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Locomotor Activity and Plasma, Red Blood Cell and Cerebral Cortex Lithium Concentration in Inbred Mice Given Lithium Carbonate

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SMITH, D. F. *Locomotor activity and plasma, red blood cell and cerebral cortex lithium concentration in inbred mice given lithium carbonate.* PHARMAC. BIOCHEM. BEHAV. 5(4) 379–382, 1976. – Inbred male C57, BALB, C3H and DBA mice received lithium carbonate in their food for 3 weeks. Their locomotor activity was measured by photocells and the concentration of lithium in their plasma, red blood cells and cerebral cortex was determined. The susceptibility of the inbred strains of mice to the activity-suppressant effect of lithium was C3H>DBA>BALB = C57. The concentration of lithium in plasma varied between 0.85–1.02 mEq/1. No relationship was found between lithium's pharmacokinetics and its effects on activity. The findings are consistent with the notion that genetic factors can influence the effects of lithium on behavior, but they do not support the hypothesis that genetic determination of lithium's uptake into cells is responsible for these effects.

Lithium Activity Inbred mice Genetics Red blood cell Cerebral cortex

THE role of genetic factors in manic-depressive illness is well-established [18,22], but their role in the effects of lithium on mania and depression has received attention only recently. Beneficial effects of lithium treatment were observed more often in manic-depressive patients with a positive family history of affective illness than in patients without such a history [14]. The best long-term prophylactic effect of lithium was found in manic-depressive patients with the highest morbidity risk of psychic disorders in their families [24]. Genetic determinants were demonstrated in the uptake of lithium into human red blood cells [4], and an association was found between clinical improvement during lithium treatment and high red blood cell lithium: plasma lithium ratios in depressed patients [12]. Mendels hypothesized that genetic determination of the uptake of lithium into cells may play a role in the effects of lithium on manic-depressive illness [13].

The present experiment was carried out to study further whether genetic factors play a role in the effects of lithium. Inbred strains of mice were used because the differences in their spontaneous activity provide a model system to test the role of genetics in the effects of drugs [3,11]; C57 mice are relatively active, BALB mice tend to be inactive, while DBA and C3H mice show intermediate levels of activity [10, 20, 21]. In the present study the activity of these inbred strains of mice was measured after long-term administration of lithium carbonate in order to determine whether there were genetic determinants in the effects of lithium on behavior. In addition, the concentration of lithium in the plasma, red blood cells and cerebral cortex in the mice was measured to test the hypothesis that genetic determination of lithium uptake into cells plays a role in the effects of lithium on behavior [13].

METHOD

Animals

Male 3–4 week old C57/B1/6J, BALB/c/A, DBA/2J and C3H/Tif mice were purchased all at once from Bomholdgaard in Ry, Denmark. The mice were housed in groups of 8 in 40 × 25 × 15 cm clear plastic cages in a thermostatically controlled room (23°C) on a 12 hr light-dark cycle (lights on 8 a.m. to 8 p.m.). They had free access to tap water and to a wet mash diet [15] containing 600 mmol Na^+/kg dry weight, 700 mmol K^+/kg dry weight, and either no lithium (control groups) or lithium carbonate (lithium groups). The concentration of lithium in the diet was increased from 20 to 40 to 80 and finally to 120 mmol/kg dry weight at 4 day intervals. The mice were weighed twice a week and their water intake was measured periodically.

Procedure

Activity tests took place after the mice had the 120 mmol lithium/kg dry weight diet for one week. The tests were carried out between 10 a.m. and 1 p.m. in a room with fluorescent light intensity of 500 footcandles and

background noice of 20 decibels produced by an electric motor. Before the activity tests the mice were placed individually for 5 min in a cylindrical glass holding cage 14 cm in dia. Then they were transferred one at a time to a 40 × 25 × 15 cm clear plastic cage placed between four equally spaced photocells located 2 cm above the floor along the longest side of the cage. Interruption of a light beam by the mouse activated an automatic counter. The total number of counts recorded during 5 min in the test cage was used as the measure of activity.

