GENETICS

Effect of Monoamine Oxidase Gene Knockout on Dopamine Metabolism in Mouse Brain Structures

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Experiments were performed on knockout Tg8 mice lacking monoamine oxidase A gene that plays a major role in dopamine catabolism. The study by the method of high-performance liquid chromatography revealed considerable regional differences in the contents of dopamine and its metabolite dihydroxyphenylacetic acid in brain structures of these animals. Tg8 mice differed from the parent C3H/HeJ strain by low level of dihydroxyphenylacetic acid in the striatum, midbrain, hypothalamus, and hippocampus and high concentration of dopamine in the striatum. No differences were revealed in the contents of dopamine and dihydroxyphenylacetic acid in the frontal cortex and amygdala. The 2.4-4.8-fold decrease in the content of dihydroxyphenylacetic acid in various brain structures was not accompanied by changes in dopamine concentration. These data reflect the effective compensation for deficiency of dopamine metabolism. Our results suggest that monoamine oxidases A and B and catechol-O-methyltransferase play different roles in dopamine metabolism in various brain structures.

Key Words: *monoamine oxidase gene knockout; dopamine; dihydroxyphenylacetic acid in brain structures*

The method of gene knockout allows breeding mouse strains with irreversible damage to specific genes. This approach opens a possibility for studying gene functions and experimental hereditary diseases [1]. The mice with monoamine oxidase A (MAO A) gene knockout [5] attract much attention. This enzyme plays a major role in the catabolism of dopamine (DA), norepinephrine, and serotonin. Moreover, hereditary disease associated with point mutation in the MAO A gene was described in humans [4]. MAO A and MAO B differ in the substrate and inhibitory specificity [8]. They are encoded by various genes that are closely localized on the X chromosome [11]. It was believed that DA is a common substrate for MAO A and MAO B. However, the relative role of these isoenzymes in animals of various species is different. Previous studies showed that in mice kept under normal conditions, DA is metabolized primarily by MAO A. MAO B is involved during compensatory changes in metabolic pathways [6]. No changes were revealed in DA content in the whole brain of adult mice with MAO A gene knockout. However, the content of its metabolite dihydroxyphenylacetic acid (DOPAC) markedly increases in these animals [5]. The intensity of DA synthesis [12] and sensitivity of dopaminergic neurons differ in various brain structures [2]. Since MAO A and MAO B have similar intracellular distribution in rodents and primates, it was hypothesized that these isoenzymes perform the same physiological functions

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[3]. Specific features of DA metabolism in brain structures during hereditary deficiency of this degrading enzyme remain unknown.

Here we studied the effect of MAO A gene knockout on the contents of DA and DOPAC and DOPAC/DA ratio in mouse brain structures.

MATERIALS AND METHODS

Experiments were performed on transgenic Tg8 mice with the irreversibly altered MAO A gene and parent strain of C3/HeJ (C3H) mice [5]. The animals were obtained from the Curie Institute (France) and maintained by selection at the Institute of Cytology and Genetics.

Experiments were performed with 4-month-old mice weighing 25 g. The animals were kept in cages (4 males and 4 females) and isolated 5 days before the experiment to exclude the "group effect". Experimental procedures were conducted according to the European Community Council Directive (86/609/EEC; November 24, 1986).

The mice were decapitated at 10.00-11.00 to measure the contents of DA and DOPAC. The midbrain, striatum, frontal cortex, amygdala, hippocampus, and hypothalamus were isolated on ice and immediately homogenized in 0.1 M HCl containing 100 ng/ml isopropyl norepinephrine as an internal standard.

