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Further definition of the effect of corticosterone on the sleep–wake pattern in the male rat

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Abstract

It is well known that the activation of the hypothalamic–pituitary–adrenal (HPA) axis can induce alterations in the sleep–wake pattern. Corticotropin-releasing factor (CRF), adrenocorticotropin, and corticosterone are involved in the activation of the axis and each one of them has shown an effect on wakefulness and sleep. Nevertheless, concerning corticosterone, the picture is still controversial. In the present study, we analyzed the effects of a low (LC, 0.2 mg), medium (MC, 2 mg), and high (HC, 4 mg) dose of corticosterone on the 24-h sleep cycle in rats. Results indicate that all doses produce an initial enhancement of wakefulness with a concomitant decrease of slow-wave sleep II (SWS II). This effect was observed within the first hour in all the doses but lasted until the third hour only after the higher doses. When plasma levels of corticosterone were analyzed by high-performance liquid chromatography (HPLC), the highest levels were observed during the first 3 h, which is coincident with an increase in the percentage of wakefulness. Nevertheless, when the overall percentage of the stages was analyzed, LC seemed to induce the opposite effect (decrease of wakefulness and increase of SWS II) than that induced by the two higher doses (increased wake time, decreased SWS II). Rapid eye movement (REM) sleep was not modified at any dose. These data indicate that corticosterone exerts an alerting effect that could be important in the initial stage of the stress response.

Keywords: Corticosterone; Sleep–wake cycle; Rat

1. Introduction

It has been suggested that, in humans, the activity of the hypothalamic–pituitary–adrenal (HPA) axis could influence some features of the sleep pattern (for review, see Friess et al., 1995). Sleep abnormalities, due to clinical alterations of the HPA axis, were restored after corticosteroid replacement therapy (Gillin et al., 1974). Conversely, patients with abnormally high levels of cortisol, slept less and had more nocturnal awakenings before therapeutic reduction in adrenal steroid production (Born and Fehm, 1998; Krieger and Glick, 1974). Furthermore, in the last decade, several reports indicate that stressful situations elicit marked alterations of the sleep pattern in rats, mainly affecting rapid eye movement (REM) sleep (Adrien et al., 1991; Bonnet et al., 1997; Bouyer et al., 1998; Rampin et al., 1991; Vázquez-Palacios and Velázquez-Moctezuma, 2000).

In addition, the administration of glucocorticoids induces a noteworthy reduction of REM sleep (Born et al., 1987, 1989, 1991; Fehm et al., 1986; Feinberg et al., 1984; Friess et al., 1994; Gillin et al., 1972), and some (Born et al., 1987, 1989, 1991; Fehm et al., 1986; Friess et al., 1994), but not all (Feinberg et al., 1984; Gillin et al., 1972) studies agree that the amount of slow-wave sleep (SWS) increased after acute glucocorticoid administration. Discrepancies may be explained by differences in the administration route used (Friess et al., 1995). Studies done in animals tend to support the findings observed in humans. Adrenocorticotropin hormone (ACTH) infusions suppressed REM sleep in intact cats (Koranyi et al., 1971) and rabbits (Cohen et al., 1970; Kawakami et al., 1965; Moser et al., 1996).

Recent studies showed that larger doses of corticosterone were able to decrease the amount of REM sleep (Bradbury et al., 1998). Nevertheless, lower doses seem to have a biphasic effect with an increase of wakefulness 6 h after the administration followed by a decrease 6 h later (Vázquez-

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Palacios and Velázquez-Moctezuma, 2000). In this study, we analyzed the variations in plasma levels of corticosterone after the administration of three different doses, as well as the effects that these treatments induce on the sleep pattern in male rats.

2. Methods

Male adult Wistar rats (300–350 g) were chronically implanted with a standard set of electrodes for electroencephalogram and the electromyogram recording. After surgery under pentobarbital anesthesia, rats were housed individually in recording cages within a sound-attenuated room. They were kept under a normal 12–12 light–dark cycle (lights on 09:00 h), with controlled temperature (22 ± 1 °C), and food and water available ad libitum.

