I. ABSTRACT

In mammals, daily (circadian) rhythms in physiology and behavior are regulated by a central clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Individual neurons in the SCN contain an autonomous molecular oscillator composed of interlocked feedback loops of clock genes. The SCN coordinates rhythmical activity within subordinate clocks that are distributed in many different tissues. These local clocks are of a similar molecular makeup to the SCN and they are responsible for driving local, tissue-specific circadian programs based on rhythmic gene expression. The circadian rhythms need to be synchronized (entrained) by external cues. For the SCN, the primary synchronizer (Zeitgeber) is the light-dark cycle perceived through retina, while for the peripheral clocks, the strongest Zeitgeber is perhaps the feeding-fasting cycle.

The experimental part of this thesis is focused on describing the effect of photoperiod on the adult as well as developing central oscillator in the SCN. Moreover, the experiments were aimed to reveal ontogenesis of the molecular core oscillator in the SCN and of the peripheral oscillator in the liver of the rat.

Previous results from our laboratory have shown that light-induced and spontaneous expression of immediate early gene c-Fos, as well as rhythm in clock protein PER1 in the SCN are strongly influenced by the photoperiod. To further analyze the effect of photoperiod, we assessed the expression of clock genes Per1, Cry1, Bmal1 and Clock by in situ hybridization in the SCN of rats maintained either on long (LD 16:8) or short photoperiod (LD 8:16) and released into darkness on the day of the experiment. Under the long period, the interval of elevated Per1 mRNA lasted for a longer and that of elevated Bmal1 mRNA for a shorter time than under the short photoperiod. Under both photoperiods, the morning and the daytime Per1 and Cry1 mRNA rise as well as the morning Bmal1 mRNA decline were closely linked to the morning light onset. Amplitude of Per1, Cry1 and Bmal1 mRNA rhythms was larger under the short than under the long photoperiod. Also, under the short photoperiod, the daily Clock mRNA profile exhibited a significant rhythm. Altogether, our data indicate that the whole complex molecular clockwork in the rat SCN is photoperiod dependent and hence may differ according to the season of the year.

Our next work aimed towards the development of the molecular oscillations. It is known that the rhythmicity in the SCN develops prenatally. We analyzed the expression of clock genes Per1, Per2, Cry1, Bmal1 and Clock in the late prenatal and early postnatal ontogenesis, namely at embryonic day (E) 19 (3 days before birth), postnatal day (P) 3 and
P10. We detected mRNA of all studied clock genes at E19; however their expression was not rhythmic. The protein products PER1, PER2 and CRY1 were undetectable at E19. Early postnatally at P3, significant rhythms in Per1, Per2, Cry1 and Bmal1 expression were present and their phase relationships were similar to those of adult rats, though with lower amplitudes. The amplitude of Per1, Per2 and Bmal1 further increased at P10, while the level of Clock mRNA remains arrhythmic throughout the development. The experiment revealed gradual development of molecular oscillations, from undetectable rhythms in 19-day old embryos to highly developed rhythms 10 days after birth.

To elucidate, when exactly during the interval E19 – P3 the rhythms of clock gene expression start to develop, we analyzed the daily profiles of Per1, Per2, Cry1, Bmal1 and Clock mRNA and also the daily profile of AVP hnRNA (an indicator of transcription driven by BMAL1/CLOCK) in 20-day old fetuses, and in newborn rats at P1 and P2. At E20, no detectable rhythms of clock genes or AVP expression were present, with the possible exception of Per1, which showed a slightly indicated rhythm. At P1, weak rhythms in Per1, Per2 and Bmal1 were present. AVP transcription was also rhythmic and its amplitude already resembled that of adults. At P2, marked rhythms of Per1, Per2, Bmal1 and a forming rhythm of Cry1 expression were present. The expression of Clock stayed arrhythmic throughout the development. The data suggest gradual forming of SCN molecular oscillations mostly postnatally.

In our previous experiments, we showed that photoperiod affects the molecular oscillator in the adult SCN. To examine when and how does the photoperiod affect the SCN clockwork during postnatal development, we analyzed the expression of Per1, Per2, Cry1 and Bmal1 in 3-, 10- and 20-day old rats maintained under either a long photoperiod (LD 16:8) or a short photoperiod (LD 8:16) and released into darkness on the day of the experiment. The photoperiod significantly affected the expression profiles of Per1 and Per2 in 20- and 10- but not yet in 3-day old rats. Expression of Cry1 was affected only in 20-day old pups and expression of Bmal1 even later. The data suggest gradual formation of photoperiodic entrainment of the SCN molecular clockwork, from no photoperiodic effect at P3 to partially developed photoperiodic entrainment mechanism at P20. The developmental interval when photoperiod begins to entrain the SCN clockwork completely might thus occur around the time of weaning.

Finally, there are almost none information about the development of circadian rhythmicity in peripheral organs, i.e., outside the SCN. Therefore we analyzed the expression of clock genes Per1, Per2, Rev-Erba, Cry1, Bmal1 and Clock in a well-studied peripheral
organ, rat liver, at P2, P10, P20 and in the liver of adult rats by real-time RT-PCR. At P2, no clear circadian rhythms in expression of clock genes were detectable and the overall daily expression was very low as compared with that in adult rats. At P10, only rhythms in Per1 and Rev-Erbα, but not in expression of other genes were present. At P20, clear circadian rhythms in Per1, Per2, Rev-Erbα and Bmal1, but not yet in Cry1 and Clock were detected. The overall daily expression of Per1, Bmal1 and Clock roughly attained the adult level while that of Per2, Rev-Erbα and Cry1 was still lower. The phase of the rhythms shifted between P10 and P20 and further between P20 and the adult stage, reflecting the change of feeding behavior during the weaning period. The data indicate that the postnatal development of the molecular clockwork in the rat liver proceeds gradually and is not completed within 20 days after birth.