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Ring A Reductions of Progestins Are Not Essential for Estrous Behavior Facilitation in Estrogen-Primed Rats

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GONZALEZ-FLORES, O., N. SANCHEZ, G. GONZALEZ-MARISCAL AND C. BEYER. *Ring A reductions of progestins are not essential for estrous behavior facilitation in estrogen-primed rats.* PHARMACOL BIOCHEM BEHAV **60**(1) 223–227 1998.—In Experiment 1 six dose levels (range 0.66-2000 μg) of progesterone (P) and two synthetic progestins with a double bond at C6: megestrol acetate (MA) and chlormadinone acetate (CA), which cannot be reduced at C5, were injected to estrogen-primed (2 μg estradiol benzoate 42 h earlier) ovariectomized (ovx) rats. The three progestins elicited significant lordosis and proceptive behaviors. Potency analysis showed that MA was the most potent progestin for stimulating estrous behavior, followed by P and CA. These results suggest that ring A reduction of progestins to 5α/5β metabolites is not essential for the facilitation of estrous behavior in ovx estrogen-primed rats. Progestins with the 3-ketone group and a double bond at C4 can also be reduced at C3 to yield 3α-hydroxysteroid metabolites potentially capable of stimulating estrous behavior. In Experiment 2, the relevance of the formation of 3α-hydroxysteroid metabolites for estrous behavior facilitation was tested by concurrently injecting indomethacin (1.5 mg), a blocker of 3α-hydroxysteroid oxidoreductase, with 400 μg of P, MA, or CA to ovx estrogen-primed rats. Indomethacin failed to block the stimulatory effect of these progestins on estrous behavior. These results suggest that 3-ketosteroid reduction is also not essential for estrous behavior facilitation by progestins. © 1998 Elsevier Science Inc.

Sexual behavior Lordosis Proceptivity Rat Chlormadinone acetate Megestrol acetate Progesterone Progestins Ring A reduction

IT is generally accepted that the binding of progesterone (P) or other progestins to the progesterone receptor (PR) is the key event for the facilitation of estrous behavior in estrogen-primed rodents (3,5,6,12). Recent studies, however, suggest that an increase in GABAergic activity in some brain regions (11,25,26,27,33) also participates in the stimulation of estrous behavior by progestins. This process appears to be triggered by the interaction of ring A-reduced P metabolites with the GABA-A receptor complex. Therefore, the reduction in ring A of P and other progestins with a 3-ketone group and a double bond at C4 through the action of two enzymes, 5α-reductase and 3α-hydroxysteroid oxidoreductase, present in limbic regions (19,28,29), appears to be of great importance for the facilitation of estrous behavior. Indeed, several findings support this idea. Thus, implants of ring A-reduced metabolites of P, i.e., 3α-hydroxy-5α-pregnan-20-one into the ventromedial hypothalamic nucleus or into the ventral tegmentum, stimulate lordosis behavior in estrogen-primed ovariectomized (ovx) rats (2,3) and hamsters (9), respectively. Moreover, despite that ring A-reduced P metabolites do not bind to the PR (21,38,44), they are more potent lordosis stimulators than P itself when IV injected to estrogen-primed rats (4). Finally, the administration of a 5α-reductase inhibitor reduces the lordogenic response to P in estrogen-primed hamsters (10).

From the above-mentioned studies, it is not, however, possible to determine whether the production of ring A-reduced metabolites is an essential or an accessory process for the facilitation of estrous behavior by progestins. To assess the im-

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portance of reduction at C5 for the stimulation of estrous behavior in rats, in Experiment 1 we tested the effect of two progestins: megestrol acetate (MA) and chlormadinone acetate (CA), which, due to a double bond at C6, are not biochemically reduced at C5 (30,31,42). This structural feature, however, does not prevent their strong binding to the PR (35). Results of Experiment 1 showed that MA and CA stimulated significant estrous behavior, thus showing that reduction at C5 is not indispensable for progestin stimulation of estrous behavior. It is, however, possible that, as P (46), MA, and CA could be directly reduced at C3, yielding 3α-hydroxysteroid metabolites capable of enhancing GABA-A receptor activity (34,47). Such putative 3α-hydroxysteroid metabolites of MA and CA would, consequently, be able to stimulate estrous behavior by such a mechanism. Therefore, in Experiment 2 we assessed the possible role of 3-ketosteroid reduction in the estrus-facilitating action of MA, CA, and P by concurrently administering indomethacin, a potent blocker of 3α-hydroxysteroid oxidoreductase (7,36,40).

