ANALYSIS OF THE MECHANISM OF THE NEUROTROPIC ACTIVITY OF CETYL γ AMINOBUTYRATE

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Experiments on albino mice showed that the cetyl ester of γ-aminobutyric acid (CGABA) completely prevents convulsions induced by thiosemicarbazide or delays their onset and increases the duration of the latent period of repeated convulsions. With respect to this effect synergism was found between CGABA and amino-oxyacetic acid. It is postulated that cetyl γ aminobutyrate, a lipophilic derivative of γ-aminobutyric acid, can penetrate through the blood-brain barrier, and its action is mediated through mechanisms connected with the metabolism of endogenous GABA.

It was shown previously that the product of esterification of γ-aminobutyric acid (GABA) by cetyl alcohol – cetyl γ aminobutyrate (CGABA) – potentiates the effect of various narcotics, induces synchronization of the EEG, selectively inhibits the investigative reaction, and increases the resistance of animals to hypoxia [1]. Since cetyl alcohol in doses of 10 to 400 mg/kg showed no evidence of central action in the writers' experiments, it can be concluded that the neurotropic activity of CGABA is due to the presence of the GABA in its molecule. Since GABA is known to penetrate with difficulty through the blood-brain barrier and that cetyl alcohol is distinguished by its high degree of lipophilicity, it has been postulated that the cetyl radical enables the compound to pass through the blood-brain barrier.

When injected into the cerebral ventricles, GABA has a protective effect against thiosemicarbazide convulsions, which are caused by the deficient formation of this acid [6, 4]. It has also been shown that the accumulation of endogenous GABA produced by amino-oxyacetic acid (AOA) [9] leads to a marked decrease in the convulsant effect of thiosemicarbazide.

It was therefore decided that the effect of CGABA be studied on the convulsant effect of thiosemicarbazide and the anticonvulsant effect of AOA.

EXPERIMENTAL METHOD

Experiments were carried out on male albino mice weighing 18-22 g. Thiosemicarbazide (TSC) was injected in doses of 10 and 15 mg/kg in a volume of 0.1 mg/10 g body weight. The latent period (time between injection of TSC and the first convulsion), the frequency of the convulsions, and the interval between the first and second convulsions were recorded. Having regard to the long latent period of TSC convulsions [4], the substances studied for their anit-TSC effect were injected intraperitoneally 30-60 min after TSC: CGABA in dose of 10 mg/kg, GABA in a dose of 250-500 mg/kg. In some experiments these substances were injected after the first convulsion. The data for the latent periods of the convulsions were analyzed by Student's method and the data for the frequency of onset of the convulsions by means of the χ^2 criterion. To determine the specifity of the anti-TSC effect, the effects of GABA and CGABA were studied on convulsions induced by leptazol (the dose in which leptazol, injected intravenously, induced clonic convulsions and clonic extension was titrated). In addition, in convulsions induced by TSC the effect of chlorpromazine

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was studied; doses of 2-4 mg/kg were used, in which chlorpromazine induces approximately the same degree of hypodynamia as CGABA in a dose of 10 mg/kg, as shown by recordings of motor activity. To determine the effect of AOA on that of CGABA, the CT_{50} index was used (the time required for convulsions to develop in 50% of mice [2]). Changes in CT_{50} of thiosemicarbazide were studied under the influence of CGABA and AOA separately and in combination. The TSC was injected subcutaneously and the other substances intraperitoneally.

EXPERIMENTAL RESULTS AND DISCUSSION

TSC in a dose of 10 mg/kg induced clonico-tonic convulsions in 95-100% of mice with a latent period of 94 (87.8-100.2) min. These figures were obtained in experiments performed in spring and summer. In later experiments, performed in winter, TSC was injected in a dose of 15 mg/kg. Some animals died during the first convulsions in a state of tonic extension. Immediately after the first convulsion, all the surviving mice developed further convulsions. As a result of injection of CGABA in a dose of 10 mg/kg, the number of animals developing convulsions fell by 50% ($\chi^2 = 4.26$, $0.01 < P < 0.05$). In the animals which developed convulsions despite treatment, the latent period was statistically significantly increased to 129 (105.3-152.7) min. This agrees with area observations of an increase in duration of the latent period of paraoxysmal discharges in the rabbit EEG [1].

If CGABA was injected immediately after the first convulsion, further convulsions were completely presented in 20% of mice, while in the other animals although convulsions were still observed, the time of their onset was postponed by a statistically significant degree: the interval between the first and second convulsions in the control was 19 (15.3-22.7) min, after injection of CGABA it was 30 (21.9-38.1) min.

The effect of CGABA described above was largely specific: it was absent against convulsions induced by leptazol. In these experiments no significant increase was found in the dose in which leptazol induces clonic convulsions, namely 55 (51.5-58.5) mg/kg, or tonic extension, namely 75 (74-76) mg/kg.

