Gonadotrophin levels in male voles (*Microtus agrestis*) reared in long and short photoperiods

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Summary. Male voles were reared from birth to the age of 56 days in photoperiods of 16L:8D or 6L:18D. In 16L:8D testes increased 10-fold in size between the age of 14 and 56 days, and there were concomitant increases in diameter of seminiferous tubules and seminal vesicle weight. Spermatozoa were present in tubules by 35 days. In 6L:18D no significant changes with age occurred in testis size, diameter of seminiferous tubules or seminal vesicle weight. LH secretion increased with age in 16L:8D, but not in 6L:18D, and pituitary and plasma levels were higher in the former than in the latter by 35 days. Pituitary levels of FSH were high up to 21 days in 16L:8D, and up to 35 days in 6L:18D. At 35 days the mean pituitary level was significantly higher in the 6L:18D than in 16L:8D. Plasma levels of FSH were significantly higher in 16L:8D at 14 and 21 days, and then gradually declined. In 6L:18D plasma FSH rose to a maximum at 35 days, but did not reach the levels occurring at an earlier age in voles in 16L:8D. The relationship between pituitary and plasma levels of FSH in the two photoperiods is compatible with the idea of a storage and a readily releasable pool of the hormone within the pituitary gland. In 16L:8D changing levels of LH and FSH seem to be due to the onset of testicular activity with consequent feedback effects of testicular hormones upon the anterior pituitary gland.

Introduction

Long days or long artificial photoperiods allow more rapid testicular growth and development in the vole (*Microtus agrestis*) than short ones (Baker & Ranson, 1932, 1933; Clarke & Kennedy, 1967; Grocock & Clarke, 1974; Grocock, 1979). The amounts of 'total gonadotrophin' and of LH in the anterior pituitary are higher in sexually mature males trapped during the breeding season than in sexually inactive animals from the field in winter (Clarke & Forsyth, 1964a; Worth, Charlton & MacKinnon, 1973). As part of a study of the causes of seasonal breeding in the vole we have measured pituitary and plasma levels of LH and FSH in voles reared from birth in long or short photoperiods.

Materials and Methods

All animals were from the closed colony maintained in the Department of Agricultural and Forest Sciences, Oxford. They were housed and fed as described by Grocock & Clarke (1974). Pregnant females were transferred from the Animal House, where the photoperiod is 16 h light: 8 h dark

(16L:8D), to light-proof cabinets in which the lighting regimen was 16L:8D or 6L:18D. The subsequent litters were reared in the photoperiod prevailing at their birth. Young were weaned at the age of 16 days, and males were kept 4–6 to a cage in the lighting conditions in which they were born. Between 10:00 and 12:00 h at the age of 14, 21, 28, 36, 42 and 56 days animals were anaesthetized with ether and terminal blood samples taken into heparinized syringes. The blood was centrifuged and the plasma stored at -20°C until assayed. Pituitary glands (including the posterior lobe) were removed immediately after death, weighed and also stored at -20°C until assayed. Carcasses were fixed in 10% formalin, and paired testes and seminal vesicles later weighed. Paraffin-wax sections of testes (8 μm) were prepared by standard methods and stained with Ehrlich's haematoxylin and eosin. The widths of 10 seminiferous tubules in each of 10 widely separated testicular sections from each animal were measured. From these values a single best estimate of width for each animal was obtained.

Pituitaries were homogenized in phosphate-buffered saline, pH 7. In agreement with the findings of Worth (1973) dilutions of plasma from castrated voles and of vole pituitary homogenates reacted with anti-ovine LH and anti-rat FSH to produce dilution curves parallel to those for ovine and rat standards (Text-fig. 1). LH and FSH were therefore measured by a heterologous double-antibody radioimmunoassay, using the ultra-microassay described by Naftolin & Corker (1971) and Worth (1973). Antiserum to ovine LH and ovine LH for iodination were kindly provided by Professor G. D. Niswender and Professor L. E. Reichert, respectively. Antiserum to rat FSH, rat FSH for iodination, and the reference preparations (ovine LH, S19; rat FSH, RP-1) were generously given by the NIAMDD, NIH, Bethesda, U.S.A. All standards and most of the unknowns were measured in triplicate. Some samples of plasma were only sufficient to allow duplicate measurements. The lowest detectable levels were 0.15 ngLH/ml and 21 ng FSH/ml. Inter- and intra-assay coefficients of variation were, respectively, 8.6 and 5.4% for LH and 7.5 and 7.8% for FSH. Data were analysed by analysis of variance and the *t* test for the significance of differences between means. Values of $P < 0.05$ have been regarded as indicating statistical significance.

