

Effect of Long-Term Pinealectomy on Serum LH Levels and on Cerebral Serotonin Metabolism in Adult Male Rats

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(Received on November 5, 1986)

M. ALDEGUNDE, R. DURAN, M. ALFONSO, I. MIGUEZ and J. VEIRA. *Effect of Long-Term Pinealectomy on Serum LH Levels and on Cerebral Serotonin Metabolism in Adult Male Rats*. Rev. esp. Fisiol., 43 (3), 351-354, 1987.

Two months after pinealectomy carried out in adult male rats previously exposed to a long photoperiod (L:D 14:10) during 10 weeks, the weight of seminal vesicles significantly increased in comparison with Sham-operated animals. Nevertheless, the weights of testes and ventral prostate did not change significantly. The serum LH concentration increased significantly by pinealectomy. The levels of serotonin and 5-hydroxyindolacetic acid significantly decreased in the hypothalamus and midbrain after pinealectomy.

Key words: Pinealectomy, Serum LH, Serotonin metabolism.

The pineal gland is considered to exert an inhibitory effect on the reproductive system. In the rat pineal gland the levels of melatonin and melatonin-synthesizing enzymes are increased during the dark periods (3). This hormone has been generally considered as the mediator of the pineal effects on the endocrine system (20), although pineal peptides and other pineal indoleamines are biologically active (16, 17, 18). However, the specific role of the pineal substances and their way of action are not yet clear.

According to TAMARKIN *et al.* (22)

pinealectomy has no marked effects on the hypothalamic-pituitary-gonadal axis in nonphotoperiodic species. However, in male and non pregnant female rats melatonin and other pineal indoleamines have been observed to exert a depressant effect on pituitary and plasma LH. Moreover, TIMA *et al.* (23) have indicated that the pineal gland may influence the rat gonadotropic-gonadal system via a serotonergic inhibitory mechanism, and MESS *et al.* (12) have concluded that the pineal gland exerts its influence through a serotonergic neural mechanism. Because of these facts, the aim of the present work is to determine the effect of long-term pinealectomy on the reproductive status and the cerebral serotonin metabolism of the adult male rat.

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Materials and Methods

Adult male Wistar rats were kept under a long photoperiod of 14 h L/10 h D (lights on at 07:00 hours) and temperature of $22 \pm 2^\circ\text{C}$ for 10 weeks. Water and food were available *ad libitum*. After this period the male rats were pinealectomized (PX) using the method of KUSZAK and RODIN (10). Rats were anesthetized with urethane (1.2 g/kg i.p.). In the sham-pinealectomized rats (SPX) surgical approaches to the pineal gland were made, but the pineal gland was not disturbed. An unoperated group was also included. The brains of PX and SPX rats were discarded when showing postoperative signs of injury.

Eight weeks after surgery animals were sacrificed at the same time every day (between 10:00 h and 11:00 h). Blood samples were collected and the serum separated and stored at -20°C until assay. Gonads, seminal vesicles and ventral prostate were dissected out and weighed. The brains were quickly removed, placed on an iced glass dish and hypothalamus and midbrain were dissected. Fluorimetric assay of serotonin (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) was carried out according to the method described by MILLER *et al.* (13). The serum LH levels were determined by a double antibody radioimmunoassay using NIAMDD rat LH kit. The second antibody (rabbit- γ -globulins) was provided by Antibody Inc., Davis, Calif. The LH was labelled in our laboratory with (^{125}I) by the chloramine T method (7). Radioactive iodine (^{125}I Na, General Radioquímica, Spain) was administered with a specific activity of 200-225 $\mu\text{Ci}/\mu\text{l}$. Iodinated hormone was purified by gel filtration using a Sephadex G-50 (Pharmacie Fine Chemicals, Sweden) column prepared in a glass pipette, and were stored at -20°C in 0.01 M sodium phosphate buffer containing 2% of bovine serum albumine (BSA fraction V, Sigma). Repurification

was performed in a column (40×1.5 cm) of Sephadex G-100 immediately before the assay. The results are expressed in ng of LH-RP-1 per milliliter of serum.

Results and Discussion

Statistical analysis of results from SPX rats versus control (unoperated) rats demonstrated that there were no significant differences between the two groups in the parameters analysed. Because of this, the significance of surgery was calculated against the values obtained in the SPX group. Moreover, no significant differences were found in the body weight of the different rat groups at the end of the experiments (table I).

The pinealectomy on adult male rats cause a significant increase in the seminal vesicle weight, but there were no significant changes in either testicular or ventral prostate weights. However, MOTTA *et al.* (14) have found that 12 days after the pinealectomy performed on adult male rats the weights of ventral prostate, seminal vesicles and testes were increased, though the increase in the testicular weight was not significant. These differences could be fundamentally explained by differences in the time of killing after pineal ablation and by differences in the experimental photoperiods.

