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Microcystin-leucine arginine inhibits gonadotropin-releasing hormone synthesis in mice hypothalamus

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ABSTRACT

Microcystin-leucine arginine (MC-LR) causes serum testosterone declines and male reproductive disorders. However, the molecular mechanisms underlying the pathological changes are still unclear. In the present study, we aimed to investigate the toxic effects of MC-LR on gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus. Our results demonstrated that MC-LR could enter GnRH neurons and inhibit GnRH synthesis, resulting in the decrease of serum GnRH and testosterone levels. The inhibitory effects of MC-LR on GnRH synthesis were identified to be associated with activation of the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA)/cAMP response element-binding protein (CREB)/c-Fos signaling pathway. With miRNA microarray analyses, we found that miR-329-3p was down-regulated most dramatically in MC-LR-treated GT1–7 cells. We then further identified that miR-329-3p regulated PRKAR1A and PRKACB expression and thus influenced GnRH synthesis. This is the first study to explore the molecular mechanism underlying the inhibitory effects of MC-LR on GnRH synthesis in the hypothalamus. Our data have provided a new perspective in the development of diagnosis and treatment strategies for male infertility as a result of dysfunction of the hypothalamic-pituitary-gonadal axis.

1. Introduction

A combination of factors including eutrophication and global warming has promoted drastic proliferation of cyanobacteria and the massive release of the associated cyanotoxins in some natural water bodies (Adamovsky et al., 2015; Chen et al., 2017; Merel et al., 2013; Wood, 2016). Microcystins (MCs) produced by *Microcystis* and *Planktothrix* have been proven to be able to inflict damage to the aquatic creatures (Ilfergane and Henkel, 2018; Kim et al., 2017; Paulino et al., 2017). As a result of the enrichment in aquatic creatures, MCs were also identified to pose a substantial health hazard to humans higher up in the food chain (Blom and Juttner, 2005; Li et al., 2011; Zhao et al., 2016). Thus far, more than 100 structural analogues of MCs have been identified, among which MC-leucine arginine (MC-LR) has drawn more attention owing to its broad distribution and potent toxicity (Blom and Juttner, 2005). MC-LR was observed to induce dysfunction of the liver, kidney, lung, pancreatic islets in experimental models (Christen et al., 2013; Ding et al., 2017; Huang et al., 2015; Li et al., 2016; Zhao et al., 2017, 2016). Moreover, further epidemiological studies revealed that MC-LR contamination of drinking water may lead to increased incidences of colorectal cancer, liver cancer, renal impairment, and diabetes in populations around the lakes or along the rivers with cyanobacterial blooms (Li et al., 2011; Lin et al., 2016; Zhao et al., 2016; Zhou et al., 2002).

We have previously reported declines in serum testosterone levels in rats following exposure to MC-LR (Li et al., 2008). Given that testosterone is produced by Leydig cells (LCs), these cells have been suggested as a potential cellular target of MC-LR (Dufau, 1988; Li et al.,

Abbreviations: AAV, Adeno-associated virus; cAMP, Cyclic adenosine monophosphate; ChIP, Chromatin immunoprecipitation; Co-IP, Coimmunoprecipitation; CREB, cAMP response element-binding protein; FBS, Fetal bovine serum; GnRH, Gonadotropin-releasing hormone; MC-LR, Microcystin-leucine arginine; PKA, Protein kinase A; PRKACB, Catalytic subunit beta of PKA; PRKAR1A, Regulatory subunit of PKA

