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Changes in the hypothalamic serotonergic function may mediate the endocrine effects of melatonin

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The effects of a single injection of melatonin on serum thyroxine, corticosterone and prolactin levels, and the associated changes in the metabolism of serotonin (5-HT) in several hypothalamic regions of male rats kept under a 14-h light 10-h dark cycle (lights on at 08:00 h), are investigated. Melatonin (500 µg/kg, s.c.) or replacing saline was injected at 18:00 h, and 8 animals from treated and control groups were killed 1 h (19:00 h), 12 h (06:00 h) and 18 h (12:00 h) later. Melatonin significantly reduced serum thyroxine, corticosterone and prolactin levels within 1 h of treatment, indicating the existence of an acute inhibitory effect on hormonal secretion. Serum prolactin levels were increased 18 h after treatment, suggesting the implication of a different long-term regulatory mechanism. Injected melatonin induced several acute changes in the metabolism of 5-HT in the hypothalamus. Thus, a significant increase of 5-HT content in the preoptic area-anterior hypothalamic (POA-AH) and medial hypothalamic regions of melatonin-treated rats was observed within 1 h of treatment. The content of 5-hydroxyindoleacetic acid (5-HIAA) increased in medial hypothalamus, and the ratio 5-HIAA/5-HT decreased in POA-AH. The results show that melatonin acutely modifies the serotonergic activity in POA-AH and medial hypothalamus, and simultaneously inhibits thyroid, adrenal and pituitary hormonal secretion. The results are discussed suggesting that the hypothalamic serotonergic system could be an ideal mechanism underlying short-term melatonin effects on endocrine function.

Key words: Melatonin, Hypothalamic serotonin, Thyroxine, Corticosterone, Prolactin, Rat.

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The pineal hormone melatonin is functionally related to neuroendocrine physiology, with obvious effects upon mammalian reproductive cycles in photoperiodic species (17). However, melatonin actions are extremely widespread and may influence other endocrine and non-endocrine functions of the organism. Thus, for example, evidence for a melatonin inhibitory effect on rodent thyroid and adrenal function has been accumulating over the last two decades (11, 26). In addition, melatonin has been shown to modulate prolactin secretion either increasing or decreasing serum prolactin levels depending upon the dose and mode of administration to rodents (4, 24).

Although melatonin effect upon mammalian endocrine systems is not disputed, basic questions on the site(s) and mechanism by which melatonin acts remains the subject of much controversy. Melatonin endocrine effects may be mediated via modulation of neurotransmitter activity regulating hormone secretion, while a hypothalamic serotonergic system transmitting melatonin information to the organism has been proposed (7, 19). In previous studies, the pineal gland via melatonin has been shown to influence the synthesis, metabolism and synaptic availability of serotonin (5-HT) in the hypothalamus (13-15). However, there are few data correlating neurochemical and endocrine measures that can throw some light on the mechanism of melatonin action. The present study investigates the effects of a melatonin injection on the metabolism of 5-HT in several hypothalamic areas as well as on the levels of thyroxine, corticosterone and prolactin in rat serum. Melatonin treatment was carried out in the late afternoon, a daily period during which melatonin is most likely to have reproducible short-term effects on brain and behavior (16). Samples were obtained at three moments: 1) an hour after treatment, when melatonin levels are high, 2) twelve hours after treatment (8th hour of dark period), when high levels of circulating melatonin are present owing to the nocturnal melatonin peak, and 3) eigthteen hours after treatment (4th hour of light period), when little amount of melatonin is present in blood (17).

Materials and Methods

Animals and treatments.– Male Sprague-Dawley rats, weighing 250-350 g, were used in this study. Animals were housed, four per cage, in climate- and illumination-controlled room (14-h light, 10-h dark cycle, lights on at 08:00 h; 21 ± 2 °C). Water and rat chow were provided *ad libitum*. Animals were allowed to acclimatize to the animal facilities for two weeks prior to their assignment to the experimental groups. One half of the animals were treated with a single injection of melatonin (500 µg/kg s.c., dissolved in 0.4 ml ethanol:saline 5:95 v/v), whilst the other animals were injected with replacing vehicle. Treatments were administered at 18:00 h. Subgroups of eight animals from each treatment group were killed by decapitation one hour (19:00 h), twelve hours (06:00 h; under dim red light) and eighteen hours (12:00 h) later. The brains were quickly removed and the hypothalamus was dissected on an ice-cold plate in preoptic area-anterior hypothalamus (POA-AH), medial hypothalamus and posterior hypothalamus (13). Tissues were frozen and stored at -80 °C until assayed.

