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Progesterone microinjections into the pontine reticular formation modify sleep in male and female rats

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Abstract

It has been reported that progesterone (P_4) induces changes in sleep, but the brain regions involved in these actions are unknown. We studied the effects of P_4 microinjections into the pontine reticular formation (PRF) upon rat sleep. Intact adult male and ovariectomized female rats were unilaterally injected with P_4 into the PRF and the sleep-waking cycle was recorded for 6 h. P_4 (1.0 and 5.0 μg/0.2 μl) did not modify sleep, but at a higher dose (7.5 μg/0.2 μl) it produced a marked decrease in rapid eye movement sleep (REM) latency in both male (55%) and female (63%) rats. A non-significant increase in the number of REM episodes was observed after P_4 administration. These findings suggest that P_4 should participate in the mechanisms related to REM initiation in the rat through its effects in the PRF.

Keywords: Progesterone; Pontine reticular formation; Steroids; Rapid eye movement sleep; Sleep-waking cycle; Brain stem

Progesterone (P_4) participates in several functions of the central nervous system (CNS) [1]. P_4 produces both short and long-term changes in the function of neurons and glial cells through different mechanisms that involve the interaction of this steroid with intracellular specific progesterone receptors (PR), modulatory sites located in neurotransmitter receptors and ionic channels [16]. By these mechanisms P_4 regulates neuronal excitability and the expression of various genes in the brain. It has also been reported that many effects of P_4 in the CNS are due to its reduced metabolites [5,18].

It has been shown that P_4 possesses hypnogenic properties in mammals [8]. Rapid eye movement sleep (REM) latency diminishes during the luteal phase of the woman menstrual cycle when P_4 levels are high [15], whereas slow-wave sleep (SWS) decreases before menses when P_4 levels are low [9]. Sleep-spindles in women also change during the menstrual cycle [10] and the sleep pattern clearly varies across the three trimesters of pregnancy [3,6]. In adult men P_4 administration diminishes SWS latency and increases Stage 2 sleep [8].

During the estrous cycle of the rat a reduction in nocturnal SWS and REM was observed on proestrus day. Both sleep phases are increased during pseudopregnancy in rats when P_4 levels are elevated [19]. The systemic administration of P_4 to male rats decreases both the amount of wakefulness (W) and the latency of SWS [13]. The brain regions involved in sleep-waking cycle changes induced by P_4 are unknown. Therefore, in order to gain an insight into the regions responsible of the effects of P_4 on sleep, we evaluated sleep-waking cycle after microinjections of this hormone into the pontine reticular formation (PRF), a key region in the regulation of sleep-waking cycle of the rat [4,7]. Since systemic administration of P_4 can modify sleep in both sexes, we decided to evaluate the effects of this steroid both in intact males and in ovariectomized females whose P_4 levels are very low and do not exhibit the variations observed during the estrous cycle [11].

Adult Wistar rats of both sexes (230–260 g) were used in the study. Female animals were bilaterally ovariectomized under ketamine (Rhoné Merieux, Querétaro, México) anesthesia (80 mg/kg, i.p.) 15 days before electrodes implantation for sleep recordings. Electrodes were surgically implanted in all rats under anesthetic conditions as mentioned above. Stainless-steel electrodes were implanted in the frontal and parietal cortices for electroencephalo-

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Table 1
Effects of P_4 microinjections into the PRF on the sleep-waking cycle of the male rat[a]

	Sham injection	Vehicle (0.2 μl)	P_4 (7.5 μg/0.2 μl)
W			
Percentage	30.6 ± 5.3	30.9 ± 5.4	29.7 ± 3.8
Number	31.3 ± 4.9	38.3 ± 2.2	41.0 ± 5.1
Mean duration	4.0 ± 0.7	3.1 ± 0.7	2.9 ± 0.5
SWS			
Percentage	67.7 ± 5.1	66.7 ± 5.4	67.8 ± 3.7
Number	34.7 ± 4.7	41.3 ± 1.6	47.8 ± 3.5
Mean duration	8.0 ± 1.1	6.1 ± 0.4	5.2 ± 0.4
Latency	16.9 ± 4.6	14.3 ± 2..8	12.3 ± 5.2
REM			
Percentage	1.7 ± 0.6	2.4 ± 0.7	2.5 ± 0.7
Number	5.0 ± 0.9	5.2 ± 0.8	8.0 ± 1.2
Mean duration	1.1 ± 0.2	1.6 ± 0.4	1.0 ± 0.1
Latency	127.3 ± 35.0	123.6 ± 19.5	57.1 ± 11.6**

[a] Values are the means ± SEM of six adult intact animals during 6 h of recording. Percentage of each phase refers to the total recorded time. Mean duration of each phase and latency are given in minutes. $^{**}P < 0.05$ compared with sham injection and vehicle (propylenglycol).

graphic (EEG) recording, and in the orbital cavity for electrooculographic (EOG) recording. Electrodes were also inserted into the neck muscles to record the electromyogram (EMG). Besides, a stainless-steel guide cannula (21-gauge) was unilaterally implanted into the PRF 4 mm above the injection site (AP: −9.3, L: 1.2, V: 9.2) [17].

