Pharmacology Biochemistry & Behavior, Vol. 37, pp. 433–438. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Effects of Progesterone on [^{35}S] *t*-Butylbicyclophosphorothionate Binding in Some Forebrain Areas of the Female Rat and Its Correlation to Aggressive Behavior

MARCELLO CANONACO,[1] ANTONELLA VALENTI AND ADRIANA MAGGI*

Zoology Laboratory, University of Calabria, 87036 Roges di Rende
**Molecular Pharmacology Laboratory, University of Milan, 20121 Milan, Italy*

Received 21 December 1989

CANONACO, M., A. VALENTI AND A. MAGGI. *Effects of progesterone on [^{35}S] t-butylbicyclophosphorothionate binding in some forebrain areas of the female rat and its correlation to aggressive behavior.* PHARMACOL BIOCHEM BEHAV **37**(3) 433–438, 1990.—The antiaggressive effects of progesterone (P) were evaluated in association with alterations in [^{35}S] *t*-butylbicyclophosphorothionate (TBPS; chloride ion channel antagonist) binding in some forebrain sites of the female rat using in vitro quantitative autoradiography. The administration of 4 mg P was followed by a reduction in the frequency of different aggressive behaviors such as circling, nose-to-nose and fighting (mostly of the defensive nature) in ovariectomized (OVX) sexually mature rats, housed in pairs, during male-female encounters. Quantitative autoradiography data revealed that the same P dose, at the forebrain level, was responsible for low [^{35}S] *t*-butylbicyclophosphorothionate binding levels in the medial preoptic area, lateral and basolateral amygdala nucleus and oriens-pyramidalis hippocampus CA1 layer, with even lower values being obtained following the in vitro addition of the potent P metabolite 5 α-pregnan-3 α-ol-20-one. These results suggest that the probable antiaggressive role of P during heterosexual encounters may be regulated by a local potent metabolite acting at the membrane site of the GABA complex.

Aggressive behavior Progesterone Quantitative autoradiography [^{35}S] *t*-Butylbicyclophosphorothionate binding

PROGESTERONE (P) has been implicated in the control of sexual behavior (18, 24, 32) as well as reduction of anxiety (1,22) and aggressive behavior (5,6) in rodents. Other probable behavioral roles of P during sexual encounters and mechanisms of action at the brain level are not fully understood. High doses of P have been shown to induce anesthesia (25) and depress wheel-running activity in food-deprived rats (21). Thus, it is feasible that the reduced anxiety and aggression could be due to the anesthetic properties of P.

At the GABA neuronal level, the potent metabolite of P, 5 α-pregnan-3 α-ol-20-one (DHP), has been described to exert a modulatory role by regulating neurotransmission and membrane excitability (13,28) in a manner similar to the barbiturates (15). There have been reports on P-induced $GABA_A$ (GABA subtype coupled to the benzodiazepine receptor and to a chloride channel supramolecular complex) receptor level changes in the different-forebrain sites of the female rodent (2,14). Recently, the binding of the cagelike convulsant *t*-butylbicyclophosphorothionate (TBPS), GABA antagonist interacting at or near the chloride ion channel receptor (9), has been shown to be displaced by DHP (7,15).

The determination of where P exerts its action at the GABA complex receptor sites would be of great importance in understanding the physiological role of GABAergic neurons in neuroendocrine (12) and behavior (6) activities. It is the aim of this paper to investigate the role played by P in the modulation of aggressive display of the female rat during male-female encounters, in absence (gonadectomized) of an endogenous source of estrogen, and correlate these results to the in vivo and in vitro P-induced TBPS receptor level changes in hypothalamic, amygdala and anterior and posterior forebrain sites. The consequential changes of the GABAergic activity due to a P-chloride ion receptor interaction could play an important role in the reduced aggressive behavior of the female rat during social-sexual activities.

[1]Requests for reprints should be addressed to Marcello Canonaco, Zoology Laboratory, University of Calabria, Via J. F. Kennedy No. 16, Roges di Rende (CS), 87036, Italy.

METHOD

Animals

Sexually immature (approximately 150 g) and mature (approximately 200 g) female rats (Sprague-Dawley, Charles River, Como, Italy) ovariectomized (OVX) 14 days prior to the hormonal treatment were maintained in Plexiglas cages. The animals were maintained either one (n = 20) or 2 (n = 24) per cage at 20–25°C under a reversed light cycle (14-10 hr bright/dim, lights on at 9:00 p.m.). All animals had free access to rat chow and water.

