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Similarity between the effects of suprachiasmatic nuclei lesions and of pinealectomy on gonadotropin release in ovariectomized, sulpiride-treated and melatonin-replaced rats

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Abstract. The aim of this study[1] was to compare the effects of pineal indole treatments on LH and FSH release in pinealectomized and suprachiasmatic lesioned and ovariectomized rats rendered hyperprolactinemic by acute sulpiride treatment. Pinealectomy or suprachiasmatic nuclei lesions in female rats both decreased plasma LH and FHS at 10, but not at 20 d after surgery, whereas the daily afternoon administration of melatonin effectively restored levels of both gonadotropins to control values. In ovariectomized rats, pinealectomy or suprachiasmatic nuclei lesions were ineffective in counteracting the high plasma levels of LH and FSH. However, sulpiride treatment in both pinealectomized and suprachiasmatic nuclei lesioned and castrated female rats significantly decreased the levels of LH and FSH, an effect which was counteracted by daily afternoon melatonin administration. Other pineal indoles tested, i.e., 5-hydroxy- and 5-methoxytryptophol, were ineffective in regulating gonadotropin levels. The results suggest that the pineal gland, through its hormone melatonin, can modulate gonadotropin secretion by acting on a dopamine mechanism independent of hypothalamic suprachiasmatic areas.
Key words. Pinealectomy; suprachiasmatic nuclei lesions; ovariectomy; LH; FSH; melatonin; sulpiride-treated rats.

Melatonin (aMT) is secreted primarily by the pineal gland, and it is well established that aMT can inhibit gonadotropin release[2,3]. Gonadotropin and prolactin (PRL) secretion are controlled by mechanisms which appear to be closely related[4–7], and an inverse relationship exists between LH and PRL levels in a number of physiological, pathophysiological and experimental situations[8–11]. Since aMT can modulate PRL secretion and, conversely, PRL can affect the pineal gland via specific receptors[12–14], it is possible that aMT and PRL may interact to exert modulatory effects on the reproductive axis. Moreover, a more complicated role for aMT in the control of reproduction is suggested by the fact that, under some circumstances, it has a stimulatory effect on LH and FHS[11,15]. The mechanisms by which aMT acts within the CNS to produce these effects are largely unknown. Studies in the Syrian hamster and in the white-footed mouse indicate that aMT affects reproduction by acting on the anterior hypothalamus (AHA) to influence the secretion of gonadotropin releasing hormone (GnRH)[16]. In sheep, there is evidence that the medial basal hypothalamus (MBH) rather than the AHA, is an important site of action of aMT[17]. Lesion studies have provided evidence that frontal afferents to the MBH are indispensable for the occurrence of these gonadotropin and PRL surges[18]. It was reported that destruction of the suprachiasmatic nuclei (SCN) induced persistent vaginal estrus, suggesting an essential role of the SCN in the stimulatory feedback action of estrogen on LH release[19]. We recently reported that lesions restricted to the SCN (SCNx) increased PRL to a degree similar to pinealectomy (Px), an effect counteracted by chronic aMT administration in the afternoon[13]. This hyperprolactinemic effect of Px or SCNx was essentially similar to the hyperprolactinemia (HPrl) obtained by acute injection of sulpiride (10 mg s.c.) at 0900 h (1 h before killing), in Px or SCNx and castrated female rats. The HPrl due to sulpiride was also counteracted by aMT[13].

Because HPrl can alter gonadotropin secretion[9], and because an association between aMT-induced reduction in plasma PRL levels and increased plasma LH has been described[11], the aim of the present study was to compare the effects of treatment with pineal indoles on LH and FHS release in SCNx or Px and OVx rats rendered hyperprolactinemic by acute sulpiride treatment, to determine whether this hypothalamic structure is required for the pineal modulation of gonadotropin release.