After a 10-day recovery period, rats were habituated to sleep recording conditions for at least 3 days. On the experimental day, rats were randomly assigned to one of the following groups: "control" (CON, 0.01 ml corn oil, $n = 12$), "corticosterone" (Sigma, St. Louis, MO) low dose (LC, 0.2 mg in 0.01 ml oil, $n = 12$), medium dose (MC, 2 mg in 0.01 ml oil, $n = 12$), or high dose (HC, 4 mg in 0.01 ml oil, $n = 12$). In a previous study (Vázquez-Palacios and Velázquez-Moctezuma, 2000) we have analyzed the effect of an LC dose (0.2 mg) that was comparable with changes in plasma levels induced by immobilization stress. In the present study, we chose higher doses of corticosterone in order to strengthen the effects on sleep. Injections were given subcutaneously just before the onset of the light period. Sleep recordings were made for 24 h immediately after each treatment.

All treatments and manipulations were applied under a dim red light. The beginning of the polygraphic recording coincided with the onset of the light period (lights on 09:00 h). Recordings were scored using the criteria of Takeuchi (1970). The presence of wakefulness, SWS I, SWS II, and REM sleep was scored in 10-s epochs. Aside from the duration of each episode, the percentage of each stage in the total recording time (24 h), the latencies to each sleep stage, and the number of awakenings were analyzed.

Twenty additional groups ($n = 5$ each) were used to determine the plasma levels of corticosterone after the administration of vehicle and of the LC, MC, or HC doses. After each treatment, rats were killed by decapitation at 1, 3, 6, 12, and 18 h after the injection and trunk blood was collected. Corticosterone was extracted from plasma and quantified by high-performance liquid chromatography (HPLC) using the modified method of Woodward and Emery (1987).

In brief, blood samples were centrifuged and plasma (1 ml) was mixed with 100 μl of a solution of 19-nortestosterone (5 μg/ml in methanol) as an internal standard. Corticosteroids were extracted into 5 ml diethyl ether-dicloromethane (60:40 v/v) by vortex mixing and immediately centrifuged for 5 min. Supernatant was vortex-mixed with 1 ml HPLC-grade water. After centrifugation, supernatant (3 ml) was evaporated at room temperature under nitrogen. The residue was redissolved in 100 μl of methanol–water (55–45 v/v). The column was equilibrated using HPLC-grade methanol and water (55/45 v/v) at a flow-rate of ml/min. Separations were made at ambient temperature and the eluate was monitored by UV detection at 250 nm. The detection limit of the assay for corticosterone was 0.05 μg/dl, using 1 ml of sample, with a signal-to-noise ratio of 2:1. This detection limit is lower than the levels of corticosterone in adrenalectomized rats (0.5 μg/dl). Interassay and intraassay coefficients of variation were determined using six plasma pools in the range of 2–200 μl/dl (covering the normal rat range and the levels observed after the administration of corticosterone). The coefficient of variation for intraday and interday precision ranged from 1.5% to 2% and from 1.4% to 3.2%, respectively.

Statistical analysis was done using a Kruskal–Wallis ANOVA followed, when significant, by a Dunn test. Percentages were analyzed using a χ^2 test. Data from HPLC were analyzed using an ANOVA test followed, when significant, by the Tukey–Kramer test (Zar, 1984).

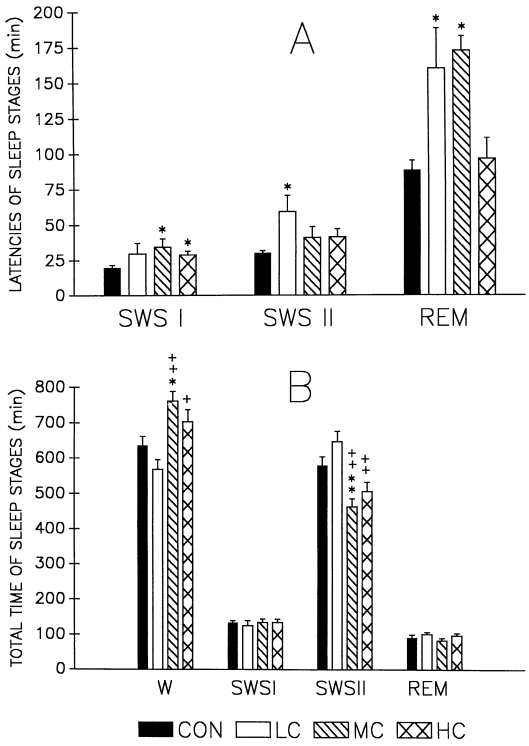

Fig. 1. Effect of LC (0.2 mg), MC (2 mg), and HC (4 mg) dose of corticosterone on latencies (A) and total time (B) of sleep stages. * $P < .05$; ** $P < .01$ compared to the CON. $^{+}$ $P < .05$; $^{++}$ $P < .01$ compared to LC group. Kruskal–Wallis test followed when significant by the Dunn test.

