Pineal influences hypothalamic Gn-RH content in the vole, *Microtus agrestis*

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Summary. Male voles reared in a stimulatory (long) photoperiod have significantly greater contents of hypothalamic Gn-RH and pituitary LH and greater testicular and seminal vesicle weights than do voles reared in inhibitory (short) photoperiods. The inhibitory effects of short photoperiod were reversed by pinealectomy.

Introduction

The vole, *Microtus agrestis*, is a seasonal breeder (Austin, 1957; Breed, 1967) and sexual development in the male vole is delayed by short photoperiods (Worth, Charlton & MacKinnon, 1973; Grocock, 1979). The pineal gland is thought to affect reproduction in a variety of seasonal breeders (see reviews by Zucker, Johnston & Frost, 1980; Reiter, 1981) and in the vole, pinealectomy or extirpation of the superior cervical ganglion renders the sexually immature male insensitive to the inhibitory effects of short photoperiods (Clarke & Farrar, 1975; Charlton, Grocock & Ostberg, 1976). Chemical sympathectomy by the use of 6-hydroxydopamine has a similar effect (Farrar & Clarke, 1976). Reiter (1973) showed that the normal gonadal regression observed in the golden hamster during the winter months is prevented by pinealectomy, suggesting that the pineal may be involved in seasonal breeding. A seasonal change in pineal morphology has been seen in voles (Lembowicz, Charlton & Grocock, 1976) and in hares (Lincoln, 1976), but the mechanisms by which the pineal may exert its antagonadal effect are not understood and so the hypothalamo-hypophysial axis was examined in this study.

Materials and Methods

Male voles were from the colony maintained in the Department of Human Anatomy, Oxford as described by Worth et al. (1973). Animals were born into short photoperiods, 8 h light:16 h dark (8L:16D; lights on 08:00 h, lights off 16:00 h GMT) and long (16L:8D; lights on 06:00 h, lights off 22:00 h GMT) photoperiods. The light intensity with the lights on was 6-9 units using a Weston Euro-Master light meter. Animals were weaned and sexes separated at 2 weeks of age. One week later animals reared in 8L:16D were randomly allocated to one of 3 groups: pinealectomy (Group 1), sham-operation (Group 2), or intact controls (Group 3). The animals reared in 16L:8D also served as intact controls (Group 4). Operations took place (between 11:00 and 13:00 h GMT)

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under ether anaesthesia. A burr hole was drilled in the skull above the region of the pineal (confluens sinuum) and the gland removed by grasping the stalk with a pair of watchmaker’s forceps. In sham-operated animals (Group 2), the superior sagittal and transverse sinuses were disrupted but the pineal gland was left intact. All animals were killed 14 days later between 11:00 and 13:00 h. Group 1 animals were examined under a dissection microscope for any pineal remnants and for any obvious brain damage. No pineal remnants or brain damage were seen.

At autopsy the testes and seminal vesicles were removed and weighed. The hypothalami and anterior pituitaries were dissected out, homogenized in 1 ml 0.1 N-HCl or phosphate-buffered saline respectively and stored at −20°C. The hypothalamic homogenates were assayed for Gn-RH as previously described (Versi, Chiappa, Fink & Charlton, 1982). Pituitary LH content was determined by the double-antibody radioimmunoassay of Niswender, Midgley, Monroe & Reichert (1968) as used previously in this laboratory by Chiappa & Fink (1977). Dilutions of vole pituitary homogenates exhibited parallelism with the LH standard (NIH-LH-S18).

Statistical comparisons were carried out by analysis of variance and Duncan’s multiple range tests (Duncan, 1955).

Results

Table 1 shows that in animals born and raised in 8L:16D the weights of the testes and seminal vesicles, the hypothalamic content of Gn-RH and the pituitary content of LH were all significantly lower than the corresponding values in voles reared in 16L:8D. The values were also significantly greater in Group 1 than in Group 2 and similar to or greater than (Gn-RH content) the values in 16L:8D animals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Photoperiod</th>
<th>No. of voles</th>
<th>Gn-RH (pg/hypothalamus)</th>
<th>LH (ng/pituitary)</th>
<th>Wt of testes (mg)</th>
<th>Wt of seminal vesicles (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8L:16D</td>
<td>12</td>
<td>684.6 ± 62.3</td>
<td>3733 ± 434</td>
<td>227.8 ± 22.5</td>
<td>22.2 ± 3.5</td>
</tr>
<tr>
<td>2</td>
<td>8L:16D</td>
<td>10</td>
<td>193 ± 25.1</td>
<td>1108 ± 177</td>
<td>40.8 ± 5.7</td>
<td>4.1 ± 1.3</td>
</tr>
<tr>
<td>3</td>
<td>8L:16D</td>
<td>18</td>
<td>175.5 ± 21.9</td>
<td>1219 ± 203</td>
<td>40.0 ± 7.4</td>
<td>2.8 ± 0.6</td>
</tr>
<tr>
<td>4</td>
<td>16L:8D</td>
<td>15</td>
<td>412.5 ± 30.1</td>
<td>3010 ± 296</td>
<td>256.8 ± 31.4</td>
<td>16.8 ± 2.6</td>
</tr>
</tbody>
</table>

