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Intracellular Progesterone Receptors are Differentially Regulated by Sex Steroid Hormones in the Hypothalamus and the Cerebral Cortex of the Rabbit

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The aim of this study was to examine the role of sex steroid hormones in the regulation of intracellular progesterone receptors (PR) in the rabbit central nervous system. We determined PR concentration in cytosol preparations from the hypothalamus, the frontal, tempo-parietal and occipital cortex, by using the specific binding of the synthetic progestin [^{3}H]ORG 2058. PR concentration was higher in the hypothalamus of intact adult females than in that of adult males and prepubertal females, whereas no significant differences were observed in the cerebral cortex of these animals. PR concentration was similar in the three cortical regions analyzed, indicating a homogeneous distribution of PR in the cerebral cortex. The administration of estradiol to ovariectomized animals increased PR concentration in the hypothalamus but not in the cortex. The administration of progesterone to ovariectomized rabbits did not modify PR concentration in any region, however when progesterone was administered after estradiol, it induced a significant diminution in hypothalamic PR concentration without effects in the cortex. These findings suggest that in the rabbit, PR are estrogen regulated in the hypothalamus but not in the cerebral cortex. In the latter, PR are not regulated by progesterone, whereas in the former the estrogen-induced PR are down-regulated by progesterone. Interestingly, hypothalamic PR constitutively expressed in ovariectomized animals are progesterone-insensitive.

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INTRODUCTION

The participation of progesterone (P_4) in several functions of the central nervous system (CNS) is well documented [1–3]. In the CNS, P_4 actions are exerted via different mechanisms that include the interaction of the steroid with either specific intracellular progestin receptors (PR) or with different proteins in the cell membrane as ligand-gated ion channels and G-protein coupled neurotransmitter receptors [4–7]. The effects mediated by intracellular PR usually last hours or days and involve changes in gene expression, whereas the membrane effects of the hormone are produced in seconds or minutes and are related to non-genomic mechanisms.

Two PR populations have been described in the CNS of cyclic ovulators as rodents [8]. One, located in the hypothalamus, is estrogen-regulated; while the other is estrogen-insensitive and is widely distributed in several brain regions such as the amygdala, cerebellum and cerebral cortex [8–10]. Previous studies have demonstrated that both intracellular PR present similar binding and physicochemical properties in the rat CNS [10].

It has been reported that PR content exhibits sex differences as well as variations during development both in the hypothalamus and the cerebral cortex of rodents [11–13]. Although the regulation of PR by estrogens has been established in the CNS of rodents, the role of P_4 in the regulation of its own receptor is not clear, thus there are data that indicate that P_4 can either increase, decrease or not affect the expression of PR [14–16].

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In the rabbit, a reflex ovulator, where P_4 seems to play an inhibitory role in female sexual behavior, as compared to its stimulatory effect reported in rodents [17, 18] there are no data on PR regulation in the CNS. Therefore, in the present study we have evaluated the participation of estradiol and P_4 on PR regulation in the hypothalamus and the cerebral cortex of the rabbit.

EXPERIMENTAL

Radioactive material and chemicals

[^{3}H]16α, ethyl-21 hydroxy-19-nor-4-pregnen-3, 20-dione (ORG 2058) (51 Ci/mmol) was purchased from Amersham International (Bucks., England). Non-radioactive steroids were obtained from Steraloids Inc. (Pawling, NY, U.S.A.) and Amersham International. All chemical reagents were of analytical grade. Radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer (model 2660) using liquifluor (Thomas Sci.) as the counting solution.

Animals and treatments

Prepubertal female as well as adult male and female New Zealand White rabbits were used throughout the study. Animals were maintained in a 12:12 h light–dark cycle with food and water *ad libitum*. When required, adult females were ovariectomized (Ovx) under ketamine anesthesia (80 mg/kg) and 1 week later they were randomly assigned to the following subcutaneous treatments: (1) daily administration of estradiol benzoate (EB) (10 μg/day) for 3 days; (2) the described administration of EB followed by 1 mg of P_4 administered on day 4; (3) a single dose of P_4 (1 mg), and (4) vehicle alone (corn oil). Twenty four hours after the last injection the animals were killed by an air injection in a lateral ear vein, and after decapitation the brain was quickly removed and the hypothalamus, the frontal, tempo-parietal and occipital regions of the cerebral cortex were dissected on ice. Each experimental group consisted of three animals, but in the case of the experiments with the hypothalamus, the tissue corresponding to three animals was pooled for each assay.

