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ANTICONVULSANT ACTION OF ETHANOLAMINE-*O*-SULPHATE AND DI-*n*-PROPYLACETATE AND THE METABOLISM OF γ-AMINOBUTYRIC ACID (GABA) IN MICE WITH AUDIOGENIC SEIZURES

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Abstract—Mice susceptible to 'audiogenic' seizures (DBA/2, 21–25 days old) were treated with either di-n-propylacetate, DPA, (200–600 mg/kg, intraperitoneally) or ethanolamine-*O*-sulphate, EOS, (7·5–15 mg/kg, intracerebroventricularly). Motor behaviour was not modified 45 min after DPA (except for slight changes after 600 mg/kg). Seizure responses to auditory stimulation were severely reduced after DPA 400 mg/kg, and totally absent after 600 mg/kg. Brain γ-aminobutyric acid (GABA) concentrations were unchanged after DPA 200–400 mg/kg, but increased by 57% after 600 mg/kg. The latter dose inhibited brain GABA-transaminase (4-aminobutyrate-2-oxoglutarate aminotransferase) activity by 33%. Kinetic studies with brain homogenates failed to show inhibition of GABA-transaminase activity by DPA (5–15 mM), but demonstrated inhibition of succinic semialdehyde dehydrogenase by substrate competition. Mice tested 24 hr after EOS injection showed mild to moderate ataxia and were completely protected against 'audiogenic' seizures. Brain GABA concentration was increased 4–10 fold. GABA-transaminase activity was inhibited by 54–58%. There was no inhibition of succinic semialdehyde dehydrogenase activity.

There is substantial evidence that γ-aminobutyric acid (GABA) is an inhibitory transmitter in many brain areas, including the cerebral cortex, hippocampus and cerebellum [1, 2]. Several types of epileptic phenomena induced by convulsant drugs can be related to impaired synthesis or synaptic efficacy of GABA (reviewed by Meldrum [3]). There is some evidence that drugs which raise brain GABA concentration by blocking its further metabolism sometimes have anticonvulsant properties. Thus ethanolamine-*O*-sulphate (EOS) which irreversibly inhibits GABA-transaminase (4-aminobutyrate-2-oxoglutarate aminotransferase, EC 2.6.1.19) [4], when injected intracerebroventricularly in mice raises the brain GABA content [5] and diminishes the hindlimb extensor phase of the maximal electroshock response [6]. The effects of EOS on the genetically-determined syndrome of audiogenic seizures in mice [7, 8] have not so far been described. Di-*n*-propyl acetate (DPA, Sodium 2-propylpentanoate) has been shown to possess anticonvulsant properties in man and in several animal test systems [9, 11]. An increase in brain GABA concentration has been demonstrated in rodents following treatment with DPA [12, 13] and this increase has been attributed to inhibition of GABA-transaminase activity. However, it has recently been shown that DPA is a relatively weak inhibitor of GABA-transaminase but a rather potent competitive inhibitor of succinic semialdehyde dehydrogenase (SSA-DH; succinate semialdehyde: NAD^+ oxidoreductase EC 1.2.1.16) the enzyme responsible for the subsequent stage in the GABA-shunt pathway [14].

In this study we have treated mice genetically susceptible to 'audiogenic' seizures with EOS or DPA and correlated subsequent behavioural changes and altered convulsant response to auditory stimulation, with changes in brain GABA concentration and changes in the cerebral activity of GABA-transaminase and succinic semialdehyde dehydrogenase. We have also conducted further *in vitro* studies of these enzymic inhibitions to clarify the mechanisms involved.

MATERIAL AND METHODS

Coenzymes, enzymes and fine chemicals were obtained from Sigma Chemical Co., Ltd., (London), 2-keto[5-^{14}C]glutaric acid (specific radioactivity 5–20 mCi/m-mol) from Radiochemical Centre, Amersham, and all other reagents from commercial sources. DBA/2 mice were purchased from Fisons Pharmaceuticals Ltd. Di-n-propylacetate was a gift from Reckitt & Colman, Hull, and ethanolamine-*O*-sulphate from Dr. L. J. Fowler (School of Pharmacy, London University).

