Concurrent decrease of vasopressin and protein kinase Cα immunoreactivity during the light phase in the vole suprachiasmatic nucleus

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Abstract

Vasopressin (AVP) is a major neuropeptide in the suprachiasmatic nucleus, the mammalian hypothalamic circadian pacemaker. Protein kinase Cα is a putatively coupled intracellular messenger. Mean numbers of AVP- and protein kinase Cα-immunoreactive neurons were determined in the suprachiasmatic nucleus of common voles, entrained to a 12:12 h light-dark (LD) cycle, at the beginning of the light period (zeitgeber time zero) and 6 h later (zeitgeber time six). At zeitgeber time zero, mean numbers of AVP- and protein kinase Cα-immunoreactive neurons were 2194 and 9897, respectively. Both numbers decreased significantly with about 40% at zeitgeber time six. This concurrent decrease was most pronounced in the dorsomedial aspect of the suprachiasmatic nucleus. These findings are consistent with the findings of a peak of AVP release in rats during the early light phase. © 1998 Published by Elsevier Science Ireland Ltd. All rights reserved

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The neuropeptide vasopressin (AVP) is produced abundantly in the main mammalian circadian pacemaker, the suprachiasmatic nucleus (SCN). AVP-immunoreactive (AVP-ir) cells are predominantly localized in the dorsomedial zone of the SCN [20]. AVP-ir neurons form a complex and dense network, contacting AVP-immunopositive neurons as well as other neurons synthetically. The neuronal AVP circuit constitutes an important output system: AVP-labeled fibers arising from the SCN project to other hypothalamic regions [7], while considerable amounts of AVP are secreted in the third ventricle in a circadian rhythmic fashion [15]. There are good indications that AVP produced in the SCN is involved in the regulation or modulation of behavioral programs. The neuronal AVP-ir population in the SCN correlates with expression of circadian rhythmicity in common voles [6], and AVP-ir neurons in the mouse SCN have been implicated in relaying timing information of circadian wheel running activity [2]. Within the SCN, AVP seems to play a role in circadian activity cycles [11], and it has been shown that AVP exerts excitatory effects on SCN neurons in vitro via the V1a receptors [8]. Activation of these receptors leads to membrane related phosphoinositol turnover and the subsequent activation of protein kinase C (PKC) [16]. PKCs are intracellular messengers, some of them dependent on calcium, and involved in a variety of processes regulating cellular excitability. Localization of the four Ca2+-dependent PKC-isozymes in the SCN has been established and they occur in the rat, mouse, rabbit and the diurnal Kusu rat, Arvicanthis niloticus, in different rates of expression [3,18]. PKCα is the isozyme that, immunocytochemically, is most abundant in the SCN of these species. PKCα is highly expressed in mouse AVP-ir SCN neurons and PKCα-ir SCN neurons are densely innervated by AVP-positive terminals [18]. The role of PKCα in the circadian pacemaker mechanism is currently unknown, but its activation could contribute to a wide variety of intracellular processes.

The common vole (Microtus arvalis) is characterized by rigid ultradian (2–4 h) rhythms in behavior [5], while the
circadian component is less stable. The large interindividual variability in expression of circadian rhythmicity under constant conditions and the relationship with the number of AVP-ir cells in the SCN [6], allows the study of the circadian output system at clock level in more detail. The rat SCN shows a peak in content and subsequent release of AVP in the early hours of the light period, while there is a decrease of AVP-ir SCN neurons measured by optical densities [13,15,17]. If it is true that PKCa is the primary PKC isoform involved in AVP signal transduction, we expect a concurrent decrease in the total number of AVP and PKCa-ir cells in the SCN during the early morning in voles with behavioral circadian rhythmicity. Therefore, the aim of this study is to investigate possible changes in AVP- and PKCa-ir cells in entrained voles, housed under light-dark (LD) conditions, and sacrificed at the immediate beginning of the light period (zeitgeber time 0, ZT 0) or 6 h later (ZT 6).

Fourteen adult male voles (3 months old), showing strong expression of circadian rhythmicity in constant conditions, were used. The animals were taken from a colony maintained in Haren that is based on individuals trapped in the Lauwersmeer (53°20′N:6°16′E). Animals were individually housed in lucite cages (25 × 25 × 30 cm) equipped with a running wheel and connected with a lucite nest box (17 × 11 × 13 cm) provided with wood shavings. Food (Hopefarms mouse pellets) and water were available ad libitum. Temperature (20 ± 1°C) and humidity (70%) were kept constant during the experiment. Animals were exposed to a 12:12 h LD cycle (lights on at 0600 h, 350 lux) for 2 weeks. After this, the animals stayed 4 weeks in constant low light conditions (LL: 2 lux), while their wheel-running activity was recorded. Subsequently, they were exposed to a 2 week LD treatment (12:12 h, lights on at 0600 h, 350 lux). Seven voles were then killed at ZT 0 while the other seven were killed at ZT 6 under deep anesthesia with 0.75 ml 0.2% sodium pentobarbital. The brains were quickly removed from the skull and subjected to immersion fixation in 4% paraformaldehyde in 0.1 M phosphate-buffer for 6 h. The brains were rinsed with 0.01 M phosphate-buffer + 0.9% saline (PBS) and cryo-protected overnight in 30% buffered sucrose at 4°C. At the level of the hypothalamus, frontal sections (25 μm) were cut with a freezing microtome close to the optic chiasm, a group of intensely stained PKCα-ir neurons (Fig. 1C) and no obvious difference was seen between dorsal, medial and ventral parts of the SCN in staining intensity and numerical density of PKCa-ir neurons. However at ZT 6, PKCa-immunoreactivity in the SCN differed considerably. The dorsolateral SCN showed a reduced PKCa-immunoreactivity, whereas in the ventromedial portion, close to the optic chiasm, a group of intensely stained PKCa-ir neurons was seen (Fig. 1D). The mean number of PKCa-ir neurons for the SCN at ZT 0 was 2194 (SEM 156) and 1270 (SEM 161) at ZT 6. The difference between the mean numbers of AVP-ir neurons at the two ZT's was significant (Fig. 2, P = 0.002, Student’s t-test, two tailed). Few scattered AVP-ir neurons were present just outside the SCN (Fig. 1B) at both ZTs.

