COMPARISON OF THE NEUROTROPIC ACTIVITIES OF γ-AMINOBUTYRIC ACID AND ITS CETYL ESTER

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The cetyl ester of γ-aminobutyric acid (CGABA), starting with doses of 5-10 mg/kg, potentiates the effect of barbiturates, depresses the investigative reflex, causes marked synchronization of the EEG, and increases the probability of survival of the animals in hypoxia. CGABA differs fundamentally in this respect from GABA, which, when administered by extracerebral routes, exhibits only a weak central effect observed in doses of 500-2000 mg/kg. Esterification of GABA by a lipophilic radical evidently improves its power of penetration into the brain.

Considering the important role of γ-aminobutyric acid (GABA) in nervous activity it is interesting to study the neurotropic activity of its metabolic products. This can be particularly important because the study of the pharmacological effects of GABA is made more difficult by its poor power of penetration through the blood-brain barrier, and it is therefore necessary to inject the GABA directly into the brain. Several types of pharmacological procedure connected directly or indirectly to GABA metabolism are known; the most widespread of them are: 1) exogenous administration of products of the Roberts cycle: GABA, succinic acid semialdehyde [1, 8]; 2) administration of amino-hydroxyacetic acid, which inhibits transaminase and raises the GABA level [2, 9, 13], or of thiosemicarbazide, which inhibits decarboxylase activity and causes GABA deficiency in the brain [3, 6]; 3) administration of substances which are substitution products of GABA increasing the degree of its penetration into the brain [10].

The object of the present investigation was to study the cetyl ester of GABA (CGABA), which was synthesized by T. V. Protopopova and N. M. Tsybina at the Institute of Pharmacology, Academy of Medical Sciences of the USSR. Because the molecule of this compound contains a lipophilic component, it is reasonable to assume that it will have neurotropic effects. To test this hypothesis, comparative studies were made of CGABA and GABA to test certain criteria of neurotropic activity.

EXPERIMENTAL METHOD

The effect of these substances were studied on: 1) the investigative reflex [5, 7]; 2) spontaneous motor activity (multichannel actograph); 3) movement coordination (revolving rod); 4) the anesthetic effect of barbiturates: effect on the duration (35 mg/kg) and strength of action (12 mg/kg, intravenously) of thiopental sodium; on the rate of onset and duration of anesthesia produced by barbital sodium (175 mg/kg, intraperitoneally) or hexobarbital (75 mg/kg, intraperitoneally); 5) the analgesic effect of morphine (5 mg/kg, intraperitoneally) and trimeperidine (2.5 mg/kg, intraperitoneally) in response to mechanical and thermal stimulation; 6) the EEG. Experiments with hexobarbital were carried out on male albino rats weighing

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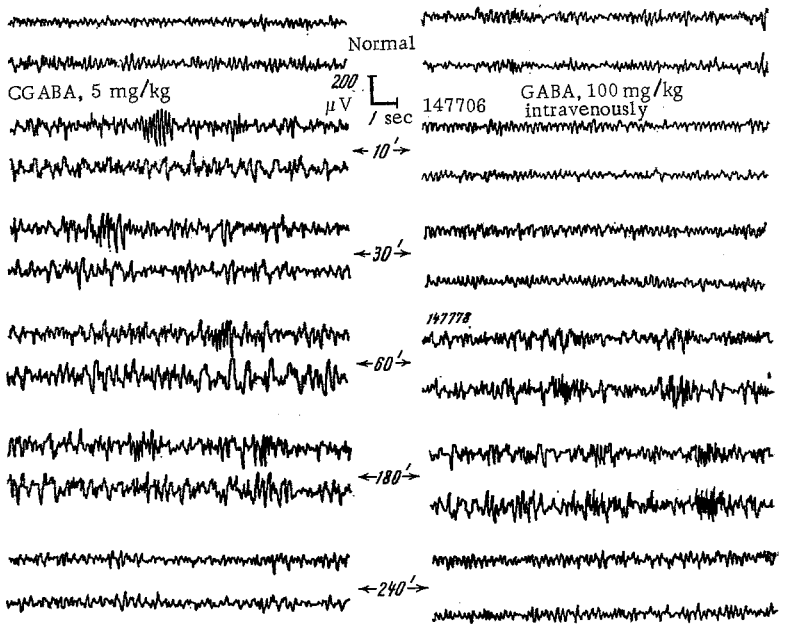

Fig. 1. Comparative intensity and times of onset of synchronizing effects of CGABA (left) and GABA (right). From top to bottom: EEG of somatosensory and visual areas. Chronic experiments on rabbits.

160-200 g; the remaining experiments were on male albino mice weighing 18.24 g. In chronic experiments on rabbits and acute experiments on curarized, unanesthetized rabbits and cats the effect of the compound was studied on the EEG of the somatosensory, association, and visual areas of the cortex, and on the transcallosal, direct cortical, and evoked responses. Allowing for the protective action of certain derivatives of "GABA shunt" in hypoxia [12], the effect of CGABA was studied on the survival of albino mice kept in a chamber with a reduced (to 8 vols% measured by the Haldane method) oxygen concentration. The acute 24-h toxicity of CGABA was determined on albino mice weighing 18-24 g. The value of LD_{50} was calculated by Litchfield's method.