Aggression Test

The effects of progesterone (P) were tested on both sexually mature and immature female rats maintained in both isolated and nonisolated conditions in order to evaluate whether the role of P on aggressive behavior, during heterosexual encounters, may be also linked to social experience (4). Only animals demonstrating sexually receptive (LQ>80) activity 48 hr after SC administration of oestradiol benzoate (10 μg) were included in the study. After a two-week resting period, animals received either 1 mg P (dissolved in sesame oil), a dose proven sufficient to block aggressive behavior (6), or 4 mg P, a dose chosen from preliminary tests that presented neither ataxia nor sedative effects, and confronted to females that only received 0.1 ml sesame oil. The treatments were administered 2 hr before lights-off in the colony room and the tests were performed 4 hr (2 p.m.) after the injection.

The behavioral tests were conducted in a neutral arena (100 × 30 × 40 cm) divided by a sliding door, in the colony room and under dim red light. The female was placed on one side of the arena, while a stimulus male was waiting on the other side, and after a five-minute habituation period the door was lifted and the animals were allowed to come in contact for not more than 10 min or until 10 mounts were attempted by the male. The overall behavioral results of the female were recorded by counting those postures displayed in aggressive behavior (5) which were circling, nose-to-nose position, raising of the back (defensive behavior) and fighting. The sexual behavior tendency as displayed by inarching of the back was also registered. The mean value of each behavioral posture was divided by the number of attempted mounts by the male and expressed in percentage.

In Vitro Quantitative Autoradiography

Following a two-week resting period only sexually mature animals maintained in nonisolated conditions, that presented reduced aggressive behavior, were used for the in vitro receptor binding study. The animals received the same P dose as above and 4 hr (2 p.m.) after injection the rats were decapitated, the brains were immediately removed, frozen using powered dry ice, mounted onto a cryostat chuck and stored in a freezer (−40°C) until sectioning. Coronal brain sections (16 μm) were thaw-mounted onto gelatin-coated slides, transferred to a vacuum desiccator for thorough drying at reduced temperature (0–5°C) for 2–4 hr and stored at −40°C until assay.

The procedures for the in vitro quantitative autoradiography were similar to those of Wamsley *et al.* (31) with some modifications. Mounted sections were subjected to 3 × 10 min preincubation washes in 50 mM ice-cold Tris-HCl buffer (pH 7.4) containing 200 mM KCl and 1 mM EDTA to remove as much endogenous ligand as possible while retaining the integrity of the brain sections. Following the washes, the sections were dried with a stream of cold air and then incubated for 90 min in the same buffer containing 2.0 nM [^{35}S] *t*-butylbicyclophosphorothionate (TBPS; 40.6 Ci/mmol, NEN). Nonspecific binding was determined in separate sections by adding 10 μM picrotoxin (Sigma). The incubation was terminated by a 20-sec rinse in ice-cold buffer, dipped in ice-cold bidistilled water and blown dry with cold air. Dried slides were transferred to cardboard film cassettes along with slides containing tritium plastic standard (Amersham) and a tritium-sensitive Ultrofilm (LKB) sheet was apposed to the sections at room temperature. After an exposure period of 16 days, the film was developed for 5 min in Kodak D-19 developer dipped in a stop solution (4% glacial acetic aid, vol/vol) for 20 sec and fixed for 2 min in Kodak Rapid Fixer at 20°C. The labelled brain sections were stained with cresyl violet for histological verification according to the atlas of König and Klippel (10).

Autoradiograms of tissue sections and Amersham plastic standards were analyzed with a Zeiss image analyzer IBAS 2000.

In Vitro Incubation With 5 α-Pregnan-3 α-ol-20-One (DHP)

In order to investigate whether the P-induced changes of [^{35}S] TBPS binding in the female rat were due to the local effect of its potent metabolite 5 α-pregnan-3 α-ol-20-one (DHP), coronal brain sections (16 μm) of control animals (n = 5) were incubated in the presence of 230 nM DHP (Sigma) ± 10 μM GABA (Sigma) according in part to the methods of Gee *et al.* (7). The final concentrations of the steroid, GABA as well as [^{35}S] TBPS incubation values for both in vivo and in vitro effects were based on preliminary autoradiography and Scatchard analysis of posterior forebrain rat sections [plates 35–43 according to the König and Klippel atlas; (10)]. DHP was initially dissolved in 95% ethanol at a concentration of 2.3 mM and diluted in Tris-HCl buffer to the desired concentration, with the final ethanol concentration in assay being 0.0095%. The brain sections were treated and analyzed in the same manner as described for the in vivo P effects.

Data Analysis

The ANOVA test was used for both behavioral and receptor studies. The significant differences, when appropriate, between control and treatment groups were established using Duncan's Multiple Range test for behavior study and Newman-Keuls' Multiple Range test for autoradiographic evaluations.