DBA/2 strain mice of either sex, 21–25 days old and weighing 7–13 g were used for all experiments unless otherwise stated. DPA (200, 400, 600 mg/kg, dissolved in saline) or saline alone, was injected intraperitoneally to groups of mice in a volume of 1% of the body weight of the animal, 45 min before auditory stimulation. EOS (7·5 or 15 mg/kg dissolved in a volume of 10 μl saline) or saline alone, was injected intracerebroventricularly—either on the left or the right side—under light ether anaesthesia 20–24 hr before auditory stimulation. After treatment all the mice were allowed food and water *ad lib.* in a constant ambient temperature of 30 to prevent a fall in body temperature.

At the appropriate time, the mice were placed under a dome (diameter 58 cm). During a 30-sec habituation period, pretest behaviour was assessed as follows: 0 = normal behaviour, 1 = walking slowly, 2 = severely reduced or absent spontaneous movements, 3 = lying on side, no spontaneous movements. Stimulation by an electric bell (Friedland Chimes, 3 in. diameter, producing 109 dB at mouse level) was applied for 60 sec or until tonic extension occurred.

The seizure response (SR) was scored as follows: 0 = no response, 1 = wild running, 2 = clonus, 3 = tonus. Only the maximum response was recorded for each animal.

Tissue preparation. At the end of 60-sec auditory stimulation or after tonic extension the mice were killed by immersion in Arcton 12 (ICI) cooled in liquid nitrogen. The brains were removed, and, while frozen, divided into two approximately equal parts and weighed. For estimation of the GABA concentration, homogenates were prepared in ice-cold 0·6 N perchloric acid solution containing 1 mM EDTA. Supernatants were removed following centrifugation, and the precipitates washed with 0·2 N perchloric acid. Washings and supernatants were combined and neutralised with 2 N KOH using phenolphthalein indicator. After 1 hr standing in ice, samples were centrifuged to remove potassium perchlorate and the solution made up to 10 ml with ice-cold glass distilled water.

For determination of *in vitro* GABA-transaminase (half) brains were homogenized in 10 vol (w/v) of ice-cold 1·5% (v/v) Triton X-100. The remainder of the 10% homogenate was then diluted to 4% by vol with ice-cold 1·5% Triton X-100 and used for the measurement of SSA-DH activity. For *in vitro* estimation of enzyme activities, whole brains of adult DBA/2 mice were used without freezing and homogenates prepared in a similar manner as for *in vivo* experiments.

GABA assay. The GABA concentration in the neutralized extracts was determined enzymically by measuring the reduction of NADP coupled to the transamination of GABA and α-ketoglutaric acid. Final concentrations in the reaction mixture (3 ml) were: 1·25 mM NADP, 2 mM α-ketoglutaric acid pH 7·2; 0·15 M pyrophosphate buffer pH 8·6; 5 mM 2-mercaptoethanol; 'Gabase' (as the source of GABA-transaminase and SSA-dehydrogenase) in a concentration to assure completed reaction within 60 min. Blanks without α-ketoglutaric acid, and standards of GABA (range: 25–500 μM) were analysed concurrently.

GABA–transaminase activity. The GABA–transaminase activity was measured according to Waksman *et al.* [15]. Final concentrations in the incubation medium (1 ml) were: 0·2 μCi 2-keto[5-^{14}C]glutaric acid, 25 mM α-ketoglutaric acid pH 6·5, 2 or 5 mM GABA 80 mM Tris buffer pH 8·2 and homogenate equivalent to 20 mg tissue. In each assay blanks with 0·2 ml of 20% trichloracetic acid added at the beginning of the incubation were analysed concurrently. For *in vitro* kinetic analysis, the buffer concentration was 40 mM and homogenate equivalent to 30 mg tissue was used. The drug was dissolved in glass-distilled water.

SSA–dehydrogenase activity. SSA–DH activity was measured according to the fluorimetric method of Kammeraat and Veldstra [16]. Final concentrations of the reaction mixture (1 ml) were: 0·33 mM NAD, 50 μM succinic semialdehyde (SSA), 4·5 mM 2-mercaptoethanol, 0·15 M pyrophosphate buffer pH 8·4, homogenate equivalent to 4 mg tissue. In each assay, blanks without SSA and standards of NADH (range: 5–100 μM) were analysed concurrently with the samples. For *in vitro* measurements of SSA DH activity 10% (w/v) homogenates were prepared in ice-cold glass distilled water and diluted to 0·1% with 0·5% Triton X-100. In the reaction mixture the pyrophosphate buffer concentration was 0·125 M and the homogenate was equivalent to 0·1 mg.