Text-fig. 1. Dilution curves of vole pituitary homogenates, vole plasma, NIH-LH-S19 and NIH-FSH-RP-1 incubated with anti-ovine LH and anti-rat FSH.

Results

Changes in testes and seminal vesicles

There were significant changes with age in the weights of testes and seminal vesicles of voles living in 16L:8D but not in those of animals from 6L:18D (Text-fig. 2). The greatest relative change in weight occurred between 14 and 21 days. Testes and seminal vesicles were significantly

Text-fig. 2. Weights of testis and seminal vesicles, seminiferous tubule diameters, and plasma and pituitary concentrations of LH and FSH in voles exposed from birth to long or short photoperiods. Values are mean $\pm$ s.e.m. for the sample sizes indicated. F ratios from analysis of variance, and degrees of freedom are also given. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

heavier in animals from 16L:8D than in those from 6L:18D, at and after the ages of 21 and 35 days respectively. The diameter of seminiferous tubules increased significantly in the 16L:8D voles from 14 to 56 days, the relative change in diameter being greater from 14 to 21 and 28 to 35 days than at other intervals. No statistically significant change in tubule diameter occurred in voles from 6L:18D. Tubules from animals living in 16L:8D were significantly wider than those of animals in 6L:18D at and after the age of 28 days. Spermatozoa were present from 35 days in testes of animals kept in 16L:8D, but none were seen in testes of the 6L:18D animals.

Pituitary and plasma levels of gonadotrophins

Pituitary weights increased significantly with age from 0.5–0.6 mg at 14 days to 0.8–0.9 mg at 56 days in each photoperiod. There were no significant differences in pituitary weight between the two photoperiod treatments. Analysis of variance showed that LH levels in the pituitary changed significantly with age in 16L:8D but not in 6L:18D (Text-fig. 2). In 16L:8D mean values were significantly higher at 35, 42 and 56 days than at 14, 21 and 28 days, or than at any age in the short photoperiod. There was no overall change with age in plasma levels of LH in either photoperiod, although in 16L:8D mean values at 42 and 56 days were significantly higher than at 28 days. Plasma levels in 16L:8D were significantly higher than in 6L:18D at 35 and 56 days. In both photoperiods there was a significant overall change with age in pituitary and plasma levels of FSH. In 16L:8D the mean pituitary level at 21 days was significantly higher than at later ages. Likewise, the plasma level of FSH in 16L:8D was highest at 21 days, differing significantly from values at 28, 42 and 56 days. In 6L:18D the mean pituitary level of FSH was significantly higher at 21 than at 14 days. Levels were sustained until 35 days, and then there was a significant decline. The mean value for plasma FSH in 6L:18D was significantly higher at 35 than at 14 or 56 days. Pituitary FSH values at 14 days appeared to be higher in 16L:8D than in 6L:18D, but the difference was not statistically significant ($P = 0.06$). However, at 21 days pituitary levels were the same in the two treatments and by 35 days the mean value in 6L:18D was significantly higher than that in 16L:6D. At 42 and 56 days means for the two photoperiods were not different. Plasma levels were significantly higher in 16L:8D than in 6L:18D at 14 and 21 days, but not thereafter.

Discussion

Two different aspects of these results deserve comment: (1) the change with age in various measures of sexual development, and (2) the effect of long or short photoperiods upon sexual development. The effect of age and photoperiod on testicular development is quite straightforward and in agreement with the results of Grocock (1979). Testes from voles living in the long photoperiod become progressively larger, and, judged by the size and state of seminiferous tubules and by seminal vesicle weight, more active than those from the short photoperiod, which do not change over the age range studied.

Age and photoperiod effects have also been demonstrated on the synthesis and release of gonadotrophins from the pituitary gland. The changes with age in the 16L:8D treatment in plasma levels of LH and FSH resemble those reported for the developing rat (Swerdlof, Walsh, Jacobs & Odell, 1971; Döhler & Wuttke, 1975; Lee, de Kretser, Hudson & Wang, 1975; Piacsek & Goodspeed, 1978). The two effects are shown most clearly for pituitary levels of LH, which increased with age in 16L:8D to become significantly higher than those of voles in 6L:18D after 28 days. Although there was no overall age effect in either photoperiod upon plasma LH values, the mean level was significantly higher in the long than short photoperiod at 35 and 56 days. It is apparent that in 16L:8D between the ages of 28 and 35 days synthesis and release of LH are appreciably increased. In the same interval there is a considerable relative enlargement of the seminal vesicles

in voles from 16L:8D. These facts presumably represent a causal sequence, the increased synthesis and release of LH causing a rise in androgen levels, which provokes seminal vesicle growth. The short photoperiod prevents this from happening. The occurrence in 16L:8D of high levels of pituitary and plasma LH while the release of androgen appears to be increasing may be caused by alteration in the sensitivity of the hypothalamo-hypophysial system to negative feedback effects of androgen, and of the pituitary to stimulation by GnRH (Davies & Bicknell, 1976; Davies, Goulden, Follett & Brown, 1976; Lincoln, 1976, 1977). The pituitary and plasma levels of LH found in this study are similar to those reported by Worth *et al.* (1973) who studied male voles from the field and the laboratory: Values changed with season or with conditions of lighting in a fashion consistent with the differences we have found between voles living in long and short photoperiods.