3. Results

Fig. 1A shows the effect of different doses of corticosterone administration on sleep latencies. The LC induced an increase of sleep latencies that reached significance in SWS II and in REM sleep. MC induced a lengthening of latencies to SWS I, and also to REM sleep. HC induced a significant increase of latencies to SWS I only. In panel B, the total time of the different stages in the 24-h sleep recording is displayed. Compared to the CON group, only MC induced a significant increase of wakefulness with a concomitant decrease in SWS II. The LC group showed the opposite tendency significant differences were found when this group was compared with the other two doses. Thus, when compared to LC, both MC and HC showed a significant increase in wakefulness time and a decrease in SWS II time.

Fig. 2 shows the temporal development of the effect of corticosterone administration on sleep. Each panel shows the total time in minutes that the Ss spent in a particular stage. Since plasmatic corticosterone changes were higher during the first 3 h, the graph displays data obtained during the first 3 h. As can be seen, during the first hour, the three doses induced a significant increase of wakefulness (W) concomitant to a decrease in SWS II. During the second and third hour after corticosterone administration, only MC and HC kept this significant increase in wakefulness and the decrease in SWS II when compared to the CON, but also when compared to LC. After the first 3 h, data were pooled in larger intervals. Initially, a 3-h interval (Hours 4–6) and thereafter in 5-h intervals. There were no significant differences in the pooled data of interval of hours 4–12 and only a slight increase in REM time after HC was observed. During the interval of Hours 7–12, LC showed an inverse effect, decreasing wakefulness, and increasing SWS II. MC group showed no differences with CON but it was different when compared to LC. The HC group only showed a significant decrease in wakefulness when compared to the CON and a decrease in SWS II when compared to LC. Significant differences appear again until the last interval (Hours 19–24), where both MC and HC showed a significant increase in wakefulness and a decrease in SWS II when compared to either with CON or LC. In addition, a significant decrease of REM was observed in both MC and HC groups.

Plasma levels of corticosterone after the exogenous administration of different doses are shown in Fig. 3.

