decreased in all regions of the brain examined. ChAc was taken as a control to exclude any general effects of neurons. There was a slight regional variation in the decrease of GAD activity which did not coincide with the regional variation in GABA levels.

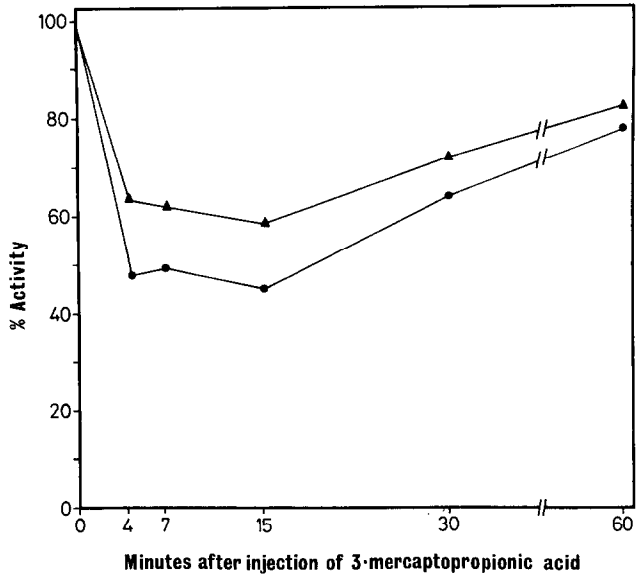

FIG. 2. Activity of glutamate decarboxylase in the rat cerebral cortex (●) and hippocampus (▲) as a function of time after administration of 3-mercaptopropionic acid (50 mg/kg). Enzyme activities were measured in 20% homogenates (final concentration) and given as % of controls injected with 0·9% NaCl. The points represent mean values from 2 or 4 animals, S.E.M. being within 10% of the mean.

The uptake of GABA in brain homogenates, presumably into synaptosomes, was studied after injection of high doses of MP (180 mg/kg) or in normal animals by including high concentrations of MP (0·1, 1 and 10 mM) in the incubation medium. We did not find any effect of MP in these experiments.

DISCUSSION

Injection of MP in doses that produce convulsions led to a rapid fall in the activity of GAD in rat brain (Fig. 2). This was accompanied by a decrease in the content of GABA in all regions of the brain investigated (Tables 1–3). The levels of GABA and GAD decreased prior to the onset of convulsions. The level of amino acids other than GABA did not change until during the convulsions.

The inhibition of GAD was easily reversible and was only detected at high tissue concentrations. This finding is in accordance with the *in vitro* studies of Lamar,[8] who showed that GAD is inhibited reversibly and competitively with respect to glutamate, and that the inhibition *in vitro* did not involve the coenzyme pyridoxal phosphate.

Our results extend the work of Rodríguez de Lores Arnaiz *et al.* who found that the level of several amino acids and enzymes changed in the cerebellum[9] and cerebral cortex[10] during convulsions induced by MP. They did not study the effects of MP prior to the onset of convulsions. Their results differ to some extent from ours. They found a significant reduction of GABA in cerebellum at low doses (75 mg/kg) of MP, but in cerebral cortex only at high doses (150 mg/kg). They found a large and significant increase in alanine in both regions of the brain and a significant fall in aspartate only in cerebellum. They did not examine taurine. Although they demonstrate in the last paper[10] that GAD is reversibly inhibited, they selected assay conditions (too low concentration of tissue and too high concentration of glutamate) that permit only a slight inhibition of GAD to be detected. This is probably the reason for their failure to demonstrate an effect of MP on GAD in nerve terminals.[10]

Rodríguez de Lores Arnaiz *et al.* ascribed the convulsive action of MP to a general disturbance of the glutamate–GABA metabolism.[9] Our results strongly suggest that the convulsions are caused by a reduction of GABA in the brain and that this reduction is caused by inhibition of GAD. Alternatively the decrease in GABA might be explained by the alleged activation of GABA aminotransferase.[9] The close correlation which exists between the levels of GAD and GABA in the brain, but not between the levels of GABA and GABA-aminotransferase[19] makes this possibility less likely.

We did not find any evidence for an inhibition of GABA uptake into synaptosomes in homogenates from animals injected with high doses of MP or in homogenates incubated with high concentrations of MP (up to 10 mM). Previously no effect on GABA uptake was obtained *in vitro* with lower concentrations (0·2 mM) of MP.[15] Since the uptake of GABA was undisturbed in animals injected with high doses of MP and since the levels of GAD returned to normal values 1 hr after injection of MP, or simply on dilution of the tissue homogenate, the loss of GABA is unlikely to be caused by rapid degenerative changes in the GABA-ergic structures. Rodríguez de Lores Arnaiz *et al.* described extensive morphological changes in the Purkinje cells in cerebellum,[9] but no such effect in the nervous elements in the cerebral cortex.[10] The reported "degenerative" reaction of the Purkinje cells[9] is therefore not representative of GABA-ergic neurons in general.

