Pharmacology Biochemistry & Behavior, Vol. 30, pp. 829–833. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Medroxyprogesterone Acetate and Tamoxifen do not Decrease Aggressive Behavior in CF-1 Male Mice[1]

NEAL G. SIMON[2] AND MICHAEL PERRY

Department of Psychology, Lehigh University, Bethlehem, PA 18015

Received 13 October 1987

SIMON, N. G. AND M. PERRY. *Medroxyprogesterone acetate and tamoxifen do not decrease aggressive behavior in CF-1 male mice.* PHARMACOL BIOCHEM BEHAV **30**(4) 829–833, 1988.—Intact CF-1 male mice were given daily injections of either medroxyprogesterone acetate (MPA, an antiandrogen), tamoxifen (TAM, an antiestrogen), or the two drugs in combination and tested for aggressive behavior toward bulbectomized stimulus males. None of the treatments decreased fighting behavior over a 20-day test period and the presence of TAM led to increased aggression even in the presence of MPA. Testis weight was reduced by MPA while both compounds decreased seminal vesicle weight. The mechanisms involved in the observed effects are considered as well as the implications of the results for the clinical use of these compounds as modulators of testosterone-facilitated behaviors.

Aggression Testosterone Antiandrogen Antiestrogen Mice

MALES that have exhibited sexually aggressive or deviant behaviors such as rape, pedophilia, or exhibitionism often have received antiandrogen therapy as part of their treatment [4, 7, 8, 23]. The basis for this intervention strategy is the well documented relationship between testosterone (T) and the display of male-typical aggressive behavior established primarily through basic research with rodents. Studies conducted over the past 10 years, however, indicate that the intracellular metabolism of T may be an important step in the facilitation of aggression. Specifically, estradiol (E_2), the aromatized metabolite of T, is now recognized as an aggression-promoting agent [2,9]. And in a recent report, it was shown that T can activate aggression in castrated male mice via either an androgenic or estrogenic pathway as a function of genotype [32].

The finding of multiple, genetically determined regulatory pathways for the activation of T-dependent aggressive behavior may have important implications for endocrinological interventions designed to reduce violent displays or sexually deviant behavior in human males. First, it suggests that antiandrogen therapy alone may be inadequate in some cases since it is possible that an estrogenic pathway also may be functional. This could account for the variable behavioral suppression obtained with medroxyprogesterone acetate (17-hydroxy-6-methyl-pregn-4-ene-3,20-dione acetate), an antiandrogen that works through androgen receptor antagonism and an antigonadotrophic action [7, 8, 27]. A second implication is that antiestrogen or combined antiandrogen-antiestrogen therapy may be useful in some cases.

In the following study we examined the effect of antiandrogen (MPA), antiestrogen (tamoxifen, TAM) or combined antagonist treatment on aggressive behavior using CF-1 male mice as a model. This strain was selected because it has both androgen- and estrogen-sensitive activational pathways [32] and thus provides a useful system for assessing the utility and/or limitations of steroid antagonists as potential aggression-reducing agents. In addition, testicular and seminal vesicle weights also were examined as an index of the drug effects on peripheral target tissues.

GENERAL METHOD

Animals

CF-1 male mice bred from stock obtained from the Charles River Breeding Laboratories (Wilmington, MA) were used. The animals were reared in same sex litters, were weaned at 21 days of age, and remained housed with littermates in 28×18×13 cm polypropylene cages lined with cedar

[1]Supported in part by BRS Grant 7R071731, NSF Grant DMB8605447, NIH Grant NS24974 and the Lehigh University Research Fund.

[2]Requests for reprints should be addressed to Neal G. Simon, Ph.D., Department of Psychology, Building #17, Lehigh University, Bethlehem, PA 18015.

