BRAIN RESEARCH 1073–1074 (2006) 348–358

available at www.sciencedirect.com

SCIENCE DIRECT®

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Temporal and spatial distribution of immunoreactive PER1 and PER2 proteins in the suprachiasmatic nucleus and peri-suprachiasmatic region of the diurnal grass rat (*Arvicanthis niloticus*)

Chidambaram Ramanathan[a,b], *Antonio A. Nunez*[a,b], *Gladys S. Martinez*[b], *Michael D. Schwartz*[a], *Laura Smale*[a,b,*]

[a]*Neuroscience Program, 108 Giltner Hall, Michigan State University, East Lansing, MI 48824, USA*
[b]*Department of Psychology, Michigan State University, East Lansing, MI 48843, USA*

ARTICLE INFO

Article history:
Accepted 16 November 2005
Available online 20 January 2006

Keywords:
Diurnal
Clock
Circadian
Peri-SCN
Lower subparaventricular zone
Suprachiasmatic nucleus

Abbreviations:
LD, light/dark cycle
DD, constant darkness
-ir, immunoreactive
ZT, Zeitgeber time
SCN, suprachiasmatic nucleus
pSCN, peri-suprachiasmatic region
LSPV, lower subparaventricular zone
sPVZ, subparaventricular zone
PVN, paraventricular nucleus

ABSTRACT

The suprachiasmatic nucleus (SCN) of the hypothalamus contains the primary circadian pacemaker in both diurnal and nocturnal mammals. The lower subparaventricular zone (LSPV) immediately dorsal to the SCN may also play an important role in the regulation of circadian rhythms. The SCN contains a multitude of oscillator cells that generate circadian rhythms through transcriptional/translational feedback loops involving a set of clock genes including *per1* and *per2*. Little is known about the temporal and spatial features of the proteins encoded by these genes in day-active mammals. The first objective of this study was to characterize the expression of PER1 and PER2 in the SCN of a diurnal rodent, the unstriped Nile grass rat (*Arvicanthis niloticus*). The second objective was to evaluate the hypothesis that a molecular clock could exist in the LSPV, where endogenous rhythms in Fos expression are seen in grass rats but not in laboratory rats. Animals were kept on a 12:12 light/dark cycle and perfused at 4-h intervals, and their brains were processed for immunohistochemical detection of PER1 and PER2. Both proteins were seen in the SCN where they peaked early in the dark phase, providing further evidence that the differences between diurnal and nocturnal patterns of behavior emerge from mechanisms lying downstream from the pacemaker within the SCN. Rhythmic expression of PER1 and PER2 was also seen in the LSPV providing support for the hypothesis that this region might participate in circadian time keeping in the diurnal grass rat. In addition, rhythms were seen lateral to the LSPV and the SCN. Results of this study are discussed in light of similarities and differences in the circadian time-keeping systems of day- and night-active animals.

* *Corresponding author*. Neuroscience Program, 108 Giltner Hall, Michigan State University, East Lansing, MI 48824, USA. Fax: +1 517 432 2744.
E-mail address: smale@msu.edu (L. Smale).

0006-8993/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.11.082

1. Introduction

The suprachiasmatic nucleus (SCN) contains the primary circadian clock and generates rhythms in physiology and behavior in both day- and night-active mammals (Hastings and Maywood, 2000; Smale et al., 2003). The SCN is a heterogeneous structure with distinct subpopulations of cells playing varying roles in the coordination of circadian rhythms. Some characteristics of SCN function, such as rhythms in uptake of 2-deoxyglucose and its control of pineal melatonin production, are fundamentally similar across species (Garidou et al., 2002; Schwartz et al., 1983). However, species vary considerably with respect to most behavioral and physiological rhythms generated by the clock. For example, rhythms in locomotor activity and sleep are reversed in diurnal and nocturnal species, as are rhythms in copulatory behavior and neuroendocrine events associated with it (McElhinny et al., 1999; Smale et al., 2003). Relatively little is known about the mechanisms responsible for either the similarities or the differences in circadian time-keeping systems of day- and night-active animals.

The data available on the molecular mechanisms responsible for generation of circadian rhythms in mice provide an opportunity to directly evaluate the hypothesis that the clock is the same in nocturnal and diurnal species (Reppert and Weaver, 2001). The clockwork is cell autonomous and comprises autoregulatory, transcriptional/translational feedback loops (Dunlap, 1999; Welsh et al., 1995). The proteins CLOCK and BMAL1 provide positive transcriptional drives to the loop. Negative feedback is mediated by the protein products of the Period (*per3*) and cryptochrome genes (*cry-2*), PER and CRY, respectively. The circadian rhythms of PER peak in the late daytime, approximately 4–6 h after the peak in their mRNA rhythms (Dunlap, 1999).

