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Effects of perinatal exposure of lithium on neuro-behaviour of developing mice offspring

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Lithium (Li) was given to female Swiss-Webster strain mice at the doses of 15 and 30 mg/kg body weight in their drinking water. Treatment started from the first day of pregnancy until the postnatal day fifteen of delivery. Thereafter, the dams were switched to plain tap water. All offspring were subjected to various tests. The rate of body weight gain was relatively slower in Li exposed pups. Furthermore, the opening of eyes and appearance of body hairs in Li exposed pups were also slower as compared to the controls. The sensory motor reflexes in Li exposed pups were found to be affected in a dose-dependent manner. Significant relative changes were also noticed in the levels of acid and alkaline phosphatases in the liver, and acetylcholinesterase in the brain tissues of the Li exposed developing offspring in a dose-dependent manner. 'Locomotor Activity Test' was performed in the male offspring only which showed a significant suppressive effect on most of the elements of this test due to Li exposure. The present Li effects in the offspring are possibly via in *utero* action and/or via mother's milk.

Keywords: Behaviour, Esterases, Lithium, Locomotor activity test, Mice offspring, Perinatal exposure, Sensory motor reflexes

Lithium (Li) is an important drug for the treatment and prophylaxis of mental disorders like mania and depression[1,2] and bipolar manic-depressive psychosis[3]. Through the use of Li and its compounds in pharmaceuticals, laboratories, ceramics and metallurgical processes, air conditioners and dehumidifiers, and lubricants brings this lightest alkali metal in close contact of human beings[4]. Li salt is easily absorbed from gut, distributed readily throughout the body, and excreted almost entirely by the kidneys[5,6]. However, prolonged treatment with therapeutic doses of Li and/or while taken as medicine, it induces substantial toxic effects[7,8]. It is considered to affect neuronal communication, cell proliferation and metabolism[9], brain, intestine, thyroid, and liver functions[10], drowsiness, slurred speech, psychomotor slowing, impaired memory, seizures, coma and death[11]. Li has also been reported to affect nerve excitation and affect the synthesis, activation, and inactivation of various neurotransmitters[12-14].

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In rodent studies Li crossed the placenta freely and caused teratogenic effects and fetotoxicity[15,16]. Fetal malformation in humans has also been reported due to Li treatment[17]. Prenatal and postnatal ingestion of Li by the nursing mouse has been reported to have toxic effects on the offspring brain and other developmental and metabolic aspects[18,19].

These findings highlight the toxicity of Li. To the author's best knowledge no precedence is available on the effect of Li consumed by the dam during pregnancy and study the behavioural and biochemical responses in the offspring. Therefore the present study has been designed to determine the teratological effects of perinatal Li exposure on the postnatally developing offspring using various behavioural and biochemical parameters.

Materials and Methods

Experimental animals—Swiss-Webster strain mice (8-9 weeks old) of either sex were housed in opaque plastic cages (three females to one male in each cage) measuring 30 × 12 × 11 cm, in the animal facility of the Biology Department, Dammam University, Dammam, Saudi Arabia. Animals were kept under 12:12 h L:D cycle at 18-22 °C. After pregnancy (appearance of vaginal plug was considered as day

one of pregnancy), the males were removed from the cages and the females were subjected to experimental treatments. The wood fillings were changed regularly and food (Pilsbury's Diet) and water were available *ad libitum* unless otherwise indicated. All experimental procedures were conducted in accordance with the institutional guidelines for the care and use of laboratory animals and were in accordance with the local animal care and ethics committee instructions.

Lithium administration—Lithium chloride (Li) of analytical grade (Riedel de Haen, Germany) was dissolved in deionized distilled water to give a dose of 15 and 30 mg/kg body weight/day. Criteria of selecting the two doses of Li were on the basis of literature reported for rodents and pilot studies conducted in our laboratory. These Li doses were calculated on the basis of average total volume of drinking water consumed by the animal in 24 h and the Li doses per day dissolved in it. These Li containing water formed the sole drinking fluid source for the experimental group of mice during the required period of the experiment. Throughout the experiment fresh drinking fluid containing Li doses were prepared and replaced every second day. The control group received deionized distilled water only. All pregnant mice were housed individually. Treatment was started from day 1 of pregnancy and was continued until postnatal day 15 (PD15) and thereafter the mothers were switched to plain deionized distilled water.

