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The adrenal gland is a source of stress-induced circulating IL-18

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Abstract

The present study compared plasma IL-18 levels between sham-operated and adrenalectomized mice following stress to investigate whether the adrenal gland contributes to the elevation of circulating IL-18 during stress. Two hours of stress provoked a robust, stressor-dependent, elevation of IL-18 mRNA and peptide in the adrenal gland in sham-operated mice. Consistently, levels of circulating mature IL-18 increased during stress and remained elevated for up to 6 h after stress. The stress-induced increase in circulating IL-18 was abolished by adrenalectomy. These findings demonstrate that the adrenal gland is required to achieve elevation of circulating IL-18 during stress.

Keywords: Interleukin-18; Immobilization stress; Adrenal gland; Adrenalectomy; HPA axis

1. Introduction

Interleukin-18 (IL-18), known as interferon-γ inducing factor, exerts pleiotropic roles as a potent pro-inflammatory cytokine. In addition to immune cells, IL-18 has been demonstrated in endocrine tissues, such as the adrenal gland, pituitary gland and neuronal cells in hypothalamus, cerebellum, hippocampus, cortex, striatum and medial habenular nucleus (Okamura et al., 1995; Conti et al., 1997; Culhane et al., 1998; Wheeler et al., 2000; Sugama et al., 2002; Nagai et al., in press). Elevated levels of IL-18 have been observed in conjunction with obesity, diabetes mellitus, ischemic heart disease, autoimmune diseases as well as infectious diseases (Grobmyer et al., 2000; Seta et al., 2000; Wong et al., 2000; Esposito et al., 2002; Mallat et al., 2002; Moriwaki et al., 2003), thus suggesting a role in some immunopathological conditions.

We previously proposed that IL-18 may possess a role in modulating the immune responses during stress (Conti et al., 1997, 2000; Sugama et al., 2000). IL-18 is constitutively expressed in the adrenal cortical cells that produce glucocorticoids and its mRNA expression is significantly increased following acute stress or ACTH administration (Conti et al., 1997, 2000; Sugama et al., 2000; Sekiyama et al., 2005). Interestingly, the murine IL-18 gene is regulated by at least two promoters named P1 and P2 (Tone et al., 1997), and the expression of IL-18 gene in the adrenal gland occurred through the specific usage of P1 and differed from that in cells of the immune system that preferentially utilized P2 (Sugama et al., 2000). On the basis of these data, we proposed a specific role for the adrenal gland in producing IL-18 during acute stress (Sugama et al., 2000). This hypothesis has been supported by recent studies demonstrating that adrenal IL-18 is secreted in its active form via ACTH and superoxide-mediated activation of caspase-1 (Sekiyama et al., 2005). However, the contribution of the adrenal gland to the elevation of plasma IL-18 levels during stress remains to be further elucidated.

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In the present study, we hypothesized that the adrenal gland is the major source of stress-induced increase in circulating IL-18 levels. To test this hypothesis, we compared the basal and stress-inducible levels of circulating IL-18 in sham-operated and adrenalectomized (ADX) mice. In addition, we compared tissue-specific induction of IL-18 in the adrenal gland and immune tissues following stressful event. The present study demonstrates the involvement of the adrenal gland in the induction of plasma IL-18 during stress.