The degree of inhibition of GAD *in vivo* is difficult to estimate, since the intraterminal concentrations of MP and glutamate are unknown, but there are reasons to believe that it would be very high. Firstly, if we presume that glutamate is uniformly distributed in the brain (10 mM) and that MP is uniformly distributed in the body (0·8 mM), the enzyme would be 80–90 per cent inhibited according to the data of Lamar.[8] Secondly, we found 50 per cent inhibition of GAD in a test system with 10 mM glutamate and where the MP concentration was diluted 5-fold (20 per cent homogenate). Thirdly, the latency before the convulsions started was about the same in rats treated with from 35 to 180 mg/kg of MP. These data could be interpreted as indicating that GAD is rapidly and nearly totally inhibited after the injection of MP and that the rate of metabolism of GABA determines when (and if) the concentration of GABA drops below the "convulsion level". The reported regional variation in reduction of GABA, probably reflects the regional variation in the rate of metabolism of GABA, which does not coincide with that of GAD. The half life for the metabolism of labelled GABA formed from labelled acetate was previously found to be about 10 min.[20] The turnover of labelled GABA injected intraventricularly has

been reported to exhibit two phases, a rapid phase with a half-life of about 30 min and a slower phase with a half-life of several hours.[21] The faster turn-over rate corresponds with the fall in GABA observed by us.

Administration of aminooxyacetic acid prior to the convulsions either delayed or completely abolished the effect of MP. These findings strongly support the concept that the convulsions are caused by a loss of GABA in the brain, since aminooxyacetic acid increases the level of GABA by blocking its metabolism.[22] Therefore it either takes a longer time before the concentration of GABA falls below the "convulsion level", or it never does so.

It is of interest to notice that aspartate and taurine decreased in all parts of the brain during convulsions, since these two amino acids also decrease during epileptic seizures.[5,6] Taurine is formed by the action of an enzyme closely related to GAD on cysteinesulfinic acid,[23] which is structurally similar to MP. Like the fall in GABA, the marked reduction of taurine could therefore possibly be caused by inhibition of synthesis, although it does not appear until during convulsions.

Small increases of glutamine and asparagine were only found in medulla-pons and cerebellum (Table 3). Other convulsive agents such as methionine sulfoximine, cause formation of ammonia in the brain leading to large increases in glutamine.[3] The dramatic changes which take place in striatum during convulsions (Table 3) are striking. They may be related to the particular role this structure plays in the regulation of motor functions. These changes may be simply caused by disturbance of the metabolic balance resulting from the convulsions.

REFERENCES

1. M. Alberici, G. Rodríguez de Lores Arnaiz and E. De Robertis, *Biochem. Pharmac.* **18,** 137 (1969).
2. O. Z. Sellinger and P. Weiler Jr. *Biochem. Pharmac.***12,** 989 (1963).
3. E. L. Peters and D. B. Tower, *J. Neurochem.* **5,** 80 (1959).
4. C. F. Baxter, *Ann. N.Y. Acad. Sci.* **166,** 257 (1969).
5. N.-M. Van Gelder, *Brain Res.* **47,** 157 (1972).
6. I. Koyama, *Can. J. Physiol. Pharmac.* **50,** 740 (1972).
7. H. Sprince, C. M. Parker, J. A. Josephs Jr. and J. Magazino, *Ann. N.Y. Acad. Sci.* **166,** 323 (1969).
8. C. Lamar Jr., *J. Neurochem.* **17,** 165 (1970).
9. G. Rodríguez de Lores Arnaiz, M. Alberici De Canal and E. De Robertis, *J. Neurochem.* **19,** 1379 (1972).
10. G. Rodríguez De Lores Arnaiz, M. Alberici De Canal, B. Robiolo and M. Mistrorigo De Paceco, *J. Neurochem.* **21,** 615 (1973).
11. G. Briel and V. Neuhoff, *Hoppe-Seyler's Z. physiol. Chem.* **353,** 540 (1972).
12. J. P. Zanetta, G. Vincendon, P. Mandel and G. Gombos, *J. Chromat.* **51,** 441 (1970).
13. F. Fonnum, *Biochem. J.* **115,** 465 (1969).
14. F. Fonnum, J. Storm-Mathisen and F. Walberg, *Brain Res.* **20,** 259 (1970).
15. L. L. Iversen and G. A. R. Johnston, *J. Neurochem.* **18,** 1939 (1971).
16. W. A. Himwich and H. C. Agrawal in *Handbook of Neurochemistry* (Ed. A. Lajtha), Vol. 1, pp. 33–52. Plenum Press, New York (1969).
17. A. J. Patel, R. Balázs and D. Richter, *Nature, Lond.* **226,** 1160 (1970).
18. T. B. Crampler and Y. H. Yoe, *Chemical Computations and Errors.* Wiley, New York (1946).
19. C. F. Baxter, in *Handbook of Neurochemistry* (Ed. A. Lajtha), Vol. 3, pp. 289–353. Plenum Press, New York (1970).
20. S. Berl, in *Metabolic Compartmentation in the Brain* (Eds. R. Balázs and J. E. Cremer), pp. 1–17. Macmillan, London (1973).
21. G. G. S. Collins, *Biochem. Pharmac.* **22,** 101 (1973).
22. K. Kuriyama, E. Roberts and M. K. Rubinstein, *Biochem. Pharmac.* **15,** 221 (1966).
23. H. C. Agrawal, A. N. Davison and L. K. Kaczmarek, *Biochem. J.* **122,** 759 (1971).