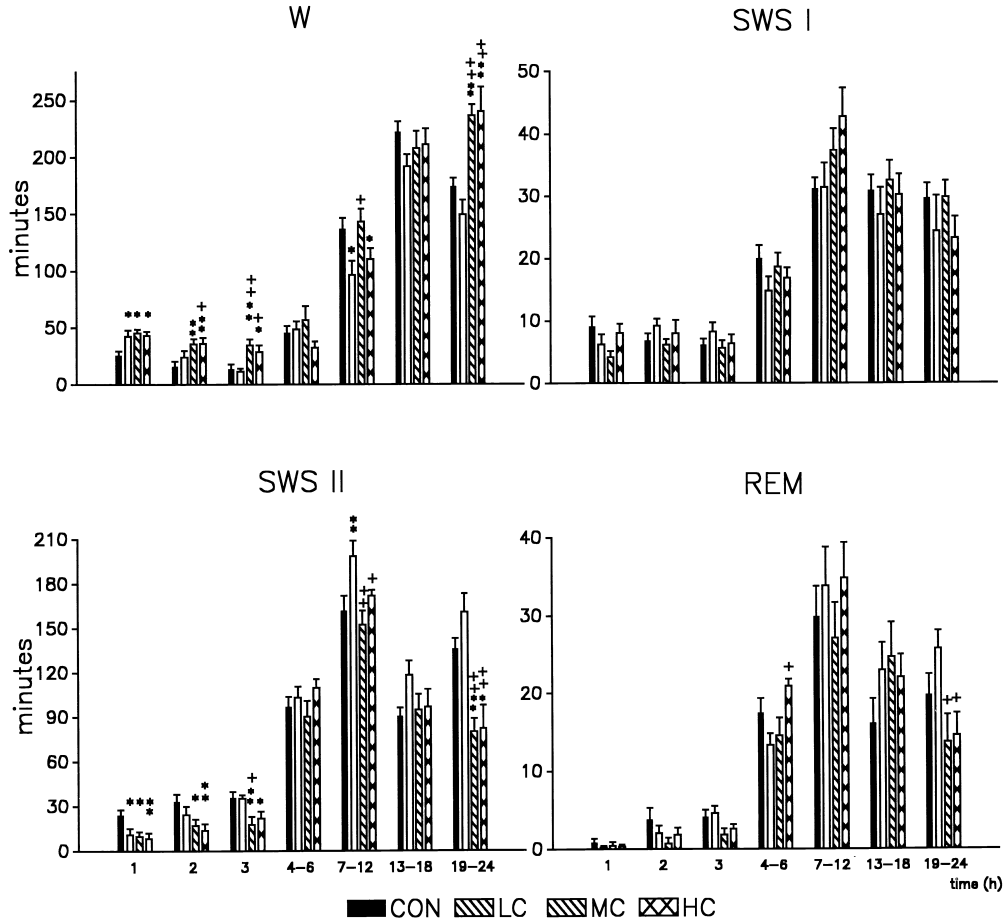

Fig. 2. Temporal variations of total time of sleep stages after the administration of LC (0.2 mg), MC (2 mg), and HC (4 mg) dose of corticosterone. * $P<.05$; ** $P<.01$ compared to the CON. $^{+}$ $P<.05$; $^{++}$ $P<.01$ when compared to LC group. Kruskal–Wallis test followed when significant by the Dunn test.

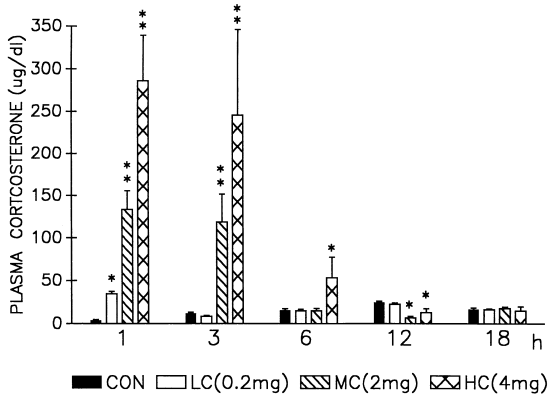

Fig. 3. Plasma levels of corticosterone determined by HPLC after the administration of LC (0.2 mg), MC (2 mg), and HC (4 mg) dose of corticosterone. * $P<.05$; ** $P<.01$ compared to the CON. ANOVA followed by Tukey test.

The administration of the HC dose (4 mg) significantly modified the plasma levels until 6 h after the injection, whereas the MC dose (2 mg) significantly modifies plasma levels only during the first 3 h. Both MC and HC doses showed a slight but significant decrease in plasma levels after 12 h of their administration. The LC dose (0.2 mg) induced a significant increase of plasma levels only during the first hour after its administration.

Table 1 summarizes the results obtained concerning other parameters recorded.

4. Discussion

The present results indicate that the effects of corticosterone administration on the wake–sleep pattern are mainly expressed in the first hours as an increase in wakefulness. This alerting effect is accompanied by a decrease of SWS II. In addition, REM sleep percentage was not affected with any of the administered doses. These data agreed with a recent study in which an immediate increase of wake was observed after forced wakefulness (Garcia-Garcia et al., 1998).

Plasma concentrations of ACTH and cortisol in humans (Gallagher et al., 1973; Krieger et al., 1971), rhesus monkeys (Kalin, 1986), and rats (Magarinos et al., 1988) exhibit circadian fluctuations that may be temporally associated with waking and sleep. Plasma concentrations of ACTH and cortisol/corticosterone display their lowest levels during early sleep and reach their highest levels around the beginning of the active period (Gallagher et al.,

Table 1
Mean (±S.E.M.) of the different phases of sleep–wake cycle for each experimental condition ($n=10$)

	Vehicle	LC (0.2 mg)	MC (2 mg)	HC (4 mg)
Wakefulness				
Total time (min)	623.67±14.74	551.2±18.63*	767.28±27.33**	702.16±28.82*
Percentage	45.31±1.33	37.90±1.29^{+}	53.27±0.23^{++}	48.5±0.24^{+}
Duration (min)	11.56±0.72	9.6±1.33	15.49±1*	17.23±1.25*
Number of episodes	56±1.67	48.62±3.18	50.62±2.87	41.62±2.06*
SWS I				
Total time (min)	117.47±4.43	117.93±8.3	133.83±9.17	136.43±8.78
Percentage	8.19±0.34	8.18±0.57	9.29±0.07	9.42±0.07
Duration (min)	2.23±0.09	1.87±0.27	2.48±0.07	2.51±0.02
Number of episodes	58.41±2.69	68.22±2.53	53.62±2.64	54.25±3.83
Latency (min)	19.97±1.71	29.99±7.41	34.49±5.97*	29.01±2.72*
SWS II				
Total time (min)	600.54±16.66	664.8±14.51*	460.93±21.0**	502.43±15.81*
Percentage	41.7±1.16	46.17±1.00^{+}	32.00±0.18^{+}	34.7±0.21^{+}
Duration (min)	8.62±0.64	6.54±0.88	8.00±0.34	7.94±0.2
Number of episodes	71.33±2.86	101.33±8.21*	57.87±2.64*	63.25±2.99*
Latency (min)	30.3±1.59	59.30±11.28*	40.95±7.63	41.33±5.76
REM sleep				
Total time (min)	100.42±4.32	104.71±5.26	78.1±8.68	106.62±7.42
Percentage	6.97±0.30	7.27±0.36	5.42±0.07	6.88±0.43
Duration (min)	2.17±0.06	1.54±0.17	1.96±0.11	1.81±0.13
Number of episodes	46.58±2.39	64.66±8.44	38.75±2.78	50.5±2.7
Latency (min)	88.06±6.57	160.25±28.28*	172.93±10.14*	95.93±14.80

