ANTAGONISM BETWEEN SODIUM HYDROXYBUTYRATE AND SOME EFFECTS OF AMINOHYDROXYACETIC ACID

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UDC 615.31:547.473.2].015.23:615.31:547.466.3

In agreement with data in the literature, a protective effect of aminohydroxyacetic acid (AHAA) was observed as an inhibitor of 4-aminobutyrate:2-oxoglutarate-aminotransferase (GABA-T) in convulsions induced in mice by thiosemicarbazide (TSC), a glutamate decarboxylase inhibitor. A similar but somewhat weaker action was exhibited by sodium hydroxybutyrate (NaHB). Meanwhile, combined administration of NaHB with AHAA reduced the intensity of its anticonvulsant effect with respect to TSC and reduced the accumulation of GABA in the brain characteristic of the action of AHAA. Competition between AHAA, NaHB, and GABA as structurally closely similar compounds for GABA-T or the GABA-ergic receptor is postulated.

KEY WORDS: GABA; aminohydroxyacetic acid; sodium hydroxybutyrate; convulsive states.

Gamma-aminobutyric acid (GABA) is known as a natural metabolite of brain tissue and an inhibitory mediator. Preparations of GABA are used at the present time for the clinical treatment of certain pathological states [5, 6]. It is accordingly interesting to study the mechanism of action of substances affecting GABA metabolism. Among them, special attention is deserved by aminohydroxyacetic acid (AHAA), an inhibitor of the enzyme GABA-T (4-aminobutyrate: 2-oxoglutarate-aminotransferase, E.C. 2.6.1.19). For this reason AHAA increases the accumulation of GABA in the brain and thus leads to the development of a number of depriming effects, including an anticonvulsant effect [2, 3]. Another substance connected with GABA metabolism is hydroxybutyric acid, one of its conversion products in the brain.

Bearing in mind the structural similarity between these substances and GABA [8, 10], it was decided to study the effect of hydroxybutyric acid on the effects of AHAA most clearly connected with its typical inhibition of GABA-T. The following effects were chosen: the protective action against seizures evoked by thiosemicarbazide (TSC), due chiefly to inadequate formation of GABA from glutamic acid, and elevation of the GABA level in the brain, one cause of this anticonvulsant effect of AHAA.

EXPERIMENTAL METHOD

Experiments were carried out on albino mice weighing 18-22 g, divided into eight groups receiving the following treatment: group 1) 0.85% NaCl (control), 2) TSC in a dose of 15 mg/kg, 3) NaHB (sodium salt of γ-hydroxybutyric acid) in a dose of 500 mg/kg, 4) NaHB combined with TSC in the above doses, 5) AHAA (Upjohn Co., USA) in a dose of 25 mg/kg, 6) AHAA and NaHB, 7) AHAA and TSC, 8) a combination of AHAA, NaHB, and TSC in the above-mentioned doses. AHAA and NaHB were injected intraperitoneally and TSC subcutaneously; in the experiments with combined administration, all the substances were injected at the same time. The solutions were made up before the experiment and injected in doses of 0.1 ml/10 g body weight. In experiments to study the convulsions and anticonvulsant effect the latent period and frequency of onset of the seizures, the severity of the chronic and tonic components, and the mortality were recorded; each group contained 40 mice. The GABA content in the brain tissue was determined in mice of

Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 79, No. 5, pp. 68-70, May, 1975. Original article submitted May 31, 1974.

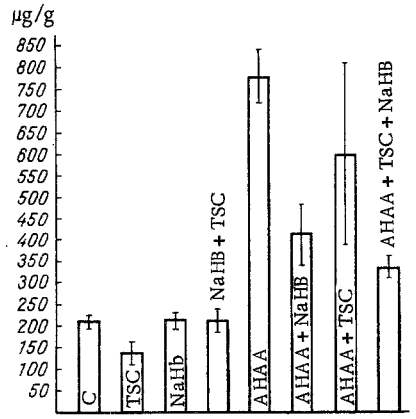

Fig. 1. Effect of TSC (15 mg/kg), NaHB (500 mg/kg), AHAA (25 mg/kg), and their combinations of GABA content in mouse brain tissue. Abscissa, substances used; ordinate, GABA content (in μg/g wet weight of tissue).

