EFFECT OF SODIUM HYDROXYBUTYRATE ON BEHAVIOR OF MICE AFTER PROLONGED ISOLATION

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Features of passive defensive behavior developing in mice kept for a long time in isolation and appearing on contact with unaggressive groups of mice can be prevented by sodium hydroxybutyrate. In a dose of 50 mg/kg, administration of which does not disturb normal motor activity, the compound suppressed alerting response, reduced the frequency of defensive postures and running away from the partner, and strengthened manifestations of "contact" behavior (approaching the partner, sniffing it). This suggests that sodium hydroxybutyrate, in small doses has a specific tranquilizing action similar in character to the effect of diazepam and other benzodiazepine derivatives. The similarity between the other neuropharmacological characteristics of sodium hydroxybutyrate and of the benzodiazepines and the possibility of common mechanisms of their tranquilizing activity are discussed.

Sodium hydroxybutyrate has found increasingly wide application in recent years in anesthesiology. So far as its use in psychoneurology is concerned, although the first reports on this matter appeared in 1962 [6], the compound has not subsequently been used on any considerable scale. One reason for this was that if given to patients with psychopathological syndromes it did not have a specific antipsychotic action. However, the ability of sodium hydroxybutyrate to increase communicativeness [2] and to reduce affective stress [12], observable even in these patients, suggests that it could be used in neurotic and neurosis-like states. One way in which a neurotic state can be simulated experimentally is by keeping animals in prolonged isolation. The changes in behavior and hormonal activity developing under such conditions are so marked that they can be described as "isolation stress" [8, 17].

The object of this investigation was to study the effects of sodium hydroxybutyrate on behavioral responses arising in mice kept in isolation when put on contact with other mice kept in a group.

EXPERIMENTAL METHOD

Male albino mice weighing 22-24 g were used. The animals were kept in isolation in cages measuring 3 × 16 × 13 cm with opaque glass windows but with free access to food and water. The isolation continued for 23 days; during that time no contact was allowed even with the attendants. A second batch of mice was kept in ordinary cages, 20 animals in each. On the 24th day each mouse kept in isolation was placed in a chamber measuring 20 × 30 × 20 cm with transparent walls, and after the lapse of the 30 min required for adaptation, another mouse previously kept in a group was placed in the same cage with it. Starting from that moment, observations were made by two experimenters on the behavior of each mouse of the pair. At the end of contact for 200 sec, during which each experimenter recorded the postures and movements of one of the two mice, the animals were returned to their original conditions. Experiments were repeated

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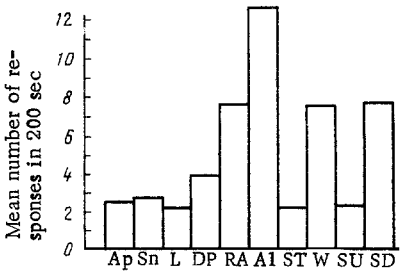

Fig. 1

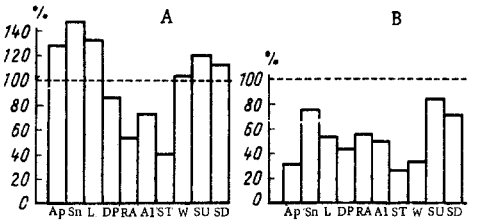

Fig. 2

Fig. 1. Chief behavioral responses of mice kept in isolation when put in contact with unaggressive mice previously kept in a group (results of observations on mice observed to give a passive-defensive reaction): AP) approaching partner; SN) sniffing partner; L) leaving partner alone; DP) defensive postures; RA) running away; Al) alertness; ST) shaking tail; W) walking around the cage; SU) standing up; SD) sitting down.

Fig. 2. Effect of sodium hydroxybutyrate in doses of 50 mg/kg (A) and 200 mg/kg (B) on manifestation of passive-defensive response after isolation. Broken line at 100% level corresponds to control. Legend as in Fig. 1.

with each pair of mice after intervals of seven days. Sodium hydroxybutyrate (sodium salt of γ-hydroxybutyric acid, synthesized at the Institute of Pharmacology, Academy of Medical Sciences of the USSR [1]) was given internally in doses of 50 and 200 mg/kg before the beginning of adaptation. To rule out any effect of habituation on the experimental results, the order of administration of the compound and water was alternated in each subgroup. Mice kept in a group were given only water. The volume of liquid administered was 0.1 ml/10 g body weight. The most clearly defined postures and movements were chosen for recording; preliminary experiments showed a sufficiently high level of correlation ($r = 0.75$–0.95) between the observations made by the two experimenters. The effect of the compound was assessed by the use of a nonparametric test [16] and the same animals, receiving water, were used as the control.

