Enhancement of serum FSH levels after pinealectomy

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Summary. The effect of pinealectomy on the circadian periodicity in serum gonadotropins was investigated in adult male rats. Pinealectomy resulted in an elevation of the serum FSH concentration 7 days after the operation. A 4-fold increase in serum FSH over 24 h with no concommitant increase in serum LH following pinealectomy suggests that the control of FSH secretion could be mediated via the pineal gland.

It has been well documentated that pineal secretions control several neuroendocrine functions, principally involving the hypothalamus[1,2] and the adenohypophysis[3]. Further, recent reports suggest that the endocrine system shows a 24-h periodicity in rats[4]. Since pineal secretions influence the neuroendocrine system, the present study was designed to investigate the direct effects of the removal of the pineal gland on the circadian periodicity of the gonadotropins.

Materials and methods. Random bred adult Holtzman male rats (280–300 g) were maintained in an air-conditioned room under controlled lighting (lights on from 05.00 to 19.00 h). Pinealectomy was performed essentially by the procedure of Kuszak and Rodin[5] under pentobarbital anaesthesia. In rats subjected to sham operation, all procedures were carried out except the removal of the pineal from its site. The rats had easy access to food and water. Both the sham-operated controls and the pinealectomized rats were sacrificed 7 days after surgery. Groups of 8 rats were sacrificed at designated times (shown in the table) so that samples were obtained at 4 h intervals over a 24-h period. Rats were decapitated within 30 sec after removal from the cage, and sera obtained from the trunk blood samples were stored frozen at −20 °C for subsequent analysis by radioimmunoassay of LH and FSH as previously reported[6], utilizing kits which were a gift from NIAMDD. The antiserum batches used for rLH and rFSH assays were NIAMDD-anti-rat LH-S4 and NIAMDD-anti-rat-FSH-S8 respectively.

The results were expressed in terms of NIAMDD rat LH RP-1 and NIAMDD rat FSH RP-1 for LH and FSH respectively. The sera of the sham-operated group and the experimental one were analyzed in the same assay. The intra-assay coefficients of variation are 5% and 8% respectively for LH and FSH assay. The data was subjected to cosinar analysis to detect the presence or absence of rhythmicity[7]. In this analysis, the cosine functions are fitted to a time series by the method of least squares. The hypothesis, amplitude = 0 is tested at 95% confidence level.

Results. As indicated in the table, a 4-fold increase in serum FSH concentration was observed throughout 24 h in pinealectomized rats as compared to the sham-operated controls. Moreover, a distinct circadian rhythm ($F > F(2.5)$ $\rho < 0.05$) with acrophase at 18.00 h and nadir at 22.00 h is observed in pinealectomized rats. In comparison with the controls, the serum FSH pattern of pinealectomized rats showed a delay in the occurrence of peak (acrophase) and trough levels.

Pinealectomy also resulted in an altered LH rhythmicity. Two distinct peak levels of serum LH were observed in pinealectomized rats; the 1st at 10.00 h and the 2nd at 18.00 h. However, the other basal LH values were slightly yet non-significantly lower than those of control rats. Serum LH pattern of pinealectomized rats also showed a shift in the occurrence of acrophase.

Discussion. The present study demonstrates the inhibitory control of FSH secretion exerted by the pineal gland in the male rat. Further, the data suggests the essential role of the pineal gland in the maintenance of rhythmicity in circulating gonadotropins.

Many investigators have demonstrated anti-gonadotropic effects of pineal compounds. However, there is a controversy regarding the exact pineal indole eliciting the antigonadotropic activity. One of the pineal secretions, melatonin, has been shown to exert anti-LH activity and was shown to act via an intermediary cell on the releasing factor neurons of the brain[8] Reiter et al[9] suggested that in immature female rats, melatonin acted at the pituitary level to inhibit the release of LH. The present data on circulating LH levels demonstrates the antigonadotropic effect of pineal secretion only at 2 time intervals ((10.00 and 18.00 h). However, the persistence of rhythm in serum LH levels with altered acrophases and changes in the rhythmic pattern (2 peak levels instead of 1) possibly suggest that pineal secretions maintain the normal rhythmic pattern in circulating LH levels.