RESULTS

Behavior Test

Tables 1 and 2 summarize the average frequencies of the agonistic postures of the female rat, for the doses of 1 and 4 mg P, exhibited during encounters with a stimulus male. Sexually mature females maintained in nonisolated conditions and treated with 4 mg P registered less social investigatory behavior of circling, $F(1,20) = 4.91$, $p<0.01$, while the immature females treated with the same dose demonstrated higher levels of nose-to-nose posture, $F(1,20) = 7.14$, $p<0.05$. The immature control animals maintained in nonisolated contitions presented overall higher behavior scores with respect to control mature animals confirming greater explorative behavior in young immature rats (11). Fighting, which in the case of the female rat is more of the defensive behavior, was less frequent following treatment with 4 mg P for both sexually mature and immature rats, $F(1,20) = 7.01$, $p<0.05$. The raising of back which may be considered a defensive and refusal to male-attempted mounts were observed less frequently in the sexually mature female following the administration of 4 mg P, $F(1,20) = 3.26$, $p<0.05$. Parallel to the low frequency of raising the back, the same animals displayed inarching of the back, $F(1,20) = 9.32$, $p<0.05$ (Table 1), however, not of the deep type observed in lordosis behavior. The animals maintained in isolated con-

TABLE 1

THE EFFECTS OF 1 AND 4 mg PROGESTERONE TREATMENT ON THE DIFFERENT AGONISTIC BEHAVIORAL POSTURES OF BOTH SEXUALLY MATURE AND IMMATURE FEMALE RATS, DURING HETEROSEXUAL ENCOUNTERS, MAINTAINED IN NONISOLATED CONDITIONS

	Groups			
Behavioral Postures	CNMA (n=4)	NMA (n=8)	CMA (n=4)	MA (n=8)
Circling	*73.5 ± 6.0	63.9 ± 7.8	59.3 ± 7.1	57.3 ± 7.6
	†70.8 ± 14.9	60.9 ± 7.2	61.3 ± 12.3	25.4 ± 9.4[2]
Nose-to-Nose	87.3 ± 13.7	71.5 ± 18.7	38.6 ± 11.1	28.7 ± 14.3
	93.5 ± 19.2	62.6 ± 11.2[1]	34.5 ± 12.1	30.3 ± 8.6
Raising of Back	75.0 ± 12.4	66.5 ± 17.0	61.0 ± 13.7	59.8 ± 14.7
	82.3 ± 14.0	68.1 ± 19.6	69.5 ± 11.9	40.8 ± 10.3[1]
Fighting	83.3 ± 10.8	69.4 ± 7.6	61.8 ± 10.2	44.0 ± 14.7
	91.5 ± 17.8	50.8 ± 11.5[1]	66.5 ± 16.9	25.4 ± 12.7[1]
Arching of Back	2.6 ± 2.4	5.5 ± 2.3	7.8 ± 3.7	6.4 ± 3.6
	4.3 ± 4.3	6.5 ± 3.2	5.8 ± 4.5[1]	30.6 ± 13.0

The frequencies of the agonistic behavioral postures in ovariectomized sexually mature and immature rats housed in pairs, for the different progesterone doses, were obtained dividing the value of each posture by the number of attempted mounts by the male and multiplying by 100. The data was analyzed using ANOVA and the significant differences, when appropriate, to the respective controls were evaluated using Duncan's Multiple Range Test. [1]$p<0.05$; [2]$p<0.01$.

Abbreviations: CNMA = immature controls; NMA = immature; CMA = mature controls; MA = mature rats. *1 and †4 mg progesterone dose.

The data are expressed in percentage (Mean ± s.e.m.)

TABLE 2

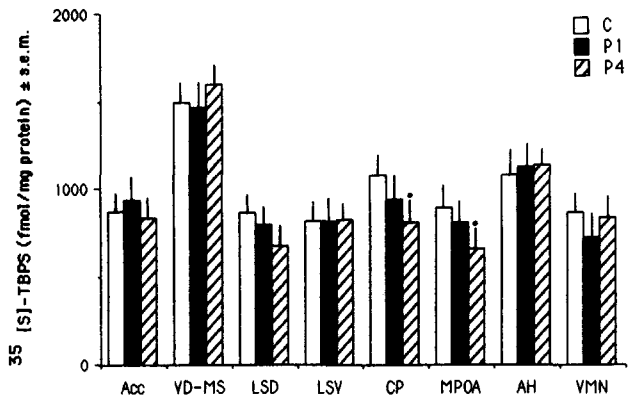
THE EFFECTS OF 1 AND 4 mg PROGESTERONE TREATMENT ON THE DIFFERENT AGONISTIC BEHAVIORAL POSTURES OF BOTH SEXUALLY MATURE AND IMMATURE FEMALE RATS, DURING HETEROSEXUAL ENCOUNTERS, MAINTAINED IN ISOLATED CONDITIONS