Materials and methods

Female Wistar rats weighing 180–200 g were used. The animals were kept throughout the study on a 12 h light-dark cycle (lights on at 07.00), and were housed 3–4 per clear plastic cage with food and water ad libitum. Rats were anesthetized with equithesin and subjected to different surgical manipulations as follows: pinealectomy (Px) or sham-pinealectomy (SPx), according to the method of Hoffman and Reiter[20]; and ovariectomy (OVx) or sham-ovariectomy (SOVx), via a classical lumbar approach. Bilateral stereotaxic lesions of the suprachiasmatic nuclei (SCNx) were made with a monopolar stainless-steel electrode insulated except for a 0.25 mm at the tip, as previously reported[13]. Briefly, stereotaxic coordinates for the lesions were anterior 7.7 mm, lateral 0.2 mm, and 0.9 mm below the dura,

according to the atlas of Paxinos and Watson. These coordinates, as well as intensity (3 mA) and duration (7 s) were determined in trial rats. In sham-lesioned animals (SSCNx), the electrode was inserted but the lesioning current was not applied. At the end of the experiments, the animals were perfused with 10% formalin and the brains were sectioned on a cryostat at 50 µm for histological examination of the lesions.

Animals (7–8/group) were classified as follows: a) Px, SCNx or OVx rats, studied at 10 and 20 d after surgery; b) Px or SCNx rats treated with pineal indoles from day 1 to day 9 post-surgery, and killed on the 10th day; and c) Px or SCNx and OVx rats injected with pineal indoles from day 1 to day 9 after surgery, rendered hyperprolactinemic with sulpiride, and killed on the 10th day. Indoles were dissolved in 95% ethanol, diluted with physiological saline to make working solutions, and administered s.c. daily in the afternoon at 18.00 h. Sulpiride (10 mg s.c.) was administered on the 10th day after surgery at 09.00 h, i.e., 1 h before killing. Serum FSH and LH levels were measured by RIA procedures (NIAMDD) and values were expressed in ng/ml of rat FSH-RP-1 and rat LH-RP-1, respectively. Interassay variances of FSH and LH levels were estimated by including a standard serum in each of six assays and were found to be 14.7% and 16.7%, respectively. Intra-assay variances were 11.7% and 12.4%, respectively (n = 6). Two-way analyses of variance followed by Bonferroni's test (BMDP-PC 90) were used for the statistical analyses.

Results and discussion

Pinealectomy in intact rats significantly decreased LH and FSH at 10 d post-surgery ($p < 0.01$), and hormone levels returned to control values at 20 d after Px (fig. 1). These effects were similar to those obtained in SCNx animals during the same periods (fig. 1). As expected, OVx significantly increased both LH and FSH at 10 and 20 d after Px in all groups ($p < 0.01$, fig. 1), whereas Px or SCNx did not affect the high levels of either gonadotropin in castrated groups. Daily afternoon administration of aMT over 9 d reversed ($p < 0.001$) the effects of pineal removal on LH in a dose-related manner (fig. 2, left). Similarly, aMT at the same doses also reversed the effects of SCNx ($p < 0.001$) on LH levels (fig. 2, left). In both cases, the lower dose of aMT used (200 µg/kg b.wt.) was enough to increase LH levels significantly. In the case of FSH, aMT administration also counteracted the effect of Px or SCNx, significantly increasing the levels of this gonadotropin (fig. 2, right), but these effects were observed in a non-dose-dependent manner. The lower dose (200 µg/kg b.wt.) produced the greatest effects (fig. 2, right). The afternoon administration of aMT for 9 d at a dose of 400 µg/kg b.wt. caused no further increase in the high gonadotropin levels found in castrated or in Px or SCNx castrated rats (table). Moreover, administration of 5-hydroxy- or 5-methoxytryptophol failed to change gonadotropin levels in any group (table).