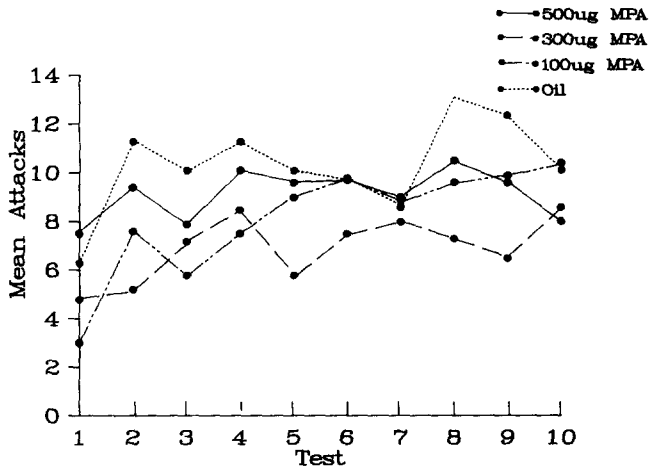

FIG. 1. The mean number of attacks exhibited by intact CF-1 males while receiving daily treatment with either oil, 100, 300, or 500 μg medroxyprogesterone acetate.

shavings. Food and water were available in excess and the colony was kept on a 12:12 hr light/dark cycle with lights on at 0700 hr. All maintenance procedures were in compliance with Federal guidelines for animal care.

Aggression Tests

The animals were housed individually when they were between 80–90 days of age and tests for aggression toward an olfactory bulbectomized stimulus male were initiated 48 hr later. Tests, conducted between 1800–1900 hr, were given every other day for 10 min over a 20-day period. Bulbectomized males were used because while neither initiating fights nor responding to attack by fighting back, they reliably elicit aggression comparable to that of an intact male [13]. This procedure allows any observed aggression to be reliably ascribed to the experimental male. A short form index, which consists of the sum of threats and biting attacks, was used to measure aggressive behavior [31]. After the final test, a random sample of 6 males from each group was autopsied to determine the morphological effects of the treatments. Testes and seminal vesicles were removed, trimmed of excess fat, and weighed wet to the nearest mg. Data were expressed as a percentage of total body weight.

EXPERIMENT 1

The mixed results obtained clinically with MPA suggest that other hormonal factors should be considered. Specifically, E_2 may function in neural tissues as an aggression-sustaining agent in intact males. The following experiment was designed to illustrate this point by exposing intact CF-1 males to MPA. Because this strain of mice exhibits male-typical aggression in response to *either* androgenic or estrogenic stimulation [32], it was predicted that MPA would not decrease the display of fighting behavior.

METHOD

Forty intact CF-1 males were divided randomly among the following treatments: either 100, 300 or 500 μg MPA/day or oil vehicle. Injections (0.05 cc) were administered SC daily beginning on the day the males were housed individually and continued throughout the test period. Intact males were used throughout the study to parallel clinical administration in human males. The dose range was selected to include the lowest and highest behaviorally effective treatments reported in rats and were adjusted for body weight [5,6].

TABLE 1

A SUMMARY OF THE EFFECTS OF MPA, TAM, MPA + TAM, OR OIL VEHICLE ADMINISTERED FOR 22 DAYS ON TESTIS AND SEMINAL VESICLE WEIGHT IN INTACT CF-1 MALE MICE

Treatment	Body Weight (g) (mean ± SEM)	Testis Weight (mean ± SEM)[1]	Seminal Vesicle Weight (mean ± SEM)[1]
MPA			
100 μg	32.1 ± 0.40	0.63 ± 0.01*	0.65 ± 0.04*
300 μg	31.0 ± 0.39	0.61 ± 0.04	0.55 ± 0.03
500 μg	32.2 ± 0.32	0.57 ± 0.02	0.46 ± 0.02
Oil	33.0 ± 0.17	0.68 ± 0.02	0.70 ± 0.03
TAM			
50 μg	34.0 ± 0.16	0.60 ± 0.02	0.57 ± 0.04†
200 μg	34.7 ± 0.35	0.58 ± 0.02	0.50 ± 0.02
400 μg	34.2 ± 0.46	0.65 ± 0.04	0.44 ± 0.04
Oil	34.0 ± 0.22	0.62 ± 0.04	0.78 ± 0.04
MPA + TAM			
500 μg + 400 μg	34.2 ± 0.33	0.67 ± 0.02	0.28 ± 0.03
Oil	33.9 ± 0.32	0.71 ± 0.04	0.62 ± 0.02

*Significant difference among groups ($p<0.05$).
[1]Data are expressed as a percentage of total body weight.