The SSA used in the assay was prepared according to Taberner *et al.* [17] and its concentration estimated enzymically by the method of Jacoby [18].

RESULTS

Pre-test behaviour and seizure response

Ethanolamine-O-Sulphate (Fig. 1). During the 30-sec habituation period the mice treated with saline showed normal spontaneous exploratory behaviour. All the animals gave a seizure response (SR) score of at least 2 (mean ± S.E.M. 2·60 ± 0·12). After EOS 7·5 mg/kg intraventricularly, 50% of the animals did not appear behaviourally different from saline-treated controls whilst the other 50% walked rather stiffly holding their hind-legs rigid (mean behaviour score 0·70 ± 0·12). The apparently unaffected animals showed a high SR score (2·60 ± 0·40, NS from saline): the affected animals were completely protected against the convulsant effects of auditory stimulation. EOS, 15 mg/kg, induced a marked change in behaviour in 80% of the mice. They had a hunched appearance with curved spine and closed or half-closed eyes and marked piloerection. Their spontaneous locomo-

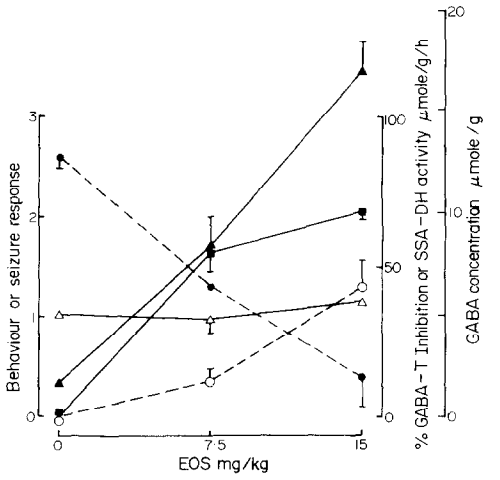

Fig. 1. Effect of intracerebroventricular injection of Ethanolamine-*O*-Sulphate (EOS) or saline in DBA/2 mice on the motor behaviour, the seizure response and on GABA concentration, GABA-transaminase and SSA-Dehydrogenase activities measured in the brain homogenates. Each point represents the mean value (± S.E.M.) of determinations in 10 animals. (▲——▲) GABA concentraton (μmole/g wet wt); (■——■) inhibition of GABA-T activity in per cent of control; (△——△) SSA-DH activity in μmole/g wet wt/h; (○——○) behaviour and (●——●) seizure responses, graded as described in Materials and Methods.

tor activity was inhibited (mean score ± S.E.M., 1·30 ± 0·26). All these animals were completely protected from seizures. However, 2 animals showed no behavioural change compared to saline-treated controls and these had SR scores of 1 and 3.

Di-n-propylacetate (Fig. 2). The saline-treated controls showed normal exploratory activity during the 30 sec habituation period and the mean seizure response ± S.E.M. was 2·80 ± 0·2. Treatment with DPA 200 mg/kg did not induce any behavioural change or significant protection against seizures (SR score, mean ± S.E.M. 2·20 ± 0·2).

After DPA 400 mg/kg the mice were substantially protected against seizures induced by auditory stimulation ($P < 0.0005$ compared with controls) but showed no evident behavioural deficit. After DPA, 600 mg/kg, behaviour in the 30 sec habituation period was not severely affected (all animals were assessed as scoring 1). The main effect was to induce either continuous fast walking round inside the dome (which continued even during the 60 sec test period) or slight ataxia. There was complete protection against seizures.

Biochemical determinations

Ethanolamine-O-sulphate (Fig. 1). In mice treated with EOS 7·5 or 15 mg/kg intracerebroventricularly the GABA concentration was increased up to 4- and 10-fold respectively compared with the concentration determined in saline injected animals.

GABA-transaminase activity was significantly inhibited at both drug concentrations. EOS, 7·5 mg/kg produced a 54% inhibition and 15 mg/kg a 68% inhibition of the enzyme activity found in the control.

The low dose of EOS did not significantly alter the SSA-dehydrogenase activity; with the high dose it was activated by 12%.

