NOOTROPIC PROPERTIES OF GABA DERIVATIVES

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A group of substances possessing what is called nootropic activity (from the Greek noos, meaning mind, knowledge) has been actively studied in recent years. It includes compounds which differ in structure: piracetam, dephenylhydantoin (DPH), pyritinol, meclofenoxate, dihydroergotoxin, and vincamine [11]. This has aroused objections: It is evidently more correct to say that these members of different pharmacologic groups possess certain common properties that determine their nootropic effect. One such property is ability to increase resistance to oxygen deficiency. The antihypoxic effect has been found to be an important prognostic sign for demonstration of the nootropic activity of derivatives of the cyclic form of gammaaminobutyric acid (GABA), namely piracetam and its derivatives [6, 8]. Derivatives of the linear form of GABA, namely sodium hydroxybutyrate, fenibut [5], and the cetyl ester (CE) of GABA [9], which are highly active antihypoxic agents from the point of view of their effect on learning when disturbed by factors causing amnesia, have not been studied. Yet it is this approach which we know to be the most appropriate for evaluation of nootropic action.

The object of the present investigation was accordingly to study a number of linear GABA derivatives on a model of conditioned passive avoidance reflex, using electroshock (ES), which disturbs mechanisms of memory trace fixation, as the amnesia-inducing agent.

EXPERIMENTAL METHOD

GABA and the following derivatives of it were tested: CE GABA, fenibut, baclofen, derivatives of the "GABA shunt" metabolite gamma-hydroxybutyric acid (GHBA), sodium hydroxybutyrate and lithium hydroxybutyrate, depakine as a substance inhibiting enzymes inactivating the "GABA shunt"; α-ketoglutarate, GABA-transaminase, and succinic semialdehyde dehydrogenase, and DPH as a substance which also inhibits this last enzyme and is regarded as a nootropic drug. Piracetam was used as the reference compound.

The antiamnesic properties of the compounds were tested on a modified model of single learning of a conditioned passive avoidance reflex (CPAR), using a box with two compartments, one illuminated, the other (initially preferred) darkened. Unavoidable painful electrical stimulation was applied to the animal in the latter compartment at the 150th-160th second of observation [10]. Preservation of the CPAR was tested after 24 h. To obtain retrograde amnesia, immediately after learning ES was applied by the transcorneal route to the rat and one of the compounds chosen for study or isotonic NaCl solution (control group) was injected intraperitoneally. Each group consisted of 10 rats and altogether 250 animals were used in these experiments. The parameter Δt, the difference between the length of stay in the darkened compartment by a given animal before learning and 24 h after learning, was analyzed. The antiamnesic effect of the drugs was manifested as lengthening of Δt. The antihypoxic action of the compound was compared on a model of hypoxic hypoxia (length of survival in a pressure chamber with an initial oxygen concentration of 8 vols. %). These experiments were carried out on 300 mice. The threshold dose in which the substance exhibited its antihypoxic effect was tested on the antiamnesic action. In some experiments with learning, doses half and twice the threshold value also were used. To estimate selectivity of the nootropic action of the drugs ED_{50} for disturbance of muscular coordination on a horizontal wire was determined.

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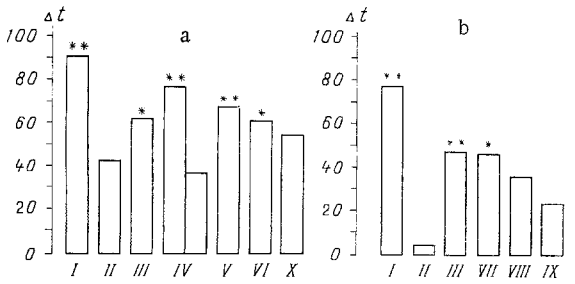

Fig. 1. Comparative activity of GABA derivatives and piracetam in the CPAR test: data obtained in winter (a) and summer (b). Δt) Difference in length of stay of animal in darkened compartment before and 24 h after learning during testing of preservation of memory trace. I) After learning, II-X) after learning, application of ES, and injection of test compounds (10 rats in each group); II) physiological saline, III) piracetam (200 mg/kg), IV) sodium hydroxybutyrate (50 and 200 mg/kg), V) lithium hydroxybutyrate (44 mg/kg), VI) CE GABA (10 mg/kg), VII) fenibut (100 mg/kg), VIII) baclofen (5 mg/kg), IX) DPH (60 mg/kg), X) GABA (150 mg/kg). Statistical significance of differences between control and experimental groups assessed by Wilcoxon–Mann–Whitney method. *P < 0.05, **P < 0.001 (compared with control series II).

EXPERIMENTAL RESULTS

Single electrical stimulation through the floor was sufficient to form CPAR: 24 h after learning, the length of time spent by the animals in the previously preferred darkened compartment was reduced and the retrograde amnesia was exhibited as preference for this "dangerous" compartment. The experiments revealed seasonal differences in the rats' passive-defensive behavior: It will be clear from the data in Fig. 1 that in the summer period ability to form CPAR was initially depressed and sensitivity to the amnesic action of ES was increased.

Irrespective of these differences in the initial parameters, piracetam in a dose of not less than 200 mg/kg reduced the intensity of retrograde amnesia. The GHBA salts, fenibut, and CE GABA also weakened the amnesic effect of ES: Whereas in the control rats exposed to ES continued to remain in the darkened compartment despite the possibility of receiving painful electric shocks there, animals receiving the test drugs spent a shorter time in the darkened compartment. In the strength of their antiamnesic action these compounds were not inferior to piracetam or they actually surpassed it (Fig. 1). Antiamnesic properties could not be found in the case of depakine, and with an increase in its dose amnesia was actually strengthened. DPH and GABA, which some workers regard as nootropic agents, did not exhibit any significant antiamnesic effect.