RESULTS AND DISCUSSION

Aggressive behavior shown during MPA treatment is depicted in Fig. 1. A repeated measures analysis of variance revealed that while the amount of fighting behavior differed across the days of testing, $F(9,324)=4.67$, $p<0.05$, there was no significant effect of MPA dosage nor was there a dose × day interaction. Because dose effects were not observed, a second analysis was conducted with the drug groups pooled. The results (not shown) were the same as above; i.e., aggressive behavior varied over the test period but was unaffected by drug treatment. Again, there was no significant drug × day interaction.

The morphologic data are summarized in Table 1. Treatment with MPA caused a significant decrease in testis weight, $F(3,20)=3.72$, $p<0.05$. Post hoc comparisons using Scheffe's test ($\alpha=0.05$) revealed that the 500 μg group was significantly lower than the oil group. In addition, a trend analysis showed that testis weight decreased linearly as a function of dosage, $F(1,20)=10.7$, $p<0.05$.

The analysis of seminal vesicle weights produced similar results. There was a significant overall difference among the four groups, $F(3,20)=9.4$, $p<0.05$. Multiple comparisons revealed that the tissues from the 300 μg and 500 μg groups were significantly lighter than those from the oil group. Further, the 500 μg dosage produced significantly greater effects than the 100 μg dose. As also seen with testis weight, a linear relationship existed between dosage and the extent

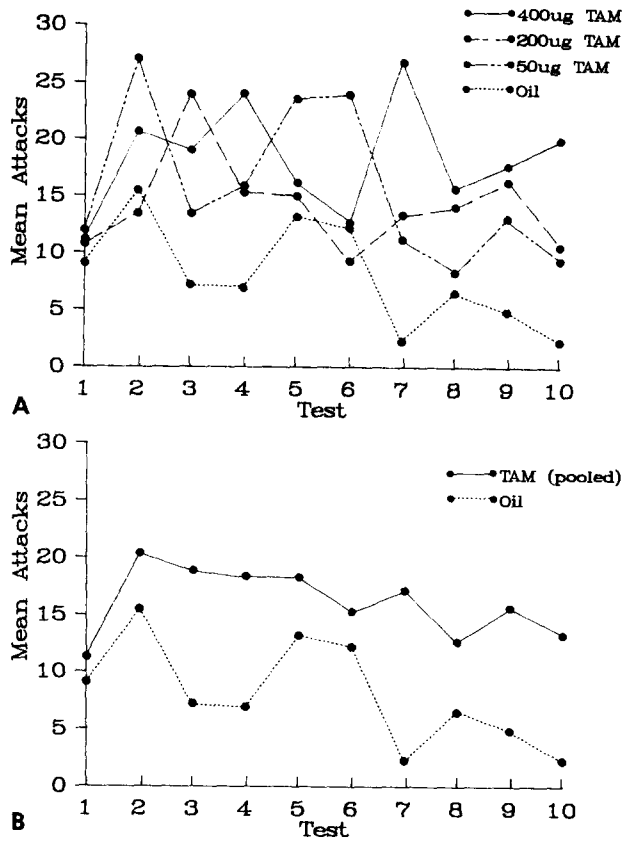

FIG. 2. The mean number of attacks exhibited by intact CF-1 males while receiving daily treatment with tamoxifen. (A) Behavior of each group is shown separately; (B) data were pooled across the 3 tamoxifen doses.

of suppression of seminal vesicle weight, $F(1,20)=28.0$, $p<0.05$.

While the morphological effects of MPA were in accord with the literature [1, 17, 22, 25], no change in aggressive behavior was noted. This lack of effect is likely due to the fact that MPA, an antiandrogen, would not interfere with an estrogen-responsive regulatory system for aggression. In the next experiment, therefore, the effect of antiestrogen treatment on fighting behavior was examined.