CGABA is a white powder with melting point 102-104°C, sparingly soluble in water but readily soluble in organic solvents. A suspension of CGABA in Tween-80, injected intraperitoneally, was used for the experiments.

EXPERIMENTAL RESULTS AND DISCUSSION

CGABA has marked neurotropic activity. Starting with a dose of 4 mg/kg it depressed the investigative reflex of mice placed in a box with a sloping grid. The value of ED_{50} by this test was 6 (3.7-9.6) mg/kg. GABA in doses up to 3 g/kg did not affect the investigative reflex. In a dose of 10 mg/kg, CGABA reduced motor activity almost threefold, from 248 (93-403) to 69 (29-109). GABA caused a statistically significant depression of motor activity starting with a dose of 250-500 mg/kg. With an increase in the dose of CGABA disturbances of movement coordination appeared. ED_{50} by this test was 45 (37-55) mg/kg, whereas GABA, even in doses of 3 g/kg, did not disturb the ability of the mice to stay on a revolving rod. In a dose of 5 mg/kg, CGABA prolonged sleep induced by thiopental sodium from 4 (2.6-5.4) to 21 (8.7-33.3) min and reduced ED_{50} for thiopental sodium from 21 (19-23.1) to 17 (16-17.9) mg/kg; in a dose of 10 mg/kg it converted a subthreshold dose of thiopental sodium in an effective dose: the percentage of animals put to sleep increased from 5-10 in the control to 70. The prolonging effect of GABA was exhibited in doses from 500 mg/kg, but the degree of prolongation of sleep was very slight – less than 9 (7.9-10.1) min. GABA caused some increase in the effectiveness of the subthreshold dose (up to 30%) in doses starting from 1 g/kg; an increase in the dose of GABA did not increase the potentiating effect. CGABA, in a dose of 10 mg/kg, increased the duration of barbital sodium anesthesia from 106 (83.1-128.9) min in the control to 139 (118.1-159.9) min and brought forward the time when the animal assumed the lateral position from 33 (28.6-37.4) min in the control to 16 (13.7-18.3) min in the animals receiving CGABA. GABA (500 mg/kg) had no significant effect on the duration or time of onset of barbital sodium anesthesia. In a dose

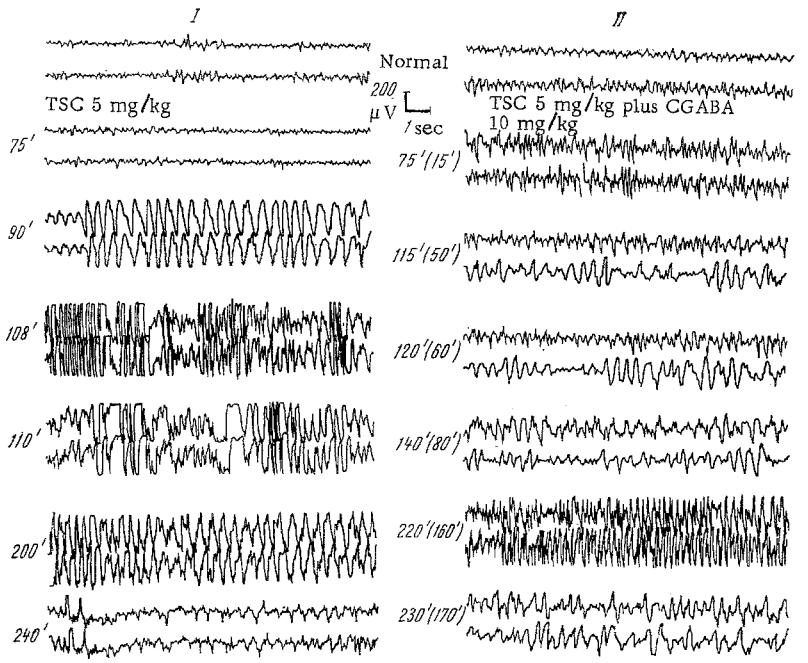

Fig. 2. Onset of paroxysms evoked by thiosemicarbazide (TSC) is delayed by CGABA: I) TSC 5 mg/kg (control). First paroxysm begins after 108 min and is followed by repetition of the attacks. II) TSC 5 mg/kg, followed after 60 min by CGABA 10 mg/kg; onset of paroxysms delayed until 220th minute, single attack. Calibration the same for all EEG's except 110' (I) and 220' (II), in which amplification is halved. EEG leads the same as in Fig. 1. Chronic experiments on rabbits.

of 10 mg/kg, CGABA quickened the onset of hexobarbital anesthesia from 196 (226.3-165.7) to 116 (81.5-140.5) sec and shortened the duration of sleep from 18 (17.3-18.7) to 37 (24.8-49.2) min. By this test GABA was ineffective. CGABA had no significant effect on the analgesic action of trimeperidine and morphine, but slightly prolonged the action of morphine.

