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EFFECT OF SODIUM *n*-DIPROPYLACETATE ON AUDIOGENIC SEIZURES AND BRAIN γ-AMINOBUTYRIC ACID LEVEL

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Abstract—A brain γ-aminobutyric acid (GABA) level increase has been observed after *n*-dipropylacetate (nDPA) treatment. A relation between brain GABA level increase and protection against seizures has been pointed out; in fact, as long as the GABA rate is higher than 1·7 μmoles/g, mice are protected against audiogenic fits.

Enzymatic studies have shown that nDPA inhibits competitively GABA-transaminase (GABA-T) in regard to GABA. Dreiding models show that there is a close structural relationship between GABA and nDPA, explaining the competitive inhibition.

AUDIOGENIC seizures in genetically sensitive mice[1,2] provide a reproducible system which avoids the toxicological drawbacks of other seizure triggering methods, such as pentetrazol injection[3] and alumina topical application[4] for the study of the biochemical parameters of epileptic fits.[5,6]

Looking for compounds which specifically inhibit convulsive fits, rather than causing a general depression of psychomotor activity, we studied sodium *n*-dipropylacetate (nDPA[4]). This substance protects rats, mice and rabbits against electroshocks and pentatetrazol induced convulsive seizures.[7–9] Moreover, we have shown that mice are protected against audiogenic seizures after nDPA treatment.[10,11] Godin *et al.*[12] observed an increased brain γ-aminobutyric acid (GABA) level in rats after nDPA administration whereas amino acid levels remained unchanged.[12]

Many studies have shown that GABA is an inhibitory transmitter of the central nervous system[12–17] and that there is a relationship between neuronal excitability and brain GABA level.[18] In view of the preliminary results of Godin *et al.*[12] indicating that nDPA inhibits the GABA transaminase, we have studied in detail the action of nDPA on this enzyme.

MATERIALS AND METHODS

Male and non-pregnant female Swiss albinos, Rb strain mice[19] (10 week-old, 20–30 g wt) which have been genetically selected for their susceptibility to an acoustic stimulus were used. Within the same stock, a resistant secondary strain was used as control. These mice were supplied by Mrs. A. Lehmann (Laboratoire de Physiologie Acoustique de Jouy-en-Josas, France).

In order to study brain metabolites at different stages of the seizure, mice were placed in a transparent plastic cage, the bottom of which was a trap-door under which

* Attachée de Recherche à l'Inserm.

there was a polyethylene vessel containing liquid nitrogen. A loud speaker, connected with a low frequency generator (1–10,000 Hz) which produces sound stimuli from 1–110 dB (c) was placed above the cage.

The threshold acoustic stimulus intensity estimated by a Philipps dB-meter, is about 100 dB, 8000 Hz. Seizures were triggered by activating the low frequency generator and at defined times the mice were dropped into the liquid nitrogen.

The brains of the frozen animals were removed and weighed while still frozen. Brains were kept frozen until the homogenization and extraction steps.

Protection tests against audiogenic seizure. Mice were tested for their susceptibility to audiogenic seizure 48 hr before the experiments. Audiogenic seizures in mice have been previously described.[5] Mice not suffering a complete fit, were excluded. The reactions of mice to acoustic stimulus were tested at various times following intramuscular injection of nDPA (dissolved in 0·9% NaCl solution) at the doses of 200 mg/kg, 300 mg/kg or 400 mg/kg.

GABA extraction and determination. Brain GABA was extracted in 0·6 N perchloric acid; GABA content in the neutralized extracts was determined as described by Sandman.[20] When the results were checked by the enzymatic method of Scott and Jacoby,[21] the values were in agreement with those obtained by chemical analysis.

Extraction of the GABA-transaminase (GABA-T). GABA-T was extracted from the brain of genetically sensitive mice by the method of Waksman *et al.*[22] with the following modifications:

(1) Dithiothreitol (10^{-4} M) was used instead of reduced glutathion to prevent inactivation.

(2) The final purification step was a chromatography on an hydroxylapatite column with a continuous phosphate buffer (pH 6·8) gradient, ionic strength 0·1 → 0·4 M. The fraction with the enzymatic activity was dialyzed overnight against phosphate buffer (pH 7·2; I = 0·01) and concentrated. The enzyme solution was made up to 30% glycerol, transferred to 1 ml vials and stored in liquid nitrogen.

