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Beware of your mouse strain; differential effects of lithium on behavioral and neurochemical phenotypes in Harlan ICR mice bred in Israel or the USA

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ABSTRACT

Animal models are crucial components in the search for better understanding of the biological bases of psychiatric disorders and for the development of novel drugs. Research, in general, and research with animal models, in particular, relies on the consistency of effects of investigated drugs or manipulations across experiments. In that context, it had been noted that behavioral responses to lithium in ICR (CD-1) mice from Harlan Israel have changed across the last years. To examine this change, the present study compared the effect of lithium treatment in ICR mice from Harlan Israel with the ICR mice from Harlan USA. The mice were treated with chronic oral lithium. Their lithium serum levels were measured and their behavior in the forced swim test (FST) was evaluated. The mice were also treated with [^{3}H]-inositol ICV and lithium injection and their frontal cortex [^{3}H]-phosphoinositol accumulation was measured. Results show that lithium serum levels in Israeli mice were significantly lower compared with the USA mice, that lithium had no behavioral effect in the Israeli mice but significantly reduced FST immobility time of the USA mice, and that phosphoinositol accumulation was much more strongly affected by lithium in the USA mice compared with the Israeli mice.
These results suggest that the Israeli Harlan colony of ICR mice changed significantly from the original ICR colony in Harlan USA and that the differences might be related to absorption or secretion of lithium.

1. Introduction

The predictive validity of animal models is critical in order to evaluate the possible effects of novel drugs and treatments (McArthur, 2010; Kara and Einat, 2013). One of the common tests in the context of affective disorders is the forced swim test (FST), where administration of antidepressant drugs as well as some mood stabilizers was repeatedly demonstrated to reduce immobility time in mice (Porsolt et al., 1977; Bersudsky et al., 2007; Can et al., 2013). We and others have repeatedly used this test to evaluate possible mechanisms of action of the mood stabilizer lithium (O'Brien et al., 2004; Bersudsky et al., 2007; Kovacsics and Gould, 2009; Toker et al., 2013, 2014) as well as to examine the effects of other compounds related to biological effects of lithium (Shtein et al.; Bersudsky et al., 2008; Cryns et al., 2008; Toker et al., 2013).

Across the years, the effect of lithium, acute or chronic, injectable or oral, was tested in a number of mice strains. Work from different laboratories shows that not all strains are equally responsive to lithium treatment in the FST (Can et al., 2013). However, the ICR (CD-1) strain has been continually reported to respond to lithium treatment in this test (Bersudsky et al., 2007; Toker et al., 2013). It was therefore surprising that initial anecdotal reports in Israel suggested that ICR mice from the breeding colony at Harlan Israel were not responding to previously effective lithium protocols in the FST. As the use of ICR mice is relatively common and lithium serves at many times as a positive control compound to assess other drugs' effects, there is a significant importance to evaluate whether these mice are in fact responsive or non-responsive to lithium treatment.

To examine this issue the present study compared the effect of lithium in ICR mice from the Harlan Israel breeding colony with ICR mice from Harlan USA breeding colony. The comparison included (1) lithium serum levels following chronic oral administration; (2) acute effect of lithium to enhance phosphoinositol accumulation in the frontal cortex and (3) effect of chronic oral lithium administration on behavior in the FST. It is important to note that the comparison was not between

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two lines of mice but between two colonies of the same strain from the same supplier. The two breeding colonies are originally from the same line and sub-strain and the only difference is the site of the colony within the same company.

2. Methods

2.1. Animals

Male, eight weeks old mice at the start of protocols were used in all experiments. Animals were maintained on a 12 h/12 h light/dark cycle (lights on between 8:00 a.m. and 8:00 p.m.) with *ad libitum* access to food and water. All tests were performed during the light phase of the cycle between 9:00 a.m. and 7:00 p.m. Two groups of ICR mice (from Harlan Israel and from Harlan USA, in a blind manner to the country origin of the mice) were allowed to acclimatize to the new environment for one week before treatment initiation. Separate cohorts of mice were used for the neurochemical analysis. All animal care and experimental procedures were in accordance with the NIH Guide for Care and Use of Laboratory Animals and were approved by the Ben-Gurion University committee for the ethical care and use of animals in research.

2.2. Lithium treatment

Chronic treatment (for serum lithium levels and for the FST): mice in the control group received powdered rodent chaw (Harlan Israel). The lithium treated group received the same powdered chaw mixed with 0.2% lithium chloride (LiCl) for 5 days followed by 0.4% LiCl for 10 additional days (O'Brien et al., 2004; Bersudsky et al., 2007; Toker et al., 2013). All groups received tap water *ad libitum* and an additional bottle containing 0.9% NaCl, to prevent electrolyte imbalance in the lithium treated mice. This oral administration protocol was previously shown to result in therapeutically-relevant lithium serum levels and to be effective in the FST (O'Brien et al., 2004; Bersudsky et al., 2007; Toker et al., 2013).

Acute treatment (for the brain phosphoinositol accumulation study): mice were treated with intraperitoneal (IP) injection of LiCl at a dose of 3.0 or 10.0 meq/kg, 10 ml/kg, or a similar volume of saline (control) 24 h prior to euthanasia and brain extraction.

