Systemic Administration of Lipopolysaccharide Increases the Expression of Aquaporin-4 in the Rat Anterior Pituitary Gland

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ABSTRACT. We investigated the effects of lipopolysaccharide (LPS)-induced endotoxemia on the expression of aquaporin-4 (AQP4) in the rat anterior pituitary gland, using the real-time polymerase chain reaction and immunohistochemistry. After intraperitoneal injection of LPS, the level of AQP4 mRNA doubled at 2, 4 and 8 hr. Immunohistochemical analysis showed an increase with time in AQP4 immunostaining in folliculo-stellate cells following LPS injection; the intensity of immunoreactivity peaked at 8 hr. At the same time, some cyst-like structures, formed by AQP4-positive cells, were observed. These findings indicate that LPS induces the expression of AQP4 in the anterior pituitary gland. The present results should provide an important key to elucidate the pathogenesis of the anterior pituitary gland during endotoxemia.

KEYWORDS: anterior pituitary gland, aquaporin-4, folliculo-stellate cell, immunohistochemistry, real-time PCR.


Aquaporin-4 (AQP4) is the predominant water channel in the brain and is expressed in astrocytes [14]. It plays important roles in the regulation of brain water homeostasis in physiological and pathological conditions [1, 3, 14]. After brain injury or in brain edema, the expression of AQP4 is up-regulated, implying that AQP4 is involved in the development [8, 14] and/or reduction [15] of brain lesions. In the anterior pituitary gland, folliculo-stellate (FS) cells comprise small populations of non-endocrine cells, which are similar in structure and function to astrocytes [6, 13]. We have previously reported that AQP4 is expressed in FS cells and might be related to water transfer within the anterior pituitary gland [9, 10]. We are not aware of any previous studies asking whether AQP4 expression is regulated in the anterior lobe and whether pathophysiological factors related to pituitary condition affect the expression of AQP4. Systemic injection of microbial toxins, such as lipopolysaccharide (LPS), which is a cell wall component of Gram-negative bacteria, is often used as an experimental model of sepsis. In the mouse, LPS can induce cerebral edema formation and up-regulate expression of brain AQP4 [2]. Previous studies of the anterior pituitary gland indicate that systemically administered LPS elicits profound changes in hormone secretion [5, 18–20] and in mRNA for cytokines [19], but there have been no reports concerning AQP4 expression in the anterior lobe after LPS injection. We therefore examined the expression of AQP4 at various time points in anterior pituitary glands of LPS-injected rats, using the real-time polymerase chain reaction (PCR) and immunohistochemistry.

A total of 48 male Sprague Dawley rats (6 or 7 weeks old) were obtained from Clea Japan (Tokyo, Japan). The rats were treated with saline containing LPS (2.5 mg/kg body weight of O127: B8, L3129, Sigma, St. Louis, MO, U.S.A.) by intraperitoneal injection. The amount of LPS is the same as earlier publications [16, 19]. The animals were then kept under observation and were put to death at different intervals after injection (2, 4, 8, 12 and 24 hr). To control for the effects of saline injection, control rats were injected with saline alone and were also sacrificed 2 hr after the injection. The Animal Care and Use Committee of Hyogo College of Medicine approved our procedures. To determine whether the expression of AQP4 genes changed in the anterior lobe, real-time PCR was performed to quantify AQP4 gene expression in the anterior pituitary glands of control (n=5) and LPS-injected rats (n=5 per time point). Total RNA of the anterior pituitary was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, U.S.A.) and validated using TaqMan Gene Expression Assays (Applied Biosystems) Rn00563196_m1 for the target gene AQP4 (RefSeq: NM_012825.2) (amplicon size=55 bp) and Rn00560865_m1 for the internal normalization gene beta-2 microglobulin (β2m) (amplicon size=58 bp). PCR commenced with an initial step of 10 min at 95°C, followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C. No false positive was detected in any non template control replicates of each gene, and all assays were performed in triplicate. Levels of AQP4 mRNA expression were shown as relative copy ratios (AQP4/ β2m). The results were expressed as mean ± SE for five rats. The data were analyzed by one-way ANOVA followed by Fisher’s least significant difference test with significant differences, assigned at the 5% probability level. For immunohistochemistry (n=3 per each group), im-
munostaining with anti-AQP4 (A5971, Sigma; diluted 1:100) or anti-S-100 protein (a marker for FS cells, Z0311, Dako
Cytomation, Glostrup, Denmark; diluted 1:4,000) antibodies was performed by the methods set out in our previous reports [9, 10]. Counter-staining was done using hematoxylin and was observed under an optical microscope.

