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Seizures induced by allylglycine, 3-mercaptopropionic acid and 4-deoxypyridoxine in mice and photosensitive baboons, and different modes of inhibition of cerebral glutamic acid decarboxylase

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Summary

1. DL-C-Allylglycine, 4-deoxypyridoxine hydrochloride and 3-mercaptopropionic acid have been studied with reference to their convulsant effects in mice and in baboons (*Papio papio*) with photosensitive epilepsy, and their action on the cerebral enzyme synthesizing γ-aminobutyric acid (L-glutamate-1-carboxy-lyase).

2. In mice, the ED_{50} values for seizures following intraperitoneal injection were allylglycine 1·0 mmol/kg body weight, 4-deoxypyridoxine 1·1 mmol/kg and 3-mercaptopropionic acid 0·27 mmol/kg. Latency to seizure onset was longest after allylglycine (44–240 min), intermediate after 4-deoxypyridoxine (9–114 min) and shortest after 3-mercaptopropionic acid (2·5–8 min).

3. In *Papio papio* intravenous administration of subconvulsant doses of allylglycine (0·87–3·1 mmol/kg), or of 4-deoxypyridoxine (0·21–0·53 mmol/kg) enhanced the occurrence and persistence of myoclonic responses to intermittent photic stimulation, and augmented the associated electroencephalographic abnormalities, without modifying their character or distribution. Higher doses produced brief seizures recurring at regular intervals, between 2–14 h after allylglycine (4·0–4·3 mmol/kg) or 1–4 h after 4-deoxypyridoxine (0·53–0·87 mmol/kg). Electroencephalographically these seizures originated unilaterally in the occipital or posterior parietal cortex.

4. In *Papio papio* photically-induced epileptic responses were enhanced 5–10 min after the intravenous injection of 3-mercaptopropionic acid (0·09–0·28 mmol/kg). A sequence of brief generalized seizures followed by complete recovery occurred 4–17 min after the injection of 3-mercaptopropionic acid (0·28–0·38 mmol/kg). Fatal status epilepticus followed the injection of 3-mercaptopropionic acid (0·57–0·75 mmol/kg). E.E.G. records showed generalized cortical involvement at the onset of the seizures.

5. L-Glutamate l-carboxy-lyase (GAD) activity was determined in whole brain homogenates from mice killed at various intervals after receiving i.p. a convulsant dose of one of the compounds. Inhibition of GAD activity was evident 30–60 min before seizure onset following allylglycine or 4-deoxypyridoxine administration, and was maximal (40–60%) just before or during seizure activity. Addition of pyridoxal phosphate to the brain homogenate relieved inhibition produced by 4-deoxypyridoxine but not that produced by allylglycine. Inhibition of GAD activity in brain homogenates from animals killed 2 or

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4 min after injection of a convulsant dose of 3-mercaptopropionic acid varied from 0–49% depending on the dose of 3-mercaptopropionic acid and the concentration of substrate in the assay system.

6. Kinetic analysis of the inhibition of GAD activity following direct addition of the compounds to mouse brain homogenates indicated that 3-mercaptopropionic acid (0·01–0·5 mM) was competitive with respect to the substrate. A comparable percentage inhibition of GAD activity was obtained only with much higher concentrations of 4-deoxypyridoxine, i.e. 10–50 mM. Allylglycine *in vitro* was a very weak inhibitor of GAD activity.

7. Three biochemically different mechanisms underlie the inhibition of cerebral GAD activity that precedes seizures induced by allylglycine, 4-deoxypyridoxine and 3-mercaptopropionic acid. The data are consistent with a critical reduction in the rate of synthesis of γ-aminobutyric acid being responsible for the onset of seizures.

Introduction

Many drugs which interfere with the synthesis of pyridoxal phosphate or with its coenzymic function produce convulsions (Holtz & Palm, 1964). That such drugs inhibit cerebral glutamic acid decarboxylase (GAD) has been repeatedly demonstrated (Killam, 1957 ; Killam & Bain, 1957 ; Tapia, de la Mora & Massieu, 1969 ; Wood & Abrahams, 1971) but because of the wide range of their other metabolic effects, the significance of this inhibition in the causation of the seizures is not clear. Recently some convulsant drugs which are not known to interfere with the coenzymic function of pyridoxal phosphate, have been shown to inhibit GAD. Thus allylglycine inhibits cerebral GAD both *in vio* and *in vitro* (Alberici, Rodriguez de Lores Arnaiz & de Robertis, 1969) and 3-mercaptopropionic acid is an inhibitor *in vitro* (Lamar, 1970).

We have previously shown (Meldrum, Balzano, Gadea & Naquet, 1970 ; Meldrum & Horton, 1971) that three pyridoxal phosphate antagonists (isoniazid, thiosemicarbazide and 4-deoxypyridoxine) enhance photically-induced epileptic responses in the baboon, *Papio papio*, and at higher doses induce seizures which on electroencephalographic records characteristically originate unilaterally in the occipital or posterior parietal cortex.