* Compared to the control group: $P<.05$, Kruskal–Wallis followed by Dunn test.
** Compared to the control group: $P<.01$, Kruskal–Wallis followed by Dunn test.
$^{+}$ $P<.05$, χ^2 test.
$^{++}$ $P<.01$, χ^2 test.

1973; Kalin, 1986; Magarinos et al., 1988). Thus, the present results support the notion of an alertness-promoting effect of corticosterone, suggested by the relationship between its circadian variations and the sleep–wake cycle. In addition, corticosterone administered to adrenalectomized rats increased the amount of active waking and decreased REM sleep during the activity portion of the light cycle compared with adrenalectomized controls (Micco et al., 1980).

In the last decade, several papers have reported the effects of stressful situations on the sleep pattern. Immobilization stress induces a noteworthy increase of REM sleep (Bouyer et al., 1998; Chastrette et al., 1988; Rampin et al., 1991). Induction of helplessness induced a delayed increase of REM sleep (Adrien et al., 1991). However, not all the stressors induced the same sleep changes. Chronic stress in rats showed an initial alerting effect with a loss of both SWS and REM sleep (Kant et al., 1995). Thus, it seems that the effects of stress on sleep depend on the characteristics of the stressor and not only on the activation of the HPA axis, whose last expression is the increase of plasmatic corticosterone levels. In addition, concerning the inhibitory effects of corticosterone on REM sleep that has been consistently reported mainly in humans (Born et al., 1987, 1989; Fehm et al., 1986), we observed a significant decrease only in the last 3 h of the 24-h recording period and only with the higher doses. These differences may be due to differences in species, dose, and administration route.

In natural conditions, aside from its normal circadian variation, the rise of plasmatic corticosterone is observed mainly after the experience of a stressful situation. The activation of the HPA axis involves the release of hormonal factors, such as corticotropin-releasing factor (CRF), ACTH, and glucocorticoids from the adrenal gland, including corticosterone. CRF has proven to be able to promote wakefulness acting both in intra- and extrahypothalamic sites within the brain and also acting in peripheral targets (for review, see Opp, 1995). Moreover, Lewis/N rats that have an hypothalamic gene defect that results in the reduced synthesis and secretion of CRF (Sternberg et al., 1989) do not display the characteristic rise of plasmatic corticosterone before the onset of darkness (Dhabhar et al., 1993). These rats spent less time awake, having also a concomitant increase of SWS (Opp, 1995). On the other hand, it is well known that corticosterone has a strong inhibitory feedback effect on CRF and, therefore, it could be expected that this inhibition of corticosterone on CRF results in an increase of SWS. This could explain the results obtained after the administration of the LC dose that, in addition, induced plasma corticosterone concentrations within the upper range of physiological variations. Concerning the results obtained with the administration of the higher doses that exceeded normal physiological range, it is possible that, as both corticosterone and CRF have alerting effects, the increase of wakefulness prevails despite of the inhibitory action of corticosterone on CRF.

Corticosterone exerts its effects in the brain acting on its receptors that have been subdivided in two classes: mineralocorticoid (MR) and glucocorticoid (GR) receptors, also known as Types I and II, respectively (for review, see Funder, 1991). It has been suggested that normal cyclic variations of corticosterone plasmatic concentrations saturate mainly MR, while higher concentrations, due to a stress response or exogenous corticosterone administration, shifts the balance between GR and MR to a predominant GR activation (De Kloet et al., 1998). As GR are widely located in the brain, it is possible that the alerting effect of corticosterone administration observed in the present study, is due to its interaction with the GR related to alerting brain structures. However, more research is needed to fully elucidate these effects.

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