Monoamine content in brain samples was measured by high-performance liquid chromatography with electrochemical detection. Monoamines were separated on a stainless column (length 60 mm, internal diameter 2 mm) packed with reversed-phase sorbent Nucleosil C_{18} (5 μ). Aqueous solution of 0.05 M potassium phosphate, 0.05 M citric acid, 0.15 g/liter sodium octyl sulfonate (Sigma), and 60 mg/liter ethylenediaminetetraacetic acid served as a mobile phase. The eluate was delivered with a pump of a Milikhrom-1 chromatograph (Nauchpribor) at a flow rate of 90 μl/min. The potential of the working electrode in an electrochemical detector was +0.6 V relative to Ag/Cl reference electrode. The method was described elsewhere [10].

The results were analyzed by two-factor ANOVA, which included 2 main factors: strain of mice (control and MAO A-knockout) and brain structure (midbrain, striatum, frontal cortex, hypothalamus, amygdala, and hippocampus). Two-factor interactions were analyzed. Inter-group differences were estimated by Student's *t* test.

RESULTS

DA content differed in various brain structures of control mice. DA content in the striatum surpassed that in the cortex, hippocampus (by 100 times), midbrain, and amygdala (by 40 times). Considerable differences were revealed in the degree of oxidative phosphorylation of DA. DOPAC content in the striatum did not differ from that in the cortex. The DOPAC/DA ratio was maximum in the frontal cortex and minimum in the striatum (Fig. 1).

MAO A gene knockout had no effect on DA content in brain structures. DA concentration moderately increased only in the striatum (by 18%). However, DOPAC content markedly decreased in most brain structures and tended to decrease in the amygdala and frontal cortex (Fig. 1). Differences were revealed in DOPAC concentration in various brain structures ($F_{5,106}$=84.5, p<0.001) in mice of different stains ($F_{1,106}$=124.7, p<0.001). Two-factor interactions were revealed ($F_{5,106}$=42.7, p<0.001).

In brain structures of Tg8 mice with reduced content of DOPAC, a relative decrease in the DOPAC/DA ratio varied from 19 (midbrain) to 39% (hypothalamus).

Our results indicate that MAO A gene knockout has various effects on DA metabolism in mouse brain structures. DOPAC content and DOPAC/DA ratio decreased in the midbrain and hypothalamus of mice locking MAO A (by 5 and 2.5 times, respectively). However, no differences were found in the frontal cortex and amygdala.

Pronounced regional differences in the influence of MAO A gene knockout on DOPAC level are in contradiction to similar changes in the metabolism of serotonin. The index of oxidative deamination of serotonin (ratio between the contents of hydroxyindoleacetic acid and serotonin) significantly differed in various brain structures. However, a relative decrease in this index was practically similar in 5 of 6 brain structures in Tg8 mice. The exception was the frontal cortex characterized by a less pronounced decrease in the index [10].

Previous studies showed that MAO B activity remains unchanged in Tg8 mice [5,7]. Considerable regional differences were revealed in the effect of MAO A gene knockout on DA metabolism. These data suggest that MAO A and MAO B play different roles in the metabolism of DA in various brain structures.

DOPAC content markedly decreased in 4 of 6 brain structures in mice lacking MAO A. It should be emphasized that DA concentration moderately increased only in the striatum. The absence of changes in DA content in most brain structures reflects effective compensation for deficiency of DA catabolism in Tg8 mice. DA serves as a substrate not only for MAO, but also for catechol-O-methyltransferase. There are various pathways for DA catabolism. Therefore, one metabolic pathway can compensate for the blockade of

