Being circadian or not: vasopressin release in cultured SCN mirrors behavior in adult voles

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We studied vasopressin (AVP) release patterns from organotypic suprachiasmatic nucleus (SCN) cultures obtained from circadian rhythmic and non-rhythmic voles. All eight SCN cultures made from non-rhythmic voles did not produce any circadian pattern in AVP release, while five out of six SCN cultures of rhythmic voles produced significant (circadian) peak values. The total amount of AVP released was 2-fold higher in SCN cultures from rhythmic vole. These data confirm our previously formulated AVP release deficit hypothesis for non-rhythmic voles, and suggest that AVP in the vole SCN plays an important role in mediating output of its circadian clock, regulating circadian organization of locomotor behavior. NeuroReport 11:3555–3558 © 2000 Lippincott Williams & Wilkins.

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INTRODUCTION

The suprachiasmatic nucleus (SCN) in the mammalian hypothalamus has been identified as a locus essential for the generation of circadian oscillations in physiology and behavior [1–3]. How and which signals from the SCN lead to circadian behavior is still an unanswered question. In this respect arginine-vasopressin (AVP), first localized in the SCN by Vandesande et al. [4], has received much attention. Circadian rhythmicity has been observed in AVP release from the SCN into surrounding hypothalamic tissue and third ventricle [5,6]. In vitro, AVP is released in a circadian fashion from cultured rat SCN neurons [7] from acute rat SCN slices [8], and from organotypic SCN slices obtained from rat pups [9,10] and vole pups [11].

Evidence exists that AVP in the rodent SCN is involved in regulating timing programs of locomotor behavior [12–14]. In the common vole, AVP in the SCN is associated with circadian locomotor behavior in vivo [13], as well as in vitro [15]. Non-rhythmic voles exhibited high numbers of AVP immunoreactive neurons in the SCN, and we hypothesized that release of AVP from such neurons is hampered (the AVP release deficit hypothesis) [13]. In this study we test this hypothesis, and question whether high AVP immunoreactivity in the vole SCN can be the result of AVP accumulation due to diminished release of the neuropeptide. Recently we have demonstrated that it is possible to culture organotypic SCN slices of adult voles [15,16]. Using this technique, we measure AVP release patterns from organotypic SCN slices of adult voles with known expression of circadian locomotor behavior.

MATERIALS AND METHODS

Fourteen adult male common voles (Microtus arvalis, 2 months of age, ~25 g) and six male rat pups (Rattus norvegicus, 7 days of age) were used in the study. Voles were obtained from our colony maintained in Haren which is based on individuals trapped in the Lauwersmeer (53°20′N;6°16′E). Voles were individually housed in transparent cages (25 × 25 × 30 cm) equipped with a running wheel and connected with a transparent nest box (17 × 11 × 13 cm) provided with wood shavings. Food (Hopefarms mouse pellets, Woerden, The Netherlands) and water were available ad lib. Temperature (20 ± 1°C) and humidity (70%) were kept constant during the experiment. Wistar rat pups were obtained from the breeding colony of the Department of Animal Physiology (University of Groningen, Haren, The Netherlands). Experiments were approved by the Animal Experimental Committee of the University of Groningen (DEC nr. 2091).

Voles were exposed to a 12:12 h light:dark (LD) cycle (lights on at 6:00 h (ZT0); 350 lux) for 2 weeks. After this, the animals stayed for 4 weeks in constant low light (LL) conditions (2 ± 1 lux). Voles were assigned to two behavioral categories: rhythmic (n = 6) and non-rhythmic (n = 8), on the basis of visual inspection of their individual wheel running activity pattern. Wheel running activity was recorded via a microswitch connected with a PC-based event
RESULTS

Examples of actograms and $\chi^2$ periodogram analyses of a rhythmic and a non-rhythmic vole are depicted in Fig. 1. The mean ($\pm$ s.e.m.) $\Delta Qp$ values of $\chi^2$ periodogram analyses were 1334$s\pm$68 ($n=6$) and 145$\pm$70 ($n=8$) for the rhythmic voles and non-rhythmic voles, respectively. A significant difference in mean $\Delta Qp$ values and therefore expression of circadian rhythmicity was found between the two groups of voles ($p<0.01$; Mann-Whitney U test). Numerous AVP immunoreactive nerve terminals and neurons were found in all SCN cultures, corroborating earlier findings [15]. AVP release patterns differed between cultures obtained from rhythmic and non-rhythmic voles (Fig. 2). Five out of six SCN cultures obtained from circadian rhythmic voles showed significant peak values (relative AVP values did exceed 3 s.d. from average) in AVP release during the 50 h sampling. The incidence of these peaks differed significantly between rhythmic and non-rhythmic voles (Fisher exact test, $p<0.05$). Time intervals between peaks varied from 18 to with a mean value of 20 h (Table 1). One culture obtained from behaviorally rhythmic voles did not show any significant peak value in AVP release. Taken together, however, post-hoc analysis of autocorrelation results obtained from the six cultures from rhythmic voles, revealed a significant variation with time (Table 1; Friedman repeated measures ANOVA, $p<0.05$).