CGABA caused characteristic changes in the spontaneous EEG: an increase in the number of slow potentials, an increase in amplitude of the dominant rhythm, and the appearance of groups of spindles. The threshold dose of CGABA for this effect was 5 mg/kg in chronic and 10-15 mg/kg in acute experiments. Synchronization of the EEG began 5-10 min after administration of the compound and it lasted 1.5-3 h (Fig. 1). CGABA increased the amplitude of the primary, transcallosal, and direct cortical responses. GABA had a synchronizing effect starting with a dose of 50-100 mg/kg. Synchronization did not take place immediately, but 30-60 min after injection of the compound (Fig. 1), and in some animals the effect was slight. The changes in the responses likewise were unstable.

CGABA increased the duration of survival of the mice during exposure to hypoxic hypoxia. In a dose of 15 mg/kg, CGABA lengthened survival from 23 (21.1-24.9) to 59 (52-66) min. The effect still continued 2 h after injection of the compound: the duration of survival of the mice in this case was 33 (27.6-38.4) min. With an increase in the dose to 25 mg/kg the protective effect lasted 4-5 h. Starting with a dose of 500 mg/kg, GABA prolonged the survival of the animals during hypoxia by less than 30%.

Esterification of the GABA molecule, as Bertelli et al. [4] have shown in the case of its methyl, isopropyl, and octyl esters, leads to the appearance of a central action. These compounds exhibit this action, unlike GABA when administered by the extracerebral root. However, their activity is low and is observed in near-toxic doses. For instance, the octyl ester of GABA, in a dose of 0.5 LD_{50}, lowers motor activity by 75%, and the methyl ester in the same dose lowers it by 50%; the activity of the ethyl ester of GABA, according to Sypniewska [11], is even lower than that of the methyl ester. This is evidently because the esters studied are not sufficiently lypophilic. Addition of a cetyl residue, which is strongly lipophilic, leads to a sharp increase in its activity. For example, in a dose of 25 mg/kg (0.14 LD_{50}) CGABA reduced motor

activity by 90%. The effective dose range of CGABA by most tests did not exceed 5-10 mg/kg; i.e., compared with the esters listed above it was 50-100 times smaller; compared with GABA, in cases in which it exhibited some activity when its dose was increased, the difference was 100 times or more. Esterification on GABA with cetyl alcohol leads to the appearance of substantially novel qualities, for example, ability to inhibit the investigative reflex in doses much smaller than those disturbing movement coordination. This is evidence of the specifically psychotropic effect of the compound, a characteristic feature, in particular, of tranquilizers of the benzodiazepine series. Another common feature with these compounds is that small doses of CGABA (0.5 mg/kg) have an activating effect on the EEG. The ability of CGABA to potentiate barbiturates is well marked, and as experiments with subthreshold doses of thiopental sodium and barbital sodium, which are not metabolized in the liver, show, central synergism plays a role in the mechanism of this potentiation. The effects described above are evidently not attributable to the cetyl moiety of the molecule, for as control experiments showed, cetyl alcohol in corresponding doses shows no sign of neurotropic activity. It may be that the effect of the cetyl ester of GABA is associated with its participation in metabolic reactions in which GABA itself takes part. This is shown indirectly by the ability of CGABA to prevent or delay the onset of paroxysmal discharges evoked by thiosemicarbazide (Fig. 2).

LITERATURE CITED

1. R. U. Ostrovskaya, N. M. Tsybina, and T. V. Protopopova, Khim.-Farmats. Zh., No. 12, 21 (1969).
2. R. U. Ostrovskaya, G. N. Artemenko, and K. S. Raevskii, Farmakol. i Toksikol., No. 2, 137 (1970).
3. C. F. Baxter, Ann. New York Acad. Sci., 166, 267 (1969).
4. A. Bertelli, L. Donati, V. Lami, et al., Internat. J. Neuropharmacol., 7, 149 (1968).
5. J. P. Boissier, C. Dumont, et al., Arch. Internat. Pharmacodyn., 133, 29 (1961).
6. K. Killam and J. A. Bain, J. Pharmacol. Exp. Ther., 119, 255 (1957).
7. P. Kneip, Arch. Internat. Pharmacodyn., 126, 237 (1960).
8. H. Laborit, Internat. J. Neuropharmacol., 3, 433 (1964).
9. H. Matthies and N. Popov, Acta Biol. Med. Germ., 20, 371 (1968).
10. J. Sieroslawska, Arch. Immunol. Ther. Exp., 13, 70 (1965).
11. M. Sypniewska, Diss. Pharm. (Krakow), 18, 229 (1966).
12. V. V. Zakusov and R. U. Ostrovskaya, in: IV Conferentia Hungarica pro Therapia et Investigatione in Pharmacologia, Budapest (1968), p. 519.
13. D. P. Wallach, Biochem. Pharmacol., 10, 323 (1961).