Behavioral observations—The pups of each experimental groups were culled to only eight per dam on PD 0 and were left with their mothers until PD 22. During this weaning period, three pups of each litter were colour marked from the others without any consideration to its sex, and were subjected to various behavioural tests under dim lighting (ca 8 lux). In all, 21 pups belonging to seven litters from each treatment category were considered. All observations were recorded on PD 1 and repeated every other day until PD 21 in the same three colour marked pups of each litter. These observations were used to measure the early development of sensory motor coordination reflexes together with morphological development in the pups. For statistical analysis, the mean of all three colour marked pups per litter was considered as a single score. Thus, seven replicates from each treatment category were considered for following observations.

Body weight—Weight is an useful indicator of development. Thus, the pups from both control and Li exposed groups were weighed every alternate day from PD 1 until PD 21.

Righting reflex—The time taken by a pup placed on its back to turn over and place all four paws on the substrate was recorded. The cut off limit for the test was 2 min.

Cliff avoidance—Pups were placed on the edge of a table top with the forepaws and face over the edge. The time taken by the pup to back away and turn from the "cliff" was recorded. The cut off time for the test was 2 min. A latency of 2 min was attributed when the animal fell from the "cliff".

Rotating reflex—The surface used to measure the rotating reflex was the same as that used for righting reflex, except that it was inclined at an angle of 30°. The pups were placed on this surface with their heads pointed downwards. The time elapsed until the pup rotate its body through 180° geonegatively and face its head upwards, was recorded as the rotating time. The cut off time of this test was also set at 2 min.

Eye opening and hair appearance—The days on which the body hair fuzz appeared, and the eyes opened were also recorded. These two parameters are also useful morphological indicators of development.

"Locomotor activity" test—After weaning at PD 22, 10 male offspring from each treated group (including representatives from each 7 litters) were subjected to 'locomotor activity' tests in an experimental wooden arena, and various elements of behaviour were observed as described by Ajarem[20] . The visual observations in the arena lasted 300 sec for each animal.

Biochemical studies—After the locomotory behavior test, one male pup was picked up at random from each group. Only the male pups were selected in order to simulate the biochemical findings with the locomotory tests since the later was also conducted only in the male offspring. Thus, seven male pups representing each litters were collected from each experimental group on PD 22, and were killed by decapitation. Their brain and liver were removed and gently rinsed in physiological saline (0.9% NaCl), and then blotted on Whatman filter paper. Their fresh weights were recorded, and organs were then frozen.

Tissue homogenate preparation—A 10% (w/v) homogenate of each frozen tissue was prepared in teflon-glass homogenizer at 4 ± 1 °C, centrifuged at 1000 *g* for 10 min to remove cell debris and the

supernatant was used for enzyme assays. The brain homogenate was prepared in an ice-cold phosphate buffer, (0.067 *M*, *p*H 7.2) and the liver was homogenized in chilled 0.25 M sucrose solution.

Enzyme estimations—The acetylcholinesterase (AChE) activity in the homogenised brain tissue was estimated by the method of Hestrin[21] using acetylchloline chloride as the substrate. The specific activity of AChE was expressed as μ moles acetylcholine chloride hydrolysed/g wet tissue weight/h at 37 ± 1 °C.

The levels of total acid phosphatase (AcP) and alkaline phosphatase (AlP) were estimated in the liver tissue homogenates using sodium p-nitrophenol phosphate as the substrate[22]. The protein content in the homogenates was estimated as per Lowry *et al*[23]. The specific activities of these phosphomonoesterases were expressed as n-moles p-nitrophenol liberated/mg protein/min at 37 ± 1 °C.

Statistical analysis—The data of body weight, dates of morphological developments, sensory motor reflexes and biochemical analyses were compared within the experimental groups by the analysis of variance (ANOVA) using Instat computer programme, and were subsequently analysed by Student-Newman-Keuls multiple comparison test. The significance levels were defined by $P \leq .05$, .01, and .001. Data of Locomotor Activity were compared within the experimental groups by the analysis of variance (ANOVA) and subsequently were analysed using Mann-Whitney U tests[24].