The expression of Per genes and their proteins appears to be an entry point for photic entrainment in nocturnal mammals. Brief exposure to light during subjective night results in a large and rapid induction of *mPer1* and *mPer2* expression (Albrecht et al., 2001; Shearman et al., 2000; Shigeyoshi et al., 1997; Takumi et al., 1998; Zylka et al., 1998). Furthermore, studies using antisense oligonucleotides have shown that *per1* and *per2* are likely to play a role in entrainment of the clock to light cues (Akiyama et al., 1999; Shigeyoshi et al., 1997; Wakamatsu et al., 2001). These findings suggest that *per1* and *per2* may be important determinants of circadian function in mammals.

Data available on the molecular mechanisms that underlie the functioning of the circadian clock in day-active rodents are relatively limited. Circadian rhythms in *per1* and *per2* mRNA in the whole SCN have been described in ground squirrels (*Spermophilus tridecemlineatus*) (Mrosovsky et al., 2001), *Arvicanthis ansorgei* (Caldelas et al., 2003) sheep (Lincoln et al., 2002), and *Arvicanthis niloticus* (Lambert et al., 2005). The rhythms in these species are similar to those seen in laboratory rats (Yan et al., 1999), mice (Hastings et al., 1999), and hamsters (Nuesslein-Hildesheim et al., 2000). However, there is currently no information on temporal or spatial characteristics of clock proteins in day-active mammals, and it remains to be determined if they are coupled to their mRNA in the same manner as in nocturnal rodents.

The hypothalamic region immediately dorsal to the SCN, which we refer to as the lower subparaventricular zone (LSPV), may also be involved in the regulation of circadian rhythms (Schwartz et al., 2004; Smale et al., 2003). The subparaventricular zone (sPVZ) as a whole, extending from the SCN to the PVN, is the major target of the SCN (Watts et al., 1987). The sPVZ in turn projects to many of the same regions as does the SCN, as well as to the SCN itself. Hence, it is in a position that may allow it to play a modulatory role, further processing temporal information originating in the SCN (Vrang et al., 2003; Watts et al., 1987). The LSPV receives inputs from many hypothalamic and preoptic areas (Moga and Moore, 1997), as well as from the retina and the SCN (Smale and Boverhof, 1999). This region exhibits a rhythm in Fos expression that differs dramatically in nocturnal lab rats compared to the diurnal species *A. niloticus* (hereafter referred as the grass rat) (Nunez et al., 1999; Schwartz et al., 2004). The number of Fos-immunoreactive cells in the LSPV of laboratory rats kept in a 12:12 LD cycle has a peak at Zeitgeber time (ZT) 01 (ZT 0—light on) and is relatively low from ZT 05 through ZT 23. By contrast, in grass rat housed in the same conditions, Fos in the LSPV rises sharply between ZT 13 and ZT 17 and remains high through ZT 1; grass rats are similar to laboratory rats in that Fos then drops and remains low from ZT 5 through ZT 13. Another difference between these species is that Fos rhythms in the LSPV persist in grass rats, but not in laboratory rat, after extended periods in constant darkness (Schwartz et al., 2004); under these conditions, the numbers of calbindin-containing cells also change as a function of time in the LSPV of grass rats but not laboratory rats. Endogenous rhythms in Fos and calbindin in the LSPV could be produced by signals from the SCN and/or from oscillator mechanisms intrinsic to the LSPV. The latter possibility predicts that elements of the molecular clock such as PER1 and PER2 should oscillate in the LSPV.

In the present study, we used immunohistochemistry to characterize the daily expression and distribution of PER1 and PER2 in and around the SCN of grass rats. Our first objective was to examine rhythms of PER1 and PER2 expression within the SCN in order to determine if the spatial and temporal patterns are the same as those seen in nocturnal animals. Our second objective was to examine the daily expression of PER1 and PER2 in the LSPV in order to evaluate the hypothesis that the endogenous rhythm in Fos in this region might be produced by a molecular oscillator intrinsic to it.

A third objective emerged from the observations that, in some animals, PER proteins were present in areas adjacent to the lateral boundary of the SCN, as well as lateral to the LSPV. To determine if this variability was associated with time of day, we extended the analysis of the effects of ZT on PER proteins to include these regions (as described below).

2. Results

2.1. SCN

The number of PER1 and PER2 immunoreactive nuclei in the SCN exhibited a clear diurnal rhythm (see Fig. 1, top two