We are now reporting experiments with three convulsant drugs that inhibit GAD in different ways. We describe their convulsant action in mice and baboons, and relate this to the time-course and biochemical features of the inhibition of cerebral GAD.

Methods

DL-C-Allylglycine (2-amino-4-pentenoic acid, mol. wt. 115), 4-deoxypyridoxine hydrochloride (mol. wt. 189·6) and 3-mercaptopropionic acid (mol. wt. 106) (all from Sigma Chemical Co., St. Louis, Missouri) were dissolved or diluted in sterile saline (0·9% w/v NaCl solution) directly prior to injection in baboons or mice.

Male white mice (strains CFW or BALB/C, wt. 20–30 g), were used for ED_{50} determinations, for *in vivo* GAD inhibition experiments, and for preparation of brain homogenates.

Mice for ED_{50} determinations were injected intraperitoneally in volumes of 0·1–0·2 ml and continuously observed for convulsions or death. Calculation of ED_{50} was by the method of Weil (1952).

For determination of *in vivo* GAD inhibition, mice were decapitated at the stated interval after i.p. drug injection. The brain was rapidly dissected out, weighed, and a 10% homogenate (w/v) prepared in ice-cold water containing 1·5% (v/v) Triton X-100 using an Ultra-Turrax (Janke & Kunkel, Kg.). The whole brain homogenate without further treatment was used as the source of the enzyme. GAD activity was measured by the production of $^{14}CO_2$ from L-glutamic acid-1-[^{14}C] (method of Roberts & Simonsen, 1963, as modified by Tapia & Awapara, 1969). The incubations were carried out in Warburg flasks, with homogenate equivalent to 30 mg tissue in a final volume of 1·1 ml. Final concentrations in the reaction mixture were 33 mM L-glutamic acid containing 0·1 μCi L-glutamic acid-1-[^{14}C], pH adjusted to 6·3 with KOH) and 73 mM phosphate buffer pH 6·3. In experiments with added pyridoxal phosphate, the final concentration of the exogenous coenzyme was 0·1 mM or 0·5 mM. Flasks were shaken for 20 min at 37° C in a water bath. The reaction was stopped by the addition of 0·2 ml 20% trichloroacetic acid (TCA), and shaking continued for a further 90 minutes. The $^{14}CO_2$ produced was absorbed in 0·1 ml of M hyamine hydroxide in methanol. This was transferred to a scintillation vial containing 10 ml of scintillation fluid (toluene/2-ethoxyethanol 7:3, with 2,5-diphenyloxazole 4 g/litre, and 1,4-di[2-(5-phyloxazolyl)]-benzene 0·1 g/litre) and counted in a Nuclear Chicago scintillation counter. Homogenates were assayed within 5 min of preparation and homogenates from control and drug-treated animals were always assayed concurrently. Samples to which TCA was added at the beginning of the incubation were carried through with each batch of assays.

For kinetic studies of GAD activity the assay conditions were the same as above, except that the final volume was 1·0 ml, and the buffer concentration 60 mM. The inhibitors were dissolved in phosphate buffer pH 6·3. GAD activity was calculated as (μmol glutamate consumed/g wet wt)/hour.

Ten adolescent baboons, *Papio papio*, from Senegal (weighing 3–6 kg), were chronically implanted with epidural electrodes as previously described (Meldrum & Horton, 1971). Animals were tested, seated in a primate chair, with polygraphic recording (Galileo, model 18B) of regional electroencephalographic activity, electrocardiogram and photic stimulation. Responses to intermittent photic stimulation, (IPS) (Dawe Strobotorch, 1202 D, at or around 25 flashes/s, 12 cm from the eye, for up to 5 min) were graded 0–4 as previously described (Meldrum *et al.*, 1970). Drugs were injected intravenously, and animals observed for seizures and tested with IPS at predetermined intervals. Retesting with another dose or drug occurred only after a minimum interval of 7 days.

Results

Seizures in mice

Table 1 gives the ED_{50} in mice for the induction of tonic seizures by each of the three drugs, and the mean latency to the first seizure. There was a wide variation in the time to seizures between drugs, and between animals. Seizures

TABLE 1. *Drug-induced seizures in mice; intraperitoneal ED_{50} (±95% confidence limits) and latency of first seizure after doses 1–2 times the ED_{50}*

	ED_{50} (mmol/kg)	Latency (min) mean±S.E. (*n*)
Allylglycine	$1{\cdot}0\pm^{0{\cdot}7}_{1{\cdot}3}$	160±12 (9)
3-Mercaptopropionoic acid	$0{\cdot}27\pm^{0{\cdot}24}_{0{\cdot}31}$	4·5±0·2 (22)
4-Deoxypyridoxine HCl	$1{\cdot}1\pm^{0{\cdot}9}_{1{\cdot}2}$	42·3±5·3 (14)

began 2–4 h after allylglycine 1·0 mmol/kg and recurred for 1–2 h; at this dose 2 out of 5 animals convulsing died after 4–5 hours. After allylglycine 1·9 mmol/kg 5 out of 6 animals died. Increasing the dose shortened the latency to death more than the latency to seizures; allylglycine 6 mmol/kg produced seizures after a mean interval of 66 min (range 44–104 min), with death at a mean latency of 83 min (range 63–105 min, $n=6$). Pretreatment with pyridoxine, 4 mmol/kg i.p., did not protect against seizures.