Results

The rate of increase in body weight of the Li treated pups lagged behind their controls from the day of their birth (PD 1) and remained so almost throughout their weaning period until PD 21. However, this effect was dose-dependent where the lower dose of Li (15 mg/kg body weight) produced a less significant (P<0.05) decline in the rate of increase in body weight from PD 13 onwards until PD 21. Whereas the higher dose (30 mg/kg) was highly significantly effective (P<0.001) as compared to their controls from the day of their birth (PD 1) until PD 21 as mentioned in the graph (Fig. 1).

The morphological developments, like eye opening and body hair appearance were also affected by Li treatment (Fig. 2). Both of these morphological parameters in the Li treated pups were significantly (P<0.05) delayed as compared to the controls as given in the figure.

Perinatal exposure of mice to Li, had a significant and dose-dependent effect on the early development of all sensory motor reflexes in the pups in the present study. Pups born to mothers treated with Li were lethargic and sluggish from the very first day (PD 1). During the first two weeks of the postnatal development, Li had significant (P<0.05) inhibitory effects on all sensory motor reflexes (Fig. 3a-c).

The levels of phosphomonoesterase enzymes in the liver of the treated pups did not remain the same as the control groups. The AlP (Fig. 4A) and AcP (Fig. 4B) were inhibited significantly (P<0.05) in the treated offspring. The AChE activity in the brain tissue of the treated pups (Fig. 5) was also found to be inhibited significantly (P<0.05).

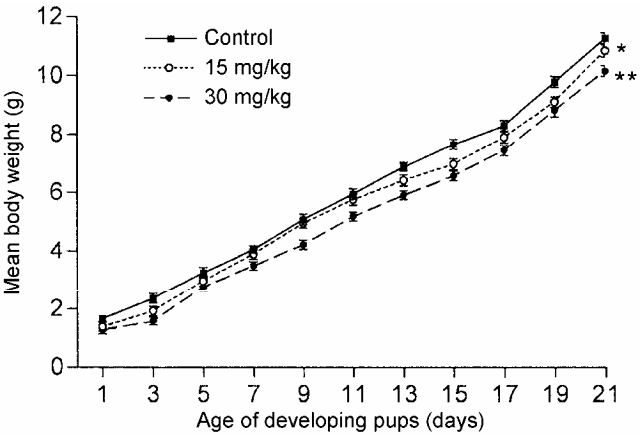

Fig. 1—Effect of perinatal lithium exposure on the body weight gain of the mouse pups. [P values: *<0.05; ** < 0.01; Student's t-test]

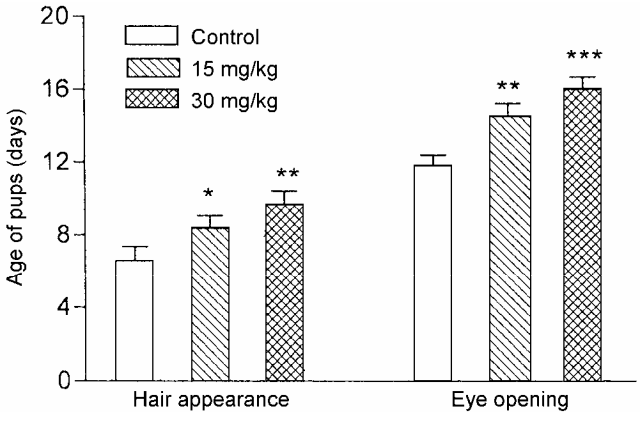

Fig. 2—Effect of perinatal lithium exposure on the hair appearance and eye opening in the mouse pups. [values are mean ± SE; P values: * < 0.05; ** < 0.01; *** < 0.001; Student's t-test]

The "Locomotor Activity" test (Table 1) showed that perinatal Li exposure had a significant inhibitory effect on the number of squares crossed and wall rears in a dose-dependent manner as compared to the controls.