METHOD

Animals and Surgical Procedures

Two-hundred ninety-nine sexually inexperienced adult Wistar female rats (240–280 g body weight) were used. They were maintained on a reverse light:dark cycle (14:10; lights off at noon) under controlled temperature (23 ± 2°C). Purina rat chow and water were available ad lib. Subjects (Ss) were ovariectomized bilaterally under ether anesthesia and housed in collective cages (four animals per cage).

Chemicals

Estradiol benzoate (EB), progesterone (P; pregn-4-ene-3,20-dione), megestrol acetate (MA; 17-hydroxy-6-methylpregna-4,6-diene-3,20-dione acetate), and chlormadinone acetate (CA; 6-chloro-17-hydroxy-pregna-4,6-diene-3,20-dione acetate) were purchased from Sigma (St. Louis, MO). Indomethacin was obtained from Research Biochemicals International (Natick, MA).

Testing Procedures

Lordosis and proceptivity were tested by placing Ss in a circular Plexiglas arena containing a sexually vigorous male. Receptivity was quantified by using the lordosis quotient (LQ = [(number of lordosis/10 mounts) × 100)]. The intensity of lordosis was quantified by determining the lordosis score (LS), as proposed by Hardy and De Bold (14). The intensity of each lordosis was rated by using a scale that ranged from 0 to 3. The incidence of proceptive behaviors (hopping, darting, ear wiggling) was also determined. A rat was considered proceptive when showing any of these behavioral patterns.

Experiment 1: Effect of P, MA, and CA on Estrous Behavior in Estrogen-Primed Rats

Treatment. Three weeks after ovariectomy Ss received a SC injection of 2 μg EB (100 μl carthamus oil) followed, 42 h later, by the injection of progestins (P, MA, CA) or vehicle (oil). Six dose levels were used for each progestin: 0.66, 3.3, 16, 80, 400, and 2,000 μg. Volume of injection was 200 μl (carthamus oil). Ss were tested at 2, 4, and 8 h following

TABLE 1

LORDOSIS QUOTIENT (LQ) AND PERCENTAGE OF PROCEPTIVE Ss OBTAINED FOLLOWING THE ADMINISTRATION OF OIL, PROGESTERONE (P), MEGESTROL ACETATE (MA), OR CHLORMADINONE ACETATE (CA) TO OVARIECTOMIZED ESTROGEN PRIMED (2 μg EB) RATS

			2 h		4 h		8 h	
Progestins	Doses (μg)	Number of Ss	LQ	% Proceptive Ss	LQ	% Proceptive Ss	LQ	% Proceptive Ss
Oil	0.2 ml	15	1 ± 5	0	1 ± 3	0	5 ± 16	0
P	0.66	15	3 ± 7	0	14 ± 29	6	7 ± 19	0
P	3.3	18	8 ± 20	5	9 ± 15	0	8 ± 13	0
P	16	20	18 ± 30	20	24 ± 34	5	15 ± 26	5
P	80	18	17 ± 32	11	41 ± 36	11	59 ± 39	22
P	400	21	23 ± 35	14	55 ± 40	28	67 ± 40	43
P	2000	12	29 ± 39	8	51 ± 38	25	61 ± 44	33
MA	0.66	8	20 ± 32	25	32 ± 43	25	9 ± 16	0
MA	3.3	6	16 ± 16	50	41 ± 38	16	35 ± 32	50
MA	16	8	16 ± 31	25	27 ± 39	25	26 ± 45	12
MA	80	10	11 ± 23	10	42 ± 37	40	34 ± 43	30
MA	400	8	12 ± 35	12	60 ± 32	62	74 ± 38	50
MA	2000	8	86 ± 35	87	85 ± 35	87	89 ± 21	87
CA	0.66	7	31 ± 43	0	26 ± 24	0	17 ± 23	0
CA	3.3	12	6 ± 18	0	18 ± 31	0	11 ± 26	0
CA	16	13	3 ± 8	0	13 ± 26	15	8 ± 18	0
CA	80	8	20 ± 31	0	36 ± 43	12	45 ± 49	12
CA	400	9	18 ± 23	0	50 ± 38	0	63 ± 42	22
CA	2000	12	34 ± 37	8	52 ± 35	33	81 ± 29	41

Values are means ± SD.