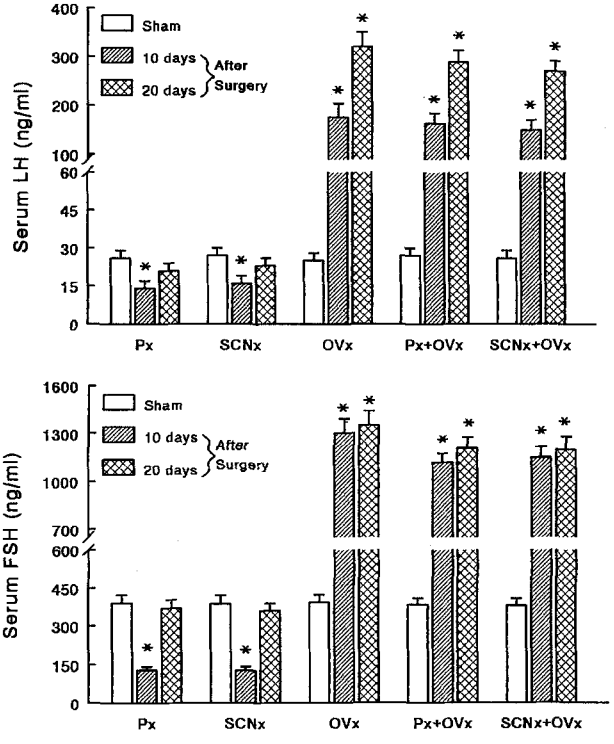

Figure 1. Mean (±SE) serum levels of LH (upper) and FSH (lower) in Px, SCNx and/or OVx rats and their respective sham-operated controls at 10 and 20 d post-surgery. *$p < 0.01$.

In an attempt to clarify whether the effects of aMT were mediated by a dopamine or dopamine-like mechanism, Px or SCNx and OVx rats were injected with aMT, 5-hydroxy- and 5-methoxytryptophol at doses of 400 µg/kg b.wt. from 1 to 9 d post-surgery, and treated with a single dose of sulpiride (100 mg s.c.) on day 10 post-surgery. The results obtained (table) showed that sulpiride treatment significantly decreased both LH and FSH levels ($p < 0.05$), whereas aMT treatment counteracted the effects of sulpiride ($p < 0.05$). Moreover, 5-hydroxy- and 5-methoxytryptophol did not modify gonadotropin levels in these groups.

Because aMT had the same effects in pinealectomized or SCN-lesioned animals, the results of the present study indicate that the pineal hormone aMT is involved in the modulation of gonadotropins regardless of the existence of an intact SCN. The other pineal indoles tested – 5-hydroxy- and 5-methoxytryptophol – did not modify gonadotropin levels in any experimental situations studied. It seems that aMT affects LH control in a more specific manner than FSH control, as dose-dependency was observed in relation to LH, but not to FSH. However, the possibility that lower doses of aMT than the ones used here may affect FSH levels cannot be

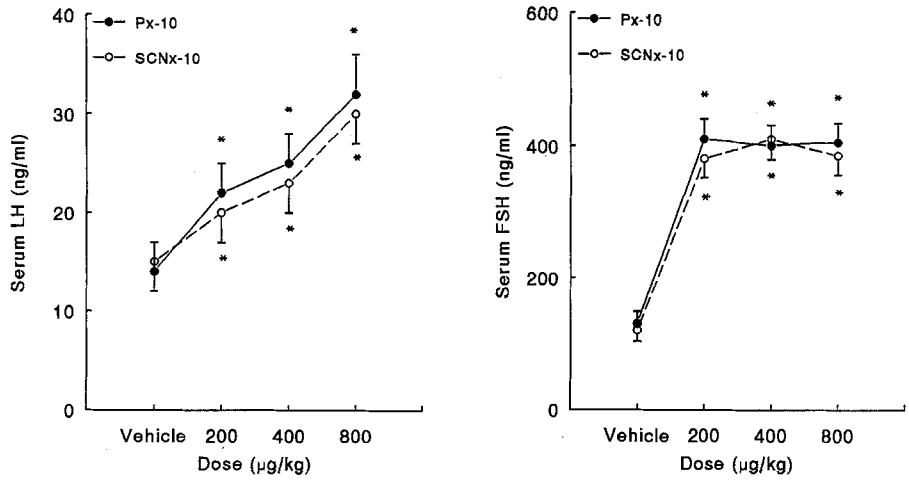

Figure 2. Effects of s.c. injections of various doses of aMT in Px and in SCNx animals. Data are expressed as mean (±SE) serum levels of LH (left) and FSH (right) in Px or SCNx rats. Significance was estimated by comparing the results obtained with drugs and vehicle alone. *$p < 0.001$.