At ZT 0 the SCN was delineated by numerous tightly packed PKCa-ir neurons (Fig. 1C), and no obvious difference was seen between dorsal, medial and ventral parts of the SCN in staining intensity and numerical density of PKCa-ir neurons. However at ZT 6, PKCa-immunoreactivity in the SCN differed considerably. The dorsolateral SCN showed a reduced PKCa-immunoreactivity, whereas in the ventromedial portion, close to the optic chiasm, a group of intensely stained PKCa-ir neurons was seen (Fig. 1D). The mean number of PKCa-ir neurons for the SCN at ZT 0 was 9897 (SEM 1030) and 6327 (SEM 826) at ZT 6. The difference between the mean numbers was significant (Fig. 2, P = 0.005, Student’s t-test, two tailed). For each animal an AVP/PKCa ratio was calculated in order to examine if the decrease in the number of immunochemically stained neurons for both antigens was proportional. The average ratio did not differ significantly between ZT 0 and 6.
(0.24 ± 0.034) and ZT 6 (0.23 ± 0.045; Student’s t-test, two tailed, P > 0.05).

This study describes significant differences in the number of AVP and PKCα-ir neurons in the SCN at two distinct time points. Several studies addressed AVP localization and content in the rat SCN as well as release at different circadian times. Immunocytochemically, Okamura [13] showed a decrease in optical density of AVP in SCN neurons from ZT 2 to ZT 8. Micropunch studies [17,22] revealed an increase in AVP content in the early morning, which corroborates with AVP release patterns in rats [15] and voles (preliminary data on AVP release from vole SCN in vitro; Gerkema et al. in preparation). Circadian rhythmic voles show also a clear decrease in the number of AVP-ir SCN neurons between ZT 0 and ZT 6. We suggest that AVP-ir neurons release AVP either synaptically or non-synaptically at their varicosities during this 6 h time interval. The decrease in AVP-ir cell bodies is the consequence of transport of AVP out of the soma to the varicosities and the terminals. The reflection of plasticity in the AVP system in the vole SCN, which can be detected by immunocytochemistry, allows the study of the functional role of AVP within the circadian clock or its output system: AVP immunoreactivity in the SCN correlates strongly with variation of expression of circadian rhythmicity [6], and, as shown in this paper, changes with the circadian phase of the pacemaker. Moreover, loss of circadian rhythmicity correlates with dramatic reduction of AVP-ir neurons in the aging vole SCN [19].

PKCα labeling in the vole SCN differed from that in rat, mouse, rabbit and the diurnal Kusu rat, Arvicomys niloticus [3,18]. In these species, PKCα-immunoreactivity was less uniformly distributed and found both in the dorsomedial and ventrolateral aspects of the SCN. However, PKCα observations were restricted to one time point in the early light period (ZT 2–3). It would therefore be of interest to determine whether comparison of different time points results in different patterns of PKCα-labeling in these species as observed here in voles.

Previously, it has been demonstrated that PKC mRNAs show circadian fluctuations [4]. Furthermore, McArthur et al. [10] found that PKCs can be activated at dusk and dawn by melatonin, which alters functioning of SCN neurons at ZT 23–0 and ZT 10–14. The function of PKCs within SCN neurons and their involvement in the intracellular signal transduction at clock level is currently under investigation. The decline in AVP-immunoreactivity in the SCN at ZT 6 as compared to ZT 0, and the concurrent decrease of PKCα-ir neurons dorsally supports the idea of direct coupling between AVP within the SCN and intracellular messengers linked to the V1a receptor. Stimulation of this receptor leads to intracellular PKCα activation, and it is known that acti-
PKCs are also known to play an important role in the stabilization of long-term potentiation (LTP), a phenomenon of long-lasting enhancement of neuronal activity after repetitive synaptic stimulation. Circadian phase dependent LTP formation has been described in the SCN of rats after tetanic optic nerve stimulation [12], suggesting that LTP may be related to adaptation mechanisms to photic stimulation. Our data are consistent with this assumption. Perhaps PKCs and LTP are linked in the ventral part of the SCN, while the low density of AVP terminals in the ventral subregion suggests that, possibly, another (neuropeptide) system is involved in the activation of PKCα in the SCN. This idea is supported by the finding that neuropeptide Y can phase shift the clock in vitro via PKC [1]. Besides NPY, serotonin is also a likely candidate, based on the temporal sensitivity of the SCN with respect to serotonin and the spatial distribution of serotonin fibers in the SCN [9].

In summary, our results point towards a proportional turnover of AVP and its putative effector system PKCα, selectively in the SCN, depending on the time of day. These findings offer the possibility for further study of the dynamics of AVP-PKC signal transduction in relation to the circadian clock function of the SCN. For example, AVP agonist and antagonist infusions into the SCN at differential ZTs could reveal the causal relation between AVP signal transduction and PKCα activation.

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