2. Materials and methods

2.1. Animals and treatments

Male C57BL6 mice, 8 weeks and 20 to 26 g, were used throughout this study. All mice were treated in accordance with the *National Institute of Health Guide for the Care and Use of Laboratory Animals* and all procedures were approved by a Nippon Medical Animal Care Committee. Experiments were performed at least 1 week after the arrival of mice at the animal facility. Stress was given by placing mice with extremities bound on the plate, combined with water immersion (temperature of the water was maintained constant at 21 ± 2 °C) (IMO + WI) (Mizoguchi et al., 2003). Immobilization stress was combined with water immersion since we found that the combined stresses gave more consistent and reproducible results than the use of simple immobilization stress in which larger individual differences in the levels of IL-18 were observed. Mice were sacrificed immediately after 1 h, 2 h IMO + WI stress or returned to their own cage, then sacrificed at 2 h and 6 h after release from 2 h period of IMO + WI stress. For adrenalectomy, mice were anesthesized with pentobarbital (120 mg/kg), and bilateral suprarenal fat pad including adrenal glands were removed with forceps after incision of skin and peritoneum. Sham operations were performed with the age-match mice. Following the ADX surgery, mice were maintained with 0.9% sodium chloride solution. Sham-operated mice were maintained with water. All mice were housed in a room maintained at 20 to 22 °C on a 12 h light–dark cycle with food and water available ad libitum. Stress experiments were performed 1 week after the ADX procedure. No significant body weight change was observed before operation (23.2 ± 0.2 g) and prior to stress experiments (23.0 ± 0.3 g). All mice survived adrenalectomy.

2.2. RIA for ACTH measurement

Plasma ACTH levels were measured using commercially available ACTH IRMA (Mitsubishi Kagaku Iatron, Tokyo, Japan) according to manufacturer's instructions in sham-operated and ADX mice at each condition as described in animals and treatments.

2.3. ELISA for IL-18

Blood was collected from cervical arteries immediately following decapitation. Plasma was obtained after the centrifugation at 5000×*g* for 10 min at 4 °C. Tissues protein were extracted from mice adrenal gland, spleen and intestine in RIPA buffer (1 × phosphate-buffered saline, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 0.1 mg/ml phenyl methyl sulphonyl fluoride, 30 μg/ml aprotinin, 1 mM sodium orthovanadata). The lysate was centrifuged and the supernatant was used for the analysis or stored at −80 °C for further analysis. The levels of IL-18 in the plasma and tissues were measured using an ELISA kit (MBL, Nagoya, Japan) according to manufacturer's instructions.

2.4. Semiquantitative real time PCR

Semiquantitative PCR was performed using Roche LightCycler equipment and LightCycler FastStart DNA Master SYBR Green I mix (Roche). Reactions were carried out in a 20 μl volume using 0.5 μM primers and 4 mM $MgCl_2$. The sequences of the primers are as follows: for GAPDH, 5′-CCTTCATTGACCTCAACTACATGGT-3′ and 5′-TCATTGTCATACCAGGAAATGAGC-3′; for IL-18, 5′-GTGACCCTCTCTGTGAAGGATA-3′ and 5′-TGTGTCCTGGAACACGTTTC-3′. PCR assays included an initial 10 min, 94 °C step to activate Taq polymerase, followed by 35–45 cycles of denaturation at 94 °C, 10 s, annealing 55 °C, 10 s, and extension 72 °C, 25 s. Standard curves were constructed using purified and sequenced IL-18 and GAPDH fragments. Results were analyzed by the second derivative methods and were expressed in arbitrary units normalized by the expression levels of the reference gene, GAPDH, quantified simultaneously with the target gene.

2.5. Western blotting

Samples (plasma) were electrophoresed on a 12% SDS-PAGE denaturing gel and electrotransferred on Hybond ECL nitrocellulose membrane (Amersham Biosciences, UK). The membrane was blocked by incubation in Tris buffer saline (TBS) solution containing 5% nonfat dry mild and 0.1% Tween 20 prior to the addition of primary antibody. The incubation of IL-18 antibody (1:2000, Santa Cruz Biotechnology, Santa Cruz, CA) was performed at room temperature for 3 h. The membrane was washed for 10 min three times in TBS/0.1% Tween 20 prior to the addition of horseradish-peroxidase-conjugated secondary anti-goat IgG (1:5000, JacksonImmunoResearch, PA, USA). The filter was incubated for 1 h and washed three times in TBS/0.1% Tween 20. Signals were detected by chemoluminescence using the ECL plus Western Blotting Detection system (Amersham Biosciences, UK) and revealed by autoradiography Kodak BioMax MS films.