Lack of Effect of Pinealectomy on Ovarian Function: Evidence for a Time-Dependent Action[1] (36049)

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When Kitay and Altschule reviewed the world literature in 1954, no clear-cut effect of the pineal over ovarian function was discernible. Since then much evidence has accumulated establishing an inhibitory role of the pineal over the gonadal axis (1–5), which may be mediated via interference with hypothalmic release of FSH-RF and LH-RF (6–9). This paper offers an explanation for the lack of positive findings from these early experiments and postulates the existence of a pineal–pituitary feedback system similar to that seen in other endocrine organs.

Materials and Methods. Female Sprague-Dawley rats (27–28 days old, weighing 50–60 g) were divided into five groups as follows: Group I served as controls; Group II were pinealectomized; Group III underwent sham pinealectomy; Group IV were ovariectomized; Group V were ovariectomized and received 42 μg of melatonin intraperitoneally daily. Rats were fed Purina Laboratory Rat Chow and provided with water *ad libitum*. All animals were maintained on a rigid diurnal cycle of illumination (lights on 5:00 a.m. to 8:00 p.m.) and randomly housed with 10–30 per group to equalize unrecognized variables. After 6 weeks, animals were sacrificed with an overdose of sodium Nembutal; and pineal, pituitary, and ovarian weights and histology were examined. Tissues were fixed in modified Bouin's solution and stained with hematoxylin and eosin (10). The brains of all animals were sectioned to determine the completeness of pinealectomy and the possibility of damage to surrounding brain tissue. Only animals in which pinealectomy was complete and in which adjacent brain structures were intact were included in the study. Ovarian RNA to DNA ratios were determined according to the Schmidt–Thannhauser procedure (11) as modified by Fleck and Munro (12). RNA was determined by a modification of the orcinol method of Morse and Carter (13) and UV absorption (14). DNA was determined by a modification of the diphenylamine method of Dische (15) and UV absorption (14). Good agreement was obtained between UV, orcinol, and diphenylamine determinations.

Results. There were no significant differences between control and sham operated animals in any of the parameters studied. Consequently, these two groups were combined in all statistical calculations.

The relationship between ovarian weight, ovarian RNA and DNA content, and the ratio of RNA to DNA in controls, sham operated and pinealectomized animals is represented in Table I. The increase in ovarian weight usually found in pinealectomized animals (1, 16–18) was not observed here. Nor was the content of RNA or the RNA/DNA index (which has been shown to be an accurate index of hormonal activity (19–23), altered. These results, together with the failure to find any changes in ovarian histology, suggest that pinealectomy had little or no effect on ovarian physiology at the time these measurements were made.

Table II shows the weights of posterior, anterior, and total pituitaries in each of the four groups studied. Neither pinealectomy nor ovariectomy resulted in any change in posterior pituitary weight. However, pinealectomy did result in a significant decrease in anterior pituitary weight ($p < .01$). As shown

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TABLE I. Rat Ovarian Weight, Total RNA and DNA and RNA/DNA Ratio ± Standard Error of the Mean.

The number of animals is indicated in parentheses. Both ovaries were combined for all determinations.

Group	Body wt (g)	Ovarian wt (mg/100 g)	RNA (μg)	DNA (μg)	RNA/DNA
I. Controls	211 ± 4.7	32.4 ± 1.1 (18)	551 ± 46	997 ± 79	0.58 ± 0.05 (12)
II. Sham operated animals	209 ± 3.7	32.3 ± 0.8 (21)	546 ± 30	940 ± 67	0.60 ± 0.04 (16)
III. Pinealectomized	210 ± 3.3	31.9 ± 1.2 (29)	565 ± 31	946 ± 66	0.61 ± 0.05 (19)

in Table III, ovariectomy was followed by an increase in pineal weight; and in three ovariectomized animals which received melatonin (42 μg) intraperitoneally for 6 weeks, there was a decrease in pineal weight rather than the anticipated increase. Present studies are now in progress to determine whether melatonin is indeed the active principle synthesized by the rat pineal.

Discussion. The increase in ovarian weight reported (1, 16) following pinealectomy was not observed here. Similar findings have been reported by Kincl and Benagiano (24). They found that pinealectomy in both rats and hamsters had no effect on gonadal development or accessory sex organ weights in either males or females autopsied 9 weeks postpinealectomy. The absence of any change in either RNA content, RNA/DNA ratio, or in ovarian histology indicated that pinealectomy had little or no discernible effect on gonadal physiology in this experiment. A possible explanation for these results may be afforded by the work of Roth (unpublished data). He found maximum changes in ovarian weight to occur 2 to 4 weeks following pinealectomy. This is the time at which most investigators have found inhibitory effects of the pineal over ovarian function. At 6 to 8 weeks, ovarian changes became negligible.