TABLE 2

RELATIVE POTENCIES OF PROGESTERONE (P), MEGESTROL ACETATE (MA), AND CHLORMADINONE ACETATE (CA) DETERMINED FOR THE LORDOSIS QUOTIENT (LQ), LORDOSIS SCORE (LS), AND PROCEPTIVITY IN OVARIECTOMIZED ESTROGEN PRIMED RATS

	Estrous Behavior					
	4 h			8 h		
Progestin	LQ	LS	Proceptivity	LQ	LS	Proceptivity
P	1	1	1	1	1	1
MA	5	2	21	4	3	3
CA	0.52	0.53	0.021	1	0.9	0.25

progestin or vehicle injections. Number of Ss used in each of the 19 groups is shown in Tables 1 and 2.

Experiment 2: Effect of Indomethacin, a 3α-Hydroxysteroid Oxidoreductase Inhibitor, on the Behavioral Response (Lordosis and Proceptivity) Induced by the SC Injection of 400μg of P, MA, or CA

Treatment. Ss were SC injected with 2 μg EB (100 μl carthamus oil) followed 42 h later, by the SC injection of the indomethacin vehicle (150 μl ethanol) and 400 μg of the progestins: P, MA, or CA (groups 1 to 3). The 400 μg dosage was selected from results obtained in Experiment 1. In groups 4 to 6 indomethacin(1.5 mg) was injected (SC) immediately before the progestins. The dose of indomethacin was selected from previous studies showing its effectiveness for inhibiting 3α-hydroxysteroid oxidoreductase in female rats (40) without causing serious side effects (13). The effect of indomethacin per se was tested in group 7 (EB plus indomethacin and oil). Number of Ss in each of the seven treatment groups is shown in Table 3.

Statistical Tests

Relative potencies of the tested progestins for inducing lordosis were calculated following the procedure described by Tallarida and Murray (41). This involved the calculation of regression lines, analysis of parallelism, determination of a common slope, and calculation of the potency of each progestin in relation to P (reference drug with relative potency = 1). Criterion for accepting parallelism of the various curves was established at a 95% confidence interval. Relative potencies of progestins for inducing proceptivity were determined as described by Tallarida and Murray (41) for quantal dose–response relations. The procedure involves the same steps as those described above for calculating the relative potency of graded responses (lordosis quotient). The only difference is that the values used for the analysis of quantal responses are not the raw data but, rather, their corresponding probit values (probability units). The effect of indomethacin on the lordogenic action of the three progestins used was analyzed by comparing groups treated with progestin plus vehicle with those injected with the same progestin plus indomethacin. A *t*-test was used for this comparison.

RESULTS

Experiment 1

Table 1 shows the LQ and proportion of proceptive Ss obtained at 2, 4, and 8 h following the administration of various doses of P, MA, or CA. Injection of the vehicle alone (carthamus oil) produced negligible lordosis behavior and no proceptivity. For the three progestins used highest LQ and proceptivity values were observed at 4 and 8 h postinjection. Both MA and CA showed monotonic dose response curves, i.e., increasing dose–response relationships, throughout the entire effective dose range employed. P reached a plateau at the dose of 400 μg, a small decrease being observed in both lordosis and proceptivity with the 2,000 μg dose.

Table 2 shows the relative potencies of the three progestins used for eliciting lordosis behavior and proceptivity. MA was

TABLE 3

EFFECT OF INDOMETHACIN (1.5 mg) ON THE ESTROUS BEHAVIOR INDUCED BY 400 μg OF PROGESTERONE (P), MEGESTROL ACETATE (MA), OR CHLORMADINONE ACETATE (CA) IN OVARIECTOMIZED ESTROGEN PRIMED (2 μg EB) RATS

			2 h		4 h	
Group	Treatment	*n*	LQ	% Proceptive Ss	LQ	% Proceptive Ss
1	P + ethanol	8	78 ± 19	87	92 ± 15	75
2	MA + ethanol	8	72 ± 20	87	92 ± 10	100
3	CA + ethanol	8	86 ± 15	87	86 ± 24	100
4	P + indomethacin	12	86 ± 25	92	100 ± 0	100
5	MA + indomethacin	12	72 ± 30	75	85 ± 26	75
6	CA + indomethacin	11	62 ± 72	54	83 ± 29	91
7	Oil + indomethacin	12	21 ± 25	25	25 ± 27	8

Values are means ± SD.

the most potent progestin for stimulating lordosis, followed by P and CA. MA also showed the greatest efficacy, i.e., it induced the largest responses, for both lordosis and proceptivity. P was almost twice as potent as CA for eliciting lordosis behavior at the 4-h test, but at 8 h both progestins were equipotent. A much larger difference in the relative potency of the three progestins was found when analyzing proceptivity. Thus, at the 4-h test MA was 21 times more potent than P and CA was almost 50 times less potent than P. This great difference in potency for eliciting proceptivity was reduced at the 8-h test. Thus, MA was only three times more potent than P which, in turn, was four times more potent than CA at this time interval.

