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IONIC REGULATION OF RECEPTION OF ^{35}S-*tert*-BUTYLBICYCLOPHOSPHOROTHIONATE BY BRAIN MEMBRANES OF INBRED MICE DIFFERING IN EMOTIONAL STRESS RESPONSE

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Binding of ^{35}S-*tert*-butylbicyclophosphorothionate (^{35}S-TBPS) reflects the function of the effector component of the supramolecular GABA-benzodiazepine receptor complex (BDRC), a Cl^--ionophore [13]. The study of reception of this ligand by brain membranes of C57BL/6 (B6) and BALB/c (C) mice, which differ in their response to emotional stress in the open field (OF) test and to administration of benzodiazepine tranquilizers [5] has shown that its binding is characterized by different degrees of dependence on the Cl^- ion concentration in the medium for animals of the inbred lines used [2]. Interlinear differences in ionic regulation of ^{3}H-diazepam reception [1, 2], discovered previously, data on the coupling of reception in benzodiazepine and Cl^--ionophore regions of BDRC [4], and also the views of other workers on the existence of a binding site for cations in BDRC [11], were taken into consideration.

In the investigation described below, to continue the study of the mechanisms of formation of hereditary differences in receptor activity of BDRC, the effect of various salts on binding of ^{35}S-TBPS by brain membranes of B6 and C mice was studied.

EXPERIMENTAL METHOD

Experiments were carried out on male B6 and C mice weighing 18-20 g, obtained from the "Stolbovaya" Nursery, Russian Academy of Medical Sciences. The mice were kept under animal house conditions at the Research Laboratory of Pharmacogenetics, Institute of Pharmacology, Russian Academy of Medical Sciences, for at least 2 weeks before the experiment began, on a standard diet, with 10 mice to a cage, and with 12 h daylight alternating

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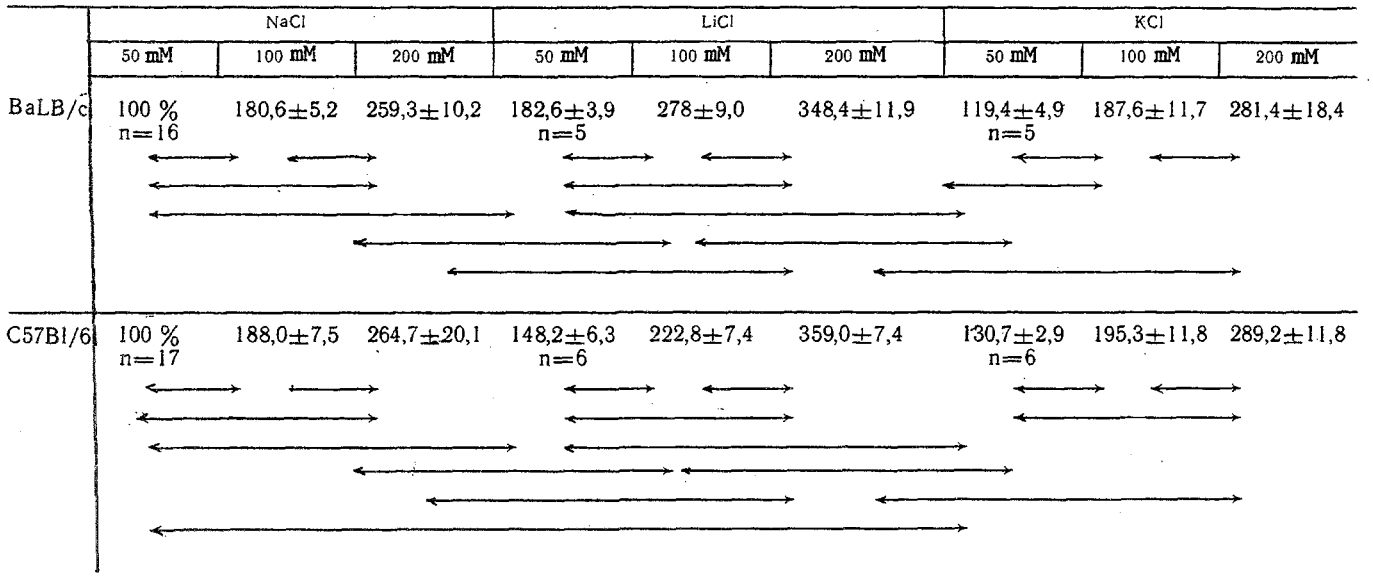
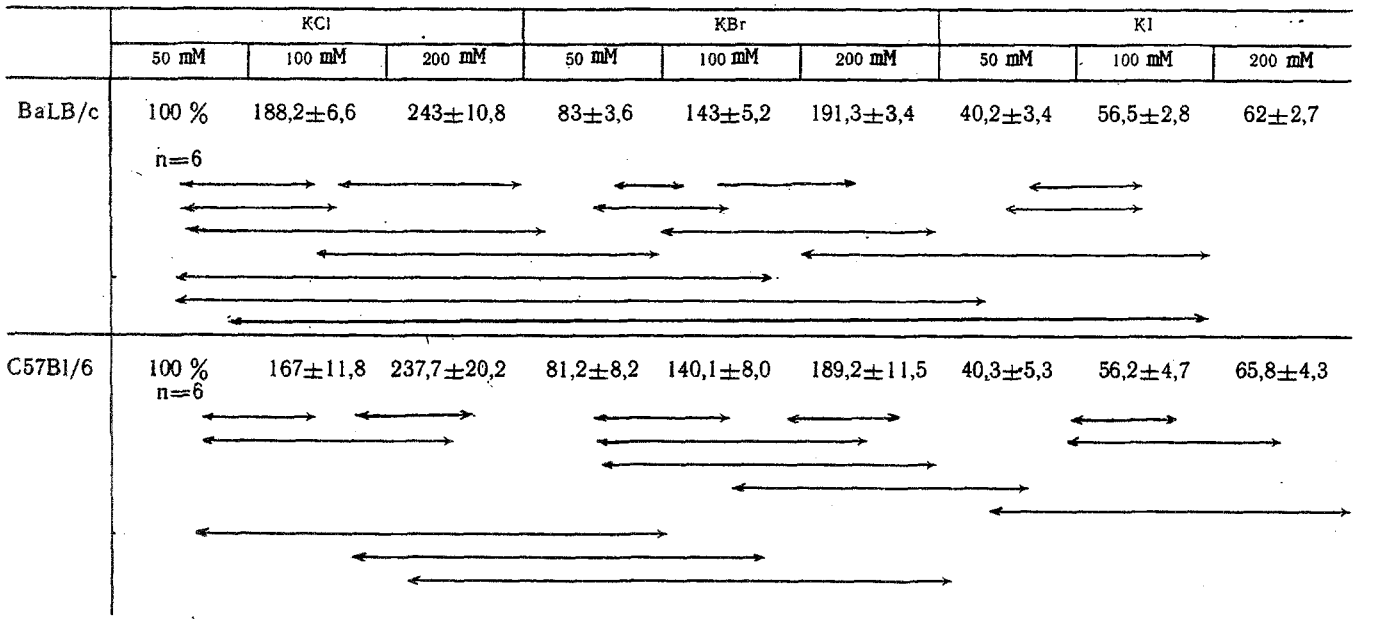
TABLE 1. Effect of Chlorides of Alkali Metals on Binding of ^{35}S-TBPS with Brain Membranes of Inbred Mice (M ± m_x)



