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ORIGINAL ARTICLE

Disrupting effects of lithium chloride in the rat ovary: Involves impaired formation and function of corpus luteum

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KEYWORDS

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Abstract Lithium is an effective drug for the treatment of bipolar disorder. Evidence suggests that lithium induces side effects on the reproductive system. We have investigated the effect of lithium chloride (LiCl) on the progesterone synthesis, the main steroid produced by corpus luteum (CL), and steroidogenic acute regulatory protein (StAR) expression, the primary mechanism of the control of CL steroidogenesis. Immature female *Wistar* rats (25-day-old) were injected with lithium chloride (2.0 mg/kg/day) or sterile distilled water for 15 days. All rats were induced with injection of pregnant mare's serum gonadotrophin (PMSG) on the 13th day of experiment and followed by human chorionic gonadotropin (hCG) 48 h later. The last injection of LiCl was at 12 h post-hCG injection. Blood and ovaries were collected at 4 h interval from 12 to 24 h post-hCG injection. Serum levels of progesterone were measured by ELISA and CL formation was determined by histological analysis. Then, StAR protein and gene expression were examined using immunohistochemistry and polymerase chain reaction. Results showed the severe changes in CL formation, progesterone secretion and StAR expression in LiCl-treated rats during luteinization. It is concluded that the CL formation and the critical step of progesterone synthesis were affected by LiCl in gonadotropin-induced rat ovary.

1. Introduction

After LH surge and ovulation, luteinization of the follicle cells occurs and the corpus luteum (CL) rapidly develops to become a highly active steroidogenic tissue (1). The reprograming of follicular cells into luteal cells requires severe changes in the expression of steroidogenic enzymes and the type of steroid produced. In rodents, CL is a substantial site of progesterone biosynthesis (2). The movement of cholesterol from the outer to the inner mitochondrial membrane is a rate limiting step in progesterone synthesis in the ovary. This translocation is mediated by steroidogenic acute regulatory protein (StAR), a phosphoprotein expressed in steroidogenic cells (3). The expression of both mRNA and protein of StAR was reported in rat (4) and human CL throughout the luteal phase (5). StAR expression coincides with the capacity of steroidogenesis and

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its expression can be used as a functional marker of CL development in rat (6).

Lithium chlorides (LiCl) is an effective drug for the treatment of bipolar disorders, a central nervous system (CNS) disease (7), however evidence suggests that metabolism, neuronal communication, cell proliferation and cell fate determination have been affected by lithium in various organisms (8). Also, lithium induced side effects on the adult male rat reproductive system (9). Ghosh et al. (1990) (10) and Sheard et al. (1997) (11) reported that lithium treatment results in marked diminution in the plasma levels of gonadotropins, prolactin and testosterone in rats and humans. In addition, lithium administration significantly decreased ovarian steroidogenic enzymes and folliculogenesis in the adult female rats (12,13). Recently we showed that the serum level of progesterone and transcript levels of key steroidogenic enzymes were altered in the gonadotropin-stimulated rats following single injection of LiCl (14). In this study, we have examined whether reproductive toxicity of lithium is associated with alterations in progesterone synthesis and the expression of StAR in the gonadotropin-induced rat following two weeks of LiCl treatment.

2. Materials and methods

2.1. Animals

Immature (25-day-old) female albino rats of the *Wistar* strain were used. All rats were housed in Plexiglas cages and kept under controlled temperature (22 ± 2 °C) and 12/12-h light–dark cycle. Animals were allowed free access to rat chow and water. The procedures were performed in accordance with institutional guidelines for animal care and use. The Animal Ethics Committee of the Department of Studies in Zoology, University of Mysore, Manasagangotri approved the experimental protocol.

2.2. Experimental design

Immature female *Wistar* rats (25-day-old) were injected intraperitoneally (i.p.) with 2.0 mg/kg/day of lithium chloride (Sigma, Germany) or sterile distilled water (0.5 ml) for 15 days.

