DISTRIBUTION OF γ-HYDROXYBUTYRIC ACID IN DIFFERENT PARTS OF THE BRAIN AND BLOOD SERUM DURING DEVELOPMENT OF ANESTHESIA IN RATS

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The dynamics of accumulation of γ-hydroxybutyric acid (GHBA) in various parts of the brain (thalamus, hypothalamus, cerebellum, corpus striatum, cerebral hemispheres, medulla, corpora quadrigemina) and in the blood serum of rats was studied by an improved gas-chromatographic method after intraperitoneal injection of sodium γ-hydroxybutyrate (1.5 g/kg). The GHBA concentration in all parts of the brain studied during induction and development of anesthesia and recovery of the animals from it was practically identical, although at all stages of anesthesia a higher level of the compound was found in the cerebral hemispheres, cerebellum, and corpus striatum. The GHBA concentration in the blood serum was constantly higher (especially in the initial period) than in the brain tissues.

KEY WORDS: *Sodium hydroxybutyrate*; *central nervous system*; *blood*; *gas chromatography*.

Despite the wide use of sodium γ-hydroxybutyrate in anesthesiology and other branches of medicine [2, 3], there are only isolated reports in the literature on the concentrations of the compound in the blood and tissues of man and laboratory animals [1, 4, 5]. The dynamics of distribution of γ-hydroxybutyric acid (GHBA) in different parts of the brain has not been studied although such data are important for the understanding of the mechanism of action of this anesthetic on the CNS.

The object of this investigation was to study the dynamics of GHBA distribution in the blood and in different parts of the brain of rats after the animals had been given an anesthetic dose of sodium γ-hydroxybutyrate. Because of the need to detect GHBA in small samples of tissue, an improved gas-chromatographic method of determining this substance was used [1, 4, 5].

EXPERIMENTAL METHOD

Experiments were carried out on 60 male albino rats weighing 140-180 g. Sodium hydroxybutyrate (20% aqueous solution) was injected intraperitoneally in a dose of 1.5 g/kg. The animals fell asleep 9-11 min after injection of the compound (the pain response and corneal reflex disappeared). The duration of sleep was 216 ± 30 min. The rats were decapitated 15, 30, 60, or 120 min after injection of the compound and on awakening. At each time at least six animals were killed. The GHBA concentration was determined in the blood serum and in different parts of the brain. The brain was washed to remove blood by perfusion of the cadaver for 10-15 min with physiological saline, and then quickly removed and frozen. The forebrain, cerebellum, hypothalamus, thalamus, corpora quadrigemina, corpus striatum, and medulla were separated in the cold. The modification of the gas-chromatographic method of determining GHBA consisted of introducing an aqueous solution of the compound mixed with a ring-forming agent (a solution of a nonvolatile acid or of its acid salt) into the doser of the chromatograph. GHBA at a high temperature (200-250°C) under such conditions is converted instantaneously into γ-butyrolactone (GBL), which was then separated on a chromatographic column and determined by means of a flame-ionization detector. By means of the suggested modification, the numerous stages of preparation of the tissue sample for analysis could be omitted and the GHBA concentration determined in small tissue samples (hundredths of a gram). To determine GHBA in the blood serum, 0.05 ml of serum was mixed with an equal quantity of ring-forming agent (20% aqueous solution of potassium acid sulfate), centrifuged for 19 min

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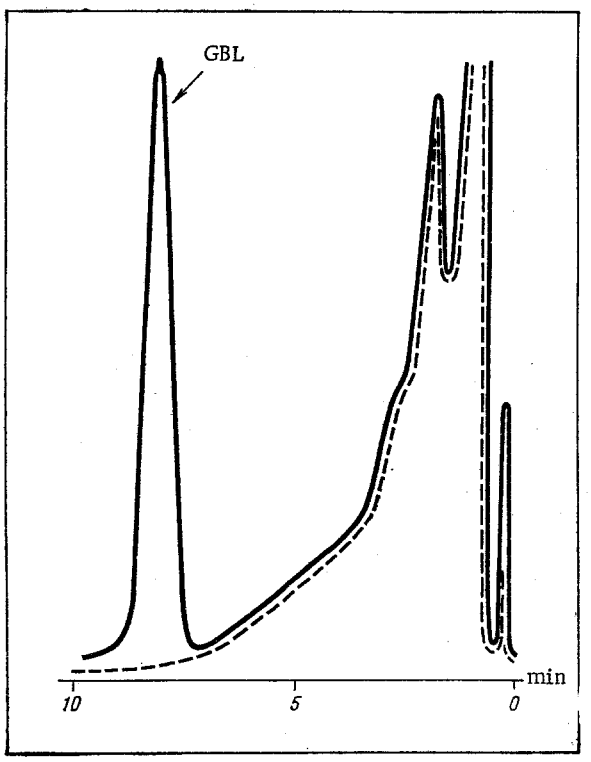