All the drugs tested increased the length of survival of the animals in the pressure chamber (Fig. 2). If the intensity of the antihypoxic effect of linear derivatives of GABA and piracetam is compared, the dose–effect relationship reveals the greater activity of the former: With an increase in the dose of sodium hydroxybutyrate by 50% above the threshold level it increased the length of survival of the mice by 90%, doubling the dose increased it by 130%, and trebling the dose increased it by 260% compared with the control. The effect of CE GABA and fenibut was to produce a rapid rise (Fig. 2). Baclofen, in a dose of only 5 mg/kg, increased the length of survival of the animals in the pressure chamber by 110%, and in a dose of 10 mg/kg by 160%. Piracetam in a threshold dose increased the duration of survival by 25%, and when given a dose of twice or three times the threshold level it increased the duration of survival by only 40% and 70% resepctively. GABA in doses of 500 and 1000 mg/kg increased this parameter by only 20%.

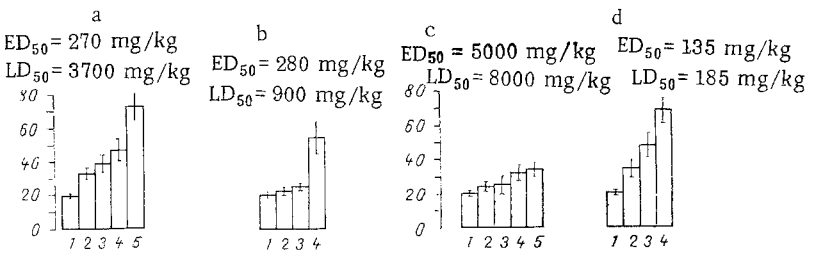

Fig. 2. Comparative activity of some GABA derivatives and piracetam in their effect on length of survival of mice in a pressure chamber (in min). a: 1) Control group, 2, 3, 4, 5) length of survival of mice receiving injection of sodium hydroxybutyrate 30 min before introduction into pressure chamber in doses of 50 (2), 75 (3), 100 (4), and 150 (5) mg/kg. b: 1) Control, 2, 3, 4,) 30 min after injection of fenibut in doses of 50, 75, and 100 mg/kg respectively. c: 1) Control, 2, 3, 4, 5) 60 min after injection of piracetam in doses of 200, 300, 600, and 1000 mg/kg respectively. d: 1) Control, 2, 3, 4) 60 min after injection of CE GABA in doses of 10, 15, and 25 mg/kg respectively. Numbers above show: ED_{50} by staying on horizontal wire test (movement coordination) and LD_{50} for corresponding drug.

The fact that the animals stayed longer in the illuminated compartment under the influence of these linear GABA derivatives can be regarded as proof of their ability to terminate the effects of ES on memory trace fixation, if the other causes of this effect are excluded. One of them could be a decrease in the animals' motor activity, when placed in the illuminated compartment at the beginning of the experiment. However, as control experiments using the Optovarimex apparatus showed, 24 h after administration of the test drugs in antiamnesic doses the number of horizontal movements made by the experimental animals was not reduced compared with the control. It was reduced only after injection of the test substance in doses several times greater (for example, when sodium hydroxybutyrate was given in a dose of 200 mg/kg or more). In this dose, incidentally, sodium hydroxybutyrate has a tranquilizing effect: The animal evidently is not afraid of painful stimulation and visits the darkened compartment — the amnesic action of ES is intensified, just as under the influence of benzodiazepine tranquilizers [13].

Since seizures evoked by electroshock are accompanied by breathing disturbances, it might be supposed that weakening of the amnesic effect of ES was due, not to the direct action of GABA derivatives on memory trace fixation, but simply to their antihypoxic activity. However, absence of the antiamnesic effect of such powerful antihypoxic agents as depakine, DPH [7], and benzodiazepine tranquilizers [1], which we discovered, is evidence that their antihypoxic properties are insufficient for manifestation of a nootropic effect.

The anticonvulsant effect of the GABA derivatives studied likewise cannot be the cause of their antiamnesic activity: All the substances tested were injected after the end of the seizure induced by ES. Anticonvulsant drugs — depakine, DPH, and benzodiazepine derivatives — have no antiamnesic properties.

This analysis enables the main causes of the antiamnesic action of linear GABA derivatives studied to be excluded except their direct effect on memory trace fixation. In the absence of any data on the mechanisms of this effect as yet, we can only postulate a possible connection with activation of macromolecular synthesis. Support for this view is given by such facts as the ability of GABA to increase transport RNA-synthetase activity [12], whereas sodium hydroxybutyrate increases the rate of uptake of leucine into brain proteins [13]; piracetam also stimulates RNA and protein synthesis [4].

It follows from the results of this investigation that the antiamnesic effect of linear GABA derivatives is combined with their antihypoxic effect. It can accordingly be concluded that they have properties which determine nootropic activity. This is rather selective, as can be judged from the ratio between the threshold antiamnesic and antihypoxic doses and those which reduce muscle tone (Fig. 2). Although linear GABA derivatives are inferior to piracetam in the degree of selectivity of their nootropic action, nevertheless these experiments revealed a definite dose range within which these substances exhibit a protective action without a depressant effect. This draws attention to the linear GABA derivatives studied in this investigation as substances with a nootropic action. The first indications have been obtained to show that there is a nootropic component in the action of the tranquilizer fenibut in neurotic disorders [2]. The results of the present experiments indicate the desirability of including sodium hydroxybutyrate in the therapeutic program for patients with initial phases of syndromes of organic psychosis, brain trauma, and other states of "cerebral insufficiency," which has hitherto been treated by piracetam alone.

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