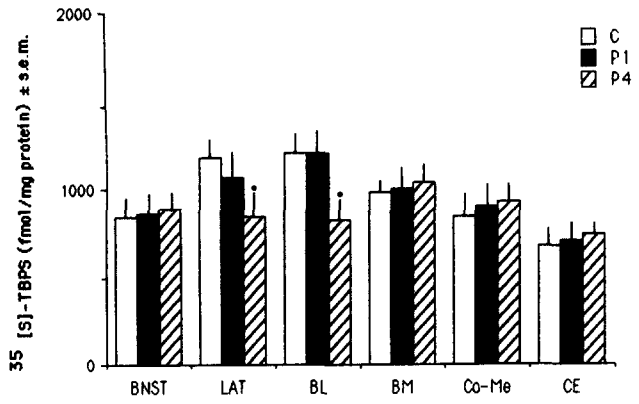
	Groups			
Behavioral Postures	CNMA (n=4)	NMA (n=6)	CMA (n=4)	MA (n=6)
Circling	*74.8 ± 8.3	54.0 ± 6.4	57.0 ± 9.2	47.8 ± 5.5
	†68.2 ± 12.9	48.1 ± 8.3	54.5 ± 13.3	32.5 ± 16.3[1]
Nose-to-Nose	63.2 ± 8.5	56.1 ± 12.2	57.5 ± 10.2	45.1 ± 10.6
	80.7 ± 13.7	63.6 ± 7.3	70.2 ± 9.6	50.0 ± 8.2
Raising of Back	83.2 ± 18.1	80.8 ± 13.2	76.2 ± 10.2	78.3 ± 7.8
	93.5 ± 14.8	84.1 ± 11.3	83.7 ± 12.9	65.8 ± 10.2
Fighting	74.7 ± 10.8	67.0 ± 9.0	66.5 ± 12.7	48.8 ± 10.7
	76.0 ± 10.7	56.0 ± 12.0[1]	72.0 ± 17.2	36.8 ± 8.3[1]
Arching of Back	3.5 ± 3.2	2.5 ± 1.9	4.2 ± 2.9	4.3 ± 2.4
	4.7 ± 4.1	4.5 ± 2.7	6.2 ± 2.9	10.6 ± 2.1

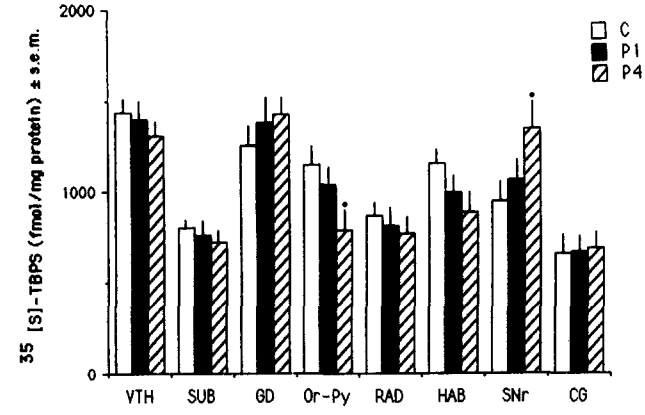
The frequencies of the agonistic behavioral postures in ovariectomized sexually mature and immature rats housed in pairs, for the different progesterone doses. See Table 1 for legend information.

Abbreviations: CNMA = immature controls; NMA = immature; CMA = mature controls; MA = mature rats. *1 and †4 mg progesterone dose.

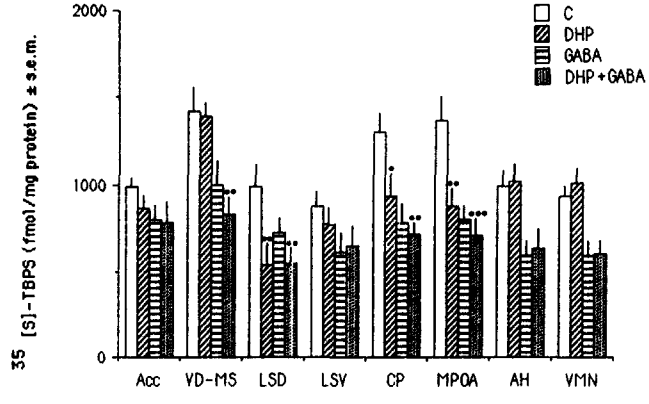
The data are expressed in percentage (Mean ± s.e.m.).