The protective effect of CGABA against convulsions induced by TSC was not, in all probability, determined by the muscle-relaxant action of the compound. As experiments on cats have shown, CGABA in a dose of 10 mg/kg has no effect on neuromuscular conduction. The value of ED_{50} with respect to muscle-relaxant action in experiments on mice (revolving rod) was 45 (37-55) mg/kg, whereas a distinct protective effect against convulsions induced by TSC in mice was observed after injection of CGABA in a dose of 10 mg/kg. Support for the view that this effect of CGABA is not due to hypodynamia was evidently given by the results of the experiments with chlorpormazine also. In doses of 2-4 mg/kg, in which chlorpromazine definitely reduced motor activity, it did not reduce the frequency of convulsions nor did it increase their latent period. The use of the CT_{50} test demonstrated mutual additive relations for CGABA and AOA. In the control, for instance, CT_{50} for thiosemicarbazide (in a dose of 15 mg/kg) was 65 min. After injections of CGABA (10 mg/kg 30 min after TSC) CT_{50} for thiosemicarbazide was increased to 82 min. Depending on the dose given, AOA could completely block the thiosemicarbazide convulsions or delay their onset. After injection of AOA (15 min before TSC) in the dose of 15 mg/kg which was used in these experiments, CT_{50} for thiosemicarbazide was increased to 94 min. With a combination of AOA and CGABA (the substances were injected in the above-mentioned doses and at certain intervals) CT_{50} increased to 170 min; i.e., the combined effect was approximately the sum of the components.

Unlike CGABA, GABA in a dose of 500 mg/kg did not reduce the percentage of animals in which TSC induced convulsions and did not increase the latent period of those convulsions.

The important role of GABA in the mechanism of nervous processes and its poor power of penetration through the blood-brain barrier explain the interest in the search for derivatives of this amino acid which, unlike GABA itself, exhibit neurotropic activity when administered by other means than into the brain. As has already been emphasized [1], CGABA, unlike esters with a shorter side chain (methy – octyl), exhibits marked neurotropic activity in doses far below toxic. It seems evident that the problem of whether the neurotropic activity of CGABA is connected with the "propulsion" of GABA through the blood-brain barrier is one of fundamental importance. Until a labeled ester of GABA is available, it is impossible to answer this question categorically, but some types of pharmacological activity can provide indirect evidence in support of this hypothesis. Convulsions induced by TSC are one of the best pharmacological tests which can usefully be employed. Hydrazides, inhibiting glutamate decarboxylase, are known to lower the brain GABA concentration [6, 7] and to disturb its membrane trasport and compartmentalization [5, 8]; a defi-

ciency of GABA formation arising in this way is regarded as the main cause of onset of the convulsions induced by TSC [10]. This explains the ability of many compounds which raise the brain GABA level and of GABA itself when injected into the cerebral ventricles to block convulsions induced by TSC. The fact that CGABA completely prevents convulsions induced by TSC or delays their onset (while GABA has no such action when administered other than into the brain), observed in the present experiments, is evidence in support of the view that the pipophilic derivative of GABA is able to penetrate through the blood-brain barrier and to exert effects characteristic of GABA in the brain. This hypothesis is also concerned by results obtained in the study of AOA, which is known to inhibit α-ketoglutarate-GABA transaminase, leading to accumulation of considerable quantities of GABA in the brain. This explains the effect of AOA against convulsions induced by TSC. AOA is also known to potentiate the effect of GABA if injected ionotophoretically into the region of cortical neurons [3]. The data obtained in the present experiments showing synergism between AOA and CGABA against convulsions induced by TSC can evidently be indirect evidence that CGABA exerts its activity through mechanisms connected with endogenous GABA metabolism.

LITERATURE CITED

1. R. U. Ostrovskaya, V. V. Parin, and N. M. Tsybina, Byull. Éksperim. Biol. i. Med., No. 1, 53 (1972).
2. D. E. Abrahams and J. D. Wood, J. Neurochem., 17, 1197 (1970).
3. I. Kelly and L. Renaud, Experientia, 27, 8 (1971).
4. A. J. Hance, W. D. Winters, P. Bach-y-Rita, et al., J. Pharmacol. Esp. Ther., 140, 385 (1963).
5. L. L. Iversen and E. A. Kravitz, J. Neurochem., 15, 609 (1968).
6. K. F. Killam and J. A. Bain, J. Pharmacol. Esp. Ther., 119, 255 (1957).
7. K. F. Killam, S. R. Dasgupta, and E. K. Killam, in: E. E. Roberts (editor), Inhibition in the Nervous System and Gamma-Aminobutyric Acid, New York (1960), p. 302.
8. P. Y. Sze and R. A. Lovell, Life Sci., 9, 889 (1970).
9. D. P. Wallach, Biochem. Pharmacol., 10, 323 (1961).
10. J. D. Wood and D. E. Abrahams, J. Neurochem., 18, 1017 (1971).