Cytosol PR binding assay

Cytosol PR concentration was measured by a method previously described by Cerbón *et al.* [10]. Briefly, tissues were homogenized in a ratio 1:3 (w/v) in TEDGDMo buffer (20 mM Tris–HCl, pH 7.4 at 4°C, 1.5 mM EDTA, 0.5 mM dithiothreitol, 10% glycerol (v/v) and 10 mM sodium molybdate) using a Polytron homogenizer (Brinkman Instruments, Westbury, NY, U.S.A.). Homogenates were centrifuged at 105,000 *g* for 1 h at 4°C in a SW 50.1 rotor (Beckman Instruments, Palo Alto, CA, U.S.A.). Kinetic parameters of [^{3}H]ORG 2058 binding were determined in cytosol aliquots incubated with increasing concentrations of [^{3}H]ORG 2058 (0.5–10 nM) in TEDGDMo buffer for 4 h at 4°C. Other cytosol aliquots were incubated with 2 nM [^{3}H]ORG 2058 in TEDGDMo buffer at 4°C for 4 h. Non-specific binding was measured in similar incubations containing a 100-fold excess of unlabeled ORG 2058. Bound and free radioligand fractions were separated by the addition of a dextran-coated charcoal suspension (0.025% dextran T-70 and 0.25% Norit-A in TEDGMo buffer) and incubated for 5 min at 4°C under continuous shaking. Following centrifugation at 800 *g* for 10 min at 4°C, aliquots of the supernatants were assayed for radioactivity. Specific binding was calculated by subtracting non-specific binding from total binding. Stereospecificity of cortical PR was assessed in cytosol preparations from intact adult female rabbits incubated for 4 h at 4°C with [^{3}H]ORG 2058 (1 nM) in the presence of a range of concentrations (10–500 nM) of different unlabeled steroids.

Saturation binding capacities and dissociation equilibrium constants (K_d) were calculated from Scatchard plots. Cytosol protein content in all samples was determined by Bradford's method [19]. The results were expressed as specifically bound fmoles of [^{3}H]ORG 2058 per mg cytosol protein. The data were statistically analyzed using a two-way analysis of variance (ANOVA) followed by a test of individual differences between means.

Radioimmunoassay for P_4

Serum P_4 concentration was measured in Ovx females treated with P_4 and vehicle as described previously [20]. Anti-P_4 serum was kindly provided by the WHO-Mexico National Reagent Production Programme. The intra- and inter-assay coefficients of variation were 7.6 and 6.5%, respectively.

RESULTS

Incubations of [^{3}H]ORG 2058 with cytosol preparations from the hypothalamus and the cerebral cortex demonstrated the presence of intracellular PR in the rabbit CNS, Scatchard plot analysis of PR binding kinetics showed the existence of high affinity binding sites in rabbit hypothalamus ($K_d = 2.4$ nM) and cerebral cortex ($K_d = 1.6$ nM). The stereospecificity of cerebral cortex PR determined by competitive binding analysis indicated that unlabeled ORG 2058 was the most potent competitor followed by R 5020, P_4 and levonorgestrel (Fig. 1). Estradiol and 5α-dihydrotestosterone did not compete for PR binding sites.

Adult female rabbits showed a similar [^{3}H]ORG 2058 binding in the frontal, tempo-parietal and occipital cortex, although no significant differences were noticed among the three cortical regions analyzed, the lowest PR concentration was observed in the occipital cortex (Table 1). PR concentration in the cortex of adult female animals did not exhibit significant differences compared to that found in adult male and prepubertal female animals (Table 1).