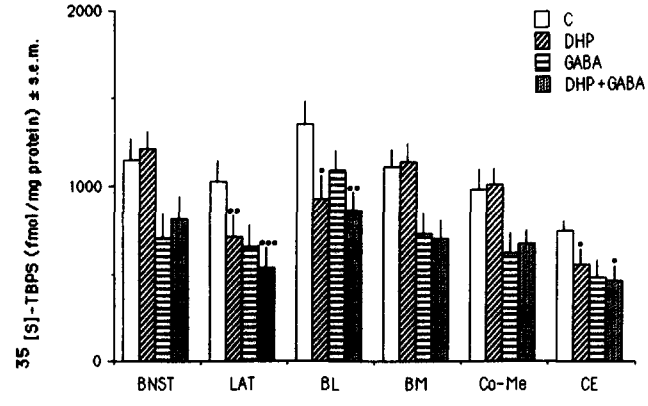


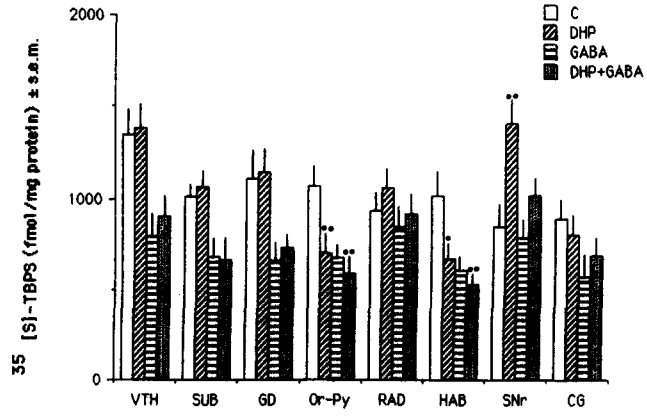




FIGS. 1–3. [^{35}S] *t*-Butylbicyclophosphorothionate binding levels for P treatment in mature female rats housed in pairs: 4 mg P (P4), 1 mg P (P1) control groups (C), as described under the Method section. Figure 1: anterior forebrain area and hypothalamus; Fig. 2: amygdala and stria terminalis; Fig. 3: hippocampus CA1 layer and posterior forebrain areas. Values are mean ± s.e.m. $^{\bullet}p<0.05$, $^{\bullet\bullet}p<0.01$, $^{\bullet\bullet\bullet}p<0.001$ vs. control (ANOVA followed by Newman-Keuls' Multiple Range test). The abbreviations used are: Acc, accumbens nucleus; AH, anterior hypothalamus nucleus; BL, basolateral amygdala nucleus; BM, basomedial amygdala nucleus; BNST, bed nucleus stria terminalis; CE, central amygdala nucleus; CG, central grey; Co-Me, cortico-medial amygdala nucleus; CP, caudate-putamen; GD, gyrus dentate; HAB, medial habenular nucleus; LAT, lateral amygdala nucleus; LSD, lateral septal nucleus dorsal; LSV, lateral septal nucleus ventral; MPOA, medial preoptic area; Or-Py, oriens pyramidalis hippocampus Ca1 layer; RAD, stratum radiatum lacunosum molecular hippocampus CA1 layer; SNr, substantia nigra pars reticularis; SUB, subiculum; VD-MS, vertical limb diagonal band-medial septal nucleus; VMN, ventromedial hypothalamic nucleus; VTH, ventral thalamic nucleus.







FIGS. 4–6. The in vitro effects of the potent P metabolite 5 α-pregnan-3 α-ol-20-one (DHP) + 10 μM GABA (DHP+GABA) on the specific binding levels of [^{35}S] *t*-butylbicyclophosphorothionate of the different control forebrain sections as described under the method section. Values are mean ± SEM $^{\bullet}p<0.05$, $^{\bullet\bullet}p<0.01$, $^{\bullet\bullet\bullet}p<0.001$ vs. control (C), whereas effects of 10 μM GABA (GABA) alone were included for confrontational purposes only. For abbreviations see Figs. 1–3.

ditions displayed more aggressive and investigatory behavior which were blocked following treatment with 4 mg P. The sexually mature animal showed less circling, $F(1,16)=4.91$, $p<0.05$, and less fighting, $F(1,16)=9.22$, $p<0.01$, whereas the sexually immature rat displayed less fighting, $F(1,16)=9.22$, $p<0.05$ (Table 2) following the same P dose.

