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Gonadal Function in Underfed Rats: II. Effect of Estrogen on Plasma Gonadotropins After Pinealectomy or Constant Light Exposure

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ABSTRACT

The effect of underfeeding on the control of plasma levels of FSH and LH was investigated in female rats after long term food deprivation. Concentrations of these hormones were measured in ovariectomized animals by radioimmunoassay, before and after injection of a single dose of estradiol. Similar measurements were made in animals which had been previously pinealectomized or exposed to continuous illumination, since such treatment partially restores activity to the quiescent reproductive tissues of rats which are maintained on a low caloric diet. While levels of plasma FSH were comparable in fed and underfed ovariectomized animals, LH was significantly lowered in the latter group. It is suggested, therefore, that the major factor contributing to reproductive dormancy in the starved animal is reduced plasma LH. This reduction was not apparently due totally to enhanced feedback sensitivity by estrogen, since it was observed in ovariectomized rats, but occurred in part through some mechanism which is sensitive to constant light exposure or pineal gland removal. Such treatment resulted in elevations of blood levels of LH, but had little effect on plasma FSH levels in underfed rats. It is therefore suggested, that energy conservation during periods of food shortage is facilitated by the establishment of control mechanisms leading to gonadal quiescence by inhibition of LH secretion. This mechanism is in part dependent upon the pineal gland for optimal function and it is potentiated by a relatively short photoperiod.

INTRODUCTION

Reproductive organ atrophy and hypofunction resulting from underfeeding occur due to aberrant neural control of the pituitary/gonadal axis, not from lack of body nutrients during the period of feed restriction (Sorrentino et al., 1971). Physiological characteristics induced by such treatment include lengthening of the diestrous interval of the vaginal cycle (Mulinos and Pomerantz, 1940), alterations of serum and pituitary levels of gonadotropins (Howland, 1971a, 1972) and decreased weight of the ovaries and uteri, with microscopic evidence of subnormal function (Piacsek and Meites, 1967). The effects of underfeeding on certain aspects of reproductive function can be reversed by maintaining the animals under continuous light conditions during treatment (Piacsek and Meites, 1967). Exposure of animals to long photoperiods results in "physiological" pinealectomy (Reiter, 1972). This observation is germane to the foregoing discussion since one of the potentiating or predisposing factors that have been reported to sensitize the pituitary/gonadal axis to suppression by the pineal gland is the animal's nutritional state. Normal development of reproductive tissues occurs in pinealectomized underfed young male rats, while sham operated control animals growing under comparable conditions demonstrate a lag in the growth of their seminal vesicles with a decrease in citric acid concentration for many weeks after initiation of the hypocaloric state (Krecek and Palaty, 1967).

Compensatory ovarian hypertrophy (COH) which is often used to detect pituitary adjustments to changes in the concentrations of circulating estrogens also fails to occur in rats when chronically (Walker and Bethea, 1977) or acutely (Howland, 1971b) underfed. Based upon the observation that, while COH does not occur in underfed rats, total castration leads to "normal" hypersecretion of luteinizing hormone (LH), Howland (1971b) suggested that the low level of feed intake results in an increased sensitivity of the hypothalamus to ovarian steroid. He confirmed this idea by

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showing that estrogen is more effective in suppressing LH levels in low feed than in high feed ovariectomized rats (Howland and Ibrahim, 1973). The COH response in rodents is influenced not only by ovarian hormones, but also by pineal gland factors, since extracts of the gland have been shown to inhibit growth in the remaining ovary in mice (Benson et al., 1971, 1972). Furthermore, the response is partially restored in growing underfed pinealectomized rats, while ovarian weight loss which normally occurs in the acutely underfed adult rat is prevented by pinealectomy, though a drastic hypertrophy is not seen (Walker and Bethea, 1977). Constant light will also stimulate COH in the underfed rat whose body weight has stabilized after the initial period of rapid loss. Based upon the observations cited above and considering the suggestion that the pineal's antigonadotropic character is enhanced by underfeeding (Sorrentino et al., 1971), we examined the interplay between underfeeding, the pineal gland and ambient light upon serum levels of the gonadotropins, FSH and LH, in the presence of estrogen, as determined by radioimmunoassay.