3-Mercaptopropionic acid had the lowest ED_{50}, the shortest latency to seizures, and the highest percentage surviving after seizures. The first seizure occurred between 2·5 and 8 min after the i.p. injection. After doses near the ED_{50}, mice convulsed 1–2 times and then recovered. After 3-mercaptopropionic acid 0·38 mmol/kg all of 6 animals convulsed but only 2 died. Doses in the range 0·80–1·9 mmol/kg were invariably fatal; death could occur at 3 min or as late as 26 min (during prolonged post-ictal depression).

Seizures began 9–114 min after 4-deoxypyridoxine (0·8–3·4 mmol/kg). The mean latency to the first seizure fell sharply with increasing dose and was 13 min ($n=6$) for 4-deoxypyridoxine 3·4 mmol/kg. Mortality was relatively high, 5 out of 6 died at 1·7 mmol/kg.

Epileptic responses in baboons

Data relating to drug doses and the time-course of the effects are summarized in Table 2.

The enhancement of myoclonic responses to IPS produced by allyglycine (0·87–3·1 mmol/kg) began within 1–2 h of the injection, reached a peak after 3–6 h, was still present at 9–12 h but had disappeared after 24 hours. One baboon showed a seizure in the absence of photic stimulation 3 h after allylglycine 1·04 mmol/kg, and several further seizures up to 10 h after the drug injection. This was an anomalous response as 5 other animals, in 9 experiments, received doses

TABLE 2. *Seizures in baboons. Drug dose-ranges enhancing photosensitivity, dose-ranges inducing seizures, and the time-course of these effects*

	Enhancing photosensitivity (mmol/kg)	Inducing seizures (mmol/kg)	Latency (min) Onset	Termination
Allylglycine	0·87–3·1		60–120	>720
		4·0–4·3	100–157	>840
3-Mercaptopropionic acid	0·09–0·28		5	10–15
		0·28–0·75	3–4	11–60
4-Deoxypyridoxine HCl*	0·21–0·53		15–30	180–300
		0·53–0·87	46–88	95–215

* Data from Meldrum & Horton, 1971.

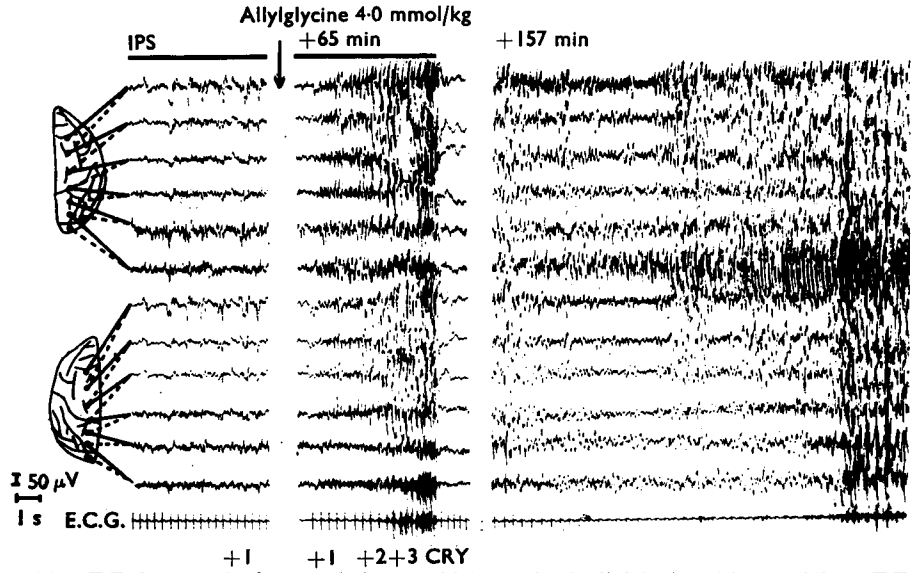

FIG. 1A. E.E.G. records from a baboon which received allylglycine 4·0 mmol/kg. E.E.G. derivations from right and left hemispheres as indicated in the diagrams. Predrug record during intermittent photic stimulation (IPS) shows eye movement artefacts anteriorly and a fronto-rolandic spike and wave associated with eyelid myoclonus, indicated as +1. IPS 68 min after the allylglycine injection readily induced myoclonus spreading to the face and trunk (+2) and limbs (+3). A generalized seizure (not shown) was provoked by IPS 127 min after drug injection. The first spontaneous seizure (at 157 min) originated posteriorly in the right hemisphere, although it was preceded by some fronto-rolandic spikes and waves.