Fig. 1

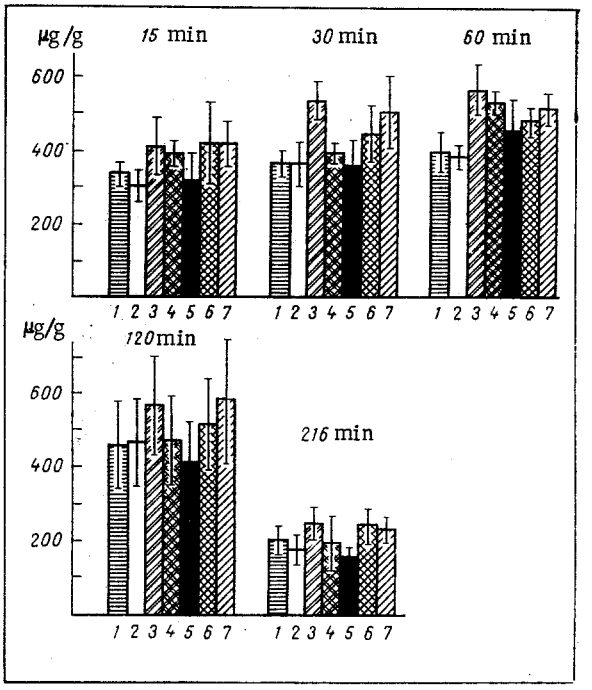

Fig. 2

Fig. 1. Gas-liquid chromatogram obtained during analysis of tissue of control animal (broken line) and of animal receiving sodium hydroxybutyrate (continuous line).

Fig. 2. Distribution of GHBA in different parts of rat brain at various times after administration of compound: 1) hypothalamus; 2) thalamus; 3) cerebral hemispheres; 4) corpora quadrigemina; 5) medulla; 6) cerebellum; 7) corpus striatum.

at 8000-10,000g, after which an aliquot (5 µl) of the supernatant was introduced into the doser of the chromatograph. To determine GHBA in the brain tissue, 0.04-0.06 g of tissue was homogenized at 0-4°C with the ring-forming agent (1:4), after which the mixture was centrifuged and analyzed in the same way as the serum. The gas-chromatographic analysis was carried out on the Khrom-2 chromatograph with flame-ionization detector. An 80 × 0.6 cm column packed with Chromosorb (grain size 60-80 mesh), coated with 20% (of the weight of the solid carrier) polypropyleneglycol adipate, was used. The temperature of the doser was 200°C and of the column 140°C. The flow rate of the carrier gas, nitrogen, was 50 ml/min, of hydrogen 50 ml/min, and of air 600 ml/min. The duration of the analysis was 9 min. A typical chromatogram obtained during analysis of GHBA in tissue is illustrated in Fig. 1. The identity of the chromatogram peak of GBL was confirmed by the addition of the pure substance to the mixture. Quantitative analysis was carried out by measuring the heights of the peaks by the absolute calibration method. Calibration graphs for determining the GHBA concentration in the brain tissue and blood serum were plotted from samples containing the corresponding biological material with the addition of known quantities of GHBA. The sensitivity of determination was 2-5 µg/sample. The relative error did not exceed ±3% (of 10 determinations).

Considerable amounts of GHBA (from 300 to 420 µg/g wet weight of tissue) were discovered in all parts of the brain 15 min after intraperitoneal injection of the compound; marked differences in the GHBA concentration between the different parts of the brain were not present in either the early or the late periods of observation (Fig. 2). Meanwhile a constantly high rate of GHBA accumulation was observed in the cerebral hemispheres, corpus striatum (basal ganglia), and also the cerebellum. In these parts of the brain the GHBA concentration reached 460-580 µg/g after 1-2 h. A somewhat smaller GHBA concentration was constantly observed in the hypothalamus, thalamus, and medulla (400-4500 µg/g 1-2 h after injection of the compound). At the time of awakening the GHBA level in all parts of the rat brain was appreciably lower (to 150-250 µg/g), but under these circumstances a higher GHBA concentration was observed in the cerebral hemispheres, corpus striatum, and cerebellum and a lower concentration in the medulla and thalamus.

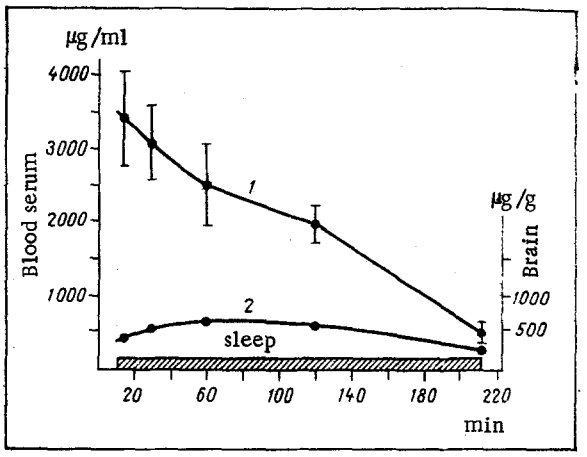

Fig. 3. GHBA concentration in blood serum (1) and cerebral hemispheres of rat (2) at various times after injection of compound.

The GHBA concentration in the blood serum of the rats rose sharply soon after intraperitoneal injection of the compound to reach a maximum after 15 min (3435 ± 647 μg/ml), after which the GHBA level fell gradually, to reach 502 ± 132 μg/ml by the time of awakening (Fig. 3).

The GHBA concentration in the blood at all periods of the anesthetic action of the compound (especially the initial period) was thus appreciably higher than in the brain tissue. This evidently indicates the presence of a blood-brain barrier for GHBA.

About 20% of animals receiving GHBA (1.5 g/kg), it will be noted, had not awakened even after 4 h. These rats after 4 h showed a much higher level of GHBA in their blood serum than the rest of the animals (1157 ± 90 μg/ml). The GHBA concentration in the brain of these rats (944 ± 166 μg/g) was also considerably higher than its level in the brain of the rest of the animals, even during the period of maximal accumulation of the compound (after 1-2 h). The results point to the absence of selective accumulation of GHBA in brain structures.

The fact that the depth and duration of anesthetic action of the compound depend on its concentration in the blood and brain necessitates a periodic check of the blood GHBA level when the compound is used under clinical conditions.

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