DIURNAL RHYTHM OF VASOTOCIN IN THE PINEAL OF THE MALE RAT

By

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ABSTRACT

The pineal content of arginine vasotocin in the male rat is subject to diurnal changes, having a high value around noon and a low value around midnight. Since in rats exposed to 24 h constant light the pineal content in arginine vasotocin decreases, and in the rats exposed to 24 h constant darkness the pineal content in arginine vasotocin increases, it appears that during the night both the synthesis and release of arginine vasotocin is enhanced.

The mammalian pineal, including the rat, contains \( \text{Pavel} \ 1965; \text{Pavel} \ & \text{Petrosescu} \ 1966; \text{Pavel et al.} \ 1975 \) and synthesizes \( \text{Pavel et al.} \ 1973; \) and in preparation) the specific nonapeptide arginine vasotocin (AVT). Most of the known biologically active compounds from the pineal show marked diurnal changes \( \text{Wurtman et al.} \ 1968; \text{Reiter} \ 1975 \). Further, Pavel (in preparation) has recently shown that the CSF concentration of AVT in man is subject to a diurnal rhythm. Hence it was of interest to see whether daily fluctuations occur in the AVT content of the pineal.

MATERIALS AND METHODS

Adult male Wistar rats were used. They were housed at constant ambient temperature of \( 22 \pm 2^\circ \text{C} \) under automatic light control which provides 12 h light, from 6:00 a.m., and 12 h darkness. The following groups of rats (each including 35 animals) were used: a) intact rats sacrificed at noon and midnight; b) hypophysectomized rats (48 h after hypophysectomized) sacrificed at noon and midnight and c) intact rats sacrificed
at noon after exposure to 24 h constant light or darkness. The pineals, which were removed within 30 seconds after decapitation, were extracted in 0.25% acetic acid (Pavel et al. 1975). The assay methods included rat antidiuretic, rat uterine and frog (Rana temporaria) bladder assays (Pavel 1965). Synthetic AVT (kindly supplied by Prof. M. Bodanszky, Case Western Reserve University, Cleveland, Ohio) with a potency of 110 ± 18 rat antidiuretic units/mg, was used as standard in antidiuretic and hydro-osmotic assays as well as for the calculation of the biological ratios. Crystalline trypsin (twice crystallized, chymotrypsin free, Serva) and sodium thioglycollate (Worthington, Biochemicals) were used for tryptic digestion and reductive inactivation of pineal extracts as previously described (Pavel et al. 1975). Since it has been recently demonstrated that both antidiuretic and hydro-osmotic activities of the pineal extracts from male rats killed around noon were completely destroyed by trypsin and sodium thioglycollate (Pavel et al. 1975), tryptic digestion and reductive inactivation was performed only on pineal extracts from rats killed at midnight.

**RESULTS**

The antidiuretic and hydro-osmotic activities of pineal extracts from intact and hypophysectomized male rats killed at noon and midnight are shown in Table 1. No rat uterine activities could be detected (i.e., less than 25 μU/mg). Both hydro-osmotic and antidiuretic activities of pineal extracts from intact rats killed at midnight were almost completely destroyed by incubation with trypsin or with 0.05 M sodium thioglycollate, i.e. that is, the antidiuretic activity was reduced to less than 5 μU/ml (the sensitivity of our method) and the hydro-osmotic activity was reduced to values near to the standard water flux of our method (3 μl/20 min.). The hydro-osmotic/rat antidiuretic ratio of pineal extracts from intact rats killed at noon and from hypophysectomized