A significant increase of AQP4 mRNA was observed in the rats that received LPS (Fig. 1). In the LPS rats, AQP4 mRNA expression at 2 hr post-injection increased significantly in the anterior pituitary gland (P<0.05) compared with controls. Significant elevated expression of AQP4 mRNA was sustained until 8 hr after the LPS injection and gradually declined thereafter. There were no significant differences between AQP4 mRNA expression levels at 12 or 24 hr and controls. By 24 hr after LPS injection, the levels of AQP4 mRNA in the anterior lobe had returned almost to control values. AQP4 immunoreactivity was observed in the parenchymal tissue of the anterior pituitary gland (Fig. 2A–F). We have reported that these AQP4-positive cells in the anterior pituitary parenchyma are FS cells [9, 10]. The present immunohistochemical analysis revealed that AQP4 immunoreactivity increased in FS cells following LPS administration (Fig. 2A–F). After LPS injection, the intensity of AQP4 immunoreactivity increased with time and peaked at 8 hr. The elevated intensity was falling at 12 hr, and no clear difference in AQP4 reactivity was observed between values at 24 hr and controls. There was no obvious difference in S-100 protein immunoreactivity in the anterior pituitary parenchyma between control and LPS-administered rats (Fig. 2a–f). These data indicate that LPS induces the expression of AQP4 mRNA and increases AQP4 immunoreactivity in the anterior pituitary gland. The enhanced levels return to control values within 24 hr. The present study therefore demonstrates a complete time course of AQP4 expression following administration of LPS. We believe this is the first report of up-regulation of AQP4 in the anterior pituitary gland. Our previous studies have shown that AQP4 protein is expressed in some FS cells and marginal layer cells in Rathke’s residual pouch, but not in any hormone-producing cells in the adenohypophysis [9, 10]. Matsuzaki et al. have reported similar immunohistochemical observations using their own AQP4 antibody [12]. In this study, the level of AQP4 transcripts was examined in whole anterior lobes, which included both FS cells and marginal cells, and we were therefore unable to determine whether mRNA for AQP4 had elevated in FS cells and/or marginal cells. However, the present immunohistochemical study demonstrated that AQP4 immunostaining was enhanced in FS cells after LPS injection, so we believe that LPS can increase the level of AQP4 mRNA at least in FS cells. FS cells are scattered throughout the anterior pituitary gland [17], and their long slender cytoplasmic processes intermingle so as to produce a three-dimensional anatomical network [4]. Our observation suggests that following LPS injection, the FS cells are stimulated to facilitate water transport in the anterior pituitary gland. Up-regulation of AQP4 in FS cells might therefore contribute to the maintenance of water homeostasis in the anterior pituitary gland after LPS administration.

Two mechanisms can be proposed to explain how peripheral LPS increases AQP4 expression in the anterior pituitary gland. First, LPS may act directly on FS cells to induce AQP4 expression, because FS cells express CD14 and Toll-like receptor-4, which are essential for LPS signal transduction [11]. Second, LPS may act indirectly; these indirect effects may be mediated by LPS-induced pro-inflammatory cytokines, such as interleukin (IL)-1β. A previous study has demonstrated that IL-1β induces the expression of AQP4 through IL-1 receptor in rat primary astrocytes [7]. In the anterior pituitary gland, robust IL-1β mRNA induction has been demonstrated at 0.5 hr after peripheral LPS injection [16, 19]. IL-1β may therefore be involved in the induction of AQP4 expression in the anterior lobe. Further studies are needed to establish the exact pathways regulating the anterior pituitary expression of AQP4 in LPS-treated rats.

Remarkably, some cyst-like structures, which have a clear lumen, were observed in the parenchymal tissue of the anterior pituitary gland at only 8 hr after LPS injection; cells forming these structures were positive for AQP4 (Fig. 3). Matsuzaki et al. have described the same structures in the normal anterior pituitary [12], but we were unable to detect them in controls or at any other time points after LPS injection in this study (data not shown). The reason for the discrepancy is unknown, but some elements that regulate the formation of cyst-like structures might exist in the healthy rat anterior pituitary gland. Our present study, which made observations at various time points after LPS administration, shows that cyst-like structure formations were caused by LPS administration; AQP4 could be related to the formation and/or disappearance of cyst-like structures. It is also suggested that water influx through AQP4 in the anterior pituitary gland is caused by systemic administration of LPS, and the water fluids move to the lumen of the cyst-like
Fig. 2. Light microscopic analysis of AQP4 (A–F)- and S-100 protein (a–f)- immunoreactivity in the parenchymal tissue of the anterior pituitary gland. AQP4 was localized to FS cells. In controls, FS cells exhibited weak labeling for AQP4 (A), moderately intense labeling at 2 hr (B) and 4 hr (C), and strong labeling at 8 hr (D). The intensity fell from 12 hr (E), and there was no obvious difference between in control values and values at 24 hr (F) after LPS injection. For S-100 protein reactivity, no clear difference was observed between in control values (a) and values at 2 hr (b), 4 hr (c), 8 hr (d), 12 hr (e) and 24 hr (f) after LPS administration. Scale bar, 50 μm.

Fig. 3. Light microscopic analysis of AQP4-immunoreactivity in the parenchymal tissue of the anterior pituitary gland at 8 hr following LPS administration. Cyst-like structures (C) were observed, and cells forming cyst-like structures were positive for AQP4. Scale bar, 100 μm.
structure, because cells forming cyst-like structures express AQP4. Future studies could use this model to examine how AQP4 expression is regulated in FS cells and influences the formation of cyst-like structures. The result could provide an important key to elucidate the pathogenesis of the anterior pituitary gland during LPS-induced endotoxemia.

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