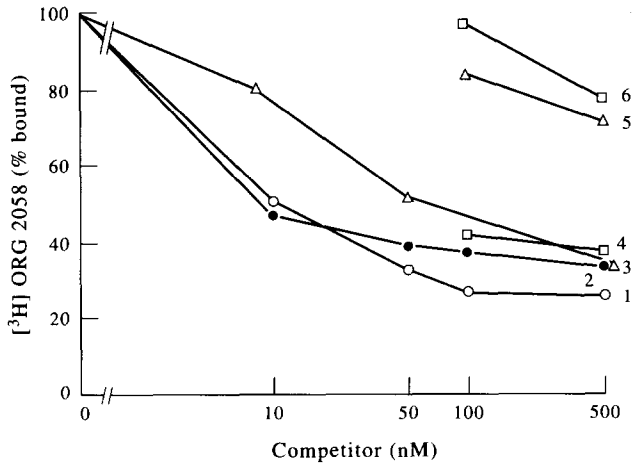

Fig. 1. Competition of non-labeled natural and synthetic steroids for intracellular PR in the rabbit cerebral cortex. PR were labeled with 1 nM [^{3}H]ORG 2058. Increasing concentrations of non-labeled ORG 2058 (1), R5020 (2), P_4 (3), levonorgestrel (4), estradiol (5) and 5α-dihydrotestosterone (6) were added. [^{3}H]ORG 2058 binding to PR in the absence of competitors was set as 100%. Results are expressed as the percentage of [^{3}H]ORG 2058 specific binding. Each point represents the mean value of 3 determinations in triplicate.

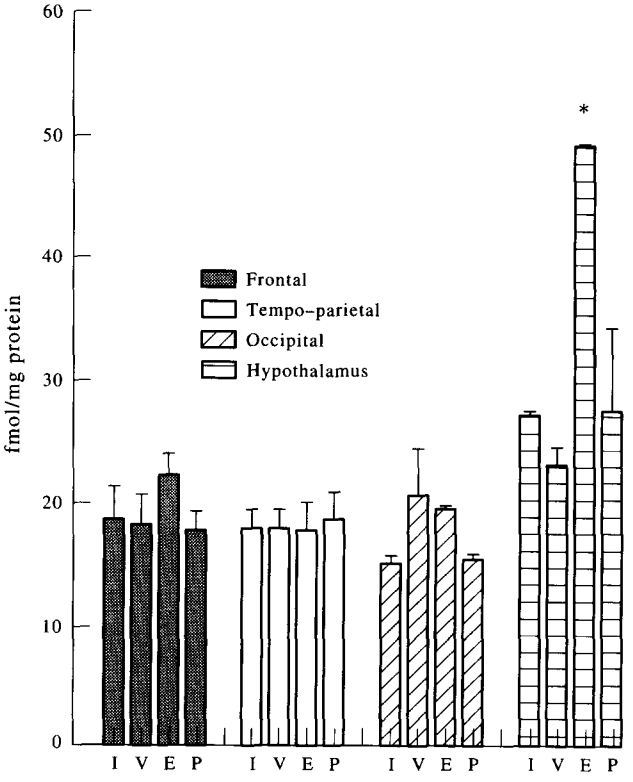

Fig. 2. Effects of EB and EB-P_4 on [^{3}H]ORG 2058 binding in the cerebral cortex and the hypothalamus of adult castrated female rabbits. Results are expressed as the mean ± SEM of 3 experiments in triplicate. (I) intact females; (V) Ovx animals treated with vehicle; (E) Ovx animals treated with EB; (P) Ovx animals treated with EB + P_4 in a sequential manner. *$P < 0.05$ as compared to the hypothalamus of I, V, and P animals.

In contrast, PR concentration in the rabbit hypothalamus, exhibited clear-cut differences among these groups. As indicated in Table 1, [^{3}H]ORG 2058 specific binding in the adult female hypothalamus was significantly higher than that found in adult male and prepubertal female animals.

Gonadectomy of adult female rabbits did not significantly change PR concentration in the different regions of the cerebral cortex (Fig. 2). In addition no differences in PR concentration were observed after the administration of EB, P_4, or the sequential treatment with EB and P_4 to Ovx rabbits (Table 2 and Fig. 2). Administration of EB to Ovx rabbits induced a marked increase on PR concentration in the hypothalamus whereas the administration of P_4 in animals primed with EB resulted in a significant diminution of PR concentration in the hypothalamus (Fig. 2).

In contrast to the diminution observed in hypothalamic PR concentration after the treatment of P_4 to EB primed animals, the single administration of P_4 to Ovx animals without EB treatment did not modify PR concentration in the hypothalamus (Table 2).

The concentration of P_4 in serum samples from Ovx rabbits treated 24 h earlier with P_4 was similar to that observed in Ovx animals treated with vehicle. In both cases P_4 concentration was low, the mean values were 0.72 and 0.50 ng/ml for the Ovx animals treated with P_4 and vehicle, respectively.