Measure of tissue tryptophan, 5-HT and 5-HIAA content.– The samples of hypothalamic tissue were homogenized in cold mobile phase, centrifuged at 2.500xg for 10 min and filtered through 0.2 µm-Millipore filters. An aliquot of the

supernatant was used for tryptophan determination following the method of BLOXAM and WARREN (5) with minor modifications. Indoleamines content in the hypothalamic areas was determined by high performance liquid chromatography with electrochemical detection as previously described (12). Filtered sample supernatant was injected into the chromatographic system equipped with a Kontron M420 solvent delivery pump, a TL-5A glassy carbon electrode (potential applied was +0.5 V vs Ag/AgCl electrode) and a LC-4B controller from Bioanalytical systems. Chromatographic separations were performed on a 5-µm Spherisorb ODS-1 reversed-phase analytical column (150 mm o.d. x 4 mm i.d.) using a mobile phase composed of 0.3 M acetic acid, 0.08 M ammonium hydroxide, 0.1 mM EDTA and 15 % methanol (v/v). All separations were performed isocratically at a flow-rate of 0.9 ml/min at room temperature. Tissue protein content was determined from aliquots of the tissue homogenates as described by BRADFORD (6).

Hormone assays.–Serum thyroxine and corticosterone levels were measured using commercial kits purchased from Pantex (Santa Monica, CA, USA) and Cambridge Medical Technical Co. (Billerica, MA, USA) respectively. Serum prolactin levels were assayed by a double antibody radioimmunoassay with reagents kindly supplied by the National Hormone and Pituitary Programme (Bethesda, MD, USA; reference preparacion rPRL RP-3). Samples were assayed within a single assay for every hormone, with an intraassay coefficient of variation of 12 % for thyroxine, 4 % for corticosterone and 6 % for prolactin. The sensitivity of the assays was 5 ng/ml for thyroxine, 0.5 ng/ml for corticosterone and 0.05 ng/ml for prolactin.

Data and statistical analysis.– All results are expressed as mean ± SEM. Statistical analyses of differences between treated and control groups at the same hour were performed using one-way ANOVA and post-hoc Student-Newman Keuls test. Significances were determined at $p < 0.05$.

Results

Table I shows the levels of thyroxine, corticosterone and prolactin in melatonin serum and vehicle-treated rats at the time-points sampled. A significant reduction in serum thyroxine and corticosterone levels

Table I. *Effect of melatonin on serum concentration (ng/ml) of thyroxine, corticosterone and prolactin in male rats.*

Melatonin (500 µg/kg, s.c.) was injected at 18:00 h. The results are expressed as means ± S.E.M. of eight data from each treatment and daytime point sampled.

Hour of day	Treatment	Thyroxine	Corticosterone	Prolactin
19:00	Saline	65.05 ± 3.80	777.2 ± 25.8	3.68 ± 0.47
	Melatonin	52.91 ± 2.92*	598.5 ± 31.7*	2.43 ± 0.29*
06:00	Saline	49.43 ± 3.38	308.5 ± 22.1	3.91 ± 0.62
	Melatonin	43.25 ± 5.01	286.2 ± 30.8	4.57 ± 0.75
12:00	Saline	57.54 ± 4.36	375.9 ± 28.3	5.94 ± 0.64
	Melatonin	52.85 ± 2.81	307.9 ± 32.9	8.31 ± 1.02**

* $p < 0.05$; ** $p < 0.01$ vs respective saline-treated group.

was observed in melatonin-treated rats an hour after treatment (19:00 h: –23 % for corticosterone; -19 % for thyroxine, $p < 0.05$ vs control group), but no significant changes were found subsequently in any of them. Serum prolactin levels were observed to decrease an hour after the melatonin treatment (19:00 h: –34 % vs control, $p < 0.05$), which contrasted with the significant increase in the levels of this hormone observed eighteen hours after the injection of melatonin (12:00 h: +51 % vs control; $p < 0.01$).

No significant differences were found in tryptophan content in the hypothalamus of melatonin and saline-treated rats (data not shown). The treatment with melatonin induced an acute increase in 5-HT levels (fig. 1) of POA-AH and medial hypothalamic areas (19:00 h: POA-AH, +25 %; medial hypothalamus, +32 %; $p < 0.05$ vs control). At this time, the 5-HIAA content increased in medial hypothalamus of melatonin treated rats (+24 %, $p < 0.05$ vs control) although neither variation in the 5-HIAA/5-HT ratio was observed. In POA-AH, a decreased 5-HIAA/5-HT ratio was found an hour after melatonin injection (saline: 1.94 ± 0.15; melatonin: 1.52 ± 0.11; $p < 0.05$). No significant change was observed in 5-HT or 5-HIAA content twelve or eighteen hours after the administration of melatonin in any of the hypothalamic areas studied.