The dose of LiCl was selected on the basis of the human therapeutic dose. Then, all rats were treated with single i.p. injection of 10 IU pregnant mare's serum gonadotrophin (PMSG) on the 13th day of experiment to induce follicular maturation and followed by single i.p. injection of 10 IU human chorionic gonadotropin (hCG) (Intervet Inc., Germany) 48 h later to induce ovulation. The last injection of lithium chloride (LiCl) or distilled water was at 12 h post-hCG injection. Rats injected only with distilled water and gonadotropins served as control group. In this model, ovulation approximately occurred at 12–14 h post-hCG injection (the oocytes were observed by applying gentle pressure to both ends of the ampulla, and placed on a slide under the stereomicroscope). All animals were killed by spinal dislocation at 4 h

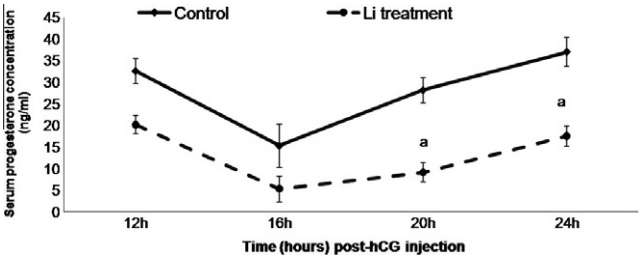

Figure 1 Serum concentrations of progesterone were determined in gonadotropin-induced rats after treatment with LiCl or distilled water (control) by ELISA. The results represent the means ± SEM of groups of eight rats. (a): Significantly different from respective control group, $P < 0.05$.

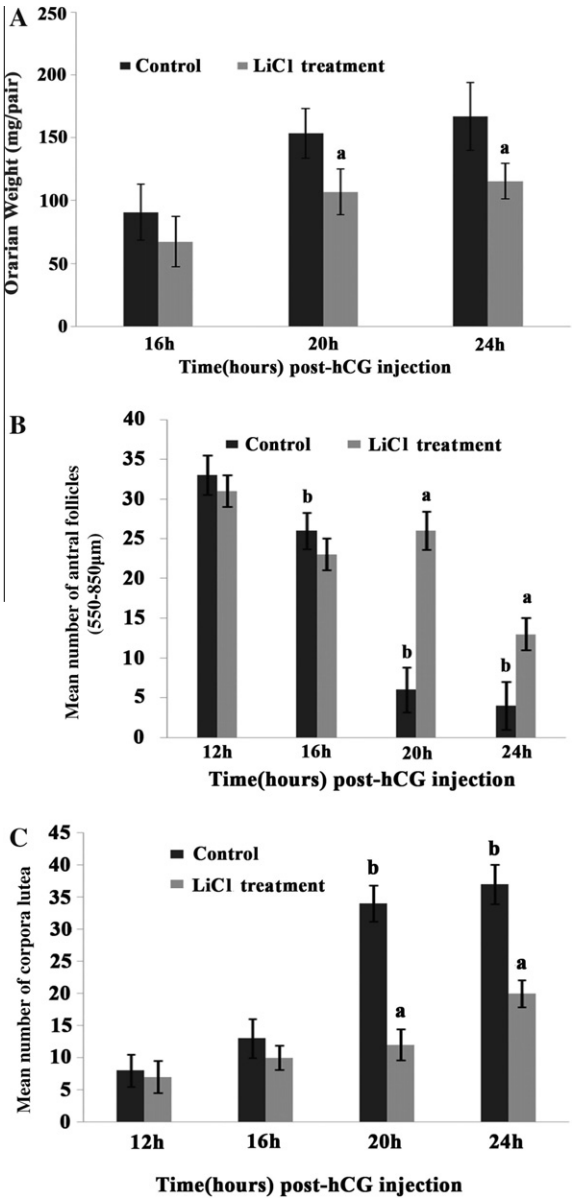

Figure 2 Effect of LiCl on the ovarian weight (A), number of antral follicles (550–850 µm) (B) and corpora lutea (C) in ovaries of gonadotropin-induced rats after treatment with either LiCl or distilled water (control). The results represent the means ± SEM of groups of eight rats. (a): Significantly different from respective control group, and (b): significantly different from control group at 12 h, $P < 0.05$.