Determination of GABA-T activity. The rate of NADH formation was measured fluorometrically in the following coupled reactions.

$$\alpha\text{-ketoglutarate} + \text{GABA} \xrightarrow{\text{GABA-T}} \text{SSA} + \text{glutamate}$$

$$\text{SSA} \xrightarrow{\text{SSADH}} \text{succinic acid}$$

$$\text{NAD} \curvearrowright \text{NADH}$$

The succinic semialdehyde dehydrogenase (SSADH) activity must be sufficient so that the first reaction is rate-limiting. The succinic semi-aldehyde dehydrogenase was extracted from the kidneys of guinea-pigs by the method of Pitts *et al.*[23]

At the beginning of any activity determination, we verified that 10 μl of the SSADH solution produced 30 nmole NADH in less than 15 min. The incubation medium contained 0·1 M Tris–HCl (pH 8·2), 1 mM NAD, 1 mM dithiothreitol, 0·1 mM pyridoxal phosphate, 1–10 mM GABA, 0·05–0·25 mM α-ketoglutarate, 10 μl SSADH, 100 μl GABA-T (100 μg/ml). To study the action of nDPA, the incubation medium contained 0·1 M Tris (pH 8·2), 1 mM NAD, 1 mM dithiothreitol, 0·1 mM pyridoxal phosphate, 1–10 mM GABA, 0·05–0·25 mM α-ketoglutarate, 10 μl SSADH, 100 μl GABA-T (100 μg/ml).

To study the action of nDPA on GABA-T, concentrations of 5 and 10 mM were used after determination of the concentrations of nDPA in the different regions of the mouse brain 45 min after intraperitoneal injection of 400 mg/kg, ^{14}C-nDPA (8 μCi/mg).

Incubations were performed at 38° in the thermostatic chamber of a Zeiss spectrofluorometer (excitation 365 nm; emission 470 nm; uncorrected values). Reaction rates were estimated from the graphic recording of the fluorescence increase.

Products. nDPA was a gift from the Laboratoire Berthier, Grenoble, France. GABA was supplied by CalBiochem, Los Angeles. α-Ketoglutarate and NAD were supplied by Boehringer, Mannheim, Germany. γ-Ethoxybutyrolactone was a gift from Dr. C. G. Wermuth. It forms succinic semialdehyde in boiling water. ^{14}C-nDPA was a gift of C.E.M., Grenoble, France.

RESULTS

Protection against audiogenic seizures. Results concerning the protection ensured by nDPA treatment are presented on Fig. 1. We noticed that the protection increases

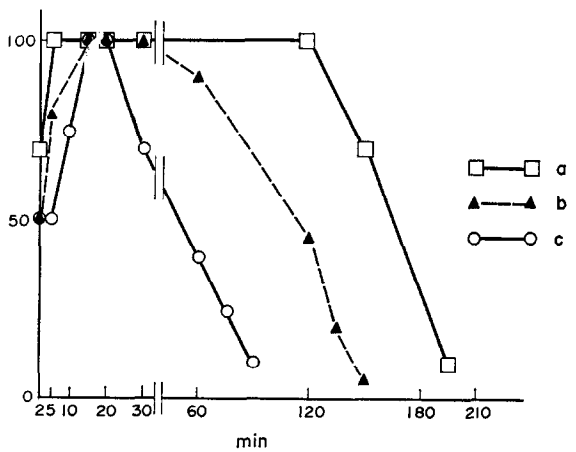


FIG. 1. Kinetic protection against audiogenic seizures after nDPA i.m. injection. (a) 400 mg/kg; (b) 300 mg/kg; (c) 200 mg/kg. Abscissa, time after injection; ordinate, percentage of full protection. Each point stands for a group of at least ten mice.

with dose. A dose of 400 mg/kg nDPA ensures a complete protection against audiogenic fits, protection which occurs quickly (5 min) and lasts for nearly 2 hr. This dose is very well tolerated, the LD_{50} amounts to 832 mg/kg.[8] Moreover we observed that there is no depression of the psychomotric activity after the nDPA administration.