It is possible that pinealectomy had an effect on ovarian function in this experiment, but that this effect was not observed at the time of autopsy (6 weeks after pinealectomy) due to compensatory changes initiated by the ovary as suggested by Roth's data. This might explain why Kincl and Benagiano (24) failed to find any effect of pinealectomy on gonadal function in animals examined 9 weeks postpinealectomy.

It is noteworthy that the effect of ovariectomy was to significantly increase pineal weight. Fiske *et al.* (25) reported a similar increase in pineal weight following ovariectomy, which was almost significant. Such results suggest the existence of a feedback mechanism between the pineal and the anterior pituitary similar to that seen in other endocrine organs. Ovariectomy results in diminished estrogen production, which, in turn, would augment pituitary gonadotropin secretion. This would then stimulate pineal activity as manifested by increased pineal weight and presumably by increased mela-

TABLE II. Rat Anterior, Posterior, and Total Pituitary Weight ± Standard Error of the Mean.

The number of animals is indicated in parentheses.

Group	Pituitary wt (mg/100 g)		
	Posterior	Anterior	Total
I. Controls	0.687 ± 0.04 (14)	4.26 ± 0.07 (15)	5.04 ± 0.13 (17)
II. Sham operated	0.681 ± 0.04 (16)	4.19 ± 0.12 (17)	4.96 ± 0.11 (21)
III. Pinealectomized	0.698 ± 0.04 (25)	3.82 ± 0.10 (25)[a]	4.69 ± 0.11 (29)[b]
IV. Ovariectomized	0.713 ± 0.06 (8)	4.11 ± 0.21 (8)	4.90 ± 0.18 (10)

[a] $p < .01$.
[b] $p < .02$.

TABLE III. Rat Pineal Weight ± Standard Error of the Mean.

The number of animals is indicated in parentheses.

Group	Pineal wt (mg/100 g)
I. Controls	0.61 ± 0.03 (17)
II. Sham operated	0.62 ± 0.04 (20)
III. Ovariectomized	0.70 ± 0.02 (11)[a]
V. Ovariectomized + melatonin	0.48 ± 0.07 (3)

[a] $p < .01$.

tonin release. This, in turn, feeds back to inhibit the increased gonadotropin levels. The fact that pineal weight remains increased at a time when compensatory ovarian changes have occurred in pinealectomized animals reflects persistent pineal stimulation from the known high circulating gonadotropin levels following ovariectomy. Other suggestive evidence for such a relationship comes from three sources: (i) the combined organ cultures of pineal and pituitary glands which show decreased pituitary FSH release in the presence of pineal tissue (20); (ii) the increase in pituitary FSH and LH following pinealectomy and subsequent decrease with pineal implants into the median eminence (17); and (iii) the fluctuation of pineal lipid content with levels of circulating gonadotropins, decreasing after hypophysectomy and increasing with pregnant mares serum and human chorionic gonadotropins (7).

The anterior pituitaries of pinealectomized animals show a significant decrease in weight compared to controls; this result is difficult to explain. If pinealectomy does stimulate FSH, ACTH, and TSH synthesis by the anterior pituitary as this and other data suggest (6, 26, 27), a concurrent increase in anterior pituitary weight would be expected. However, this apparent discrepancy has also been observed by Roth (unpublished data).

The increase in anterior pituitary weight reported by Wurtman *et al.* (1) in pinealectomized rats is open to question. He compares pinealectomized animals to shams, not to controls. However, since his shams show a marked decrease in anterior pituitary weight from the control animals, in his paper it appears as if the anterior pituitary weight of pinealectomized rats has increased. In actuality, his data show a slight decrease after pinealectomy compared to controls. In our experiments all parameters examined in sham operated animals were similar to controls as one would expect. It seems unlikely that minor differences in experimental technique between our and Wurtman's results can explain the discrepancy in anterior pituitary weights in sham operated animals. A true increase in pituitary weight following pinealectomy has been reported by several investigators (3, 28). However, an equal number of investigators have failed to find any changes in pituitary weight following pinealectomy (29–31). It is likely that such changes are based on a complex set of interactions between exposure to light, pineal physiology, circulating levels of sex steroids, and neuronal input from the limbic system thereby making any change whatsoever difficult to interpret.

Summary. The interrelationship between the rat pineal, ovary, and hypophysis was examined utilizing organ weight, histology, RNA content, and RNA:DNA ratio as an index of synthetic activity. Six weeks post-pinealectomy, no change in ovarian function was observed utilizing the above parameters. A time-dependent action of pinealectomy on ovarian activity is postulated. Following ovariectomy a significant increase in pineal weight was seen. This suggests the existence of a feedback mechanism between the pineal and anterior pituitary similar to that of other endocrine organs.

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