Discussion

It is evident from the present results that the perinatal exposure of female mice to Li, influences the rate of physical maturation, sensory motor reflexes and the level of enzyme activities in liver and brain tissues of the pups at different developmental stages during the weaning period. Further, after the

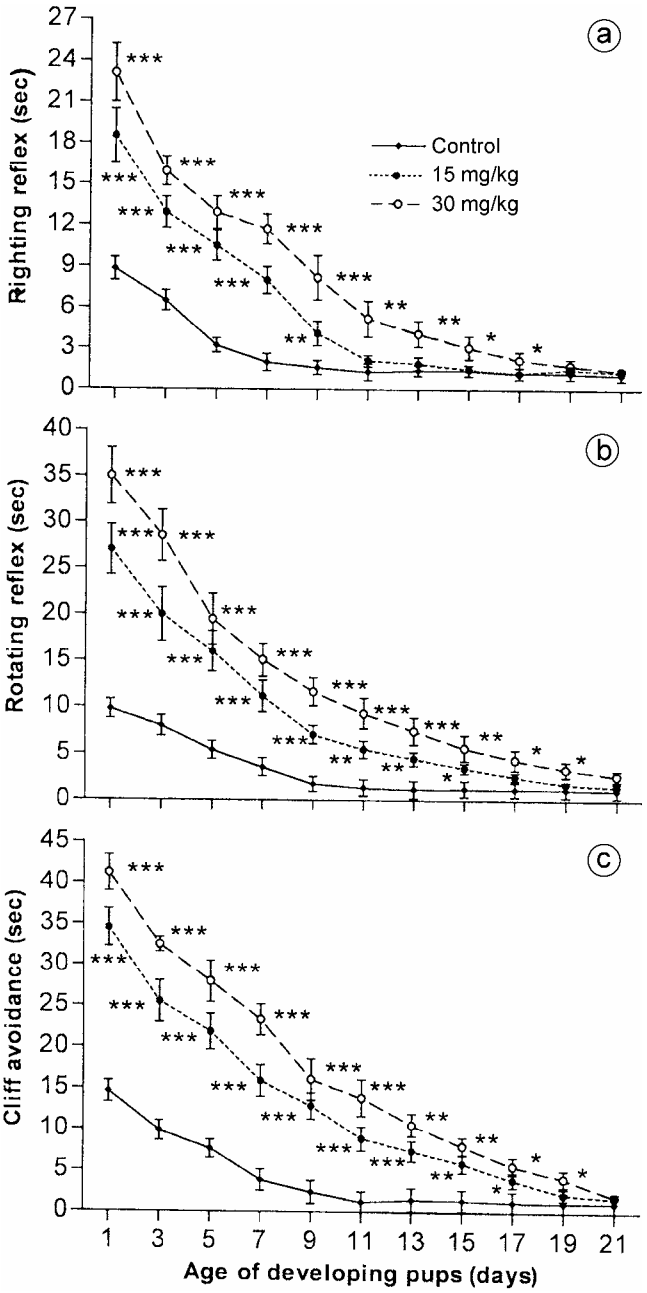

Fig. 3—Effect of perinatal lithium exposure on the mean righting reflex (a), mean rotating reflex (b) and mean cliff avoidance activity (c) of the mouse pups. [*P* values: * < 0.05; ** < 0.01; *** <0.001; Student's *t*-test].

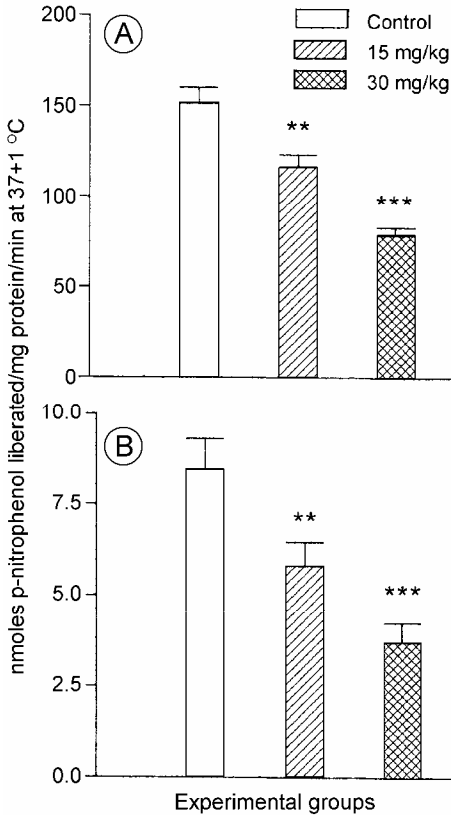

Fig. 4—Effect of perinatal lithium exposure on the activity of acid phosphatase (A) and alkaline phosphatase (B) in liver of the mouse offspring after weaning age. [*P* values: ** < 0.01; *** < 0.001; Student's *t*-test].