Determination of brain GABA level after an acoustic stimulus. There is no significant difference between the brain GABA level of genetically sensible and normal mice from the same strain (Table 1). The outcome of tonic fits is accompanied by a decrease of brain GABA level of approximately 18 per cent.

Effects of nDPA treatment on brain GABA level. Brain GABA level increases after an intramuscular nDPA injection (400 mg/kg) as can be seen in Table 1, and Fig. 2. The highest level was reached after 30 min (37 per cent; $P \leqslant 0{\cdot}05$). The return to the

TABLE 1. BRAIN GABA LEVEL OF SENSITIVE MICE

	μmoles/g wet wt ± S.D.
Normal mice (same strain) (10)	1·70 ± 0·20
Normal mice after nDPA treatment (10)	2·27 ± 0·04*
Sensitive mice (20)	1·60 ± 0·04
Sensitive mice after nDPA treatment (17)	2·24 ± 0·08†
Sensitive mice 10 sec after an audiogenic tonic seizure (9)	1·38 ± 0·21†

* $P < 0·09$; † $P < 0·05$.
Values in parentheses indicate the number of experiments.

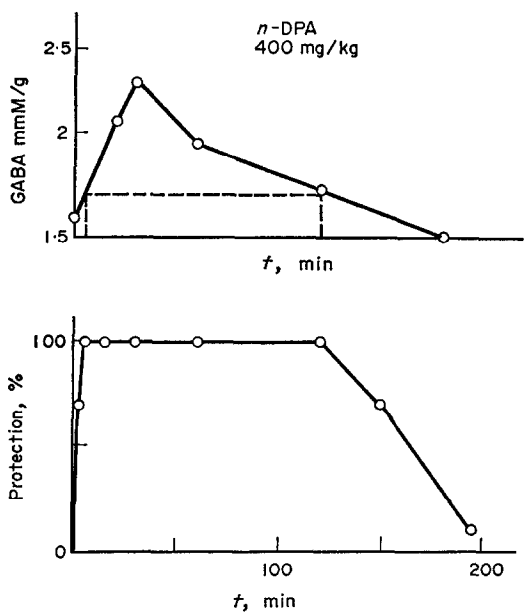


FIG. 2. Relationship between brain GABA level and protection against audiogenic seizure after nDPA treatment.

control level was accomplished after 180 min. Fifteen min after an intramuscular injection of nDPA, (200 mg/kg) the brain GABA level was 1·73 ± 0·01 μmole/g ($P < 0·005$). The return to the control level was accomplished after 1 hr (1·64 ± 0·05).

Study of the GABA-T inhibition by nDPA. We have studied the inhibition of GABA-T by nDPA on each of the substrates of the reaction of transamination, GABA and α-ketoglutaric acid.

The graphic study is presented on Figs. 3–5. There is a competitive inhibition between GABA and nDPA while the K_m- and K_i-values are equal: $1·4\ 10^{-3}$ M (Figs. 3 and 4). The K_m value of α-ketoglutarate is 10^{-4} M and the inhibition by nDPA is not competitive (Fig. 5).

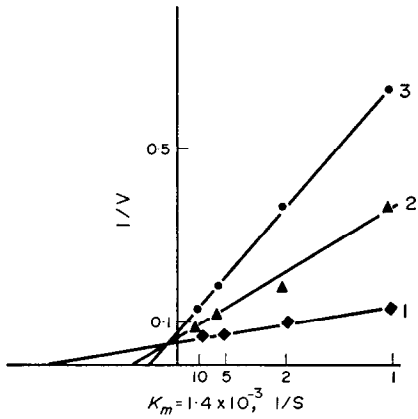


FIG. 3. Graphic study of GABA–nDPA competition at the GABA-T level ($1/v = F(1/s)$ for several nDPA concentrations. (1) no DPA; (2) 5×10^{-3} M; (3) 10^{-2} M. Abscissa ($1/s$) s = GABA concentration in mM; ordinate, ($1/v$) v = arbitrary fluorometric units.