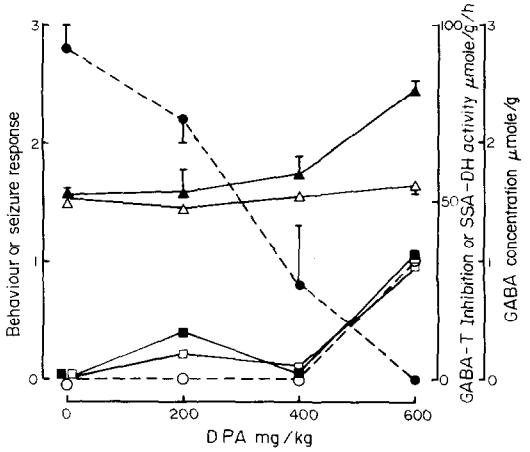

Fig. 2. The effect of Di-*n*-propylacetate (intraperitoneal in DBA/2 mice) on the behaviour, and on seizure response after auditory stimulation, and on the cerebral GABA concentration and activities of GABA-T and SSA-DH. Controls treated with 0·9% NaCl (i.p.). (O– –O) behaviour and (● – –●) seizure response graded as described in Materials and Methods; (▲——▲) GABA content in μmole/g wet wt GABA-T inhibition (per cent of control) in the presence of (■——■) 5 mM GABA and (□– –□) 2 mM GABA; (△——△) SSA-DH activity in μmole/g wet wt/h. Each point represents the mean value ± S.E.M. of determinations performed in 5 animals.

Di-n-propylacetate (Fig. 2). After low doses of DPA (200 and 400 mg/kg) the GABA concentration was not significantly different from the concentration determined in control animals (+1% after DPA 200 mg/kg and +11% after 400 mg/kg). However, it increased by 57% after DPA 600 mg/kg. DPA 600 mg/kg produced an inhibition of 33% of the GABA-transaminase activity found in the control; 400 mg/kg did not modify GABA-transaminase activity, and 200 mg/kg inhibited it by 7–13%. The activity of GABA-transaminase was not significantly different when 2 or 5 mM GABA was used as substrate (at any dose of DPA). The SSA-DH activity after DPA treatment was not different from that of the control experiments.

In vitro experiments showed that DPA at 5×10^{-3} M produced an 89% inhibition of SSA-DH activity (assayed with 5 μM succinic semialdehyde, SSA). Kinetic analysis of the *in vitro* inhibition of SSA-DH activity by DPA was carried out using various substrate and inhibitor concentrations (Fig. 3). In the Lineweaver–Burk plot (1/v against 1/S) the straight lines intersecting on the ordinate indicate competition between DPA and SSA for the active site of SSA-DH (Fig. 3a). A Dixon plot (Fig. 3b) shows, for low doses of DPA, straight lines intersecting just after the vertical axis, consistent with competitive inhibition. The apparent K_i for DPA is less than 10^{-3} M.

In vitro kinetic studies of the effect of DPA (5–15 mM) on GABA-transaminase activity failed to show inhibition (slight inhibition was seen with DPA 20 mM) (Fig. 3c). This weak or absent inhibition is confirmed by the Dixon plot in which different substrate concentrations give lines parallel to the abscissa (Fig. 3d).

DISCUSSION

The biochemical findings in the brains of mice given EOS intracerebroventricularly are closely similar to the observations of Fowler [5] in rats receiving EOS intracisternally. He described a 65–80% inhibition of GABA-transaminase, 8–24 hr after EOS 2 mg/kg, associated with a raised brain GABA concentration, which sometimes exceeded 200% of control values. We find, using higher doses of EOS (7·5–15 mg/kg), a similar inhibition of GABA-transaminase at 24 hr (55–70%) and rather greater changes in brain GABA concentration (increased 5–10 fold). However, a 10-fold increase in brain GABA concentration has been referred to by Fowler [5] as following injection of higher doses of EOS, and a similar increase has been observed [19] in mice receiving hydrazinopropionic acid (which potently inhibits GABA-transaminase). That EOS does not inhibit SSA-DH has not previously been reported. This finding supports the suggestion that EOS, being an active-site directed inhibitor, is the most specific GABA-transaminase inhibitor currently available. Unlike hydrazines or pyridoxine-antagonist inhibitors of GABA-transaminase, it does not inhibit glutamic acid decarboxylase (L-glutamate 1-carboxy-lyase; EC 4.1.1.15) or other transaminases (such as alanine aminotransferase or aspartate aminotransferase) [3, 4].