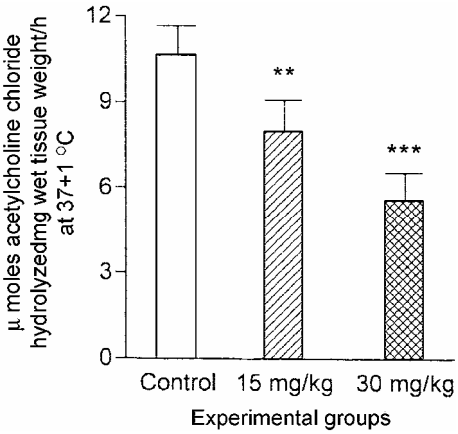

Fig. 5—Effect of perinatal lithium exposure on the activity of acetylcholinesterase in the brain of the mouse pups after weaning age. [*P* values: ** < 0.01 and *** < 0.001; Student's *t*-test).

Table 1—Effect of perinatal lithium exposure on the locomotor activity of male mouse offspring after weaning age.

Treatment group	Median number (with ranges) of acts and postures					
	Number of squares crossed	Wall rears	Rears	Wash	Locomotion duration (sec)	Immobility duration (sec)
Control	254 (191-327)	26 (19-28)	9 (4-13)	7 (3-15)	239 (202-300)	61 (0-98)
Dose – I (15 mg/kg)	115* (113-136)	13* (9-19)	6* (1-9)	6 (5-12)	129* (119-218)	131.5* (82-181)
Dose – II (30 mg/kg)	79** (61-192)	8* (6-14)	2* (0-7)	5 (0-13)	114** (91-192)	186** (108-209)

P values:* <0.05;**<0.001 statistically significant from the control by Mann-Whitney U-test.

weaning period, in the male offspring, various behavioural indices are also affected in the locomotor activity test. This may possibly be due to the fact that Li was available to the developing fetus *in utero* as well as in the milk of the lactating mothers during the weaning period. It is known that pregnant females have the capability to transfer the Li to the offspring via milk[19]. Also, it is well established that significant quantities of compounds that are given to mothers in late pregnancy may be transmitted to the offspring *in utero* and/or during lactation[25-32]. It is therefore possible that the above discussed factors may singly or together affect the growing offspring during the weaning period and ultimately producing lasting behavioural effects.

In the present study, Li exposure alters the level of phosphomonoesterase enzymes (AcP and AlP) in the liver, and AChE enzyme in the brain tissue off the offspring. This biochemical damage may be due to Li presence in the offspring either *in utero* and/or in milk. Earlier reports have also confirmed that Li exposure during the gestational period alters the level of some hepatic enzymes in the offspring[18,19]. Thus, alterations in the level of AcP and AlP of the liver in the present study, might have lead to variations in the phosphate pool of the pups through early development, which ultimately lead to disturbed energy source available to the animals with consequent disturbance in its metabolism[33]. This is probably reflected in the form of disturbed physical maturation (body weight gain, eyes opening, body hair appearance), sensory motor reflexes and behavioural activities.

AChE is an important neurotransmitter that has been implicated for behaviour[34]. It has been reported that alterations in brain enzymes are among the factors responsible for disturbances in behavioural activity of affected animals[28,29,35-38]. Li is considered to affect neuronal communication[9] and encounter early neurological features, including drowsiness, psychomotor slowing, and impaired memory and, in severe cases, seizures, coma and death[11] and affect nerve excitation through the synthesis, activation, and inactivation of various neurotransmitters[13,14]. Thus, Li exposure in the present study, could have produced developmental abnormalities in the brain of the treated offspring and in the level of AChE activity that may have brought the observed teratological and behavioural effects in the offspring. However, it is reasonable to mention here that evaluation of more neurotransmitters like catecholamines (viz. serotonin, norepinephrin, dopamine, etc.) in the brain regions can give better explanation for results on behavioural patterns. Also, the present study strongly supports the earlier findings that AcP, AlP and AChE could be used as convenient marker enzymes in teratological studies of not only the adult mice[28] but also that of the young pups through their early postnatal developing stages[29].

In summary, because of the risk of teratogenicity, Li should be avoided during the period of organogenesis and if possible throughout pregnancy. Such exposure to Li during the sensitive developmental stages might be harmful with severe clinical and teratological consequences to the offspring.

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