Progesterone Effects on [^{35}S] TBPS Binding

The in vivo administration of 4 mg P, but not 1 mg P, had

significant inhibitory effects on the binding of [^{35}S] TBPS binding in hypothalamic, amygdala, hippocampus and some anterior and posterior forebrain nuclei that are reported in Figs. 1–3. The specifically bound [^{35}S] TBPS to the GABA-regulated chloride ionophore, under the above conditions, represented 60–75% of total binding in the brain areas studied. Reduced [^{35}S] TBPS binding levels ($p<0.05$) were obtained for the medial preoptic area (MPOA) and caudate putamen (CP) brain sites (Fig. 1). The same P dose was responsible for the low TBP receptor levels in the lateral (LAT) and basolateral amygdala (BL) nucleus (Fig. 2). A reduced TBP receptor concentration ($p<0.05$) was also encountered in oriens-pyramidalis hippocampus CA1 layer (Or-Py), whereas high receptor levels were obtained in the substantia nigra pars reticularis (SNr). The in vitro addition of the P metabolite DHP accounted for even greater effects in the same forebrain areas as well as other brain sites (Figs. 4–6). Lower [^{35}S] TBPS values were demonstrated not only in the MPOA ($p<0.001$) and CP ($p<0.01$), but also in the VD-MS and LSD ($p<0.01$) brain sites in the presence of DHP and GABA (Fig. 4). In the amygdala, the LAT nucleus ($p<0.001$) was the only brain site that presented low [^{35}S] TBPS in a GABA-dependent manner, whereas the BL ($p<0.01$) and also the central amygdala nucleus (CE; $p<0.05$) displayed DHP-induced changes. The DHP activity at the Or-Py level was not of the GABA-dependent ($p<0.01$) type even though lower values were obtained in presence of GABA, while the DHP effect at habenular nucleus ($p<0.01$) required the addition of GABA (Fig. 6). Interestingly, the in vitro DHP as well as the in vivo P effects at the substantia nigra pars reticularis (SNr) site were not of the inhibiting type and furthermore the addition of GABA did not have any enhancing activity, rather it blocked the DHP effect (Fig. 6).

DISCUSSION

In our study 4 mg P induced a reduction of aggressive displays in ovariectomized rats as measured in male-female encounters. In contrast to an earlier study in hamsters (6), 1 mg was ineffective. Interesting to note is that the antiaggressive P effects are substantially greater in mature animals housed in pairs not only with respect to immature animals, maintained in same conditions but are even further evident with respect to rats housed in isolated conditions. These data lend support to the suggestion that social learning processes are influenced during development by social contact (4, 18, 30).

At the brain level of sexually mature animals, it has been shown that the MPOA is a critically important site involved in sex steroid-mediated behavior and that the control of sexual activity, at the MPOA area, is also social contact dependent (11). Olster and Blaustein (18) have also demonstrated that immature animals contained low P receptor levels in the MPOA and this low receptor level may account for the reduced P antiaggressive effects in immature rats. However, the addition of P to the MPOA is able to inhibit aggressive behavior in the female animal (29) suggesting that this forebrain site is very actively involved in the elimination of the agonistic component and facilitation of lordosis behavior. The fact that the antiaggressive role of P was not E dependent tends to also imply that either P receptors not induced by E are involved (19), or that P may be acting in a nontraditional manner [e.g., nongenomic; (2)].

The inhibitory P effects on TBPS binding levels in the hypothalamic and amygdala forebrain areas indicate that a P-chloride ion channel receptor interaction in these sites may be an important mechanism involved in the control of aggressive behavior. At the hypothalamic level it has been shown that elevated receptor sites of the GABA complex such as $GABA_A$ receptor in the MPOA (key reproductive brain site) are correlated to P-induced antiaggressive activity in the female hamster (2), while lesions in this area increased sexual behavior (20); this suggests that P is activating the inhibitory component (GABAergic activity) in this forebrain area which could very well be responsible for the blocking of aggressive behavior during sexual encounter and subsequently, together with the ventromedial hypothalamic nucleus, involved in the maintenance of lordosis behavior (29).

At the amygdala level the reduced chloride receptor activity was encountered in the BL and LAT brain areas. It has been shown that longer term P treatment rapidly inhibits amygdala kindling in young rats (8) and lesions in the medial-cortical (Me-Co) and BL of the female rat proved to be inhibitory for the regulation of aggressive behavior (27,30), while lesions in the posterior area of the LAT promoted sexual receptivity (16). As for the MPOA area, also for the amygdala nucleus the P-induced low TBPS receptor levels could be linked with an increased GABAergic inhibitory activity thus producing an antiaggressive effect. Changes of TBPS receptor levels following P treatment were also encountered in other forebrain sites suggesting that there may be more than the hypothalamic and amygdala nuclei involved with the control of aggressive behavior. Two areas noteworthy of mentioning are the CP and Or-Py, posterior forebrain sites involved with convulsive seizure activity. O'Connor *et al.* (17) have shown low binding levels of the convulsant [^{3}H] *t*-butylbicycloorthobenzoate in the CP of the female rat treated with estradiol. This same steroid has furnished anticonvulsive activity against picrotoxin-induced seizures (26). Furthermore, it has been shown that the administration of more than one P dose is also implicated in the enhancement of $GABA_A$ receptor levels in CP and Or-Py (2). Combined with our data, it is interesting to consider that the protective role of progesterone, during agonistic events, involves the GABAergic complex and in particular is aimed at the chloride ion receptor site of the various forebrain areas in a manner similar to the barbiturates (15).