Table 1. [^{3}H]ORG 2058 specific binding in the hypothalamus and several regions of the cerebral cortex of male and female intact rabbits

	Prepubertal females	Adult females	Adult males
Hypothalamus	12.96 ± 3.22	26.75 ± 0.32*	17.36 ± 2.82
Frontal cortex	20.94 ± 2.81	18.85 ± 1.81	21.24 ± 1.06
Tempo-parietal cortex	19.72 ± 1.78	17.81 ± 1.60	17.45 ± 1.76
Occipital cortex	17.25 ± 2.67	14.88 ± 0.69	15.00 ± 3.44

Results are expressed as fmol/mg protein and represent the mean ± SEM of 3 experiments performed each one in triplicate (3 animals per group). *$P < 0.05$ compared to the hypothalamus of prepubertal females and adult males.

Table 2. [^{3}H]ORG 2058 specific binding in the hypothalamus and the cerebral cortex of Ovx rabbits treated with P_4

	Vehicle (corn oil)	P_4 (1 mg)
Hypothalamus	21.3 ± 3.7	18.1 ± 3.0
Cerebral cortex	9.6 ± 1.9	13.8 ± 3.4

Results are expressed as fmol/mg protein and represent the mean ± SEM of 3 experiments performed each one in triplicate (3 animals per group). No significant differences were found between the group treated with P_4 and vehicle group.

DISCUSSION

The results demonstrate the presence of specific intracellular PR in different regions of the cerebral cortex and the hypothalamus of male and female rabbits. The binding characteristics and stereospecificity of cortical PR are similar to those found in the cortex of other mammals as well as those described in other peripheral P_4 responsive tissues [5, 21].

The lack of differences in PR concentration of the frontal, tempo-parietal and occipital cortex indicates a homogeneous distribution of intracellular PR, at least in prepubertal and adult animals. It is interesting to mention that Shugrue *et al.* [22] have reported changes in the content and distribution of cortical PR in the mouse developing brain.

The finding that PR concentration in different cortical regions was similar in male and female (both prepubertal and adult) rabbits, suggests that this PR population is not regulated by sex steroid hormones. In contrast, PR concentration in the rabbit hypothalamus exhibited a clear-cut sexual dimorphism, being higher in females than in males. These data confirm and extend similar results found in rodent hypothalamus [23, 24].

A higher hypothalamic PR concentration was also noticed in adult female rabbits than in prepubertal animals. This finding suggests an estrogen modulation of PR and is in line with the low content of these receptors observed in the hypothalamus of immature guinea pigs that may explain the lack of lordosis behavior reported in these young animals after the treatment with EB [25]. Furthermore, the administration of EB to Ovx rabbits induced a significant increase in hypothalamic PR concentration.

The results also revealed that PR concentration in estrogen-primed rabbits, significantly decreased after P_4 treatment in the hypothalamus, whereas no changes were observed in the different cerebral cortex regions, suggesting that after estrogen priming, PR are down-regulated by P_4 in the rabbit hypothalamus but not in the cerebral cortex. Although the diminution of hypothalamic cytosolic PR concentration produced by P_4 could be due to a tight nuclear binding produced by the hormone or to the occupancy of PR by P_4, these possibilities are unlikely, since P_4 concentration in Ovx animals treated with a single dose of P_4 was similar to that found in Ovx animals treated with vehicle. Furthermore, in guinea pig hypothalamus neither depletion of cytoplasmic PR nor an increased concentration of plasmatic P_4 as compared to the oil injection were found, 24 h after P_4 administration [26].

Interestingly, the administration of P_4 to Ovx animals without EB priming did not modify PR concentration in the hypothalamus, suggesting that constitutively expressed PR are insensitive to P_4 regulation. The mechanisms involved in the different regulation of PR by P_4 in the hypothalamus of Ovx EB-primed rabbits and Ovx non-EB-primed animals require further research.

Recently, Savouret *et al.* [27] have demonstrated that the estrogen and P_4 responsive elements in the rabbit PR gene are located in the same region. Whether the different steroid hormone regulation of PR in the CNS is due to the interaction among various tissue-specific factors which results in a differential PR gene expressions is still unknown.

The overall results demonstrate that in the rabbit, hypothalamic PR have a dual estrogen–progesterone regulation whereas cortical PR are steroid hormone-insensitive.

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