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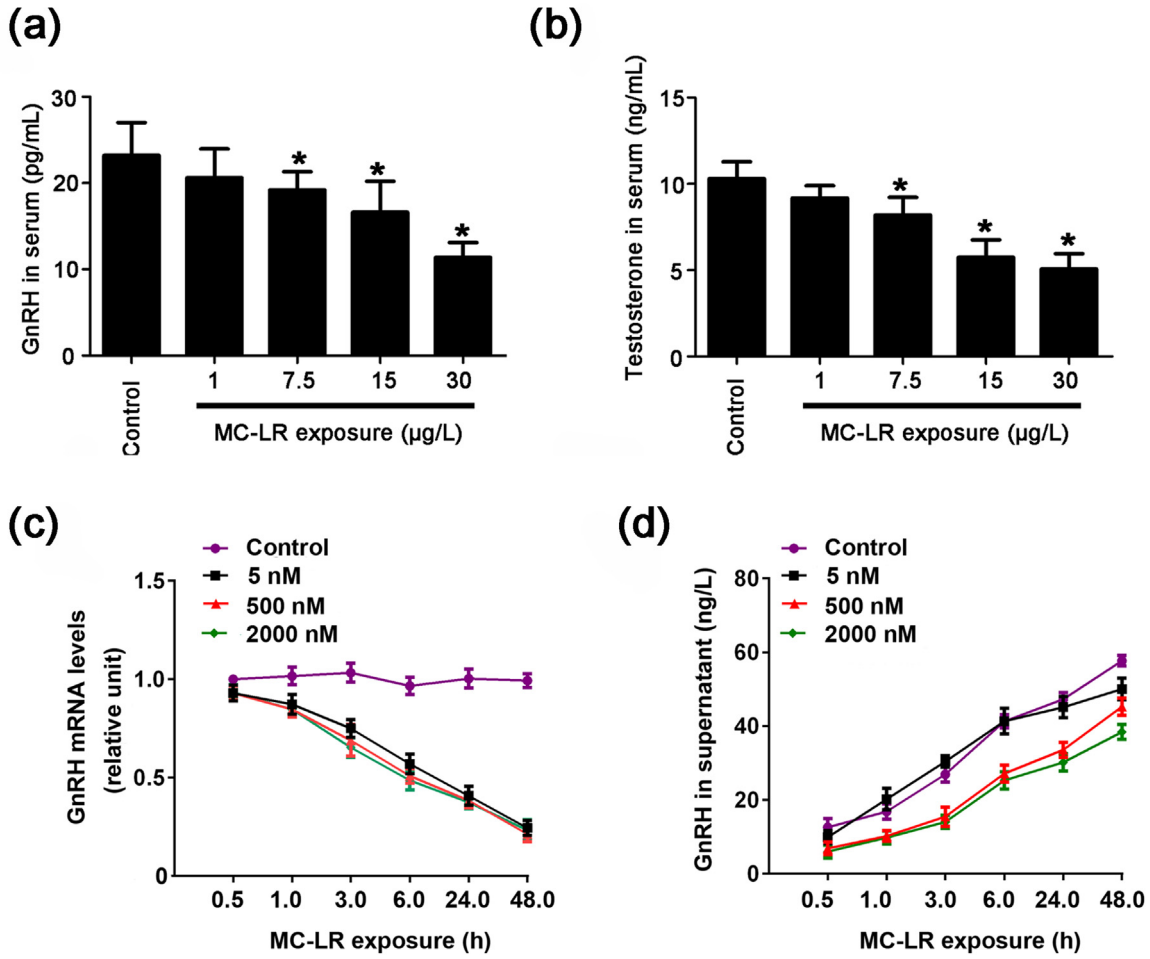

Fig. 1. MC-LR inhibits gonadotropin-releasing hormone (GnRH) synthesis in GT1–7 cells. Mice were provided with drinking water containing various concentrations of MC-LR for 180 consecutive days. (a) Serum GnRH levels were measured by HPLC-MS. Data are presented as means ± SEM (n = 10). *$p < 0.05$, vs control. (b) Serum testosterone levels were detected by chemiluminescence kits. Data are presented as means ± SEM (n = 10). *$p < 0.05$, vs control. (c, d) GT1–7 cells were treated with various concentrations of MC-LR for various lengths as indicated. The relative mRNA levels of GnRH were detected by qRT-PCR (c); levels of GnRH in supernatant were measured by HPLC-MS (d).

2008). However, our further study has demonstrated that MC-LR was not able to enter into LCs to inhibit testosterone synthesis, thereby alluding to the fact that MC-LR may decrease testosterone levels by damaging the upstream endocrine system (Wang et al., 2013). The hypothalamic-pituitary-gonadal axis is an important endocrine regulatory pathway in humans. Gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus can produce GnRH to regulate the synthesis of testosterone by LCs (Counis et al., 2005). Disruption of GnRH synthesis may induce abnormal sexual maturation and infertility. We have previously observed decreased levels of serum testosterone, LH, and GnRH in mice following acute or chronic exposure to MC-LR, suggesting that MC-LR may disrupt testosterone synthesis in LCs through inhibiting GnRH synthesis in the GnRH neurons (Ding et al., 2017; Wang et al., 2016).

In this study, we aimed to further investigate the specific molecular mechanism underlying reduced GnRH synthesis in the GnRH neurons after exposure to MC-LR. Moreover, we were also interested in exploring the effect of MC-LR on miRNA expression and understanding whether this could provide a mechanistic explanation for the decline of GnRH. To our knowledge, this is the first study that demonstrated altered expression of miRNAs in MC-LR-treated GnRH neurons. Our study revealed that miR-329-3p was most dramatically down-regulated. Furthermore, miR-329-3p was found to be able to regulate GnRH synthesis by targeting the regulatory subunit of PKA (PRKAR1A) and the catalytic subunit beta of PKA (PRKACB).

2. Materials and methods

2.1. Main chemicals

MC-LR was purchased from Alexis Biochemicals (Lausen, Switzerland). MC-LR (1 mg) was dissolved in 100 μL DMSO and further diluted to 1 mL with DMEM (Gibco) to prepare the stock solution (1 mM). The luciferase assay system was purchased from Promega (Fitchburg, WI). The PKA kinase activity kit was obtained from Enzo Life Sciences (Farmingdale, NY). The antibodies used in this research are listed in Supplementary Table S1.

2.2. Cell culture and viability test

GT1–7 cells, a well-established mouse cell line that shares many of the properties of GnRH neurons, have been extensively used as a surrogate for primary GnRH neurons in the literature (Glidewell-Kenney et al., 2013; Hoffmann et al., 2016). Cell viability was evaluated by the Cell Counting Kit-8 (CCK-8) test according to the manufacturer's instructions.

2.3. Quantitative RT-PCR (qRT-PCR)

Total RNA from GT1–7cells and the hypothalamus tissue was isolated using Trizol reagent according to the manufacturer's protocol