Legend: n) number of animals; ⇔) statistically significant differences ($p < 0.05$).

TABLE 2. Effect of Halides of Potassium on Binding of ^{35}S-TBPS with Brain Membranes of Inbred Mice (M ± m_x)



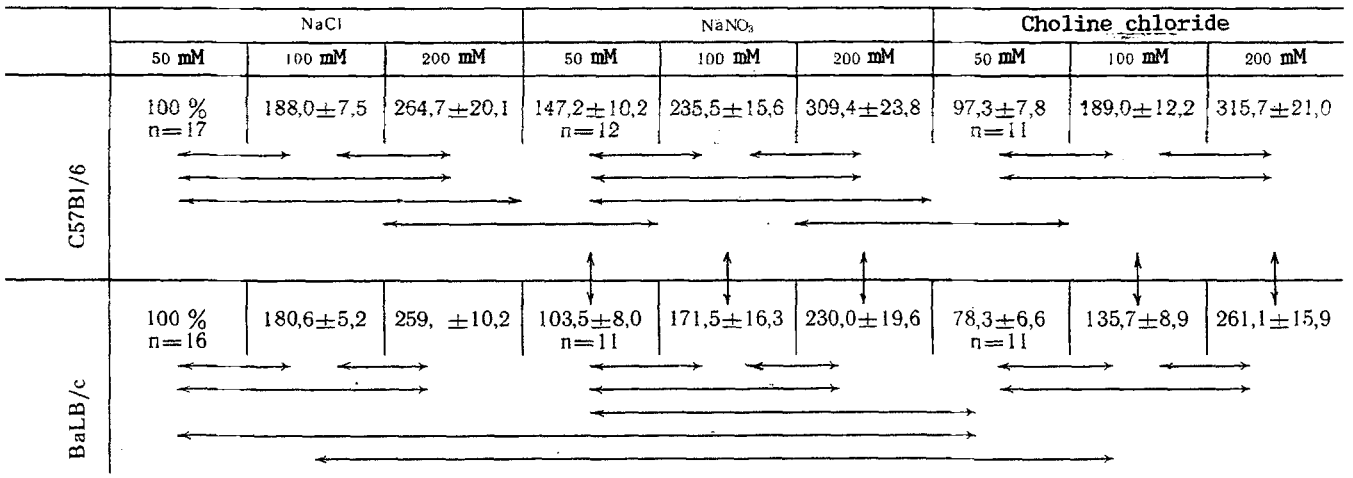
Legend: n) number of animals; ⇔) statistically significant differences ($p < 0.05$).

with 12 h darkness. The membrane fraction of the brain was isolated and radioligand binding carried out by methods described previously [2]. ^{35}S-TBPS (specific activity 87.7 Ci/mmole, from "New England Nuclear," USA), was used as the ligand. The results were subjected to statistical analysis by Student's test for untied and tied pairs.