Discussion

The present study confirms previous observations on the effectiveness of melatonin to change the endocrine and neural function when administered in the last phase of the scotoperiod and usually within an hour of injection (16, 24). A single injection of melatonin induced an acute decrease in corticosterone and thyroxine levels in serum, which agrees with previous findings on the effect of injected melatonin, as well as with the increased levels of corticosterone and thyroxine

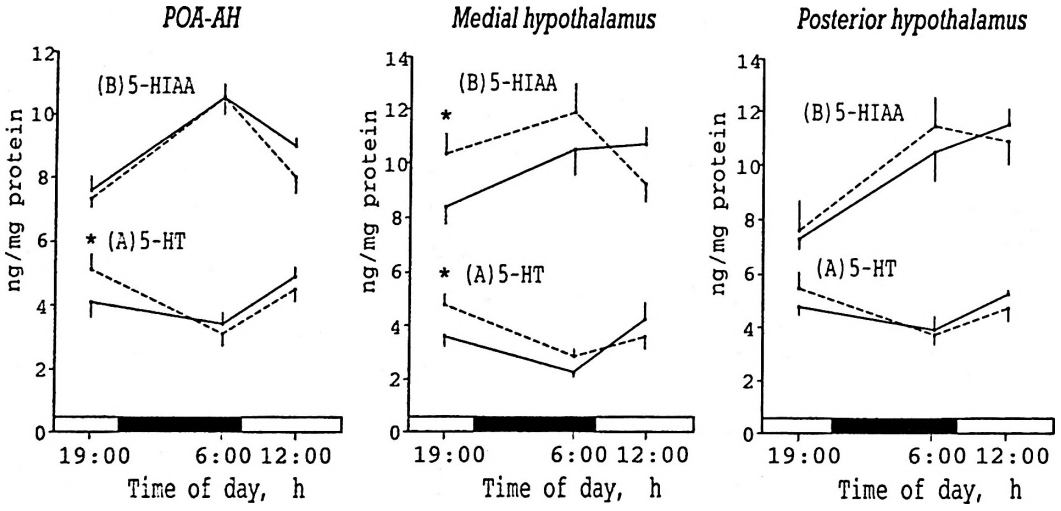

Fig. 1. *Effect of a single injection of melatonin on the levels of 5-HT and 5-HIAA in different hypothalamic regions of male rats.*
Melatonin (dotted line) or saline (continuous line) were injected at 18:00 h. Data are means ± S.E.M. of 7-8 data from each treatment and daytime point sampled. * $P < 0.05$; vs respective saline-treated group.

after pinealectomy in rodents (11, 26). Moreover, similar changes were reported to occur in the secretion of pituitary adrenocorticotropin (ACTH) and thyrotropin-stimulating hormone (TSH) after melatonin treatments (1, 11). Indeed, melatonin has been shown to have an inhibitory influence on the secretion of hypothalamic thyrotropin-releasing hormone (TRH) (25), suggesting that at least part of the effect of the pineal hormone on adrenal and thyroid function may be mediated by the hypothalamic structures.

Melatonin has been shown to influence circulating prolactin levels, although the complexity of this interaction is shown by the different responses obtained from each experimental design (4, 16, 24). In our experimental conditions, melatonin inhibited circulating prolactin levels within an hour of treatment. This result agrees with other studies where an inhibitory effect of melatonin on prolactin secretion in pituitary-grafted rats was observed (9), suggesting that melatonin directly regulates the secretion of the pituitary lactotrophe cells. However, our data show also an important increase in prolactin levels eighteeen hours after the injection of melatonin. This indicates that a dual mechanism may mediate the effects of melatonin on prolactin secretion, which involves an acute inhibitory action on the pituitary cells but also a delayed stimulatory component probably by acting on the hypothalamic centers controlling prolactin secretion. Moreover, the short-term -inhibitory- and long-term -stimulatory- effects of melatonin on prolactin were associated respectively with the lowest and the highest daily prolactin levels in serum, suggesting an interaction between injected melatonin and the daily fluctuation of prolactin secretion that could mediate melatonin effects.

In the present study, melatonin increased the content of 5-HT in the POA-AH within an hour of treatment. This result, together with the lack of changes in 5-HIAA content and the decreased 5-HIAA/5-HT ratio, indicates that melatonin induced a decreased serotonergic activity in the POA-AH. In contrast, concurrent increases in 5-HT and 5-HIAA content were observed in medial hypothalamus, suggesting the existence of an increased 5-HT turnover and neuronal activity. All these changes support a short-term melatonin influence on 5-HT metabolism in hypothalamic terminal regions, which is independent of changes in the availability of tryptophan and probably involves differential local alterations in 5-HT synthesis and release processes. These results agree with previous works showing that both melatonin and pinealectomy modify differentially the metabolism (13, 14) and uptake/release of 5-HT (15) in neuronal terminals of the POA-AH and medial hypothalamus.

