Changes in expression of steroidogenic acute regulatory protein (StAR) gene in lithium-treated rats during luteinization

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Abstract
Background: The rate limiting step in progesterone synthesis by corpus luteum appears to be the movement of cholesterol from the outer to the inner mitochondrial membrane that is facilitated by a novel protein named steroidogenic acute regulatory (StAR) protein. Recently we have found a reduction in StAR protein expression following lithium chloride (LiCl) treatment in gonadotropin-stimulated rat. In the present study, the effect of lithium on the expression of StAR gene and serum levels of progesterone were investigated. Methods: Immature 23 days old Wistar rats were induced with injection of pregnant mare’s serum gonadotrophin (PMSG) followed by injection of human chorionic gonadotropin (hCG). Then, rats were injected with either LiCl or saline at 12 hours post-hCG injection. Ovaries were collected at 4-hour interval from 8 to 24 hour post-hCG injection. Expression of StAR gene was determined by semi-quantitative RT-PCR. In addition, serum levels of progesterone were measured by ELISA. Results: Our results showed that in response to the gonadotropin treatment, serum levels of progesterone increased in both control and LiCl-treated animals, but it significantly decreased in LiCl-treated animals as compared with the control. Also significant decreases were observed in expression of StAR gene in early stage of luteinization following LiCl treatment. Conclusion: It is concluded that LiCl is an effective suppressor of StAR expression, and also the critical step of the progesterone synthesis was affected by LiCl treatment in gonadotropin-stimulated rat.

Key words: Lithium Chloride, Corpus luteum, StAR gene, Progesterone.
samples were collected by cardiac puncture. Serum was separated by centrifugation and stored at -20°C until used for subsequent determination of hormonal parameters. The ovaries were rapidly removed, washed in a cold saline solution, snap-frozen in liquid nitrogen and stored at -80°C until they were used in RNA extraction.

**Hormone assay**

Serum levels of progesterone were determined by ELISA using a commercial kit (Progesterone / Progesteron, IBL, Germany) according to the manufacturers’ protocols.

**RNA isolation and semi-quantitative RT-PCR**

Total RNA was extracted using the RNeasy mini kit (Qiagen, Germany) and the reverse transcription (RT) reaction was performed with QuantiTect Reverse transcription kit (Qiagen, Germany) according to the manufacturer’s instructions. Polymerase chain reaction (PCR) was performed with 2 microlitre of cDNA preparation, using specific primers that were previously published and checked on the basis of gene sequences identified with the BLAST search for STAR (Motuh et al., 2006) as follows: sense 5’-CATCCAGCAAGGAGGAAG-3’ and antisense 5’-CGTGAGTTGGCTTGTGAGG-3’. The conditions used for PCR were as follows: 94°C for 2 min, 23-cycles (94°C for 30 sec, 64°C for 30 sec, and 72°C for 30 sec), and then 72°C for 10 min. Also the amount of quantified mRNA was normalized by Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA as an internal control using the following primers: sense 5’-CAAGGTCATCCATGACAACCTTGTAG-3’, antisense 5’-GTCCACCAACCTGCTGTAG-3’. The PCR condition used for GAPDH was 94°C for 3 min, and 25cycles of 94°C for 30 sec, 56°C for 30 sec and 72°C for 45 sec. The cDNA amplified was electrophoresed in 1% agarose gel and stained with ethidium bromide. The band image was quantified using Image J software (version 1.43). The amount of quantified mRNA was normalized by GAPDH mRNA as an internal control.

**Statistical analysis**

Experiments were repeated at least two times with eight animals in each treatment and time point. One-way ANOVA was used to analyze differences between groups, with P < 0.05 considered statistically significant. Tests were performed using SPSS software (Software program, version 13, SPSS, Inc., Chicago, USA).

**RESULTS**

Serum levels of progesterone

Result showed that a significant decrease was observed at 16 h in plasma progesterone concentration and then increased over the time (at 20 and 24 h) following hCG injection in control. Although, serum progesterone concentration was lower, 3- and 2.1-fold at 20 and 24 h, in LiCl-treated rat as compared with control (Fig 1).

**StAR gene responses**

In order to provide insights into how LiCl may affect on the progesterone production, the expression of StAR gene was determined by semi-quantitative RT-PCR. As shown in Figure 2, the luteal expression of StAR mRNA, a well-known marker for luteal phase, was markedly increased by hCG after ovulation (16, 20 and 24 h post-hCG injection). While significant decreases were observed in STAR gene at 20 and 24 h after LiCl treatment (Fig. 2A and B).

**DISCUSSION**

As shown in figure 1, serum progesterone concentrations was decreased at 16 h and followed with the dramatically rises at 20 and 24 h that suggests the process of ovulation (at 16 h), and then the beginning of luteinization and progesterone biosynthesis (at 20 and 24 h). Thus, a positive correlation was revealed between the serum progesterone concentration and the time after ovulation in control rats.

Increase in progesterone concentrations was coincident with the significant increase in the Star gene expression at 20 and 24 h post-hCG injection that indicate a critical time point for luteinization. The significant decrease was observed in the serum progesterone levels between 16 and 24 h following LiCl treatment (Fig. 1). PRL the pituitary hormone affects the formation and function of CL in rat and removal of the endogenous PRL in pregnant rat results in failure of luteal development and reduced progesterone secretion by the CL. Previous studies showed that the plasma levels of gonadotropins and PRL were significantly reduced by lithium in rats and humans. Therefore, it is conceivable that PRL levels could be affected by lithium and led to the changes in the process of luteinization.

The decrease in serum levels of progesterone in response to LiCl treatment could be the result of a change in the levels of STAR mRNA. The Formation of the CL is accompanied by a dramatic increase in the expression of this gene in earlier studies. Our results showed that STAR expression increased between 16 and 24 h in control rats and this increase was positively correlated with the progesterone concentration (Fig. 1 and 2A). The significant decreases were observed in STAR expression at 16 and 20 h in LiCl treatment that could be due to effect of LiCl on the levels of pituitary hormons vis.PRL and LH (Furthermore, Wnt4 repressed steroidogenesis in adrenocortical and Leydig cell lines by reduced progesterone secretion. Consistent with this finding that Li mimics Wnt/β-catenin signaling, the effects of LiCl treatment on steroidogenesis genes expression could be due to activation Wnt signaling pathway.
It is concluded that the effect of lithium on the CL is reflected in the reduction of progesterone which could be attributed to the interference on steroidogenesis as evident from the decreased expression of STAR gene.

**LEGENDS OF FIGURES:**

**Figure 1** Serum progesterone concentrations at the multiple times after hCG injection in control and LiCl-treated groups. 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 hours, P < 0.05.

**Figure 2** Expression of STAR mRNAs in ovary was determined by RT-PCR (A). Semi-quantitative RT-PCR analysis of STAR mRNA levels in ovary (B). Relative levels of mRNA are expressed as the ratio of the target genes relative to GAPDH in each sample. 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 hours, P < 0.05.
REFERENCES