MATERIALS AND METHODS

Charles River CD (outbred albino) derived rats from our colony were underfed from birth to determine the effects of long term food restriction on feedback sensitivity. Breeding was synchronized so that pups of the same age could be utilized in the same experiment. At parturition, 6 female pups were randomly redistributed to each nursing mother. Pups from the underfed group were then removed from the mother for 12 h of each day until weaning, which occurred at three weeks of age for all rats. They were then offered LabBlox (Wayne Company) *ad libitum* for three days, during which time total consumption was monitored. Thereafter, the daily amount delivered to the underfed group was adjusted to equal one half of the amount consumed per day by their fully fed counterparts. This amount was regularly increased as the consumption of the fed group increased during growth. Similarly, when the weights of the control group stabilized and their food consumption leveled off, one half of that amount was fed as the maintenance quantity for the underfed animals. Testing of feedback sensitivity was begun after 7 months of such treatment.

Another experimental group was constructed to determine the effect of food deprivation on hormonal feedback sensitivity after full growth had been achieved through optimal feeding. In this case, litters were prepared as above, except that full feed was allowed until the animals had reached 6 months of age. Rats designated to be underfed were then allowed one half of the feed consumed by the control group. After 30 days, feedback sensitivity determinations were performed.

Animals of both long and short term food restricted and fully fed groups were pinealectomized to test the effect of such treatment on estrogen suppression of pituitary gonadotropin secretion. After weaning, the pineal gland was surgically removed from all test animals, therefore, these groups could also be considered long term pinealectomized, since 6 more months passed before feedback determinations were performed. Short term underfed animals, however, were pinealectomized directly before being placed on restricted rations, and all such animals in this group were only pinealectomized one month prior to testing.

Inhibition of pituitary FSH and LH secretion by estrogen was finally tested in all animals at 7 months of age. All animals had been previously ovariectomized when they were 4 months old. Initially, blood was collected by heart puncture while the rats were lightly etherized at 1400 h on the day preceeding estrogen administration. This was done to provide information about serum gonadotropin levels before the steroid was given. At 1000 h of the next day, estradiol valerate dissolved in sesame oil was injected subcutaneously at a dose of 7 ug/100 gm body weight. Twenty eight hours later, at 1400 h of the final day of the experiment, blood was again drawn by heart puncture and later analyzed for gonadotropin content with the presteroid samples. Control animals received only the oil vehicle to determine if the gonadotropin titers were nonspecifically altered.

Plasma levels of LH were determined by the method of Niswender et al. (1968) with slight modifications. These included a final dilution of the antiserum to 1:300,000, the addition of 0.18 ng/tube of radioiodinated hormone and the utilization of Rat LH-RP-1 standard. In this system, approximately 30 percent of the radioiodinated hormone was bound in the absence of unlabeled hormone and the cross-reactivity of FSH and TSH with the LH antiserum was <0.32 percent and <0.59 percent, respectively, at 50 percent bound.

Plasma FSH levels were determined using the rat immunoassay kit materials provided by the NIAMDD, in which 18 percent of the labeled hormone was bound in the absence of added hormone. The final dilution of FSH antibody was 1:6300; Rat FSH-RP-1 was used as standard. Cross reaction of LH and TSH with the antiserum was not detectable (<0.2 percent) at the midpoint of the curve. Sheep anti-rabbit gamma globulin (final dilution, 1:100) which was produced in our laboratories was used to separate bound hormone from free in both assay systems. Plasma sample volumes for LH and FSH were 50 ul and 20 ul respectively. Determinations were done in duplicate. The within assay variation was 4.8 percent for LH and 5.1 percent for FSH.

The statistical significance of differences between treatment means was determined by analysis of variance.

