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SEIZURE PRONENESS AND NEUROTRANSMITTER UPTAKE

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The ability of midbrain homogenates from two strains of mice to accumulate several putative neurotransmitters, or their precursor in the case of acetylcholine, has been examined. The high-affinity transport mechanisms toward glutamate, GABA, dopamine, and glycine were similar in both strains. The seizure-prone DBA/2IBG strain had a significantly higher capacity to transport choline than did the relatively seizure-resistant C57BL/6 IBG mice. However, no difference in the density of muscarinic binding sites in the two mouse strains was found.

INTRODUCTION

Mouse strains may have widely differing susceptibility to seizures. Thus, the induction of convulsions, either by pharmacological or audiogenic means, is more readily accomplished in the DBA/2J line than in C57BL/6J mice (1). This differential sensitivity is maximal at 21 days age when the convulsive proneness of DBA mice is greatest (2, 3). The fact that these mice are excessively liable to seizures induced by a variety of con-

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vulsive stimuli suggests a generalized cerebral hypersensitivity rather than a difference in a sensory modality (4).

Several neurotransmitter-related enzymes have been found to have differing levels in these strains of mice (5–7). A relation between seizure susceptibility and reduced levels of monoamine transmitters has been proposed (5, 8), but changes in cholinergic system enzymes have also been suggested as the underlying cause of audiogenic seizure sensitivity (9, 10).

In this study we have measured the activity of the high-affinity transport system toward putative neurotransmitters or their precursors in these two mouse strains. While not confined to the presynaptic area, sodium-dependent high-affinity uptake systems are characteristic of and localized to nerve tissue (11, 12). It was felt that measurement of such movements of neurotransmitters, rather than assay of their concentrations and of the levels of their associated enzymes, might be a functional index of inter-strain differences more closely related to excitability. Both mouse lines concentrated four putative neurotransmitters from around 10^{-8} M concentration to a very similar extent. However, the seizure-prone DBA mice exhibited a considerably higher capacity for choline accumulation than did the resistant C57 mice.

EXPERIMENTAL PROCEDURE

C57BL/6IBG (C57) and DBA/2IBG (DBA) mice were obtained from the Institute for Behavioral Genetics, University of Colorado, Boulder, through the courtesy of Dr. K. Schlesinger. They were derived from original strains developed in the Jackson Laboratories, Bar Harbor, Maine. Care was taken not to expose experimental mice or their mothers to environmental stresses such as excess noise or cold. All determinations were carried out simultaneously on both mouse strains and on the F_1 hybrid, in order to allow optimal evaluation of strain differences. To minimize the effects of diurnal variation, animals were always sacrificed between 10 AM and 2 PM. At 21 days of age, animals were decapitated, the brains removed, and the cortex and cerebellum carefully dissected away and discarded. The remaining tissues were rapidly weighed and then homogenized in 19 volumes of 0.32 M sucrose.

Incubation. The standard incubation medium consisted of Krebs-Ringer buffer containing 121 mM NaCl, 4.0 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1 mM ascorbic acid, 0.015 mM EDTA, 20 mM glucose, and 40 mM Tris HCl, pH 7.6. An inhibitor of monoamine oxidase (nialamide) was also present (7.5×10^{-5} M) as was aminooxyacetic acid (1×10^{-5} M), an inhibitor of γ-aminobutyric acid transaminase. To this medium was then added a single radioactive compound (New England Nuclear, Boston, Massachusetts). Final μM concentrations of each compound were: DL-[3-^{3}H]glutamic acid 0.01 (8.2 Ci/mmol); [2,3-^{3}H]GABA, 0.01 (10 Ci/mmol); DL-[7-^{3}H]norepinephrine, 0.013 (7.5 Ci/mmol); [*methyl*-^{3}H]choline chloride, 0.017 (2.34 Ci/mmol) and [*ethyl*-1-^{3}H]3,4-dihydroxyphenylethylamine (dopamine) 0.0125 (8.0 Ci/mmol). Radioactive choline was used (rather than acetylcholine) since it is relatively stable and specifically transported by cholinergic neurons (13).