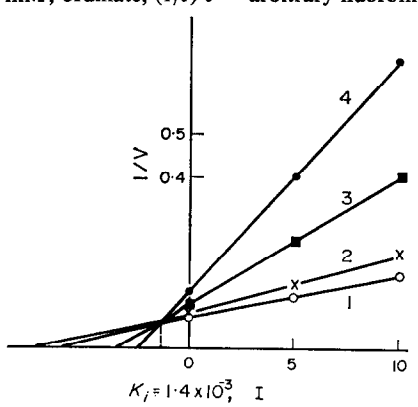


FIG. 4. Graphic determination of the K_i in the GABA–nDPA competitive-inhibition at the GABA-T level. $(1/v) = f(\mathrm{I})$ for several GABA concentrations. (1) 10^{-3} M; (2) 2×10^{-3} M; (3) 5×10^{-3} M; (4) 10^{-2} M. Abscissa, I, nDPA concentration in mM; ordinate, ($1/v$), v: arbitrary fluorometric units.

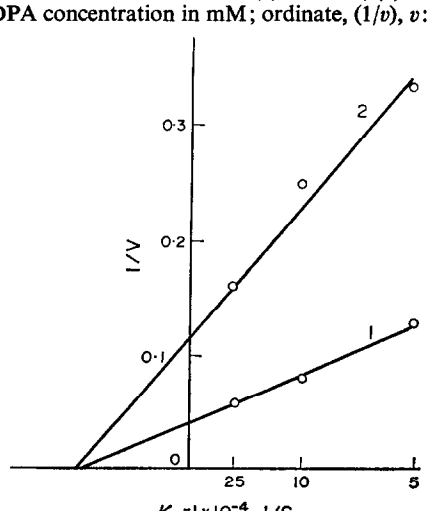


FIG. 5. Graphic study of α-ketoglutarate–nDPA competition at the GABA-T level. $(1/v) = f(1/s)$ for several nDPA concentrations. (1) no DPA; (2) 5×10^{-3} M. Abscissa, ($1/s$), α-ketoglutarate concentration (10^{-5} M); ordinate, ($1/v$), v = arbitrary fluorometric units.

DISCUSSION

Taking account of the part ascribed to GABA in the central nervous system, it is interesting to note a decrease of the cerebral GABA rate of approximately 17·5 per cent during the tonic phase. Though this variation is not significant inside the whole brain, it may be within some regions of the central nervous system. Various authors have observed a decrease of the GABA biosynthesis in the brain after the administration of convulsing hydrazides.[24-27] These hydrazides inhibit the action of enzymatic systems, the coenzyme of which is the pyridoxal phosphate, such as the glutamate decarboxylase (GAD) that produces the GABA and the GABA-transaminase (GABA-T) that catabolizes it. Nevertheless the enzyme–coenzyme bond is in the case of GAD more labile than that of the GABA-T and thus explains a preferential effect on the GABA synthesis. However, Tapia and Awapara[28] have observed that after the administration of hydrazone γ-glutamyl pyridoxal phosphate, an inhibitor of GAD, the inhibition of GABA synthesis is maximum during convulsions. Lehmann has observed that the threshold of the audiogenous fit was also lowered by hydrazides.[19]

Let us recall that Sze[29] determined the rate of some amino acids, among which GABA in the brain of a stock of resistant 19-day-old mice that undergo "priming". He noticed that the only change observed during the development of the sensibilization to the acoustic stimulus, that is between 10 min and 20 min after the priming, was a brain GABA decrease. Of course, this observation does not solve the problem about the involvement of that temporary and immediate decrease of the GABA rate in the brain on the development of the sensitiveness to an audiogenic fit. These facts seem to give evidence to the idea that the decrease of the GABA rate could promote the triggering of the convulsive seizure.

Though we did not notice any change in the GABA rate among the susceptible or the resisting mice, it appeared to be worth while to research if any variation of the GABA level would be responsible for the protection against the triggering of audiogenic fits. Some authors have in fact pointed out that a brain GABA increase occurred consecutively to an administration of amino-oxyacetic acid and hydroxylamine, inhibitors of the GABA-T; according to these authors, the GABA increase should be related to a decrease of neuronal excitability.[16]

It has been pointed out that convulsions consecutive to electroshocks on the cat were lessened or even suppressed by an administration on the cortex or by an intraventricular injection of GABA.[27,30] The direct study of the effect of GABA upon the convulsive fits cannot be realized because this amino acid, injected by an enteral, or parenteral way does not cross the blood-brain barrier. Godin *et al.*[12] have demonstrated an increased GABA rate in the brain after the administration of nDPA by the intraperitoneal way; it appeared interesting to research if there were no correlations between the endogenous increase of GABA and the protection against the fits.