These data show a temporal correlation between changes in endocrine secretion and serotonergic function which occurred shortly after melatonin administration. Serotonin is involved in the regulation of pituitary ACTH and TSH secretion, predominantly through the control exerted on the hypothalamic secretion of TRH and CRF (21). Most reported studies support an inhibitory role of 5-HT in TSH-thyroxin secretion, as well as a stimulatory 5-HT influence on ACTH-corticosteroid secretion (21). However, an inhibitory role of 5-HT on ACTH secretion has been also described (22). Our data agree with the idea that the melatonin-induced inhibitory effect on thyroxine and corticosterone levels could be mediated by an increased serotonergic activity at the level of the mediobasal hypothalamic region, which contains the paraventricular TRH and CRF neuronal cell bodies (18, 21).

The regulation of prolactin secretion

from the anterior pituitary is dominated by a potent inhibitory dopamine (DA) influence arising from the tuberoinfundibular tract (2). Evidence has been accumulating that involves to 5-HT in the stimulatory release of prolactin either by stimulating the release of a prolactin-releasing factor or by inhibiting DA release (21). The serotonergic pathway thought to be responsible, probably terminates in the mediobasal hypothalamus, but evidence shows that the anterior hypothalamus is a serotonergic site that influences prolactin release (18). These data suggest that the decreased serotonergic activity found in POA-AH of melatonin-treated rats could mediate the short-term melatonin inhibitory effect on prolactin secretion. However, the changes observed in serum prolactin eighteen hours after melatonin injection seem to be independent of alterations of 5-HT function. Other possibilities must be considered since there is a multitude of substances responsible for regulation of prolactin release, some of which are also influenced by melatonin (25).

In rodents, it is known that the preoptic, suprachiasmatic and medio-basal regions of the hypothalamus, as well as the pars tuberalis of the anterior pituitary, exhibit high density of melatonin receptors (20) which mediate the circadian and seasonal timing of a number of physiological processes (17). In the present study, melatonin modulation of hormonal and neural function was demonstrated during the light hours of the light-dark cycle, a time when endogenous secretion of the hormone by the pineal is normally suppressed, but in which the highest expression of melatonin receptors exists (10). The activation of melatonin receptors has been shown to inhibit DA release in the retina (8), and to decrease cAMP formation in the pars tuberalis (23). Melatonin appears also to modulate hypothalamic DA release via a Ca^{++}-dependent mechanism (27). In agreement with this, the present results suggest that some of the melatonin-induced changes in hormonal function could be mediated through the serotonergic system of the hypothalamus. Whether, this effect is conducted directly through activation of melatonin receptors or indirectly through modulation of intracellular Ca^{++}-calmodulin binding and ATPase activity (3), needs to be elucidated in more precise studies.

J. M. MÍGUEZ y M. ALDEGUNDE. *Los cambios de la función serotoninérgica hipotalámica pueden mediar los efectos endocrinos de la melatonina.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 52 (4), 239-245, 1996.

Se estudian los efectos inducidos por la administración subcutánea de melatonina en los niveles de tiroxina, corticosterona y prolactina en suero, así como los cambios producidos en el metabolismo de la serotonina (5-HT) en varias regiones del hipotálamo de rata. La melatonina (500 µg/kg, s.c.) se administra en la última fase del escotoperiodo y los animales de los grupos tratado y control inyectado con salino se sacrifican 1 h (19:00 h), 12 h (06:00 h) y 18 h (12:00 h) después. Se observa un efecto inhibitorio de la melatonina sobre los niveles de tiroxina y corticosterona una hora despues del tratamiento. También los niveles de prolactina disminuyen inmediatamente después de la inyección de melatonina, aunque se incrementan, con respecto a los controles, a las 18 h del tratamiento. Las ratas tratadas con melatonina incrementan los niveles de 5-HT en el área preóptica-hipotálamo anterior e hipotálamo medio, así como los niveles del ácido 5-hidroxindol-acético en el hipotálamo medio, 1 h después de la inyección. Estos datos confirman que la melatonina modifica de forma aguda y diferencial el metabolismo serotoninérgico en el hipotálamo anterior y medio, al mismo tiempo que inhibe la secreción hormonal tiroidea, adrenal e hipofisaria. Los resultados se discuten en el sentido de que los cambios en la función serotoninérgica hipotalámica podrían actuar como un mecanismo mediador de los efectos endocrinos inducidos por la melatonina a corto plazo.

Palabras clave: Melatonina, Serotonina hipotálamica, Tiroxina, Corticosterona, Prolactina, Rata.

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