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MECHANISM OF THE ANTIHYPOXIC EFFECT OF VALPROATE

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Many GABA-ergic substances are known to increase the resistance of the organism to hypoxia. Sodium hydroxybutyrate has been studied in the greatest detail in this respect [4, 6]. The antihypoxic acitivity of fenibut* [3] also has been described; ability to increase the survival rate during exposure to hypoxia and to restore brain functions, disturbed by hypoxia, to normal are characteristic features of piracetam, a compound which, from the chemical point of view, is a cyclic derivative of GABA [5, 10]. The benzodiazepine tranquilizers, whose action in the modern view is due largely to potentiation of GABA-ergic inhibition also possess antihypoxic activity [1, 2, 13].

The object of the present investigation was to study the effect on resistance to hypoxia of the antiepileptic agent valproate, the anticonvulsant properties of which are associated by most workers with the accumulation of GABA because of inhibition of α-ketoglutarate-GABA transaminase (GABA-T) [14], and to compare the action of valproate and of some GABA derivatives or of pharmacologic agents interfering with the course of different stages of the "GABA shunt."

EXPERIMENTAL METHOD

To estimate the effect of the various substances tested the following parameters were used: the length of survival of mice exposed to hypoxic normobaric hypoxia, the concentrations of lactate and pyruvate in brain and heart tissues, the calculated value of the hypoxic lactate excess (after Huckabee [11]); the dynamics of changes in the ECG of the rats during exposure to hypoxic hypoxia. Hypoxic normobaric hypoxia was induced in mice by placing them (one at a time) in airtight containers with a capacity of 250 ml, in which the initial O_2 concentration in the inspired air was 8 vol. %. To reproduce the conditions of hypobaric hypoxic hypoxia, mice (12 animals at the same time, six control and six receiving the preparation) were placed in a pressure chamber, and raised to an "altitude" of 10,500 m at the rate of 1000 m/min (exposure 10 min, "descent" in the course of 3 min). The concentrations of lactate [7] and pyruvate [9] in the brain and heart tissues were determined in mice in four series of experiments: series I) the mice were kept under conditions of normal respiration and received no drugs, II) the mice were exposed to normobaric hypoxic hypoxia and

*β-Phenyl-γ-aminobutyric acid.

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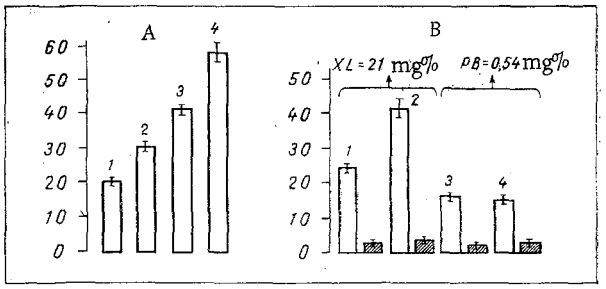

Fig. 1

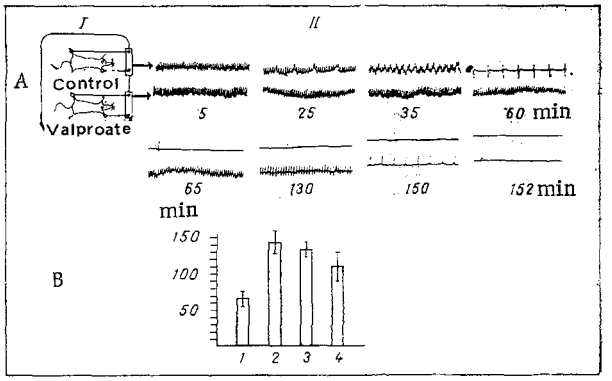

Fig. 2

Fig. 1. Protective effect of calcium valproate against hypoxic hypoxia. A) Dose-dependent increase in length of survival of mice in airtight chamber. Ordinate, time (in min). 1) Control, 2-4) administration of calcium valproate in doses of 100, 200, and 400 mg/kg respectively, 30 min before hypoxia; B) effect of valproate (300 mg/kg, 30 min before sacrifice) on concentration (in mg %) of lactate (unshaded columns) and pyruvate (shaded columns) and on calculated value of hypoxic lactate excess in mouse brain tissue (XL). 1, 3) Control; 2, 4) hypoxia. 1, 2) Without valproate; 3, 4) with calcium valproate.

Fig. 2. Ability of valproate (compared with certain other GABA-ergic substances) to delay development of arrhythmias and complete disappearance of the ECG due to hypoxia. A: I) Airtight chamber (initial O_2 concentration 8 vols. %), II) ECG (lead II); B: 1) control, 2) valproate (300 mg/kg), 3) sodium hydroxybutyrate (300 mg/kg), 4) piracetam (500 mg/kg); ordinate, time of preservation of ECG in airtight chamber (in min).

received no drugs, III) the animals received the test substance during normal respiration, IV) the animals received the test substance 30 min before being placed in the pressure chamber. The ECG was recorded in rats kept two at a time in a pressure chamber with a capacity of 0.8 liters, initially containing O_2 in a concentration of 8 vols. %. The ECG was recorded on the Alvar Riega-VIII electroencephalograph. All test substances were injected intraperitoneally.

EXPERIMENTAL RESULTS

Sodium valproate (from Labaz, France) or calcium valproate (from Germed, East Germany), in doses of 50-400 mg/kg, significantly prolonged the survival of animals placed in an airtight chamber. The antihypoxic effect of valproate was distinctly dose-dependent (Fig. 1A) and, what is important, it was exhibited in response to administration of the drug starting with a small dose (100 mg/kg), in which it had no general depressant effect: ED_{50} for valproate by the revolving rod test is 390 mg/kg.