<table>
<thead>
<tr>
<th>Method of assay</th>
<th>Endocrine structure</th>
<th>Experimental procedure</th>
<th>Hour of day</th>
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<tr>
<td></td>
<td></td>
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<td>noon</td>
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<tr>
<td>Rat antidiuresis</td>
<td>pineal</td>
<td>intact</td>
<td>6 ± 1</td>
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<tr>
<td>Frog bladder</td>
<td>pineal</td>
<td>intact</td>
<td>996 ± 130</td>
</tr>
<tr>
<td>Rat antidiuresis</td>
<td>pineal</td>
<td>hypox</td>
<td>7 ± 1.5</td>
</tr>
<tr>
<td>Frog bladder</td>
<td>pineal</td>
<td>hypox</td>
<td>1176 ± 165</td>
</tr>
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rats killed at noon and midnight were 166, 168 and 170, respectively, whereas the hydro-osmotic/rat antidiuretic ratio of pineal extracts from intact rats killed at midnight, was 17. The hydro-osmotic/rat antidiuretic ratio found for the synthetic AVT by the same assays was 175. The hydro-osmotic activities of pineal extracts from intact rats killed at noon after exposure to 24 h constant light or darkness, were 550 ± 65 (SEM) μU AVT/mg and 1975 ± 185 (SEM) μU AVT/mg, respectively.

**DISCUSSION**

The hydro-osmotic/rat antidiuretic ratios of pineal extracts from intact rats killed at noon and from hypophysectomized rats killed at noon and midnight were not significantly different from the same activity ratio of 175 that we found for synthetic AVT by the same assays. This ratio is extremely specific for AVT (Sawyer 1961). However, the hydro-osmotic/rat antidiuretic ratio of 17, found in the pineal extracts from intact rats killed at midnight, differs significantly from that of synthetic AVT, indicating the presence of an additional antidiuretic activity. Since in hypophysectomized rats the nocturnal increase in the antidiuretic activity is completely abolished and since cultured cells from the human (Pavel et al. 1973) as well as from the rat pineal (Pavel et al., in preparation) apparently synthesize only AVT, it is clear that the nocturnal increase in the antidiuretic activity, reflects only an increased storage by the pineal of arginine vasopressin (AVP) which is released from the neurohypophysis during the night, and not an endogenous pineal rhythm. Thus, recently Rosenbloom & Fisher (1974), have demonstrated by radioimmunoassay that bovine pineals obtained from the slaughterhouse (at which the animals were killed both during the day and night) contain approximately equal amounts of AVP and AVT whereas in the pineals of rats killed during the day (Rosenbloom & Fisher 1975) no radioimmunoassayable AVP could be detected.

The antidiuretic assay of pineal extracts measures both the antidiuretic activity of AVP and AVT whereas the hydro-osmotic assay, the most specific biological assay for AVT (Sawyer 1961), measures in the present experimental conditions only AVT. Indeed, since AVP has less than 1 % of the hydro-osmotic activity of AVT (Sawyer 1961), even higher concentrations of AVP than those reported in the present experiments would not affect bladder permeability. Also oxytocin, if present in the pineal extracts, would be there only at concentrations less than 25 μU/mg (the sensitivity of our method). Since the hydro-osmotic activity of oxytocin is equal to that of uterine activity (Sawyer 1961) it is clear that such concentrations of oxytocin would not be able to affect bladder permeability. Furthermore, the complete inactivation of
the hydro-osmotic activity by trypsin, indicates that the activity is due exclusively to a basic peptide (Du Vigneaud et al. 1953). Oxytocin is not affected by tryptic digestion since it contains neither arginine nor lysine (Du Vigneaud et al. 1953). On the other hand, no substances known in the pineal, nervous system or CSF, such as acetylcholine, noradrenaline, 5-hydroxytryptamine (Ishii et al. 1962) angiotensin II, bradykinin, adrenaline (Rasmussen et al. 1963) or melatonin (Pavel, unpublished results), affect bladder permeability. Therefore, in a mixture containing low concentrations of AVP, AVT and oxytocin, as in the case of pineal extracts, the hydro-osmotic assay measures selectively only the activity of AVT. Since during the night AVT is released into the CSF of man (Pavel, in preparation), the nocturnal decrease in the pineal hydro-osmotic activity of rats, most likely reflects an increased release of AVT into the CSF.

On the other hand, since in rats exposed to 24 h constant light the pineal hydro-osmotic activity decreases whereas in rats exposed to 24 h darkness the pineal hydro-osmotic activity increases, it appears that during the night in the dark both the synthesis and release of AVT is enhanced.

REFERENCES


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