INCREASE IN RESISTANCE OF MICE TO HYPOXIA PRODUCED BY TRANQUILIZERS OF THE BENZODIAZEPINE SERIES

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The effect of diazepam (Seduxen), chlorodiazepoxide (Librium), and nitrazepam (Mogadon) on life span was studied in albino mice kept in a closed chamber with reduced oxygen concentration (8.7 vol.%). The drugs listed above increase the life span of mice under these conditions, diazepam being most effective. The protective effect of this lasts over 4-5 h and has considerable therapeutic latitude: it is exhibited in a dose of 10 mg/kg, or 1:24 or LD_{50} for diazepam. It is postulated that this property of diazepam may render it useful in clinical practice for the treatment of hypoxic states. The tranquilizer meprobamate, a propandiol derivative, like the neuroleptic chlorpromazine, has no marked protective action under hypoxic conditions.

Benzodiazepine derivatives have recently found application not only in psychiatric practice [1, 12, 13], but in other fields of medicine, notably in anesthesiology, for premedication and the induction and maintenance of anesthesia [14-16]. Surgical operations are frequently accompanied by hypoxia through spasm of the peripheral blood vessels. Hypoxia can also develop at certain stages of an operation, for example if the circulation is excluded. It is therefore desirable that all substances used during operations should be studied from the point of view of their effect on hypoxia. With regard to anesthetics such as diethyl ether and barbiturates, which have been in use for a long time, it is known that they have mainly an adverse effect on the resistance of animals to hypoxia [8, 10, 17]. This effect depends, first, on their ability to disturb synthesis of high-energy phosphorus compounds, giving an "uncoupling" effect [9], and second, on circulatory failure and depression of respiration. Superficial barbiturate (but not ether) anesthesia is sometimes accompained, admittedly, by a slight increase in resistance to hypoxia, evidently due to a reduction of the energy expenditure, to prevention of hypoxic convulsions, and to hypothermia [4, 11,18]. However, this effect appears if barbiturates are given in near-toxic doses [6]. Sodium hydroxybutyrate is more effective and has greater therapeutic latitude than the barbiturates [3, 5, 7]. Compounds of the benzodiazepine series have not been specially investigated in this direction.

The object of the present investigation was to study the effect of certain tranquilizers of the benzodiazepine series on the resistance of animals to hypoxia. Comparative studies were made of meprobamate and chlorpromazine.

EXPERIMENTAL METHOD

Hypoxia of respiratory type was induced by lowering the partial pressure of oxygen in the inspired air to 8.7 vol.% (by Haldane's method). The tests were carried out on albino mice kept in 1750-ml exsiccators, in which some of the air was replaced by nitrogen supplied from a cylinder through a meter. The

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exsiccator cocks were then closed and the time noted when hypoxic convulsions appeared and respiratory movements stopped. The life span of mice not receiving the drugs (control) and of mice receiving one of the test drugs at various times (30 min, 1-6 h) before hypoxia was determined.

At least 16 mice were used in each series. All the experiments were carried out in February or March, a very important factor for the control level: in winter and spring, the life span under hypoxic conditions is somewhat longer than in summer. Diazepam, as Seduxen solution (Richter) in ampules, cholordiazepoxide, as Elenium (Polfa) tablets, and nitrazepam, as a powder, were given in doses of 10 mg/kg, meprobamate (powder) in doses of 50 and 100 mg/kg, and chlorpromazine in doses of 5 and 20 mg/kg body weight. All the drugs were injected intraperitoneally: diazepam and chlorpromazine as solutions, and chlordiazepoxide, nitrazepam, and meprobamate as suspensions in Tween-80 or starch.