This medium was gassed with 95% O_2–5% CO_2 and 0.9 ml of this was mixed with 0.1 ml of a 5% (v/v) tissue homogenate in sucrose. Incubation was at 37°C for 5 min with continuous shaking. To allow for non-energy-dependent neurotransmitter binding, identical mixtures were held at 0°C, and these served as controls. In the case of dopamine, incubations were performed in dim light in order to retard photodecomposition.

All samples were then centrifuged at 0°C and 28,000 *g* for 10 min. Supernatants were drawn off for determination of radioactivity remaining unbound to particulate matter. Pellets were resuspended in 4 ml isotonic (0.14 M) NaCl and recentrifuged (28,000 *g*, 10 min). The washed pellets were then dissolved in 0.5 ml tissue solubilizer (NCS, Amersham Searle, Arlington Heights, Illinois) at 45°C. Radioactivity in these samples were determined. It was thus possible to calculate what percentage of the total radioactive compound in each incubation tube was actively taken up by the particulate fraction. Final results were expressed as picomoles taken up/minute/gram wet tissue.

Binding. Dissected tissue was weighed and frozen at −80°C before homogenization in 20 volumes 0.32 M sucrose and centrifugation (40,000 *g*, 10 min). Precipitates were resuspended in 20 volumes cold distilled water and recentrifuged. The final pellet was refrozen, thawed, and suspended in 20 mM Tris HCl, pH 7.1, to a final concentration of 15 mg original tissue/ml. Binding assay was carried out in 1 ml Tris HCl buffer at 30° for 15 min. The reaction mixture consisted of 0.1 ml membrane suspension and 0.05 μCi of quinuclidynyl benzilate (QNB) (New England Nuclear Corp.) of specific activity 19.8 Ci/mmol. This is a potent muscarinic antagonist and can be used to reflect the density of cholinergic muscarinic receptor sites. Control tubes also contained 10^{-4} M atropine. At the end of incubation, tubes were centrifuged (40,000 *g*, 10 min) and supernatants collected to assay unbound radioactivity. Pellets were washed once in 4 ml Tris HCl buffer, recentrifuged, and dissolved as described above. Nonspecific counts (bound in the presence of atropine) were always below 15% of total bound radioactivity.

RESULTS

The transport systems toward GABA, glutamate, and glycine in the two parent mouse strains differed in uptake capacity by less than 6% and were thus very similar in intensity (Table I). Dopamine accumulation was around one third greater in the DBA/2J mice, but this was not statistically significant. The greatest difference between the two mouse lines lay in their relative ability to concentrate radioactive choline. This was 40% greater in the C57B1/6J line than in the DBA/2J mice, and this was highly significant ($P < 0.001$, Students two-tailed *t* test). The F_1 generation produced by crossing these strains had an uptake capacity toward choline that was intermediate between the parent lines and was significantly different from both ($P < 0.02$). Other values for the F_1 strain were not greatly different from the original strains except that dopamine uptake was markedly low in the F_1 cross.

In view of the large difference in ability to accumulate choline, membrane preparations from midbrain homogenates of the parent strains were prepared and assayed for their ability to bind [^{3}H]QNB. No significant

TABLE I

HIGH-AFFINITY UPTAKE OF PUTATIVE NEUROTRANSMITTERS OR THEIR PRECUSORS BY HOMOGENATES OF MIDBRAIN REGIONS OF SEVERAL STRAINS OF MOUSE AT 21 DAYS OF AGE[a]

	Transport (pmol/min/g wet tissue)		
^{3}H compound	DBA/2IBG	C57BL/6IBG	Fl Cross
Choline	25.7 ± 0.6	36.0 ± 1.3*	30.9 ± 1.4*
GABA	65.1 ± 1.2	63.2 ± 1.7	62.6 ± 1.8
Dopamine	294.0 ± 26.0	220.0 ± 40.0	209.0 ± 30.0
Glutamate	239.0 ± 10.0	253.0 ± 19.0	236.0 ± 6.0
Glycine	27.3 ± 1.0	28.9 ± 1.5	24.1 ± 0.4

[a] Standard errors of the mean are presented. * indicates that value differs significantly from the corresponding value for DBA/2J mice ($P < 0.05$, Students two-tailed t test). Each data point represents the mean of figures derived from 6 to 19 individual mice. Incubation was 5 min at 37°. Details of the procedure are given in the text.