EXPERIMENTAL RESULTS AND DISCUSSION

The response of one mouse to another differs sharply, depending on whether it has previously been kept in isolation or in a group. Whereas the behavior of a mouse kept previously in a group, when placed in contact with a "partner," is unchanged (gait, standing on its hind limbs, sitting, licking its fur), the mouse previously kept in isolation and put in contact with another mouse shows a response characterized by definite changes in behavior. The character of this response differs from one isolated mouse to another. Workers who have studied this problem in the past have paid particular attention to the appearance of aggressive behavior in these animals [18]. However, as a detailed study [11] has shown, aggressiveness appears in approximately 25-30% of isolated mice, a small proportion of mice (about 15-20%) do not respond at all to the partner mouse, and, finally, the majority (50-60%) of isolated mice respond to the introduction of a partner, previously kept in a group, by an alerting response, by running away from the partner, or by a defensive posture (Fig. 1). The study of the effect of sodium hydroxybutyrate on this form of passive-defensive behavior, which many workers describe by the term "timidity," was given special attention in the present investigation.

In a dose of 50 mg/kg, sodium hydroxybutyrate caused a statistically significant ($P < 0.02$) decrease in the frequency of appearance of the clearest features of passive-defensive behavior, such as running away from the partner, alertness, and shaking the tail (Fig. 2). The frequency of appearance of defensive postures also was reduced. It is important to emphasize that, in this dose, the compound did not reduce the frequency of walking around the cage or standing on the hind limbs, compared with the control, but on the contrary it actually increased the communicativeness of the isolated relative to its partner: the number of approaching movements and sniffings was increased (Fig. 2).

In a larger dose (200 mg/kg) sodium hydroxybutyrate also reduced the visible manifestations of passive-defensive behavior (alertness, running away from the partner, shaking the tail), but this effect was not so selective as when the smaller dose was given: it was also exhibited with respect to such features as walking around the cage, approaching the partner and leaving it alone, but to a lesser degree with respect to standing on the hind paws.

Behavior of the mice kept in a group was identical regardless of whether their partners were mice receiving sodium hydroxybutyrate or mice receiving water. Consequently, the changes in behavior of the isolated mice were in fact due to the compound itself and not to changes in the partner's behavior.

These results show that sodium hydroxybutyrate definitely reduces the degree of manifestation of certain behavioral reactions developing in mice during prolonged isolation. After administration of a small dose of the compound its effect shows considerable selectivity. This is shown by preservation not only of motor activity, but also of the normal ratio between the slow and paradoxical phases of sleep as determined at the Institute of Pharmacology, Czechoslovak Academy of Sciences, by Kadlecova. These facts suggest that sodium hydroxybutyrate, in small doses, can inhibit the manifestigation of alarm and anxiety and that this property is not the result of the general sedative action of the compound, but of a more selective tranquilizing effect. Several other depriming substances also modify the behavior of isolated mice [11]. However, the effect of these drugs and of sodium hydroxybutyrate is different. Chlorpromazine, for instance, although reducing manifestations of timidity, nevertheless gave this effect starting with doses of 7.5 mg/kg, which inhibited general motor activity. Some manifestations of timidity were abolished by small doses of barbital (20 mg/kg), but under these circumstances the mice developed signs of aggression which had previously been absent. The greatest similarity with sodium hydroxybutyrate is shown by chlordiazepoxide, which not only suppresses various passive-defensive responses, but also increases the manifestations of communicativeness.

Mention must also be made of other evidence of the similarity between sodium hydroxybutyrate and the benzodiazepine derivatives. Of the many neurotropic drugs, only these compounds do not inhibit the phase of paradoxical sleep, whereas the barbiturates, neuroleptics, and meprobamate reduce its intensity [9]. Certain common brain structures are evidently implicated in the mechanism of the effects of the benzodiazepine derivatives and of sodium hydroxybutyrate. For instance, they have been shown to inhibit the conduction of impulses along interzonal, interhemispheric, and other cortico-cortical connections but to have a less marked effect on ascending reticulo-cortical projections. Benzodiazepine derivatives and sodium hydroxybutyrate differ in this respect also from the barbiturates, the effect of which on the cortex is less selective, and from the phenothiazine neuroleptics, for which no direct cortical effect has been demonstrated [3, 5].

The possibility of certain common biochemical mechanisms in the action of sodium hydroxybutyrate and the benzodiazepine derivatives cannot be ruled out. γ-Aminobutyric acid is known to be metabolically closely connected with γ-hydroxybutyric acid [7, 14]. On the other hand, the work of Saad [15] has shown that diazepam raises the brain γ-aminobutyric acid level. Interference with γ-aminobutyric acid metabolism is possibly one of the common links in the chain of metabolic changes that arise after administration of sodium hydroxybutyrate and diazepam. The possibility cannot be ruled out that they have a similar action also on certain stages of carbohydrate metabolism. Both benzodiazepines and sodium hydroxybutyrate lower the lactate concentration in the brain [4, 19]. Since injection of lactate in man gives rise to a sense of anxiety and stress [10, 13], it may be that the ability of these substances to lower the lactate level is one cause of their tranqulizing action.

In conclusion it must be emphasized that the experimental model suggested above may prove convenient for the discovery of drugs reducing the sense of anxiety. It can be used to study the physiological and biochemical mechanisms of the anxiety-relieving effect.

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