Age and photoperiod had an influence upon pituitary and plasma levels of FSH in 16L:8D and 6L:18D. By the age of 21 days the pituitary seems able to synthesize FSH equally well in long and short photoperiods. However, its capacity to release FSH is, at early ages, appreciably greater in 16L:8D than in 6L:18D. After the age of 21 days synthesis and release decline in voles from the long photoperiod, while synthesis is sustained in the short photoperiod so that by the age of 35 days pituitary levels of FSH are higher in 6L:18D than in 16L:8D. Plasma FSH values in 6L:18D are greater at 35 days than at other ages, but do not reach the level found at 21 days in 16L:8D. In the long photoperiod testes and seminiferous tubules undergo considerable relative change in size between 14 and 21 days. These testicular changes may be caused by the higher release of FSH, which initiates seminiferous tubule growth and promotes spermatogenesis (Raj & Dym, 1976; Courot *et al.*, 1979). Negative feedback of a substance (inhibin?) arising from seminiferous tubules may then cause the reduction in FSH secretion and release observed in 16L:8D after the age of 21 days (Cahoreau, Blanc, Dacheux, Pisselet & Courot, 1979; Davies, Main, Laurie & Setchell, 1979; Sheth, Joshi, Moodbidri & Rao, 1979). Androgen from the interstitial tissue may be sufficient to maintain spermatogenesis once it has been established (Dym *et al.*, 1979).

The contrast between the long and short photoperiods in the relationship between pituitary synthesis and release of FSH suggests the occurrence within the pituitary of a storage and a readily releasable pool of FSH, as has been suggested for LH (Lincoln, 1976, 1977; Pickering & Fink, 1979). This is inferred from the observation that in 16L:8D at 21 days synthesis and release of FSH are high, but in 6L:18D synthesis at 21, 28 and 35 days is essentially the same as at 21 days in 16L:8D, but release is very much lower. The size of each pool, and the transfer of hormone from one to the other, could be regarded as being influenced by photoperiod and also by feedback from the testes. Long photoperiods allow both pools to be large at an early age before the testes develop; short photoperiods can sustain a storage pool for a while. The slight, though statistically significant, rise and fall in plasma FSH in 6L:18D suggests some limited capacity to release FSH, which may be reduced by negative feedback from the testes. The hypothalamo-hypophysial system is thought to be very much more sensitive to negative feedback effects of gonadal hormones in non-stimulatory than in stimulatory photoperiods (for review, see Clarke, 1981). Methods other than those used here for the assessment of testicular activity would be necessary to disclose the existence and source of such a feedback from the testes of voles in 6L:18D.

The present results for the first 56 days of life afford some insight into changing gonadotrophin output in males during the transition under a long photoperiod from the pre- to post-pubertal state, as occurs under field conditions each spring for young of that year. Voles exposed from birth to short photoperiods have a low output of gonadotrophins and remain in a prepubertal state; they may be representative of wild voles that are born late in the breeding season and in which sexual development is inhibited by the onset of unfavourable autumn conditions (Clarke & Forsyth, 1964b; Clarke & Kennedy, 1967). In a number of mammals mean pituitary and plasma levels of FSH and LH are higher in the breeding than the non-breeding season (see Clarke, 1981). Changes in the frequency of plasma pulses of LH occur in sheep as they pass from a non-stimulatory to a stimulatory photoperiod (Lincoln & Short, 1980), but the sampling techniques used for sheep may be very difficult to apply to small mammals such as the vole.

We are indebted to Dr G. Fink, Miss Vivienne Langford, Mr R. Laynes, Dr P. C. B. MacKinnon, Mrs Susan Wong and Miss Janet Stewart for help with this investigation; Professor G. D. Niswender, Professor L. E. Reichert, and the N.I.A.M.D., for the supply of hormones and antibodies; and to the Smith Kline & French Foundation and the Royal Society for grants covering part of the cost of materials and equipment. The investigation was carried out under a grant to J.R.C. from the Medical Research Council (MRC G.974/297/B).

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Received 12 May 1982