Statistics (analysis of variance and Duncan’s multiple range test):
Group 1 versus Group 2  
Group 4 versus Group 3  
Group 1 versus Group 4  

Discussion

These results show that the accelerated sexual development in male voles reared in long photoperiods compared with those reared in short photoperiods was associated with greater contents of hypothalamic immunoreactive Gn-RH and pituitary LH. The inhibitory effect of short photoperiods is overcome by pinealectomy after only 2 weeks. The fact that pinealectomized animals had raised levels of pituitary LH, and large testes and seminal vesicles, suggests that during these 2 weeks there may have been increased LH and Gn-RH release. To account for this increased release and content of hypothalamic Gn-RH in pinealectomized voles (treble that measured in sham-operated animals) there must presumably have been a considerably increased synthesis in just 2 weeks. It would therefore appear that the pineal inhibits both the synthesis and release of Gn-
RH, although it is possible that the pineal primarily inhibits synthesis of Gn-RH; the presumed increased release in pinealectomized animals could be due to ‘overflow’. It is also possible that the vole pineal may inhibit Gn-RH synthesis but that this effect is not modulated by photoperiod. The pinealectomized animals had significantly higher hypothalamic Gn-RH levels than did controls reared in long photoperiods, suggesting that the pineal may inhibit Gn-RH synthesis even in a stimulatory photoperiod.

How the pineal may mediate its antigonadal effect is not clear. Since the pineal secretes melatonin, much research has centred around the effects of this substance on the hypothalamo-hypophysial axis. The results, however, have been equivocal. Rat hypothalami incubated with melatonin released Gn-RH into the perfusion medium (Kao & Weisz, 1977). In the rat, melatonin inhibits the action of Gn-RH on the pituitary in vitro (Martin, Engle & Klein, 1977; Symons, Pryde, Laud & Arendt, 1981; Martin & Sattler, 1982) and in vivo (Martin, McKellar & Klein, 1980). However, in-vivo studies showed no effect of melatonin on the pituitary response to Gn-RH in sheep (Symons & Arendt, 1982) or man (Weinberg, Weitzman, Fukushima, Cancel & Rosenfeld, 1980). It is possible that a pineal substance could have its effect by modulating the action of Gn-RH on the pituitary. If this were the case, then the results of the current vole study could be explained without resorting to the suggestion that Gn-RH release is increased by pinealectomy. In the vole, preliminary studies indicate an antigonadal effect of melatonin (H. M. Charlton & C. A. Grocock, unpublished observations) but its effect on the hypothalamo-hypophysial system has not been investigated.

The golden hamster (Mesocricetus auratus) is a long-day seasonal breeder and in the laboratory the testes of animals transferred from long to short photoperiods regress. Pickard & Silverman (1979) showed that, under such conditions, hypothalamic Gn-RH content was increased and they suggest that this is due to decreased release. In the present study, voles reared in the short photoperiod had less hypothalamic Gn-RH than those reared in the long photoperiod but this does not necessarily indicate a difference between the vole and the hamster. In the hamster, the animals were transferred into short photoperiods at 10–12 weeks of age whereas animals in the present study were born into the lighting regimen and killed after 5 weeks. In the vole, therefore, testicular development was inhibited while in the hamster the testes had developed and then regressed.

We thank Dr G. D. Niswender, Dr T. M. Nett, Dr A. F. Parlow, Dr L. E. Reichert, Jr and the National Pituitary Agency of NIAMDD (Baltimore, Maryland) for radioimmunoassay materials; and Dr H. Gregory, Dr J. Gormley and the late Dr A. L. Walpole (I.C.I. Pharmaceuticals) for synthetic Gn-RH. This work was supported by grants from the M.R.C. to H.M.C. and G.F., and E.V. was in receipt of an M.R.C. Scholarship.

References


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Received 19 July 1982