TABLE II

MUSCARINIC RECEPTOR DENSITY OF MEMBRANES PREPARED FROM 21-DAY-OLD MICE, DETERMINED BY ABILITY TO SPECIFICALLY BIND [^{3}H]QNB[a]

[^{3}H]QNB bound (pmol/g original wet tissue)	
DBA/2IBG	C57BL/6IBG
81.6 ± 3.0	72.4 ± 6.7

[a] Standard errors of the mean are presented. Each data point represents the mean of figures derived from 6 individual mice. Incubation for 15 min at 30°. Details of the procedure are given in the text.

differences in the binding capacity of membrane preparations from the two mouse strains was found (Table II).

DISCUSSION

Previous data from this laboratory have shown that the transport mechanisms for choline, GABA, glutamate, and glycine are all highly specific (14). The dopamine uptake system, however, seems to be partially indistinguishable from uptake systems relating to norepinephrine and serotonin (12, 15). The sodium dependence of high-affinity uptake by mouse

brain homogenates under our conditions has also been previously established (14). The short incubation time used minimized metabolic conversion of these compounds (11, 16, 17), and their low concentration ensured that any uptake was largely due to high-affinity mechanisms. An advantage of using homogenates to study such energy-dependent uptake, as opposed to purified synaptosomes is that there is no preparative loss of tissue. Thus, the ability of various mouse strain brain regions to accumulate neurotransmission-related compounds could be precisely compared. The whole brains minus neocortex and cerebellum were employed in this study since audiogenic seizure induction in mice appears to involve solely subcortical mechanisms (18, 19). Our data clearly show a major difference in choline uptake by the two mouse strains. The F_1 hybrid which has an intermediate seizure susceptibility (8) also had an intermediate choline transport capacity. Audiogenic seizure proneness appears to be an autosomal recessive (1, 2). More extensive cross breeding studies would be needed to determine whether this was linked to cholinergic transport capacity. These differences could have several underlying explanations. Relatively greater reuptake of acetylcholine from the synaptic cleft by the C57 mice could account for the lesser excitability of this strain, since acetylcholine tends to be excitatory centrally (20). The choline acetyltransferase activity in the cochlea of seizure-prone Swiss albino (rb) mice has been found to be 50% higher than in the corresponding seizure-resistant line (10). Scopolamine, a muscarinic antagonist, has been shown to increase locomotor activity in rats (21). Thus, reduced cholinergic drive can also have excitatory effects. In support of this is the finding that the binding of [^{3}H]QNB in the amygdaloid regions of the rat is depressed after kindling (22). However, another possibility is that the cholinergic synapses in the C57 mice are anatomically positioned so as to reduce overall excitability. Kellogg (3) has suggested, in the case of serotonin neurons, that higher turnover rates in seizure-prone mice in some circumstances reflect an ineffective compensation for a deficiency in density of serotonergic neurons.

The greater ability of homogenates from DBA mice to transport dopamine, while not significantly different from the C57 mice ($P = 0.1$), was in accord with other data showing an inverse correlation between monoamine levels and audiogenic seizure susceptibility in these strains (23).

Overall convulsive tendency appears to be related to levels and metabolic parameters of a wide variety of neurotransmitter candidates. Although a single biochemical change may be the primary cause of differences in convulsion susceptibility, this may also lead to many secondary modifications. For example, the reduction in dopaminergic drive caused

by reserpine can enhance the intensity of choline uptake in the corpus striatum (24). It is thus not possible to attribute seizure proneness to a single metabolic event at this time.

The data from the [^{3}H]QNB binding assay suggest that the major difference between the strains lies in their relative cholinergic uptake capacity rather than in density of muscarinic, postsynaptic receptor sites. The DBA mice had a slightly higher binding site value than the C57 mice, but this 12% difference was not significant. Thus, in animals of this age, there was no indication of regulation of the number postsynaptic receptor sites by differences in presynaptic cholinergic activity. Since the seizure susceptibility of these strains becomes very similar in older animals, it would be of interest to know whether this is related to an eventual compensatory reduction of muscarinic receptors in the DBA mice.

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