GENETICS

Effect of Thyroxin on Behavior of Mice with Inherited Difference in Predisposition to Catalepsy

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We studied effects of chronic thyroxin administration (2 mg/l, for 60 days) on motor activity, anxiety and depression-like behavior in cataleptic (ASC/Icg) and non-cataleptic (AKR/J) strains of mice. No effects of thyroxin on anxiety indicators in "open field" and "light/dark" tests were revealed in mice of the strains under study. At the same time, thyroxin increased moveability in the "open field" test in AKR/J mice and produced an antidepressant effect in the "forced swimming" test in animals from ASC/Icg strain. Obtained results are indicative of the role of inherited predisposition to catalepsy in determining the sensitivity to thyroid hormones.

Key Words: *thyroxin; open field; light/dark; forced swimming; mice.*

Thyroid hormones are used for treatment of patients with depression. High doses of thyroxin produce therapeutic effect in some patients with depression [4] and low doses of triiodothyronine enhance the action of tricyclic antidepressants [12]. However, mechanisms of individual sensitivity to thyroid hormones and of their antidepressant action are poorly understood. It is believed that individual sensitivity to thyroid hormones is determined by genetic factors [5]. Experimental data on the influence of thyroid hormones on immobility time in forced swimming test (which is the main "depression" indicator) are controversial: lack of thyroid hormones increased [10], reduced [7] or did not affect [14] this index in rats.

An association was found out between inherited predisposition to freezing response (catalepsy), "depressive" features of behavior and thyroid hormones [9]. ASC/Icg (Antidepressant Sensitive Catalepsy) mice, selected for high predisposition to catalepsy from the population of hybrids between CBA/Lac (cataleptic) and AKR/J (non-cataleptic) lines, differ from parental lines by pronounced "depressive" features: decreased exploratory and motor activity in the open field and high immobility in the forced swimming test [1]. It was demonstrated that thyroxin inhibits catalepsy in cataleptic ASC/Icg mice but enhances catalepsy in animals from the non-cataleptic strain AKR/J [2].

The aim of the study was to compare the long-term effect of chronic thyroxin treatment on animal behavior in cataleptic ASC/Icg mice and in a catalepsy-resistant parental strain AKR/J in "open field" test, "dark/light" test and "forced swimming" test.

MATERIALS AND METHODS

Mature male mice from AKR/J (n=73) and ASC/Icg (n=75) strains were used. The AKR/J strain has been maintained by close inbreeding in the Institute of Cytology and Genetics of the Siberian Division

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of the Russian Academy of Sciences for 40 years. ASC/Icg strain was derived in Laboratory for Neurogenomics of Behavior of the Institute of Cytology and Genetics of the Siberian Division of the Russian Academy of Sciences [8]. Experiments were performed using ASC/Icg mice from the 12-th generation of selection. Mice were 2 months old and weighted 22±2 g by the beginning of experiment. AKR/J (n=21) and ASC/Icg (n=20) mice received water (2 months) which contained thyroxin in concentration 2 mg/l. It was established this concentration produces a 6-fold increase of thyroxin blood concentration [11]. Water was replaced every 2 days. The other mice were used as control and received water during experiment. During the whole procedure animals were kept 4-5 animals per cage at 22°C and natural illumination. 2 days before test thyroxin was replaced by water, animals were placed in individual cages in order to get rid of group effect and kept in such a way up to the completion of experimental period. We conducted no more than on test per day. Animal keeping and all experimental procedures were conducted according to international rules for animal treatment (European Union Directive No. 86/309, issued 24 December 1986).

Animal movements in the "open field" test, "forced swimming" test and "light/dark" test were recorded automatically and were analyzed using EthoStudio tracking system [3].

The "open field" test was conducted in an open arena with dimensions 80*80*20 cm, made of white plastic and illuminated with a 200 W lamp. Arena was divided into 64 quadrants (10×10 cm) with a highlighted central area (40×40 cm). A mouse was placed on the arena near the wall equidistant from the two corners. The number of crossed quadrants, number of rearings and the time spent in the center were recorded for 5 min and interpreted as anxiety indicators [13].

The "forced swimming" test was done at 25°C using a plastic chamber 18×18×22 cm $^3/_4$ full with water. After 40 sec of adaptation the immobility time (sec), during which the mouse did not try to get out of the tank, was recorded for 3 minutes. The mouse was considered to be immobile when its displacement speed in the water was less than 2 cm/sec [6].

The "light/dark" test was done in a box which consisted of an open chamber (27×27×27 cm) illuminated by a 200 W lamp and a closed (dark) chamber (27×18×27 cm). The compartments were connected by a square porthole 7×7 cm. The mice were placed into the light chamber with its face looking towards the dark chamber. The number of light/dark transitions and the time spent in the dark chamber were recorded for 5 min. These indices serve as anxiety measure [13].

Behavioral data were expressed as $m \pm SEM$ and were analyzed using two-way analysis of variance (ANOVA) with consequent Fisher's multiple comparison test. The impact of thyroxin on anxiety in the open field test was estimated after data correction for motor activity using ANCOVA.