After surgery the animals were allowed 5 days to recover in their home-cage with food and water ad libitum, in a 12:12 h light/dark cycle with lights on at 07:00 h. During the following 5 days they were habituated to the recording conditions. Once the habituation period was completed, six rats of each sex were unilaterally injected with 1.0, 5.0 and 7.5 μg of P_4 (Steraloids, Pauling, NY) in a total volume of 0.2 μl using an injection needle (27-gauge) inserted into the guide cannula connected through a polyethilene tube to a 5 μl Hamilton syringe. The sleep study was carried out for 3 consecutive days: on day 1 of the experiment a baseline sleep recording was performed following a sham injection procedure (just insertion of the needle but no injection). On day 2 the animals were randomly injected with vehicle (propylenglycol) or with P_4. On day 3 the animals previously injected with vehicle received P_4 while those which had received the hormone were injected with vehicle. Immediately after each injection (at 08:00 h) a polygraphic recording was performed for 6 h. The records of the sleep-waking cycle were analyzed visually by a scorer blind to the treatments following established criteria for W (mixed-frequency, low voltage EEG, variable eye movements and high neck EMG), SWS (low-frequency, high voltage EEG, sleep spindles, scarce eye movements and medium muscle tone), and REM (mixed-frequency, low voltage EEG, rapid eye movements and low EMG neck tonus).

At the end of the third recording, Direct blue (Sigma) (1.2 μg/0.2 μl) was injected in order to localize the injection site. When the sleep study was completed animals were injected with an overdose of sodium pentobarbital and transcardially perfused with saline and 3.7% formalin. The brains were removed and postfixed in 3.7% formalin. The injection sites were verified in 80 μm coronal slices by using the atlas of Paxinos and Watson [17].

We evaluated latency (in the case of sleep phases), percentage, number of episodes and mean duration of W, SWS and REM. Repeated measures analysis of variance and post-hoc Student's *t*-test were used for statistical evaluation. Probability values were calculated by using the Prism 2.01 program (Graph Pad, CA).

P_4 microinjections into the PRF (1.0 and 5.0 μg/0.2 μl) did not modify sleep-waking cycle (data not shown) but at a higher dose (7.5 μg/0.2 μl) produced a marked decrease in the latency to REM in male (55%) and female (63%) rats as compared with the vehicle. P_4 did not modify the percentage or the duration of this sleep phase (Tables 1 and 2). Interestingly, a tendency towards an increase in the number of REM episodes both in males (55%) and females (39%), compared with the vehicle, was also observed with the highest dose of P_4; although these changes were not statistically significant (Tables 1 and 2). Neither W nor SWS was affected by P_4.

All P_4 microinjections were located in the nucleus reticularis pontis caudalis (Fig. 1) of the PRF [17]. Although slight differences were noticed in the injection sites, the electrographic recording of the animals was similar in all cases.

Our results suggest that P_4 should be involved in REM

Table 2
Effects of P_4 microinjections into the PRF on the sleep-waking cycle of the female rat[a]

	Sham injection	Vehicle (0.2 μl)	P_4 (7.5 μg/0.2 μl)
W			
Percentage	25.5 ± 2.8	27.9 ± 2.5	20.4 ± 1.0
Number	26.7 ± 3.7	25.7 ± 2.1	25.7 ± 4.6
Mean duration	4.1 ± 1.1	4.2 ± 0.5	3.5 ± 0.7
SWS			
Percentage	69.7 ± 2.7	67.7 ± 2.4	73.3 ± 1.8
Number	34.2 ± 4.0	32.0 ± 2.0	35.8 ± 4.0
Mean duration	8.1 ± 1.1	8.1 ± 0.6	7.9 ± 0.7
Latency	15.6 ± 4.5	22.0 ± 3.8	11.1 ± 4.2
REM			
Percentage	4.8 ± 0.5	4.3 ± 0.5	6.1 ± 1.0
Number	10.2 ± 1.3	8.5 ± 1.2	11.8 ± 1.6
Mean duration	1.8 ± 0.2	1.9 ± 0.1	1.9 ± 0.1
Latency	116.5 ± 18.2	83.8 ± 9.3	43.5 ± 6.1**

[a] Values are the means ± SEM of six ovariectomized animals during 6 h of recording. Percentage of each phase refers to the total recorded time. Mean duration of each phase and latency are given in minutes. $^{**}P < 0.05$ compared with sham injection and vehicle (propylenglycol).