Table. Comparison of the effects of indole administration on mean (±SE) serum levels of LH and FSH in control and in experimental groups (S = sulpiride). Indoles were injected (400 μg/kg, s.c.) in the afternoon for 9 d starting 1 d after surgery. Sulpiride (100 mg, s.c.) was injected at 09.00 h on the 10th day, and samples were collected 1 h later. Data are expressed as ng/ml of LH-RP-1 and FSH-RP-1, respectively.

Experimental group	Vehicle		Melatonin		5-hydroxytryptophol		5-methoxytryptophol	
	LH	FSH	LH	FSH	LH	FSH	LH	FSH
Control	26 ± 2.2	390 ± 27.2	25 ± 3.2	374 ± 28.4	26 ± 2.1	400 ± 32.4	23 ± 1.9	380 ± 35.3
Px	14 ± 3.4[a]	130 ± 12.6[a]	25 ± 3.3[c]	405 ± 31.7[c]	16 ± 3.2	126 ± 10.2	13 ± 2.7	129 ± 13.2
SCNx	15 ± 3.2[a]	128 ± 14.5[a]	23 ± 3.4[c]	410 ± 34.6[c]	14 ± 2.9	112 ± 11.3	15 ± 4.2	137 ± 11.7
OVx	175 ± 22.1[a]	1300 ± 68.4[a]	166 ± 18.4	1340 ± 79.2	169 ± 22.1	1380 ± 98.7	186 ± 22.5	1190 ± 70.2
Px + OVx	163 ± 26.8[a,b]	1120 ± 57.5[a,b]	188 ± 21.6	1290 ± 77.5	158 ± 27.3	1260 ± 67.6	151 ± 17.4	1200 ± 81.6
SCNx + OVx	150 ± 21.2[a,b]	1150 ± 67.2[a,b]	146 ± 20.3	1320 ± 75.8	163 ± 21.4	1310 ± 78.1	158 ± 20.4	1180 ± 63.4
Px + OVx + S	100 ± 19.7[a]	710 ± 33.6[a]	166 ± 24.2[c]	1187 ± 72.2[c]	98 ± 17.6	805 ± 36.6	112 ± 18.7	790 ± 37.2
SCNx + OVx + S	94 ± 17.6[a]	890 ± 48[4a]	148 ± 26.7[c]	1227 ± 86.1[c]	92 ± 20.2	830 ± 41.6	108 ± 23.2	1050 ± 51.7

[a]$p < 0.01$ vs control; [b]$p < 0.05$ vs sulpiride groups; [c]$p < 0.05$ vs vehicle.

ruled out. Nevertheless, the lower dose of aMT used (200 μg/kg b.wt.) is low enough to raise the possibility of a physiological rather than a pharmacological action on gonadotropin regulation. A decrease in LH (and also FSH) in sulpiride-induced HPrl has been previously reported[9]. HPrl may therefore induce anovulation due to impaired LH secretion caused by the suppression of LHRH release, caused in turn by an increase in dopamine turnover in the MBH. The inhibitory effect of PRL on LH secretion is mediated by tuberoinfundibular dopaminergic neurons in the lateral palisade zone of the median eminence (ME)[9]. In a previous report, we found that aMT administration (with the same protocol as used here) counteracts sulpiride-induced HPrl in Px and OVx rats[13]. The aMT-induced increase in plasma LH and FSH described here was associated with the previously described reduction in plasma PRL levels, in accordance with a recent study in Px and SCNx rats[11]. Moreover, aMT administration also counteracted the decreased gonadotropin levels in Px or SCNx rats, whereas no change in gonadotropin levels was detected during aMT treatment in sham-operated animals. Lesions in the medial basal part of the suprachiasmatic area (MBSC) decrease LH and FHS, and significantly disrupt steroid-induced surges of PRL and gonadotropin release[18]. MBSC-lesioned rats exhibit persistant vaginal estrus, as observed in SCNx rats[18,19]. Other authors have reported an increase in plasma LH levels after Px, and a reduction in plasma LH levels after aMT treatment in control rats[21–23]. This difference between the response of LH to aMT may have several causes aside from the differences in strain or dose of aMT used, such as time of aMT administration, age, and the route of administration.