RESULTS

Behavioral data from the "open field" and "light/dark" tests are presented in the Table 1. A significant effect of genotype on vertical activity ($F_{1,72}$= 14.34; p<0.001) was revealed in the open field. No effect of thyroxin ($F_{1,72}$=1.65; p>0.05) and of genotype-thyroxin interaction ($F_{1,72}$=0.362; p>0.05) on the number of rearings was found. A significant effect of genotype ($F_{1,72}$=39.4; p<0.001) or thyroxin ($F_{1,72}$=11.26; p<0.01) on motor activity in the open field was demonstrated, however no genotype-thyroxin interaction was revealed ($F_{1,72}$=1.29; p>0.05). Control AKR/J mice moved substantially more than

TABLE 1. Behavior of Mice in "Open Field" Test and "Light/Dark" Test ($m \pm SEM$)

Index	AKR/J mice		ASC/Icg mice	
	control	thyroxin	control	thyroxin
Open field				
number of crossed quadrants	152.0±14.7	217.0±18.4**	77.6±8.7**	109.7±12.9
time spent in the center, s	7.6±1.8	16.7±2.7*	10.0±2.4	20.1±4.8*
vertical activity	19.0±2.2	26.0±6.4	7.0±1.4*	9.6±1.7
Light/dark test				
number of transitions	7.75±1.13	9.35±0.85	5.5±0.7	7.16±0.56
time spent in the dark chamber, s	126.5±17.9	157.4±13.4	127±23	164±16

Note. *p<0.05, **p<0.01 compared to corresponding control group; *p<0.05, **p<0.001 compared to control AKR/J mice.

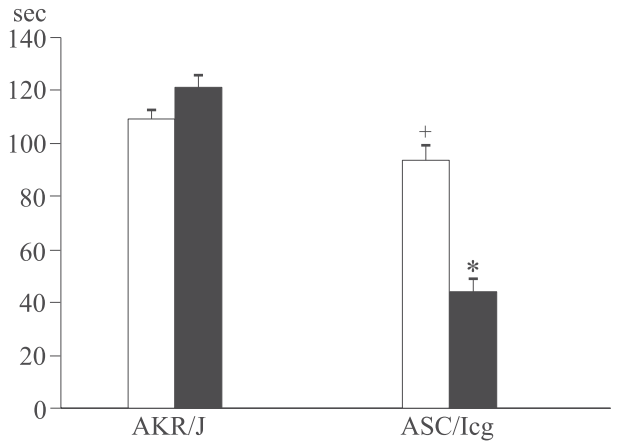

Fig. 1. Immobility time in the "forced swimming" test for control (light bars) and thyroxin-treated (dark brars) AKR/J and ASC/Icg mice. *$p<0.001$ compared to corresponding control group; $^{+}p<0.01$ compared to control AKR/J mice.

ASC/Icg mice ($p<0.001$). Chronic thyroxin administration increased motor activity only in AKR/J mice ($p<0.01$). We did not reveal any effect of genotype ($F_{1,72}=0.89$; $p>0.05$) or genotype—thyroxin interaction ($F_{1,72}=0.023$; $p>0.05$) on the time spent in the center, but the effect of thyroxin was found ($F_{1,72}=9.65$; $p<0.01$). Thyroxin administration increased the time spent in the center ($p<0.05$) in mice of both lines. However this is not an indicator of anxiety reduction, but a result of mobility increase, as far as the difference disappeared after the time spent in the center had been corrected for the number of crossed quadrants ($F_{1,71}=2.56$; $p>0.05$).

Chronic thyroxin administration did not affect anxiety indices in the "light/dark" test ($F_{1,72}=3.62$, $p>0.05$ for transitions between chambers; $F_{1,72}=3.58$, $p>0.05$ for the time spent in the dark chamber). Effect of genotype on the number of transitions between chambers was demonstrated ($F_{1,72}=6.67$, $p<0.05$).

We revealed a significant effect of genotype ($F_{1,112}=80.69$, $p<0.001$), thyroxin ($F_{1,112}=13.39$, $p<0.001$), and genotype—thyroxin interaction ($F_{1,112}=35.64$, $p<0.001$) on immobility in the "forced swimming" test (Fig. 1). Chronic thyroxin administration did not affect immobility in AKR/J ($p>0.05$) mice but led to its significant reduction in mice from ASC/Icg strain ($p<0.001$).

The main result of the study is the demonstration of opposite effects of thyroxin on motor activity in the "open field" test and on immobility in "forced swimming" test in mice with inherited differences in predisposition to catalepsy. Chronic thyroxin administration substantially enhanced motor activity but did not affect immobility in the "forced swimming" test in animals from the strain AKR/J, resistant to catalepsy. To the contrary, the hormone specifically reduced the immobility time in the "forced swimming" test, while not affecting total motor activity, in mice from the cataleptic strain ASC/Icg.

The number of transitions between chambers and the time spent in the dark chamber are considered to be anxiety indicators in the "light/dark" test [13]. We found that thyroxin does not affect these indices in both strains. These data are in line with observation that lack of thyroxin as well as its excess does not affect anxiety indices in rats [9].

Thus, we revealed the association of chronic thyroxin administration effect on motor activity in the open field and on immobility upon forced swimming with inherited predisposition to catalepsy. It was previously found out that thyroxin produced anticataleptic effect in mice from ASC/Icg strain and enhanced catalepsy in AKR/J mice [2]. The antidepressant and anticataleptic effects of thyroxin on the cataleptic strain ASC/Icg probably consist in recovery of brain functions which were disrupted during breeding for catalepsy. The ASC/Icg can be suggested as a model for investigation of behavioral effects of thyroid hormones.

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