The pineal hormone, melatonin, has also been shown to have anti-FSH activity in retarding the compensatory ovarian hypertrophy in rats and preventing the associated elevation in serum FSH[10]. However, Vaughen et al.[11] demonstrated the same anti-FSH effects by administering melatonin-free pineal extracts. Fraschini and Martini[12,13] described 2 distinctly separate channels according to which 1. the pineal exerts control over the anterior pituitary gonadotropins, and 2. 5-methoxytryptophol and serotonin are considered to be envoys for the control of FSH secretion[14]. Though there is a controversy as to which pineal indole controls anti-FSH activity, there are equivocal findings that pineal secretions exert anti-gonadotropic effects[13,15,16]. The results of the present study confirm the previous findings that the pineal controls FSH secretion. Kamberi et al.[14] hypothesized that pineal secretions control

Serum FSH and LH levels and chronogram in sham-pinealectomized and pinealectomized rats over 24 h

Groups	02.00 h	06.00 h	10.00 h	14.00 h	18.00 h	22.00 h	Amplitude	Phase	F^+
Serum FSH (ng/ml)									
Sham controls	86±3	100±6	106±5	117±5	110±6	114±9	10	0 h 51 min	1.8
Pinealectomy	533±43	552±14	535±49	548±37	656±20	486±57	31.7	4 h 50 min	3.5*
Serum LH (ng/ml)									
Sham controls	39±4	72±5	56±2	38±6	42±5	38±4	14.3	1 h 17 min	12.7**
Pinealectomy	34±5	28±2	115±14	25±2	104±16	26±4	22.6	4 h 44 min	10.4**
							21.4	2 h 50 min	11.0**

Values are expressed as mean ± SEM of 8 observations. F^+ $F_{2,61}$ (0.05 or 0.01) = *$p < 0.05$; **$p < 0.01$.

the discharge of hypothalamic FSH releasing factor. Recent evidence on the control of FSH secretion shows that inhibin, a non-steroidal hormone secreted by the gonads, selectively controls FSH secretion[17]. It would be interesting to study whether pineal control of FSH secretion is mediated through the gonadal inhibin.

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PRO EXPERIMENTIS

Fluorometric estimation of dead cells in cell suspensions[1]

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Summary. An objective vitality test is proposed. It is based on the fluorescence increment of ethidium bromide in the presence of dead cells, which is proportional to cellular DNA under conditions previously defined.

Estimation of the proportion of dead cells in a given cell suspension is a very common procedure in experimental biology and medicine. Dye exclusion techniques[2] are most frequently used, but require subjective counting. Among various dyes excluded from living cells, ethidium bromide (EBr) offers the possibility of developing an objective method because it reacts quantitatively with nucleic acids[3]. Edidin[4] was the first to evaluate titers of cytotoxic antisera using the fluorometric measurement of EBr stained cell suspensions. His method, however, was not based on a true quantitative estimation of deoxyribonucleic acid (DNA) and was neither intended nor suited for the calculation of absolute or relative cell numbers. By making use of recent developments in the fluorometry of nucleic acids by EBr[5-7], especially dissociation of nucleoproteins by heparin and digestion of ribonucleic acid, we arrived at conditions where the DNA of dead cells (inside or outside the cell) is detected specifically and quantitatively. The proposed procedure may be used to carry out, in cell suspensions: a) relative counts of dead and living cells, and b) quantitative estimations of DNA or (by a factor) of the total number of cells present.

Materials. Ascites cells of a mouse sarcoma induced by UV light (UVT 14306)[8] were carried in the inbred strain XVII/Bln. Fluorometric measurements were performed with a Beckman SF 1078 spectrofluorometer.

Solutions (numbers refer to the table): (1) Cell suspension (about 10^6/ml) in PBS-GS, washed once. (2) Ribonuclease (RNase, EC 2.7.7.16, Ferak, Berlin), 50 µg/ml in PBS. (3) Heparin (Polfa, Warsaw), commercial solution (5000 IU/ml) diluted 1:300 with PBS. (4) Digitonin (VEB Ysat, Wernigerode), 1.5 mg/ml in ethanol. (5) PBS (Dulbecco's phosphate buffered saline)[9], pH 7.5. (6) PBS-GS (PBS plus 2 mg/ml glucose and 1% inactivated fetal calf serum from Flow Labs., Bonn). (7) Ethidium bromide (Serva, Heidelberg), 25 µg/ml in PBS. Solutions 2,4 and 7 can be stored at +4 °C for 1 month.

Proposed procedure. Assay mixtures according to the table, but without EBr, are prepared and incubated in a waterbath at 37 °C for 20 min. Thereafter EBr is added. As soon as constant temperature is reached, mixtures can be measured. Excitation is set at 365 nm, emission at 590 nm. In the case of filter fluorometers, the filter combination of Boer[10] can be used. The fluorometer reading of mixture A (F_A) is adjusted to the full scale of the instrument.

Assay mixtures

Mixture	1 Cell suspension	2 RNase	3 Heparin	4 Digitonin	5 PBS	6 PBS-GS	7 EBr
A	0.5	0.5	0.5	0.01	0.5	–	0.5
B	0.5	0.5	0.5	–	0.5	–	0.5
C	–	0.5	0.5	–	0.5	0.5	0.5

Volumes in ml, total volume 2.5 ml (in 4 ml cuvettes). Numbers of solutions refer to 'materials' section, where concentrations are given