If we examine simultaneously the effects of nDPA on the behaviour of sensible mice (Fig. 2) and the GABA level, we become aware that a relation exists between brain GABA level, and the protection against convulsive fits. Thus 20 min or 30 min after nDPA treatment, the brain GABA rate increases in a significant manner; increase that corresponds to the total protection of the mice. As long as the GABA rate in the brain of those mice remains superior to 1·7 μM/g, no audiogenic fit appears: at a rate under 1·7 μM/g fits appear again.

Our results thus give evidence to the role of GABA in the protection against the

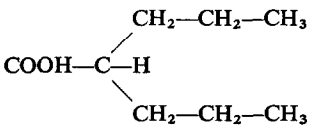
triggering of audiogenic fits. Taking account of the experiences realized in our laboratory by Godin *et al.*[12] *in vivo* and *in vitro*, this effect of nDPA may be compared with that of hydroxylamine,[31] of amino oxyacetic acid[16] and of hydrazino propionic acid[32] which, *in vivo*, are powerful inhibitors of the GABA-T and promote an increase of the cerebral GABA rate.

Preliminary tests have demonstrated that nDPA inhibits the GABA-T action and we have endeavoured to study in a more accurate way the nDPA action on that enzyme. In fact, the GABA increase could be explained by a slackening of its catabolism consecutive to a GABA-T inhibition.

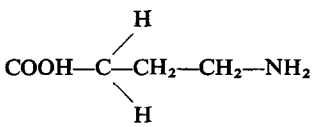
Our study has given evidence that nDPA inhibits competitively GABA-T in regard to GABA.

While researching the mechanisms of this inhibition, we have thought of a structural analogy between GABA and nDPA. This analogy is noticed on the following formulas as well as the GABA-hydrazinopropionic acid analogy.

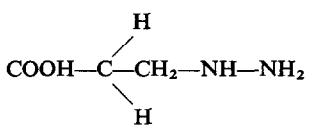
nDPA



GABA



Hydrazinopropionic acid



The GABA–nDPA structural analogy is more clearly understood by the analysis of the Dreiding's models (Fig. 6).

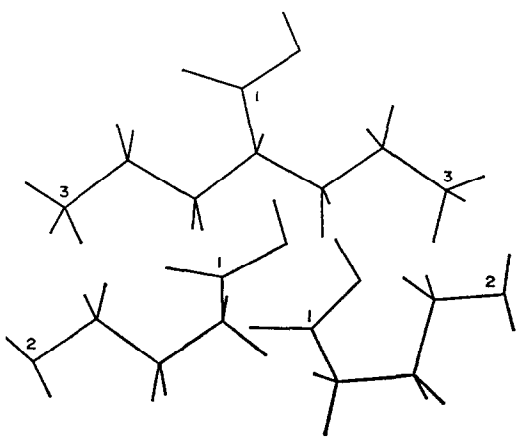
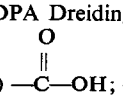


FIG. 6. Photographic picture of GABA and nDPA Dreiding models. Top, 1 molecule of nDPA; bottom, 2 molecules of GABA. (1)  ; (2)  ; (3)  .

Thus, for the first time we have pointed out a physiological process of the action of an anticonvulsant.