At 10–11 a.m. on the day after the activity test, blood samples were drawn from the vena cava of the mice under ether anesthesia into preweighed heparinized test tubes. Then the mice were decapitated and samples of the left cerebral cortex were taken and placed in preweighed test tubes. The blood samples were centrifuged at 1,000 G for 30 min. Duplicate samples of plasma were diluted 1:40 (v:v) in demineralized water for lithium determination by flame photometry [2]. The rest of the plasma was removed from the red blood cells and discarded. The red blood cells were weighed and then broken by addition of 6% butanol 1:19 (w:v) [7], vigorously shaken and stored at 4°C overnight. The next day the samples were shaken vigorously and centrifuged at 3,000 G for 15 min at room temperature. Duplicate samples of the supernatant were diluted 1:40 (v:v) in demineralized water for lithium determination by flame photometry using acetylene gas. A correction for trapped plasma volume of 3% was used in the calculation of red blood cell lithium concentration [5]. Cerebral cortex samples were cooked for 1 hr in 1 ml concentrated nitric acid in a boiling water bath. Then they were diluted with demineralized water to a final volume of 1:20 (w:v), shaken vigorously and centrifuged at 3,000 G for 15 min. Duplicate samples of the supernatant were diluted 1:40 (v:v) in demineralized water for lithium determination by flame photometry using acetylene gas. Samples of red blood cells and cerebral cortex from control mice were used to make standard solutions for the lithium determinations.

Statistical significance of results was determined by 1-way and 2-way analysis of variance and Student's *t*-tests.

RESULTS

All groups of mice showed a weight gain of 1–2 g per day. Lithium administration did not significantly affect body weight gain. Water intake averaged 6–11 ml/mouse/day in control groups as well as in BALB, C3H and DBA lithium groups, while water intake increased significantly ($p<0.05$) to 24 ml/mouse/day in the C57 lithium group prior to the activity test.

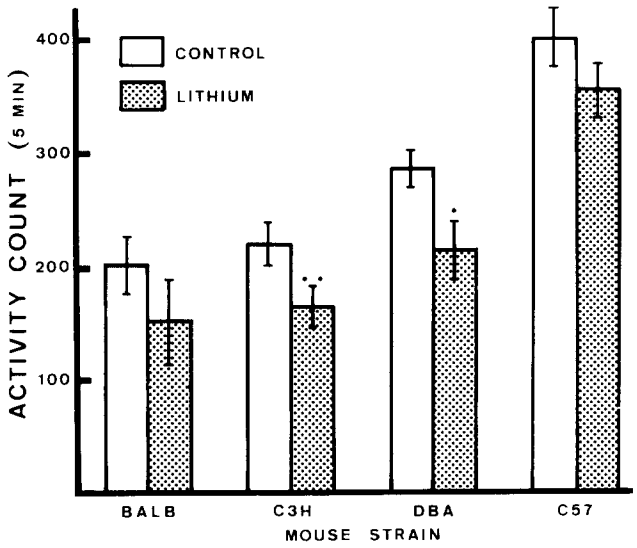

FIG. 1. Effect of long-term lithium carbonate administration on spontaneous activity in four inbred strains of mice. Values are means ± SEM for 8 mice per group. * and ** indicate significant difference from control group (p's<0.025 and 0.01, respectively).

Figure 1 shows the results of the activity tests. As expected, there were significant differences in activity among the inbred strains of mice, $F(3,56) = 41.0$, $p<0.001$. In the control groups, C57 mice were significantly more active than all other groups (p's<0.01), while DBA mice were significantly more active than the C3H and BALB mice (p's<0.05). Lithium administration significantly reduced activity, $F(1,56) = 12.3$, $p<0.005$; it was significantly decreased in the C3H and DBA mice (p's<0.01 and 0.025, respectively), but not in BALB and C57 mice compared to the respective control groups.

