Lack of circadian patterns in vasoactive intestinal polypeptide release and variability in vasopressin release in vole suprachiasmatic nuclei in vitro

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Abstract

Organotypic hypothalamic cultures of neonatal rats comprising the suprachiasmatic nuclei (SCN) produce stable 20 h release patterns of vasoactive intestinal polypeptide (VIP) and arginine-vasopressin (AVP). Compared with rats, voles show variably expressed circadian activity patterns. In this study we measured neuropeptidergic release patterns in organotypic SCN cultures of neonatal common voles (Microtus arvalis, n = 6). Slices were prepared at postnatal day 6. After 14 days of incubation, 2 h samples of medium were collected during 50 h. None of the vole SCN slices showed a circadian modulation in VIP release. Peaks in AVP occurred, 20 h apart from each other, in four of six vole SCN slices. These findings contrast with the concurrent release patterns of VIP and AVP in rat SCN slices. The results suggest an independent role of both neuropeptides in the oscillatory output pathways of the circadian pacemaker in the common vole.

Keywords: Circadian; Ultradian; Vasopressin; Vasoactive intestinal polypeptide; Vole; Suprachiasmatic nucleus; Organotypic

Organotypic hypothalamic slice cultures from 6-day-old rat pups are capable of regular release of neuropeptides-like vasoactive intestinal polypeptide (VIP) and arginine-vasopressin (AVP) [10,11]. These slices contain the suprachiasmatic nuclei (SCN), the primary locus of the mammalian circadian timing system [8,9]. If suprachiasmatic cultures are prepared from neonatal rat pups, release patterns of both VIP and AVP are already organized in a circadian fashion. Rats are known for a strictly circadian organization of physiology and behavior.

On the contrary, some other rodent species, like the microtine herbivore voles show a large interindividual variability in the expression of circadian rhythmicity in behavior [2]. Moreover, differences that occur in immunoreactivity of AVP-SCN cells are negatively correlated with the degree of circadian activity patterns [3,5]. In this study we address the question how generalisable a circadian release pattern of VIP and AVP is, in brain slices from rodent pups. We report on neuropeptide release from slices obtained from common vole (Microtus arvalis) pups. In addition, we wanted to assess whether variability in the circadian patterns of immunoreactivity of neuropeptides in adult voles in vivo [3,5], is already reflected in neuropeptide release from vole pup suprachiasmatic slices.

Laboratory reared common voles were used. The breeding colony was based on individuals trapped in the Lauwersmeer, Netherlands (53°20' N; 6°16' E). From two females we obtained six pups. Brains from 6-day-old pups of voles (n = 6; body weight 3.2–3.6 g), and of rats (Rattus norvegicus; n = 2), were cut coronally into 375-μm thick slices. From these, small tissue blocks of 500 μm by 500 μm, centered around the SCN, were prepared and cultured on coverslips in tubes (Nunc), placed in a roller drum (10 rev./min) at 36°C. Culture medium, 700 μl per tube (50%: Eagle's basal medium (Gibco) + 4.16 mM NaHCO3 + 60.55 mM D-Glucose; 25%: Hanks balanced salt solution; 25% Horse serum) was exchanged after 8 days, and replaced by...
sample medium (as culture medium but only 10% Horse serum) 12 h before sampling. At intervals of 2-h, 25 samples were taken from day 12–14 after preparation of the slices, and replaced by fresh sample medium. Samples were denatured by adding 70 \mu l 0.1 N HCl, and heating at 95°C during 10’. Samples were dried in a speedvac concentrator and stored at 4°C before determination of VIP and AVP content by enzyme immunoassay as described before [10,11]. Concurrently with the preparation of vole brain slices, also slices from two 6-day-old Wistar-Imamichi rat pups were prepared, sampled and assayed for VIP and AVP. Timing of peaks in neuropeptide release were calculated by determining when the derivative of the release with respect to time equaled zero. Besides visual inspection from intervals between consecutive peaks, autocorrelation was applied to determine periods of rhythms in peptide release.

Judged from phasecontrast microscope inspection, all vole hypothalamic slices contained SCN tissue and morphologically developed similarly, as described in detail elsewhere [14]. The vole SCN cultures, however, were smaller in surface and consisted of multiple cell layers, compared with the rat brain cultures. The simultaneous measurements of VIP and AVP release patterns in the six individual vole brain cultures are presented in Fig. 1. Maximal VIP release varied between 5.8 and 2.8 fmol/2 h, the minimal between 2.6 and 1.9 fmol/2 h. The amplitude, expressed as the quotient of maximal and minimal release, varied between 2.3 and 1.5. The VIP release patterns showed no clear peaks and troughs in the 16–20 h range (Fig. 1, left). Instead, several records in Fig. 1 are suggestive of a pulsatile VIP release at a higher frequency. The average production of VIP in the slices was 34 fmol/24 h (SD 6.6).

Peak AVP levels of the same vole SCN cultures ranged from 13 to 23 fmol/2 h, trough values from 1.6 to 9 fmol/2 h. The amplitude of AVP release, indicated by the quotient of maximal and minimal release, differed considerably between cultures, from 8.7 (Fig. 1, culture 1) to 2.1 (Fig. 1, culture 6). From the six cultures of vole SCN, three (Fig. 1, culture 1–3) showed two full cycles in AVP release, with periods that varied between 16 and 20 h (mean 18.0 h; SD 1.16; n = 6). One slice (Fig. 1, culture 4) showed only one cycle in AVP release, with a 20-h period, and the two other slices did not reveal any such cycle. The mean total amount of AVP, produced per slice was 118 fmol/24 h (SD 27); the three vole cultures with a circadian release pattern lagged behind compared with the three others (on average 90 ± 14 fmol/24 h versus 130 ± 21 fmol/24 h).

