Abstract

All mammals exhibit daily rhythms which persist in non-periodic environment with a period close to 24h. These rhythms are entrained to the 24h day mostly by the light-dark cycle. These circadian rhythms are controlled by a clock (pacemaker) located in the suprachiasmatic nuclei (SCN) of the hypothalamus. In the rat, the circadian clock within the SCN develops gradually from prenatal to postnatal period and is supposed to be synchronized mainly by maternal signals. However, the rat SCN is sensitive to light immediately after birth. **The aim** of the present work was to investigate the mechanism of entrainment of the circadian clock within the rat SCN during fetal and early postnatal development. The specific questions were whether and when the immature fetal and neonatal molecular SCN clocks can be reset by maternal cues, and whether and when the external light-dark cycle (LD) can affect the developing circadian rhythms. The role of light-dark cycle in the development of the photoperiodic entrainment during early postnatal period was also examined. **Experiment no. (1):** Pregnant rats were maintained under a light – dark regime with 12 h of light and 12 h of darkness (LD12:12). At gestational day 20 (E20), the fetuses were sampled throughout the day under either LD12:12 or constant darkness (DD). The daily profile of *c-fos* gene expression was determined by *in situ* hybridization. **Experiment no. (2):** Pregnant rats were maintained under LD 12:12 until the day of delivery, then released into DD and the pups were sampled in DD at postnatal day 3 (P) 3. The daily profiles of clock gene expression within the SCN of pups maintained in DD were compared with those of pups kept under LD12:12 (Sládek, *et al*, 2004). **Experiment (3)**: Pregnant rats were maintained under a long photoperiod (LD 16:8) and their pups were sampled under the same LD regime at P3 and P10. The daily profiles of clock gene expression within the SCN of pups maintained under LD16:8 were compared with those of pups sampled under DD (Kováčiková *et al*, 2005). **Experiment (4):** Pregnant rats were maintained under LD12:12. They were exposed to a 6 h delay of the dark period at different stages of the fetal SCN development and, thereafter, they were released into constant darkness. Adult male rats maintained under the same LD regime were exposed to an identical shifting procedure. Daily rhythms in spontaneous *c-fos, Avp*, *Per1* and *Per2* expression were examined within the adult and newborn rat SCN. **Result (1)**: A clear rhythm of *c-fos* gene expression was detected at E20. **Result (2)**: In pups at P3, significant rhythms in *Per1, Per2*,

Cry1 and *Bmal1* expression were detected under DD. The phase of the rhythms in clock gene expression were not different under LD and DD conditions, however, slight differences in the time of the rise and the decline *of Per1*, *Per2* and *Cry1* mRNA levels between the pups maintained under DD and those maintained in LD were detected. **Result (3**): In 10-day-old pups maintained under LD16:8, presence of the LD cycle induced an advance of the rise in *Per1*, *Per2* and *Bmal1*, expression as compared to pups released into DD. The effect on *Cry1* expression was only suggested. However, the presence of the LD cycle at P3 induced only slight advance of the rise in *Per1* and *Per2* mRNA, but not of *Cry1* and *Bmal1* mRNA. **Result (4)**: Exposure of adult rats to a 6-h phase delay of the dark period (shifting procedure) induced a significant phase delay of locomotor activity within 3 days after the phase shift as well as a delay in the rhythms of *c-fos* and *Avp* expression within three days and *Per1* and *Per2* expression within five days after the shift. Exposure of pregnant rats to the shifting procedure at E18, but not at E20, phase delayed the rhythm in *c-fos* and *Avp* expression in the SCN of newborn pups at P0-1. The shifting procedure at E20 did, however, induce a phase delay of *Per1* and *Per2* expression rhythms at P3 and P6. Hence, five days were necessary for phase-shifting the pups' SCN clock by maternal cues, while only three days were necessary for phase-shifting the maternal SCN by photic cues. From the present studies we can **conclude** that: **(1)** Expression of *c-fos* gene exhibits circadian rhythmicity within the SCN at E20, which indicates a significant rhythm in the neuronal activity during the fetal SCN development. **(2)** Absence of the LD cycle does not affect the phase of the developing rhythms in expression of clock genes within the SCN at P3. Therefore, the phase of the developing circadian clock is primarily set by maternal cues. **(3)** At P10, presence of the LD cycle modulates the photoperiodic entrainment of the rhythm in clock gene expression, namely the *Per1*, *Per2* and *Bmal1* mRNA rhythms in pups maintained under a long photoperiod. At P3, the modulation was only marginal. Thus, presence of the LD cycle facilitates entrainment to the long photoperiod during early postnatal development. **(4)** The SCN clock is capable of significant phase shifts at fetal developmental stages when no or very faint molecular oscillations can be detected. This finding suggests that maternal cues are the most important cues for entrainment of the developing clock during prenatal and early postnatal development.