RESULTS

No significant differences in response to testing between the long and short term feed restricted animals were evident, therefore, they were used together in composing the entire

underfed group. Despite the marked difference in body weight between fed and underfed animals, there was no consistent alteration in plasma gonadotropin levels which could be correlated with such changes, i.e., gonadotropin titers were not necessarily lower in smaller animals. Also, pineal removal or exposure to continuous illumination had different effects on plasma hormones in the two feed groups. Measurements of these compounds were made for these two major categories of animals and the result of multiple comparisons are reported in Table 1. Generally, plasma levels of gonadotropins were elevated in underfed when compared with fed animals. This was true not only in untreated animals, but even in pinealectomized and constant light exposed groups, where treatment changed hormone levels from those measured in untreated rats. For example, underfed intacts had significantly greater levels of plasma FSH ($P<0.001$) and LH ($P<0.05$), than fed pinealectomized rats. Similarly, underfed pinealectomized animals displayed increased levels of the two gonadotropins (FSH, $P<0.02$; LH, $P<0.05$) when compared with the same fed pinealectomized group. Underfed animals which were kept in constant light followed the same trend when compared with their fed counterparts (FSH, $P<0.01$; LH, $P<0.05$). Only in the case of LH, did titers of the pituitary hormone from fed rats significantly ($P<0.02$) exceed those measured from feed restricted ones. With regard to the effects of pinealectomy and constant light exposure upon gonadotropin levels in the same group, the two treatments tended to change the concentration of hormone from the control value, in the same direction. For example, those treatments generally caused plasma hormone values to become reduced in these ovariectomized animals. In the fed groups, the most significant change was seen, with reduced titers of LH found in pinealectomized ($P<0.001$) and constant light exposed animals ($P<0.01$). FSH values were changed in the same direction by treatment in the fed group, however, the underfed animals responded less strongly. Similar effects were evident when comparing mean values and these are also expressed in Table 1.

Upon the administration of estradiol to fed and underfed rats, gonadotropin levels were generally lowered except in the case of LH for underfed intact rats. While there was generally greater than a 50 percent reduction in FSH and LH due to estrogen administration, approximately 75 percent remained in the underfed intact animals after the steroid was given. There was a significant difference ($P<0.05$) in the LH suppression response in underfed animals, however, if they were maintained in constant light, with post-estrogen LH values in some of these rats falling to as low as 10 percent of their controls. A similar but attenuated response was measured in the underfed pinealectomized

TABLE 1. Effect of caloric restriction and estrogen treatment on plasma gonadotropin levels in ovariectomized adult rats which were pinealectomized, exposed to constant light or untreated. Values bearing the same superscript are significantly different (α=p<.05; ψ=p<.01; β=p<.001). N = 9 rats per group. Values represent means ± standard error.

Group	Plasma gonadotropin levels before estrogen (ng/ml) FSH	LH	Plasma gonadotropins percent of control value after estrogen FSH	LH
Fed	1848.0 ± 376.1	2750 ± 196.1$^{\beta,\psi}$	57.7 ± 15.4	32.6 ± 13.3
		p<.02		
Underfed	2098.7 ± 235.8	1354 ± 263.7	47.7 ± 6.0	73.9 ± 20.4$^{\alpha}$
Fed Pinealectomized	984.0 ± 83.4	497 ± 62.7$^{\beta}$	77.2 ± 8.4	81.7 ± 10.3
	p<.02	p<.05		
Underfed Pinealectomized	1611.6 ± 107.6	1694 ± 211.7	54.0 ± 7.6	48.7 ± 10.3
Fed Constant light	1070.0 ± 50.0	867 ± 99.8$^{\psi}$	119.6 ± 12.5	53.2 ± 7.7
	p<.01	p<.05		
Underfed Constant light	1919.0 ± 141.3	2488 ± 489.6	64.0 ± 5.7	19.0 ± 4.4$^{\alpha}$

group. The response was seemingly unusual, because in all other groups, the removal of the pineal gland or exposure of the animals to continuous illumination, decreased the effectiveness of estrogen in suppressing plasma levels of FSH and LH.

The withholding of food itself caused a significant reduction in the amount of LH but not FSH that could be measured in the plasma of ovariectomized rats. Pinealectomy or constant light tended to reduce these values in all animals, but the effect was more profound in the fully fed animals. Estrogen administration further reduced the levels of FSH and LH in all groups of all treatments, however, the degree of suppression by the steroid upon the pituitary hormone was modified by pinealectomy and more profoundly by exposure to constant light.