The fact that greater P effects are evidenced in the above forebrains as well as other sites in the presence of the metabolite DHP plus GABA confirm that the regulatory P behavioral effect is very probably mediated by this potent metabolite at the receptor membrane level, at least in some brain sites. Fluctuating levels of DHP have been demonstrated in brain areas and ovarian plasma during estrous cycle and stressful conditions (1), whereas the administration of DHP to the female guinea pig increased lordosis behavior (3). It is not surprising to observe that the GABA component plays an important role on DHP activity, in most but not all forebrain areas (work to be submitted), since positive efficacy effects of GABA on both TBPS (7) and benzodiazepine [another GABAergic component; (9)] receptor activity have already been demonstrated. In this context, plus the in vivo generation of DHP at neural sites that contain high content of 5 α-reductase and 3α-oxidoreductase (23), it may be tempting to suggest that the antiaggressive P effects, at least in some brain sites, are DHP regulated in a GABA-dependent manner and that this interaction is occurring via a distinct membrane-bound 'steroid site' coupled to the GABA complex (7).

In summary, high doses of P were responsible for the blocking of the agonistic component in the female rat during heterosexual encounters, as well as the lowering of [^{35}S] TBPS binding levels in the different brain areas, indicating that P may be inhibiting aggressive behavior via its interaction with the chloride ion channel receptor component of the GABA complex. Furthermore, the greater receptor level changes in the hypothalamus, amygdala and other various forebrain sites being DHP-induced, even though basically in a GABA-dependent manner, suggests that the P behavior effects could very probably be due to the activity of this potent metabolite DHP.

ACKNOWLEDGEMENTS

We are grateful to Marcello Bernardo for his assistance in the elaboration of statistical data (Macintosh SE computer) and preparation of the manuscript. This work was supported in part by the Pharmaceutical Company Hoffmann-La Roche Milan and M.P.I. 40 and 60%.