REFERENCES

1. R. G. BUSNEL, *Mouse News Letts* **21**, 42 (1959).
2. A. LEHMANN and R. G. BUSNEL, in *Acoustic Behaviour of Animals* (Ed. R. G. BUSNELL) p. 244. Elsevier, Amsterdam (1963).
3. C. AJMONE-MARSAN and F. MAROSSERO, *Electroencephal. Clin. Neurophysiol.* **2**, 133 (1950).
4. L. M. KOPELOFF, E. BURRERA and N. KOPELOFF, *Am. J. Psychiat.* **98**, 881 (1942).
5. A. LEHMANN, Thèse de Doctorat ès-Sciences Naturelles, Paris (1964).
6. A. LEHMANN, *Agressologie* **211**, 221, 311 (1964).
7. G. CARRAZ, H. MEUNIER, Y. MEYNIER, P. EYMARD and M. EYMARD, *Therapie* **18**, 435 (1963).
8. G. CARRAZ, in *Pharmacodynamie de l'Acide Dipropylacétique (ou Propyl-2-Pentanoïque) et de ses Amides*, Imprimerie Eymond, Grenoble (1968).
9. S. LEBRETON, G. CARRAZ, H. BERIEL and H. MEUNIER, *Therapie* **19**, 451 (1964).
10. S. SIMLER, H. RANDRIANARISOA, A. LEHMANN and P. MANDEL, *J. Physiol.* **60**, 547 (1968).
11. S. SIMLER, H. RANDRIANARISOA, A. LEHMANN and P. MANDEL, in *Pathogenesis of Epilepsy* (Eds. G. USUNOFF, E. ATSEV, I. DOSSEVA and S. DIMOV) p. 59. Publishing House of the Bulgarian Academy of Sciences, Sofia (1971).
12. Y. GODIN, L. HEINER, J. MARK and P. MANDEL, *J. Neurochem.* **16**, 869 (1969).
13. K. A. C. ELLIOTT and R. LOVELL, *Fedn Proc.* **21**, 364 (1962).
14. K. KRNJEVIC and S. SCHWARTZ, *Exp. Brain Res.* **3**, 320 (1967).
15. K. KRNJEVIC, *Nature* **228**, 119 (1970).
16. K. KURIYAMA, E. ROBERTS and M. K. RUBINSTEIN, *Biochem. Pharmac.* **15**, 221 (1966).
17. P. B. MOLINOFF and E. A. KRAVITZ, *J. Neurochem.* **15**, 391 (1968).
18. K. F. KILLIAM and J. A. BRAIN, *J. Pharmac. exp. Ther.* **19**, 255 (1957).
19. A. LEHMANN, *J. Physiol.* **55**, 282 (1963).
20. R. P. SANDMAN, *Analyt. Biochem.* **3**, 158 (1962).
21. E. M. SCOTT and W. B. JACOBY, *J. biol. Chem.* **234**, 932 (1959).
22. A. WAKSMAN and E. ROBERTS, *Biochemistry* **4**, 2132 (1965).
23. N. PITTS JR., C. QUICK and E. ROBINS, *J. Neurochem.* **12**, 93 (1965).
24. E. ROBERTS, *Fourth. Int. Neurochem. Symp.* p. 218. Pergamon Press, Oxford (1960).
25. E. ROBERTS, C. F. BAXTER and E. EIDELBERG, *Proc. Second Int. Meeting Neurobiol.* p. 392. Elsevier, Amsterdam (1960).
26. K. F. KILLIAM, *J. Pharmac. exp. Ther.* **119**, 255 (1957).
27. K. F. KILLIAM and J. A. BAIN, *J. Pharmac. exp. Ther.* **119**, 263 (1957).
28. R. TAPIA and J. AWAPARA, *J. Proc. Soc. exp. Biol. Med.* **126**, 218 (1967).
29. P. Y. SZE, *A.A.A.S. Meeting*, Boston, Mass. (1969).
30. S. R. DASGUPTA, E. L. KILLIAM and K. F. KILLIAM, *J. Pharmac. exp. Ther.* **122**, 16A (1958).
31. K. F. KILLIAM, S. A. DASGUPTA and E. K. KILLIAM, in *Inhibition in the nervous System and GABA* (Ed. E. ROBERTS) p. 302. Pergamon Press, Oxford (1960).
32. C. F. BAXTER and E. ROBERTS, *Proc. Soc. exp. Biol. Med.* **101**, 811 (1959).
33. N. M. VAN GELDER, *J. Neurochem.* **16**, 1355 (1969).
34. G. CARRAZ, A. DAMEY, J. P. SCHNETZLER and J. BONNIN, *J. Med. Lyon* **46**, 705 (1965).