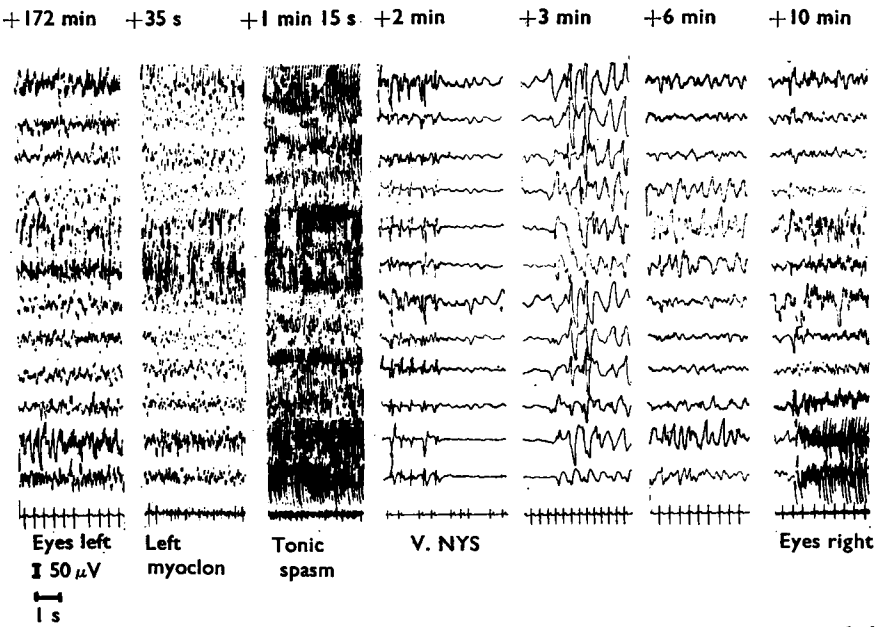

FIG. 1B. E.E.G. records illustrative of the prolonged sequence of seizures (same baboon as Fig. 1A) after administration of allylglycine 4·0 mmol/kg. E.E.G. derivations as for Figure 1A. At 172 min after the drug injection (and 9 min after the preceding seizure) spike discharges become sustained posteriorly in the right cortex. This activity spreads anteriorly 35 s after the first record. Myoclonus, initially unilateral, leads at 1 min 15 s into a symmetrical tonic spasm, terminating with slow rhythmic myoclonus, and then vertical nystagmus during the post-ictal depression (+2 min). Recovery is through various delta rhythms to faster activity. At 182 min after the drug injection a sustained spike discharge in the left hemisphere posteriorly, initiates another seizure.

in the range 1·22–3·10 mmol/kg, and none showed seizures in the absence of IPS (although all, except one receiving 1·39 mmol/kg, showed seizures in response to IPS). However, after allylglycine (4·0–4·3 mmol/kg) seizures recurred spontaneously, beginning 100–157 min after the injection and continued at mean intervals of 10 or 14 min (range 3–39 min) for more than 9–12 hours. On the E.E.G., these seizures characteristically began with an augmenting burst of spikes posteriorly, usually predominating on one side. This was associated with conjugate deviation of the eyes to the opposite side. Inter-hemispheric spread usually preceded generalization to the anterior neocortex. Widespread myoclonus was followed by a brief tonic spasm, rhythmic myoclonus and post-ictal depression (see Figure 1).

Myoclonic responses to IPS were enhanced 5–10 min after 3-mercaptopropionic acid (0·09–0·28 mmol/kg) but had returned to control levels at 15 minutes. Myoclonic responses continued beyond the end of IPS after 0·18–0·28 mmol/kg. One baboon receiving 0·28 mmol/kg and both animals receiving 0·38 mmol/kg showed 3–6 brief seizures, in the absence of IPS, beginning 4 min and ending 9–17 min after the i.v. injection. In the minute before the first 'spontaneous' seizure, isolated spikes and bursts of spikes and waves occurred, sometimes bilaterally and sometimes unilaterally, in various cortical regions, associated with eyelid myoclonus or jerks of the head or other parts of the body (see Figure 2). The tonic phase of the seizure was preceded by 5–15 s of irregular generalized spikes and waves and generalized myoclonus. The last brief seizure in the sequence was followed by a brief post-ictal silence, then delta activity. The rapid return to normal rhythms was interrupted by a few bursts of spikes and waves. IPS at 15 or 30 min failed to induce any myoclonic responses.