Since valproate inhibits not only GABA-T, but also succinic semialdehyde dehydrogenase (SSDH) [1], and since the role of inhibition of each of these enzymes in the mechanism of the antihypoxic activity of valproate has not previously been investigated, it was important to compare valproate with typical GABA-T inhibitors, namely aminohydroxyacetic and aminohydroxybutyric acids and hydroxylamine [8], and with diphenylhydantoin, another SSDH inhibitor [15]. It was shown that none of the typical GABA-T inhibitors studied had any protective action on the model of hypoxic hypoxia used, although as GABA-T inhibitors and, consequently, as GABA accumulators, they were considerably more active than valproate. Whereas valproate increased the GABA concentration in brain tissue by 37% even in a dose of 400 mg/kg [14], aminohydroxyacetic acid, in the dose used (25 mg/kg), raised the GABA level by 270% compared with its initial value [8]. Diphenylhydantoin, on the other hand, had an antihypoxic effect: in a dose of 40 mg/kg it prolonged the survival of mice in an airtight chamber on average to 27.0 ± 2.3 min, and in a dose of 60 mg/kg it prolonged their survival to 40.5 ± 3.0 min, compared with 20.0 ± 1.1 min in the corresponding test on the control group. The protective effect of diphenylhydantoin was demonstrated particularly clearly against hypobaric hypoxia: the number of surviving animals in the control group averaged 20%, but diphenylhydantoin in doses of 15, 30, and 60 mg/kg, increased the survival rate to 50, 70, and 84% respectively.

To compare the intensity of the antihypoxic effect of valproate and of antihypoxic agents of the GABA-ergic series described previously, it must be pointed out that if sodium hydroxybutyrate was injected in doses of 50, 100, 300, and 500 mg/kg the mean length of survival of the mice in an airtight chamber was increased to 33, 46, 60, and 41 min respectively (20.0 ± 1.1 min in the control). The sodium salt of succinic semialdehyde (100, 300, and 500 mg/kg) lengthened survival to 45, 90, and 75 min. Piracetam was less active than all the substances listed above: in doses of 300, 500, and 1000 mg/kg it increased the mean life span to 25, 27, and 34 min respectively [5].

Except piracetam, all the substances studied also exerted a protective effect against hypobaric hypoxia. Sodium hydroxybutyrate, in doses of 80, 150, and 400 mg/kg, increased the survival rate to 52, 84, and 68% respectively, whereas valproate in doses of 200 and 300 mg/kg increased the survival rate to 50 and 48%. The survival rate of mice in the control group, under the conditions of this model, was 15%. A special feature of the action of valproate was the rather shorter duration of the protective effect against hypobaric hypoxia than against normobaric hypoxia.

Valproate delayed the development of disturbances of the cardiac rhythm due to hypoxia and increased the total duration of preservation of the ECG in rats kept in an airtight chamber (Fig. 2). Similar data were obtained as a result of administration of sodium hydroxybutyrate, but piracetam had rather weaker activity.

As was shown previously for sodium hydroxybutyrate and succinic semialdehyde [4], valproate prevents the increase in the lactate concentration in brain tissue characteristic of hypoxia (Fig. 1B). Similar results were obtained with heart muscle tissue. In animals receiving valproate not only was the accumulation of lactate due to hypoxia prevented, but its background concentration was actually lowered below the control level (Fig. 2b, 3). It can be tentatively suggested that this was due to an improvement in the conditions for conversion of lactate under the influences of valproate. It has been shown [12] that SSDH is a more sensitive enzyme to the action of valproate than GABA-T. The inhibition constants of these enzymes *in vitro* are 22.9 and 0.5 mM respectively. Whereas the doses in which valproate inhibits GABA-T *in vivo* are 400-600 mg/kg [14], it can be postulated that a dose of valproate of 50 mg/kg, in which it exhibits an initial antihypoxic effect, is quite sufficient to inhibit SSDH. The conditions are thereby created for a shift of the "GABA shunt" reactions toward reduction of succinic semialdehyde into γ-hydroxybutyric acid (GABA). Since in the course of this reaction the reduced form of NAD-dependent dehydrogenases is converted into the oxidized form, the reserves of additional hydrogen acceptors are increased, with the result that the lactate concentration in the tissues falls and functional disturbances caused by its accumulation are prevented.

An important role in the mechanism of the antihypoxic activity of valproate is thus played by a shift of the "GABA shunt" reactions toward the formation of succinic semialdehyde, with its subsequent reduction to GHBA, whereas accumulation of GABA itself is not accompanied by any increase of resistance to hypoxia. This is demonstrated by the fact that valproate has a distinct antihypoxic effect, similar to that of succinic semialdehyde and sodium hydroxybutyrate, even in small doses, and by the fact that diphenylhydantoin, another SSDH inhibitor, has a similar effect, whereas the substances listed above, which cause marked accumulation of GABA, have no such effect. Although the mechanism of the antihypoxic activity of valproate requires further study, the fact that it does have such an effect is itself interesting. The well-marked antihypoxic activity of valproate, much greater than that of piracetam and, with respect to some parameters, actually stronger than the effect of sodium hydroxybutyrate, an active antihypoxic agent, demonstrates the potential value of research into new aspects of the use of valproate in clinical neurology and, in particular, in anesthesiology and resuscitation practice.

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