2.3. Brain phosphoinositol accumulation

[^{3}H]-inositol intracerebroventricular (ICV) injection: mice were anesthetized with 20% isoflurane (diluted in propylene glycol). An incision was made above the bregma and a 25 gauge needle was used to create a hole in the scalp above the lateral ventricle, 0.2–0.3 mm posterior to bregma and 1 mm lateral to the midline. A Hamilton syringe with a 27 gauge needle was used to administer 4 μCi [^{3}H]-inositol in 1 μl of inositol [20 mg/ml in artificial cerebrospinal fluid (aCSF)] at a rate of 0.5 μl/20 s.

Brain phosphoinositol accumulation was assayed according to Whitworth and Kendall (Whitworth and Kendall, 1988) with minor modifications. In brief, mice were given an ICV injection of [^{3}H]-inositol and sacrificed by cervical dislocation followed by immediate decapitation. Their brains were quickly dissected on ice to separate the frontal cortex and samples were then sonicated in 1 ml ice-cold perchloric acid (PCA, 10% w/v) for 20–30 s to extract the [^{3}H]-inositol phosphates. Sonicated samples were neutralized with KOH (1.5 M) and left on ice for at least 20 min before centrifugation at 2000 g for 20 min. Then the supernatant was added to 3 ml Tris buffer (50 mM, pH 7.4), mixed and taken for analysis of total [^{3}H]-inositol phosphates accumulation by anion exchange chromatography on Dowex chloride columns. The columns were washed with 15 ml H_2O before elution of the [^{3}H]-inositol phosphates with 5 ml HCl (1 M). Radioactivity of [^{3}H]-inositol phosphates was assessed by liquid scintillation counting. The results were calculated per mg protein in the fraction. Protein concentration was assayed by the Bradford method (Bradford, 1976).

2.4. Forced swim test (FST)

Animals were individually placed for a 6-min session in a glass cylinder filled with water at 24 ± 1 °C such that the mouse could not touch the bottom or climb out of the cylinder. The session was digitally recorded and analyzed later by an experimenter who was blind to the treatment (Y.S.). Immobility (floating) time was scored from recordings for the last 4 min of the test. Immobility was defined as the time spent by a mouse floating, making only those movements necessary to keep its head above the water.

2.5. Data analysis

Lithium serum levels and behavior in the FST were analyzed using Student's t-test. Phosphoinositol accumulation data were analyzed using a factorial ANOVA followed by LSD post-hoc comparisons. Significance was assumed at $p < 0.05$.

3. Results

3.1. Serum Lithium levels

Chronic administration of oral lithium to ICR mice resulted in detectable serum lithium levels in both the USA and the Israeli ICR mice but the levels in the USA mice were significantly higher compared with the mice from Israel [0.85 ± 0.12 (n = 10) and 0.33 ± 0.08 meq/l (n = 9, because we were not able to withdraw blood from one mouse), respectively; $t(17) = 10.5$, $p < 0.001$].

3.2. Frontal cortex [^{3}H]-phosphoinositol accumulation

Despite the well-known effect of lithium on the PI cycle, an acute administration of 10 mEq lithium to the Israeli ICR mice did not affect frontal cortex [^{3}H]-phosphoinositol accumulation. The same regimen and dose of lithium significantly increased frontal cortex [^{3}H]-phosphoinositol accumulation in the USA ICR mice [Fig. 1; ANOVA: Origin effect — $F(1,31) = 28.7$, $p < 0.0001$; Lithium effect — $F(2,31) = 7.99$, $p < 0.003$; Origin × Lithium Interaction — $F(2,31) = 8.37$, $p < 0.001$; see figure for post-hoc comparisons].

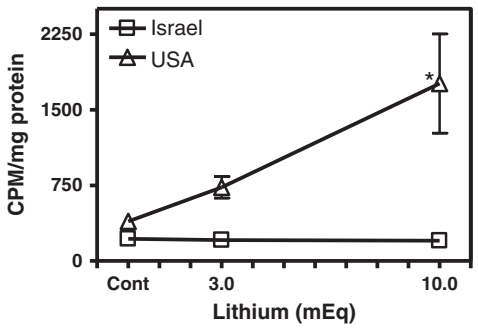

Fig. 1. Dose response of frontal cortex phosphoinositol accumulation following acute lithium administration. ICR mice from Israel and the USA were injected IP with 0 (Cont, n = 7 and n = 6 for the Israeli and the USA mice, respectively), 3 (n = 7 and n = 6 for the Israeli and the USA mice, respectively) or 10 (n = 6 and n = 5 for the Israeli and the USA mice, respectively) meq/kg of lithium chloride 24 h prior to euthanasia. Four μCi [^{3}H]-inositol in 1 μl of 20 mg/ml inositol in artificial cerebrospinal fluid (aCSF) were administered ICV 20 h prior to sacrifice. Results are presented as mean ± S.E. ANOVA: Origin effect — $F(1,31) = 28.7$, $p < 0.0001$; Lithium effect — $F(2,31) = 7.99$, $p < 0.003$; Origin × Lithium Interaction — $F(2,31) = 8.37$, $p < 0.002$; Fisher's LSD post-hoc analysis: USA mice control *vs.* 10 meq/kg lithium, $p < 0.0001$.

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