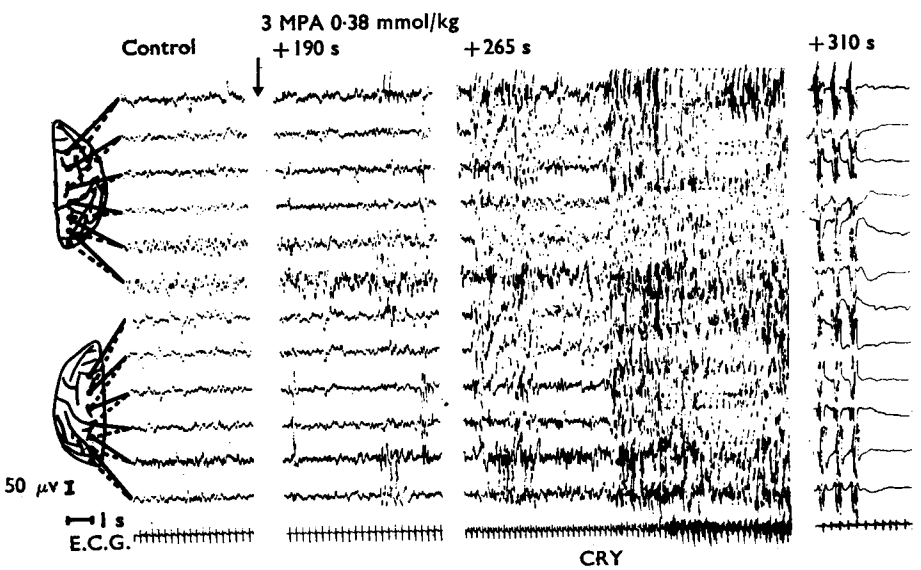

FIG. 2. E.E.G. records to show the onset of seizure activity following 3-mercaptopropionic acid (0·38 mmol/kg, i.v.) in the same baboon as in Figure 1. At 190 s after the injection spikes and waves appear singly and in bursts in frontal, parietal *or* occipital regions. At 265 s such activity becomes symmetrical and sustained, and is accompanied by generalized myoclonus, which evolves into the first of a sequence of tonic spasms. Each brief seizure terminates with rhythmic myoclonus and post-ictal depression (as at 310 seconds).

3-Mercaptopropionic acid 0·57 and 0·75 mmol/kg proved fatal. A repetitive series of brief seizures started 3 min after the injection, and continued at 1–2 min intervals to a total of 30 seizures in 40 min (0·57 mmol/kg) and 20 in 27 min (0·75 mmol/kg). In one case the second seizure and in the other the third and fourth seizures were atypical in that they began with a brief lateral deviation of the eyes. Normally each seizure began with symmetrical jerks involving the trunk and limbs and a cry, followed by a tonic spasm and mydriasis, then myoclonus ending with post-ictal depression at which time a vertical nystagmus was often seen. The intervals between the seizures became briefer, and the tonic phase less apparent, so that ultimately the E.E.G. showed continuous irregular seizure activity or intermittent generalized polyspikes and waves. Rectal temperature rose above 39·5° C, respiratory failure and cardiac arrest occurred after 60 min (0·57 mmol/kg) or 44 min (0·75 mmol/kg).

The doses of 4-deoxypyridoxine enhancing photosensitivity (Table 2) and inducing seizures in baboons have been described previously (Meldrum & Horton, 1971). The most characteristic feature of these seizures is their focal origin in one occipital cortex and their slow progressive generalization to the rest of the cortex ; in this respect they closely resemble those seen after allylglycine (illustrated in Figure 1).

In vivo *determination of GAD activity*

The results of GAD assays in brain homogenates from mice killed at various times after the i.p. injection of convulsant doses of the three drugs are shown in Figure 3. Control homogenates consumed 18–26 (μmol glutamate/g wet wt)/h without additional pyridoxal phosphate, and 31–45 (μmol glutamate/g wet wt)/h with pyridoxal phosphate 0·1 mM added.

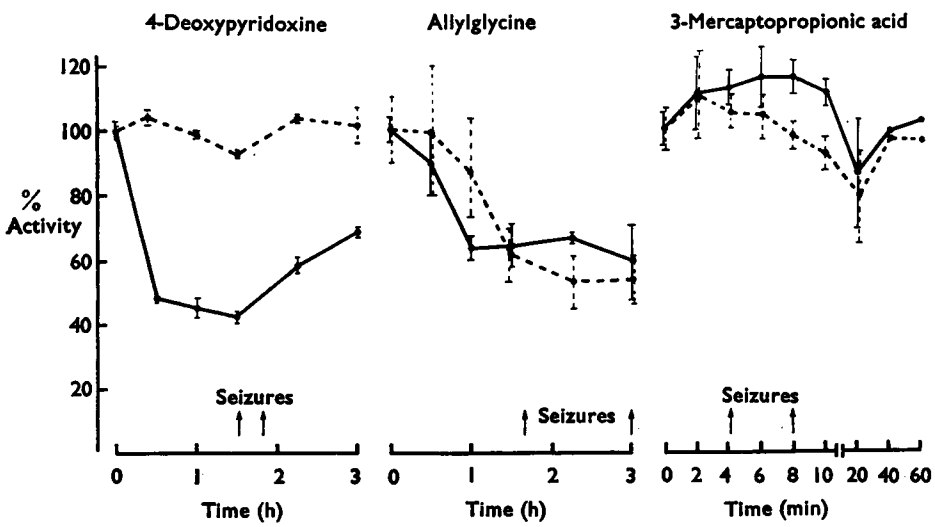

FIG. 3. Graphs showing glutamic acid decarboxylase activity (as % of control activities ±S.E.M.) in whole brain homogenates of mice killed at various intervals after the i.p. injection of (A) 4-deoxypyridoxine 1·18 mmol/kg, (B) allylglycine 1·74 mmol/kg and (C) mercaptopropionic acid 0·28 mmol/kg. Dotted lines refer to homogenates with additional pyridoxal phosphate (final conc. 0·1 mM); continuous lines refer to homogenates without additional pyridoxal phosphate. Duration over which seizures were observed is indicated by arrows.