Table 1 presents the data on the concentration of lithium in plasma, red blood cells and cerebral cortex in the lithium groups. The concentration of lithium in plasma differed significantly between the groups, $F(3,28) = 2.99$, $p<0.05$; it was significantly higher in the C3H mice than in the DBA and BALB mice (p's<0.05) and also higher in the C57 mice compared to the DBA mice ($p<0.05$). There were no significant differences between the groups in the red

TABLE 1

LITHIUM CONCENTRATION IN PLASMA, RED BLOOD CELLS (RBC) AND CEREBRAL CORTEX IN INBRED STRAINS OF MICE GIVEN LITHIUM CARBONATE. VALUES ARE MEANS ± SD FOR 8 ANIMALS

Strain	Plasma Li^+ mEq/l	RBC Li^+ mEq/kg	Cortex Li^+ mEq/kg	RBC:Plasma Li^+ ratio	Cortex:Plasma Li^+ ratio	Cortex:RBC Li^+ ratio
BALB	0.87 ± 0.13	1.01 ± 0.09	1.30 ± 0.29	1.18 ± 0.12	1.55 ± 0.50	1.30 ± 0.36
C3H	1.02 ± 0.17*†	1.18 ± 0.16	1.37 ± 0.35	1.17 ± 0.10	1.34 ± 0.26	1.16 ± 0.25
DBA	0.85 ± 0.11	1.09 ± 0.23	1.23 ± 0.20	1.23 ± 0.12	1.47 ± 0.29	1.15 ± 0.17
C57	1.00 ± 0.16*	1.20 ± 0.13	1.40 ± 0.20	1.21 ± 0.09	1.42 ± 0.21	1.18 ± 0.18

* = significantly greater than in DBA mice ($p<0.05$).
† = significantly greater than in BALB mice ($p<0.05$).

blood cell lithium concentration, the cerebral cortex lithium concentration, the red blood cell lithium: plasma lithium ratio, the cerebral cortex lithium: plasma lithium ratio, and the cerebral cortex lithium: red blood cell lithium ratio.

DISCUSSION

The main finding in this study is the difference in the effects of lithium on activity in inbred mice; the susceptibility of the mice to the activity-suppressant effect of lithium was C3H>DBA>BALB ≈ C57. This finding supports the notion that genetic factors play a role in the effects of lithium on behavior.

A relationship failed to occur between lithium pharmacokinetics and the effects of lithium on activity. The concentration of lithium in plasma was unrelated to the effects of lithium on activity since the DBA and C3H mice showed the most pronounced reduction in activity during lithium administration, but had the lowest and highest plasma lithium concentrations, respectively. In addition, the concentrations of lithium in red blood cells and cerebral cortex as well as the ratios of the lithium concentration in plasma, red blood cells and cerebral cortex failed to differ between the groups. Consequently, these measures of lithium pharmacokinetics were not related to the differences in the effects of lithium on activity in the mice. The present findings provide no support for the hypothesis that genetic determination of the uptake of lithium into cells plays an important role in the effects of lithium on behavior [13]. My results in mice agree with studies in man that failed to find a relationship between lithium uptake into red blood cells and the effects of lithium on manic-depressive illness [17,23].

Of course, the failure of a relationship to be found between lithium pharmacokinetics and genetic differences in lithium's action on behavior does not prove that such a relationship does not exist [8]. Nevertheless, I think another hypothesis ought to be considered to account for the genetic differences in lithium's effects on behavior. This hypothesis assumes that there is a relationship between behavior and genetic differences in biogenic amines. Such a relationship has in fact been observed in inbred strains of mice [1,9]. Lithium affects the metabolism of biogenic amines [6, 16, 19], but it is not yet known whether genetic factors play a role in these effects. It is interesting to speculate, however, that genetic determination of lithium's effects on biogenic amines might occur and could play a role in genetic differences in lithium's action on behavior. This hypothesis can be tested in inbred strains of mice.

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