Previous studies reported that aMT administration to pituitary-grafted rats increases PRL and decreases plasma LH levels[11], an effect that may be due to the action of aMT as the hypothalamic level, and that may have involved counteraction of the effects of hyperprolactinemia on the hypothalamus. Moreover, afternoon injections of aMT significantly decreased circulating levels of PRL whereas FSH was enhanced[24]. Melatonin

may have affected gonadotropin secretion at the pituitary level by counteracting the desensitizing effect on HPrl on the pituitary response to LHRH[9]. According to previous reports, SCN lesions in OVx rats blocked progesterone-induced gonadotropin surges, and these lesions decreased LHRH content of the tissue rostral and caudal to the lesion, although no significant changes in tonic levels of gonadotropins were found[25]. The time after lesioning in these studies (up to 7 d) may be an important factor; in the present study, the decrease in both LH and FSH levels disappeared at 20 d, indicating an acute effect of Px or SCNx. The time elapsed after manipulation of the pineal or pineal-related brain areas may therefore be critical in animal experimentation. The fact that the SCN was not necessary for aMT to counteract the effects of Px or SCNx in normal or castrated rats points toward alternative mechanisms of aMT control of gonadotropin, e.g. hypothalamic or hypophyseal pathways, as aMT binding sites in other areas besides the SCN (i.e. the ME and MBH) have been described at the hypothalamo-hypophyseal level[26].

The effects of aMT seen here suggest that this hormone can interfere with the control of gonadotropin synthesis and/or secretion by dopamine, as it does with PRL. Because we did not measure hypophyseal content of LH and FSH in the experimental groups, we cannot determine whether the changes in plasma gonadotropin levels were due to changes in their rate of synthesis. aMT may play an important role in determining the quality of LH and FSH normally stored within the pituitary gland[6]. Melatonin implants[15] placed in the MBH increased the blood concentration of FSH and markedly decreased the plasma concentration of PRL. The mechanisms by which aMT acts within or close to the MBH to influence the secretion of gonadotropins (and PRL) are still unresolved. Exposure to aMT must in some way influence the secretion of LHRH, and the modes of action proposed include an influence on the activity of catecholaminergic neurons, which regulate the pulsatile secretion of LHRH, on dopaminergic neurons[11,27], and an indirect action on the hypothalamus via the pars tuberalis[15], where ^{125}I-aMT binding sites have recently been described[28].

Recent studies have reported[7] that aMT administration decreases dopamine concentration in the median eminence (ME) concomitantly with the suppression of pituitary and plasma PRL. These observations suggest that daily afternoon aMT injections inhibit PRL secretion and interfere with LH cycles, despite the decreased dopamine activity in the ME. Such findings support the view that sites other than the ME are the loci of aMT action, and indicate that aMT may act at multiple sites within the CNS to elicit different responses[15].

The near failure of 5-hydroxy- and 5-methoxytryptophol to affect gonadotropin levels in sulpiride-treated rats and in Px or SCNx and OVx rats indicates that these hormones are not involved to a significant extent in the pineal regulation of gonadotropin secretion, at least at the doses and in the acute hyperprolactinemic model used here.

In conclusion, aMT probably affects common neural systems involved in the control of PRL and LH. The results of this paper raise questions about the terminology of pineal gland function, and perhaps the terms used to assign the roles of antigonadotropin, progonadotropin or counterantigonadotropin to aMT should be revised. We agree with Kennaway[29] that various species use the pineal gland's hormonal output in different ways, i.e., to produce gonadal involution or gonadal recrudescence, depending mainly on geographical origin and length of gestation. The generalization that melatonin's primary function is to cause gonadal involution may be inappropriate. Physiologically, aMT contributes to the proper timing of gonadotropin surges, and facilitates regular estrous cycles.

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