After allylglycine (1·74 mmol/kg) mice showed 1–3 seizures at various intervals from 95–180 min after the injection. Cerebral GAD activity in mice killed at 90 min (i.e. just before the earliest seizures observed at this dose level) was inhibited by about 35%. This inhibition was not relieved by pyridoxal phosphate 0·1 mM (shown in Fig. 3); 0·5 mM pyridoxal phosphate (not shown) gave similar results to 0·1 mM.

After 4-deoxypyridoxine (1·18 mmol/kg) the mice had one seizure (between 92 and 112 min) and then recovered. GAD activity measured without the addition of pyridoxal phosphate declined rapidly during the first 30 min and then more slowly, reaching 58% inhibition at 90 minutes. After the seizure there was some recovery of GAD activity. The addition of pyridoxal phosphate (0·1 mM) restored GAD activity to control levels (except at 90 min when a 7% inhibition was seen).

Mice showed 1 or 2 seizures between 4 and 8 min after 3-mercaptopropionic acid (0·28 mmol/kg). Before and during this period there was a significant increase ($P<0·01$ by analysis of variance) in GAD activity (up to 16% at 8 minutes). At 20 min there was a slight fall subsequently followed by a return to normal. The early activation of GAD was not significant when homogenates were assayed after the addition of 0·1 mM pyridoxal phosphate.

Determination of GAD activity (with a glutamate concentration of 5 mM and brain homogenate equivalent to 60 mg of tissue) was also made after 3-mercaptopropionic acid 1·13 mmol/kg, which induced convulsions between 2 and 4 min and death usually between 4 and 5 min after injection. Animals killed at 2 min and 4 min showed a mean reduction in GAD activity, compared with concurrent controls, of 42% and 49% respectively ($P<0·001$, Student's t-test) in the absence of exogenous pyridoxal phosphate, and 53% and 60% respectively in the presence of 0·5 mM pyridoxal phosphate ($P<0·001$, Student's t-test).

In vitro *kinetic analysis of GAD inhibition*

Table 3 shows that 3-mercaptopropionic acid is a potent inhibitor of GAD *in vitro*, producing a 72% inhibition at 10^{-4}M, with 15 mM glutamate. Determinations at various substrate concentrations gave the plot of $1/v$ against $1/S$ shown in Figure 4A. The straight lines intersecting on the vertical axis indicate competition between 3-mercaptopropionic acid and glutamate for the active site of the enzyme. A Dixon plot (Fig. 4C) produced straight lines intersecting close to the vertical axis, consistent with competitive inhibition. The apparent Ki for 3-mercaptopropionic acid is less than 10^{-5}M.

Table 3 shows that allylglycine was much less potent than 3-mercaptopropionic acid as an *in vitro* inhibitor of GAD. Even 10^{-1}M allylgylcine produced only

TABLE 3. In vitro *determinations of GAD activity in whole brain homogenates in the presence of various concentrations of 3-mercaptopropionic acid, allylglycine and 4-deoxypyridoxine, expressed as % of the activity in control homogenates determined concurrently*

3-Mercaptoprop. (M)	GAD activity % of control	Allylglycine (M)	GAD activity % of control	4-Deoxypyrid. (M)	GAD activity % of control
5×10^{-4}	7·5	$1·2\times10^{-1}$	68·6	10^{-1}	0·4
10^{-4}	28·4	10^{-1}	68·8	5×10^{-2}	6·8
10^{-5}	81·0	6×10^{-2}	75·4	2×10^{-2}	54·1
5×10^{-6}	93·4	2×10^{-2}	85·7	10^{-2}	88·2

Final glutamate concentration was 15 mM.

