論 文 の 内 容 の 要 旨

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論文題目　Studies on the Role of Hypothalamic Cyclooxygenase-2-Related Signaling in Response to Acute Stresses
（急性ストレス反応における視床下部シクロオキシゲナーゼ－２関連シグナルの役割に関する研究）

Stress is a term that is widely used in modern society. Stress activates two main physiological pathways: the activation of the autonomic nervous system and hypothalamic-pituitary-adrenal (HPA) axis, which results in an increase in plasma glucocorticoids levels. HPA axis controls reaction to stress and regulates many body processes, including the immune system, digestion, energy storage and expenditure, sexuality, and mood and emotions. Activation of the HPA axis is considered as the key way to response to stresses to maintain the steady states in the organism. There is a large body of evidence to support that activation of the HPA axis is regulated by signaling through molecules like prostaglandins (PGs), especially PGE2, in the brain. PGs are produced by metabolism of arachidonic acid by cyclooxygenase (COX), which exists in two distinct isoforms, COX-1 and COX-2. COX-1 has the characteristics of a housekeeping gene, which is constitutively expressed in most cells and tissues, whereas

COX-2 is a rate-limiting enzyme in PGs synthesis, which is generally expressed at low basal levels and rapidly induced in response to various stress stimuli. Furthermore, our laboratory has previously shown that glucocorticoids maintain gonadotropin secretion through the suppression of COX-2 under various acute stress conditions, suggesting that COX-2 plays a role as a common mediator of stresses in the brain. However, the mechanisms by which different acute stresses converge to regulate COX-2 and PGs synthesis and the relationship between COX-2 and PGs synthesis and activation of the HPA axis under various acute stress conditions are not fully understood. This dissertation was conducted to investigate the relationship and mechanism between hypothalamic COX-2-related signaling and activation of the HPA axis under three different acute stress conditions, namely infectious, hypoglycemic and restraint stresses.

In Chapter 1, I evaluated the involvement of hypothalamic COX-2-related signaling in activation of the HPA axis. Three different acute stresses, i.e. infectious (lipopolysaccharide, LPS), hypoglycemic (2-Deoxy-D-glucose, 2DG) and restraint stresses, were applied to male intact and adrenalectomized (ADX) rats. First, I used an unselective COX inhibitor (indomethacin) or a selective COX-2 inhibitor (NS-398) to elucidate the roles of COX-2-related signaling pathway in activation of the HPA axis under three different acute stress conditions. Subsequently, the gene expression of corticotrophin-releasing hormone (CRH), COX-2, microsomal prostaglandin E synthase-1 (mPGES-1), and interleukin-1β (IL-1β) in the hypothalamus were examined by real-time RT-PCR using intact and ADX rats. The results showed that both indomethacin and NS-398 significantly attenuated the increase of serum corticosterone levels after LPS and restraint stresses, but not after 2DG injection. All the three acute stresses induced significant increases in serum corticosterone levels in intact rats at the both time points examined (30 and 120 min), while COX-2 and mPGES-1 mRNA levels in the hypothalamus were not increased at 30 min in intact rats. CRH mRNA levels were not changed throughout the experimental period under all the three acute stress conditions. COX-2 and mPGES-1 mRNA levels were significantly increased 120 min after LPS injection, but were not up-regulated after 2DG and restraint stresses in intact rats. In

ADX rats, only LPS and restraint stresses increased CRH mRNA levels at 120 min, which was completely restored by corticosterone treatment. COX-2 and mPGES-1 mRNA expression was significantly increased 120 min after 2DG and restraint stresses, which was blocked by treatment with corticosterone. In contrast, following LPS injection, COX-2 and mPGES-1 mRNA levels in ADX rats were not different from those in intact rats at both 30 and 120 min. IL-1β mRNA levels in the hypothalamus in intact rats were increased at 120 min only by LPS injection, though those in ADX rats were increased by all the three acute stress stimuli. These results suggest that the relationship between COX-2-related signaling and activation of the HPA axis is stress-specific, and that COX-2-related signaling preferably mediates infectious and restraint stresses. Furthermore, the expression of COX-2 and mPGES-1 mRNA under the infectious stress condition were not negatively regulated by endogenous glucocorticoids, likely due to an increase in IL-1β levels.

The results in Chapter 1 suggest that activation of the HPA axis is dependent on COX-2-related signaling under infectious and restraint stress conditions, but less dependent on it under hypoglycemic stress condition. In Chapter 2, I evaluated the mechanisms underlying a stress-specific relevance between COX-2-related signaling and activation of the HPA axis. First, I outlined the stress-induced pattern of neuronal activation via the expression of the immediate early gene product c-Fos by immunostaining in the brain under infectious, hypoglycemic and restraint stress conditions. Subsequently, I used a selective COX-2 inhibitor (NS-398) to elucidate the roles of COX-2-related signaling pathway in the neuronal activation. The results showed that all the three acute stresses increased the number of c-Fos-immunoreactive (c-Fos-IR) cells in several brain regions, including the paraventricular hypothalamic nucleus (PVN), supraoptic nucleus (SON), arcuate nucleus, central amygdaloid nucleus, medial amygdaloid nucleus, hippocampus and piriform cortex. Quantitative analysis showed that the number of c-Fos-IR cells in the PVN and SON at 120 min were higher than those of other 2 time points (0 and 30 min) under all the three acute stress conditions. Moreover, LPS and restraint stresses increased the number of c-Fos-IR cells in the ventromedial

hypothalamic nucleus (VMH), while 2DG injection increased it in the lateral hypothalamic area (LHA), supporting the notion that the VMH and LHA play a role as satiety and feeding centers, respectively. In the PVN, NS-398 did not decrease the number of c-Fos-IR cells at any time points after three different acute stresses. Furthermore, in the SON, the number of c-Fos-IR cells in NS-398-treated rats was significantly higher than those in vehicle-injected rats at 30 min after LPS and restraint stresses, whereas there was no such difference at any time points after 2DG stress. These results indicate that, among the brain regions responding to stresses, the PVN and SON are strongly activated by stresses. In addition, it is suggested that, while CRH neurons in the PVN are involved in activation of the HPA axis, oxytocin neurons, which are known to be inhibitory to the HPA axis, in the SON as well as PVN are suppressed through COX-2-related signaling and involved in the activation of the HPA axis under infectious and restraint stress conditions. This stress-specific difference in the activation of the brain nuclei following treatment with COX-2 inhibitor may at least partially account for a stress-specific relevance between COX-2-related signaling and activation of the HPA axis.

In conclusion, one of the major findings of this thesis is that activation of the HPA axis is at least partially dependent on COX-2-related signaling under infectious and restraint stress conditions, while less dependent on it under hypoglycemic stress condition. The mechanisms underlying this stress-specific relevance between COX-2-related signaling and activation of the HPA axis may involve stress-specific activation of CRH neurons in the PVN and oxytocin neurons in the PVN and SON. The second major finding of this thesis is that endogenous glucocorticoids negatively regulate COX-2-related signaling under hypoglycemic and restraint stress conditions, but not under infectious stress condition, probably because infectious stress stimulation is mediated by cytokines including IL-1β in the hypothalamus. Thus, the present study revealed that, although COX-2-related signaling is potentially up-regulated by all the three acute stresses used, the role and regulatory mechanism of it varies among stresses, which may be important for animals to effectively respond to each stress condition.