Experiment 2

Table 3 shows the effects provoked on both lordosis and proceptive behavior by the administration of 400 μg of P, MA, or CA alone (groups 1 to 3) and combined with 1.5 mg of indomethacin (groups 4 to 6). As in Experiment 1, the administration of progestins at this dose induced intense lordosis and proceptive behaviors. The concurrent administration of indomethacin failed to inhibit lordosis or proceptive responses to all three progestins. Indomethacin, at the dose used, did not produce any overt changes in locomotor behavior or gastrointestinal motility. Indomethacin per se failed to induce significant lordosis or proceptive behavior (group 7).

DISCUSSION

Kincl (20) has previously studied the effect of a progestin with a double bond at C6 on lordosis behavior. He concluded that CA possessed less than 25% of the potency of P to induce the "copulatory reflex" in estrogen-primed guinea pigs. In the present study, CA was indeed less potent than P to stimulate estrous behavior (particularly the proceptive component), a finding consistent with the idea of the importance of ring A reduction at C5 for estrous behavior facilitation. However, MA, which has the same structural impediment for ring A reduction than CA, was considerably more potent than P for stimulating estrous behavior. These results strongly suggest that full estrous behavior can be stimulated in estrogen-primed rats even when 5α and 5β reduced progestin metabolites are not produced. This conclusion is also supported by the fact that R5020, despite its limited reduction at C5 [due to the presence of a double bond at C9; (35)], potently stimulates estrous behavior in estrogen-primed rodents (5,6,43). Our results also suggest that 3-ketosteroid reduction is not essential for the display of estrous behavior following the administration of P, MA, or CA to estrogen-primed rats. Thus, indomethacin, at dosages known to block 3-ketosteroid reduction in vivo (45), failed to decrease the lordogenic action of the progestins used. This result agrees with the previous observations of Rodríguez-Sierra and Komisaruk (37) and Hall and Luttge (13), who failed to block the lordogenic effect of P in ovx, EB-primed rats by the previous administration of similar or larger dosages of indomethacin than the one used in this study.

It is theoretically possible that MA or CA facilitated estrous behavior by stimulating the synthesis of 3α-hydroxysteroid metabolites, i.e., 3α-hydroxy-5α-pregnan-20-one from the adrenal glands or the brain itself. Thus, it is well established that the brain can synthetize 3α-hydroxy-5α-pregnan-20-one (1,19) and that this progestin is normally secreted by the adrenal gland of the rat (17). However, brain 3α-hydroxysteroid oxidoreductase activity is not affected in rats by either castration, adrenalectomy or the administration of progestins (8). Moreover, brain 3α-hydroxysteroid oxidoreductase activity is not altered by the drastic changes in progesterone concentration occurring during pregnancy (8,19,24). Furthermore, in vitro studies have shown that progestins with a 3-ketone group and a double bond at C4, if anything, inhibit rather than enhance the activity of 3α-hydroxysteroid oxidoreductase (7,32). From the above-mentioned data it appears unlikely that the progestins with a 3-ketone group and a double bond at C4 used in this study acted through the endogenous production of 3α-hydroxy-pregnanolones which, in turn, would facilitate lordosis by interacting with the GABA-A receptor complex. This conclusion is also supported by our finding that indomethacin, an inhibitor of the conversion of P to 3α-hydroxysteroid derivatives (40,45), failed to interfere with the estrus-facilitating action of P, MA, and CA in our estrogen-primed rats.

The present results may imply that strong binding to the PR, as that occurring with MA and CA, is sufficient to facilitate normal estrous behavior in estrogen-primed rats. The activation of GABA-A receptors by ring A-reduced P metabolites, though probably important under normal conditions, is not an essential but, rather, an accessory process for the facilitation of estrous behavior by progestins. It is, however, possible that MA or CA, aside from binding to the PR, may have interacted with the GABA-A receptor complex to facilitate lordosis. Although pregnanes reduced in ring A are the most potent ones for modulating GABA-A receptors (15,16,23), progestins with a 3-ketone group and a double bond at C4 can also activate the GABAergic system. Thus, P per se enhances muscimol binding in a preparation of rat cerebral cortex membranes (22), it increases GABA-induced chloride currents in voltage-clamped neurons (48), and it increases the effect of GABA agonists on neuronal firing in the cerebellum (39). Indeed, in some brain areas, i.e., frontal cortex, P is more potent than its ring A-reduced metabolites to enhance muscimol binding to the GABA-A receptor (18).

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