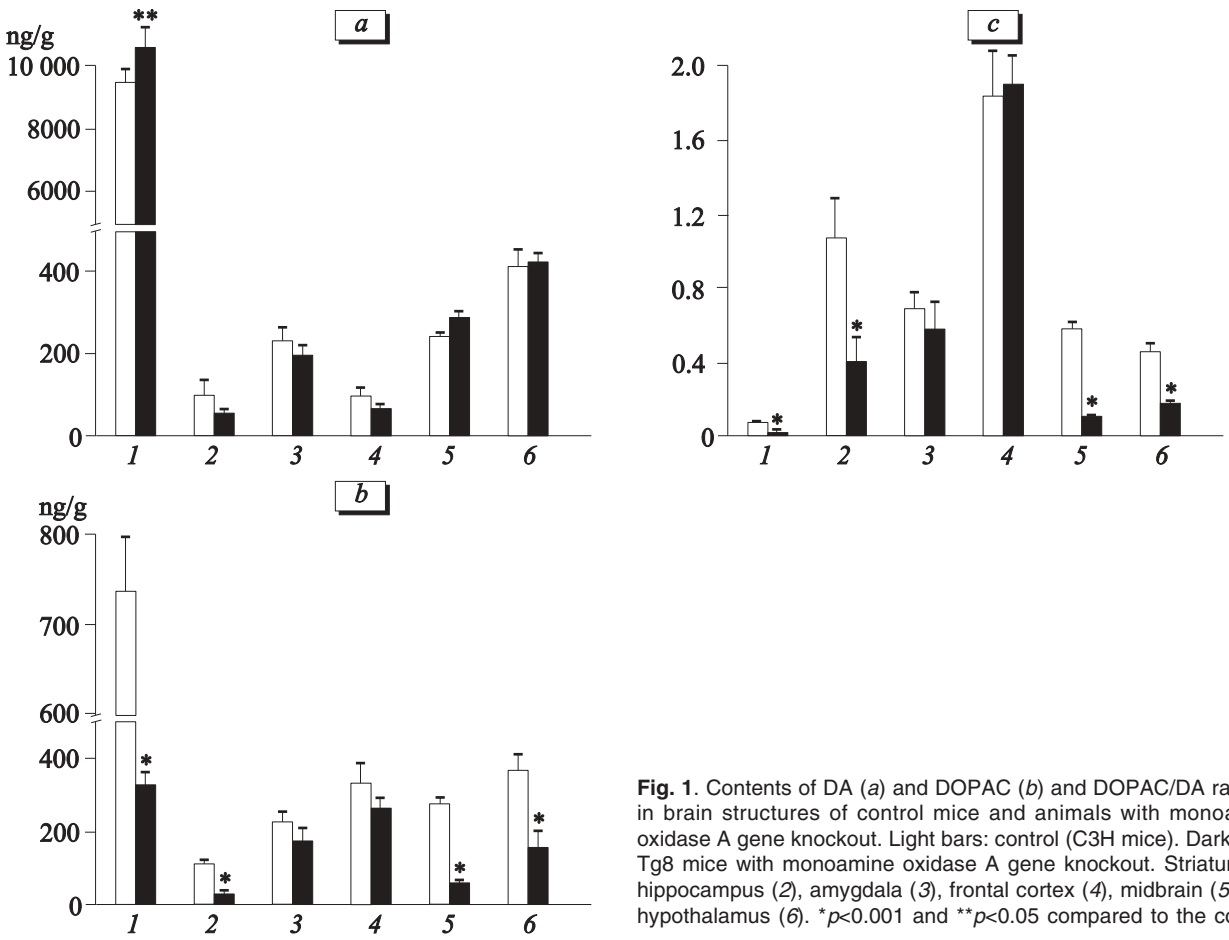

Fig. 1. Contents of DA (*a*) and DOPAC (*b*) and DOPAC/DA ratio (*c*) in brain structures of control mice and animals with monoamine oxidase A gene knockout. Light bars: control (C3H mice). Dark bars: Tg8 mice with monoamine oxidase A gene knockout. Striatum (*1*), hippocampus (*2*), amygdala (*3*), frontal cortex (*4*), midbrain (*5*), and hypothalamus (*6*). **p*<0.001 and ***p*<0.05 compared to the control.

another. Our assumption is confirmed by published data that the content of desaminated metabolites decreases, while the concentration of methylated catecholamine metabolites increases in men with mutation of the MAO A gene [9].

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