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Effect of Melatonin on the Luteinizing Hormone Release Induced by Clomiphene and Luteinizing Hormone-Releasing Hormone[1] (3928)

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(Introduced by V. G. Foglia)

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Much experimental evidence indicates that the pineal gland is involved in the regulation of reproductive function (1). This effect appears to be mediated, at least partially, by melatonin (2). It is well known that melatonin has a definite inhibitory influence on reproductive function (3, 4). For instance, it has been demonstrated that the injection of melatonin into the third ventricle is followed by a decrease in the luteinizing hormone (LH) and follicle-stimulating hormone (FSH) plasmatic concentrations (4, 5).

Luteinizing hormone-releasing hormone (LH-RH) and clomiphene citrate are known to stimulate the release of LH in the rat. LH-RH stimulates the synthesis and release of LH at the anterior pituitary level (6), whereas there is evidence that clomiphene exerts its principal effect on the hypothalamus (7, 8).

The aim of the present study was to obtain information on the site or mechanism by which melatonin modifies the reproductive function. This was performed by studying the effect of the pineal principle on the LH release in response to LH-RH and clomiphene.

Material and Methods. Adult male rats, from the strain of the Institute of Physiology of the Buenos Aires Medical School maintained in cycles of 14 hr of light and 10 hr of darkness and fed Purina laboratory chow *ad libitum,* were used for all experiments.

Experiment 1. The effect of melatonin and clomiphene on LH secretion were studied in the following groups of rats: (i) control; (ii) treated with clomiphene citrate (0.01 mg/100 g of body weight/day im, for 6 days); (iii) treated with melatonin (1 mg/day sc, dissolved in ethanol 10%, for 6 days); and (iv) treated with clomiphene and melatonin (the drugs were simultaneously administered with the same regimens and doses as described for groups 2 and 3). Controls received only the vehicles.

Twenty-four hours after the last injection, the animals were killed by decapitation, blood samples were taken from the trunk for the LH assay, and the seminal vesicles and ventral prostate weights were recorded. Blood samples were allowed to clot at 4° and centrifuged, and the serum was separated and kept frozen until it was assayed. The concentration of LH in serum from individual rats was assayed in duplicate by means of the double-antibody radioimmunoassay as described by Niswender *et al.* (9). The results were expressed in terms of nanograms of NIAMD-RAT-LH-RP-1/ml of serum. The statistical analysis of the data, planned in the design of the experiment, consisted of the comparison of each experimental group vs control by means of Student's *t* test.

Experiment 2. In this series the effect of pretreatment with melatonin on the LH release after LH-RH administration was studied. Rats were treated with 1 mg of melatonin sc (dissolved in 10% ethanol) for 6 days. Control rats were injected with the vehicle. Twenty-four hours after the last injection, the animals were anesthetized with 0.25 ml of 25% urethane/100 g of body weight. Basal blood samples were withdrawn from the jugular vein, and immediately 75 ng of LH-RH dissolved in 0.5 ml of saline as injected into this vein. Blood samples were taken 20 and 60 min after the injection, and LH concentration was deter-

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mined as described above. Differences in serum LH concentrations before and after the injection of LH-RH were calculated in each animal.

Experiment 3. In this experiment rats were anesthetized as described previously, and after taking basal blood samples through the jugular vein one group was injected into this vein with 75 ng of synthetic LH-RH (dissolved in 0.5 ml of saline) plus 500 ng of melatonin (dissolved in 0.5 ml of ethanol 10%). The other group was injected with 75 ng of LH-RH plus 0.5 ml ethanol 10%. Blood samples were taken 20 and 60 min after the injection, and LH was assayed as described in Expt. 1. Differences in serum LH concentrations were also calculated as in Expt. 2.

Results. Effect of melatonin and clomiphene on LH serum levels. As can be seen in Table I, the daily administration of 1 mg of melatonin for 6 days produced a significant decrease in the serum LH levels as well as in the seminal vesicles and ventral prostate weights. On the contrary, the daily injection of 0.01 mg/100 g of body weight of clomiphene for 6 days resulted in an increase of LH levels and in the accessory sex gland weights. Simultaneous treatment with melatonin and clomiphene produced an inhibitory effect on LH levels and on seminal vesicles and prostate weights similar to those observed with melatonin alone.

Effect of melatonin on the LH release after the administration of the LH-RH. Neither the daily pretreatment with 1 mg of melatonin (Table II) nor its simultaneous iv administration with LH-RH (Table III) modified the LH release produced by the hypothalamic hormone.

Discussion. Much evidence indicates that the secretion of LH in rats is affected by pinealectomy or administration of melatonin. For instance, the LH content in the pituitary increases after pinealectomy (10, 11) and decreases in plasma when melatonin is injected into the third ventricle (4).

The results presented here show that daily administration of 1 mg of melatonin for 6 days produced a significant decrease in serum LH levels as well as in the seminal vesicles and ventral prostate weights. On the other hand, neither the pretreatment with melatonin nor its simultaneous iv ad-

TABLE I. EFFECT OF MELATONIN AND CLOMIPHENE ON SERUM LH AND ACCESSORY SEX GLANDS.