The behavioural effects of EOS were similar to those described by Baxter *et al.* [6]. The individual

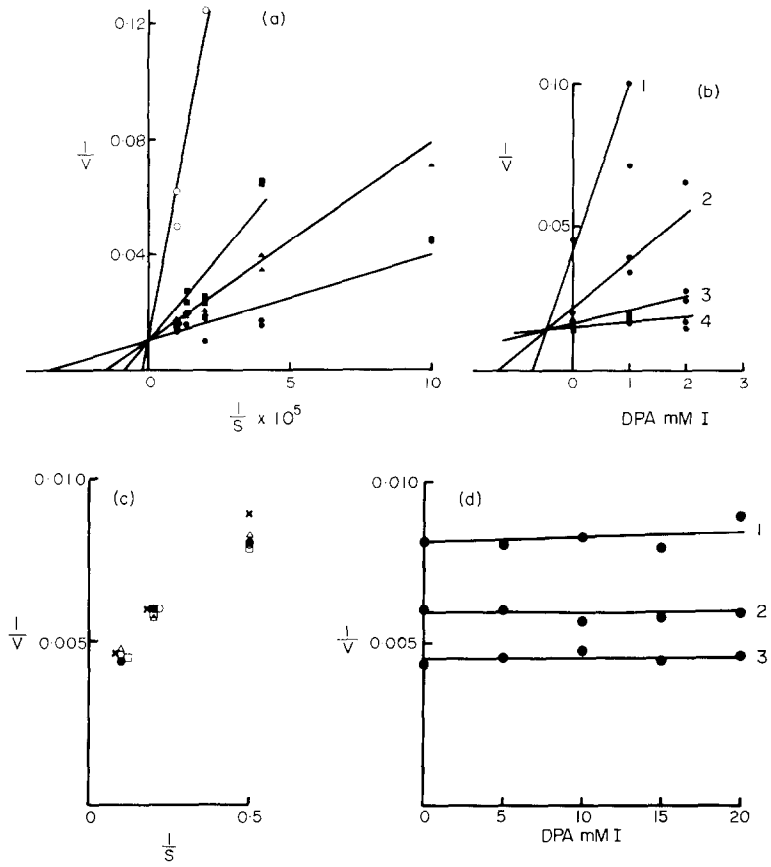

Fig. 3. Effect of Di-*n*-propylacetate on the *in vitro* kinetic analysis of cerebral SSA-dehydrogenase and GABA-transaminase activities. Lineweaver Burk plots in (a) of 1/v (v = μmole NAD consumed/g wet wt/hr) against 1/S (S = SSA concentration in μM) and in (c) of 1/v (v = μmole α-ketoglutarate consumed/g wet wt per hour) against 1/S (S = GABA conen in mM). Di-*n*-propylacetate concentration (in mM): ●—— ● no inhibitor, ▲ —▲ 1, ■ —■ 2, ○ —○ 5, △ —△ 10, □ —□ 15 and × —× 20. (b) and (d) are Dixon plots of 1/v against I (I = DPA concentration in mM) for various substrate concentrations. SSA concentrations (b): 1) = 10^{-6} M; 2) = $2{\cdot}5 \times 10^{-6}$ M; 3) = $7{\cdot}5 \times 10^{-6}$ M; 4) = 10^{-5} M. GABA concentrations (d): 1) = 2×10^{-3} M; 2) = 5×10^{-3} M; 3) = 10^{-2} M.

variation is presumably the result of differing areas of diffusion of drug from the injection site. When even slight behavioural changes were apparent all phases of the seizure response to auditory stimulation were absent. This is an extremely potent anti-convulsant effect and contrasts with the weak protection against maximal electroshock observed in mice up to 36 hr after comparable doses of EOS [6].