All SCN cultures made from non-rhythmic voles failed to show significant peak values of AVP release. No rhythmic pattern in AVP release emerged from the SCN slices of the eight non-rhythmic voles, as revealed by the absence of significant outcomes of autocorrelation and confirmed by subsequent post-hoc analysis (Friedman repeated measures ANOVA, $p>0.05$). Five of six rat pup cultures showed significant peak values in AVP release over 50 h with an average period of 22 h. Autocorrelation and post-hoc analysis (Friedman repeated measures ANOVA, $p<0.05$) of the mean relative values of all six cultures revealed a significant circadian variation in relative AVP release (Table 1).

The total amount of AVP release for the rhythmic and non-rhythmic vole cultures was obtained by summation of all AVP sample concentrations for both behavioral categories. Interestingly, absolute levels of AVP production in the cultures, notwithstanding variability of morphological features such as size and number of cell layers, were about 2-fold higher in SCN cultures from rhythmic voles compared to AVP release in cultures made from non-rhythmic voles (Mann-Whitney U test, $p<0.005$, Table 1). Levels of AVP production in the cultures from rhythmic voles did not differ from those obtained in rat pup cultures (Mann-Whitney U test, $p>0.05$). AVP release patterns from rat cultures were similar to those from cultures of rhythmic voles, although with a period that tended to be slightly shorter, but was not significantly different (22 h; Mann-Whitney U test, $p>0.05$; Table 1). Rat cultures differed in the incidence of significant peaks in AVP release from non-rhythmic voles (Fisher exact test, $p<0.005$), but not from rhythmic voles (Table 1).

DISCUSSION

A striking difference has been found in AVP regulation within the SCN between behaviorally rhythmic and non-rhythmic voles. Our data in this study demonstrate a strong correlation between behavioral rhythmicity and in vitro AVP release, which supports the AVP release deficit.
hypothesis [13]. Previously we successfully cultured the SCN of adult rodents in an organotypic slice preparation [16] and we found a difference in number of AVP immunoreactive neurons in the SCN \textit{in vitro} between non-rhythmic and rhythmic voles [15]. Now, we show that SCN cultures obtained from such non-rhythmic voles lack AVP release peaks and exhibit a constant and low level of AVP release \textit{in vitro}.

Fig. 1. Examples of actograms of a rhythmic vole (a) and a non-rhythmic vole (b) and the result of $\chi^2$ periodogram analyses of the same rhythmic vole (c) and non-rhythmic vole (d). Tilted line in (a) indicates the precision of activity onset in rhythmic voles, while in (b) no activity onset could be determined. In (c) and (d) the period length is indicated in hours.

Fig. 2. Examples of AVP release from organotypic slice cultures obtained from (a) a rhythmic vole, (b) a non-rhythmic vole, and (c) a rat pup. Sample points were 2 h apart. Percentages were calculated by dividing values by the mean value over 50 h. Circadian period in (a) is 20 h and in (c) 22 h.
AVP content and release in the rat SCN has been reported to show a circadian oscillation under constant light conditions in vivo and in vitro [6,7,9,10,20,21]. It should be noted, however, that in vitro AVP release patterns consistently deviate from 24 h in a species-specific way. This is true for rat pups [9], vole pups [11] and adult voles (this study). Results obtained from non-rhythmic voles, however, contrast strongly with these findings. Some AVP release still takes place in the SCN of non-rhythmic voles, but without circadian modulation and in significantly lower amounts compared with rhythmic voles. In retrospect, an AVP release deficit may already be present in vole pups, since one-third of organotypic SCN cultures of these pups failed to show circadian fluctuations [11].

Whether the AVP release deficit in the SCN of non-rhythmic voles is the cause of non-rhythmic locomotor behavior remains to be investigated. AVP-deficient rats still exhibit circadian rhythmicity [22], indicating that the AVP release deficit hypothesis in voles cannot be generalized widely across rodents. Furthermore, the same deviation in release mechanism which impairs AVP release in voles could also impair other neurochemical output of the SCN (such as monoamines, or other neuropeptides), critically involved in the organization of vole circadian behavioral patterns. However, it might be speculated that a deficit in the core molecular mechanism, regulating rhythmic output from the SCN like in Clock/Clock mutant mice causes such a low AVP release, since recent studies indicate that rhythmicity of AVP mRNA in the SCN is apparently driven by the core circadian clock loop [23,24]. One argument against this idea is that acute SCN slices from non-rhythmic voles show circadian patterns in multi-unit activity as seen in slices obtained from circadian rhythmic voles [25].

In any case, future studies should reveal what mechanism in this AVP system is disturbed or downregulated. It would therefore be useful to know whether expression of the AVP mRNA is altered in non-rhythmic voles, and whether the AVP immunoreactivity in SCN neurons reflects different stages of AVP processing in the cell. Such as, for example, cleavage from neurophysin. Perhaps also, rapid transport of AVP down to the axon is slowed down. Moreover, our results also provide new insight in the interpretation of high levels of neuropeptide-immunostaining in the SCN. Obviously such high immunostaining levels are indicative for low release rather than a continuous highly productive peptidergic system.

**CONCLUSION**

AVP release patterns obtained in organotypic SCN cultures of adult common voles confirmed the AVP release deficit hypothesis for non-rhythmic voles [13]. Moreover, AVP release from the isolated pacemaker in vitro does reveal the behavioral patterns in the intact animal. AVP in the SCN might be highly important in regulating timing of locomotion in voles. Clearly the common vole represents a unique model to unravel the connection between the circadian pacemaker and timing of locomotor behavior.

**REFERENCES**


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