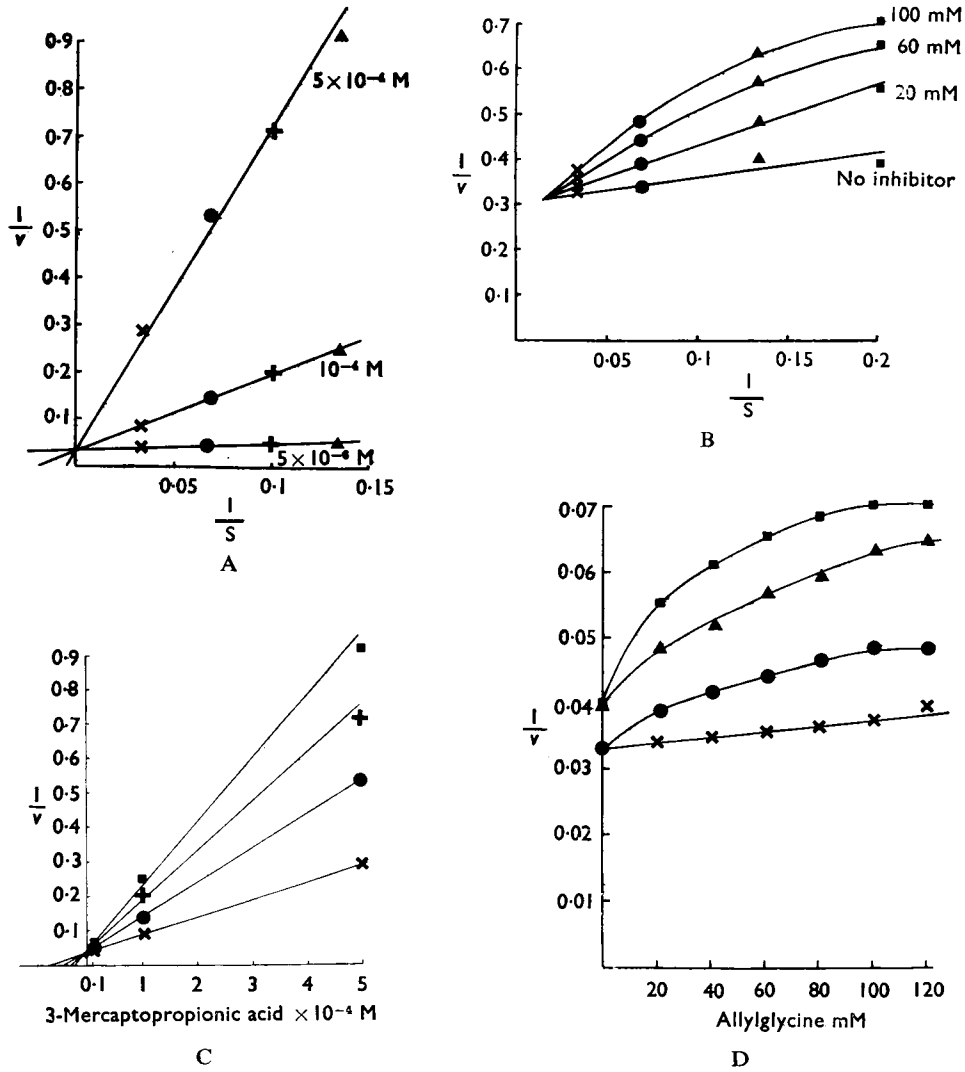

FIG. 4. Kinetic analysis of inhibition of cerebral glutamic acid decarboxylase by 3-mercaptopropionic acid (A and C) and allylglycine (B and D). Graphs A and B are Lineweaver-Burk plots of $1/v$ (v=(μmole glutamate consumed/g wet weight)/h) against $1/S$ (S=glutamate concentration in mM) for 3 inhibitor concentrations (3-mercaptopropionic acid 5×10^{-6}—5×10^{-4}M; allylglycine 20–100 mM). Graphs C and D are Dixon plots of $1/v$ against I (inhibitor concentration) for various substrate concentrations. Glutamate concentrations: 5 mM ■, 7·5 mM ▲, 10 mM +, 15 mM ● and 30 mM ×.

31% inhibition of GAD activity (with 15 mM glutamate). A Dixon plot (Fig. 4D) gave hyperbolic lines, suggesting that the inhibition is not purely competitive or non-competitive. Figure 4B shows that a plot of $1/v$ against $1/S$ failed to give straight lines indicating a departure from classical Michaelis-Menten kinetics.

A high concentration of 4-deoxypyridoxine is required *in vitro* to demonstrate GAD inhibition (see Table 3). With 4-deoxypyridoxine 10^{-1}M this inhibition becomes complete.

Discussion

The experiments described here show that the three drugs at dose-levels inducing convulsions inhibit cerebral glutamic acid decarboxylase activity with a time-course that corresponds to the time-course of the seizures. They also indicate that enzyme inhibition estimated only *in vitro* or by the usual *in vivo* method may be misleading; to obtain a valid picture both types of experiment must be considered in the light of the kinetics of the inhibitory action.

If i.v. injections in baboons and i.p. injections in mice are compared, doses producing seizures and the latency to seizure onset were similar for each of the drugs, except that proportionately higher doses of allylglycine were required to induce seizures in the baboon. However, over a wide sub-convulsant dose-range allylglycine produced a stable and long-lasting enhancement of photically-induced epileptic responses resembling in this respect 4-deoxypyridoxine and isoniazid (Medrum & Horton, 1971; Meldrum *et al.*, 1970). Also the E.E.G. pattern of the seizures after allyglycine was identical to that seen after 4-deoxypyridoxine or other drugs known to interfere with the synthesis or coenzymic function of pyridoxal phosphate, that is a repetitive sequence of brief seizures with intervening recovery of E.E.G. activity, each seizure originating unilaterally in the posterior parietal or occipital neocortex (Meldrum & Horton, 1971; Meldrum *et al.*, 1970). For 3-mercaptopropionic acid the latency of seizures after i.p. injection in mice as for i.v. injection in baboons was 4 min which is substantially shorter than the 15–20 min reported for rats (Lamar, 1970). Nevertheless it is longer than the 4–19 s latency for seizures in baboons after bicuculline (Meldrum & Horton, 1971). Following 3-mercaptopropionic both mice and baboons showed either a rapid return to normal or died directly after a brief period of seizure activity, in contrast to the more sustained effects of 4-deoxypyridoxine or allylglycine.