EXPERIMENTAL RESULTS AND DISCUSSION

Mice not receiving any of the drugs, when placed in a chamber with an oxygen concentration reduced to 8.7 vol.% died after 23 ± 1 min. The first of every group of 4 mice placed in the exsiccator died after 17 ± 0.6 min, and the rest after 28 ± 2 min. Diazepam, if injected 30 min before the beginning of exposure to hypoxia, more than doubled the life span of the animals: in the mice of this series it was 52 ± 2.7 min, the first mice dying after 38 ± 4.1 min and the rest after 66 ± 4.2 min. The effect of diazepam also was observed when the drug was injected 1 h before hypoxia: in this case the life span was 51 ± 3.1 min. The ability of diazepam to increase the life span of mice under hypoxic conditions still persisted 3 or 4 h after its administration, when the life span was 38 ± 4.2 and 38 ± 4.6 min respectively. The effect was no longer observed 5-6 h after injection of the drug. A similar effect, although somewhat less marked and of shorter duration, on the survival of the mice under these conditions was shown by the other two benzodiazepine derivatives: chlordiazepoxide and nitrazepam. When these were injected 30 min before hypoxia they increased the life span of the mice to 36 ± 5.1 and 34 ± 2.1 min respectively.

To determine whether ability to prolong life during exposure to hypoxia is characteristic of compounds of the benzodiazepine series only, or whether it is found also in tranquilizers of different chemical structure, experiments were carried out with meprobamate. These showed that meprobamate causes no significant increase in the life span of the mice under these conditions: after administration of meprobamate in a dose of 50 mg/kg, the life span was 29 ± 3 min, and after a dose of 100 mg/kg it was 26 ± 2 min. It can be concluded that the anticonvulsant activity common to both groups of tranquilizers is not the cause of the observed effect of the benzodiazepine derivatives. The decrease in motor activity likewise could not be an important cause of this effect, as the negative results of experiments not only with meprobamate, but also with chlorpromazine showed. Chlorpromazine, in doses of 5 and 20 mg/kg, sharply inhibiting motor activity, produced no significant increase in the life span of the mice: with a dose of 5 mg/kg this was 27 ± 5 min, only slightly greater than the control, while with a dose of 20 mg/kg it was less than the control (12 ± 1.5 min). It can be assumed that the anticonvulsant effect and the hypodynamia, playing an important role in the mechanism of action of barbiturates in hypoxia [18], are unimportant in the case of benzodiazepine derivatives. Their protective effect under hypoxic conditions must evidently be due to their specific interference in metabolism, so that the sensitivity of the tissues to oxygen deficiency is reduced. Preliminary observations suggest that an increase in the resistance of the brain and, in particular, of the cortical structures to hypoxia plays a definite role in the mechanism of this effect. For example, experiments on curarized rabbits showed that the electrocorticogram and the direct cortical response disappeared in the control animals 2-3 min after exclusion of respiration. In animals receiving diazepam or chlordiazepoxide, on the other hand, the time of disappearance of cortical potentials was delayed until 5-6 min.

Of the 3 benzodiazepine derivatives studied, diazepam had the greatest protective effect under hypoxic conditions. It is important to emphasize that diazepam is effective when given in doses much below the toxic level. Whereas LD_{50} of diazepam for mice by intraperitoneal injection is 240 (129-300) mg/kg [2], a dose of 10 mg/kg is sufficient to more than double the life span during exposure to hypoxia, giving a mean ratio of 24:1 between these doses. Barbiturates do not give such as definite effect; in the writers' experiments with nembutal, for example, the life span during hypoxia was increased by only 30-40%, and this effect appeared with a dose of 50 mg/kg, whereas LD_{50} for this compound is 110 (93-129.7) mg/kg. Even sodium hydroxybutyrate, which has proved its worth in resuscitation as an effective drug for the treatment of hypoxic states, has a lower index of antihypoxic action than diazepam. The survival period of the mice during hypoxia was doubled by a dose of 500 mg/kg, approximately one-tenth of its LD_{50}. Diazepam differs

from sodium hydroxybutyrate also in giving a longer effect: hydroxybutyrate exerts the action described for not more than 1-1.5 h, compared with 4-5 h for diazepam. The facts described in this paper suggest that the ability of diazepam to increase resistance to hypoxia justifies its use in clinical practice.

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