the same groups; each group consisted of seven or eight animals. The mice were decapitated 60 min after injection of the preparations, the brain was quickly removed and frozen with liquid nitrogen, and the GABA content was determined by paper chromatography [1]. The results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS AND DISCUSSION

TSC, in the dose used, evoked convulsions in 98% of the animals, half of which died in the presence of severe clonicotonic convulsions. The latent period of these convulsions varied between 50 and 90 min (mean 77 min). NaHB appreciably weakened the convulsant effect of TSC: in 30% of mice the seizures were completely prevented (total absence of seizures during observation on mice for 5 h); in the remaining 70%, although seizures occurred, they were much weaker and the latent period was significantly lengthened (to 170 min). AHAA, in the dose used, completely prevented the paroxysmal effect of TSC: in all 100% of mice in observations lasting 5 h not only were no seizures, but not even any slight spasms were observed. However, the protective effect of AHAA against seizures induced by TSC was much weaker if the animal received NaHB together with AHAA. In this case AHAA no longer gave 100% protection against seizures evoked by TSC; almost half of the animals developed convulsions, although after a longer latent period than in the control.

Since a fall in the GABA level in the brain tissue is one of the main causes of the convulsant effect of TSC, and since their effect on this process could be one reason for the protective action of the tested drugs in convulsions, the GABA content was determined in the brain of the animals of all eight groups studied. The first result of these experiments (Fig. 1) was to show that TSC significantly lowered the brain GABA level. This is in agreement with data showing inhibition of glutamate decarboxylase activity by TSC. AHAA, as a powerful inhibitor of GABA-T, by delaying conversions of GABA, increases its content in the brain tissue and, if given together with TSC, it prevents the fall in the GABA content characteristic of the action of this substance. The reason is evidently that AHAA inhibits GABA-T activity much more than TSC inhibits glutamate decarboxylase activity; for that reason the increase in the level of GABA in the brain is much greater than its deficiency. At the same time, it was shown that NaHB also reduces the degree of decrease in the GABA level caused by TSC. This is presumably connected with the ability of NaHB to inhibit GABA-T activity. NaHB has been shown to be a competitive inhibitor of GABA-T in experiments in vitro [8]. Since this inhibition is only slight in degree (only 18%, even with a concentration of NaHB of 10^{-2} M), in the present experiments NaHB did not increase the GABA level in the control animals; however, under conditions of deficient GABA formation, the NaHB competing with it possibly inhibited GABA-T by a greater degree, with a consequent more marked increase in the brain GABA level.

It is important to emphasize that combined administration of AHAA and NaHB led to a sharp decrease in the accumulation of GABA in the brain tissue characteristic of AHAA (Fig. 1). This decrease took place both in response to injection of a combination of NaHB and AHAA only, or a combination of NaHB with AHAA and TSC. In both cases the addition of NaHB almost halved the accumulation of GABA taking place under the influence of AHAA. This was presumably connected with a decrease in the quantity of AHAA reacting with GABA-T, as a result of competition for the enzyme with NaHB. The latter, although a weaker inhibitor of GABA-T than AHAA, could nevertheless compete with it for the enzyme, for it was injected in a dose 20 times larger than the dose of AHAA.

The results thus demonstrated the antagonistic action of NaHB on the effects of AHAA. This action was manifested as an influence both on the anticonvulsant activity of AHAA and on its ability to cause accumulation of GABA in the brain tissue. NaHB weakened the protective effect of AHAA against TSC-induced convulsions and reduced the degree of the characteristic accumulation of GABA in the brain. The inhibitory effect of NaHB on GABA accumulation in the brain tissue is conjecturally based on the structural similarity between GABA, NaHB, and AHAA, which could be responsible for their competition for the active centers of the GABA-T enzyme. The fact that in the group of animals receiving AHAA and NaHB, TSC nevertheless

evoked convulsions in some mice, although the GABA level in the brain of the animals of this group was not lowered, is in agreement with views [4, 5] indicating the absence of complete correlation between the degree of predisposition of the brain to seizures and the GABA level. Weakening of the anticonvulsant effect of AHAA by NaHB could also be the result of a factor such as competition of NaHB and AHAA with GABA for the GABA-ergic receptor.

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