The increase in the seminal vesicle and ventral prostate weights is a good index of the LH liberation. Evidently, this fact could be the cause of the significant increase observed in the seminal vesicle weight two months after the pinealectomy, since in the present study a significant increase in the serum LH was observed (table I). These results are in agreement with previous reports (2, 5, 6). In the same way as the changes in the seminal vesicle and ventral prostate weights are a good index of the LH liberation, the changes in testicular weights are an index of the FSH liberation. Because of this

Table I. *Weights (g) of testis, prostate gland, seminal vesicles and serum LH concentration, in Control (unoperated), SPX and PX male rats.*
Grams per 100 g b.w. are given in parentheses. Mean $\pm$ S.E.M.

	Control (n = 5)	S PX (n = 6)	PX (n = 5)
Testicular weight	3.29 $\pm$ 0.32 (0.82 $\pm$ 0.05)	3.41 $\pm$ 0.35 (0.77 $\pm$ 0.04)	3.39 $\pm$ 0.34 (0.80 $\pm$ 0.04)
Seminal vesicular weight	0.52 $\pm$ 0.12 (0.13 $\pm$ 0.02)	0.54 $\pm$ 0.06 (0.12 $\pm$ 0.02)	0.83 $\pm$ 0.10* (0.19 $\pm$ 0.02*)
Prostate gland weight	0.52 $\pm$ 0.07 (0.12 $\pm$ 0.01)	0.57 $\pm$ 0.08 (0.14 $\pm$ 0.02)	0.56 $\pm$ 0.08 (0.13 $\pm$ 0.01)
Rat body weight at the end of the experiment	328 $\pm$ 19	430 $\pm$ 110	411 $\pm$ 109
Serum LH (ng-RP-1/ml)	32.5 $\pm$ 3.5	30.0 $\pm$ 1.0	41.3 $\pm$ 5*

* P < 0.05. Significance of differences between pX and SPX.

fact, it could be deduced initially that the pinealectomy in our experimental conditions did not cause any significant changes in the FSH secretion. However, the lack of significant changes in testicular weight between SPX and PX rats may be due to the long photoperiod of preadaptation (14 hr L/10 hr D, during 10 weeks). The exposure to prolonged photoperiods depresses the activity of the pineal gland, which may be responsible for the testicular development reaching its highest level before pinealectomy; therefore, a possible rise in the FSH level caused by pinealectomy might not affect a high testicular development. This hypothesis is supported by previous re-

ports, in which an increase in the levels of both pituitary and circulatory FSH (2, 5), was found.

Many works have suggested that the melatonin effects on the reproductive system are mediated by the Central Nervous System and the changes in the released pituitary hormones may be the result of changes in the brain monoaminergic system activity. Pinealectomy to affect has been previously reported the content and metabolism of the monoamines in some regions of the brain (1, 15, 21). Since FRASCHINI *et al.* (6) have suggested that the pineal indolic compounds could act on the hypophysis by means of receptors localized in the median eminence and midbrain, and MESS *et al.* (12) have indicated that the pineal gland exerts its influence through the serotonergic system, in the present study, 5-HT and 5-HIAA levels in the hypothalamus and midbrain have also been studied (table II). In both cases, a significant fall in the 5-HT and 5-HIAA levels has been observed, which indicates an impaired production that could reflect a lower serotonin utilization. Some investigators have suggested that serotonin exerts an inhibitory influence on the LH release (4, 8, 9, 11,

Table II. *5-HT and 5-HIAA concentrations (μ g/g) in the hypothalamus and midbrain in SPX and PX male rats (mean $\pm$ S.E.M.).*

	Substance	SPX (6)	PX (5)
Hypothalamus	5-HT	1.41 $\pm$ 0.08	1.00 $\pm$ 0.09*
Midbrain	5-HT	1.07 $\pm$ 0.05	0.81 $\pm$ 0.02*
Hypothalamus	5-HIAA	1.50 $\pm$ 0.11	0.90 $\pm$ 0.08*
Midbrain	5-HIAA	1.12 $\pm$ 0.08	0.83 $\pm$ 0.07*

* P < 0.05. Significance of differences between SPX and PX.

19). These facts could suggest that the increased LH levels caused by pinealectomy may be produced by the lack of an inhibitory influence mediated by the serotonergic system on the hypothalamus and/or midbrain.

Acknowledgements

The authors wish to express their gratitude to the U.S.A. National Pituitary Agency, Pituitary Hormone Distribution Program NIAMDD, for the provision of hormone RIA kit.

Resumen

Dos meses después de la pinealectomía realizada en ratas macho adultas previamente expuestas a un largo fotoperíodo (D:N 14:10) durante 10 semanas, el peso de las vesículas seminales está significativamente aumentado con respecto a los animales de operación simulada. Sin embargo, los pesos de los testículos y de la próstata ventral no experimentan variación significativa. Después de la pinealectomía la concentración de la LH sérica aumenta significativamente y los niveles de serotonina y de ácido 5-hidroxiindolacético disminuyen significativamente en hipotálamo y en cerebro medio.

Palabras clave: Pinealectomía, LH sérica, Metabolismo serotonina.

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