EXPERIMENTAL RESULTS

The data given in Table 1 demonstrate the comparative efficacy of chlorides of the alkali metals (Na^+, Li^+, K^+) in stimulating binding of ^{35}S-TBPS with brain membranes of inbred mice. It will be seen that all three salts, de-

TABLE 3. Effect of Different Sodium Salts on Binding of ^{35}S-TBPS with Brain Membranes of Inbred Mice (M ± m_x)



Legend: n) number of animals; ⇔) statistically significant differences ($p < 0.05$).

pending on their concentration, increased the level of reception in mice of both lines. Lithium chloride was found to have maximal stimulating capacity, and KCl differed significantly in its effect from NaCl only in a concentration of 50 mM. No interlinear differences were found in the relative stimulating ability of similar KCl and NaCl concentrations. Conversely, C mice exhibited high activity of LiCl, especially in concentrations of 50 and 100 mM.

To compare the effect of anions on binding of ^{35}S-TBPS we used KCl, KBr, and KI. The experiments showed that of the anions used, the highest stimulating capacity for both lines of mice was possessed by Cl^- ions (Table 2). Addition of KI appreciably lowered the level of reception compared with KCl and KBr, without any marked interlinear differences. However, whereas with C animals replacement of Cl^- by Br^- regularly reduced the stimulating activity of all concentrations used, in B6 animals this parameter was unchanged.

To answer the question of the relative importance of the anionic and cationic components of the increase in binding of ^{35}S-TBPS with brain membranes, a comparative study was made of the stimulating ability of NaCl, $NaNO_3$, and choline chloride (Table 3). Replacement of NaCl by $NaNO_3$ did not change the stimulating capacity of all concentrations used with C mice. On the addition of choline chloride in concentrations of 50 and 100 mM binding of the radioligand was less, but with 150 mM it was the same as after addition of similar NaCl concentrations. Comparative analysis of the stimulating capacity of $NaNO_3$ and choline chloride showed that only in a concentration of 50 mM did choline chloride stimulate binding of the radioligand less strongly, whereas activity of the other concentrations of the two salts was similar. Conversely, in B6 animals addition of $NaNO_3$ instead of NaCl increased the level of reception in concentrations of 50 and 100 mM appreciably more strongly. However, by contrast with C mice, the stimulating activity of all concentrations of choline chloride used did not differ from activity of the corresponding concentrations of NaCl. Comparison of ability of choline chloride and $NaNO_3$ to increase binding of the radioligand revealed stronger stimulating activity of $NaNO_3$ in concentrations of 50 and 100 mM. Interlinear differences in this case consisted of a significantly higher stimulating activity of all concentrations of $NaNO_3$ for B6 mice compared with C, and weaker ability of 100 mM choline chloride to increase binding of the radioligand in C mice.

On the whole, the results are in agreement with those of other investigations [7, 12]. However, our analysis cast some doubts on the validity of the traditional interpretation of the mechanism of ionic regulation of reception in the region of BDRC, which has usually been regarded in connection with the ability of ions to pass through the corresponding ionic channels. In fact, in some cases high correlation was observed with this parameter [7], but in other studies this was not the case [10]. In our view the order of activities of the anions ($Cl^- > Br^- > I^-$) which was obtained can hardly be explained simply by the different affinity of both other ions for the Cl^--ionophore, more especially because permeability of the channel for Br^- and I^- ions is higher than for Cl^- [6]. Examination of the action of the ions from the standpoint of their ability to modify the charge on the membrane may be a more promising approach. The validity of this argument is confirmed by data given in Table 3. It can be concluded from their

analysis that characteristics of the ions are more important for the regulation of binding of ^{35}S-TBPS in B6 mice, but the cationic component is more important for C animals.

The order of activities of the cations ($Li^+ > K^+ > Na^+$) likewise does not agree with their relative ability to pass through the ionic channel [3]. However, considering the degree of hydration of these ions and, most important, their ability to lose (K^+) or not to lose (Na^+, Li^+) their hydration shell [3], the arrangement of the ions coincides fully with the various activities we obtained. With this approach the decisive factor, given an equal charge, is most probably the density of the charge on the ion surface. This conclusion is confirmed by examination of the biological membrane as an ion-exchanger with negative surface charge. In such a model system, the cations are arranged in the following order of activity of surface interaction: $Li^+ > K^+ > Na^+$ [8, 9].

Thus our findings as a whole are evidence that receptor restructuring in the Cl^- ionophore region of BDRC may be effected through electrostatic interaction between ions and the membrane surface. The possibility cannot be ruled out that interlinear differences discovered in the character of reception of the ligand with the membrane, are associated with an unequal surface charge in the animals of the genotypes used.

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