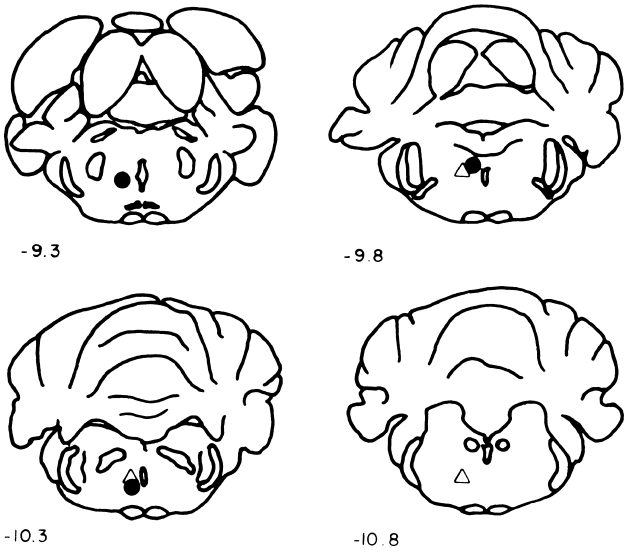

Fig. 1. Location of P_4 (7.5 μg/0.2 μl) microinjection sites in the PRF of male (●) and female (△) rats. Schemes and coordinates were taken from the atlas of Paxinos and Watson [17]. Two injections were located in each marked site. All sites corresponded to the nucleus reticularis pontis caudalis.

regulation through its effects in the PRF. The reduction of REM latency observed during the luteal phase of the woman menstrual cycle [15] when P_4 levels are high is in line with our data since we found that P_4 microinjections into the PRF markedly diminishes REM latency in rats. This reduction as well as the tendency towards an increase in the number of REM episodes after P_4 administration suggest that this steroid hormone in the PRF should be involved in the regulation of REM initiation but not in its maintenance. In addition, that percentage or duration of REM was not affected by P_4.

P_4 induced very similar effects both in male and female rats suggesting that the mechanisms involved in the regulation of REM by P_4 at the PRF level are similar in both sexes. Although all the injections were located in the nucleus reticularis pontis caudalis of the PRF (Fig. 1), the possibility that the induction of REM by P_4 could be due to the activation of other regions such as the nucleus reticularis pontis oralis, produced by the diffusion of the hormone from the injection site cannot be discarded and deserves further investigation.

In contrast with our results, a delay in REM latency after the administration of high doses of P_4 (180 mg/kg, i.p.) was reported in male rats [13]. This difference could be due to both the doses and the route of administration of the hormone. It is also possible that P_4 modulates REM initiation through different pathways in the brain.

Interestingly, it has been shown that the systemic administration of P_4 to male rats produces, like benzodiazepines, both an increase in pre-REM and a reduction in SWS latency [13]. However, we did not find any P_4 effect upon SWS. These different effects could be due to the doses used in both studies. It would be interesting to assess the effect of higher doses of P_4 in PRF and other brain areas upon sleep-waking cycle of the rat.

The mechanisms involved in the induction of REM by P_4 in the PRF are unknown. P_4 effects may be produced through its interaction with PR and the subsequent activation of specific genes in the PRF. This may be supported by a recent report which indicates the presence of PR in brain stem reticular formation of the rat [12].

Possibly, P_4 effects on REM latency may not be directly produced by the hormone but indirectly through its A-ring reduced metabolites such as 5α- and 5β-pregnanolone, which possess hypnogenic properties [13,14]. Hitherto P_4 metabolism in the PRF is unknown and deserves further investigation. The effects of P_4 on REM may be mediated through the activation of cholinergic neurotransmission in the PRF since there is extensive information about the key role of acetylcholine in the initiation and maintenance of REM [2]. The knowledge of the role of P_4 in cholinergic neurotransmission regulation in the PRF could be determinant in elucidating the mechanisms involved in the induction of REM by P_4.

In conclusion, our data indicate that the PRF is involved in the induction of REM by P_4 in both male and female rats.

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