Autocorrelation analysis of the VIP release data resulted in an absence of periodicity (Fig. 2A; Friedman two-way ANOVA, non-significant). In contrast, autocorrelation of the AVP release data revealed a significant variation (Friedman two-way ANOVA, P < 0.005). A periodicity around 20 h was indicated in four SCN cultures. Although this periodicity lacked in the autocorrelation analysis of two other cultures, the mean plot of autocorrelation of the AVP data peaks at 20 h (Fig. 2B).

In two rat brain slice cultures, the mean daily VIP release amounted 40 ± 3.0 fmol, the AVP release 90 ± 7.3 fmol. Whereas these numbers did not differ substantially from the values obtained in vole brain cultures, the temporal patterns of VIP and AVP release in these rat cultures, however, revealed outspoken and concurrent circadian patterns of VIP and AVP release, with identical periods varying from 18 to 20 h (mean 19 h; SD 1; n = 4, both for VIP and AVP). Peak VIP levels ranged from 4 to 6 fmol/2 h, trough values from 2 to 2.5 fmol/2 h; peak AVP levels varied from 9 to 13 fmol/2 h, trough values from 4.5 to 7 fmol/2 h. Autocorrelation analysis of the release patterns in rat cultures showed clear rhythmicity in the 20 h range, both for VIP and AVP (Fig. 2C,D).

In two earlier studies, all surviving organotypic rat SCN cultures showed circadian rhythms in VIP and AVP release [10,11], although with remarkably short periods [10]. Period length was very similar for VIP and for AVP (mean 21.4 h; SD 3.9). A stable phase relationship persisted in vitro between the two peptide release rhythms [10]. Identical results were obtained in the two rat brain cultures in this
Early studies on VIP and AVP release in rat brain slices showed considerable variability in the range of AVP and VIP levels [10,11]. In comparison, we obtained relatively low levels of VIP, and thus also low VIP/AVP ratios. We think these differences are due to the use of different rat strains. The Wistar-Imamichi rats we used in this study showed low VIP levels in other ongoing experiments also, compared with the results obtained in rats from the Sapporo colony, used in the earlier studies [10,11].

First of all, this study revealed that organotypic SCN cultures from neonatal vole show no circadian pattern of VIP release. This result differs from earlier studies in rats [10,11], as well as from the results obtained in rat brain slices in this study. The possibility of faster, ultradian VIP release patterns cannot be completely excluded, but the 2-h sampling procedure did not allow for a more detailed analysis. Collection of neuropeptide release data from vole hypothalamic slices, allowing for a higher resolution of analysis, would be a worthwhile enterprise, in view of the 2–4-h ultradian feeding rhythms in microtine species [2].

In this study we could establish rhythmic AVP release in brain slices obtained from pups in a second rodent species. Part of the vole SCN cultures produced AVP in a rhythmic circadian fashion, at an age at which no circadian behavior patterns are expressed. In some other vole SCN cultures no circadian pattern of AVP was observed. Such variability in the expression of circadian rhythmicity is also characteristic for the locomotor pattern of adult voles [2]. It should be noticed, however, that absence of circadian AVP release patterns in SCN slices also has been reported in rats, although in a seemingly lower incidence, compared with common vole SCN slices. While the studies that report on the simultaneous VIP and AVP release in vitro only describe circadian rhythm patterns [10,11], earlier studies also mention the absence of rhythmicity in AVP release in part of the rat SCN slices [13].

In future studies of adult vole SCN cultures, it remains to be established whether there is a causal relationship between the findings of labile circadian rhythmicity in the SCN and behavior. Even correlational evidence will require organotypic slice cultures from individuals that previously have been analyzed in a behavioral study. VIP and AVP are prominently involved in neuronal [6] and humoral [7,10,11] output from the SCN. Most output projections from the SCN consist of VIP and AVP fibers [6,15]. Studies on the humoral output of the SCN in relation to behavior have become especially relevant since the finding of restoration of behavioral rhythmicity in SCN lesioned animals by transplantation of fetal SCN tissue in a capsule that prevents synaptic communication [12].

The amount of AVP released by vole cultures in a noncircadian fashion was relatively large, compared with the circadian release patterns. This finding is of interest in view of the results obtained in behaviorally non-rhythmic, adult voles that showed larger number of immunoreactive AVP cells in the SCN, at least in the early morning [3]. The period of AVP release in rhythmic vole cultures was similar to those of rat cultures. In multi-unit activity (MUA) measurements in acute vole SCN slices, also periods of about 20 h have been observed [1]. Several explanations are possible for this consistent deviation in vitro from behavioral measurements with a circadian period from 23 to 25 h [3]. The number of neurons in SCN slices is reduced, compared with the in vivo situation, and of course afferent connections are eliminated; both factors may lead to a reduction of period length [10]. Remarkably, in rats a MUA pattern has been observed in vitro with a period of 24 h [4].

Whereas the rat SCN cultures show a synchronized release rhythm of VIP and AVP, none of the common vole SCN cultures show a circadian VIP rhythm and only part of these vole cultures show an AVP rhythm. In voles, a parallel release pattern of the two neuropeptides is thus lacking completely, suggesting an independent role of both neuropeptides in the oscillatory output pathways of the circadian pacemaker in the common vole. Taken together, this study underlines that SCN cultures from rodent pups can reveal rhythmic properties of circadian clocks that can differ within and between species.

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