An increase in brain GABA concentration has been reported for rats receiving DPA 200 or 400 mg/kg i.p. (+30% and +46% respectively) [12] or for mice receiving DPA 400 mg/kg i.p. (+34–40%) [13]. We have not found significant increases in GABA after such doses of DPA, but 600 mg/kg produced a greater augmentation (+57%). An *in vivo* inhibition of GABA-transaminase (such as we observe in brain homogenates from mice treated with DPA 600 mg/kg i.p.) was not detected by Godin *et al.* [12]. The latter authors reported that 25 mM DPA inhibited GABA-transaminase *in vitro* by 37·6%. In cats DPA 200 mg/kg gives peak plasma concentrations in the range 0·3–0·5 mg/ml [11]; assuming the drug has unimpeded access to the brain cerebral concentrations of DPA would be 2–3 mM, and if the concentrations remain proportional, 600 mg/kg would give a cerebral concentration of 6–9 mM. (Preliminary experiments [20] with ^{14}C-DPA have suggested that mouse brain concentrations of DPA may be higher than this, possibly 20 mM after DPA 400 mg/kg, but the experiments did not exclude the possibility of the accumulation of metabolites of DPA).

We are unable to show *in vitro* inhibition of GABA-transaminase activity by apparently appropriate concentrations of DPA (i.e. 5–15 mM). How inhibition of GABA-transaminase *in vivo* arises is unknown. The claim that DPA *in vitro* is a competitive inhibitor of GABA-transaminase [13], with a K_i value of $1{\cdot}4 \times 10^{-3}$ M, is probably erroneous and arose because the assay system employed a linked reaction including SSA-DH, and, as shown by Harvey *et al.* [14], and confirmed by us, DPA potently inhibits SSA-DH. DPA is competitive with succinic semialdehyde with an apparent K_i less than 10^{-3} M (i.e. adequate to account for the apparent findings of Simler *et al.* [13] with GABA-transaminase). The concentration of DPA required to produce an approximately 90% inhibition of SSA-DH (5 mM) is probably achieved in the brain after DPA 600 mg/kg. However,

with our assay conditions it was not possible to demonstrate SSA-DH inhibition in brain homogenates from such animals, presumably because of the competitive nature of the inhibition and the dilution of the DPA in the final reaction mixture. As the reaction catalysed by GABA-transaminase is a reversible equilibrium reaction an increase in the concentration of succinic semialdehyde following inhibition of SSA-DH could presumably increase brain GABA content. The failure of GABA to accumulate in the brain after DPA, 200 or 400 mg/kg, indicates either that any inhibition of GABA-transaminase or of SSA-DH is insufficient to lead to an accumulation of GABA, or that the flux through the GABA shunt is reduced. The latter possibility, as it would indicate a reduced rate of synthesis of GABA would be expected to increase the probability of seizures [3].

That DPA 400 mg/kg protects mice for up to 2 hr against seizures induced by auditory stimulation (in the absence of any 'psychomotor' changes) has been previously reported [13]. We find that protection is not quite complete after DPA 400 mg/kg, but is total after 600 mg/kg, however, the higher dose produced slight changes in motor activity.

It has been claimed that the anticonvulsant action of DPA is a consequence of the increased concentration of GABA in the brain [12, 13]. The demonstration of a clear anticonvulsant action associated with insignificant changes in GABA concentration (following DPA 400 mg/kg) makes it unlikely that there is a causal relationship between these effects. The structural similarity of DPA and GABA makes it possible that DPA will act on receptors for GABA or other amino acids, or on carriers involved in reuptake or transport of GABA and related compounds.

Although the changes in brain GABA concentration are very much greater after EOS, it is not proven that the behavioural changes and anticonvulsant effects of EOS are a direct consequence of such increases in brain GABA concentration. One problem concerns the compartment in which the increase occurs. If the increase is primarily in GABA-ergic synaptic terminals, enhanced release of GABA could lead to more effective inhibitory synaptic transmission. Synaptically released GABA is inactivated by reuptake into glia and neurones, especially inhibitory interneurones [21–23]. Inhibition of GABA-trans aminase (in the mitochondria of neurones and glia) may produce an accumulation of GABA in neurones or in glia. Even if the accumulation is primarily in glia this may be of physiological significance, as a release of GABA from glia has been demonstrated [24]. The possibility that anticonvulsant effects could be a secondary or indirect consequence of changes in brain GABA content is suggested by the observations that (a) many drugs modifying brain GABA metabolism also alter brain amine concentrations [25] and (b) that in mice receiving EOS intracerebroventricularly the rise in brain GABA concentration is followed by an increase in brain 5-hydroxytryptamine concentration [6]. A relationship between the concentration in the brain of 5-HT or other amines and seizure threshold has frequently been demonstrated [26, 27], and a role for such changes in anticonvulsant drug action has been proposed [28–31].