REFERENCES

1. Backstrom, T.; Bixo, M.; Hammarback, S. Ovarian steroid hormones. Effects on mood, behavior and brain excitability. Acta Obstet. Gynecol. Scand. (Suppl.) 130:19–24; 1985.
2. Canonaco, M.; O'Connor, L. H.; Pfaff, D. W.; McEwen, B. S. Longer term progesterone treatment induces changes of $GABA_A$ receptor levels in forebrain sites in the female hamster: quantitative autoradiography study. Exp. Brain Res. 77:407–411; 1989.
3. Czaja, J. A.; Goldfoot, D. A.; Karavolas, H. J. Comparative facilitation and inhibition of lordosis in the guinea pig with progesterone, 5α-pregnane-3, 20-dione, or 3α-hydroxy-5α-pregnane-20-one. Horm. Behav. 5:261–274; 1974.
4. Dessi Fulgheri, F.; Lupo, C. Hypothalamic testosterone metabolism during the development of male rats reared in different social environment. Monit. Zool. Ital. 14:104–105; 1983.
5. Floody, O. R.; Pfaff, D. W. Aggressive behavior in female hamsters: the hormonal basis for fluctuations in female aggressiveness correlated with estrous state. J. Comp. Physiol. Psychol. 91:443–464; 1971.
6. Fraile, I.; McEwen, B. S.; Pfaff, D. W. Progesterone inhibition of aggressive behavior in hamster. Physiol. Behav. 39:225–229; 1987.
7. Gee, K. W.; Chang, W. C.; Brinton, R. E.; McEwen, B. S. GABA-dependent modulation of the Cl^- ionophore by steroids in the rat brain. Eur. J. Pharmacol. 136:419–423; 1987.
8. Holmes, G. L.; Weber, D. A. The effect of progesterone on kindling: a developmental study. Dev. Brain Res. 161:45–53; 1984.
9. Johnston, G. A. R. Multiplicity of GABA receptors. In: Olsen, R. W.; Venter, J. C., eds. Benzodiazepine/GABA receptors and chloride channels: Structural and functional properties. New York: Alan R. Liss, Inc.; 1986:57–72.
10. König, J. F. R.; Klippel, R. A. The rat brain: A stereotaxic atlas of the forebrain and lower parts of the brain stem. New York: Robert E. Kreiger Publishing Company Inc.; 1963.
11. Leedy, M. G.; Vela, E. A.; Popolow, H. B.; Gerall, A. A. Effect of prepuberal medial preoptic area lesions on male rat sexual behavior. Physiol. Behav. 24:341–346; 1980.
12. McCann, S. M.; Rettori, J. Gamma aminobutyric acid (GABA) controls anterior pituitary hormone secretion. In: Racagni, G.; Donoso, A. O., eds. GABA and endocrine function. New York: Raven Press; 1986:173–189.
13. McEwen, B. S.; Biegon, A.; Fischette, C. T.; Parsons, B.; Rainbow, T. C. Toward a neurochemical basis of steroid hormone action. In: Martini, L.; Ganong, W. F., eds. Frontiers in neuroendocrinology. New York: Raven Press; 1984:153–176.
14. Maggi, A.; Perez, J. Progesterone and estrogens in rat brain: modulation of GABA (gamma-aminobutyric acid) receptor activity. Eur. J. Pharmacol. 103:165–168; 1986.
15. Majewska, M. D.; Harrison, N. L.; Schwart, R. D.; Barker, T. L.; Paul, S. M. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232:1004–1007; 1986.
16. Mascò, D. H.; Carrer, H. F. Sexual receptivity in female rats after lesion or stimulation in different amygdaloid nuclei. Physiol. Behav. 24:1073–1080; 1980.
17. O'Connor, L. H.; McEwen, B. S. Comparisons of effects of estradiol on GABA receptors and GABA-regulated chloride ionophore binding sites using quantitative autoradiography. Soc. Neurosci. Abstr. 12: 168.7; 1986.
18. Olster, D. H.; Blaustein, J. D. Development of progesterone-facilitated lordosis in female guinea pigs: relationship to neural estrogen and progestin receptors. Brain Res. 484:168–176; 1989.
19. Parsons, B.; Rainbow, T. C.; MacLuskey, N. J.; McEwen, B. S. Progestin receptor levels in rat hypothalamic and limbic nuclei. Neuroscience 2:1446–1452; 1982
20. Powers, B.; Valenstein, E. S. Sexual receptivity: facilitation by medial preoptic lesions in female rats. Science 175:1003–1005; 1972.
21. Rodier, W. I., III; Segal, S. The effect of progesterone on the activity-wheel running of ovariectomized rats. Horm. Behav. 9: 214–221; 1977.
22. Rodriguez-Sierra, J. F.; Hagley, M. T.; Hendricks, S. E. Anxiolytic effects of progesterone are sexually dimorphic. Life Sci. 38:1841–1845; 1986.
23. Rommerts, F. F. G.; Van der Molen, H. J. Occurrence and localization of 5 α-steroid reductase, 3 α- and 17 β-hydroxy steroid dehydrogenase in hypothalamic and other brain tissue of the male rat. Biochim. Biophys. Acta 248:489–502; 1971.
24. Rubin, B. S.; Barfield, R. J. Induction of estrous behavior in ovariectomized rats by sequential replacement of estrogen and progesterone to the ventromedial hypothalamus. Neuroendocrinology 37:218–224; 1983.
25. Seyle, H. Correlation between the chemical structure and pharmacological actions of the steroids. Endocrinology 30:437–453; 1942.
26. Schwartz-Giblin, S.; Korotzer, A.; Pfaff, D. W. Steroid hormone effects on picrotoxin-induced seizures in female and male rats. Brain Res. 476:240–247; 1989.
27. Shibata, S.; Yamashita, K.; Yamamoto, E.; Ozaki, T.; Ueki, S. Effects of benzodiazepine and GABA antagonists on anticonflict effects of antianxiety drugs injected into the rat amygdala in a water-lick suppression test. Psychopharmacology (Berlin) 98:38–44; 1988.
28. Smith, S. S.; Waterhouse, B. D.; Chapin, J. K.; Woodward, D. J. Progesterone alters GABA and glutamate responsiveness: a possible mechanism for its anxiolytic action. Brain Res. 400:353–359; 1987.
29. Takahashi, L. K.; Lisk, R. D. Diencephalic sites of progestin action for inhibiting aggression and facilitating sexual receptivity in estrogen-primed golden hamsters. Endocrinology 116:2393–2399; 1985.
30. Vochteloo, J. D.; Koolhaas, J. M. Medial amygdala lesions in male rats reduce aggressive behavior: Interference with experience. Physiol. Behav. 41:99–102; 1987.
31. Wamsley, J. K.; Gee, K. W.; Yamamura, H. I. Comparison of the distribution of convulsant/barbiturate and benzodiazepine receptors using light microscopic autoradiography. Life Sci. 33:2321–2329; 1983.
32. Whalen, R. E. Estrogen-progesterone induction of mating behavior in female rats. Horm. Behav. 5:157–162; 1974.