DISCUSSION

These data do not support the contention that underfeeding enhances LH feedback sensitivity to estrogen (Howland and Ibrahim, 1973) because serum LH was reduced in ovariectomized underfed rats. Furthermore, when estrogen was administered to these animals, the degree of LH suppression was far less than in the fed animals. Strain and age differences, but more importantly, perhaps, the differences in feeding schedules, could account for the apparent discrepancies in feedback sensitivity data. Howland and Ibrahim's (1973) animals were underfed for a short period of time preceeding testing, while the majority of ours were tested after life long feed restriction. The adaptation of homeostatic maintaining systems to acute food deprivation may result in a quite different response when tested, from one which would result in a system which has developed under conditions of chronic food restriction.

The reduced levels of serum LH in ovariectomized underfed rats may reflect, at least in part, the activity of the pineal gland, since titers of the pituitary hormone were elevated in pinealectomized and constant light exposed ovariectomized underfed animals. This conclusion is supported by our previous observation of enhanced COH in underfed rats which were kept in constant light or pinealectomized when compared with similar pineal intact animals on a 12 h photoperiod (Walker and Bethea, 1977). Javoy et al. (1970) demonstrated that pinealectomy in castrate female rats caused a very sharp decrease in endogenous serotonin in the hypothalamus, but not in other brain structures. Since serotonin is inhibitory to LH release and pineal indoleaminergic material interferes with the regulation of LH secretion (Moszkowska et al., 1971); since melatonin is taken up selectively in the basal hypothalamus (Anton-Tay and Wurtman, 1969) and since it increases serotonin in this region of the brain when administered systemically (Anton-Tay et al., 1968), depression of LH in the underfed castrate rat may be due, in part, to the enhanced influence of the pineal gland upon the hypothalamus under conditions of food restriction. That there is a more profound effect of light on LH secretion than pinealectomy, suggests that the latter is not at play alone in reducing LH secretion during underfeeding. Indeed, environmental lighting, while controlling certain aspects of pineal activity (Axelrod et al., 1965), thereby affecting pituitary function indirectly, probably influences pituitary gonadotropin secretion in a more direct manner as well. The basis for this conclusion comes from several sources (Blumck, 1958; Moore et al., 1968; Moore and Lenn, 1972). These report that retinohypothalamic fibers project to the suprachiasmatic nucleus, thereby potentially influencing cells of the hypophysiotrophic area directly. Hence, the effect of light encompasses both the pineal influence as well as direct retinohypothalamic neuronal influence on the hypothalamo-hypophysial-gonadal axis. This would logically account for the greater change in LH levels in response to light, than to pinealectomy alone.

While interplay between pineal hormones or other photosensitive compounds of the brain and estrogen to produce feedback effects on pituitary gonadotropin secretion during underfeeding have not been reported, melatonin has been shown to differentially interact with gonadal steroids to alter the activity of the reproductive system. For example, melatonin or serotonin treatment potentiates estrogen sterilization in male rats, while in contrast, melatonin acts as a protective rather than an potentiative agent for the steroid in females (Reiter et al., 1975). In chronically underfed female rats, some substance, ostensibly produced in the pineal gland could antagonize the effect of estrogen to suppress LH levels to any greater degree. To say, however, that the capacity of the system to diminish levels of this hormone in the blood is lost because they are at absolute basal levels, is disputed by the fact that in constant light exposed animals, they fall

approximately 50 percent more than in the postestrogen treated control.

While the majority of studies on the nature of pineal gland principles demonstrate their antigonadotropic properties, the existence of a progonadotropic pineal polypeptide (F_2 fraction) that is biochemically quite stable, has been confirmed (Moszkowska et al., 1971). Perhaps the effect of underfeeding upon reproductive tissue atrophy is not so much due to the absence of FSH, since its levels have been shown to be slightly elevated in underfed animals (Piacsek and Meites, 1967; Howland, 1971b; Howland and Ibrahim, 1973), but due instead, to the absence or ineffectiveness of this pineal principle in the underfed castrate rat. Decline in reproduction function does not seem to be due to an enhanced sensitivity to feedback suppression by estrogen, since in both fed and underfed groups, plasma FSH levels decrease to approximately the same value. While the greatest decrease did occur in the underfed intact group, the difference was not significant. Slight enhancement of the sensitivity of FSH feedback suppression, however, may be indicated by this change and may play some role in underfeeding induced reproductive dysfunction.

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