Group and[a] treatment	Mean serum LH[b] concentration ± SE	Seminal vesicles wt (mg/100 g of body wt) ± SE	Ventral prostate wt (mg/100 g body wt) ± SE
Control (9)	34.5 ± 5.1	160 ± 7.8	95.4 ± 6.08
Melatonin (7)	14.5 ± 4.3*	70 ± 4.2***	47.5 ± 3.57***
Clomiphene citrate (9)	185.0 ± 58.1**	250 ± 8.0***	128.8 ± 7.35****
Clomiphene citrate plus[c] melatonin (9)	17.0 ± 4.5**	85 ± 8.1***	55.5 ± 5.71***

[a] Melatonin: 1 mg/day/rat sc; clomiphene citrate: 0.01 mg/100 g of body weight/day im. Duration of treatment: 6 days. The number of rats is in parentheses. Controls were injected with the solvents only.
[b] The values are in ng/ml of NIAMD-Rat-LH-RPI.
[c] Melatonin and clomiphene were simultaneously injected.
* $P < 0.01$; ** $P < 0.02$; *** $P < 0.001$; and **** $P < 0.005$, as compared with corresponding controls.

TABLE II. EFFECT OF THE PRETREATMENT WITH MELATONIN ON THE RESPONSE TO SYNTHETIC LH-RH.

Pretreatment[a]	Injection[b]	Mean serum LH concentration ± SE[c]			Serum LH increase (ng/ml ± SE)	
		Before injection	After injection			
			20 min	60 min	20 min	60 min
Control	LH-RH (10)	30.2 ± 2.8	377.1 ± 59.2	97.1 ± 23.1	346.9 ± 40.8	66.8 ± 22.1
Melatonin	LH-RH (12)	18.8 ± 2.2*	381.0 ± 66.9	95.3 ± 22.0	362.2 ± 60.9	76.5 ± 20.0

[a] Melatonin dissolved in ethanol 10% was injected sc (1 mg/day) for 6 days. Controls received the vehicle.
[b] LH-RH was injected iv in the dose of 75 ng. The number of rats is in parentheses.
[c] Values are in ng/ml of NIAMD-Rat-LH-RPI.
* $P < 0.002$.

TABLE III. EFFECT OF MELATONIN ON THE RESPONSE TO SYNTHETIC LH-RH.

Injection[a]	Mean serum LH concentration ± SE[b]			Serum LH increase (ng/ml ± SE)	
	Before injection	After injection			
		20 min	60 min	20 min	60 min
LH-RH 75 ng plus ethanol 10% (10)	32.3 ± 3.0	325.3 ± 42.8	143.3 ± 23.0	293.0 ± 33.2	111.0 ± 19.2
LH-RH 75 ng plus Melatonin 500 μg (13)	28.4 ± 2.7	285.0 ± 30.2	128.4 ± 32.8	256.1 ± 28.3	100.1 ± 25.2

[a] The number of rats is in parentheses. Melatonin dissolved in ethanol 10% was injected simultaneously iv with LH-RH.

[b] The values are in ng/ml of NIAMD-Rat-LH-RPI. There are no significant differences between the groups.

ministration with synthetic LH-RH modified the magnitude of the LH-release in response to this hypothalamic hormone. These findings confirm previous reports indicating that melatonin is able to inhibit LH secretion and also indicate that the anterior pituitary gland is not the site of this inhibition.

There is experimental evidence suggesting that clomiphene stimulates LH-release by acting at hypothalamic level (7, 8). The previous reports showing that the administration of clomiphene results in an increase in LH release are confirmed by the present study, in which male rats treated with clomiphene for 6 days showed a significant increase in the serum LH levels as well as in the seminal vesicles and ventral prostate weights.

Our results also show that in the group treated with melatonin and clomiphene, serum LH levels and accesory sex glands weights were as low as those found in the group treated with melatonin alone, being significantly lower than in control rats. These results indicate that the stimulatory effect of clomiphene on LH secretion can be nullified by melatonin.

The fact that melatonin did not modify the magnitude of the LH-release in response to LH-RH at pituitary level, but was able to inhibit the effect of clomiphene, apparently exerted at the hypothalamic level, seems to indicate that the hypothalamus may be the site of the inhibitory effect of melatonin on LH secretion. This view is supported by previous reports in which it has been observed that melatonin causes a decrease of LH levels when injected into the third ventricle but not when infused directly into the pituitary (4).

Summary. The effect of melatonin on the LH-release response after the administration of synthetic LH-RH and clomiphene citrate was investigated in adult male rats. The sc administration of melatonin (1 mg/day) for 6 days produced a significant decrease in serum LH levels and in seminal vesicles and ventral prostate weights. On the other hand, the daily injection of 0.01 mg/100 g of body weight of clomiphene citrate during 6 days significantly stimulated LH levels and the weights of the accessory sex glands. Simultaneous treatment with melatonin and clomiphene produced an inhibitory effect similar to that obtained with melatonin alone. Neither pretreatment with melatonin (1 mg/day, sc for 6 days) nor its simultaneous iv administration (500 μg) with 75 ng of LH-RH modified the LH-release in response to the hypothalamic hormone. The fact that melatonin was able to suppress the effect of clomiphene, which is probably exerted at the hypothalamic level, but not the action of LH-RH on the pituitary, appears to indicate that the hypothalamus may be the site involved in the inhibitory effect of the pineal principle on the reproductive function.

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