Abstract

Circadian clock in the suprachiasmatic nucleus of the hypothalamus (SCN) regulates daily rhythms in behavior and physiology and is an important part of the mechanisms regulating mammalian homeostasis. SCN are synchronized with a 24hour cycle mainly by light, but they can also be regulated by a variety of nonphotic signals, such as growth factors, opioids, cytokines, or lipopolysaccharide (LPS), which act by inducing the JAK/STAT signaling pathway. STAT family proteins (i.e. signal transducers and activator of transcription) regulate many aspects of cellular physiology, from growth and differentiation to immune response. However, the JAK/STAT signaling pathway has not yet been studied in the SCN and the function of STAT proteins in the SCN has not yet been clarified.

In the first part of the thesis, we focused on localization of STAT3 and STAT5 proteins in the rat SCN and determination of rhythm in proteins and mRNA. Our experiments showed the daily rhythm in the levels of STAT3 protein in SCN astrocytes of rat with low but significant amplitude and with maximum in the morning. In addition, we revealed strong but nonrhythmic expression of STAT5A protein in astrocytes and STAT5B protein in nonastrocytic cells of SCN. It was also found that *Stat3* mRNA show, similarly to protein, circadian rhythm in SCN of rats with high levels during the day and low levels during the night, and therefore rhythmic expression of transcription factor STAT3 is controlled by circadian clocks.

In the second part of this thesis, we examined the effect of LPS (1 mg/kg) applied during the day or night on behavioral changes by monitoring the rhythm of locomotor activity in rats. The research shows that the recovery of locomotor activity rhythm took longer in animals that were injected with LPS during the night. We also studied immediate changes in the expression of the clock genes *Per1*, *Per2* and *Rev-erbα* and in the levels of phosphorylated kinase ERK1/2 and GSK3β in SCN in response to day or night stimulation with LPS. These factors are sensitive to external cues and fuction as the molecular entry into the circadian clockwork. Slight and transient changes were detected in the expression of selected clock genes and in the levels of kinases in tissue slices after LPS application compared to controls. We also looked at whether systemic application of LPS affects expression of *Stat3* mRNA. It was shown that LPS also triggers changes in the expression of *Stat3* in SCN depending on the time of its administration. We also focused on investigating the effect of LPS on activation of STAT3 protein. Acute administration of LPS during the day or night induced STAT3 phosphorylation on

tyrosine (Y705; pSTAT3(Y)) while STAT3 phosphorylation on serine (S727; pSTAT3(S)) was induced only with daily LPS administration. STAT3 phosphorylation on tyrosine (Y705) persisted elevated even after 24 hours of daily LPS administration, but decreased within 8 hours after LPS administration at night. Thus, the results of these experiments showed that systemic inflammatory stimulation with endotoxin LPS induces phosphorylation of STAT3 protein in SCN depending on the time of day.

In summary, our data point to the role of STAT3 in the circadian clock response to inflammatory reaction caused by LPS, providing further evidence of the interaction of the circadian system with the immune system. These results also confirm that the sensitivity of the organism to inflammatory stimuli depends on the time of day.

Keywords: circadian rhythm, lipopolysaccharide, STAT proteins, suprachiasmatic nuclei