The correlation between changes in motor behaviour and inhibition of GABA-transaminase activity that is apparent in mice receiving EOS or high doses of DPA suggests that acute neurological toxicity of these and perhaps other anticonvulsant drugs, may be related to altered GABA metabolism.

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REFERENCES

1. K. Krnjević, *Physiol. Rev.* **54,** 418 (1974).
2. D. R. Curtis and G. A. R. Johnston, *Ergebn. Physiol.* **69,** 97 (1974).
3. B. S. Meldrum, *Int. Rev. Neurobiol.* **17,** 1 (1975).
4. L. J. Fowler and R. A. John, *Biochem. J.* **130,** 569 (1972).
5. L. J. Fowler, *J. Neurochem.* **21,** 437 (1973).
6. M. G. Baxter, L. J. Fowler, A. A. Miller and J. M. G. Walker, *Br. J. Pharmac.* **47,** 681P (1973).
7. A. Lehmann, *Aggressol.* **5,** 311 (1964).
8. R. L. Collins, In *Experimental Models of Epilepsy* (Eds. D. P. Purpura, J. K. Penry, D. B. Tower, D. M. Woodbury, and R. Walter) p. 347, Raven Press, New York (1972).
9. H. Meunier, G. Carraz, Y. Meunier, P. Eymard and M. Aimard, *Thérapie* **18,** 435 (1963).
10. E. Volzke and H. Doose, *Epilepsia* **14,** 185 (1973).
11. H. Van Duijn and M. K. F. Beckmann, *Epilepsia* **16,** 83 (1975).
12. Y. Godin, L. Heiner, J. Mark and P. Mandel, *J. Neurochem.* **19,** 869 (1969).
13. S. Simler, L. Ciesielski, M. Maitre, H. Randrianarisoa and P. Mandel, *Biochem. Pharmac.* **22,** 1701 (1973).
14. P. K. P. Harvey, H. F. Bradford and A. N. Davison, *Febs Lett.* **52,** 251 (1975).
15. A. Waksman, M. K. Rubinstein, K. Kuriyama and E. Roberts, *J. Neurochem.* **15,** 351 (1968).
16. C. Kammeraat and H. Veldstra, *Biochim. biophys. Acta* **151,** 1 (1968).
17. P. V. Taberner, J. E. G. Barnett and G. A. Kerkut, *J. Neurochem.* **19,** 95 (1972).
18. W. B. Jacoby, In *Methods in Enzymology*, 5, (Eds. S. P. Colowick and N. Kaplan) p. 765. Pergamon Press, New York (1962).
19. N. M. Gelder, *J. Neurochem.* **15,** 747 (1968).
20. L. Ciesielski, M. Maitre, C. Cash and P. Mandel, *Biochem. Pharmac.* **24,** 1055 (1975).
21. F. Schon and L. L. Iversen, *Brain Res.* **42,** 503 (1972).
22. T. Hökfelt and A. Ljungdahl, *Exp. Brain Res.* **14,** 354 (1972).
23. L. L. Iversen and J. S. Kelly, *Biochem. Pharmac.* **24,** 933 (1975).
24. M. C. W. Minchin and L. L. Iversen, *J. Neurochem.* **23,** 533 (1974).
25. N. H. Yessian, A. R. Armenian and H. Ch. Buniatian, *J. Neurochem.* **16,** 1425 (1969).
26. W. O. Boggan, In *Serotonin and Behaviour*, (Eds. J. Barchas and E. Usdin) p. 167, Academic Press, New York (1973).
27. B. S. Meldrum, G. Anlezark, E. Balzamo, R. W. Horton and M. Trimble, In *Advances in Neurology*, vol. 10, (Eds. B. S. Meldrum and C. D. Marsden) p. 147, Raven Press, New York (1975).
28. D. D. Bonnycastle, N. J. Giarman and M. K. Paasonen, *Br. J. Pharmac. Chemother.* **12,** 228 (1957).
29. E. G. Anderson, S. D. Markowitz, and D. D. Bonnycastle, *J. Pharmac. exp. Therap.* **136,** 179 (1962).
30. W. D. Gray and C. E. Rauh, *J. Pharmac. exp. Therap.* **177,** 206 (1971).
31. H. Meyer and H. H. Frey, *Neuropharmac.* **12,** 939 (1973).