Genetic Differences in the Synthesis and Reception of Noradrenaline in the Mouse Brain and Behavior in a Novel Environment

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The activity of tyrosine hydroxylase, the key enzyme in catecholamine biosynthesis, was studied along with adrenoceptor density in the brains of male CBA/Lac, BALB/cLac, and C57BL/6J mice, which show different responses to novel environments. C57BL mice showed the highest level of movement activity and the lowest level of emotionality in a novel environment. Mice of this line also showed the highest brainstem tyrosine hydroxylase activity. At the same time, the density of β-adrenoceptors in the cortex and hypothalamus of C57BL mice was lower than in the other two lines of mice, while the density of α2-adrenoceptors in these parts of the brain was lower than in CBA mice. In BALB mice, movement activity was twice as high as in CBA mice, while levels of emotionality were similar in these two lines. Tyrosine hydroxylase activity was higher in the cerebral cortex of BALB mice, while the density of α2-adrenoceptors was lower than in CBA mice. These results show that increased investigative activity and decreased emotionality were seen in animals with higher levels of noradrenaline synthesis and decreased density of adrenergic receptors in the brain.

KEY WORDS: Tyrosine hydroxylase, adrenergic receptors, brain, open field test, mouse lines.

Many data have been obtained indicating that the brain noradrenergic system is involved in controlling behavior linked to stress and anxiety [3-5, 7-10, 12, 14]. The main accumulations of noradrenergic neuron bodies occur in the locus coeruleus of the brainstem. Axons from these neurons innervate the cortex and subcortical structures, including the hypothalamus, via monosynaptic pathways [11]. The activity of noradrenergic transmission is determined by two major factors—mediator synthesis and release into the synaptic cleft and its interaction with the three main types of receptors, α1-, α2-, and β-adrenoceptors [2]. The activity of the key enzyme in catecholamine biosynthesis, tyrosine hydroxylase, is an important factor of the function of the presynaptic part of this system. The activity of this enzyme correlates with the discharge frequency of noradrenergic neurons and mediator metabolism [15]. The postsynaptic effects of the mediator depend largely on the density of its receptors. The noradrenergic system of the brain is activated in novel environments. However, it remains unclear how the properties of the pre- and postsynaptic components of this neuromediator system can affect the features of an individual’s behavioral reactions to a novel stress stimulus. Inbred mouse lines provide a good model for inherited differences in reactions to stress stimuli. Thus, the aim of the present work was to analyze behavior in a novel environment in three lines of mice and the measure tyrosine hydroxylase activity and adrenergic receptor density in the brain.

METHODS

Studies were performed using adult male CBA/Lac, BALB/cLac, and C57BL/6J mice. Animals were
kept with free access to water and feed, in a natural light regime, at a temperature of 22-24°C.

Mice were assessed in an open field test after 17:00 on each of three successive days: mice were placed in a brightly illuminated arena of size 70 x 70 cm, divided into squares of 10 x 10 cm, for 3 min. The latent period of the onset of movement activity was recorded, along with the amount of horizontal movement activity (number of squares crossed) and the number of defecations.

Mice were decapitated and the cortex, hypothalamus, and brainstem, including the hindbrain and pons, were removed quickly in the cold. Homogenates of these brain regions were used for assay of tyrosine hydroxylase activity, performed at a saturating concentration of L-tyrosine (0.8 mM) and cofactor, dimethyltetrahydropteridine (0.8 mM) [1]. Protein concentrations were estimated as described by Lowry et al. Specific membrane binding in the cortex and hypothalamus was assayed using 0.4 nM [3H]prazosin, 1.6 nM [3H]clonidine, and 1.6 nM [3H]dihydroalprenolol (all labeled ligands were obtained from Amersham) and results were used to determine the densities of α1-, α1-, and β-adrenoceptors respectively in these brain structures [13]. Data were analyzed statistically using Student’s t test.

RESULTS

Mice of the three lines studied here showed marked differences in behavior in the open field test (Fig. 1). The latent period for the onset of movement activity in CBA mice in the novel environment was 15 times longer than that in C57BL mice, while the number of defecations was five times greater; conversely, the number of square crossings was three times lower. BALB animals performed the same number of defecations as CBA mice, while BALB mice were between the other two lines for the other two parameters.

Marked interline differences were also seen in tyrosine hydroxylase activity in the brain (Fig. 2). Enzyme activity was highest in C57BL mice in all parts of the brain studied. Animals of this line had cortex and brainstem enzyme activity 1.5 times higher than in CBA mice. Interline differences in enzyme activity in the hypothalamus did not reach statistical significance. BALB mice had enzyme activity in the cortex similar to that in C57BL mice, while tyrosine hydroxylase activity in the brainstem was the lowest of all three lines. Enzyme activity in the brainstem of BALB mice was more than 1.5 times lower than in CBA mice and 2.5 times lower than in C57BL mice.

Mouse lines also showed significant differences in terms of the density of adrenergic receptors in the hypothalamus and cerebral cortex (Fig. 3). The densities of α1- and α2-adrenoceptors were two times lower in C57BL than in CBA mice; additionally, α1-adrenoceptor density was 23% lower in the hypothalamus. BALB mice had similar numbers of α2-adrenoceptors as C57BL mice in both parts of the brain, while the number of β-adrenoceptors in the hypothalamus was identical to that in CBA mice. The densities of β-adrenoceptor in the cortex and α1-adrenoceptor in the hypothalamus in BALB mice were between the densities in CBA and C57BL mice.