The nature of the inhibition of cerebral GAD activity following systemic administration of allylglycine remains obscure. The enhanced photosensitivity and the E.E.G. pattern of the seizures in baboons focuses attention on the synthesis and coenzymic function of pyridoxal phosphate. As the inhibition was still present after the addition of pyridoxal phosphate to brain homogenates from allylglycine-treated mice interference with the synthesis of pyridoxal phosphate cannot be responsible for it. Combination of allylglycine (or a compound derived from it) with the apoenzyme, preventing the normal binding of the coenzyme, remains a possible mechanism. The very weak inhibition shown in the *in vitro* studies is evidence against allyglycine competing with the substrate *in vivo* for the active site of the enzyme. The weak *in vitro* action of allylglycine and the length of the latent period *in vivo* are consistent with a metabolite of allylglycine being responsible for the GAD inhibition *in vivo*. Inhibition by allylglycine of protein synthesis in brain fractions has recently been demonstrated *in vivo* and *in vitro* (Alberici de Canal & Rodriguez de Lores Arnaiz, 1972). The relationship of this to the convulsant or GAD inhibitory action of allylglycine is not known.

The *in vitro* studies show that 3-mercaptopropionic acid is a fully competitive inhibitor of GAD, confirming the observation of Lamar (1970). It therefore follows that when 3-mercaptopropionic acid is administered *in vivo* and GAD activity estimated by addition of excess substrate to the brain homogenate, the true *in vivo* inhibition will be underestimated to an extent depending on the

change in the ratio of inhibitor to substrate. In fact no inhibition was seen after convulsant doses of 3-mercaptopropionic acid with assay conditions revealing marked inhibition after allylglycine or 4-deoxypyridoxine. Reduction of the substrate concentration and an increase in the dose of 3-mercaptopropionic acid however revealed a substantial degree of inhibition only 2 min after i.p. administration.

That 4-deoxypyridoxine inhibits GAD activity by interfering with the availability or coenzymic function of pyridoxal phosphate is widely accepted (Holtz & Palm, 1964) and was confirmed in our *in vivo* experiments by the absence of inhibition when pyridoxal phosphate was added to the brain homogenates. Pyridoxal phosphate antagonists may influence the activity of other cerebral decarboxylases and transaminases: the other biochemical effects of allylglycine and 3-mercaptopropionic acid have not been extensively investigated, but allylglycine appears to have little effect on cerebral transaminases but does produce slight inhibition of glutamine synthetase (Alberici *et al.*, 1969). 3-Mercaptopropionic acid is also a competitive inhibitor of bacterial GABA-transaminase, but does not affect glutamine synthetase (Lamar, 1970).

There is substantial evidence that glycine and γ-aminobutyric acid (GABA) act as inhibitory transmitters in the central nervous system. As allylglycine and 3-mercaptopropionic acid have some structural resemblances to these compounds it is necessary to consider whether they could act on synaptic transmission by mechanisms additional to their action on the synthesis of GABA. Iontophoretic studies with allylglycine in goldfish (Roper, 1970) indicated that convulsant systemic doses of allylglycine did not block strychnine-sensitive inhibitions, a result consistent with our observations in the baboon where the seizure pattern differs totally from that seen after strychnine. Iontophoretically applied allylglycine had a direct inhibitory effect resembling that of glycine, but this is unlikely to be related to the seizure induction. There is no information available about iontophoretic studies with 3-mercaptopropionic acid.

GABA probably acts as the inhibitory transmitter in several physiologically distinct systems; these include the intracortical inhibitory system of Krnjević, Randić & Straughan (1966a,b) some systems of collateral inhibition mediated by interneurones (Curtis, Duggan, Felix, Johnston & McLennan, 1971; Davidson & Southwick, 1971; Duggan & McLennan, 1971) and 'presynaptic' inhibition on afferent pathways (Curtis, Duggan, Felix & Johnston, 1971; Davidson & Southwick, 1971). Involvement of the latter two systems could explain the enhancement of all forms of 'reflex epilepsy' (i.e. seizures provoked by sensory stimulation) seen after pyridoxal phosphate antagonists (Pfeiffer, Jenney & Marshall, 1956; Lehmann, 1964; Meldrum *et al.*, 1970) and the GAD inhibitors described here.

The turnover of GABA is unlikely to be the same in the physiologically different inhibitory systems in which it is involved. Measurements in rabbits, in the 25 min pre-ictal period after administration of methoxypyridoxine (Hassler, Hassler & Okada, 1972) show a rapid fall in GABA concentration in brain areas with high GABA levels (caudate, putamen, superior colliculus and thalamus) but no change in other areas (including dorsal hippocampus and sensorimotor cortex). If 3-mercaptopropionic acid is producing seizures by an action on GABA synthesis, the rapidity of onset of the seizures implies that in certain areas the amounts of GABA available can be critically reduced in 1–3 minutes.

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