EXPERIMENT 2

Tamoxifen (2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-N,N, dimethylethanamine) is a potent antiestrogen that works by competing for target tissue receptors. It blocks the ability of E_2 to activate lordosis in rats [15] and also has been used clinically to treat estrogen-dependent tumors [21, 28, 33]. Based on these data, it was felt that TAM would suppress aggression if E_2 was the primary activating agent. While such findings would be consistent with results obtained with some strains of mice [10,20], it was not necessarily expected that TAM would decrease aggression in CF-1 males. This is because castrated males from this strain have the capacity to exhibit fighting behavior in response to specific androgenic stimulation [32].

METHOD

When the males were individually housed, they were divided among the following treatments (n=10/group): daily injections of either 50, 200, or 400 μg tamoxifen citrate (Sigma) or oil vehicle only. These doses were derived by converting an effective dose for suppression of estrogen-dependent behavior in female rats (approximately 5 mg/kg [15]) and then expanding the range to include lower (1.4 mg/kg) and higher (11.4 mg/kg) values. All other aspects of the procedure were as described in Experiment 1.

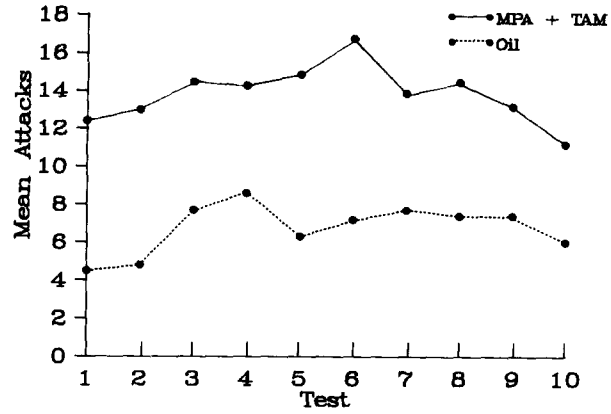

FIG. 3. The mean number of attacks exhibited by intact CF-1 males while receiving daily treatment with 500 μg MPA + 400 μg TAM.

RESULTS

The behavioral findings are shown in Fig. 2. A repeated measures analysis of variance revealed that fighting behavior differed significantly across the days of testing, $F(9,324)=2.31$, $p<0.05$. However, there were no significant differences among the groups nor was there a significant drug × day interaction. A subsequent analysis, in which the 3 TAM doses were pooled, did show a significant drug effect, $F(1,18)=4.98$, $p<0.05$. Interestingly, the TAM group showed increased levels of fighting behavior relative to the vehicle group.

Tamoxifen did not suppress, and may have increased aggression in CF-1 males. The failure of antiestrogen treatment to modulate fighting behavior suggests that a functional androgen-sensitive regulatory pathway is sufficient to maintain normal levels of aggression. Alternatively, TAM may be acting as an estrogen receptor agonist under these conditions or could, in some fashion, be facilitating the androgen-based system.

The effects of TAM on testis and seminal vesicle weights are shown in Table 1. A one-way analysis of variance demonstrated that testis weight was not significantly different among the groups. Seminal vesicle weight was significantly decreased by TAM, $F(3,20)=15.9$, $p<0.05$. Post hoc comparisons using Scheffe's test showed that the 3 TAM groups, while not differing from each other, had significantly smaller seminal vesicles in comparison to the oil group. Whether there was a dose-related effect of TAM was examined through a trend analysis. The results showed a significant linear relationship between the decrease in tissue weight and treatment dosage, $F(1,20)=43.05$, $p<0.05$. The decreased size of an androgen-dependent target tissue in response to antiestrogen treatment is consistent with the observation that estrogens can induce cellular hypertrophy in the seminal vesicles [3,24].

EXPERIMENT 3

The display of aggression by CF-1 male mice apparently can be sustained by either androgens or estrogens alone. Therefore, whether a combined antiandrogen-antiestrogen regimen would decrease fighting behavior was examined in the following experiment. The highest doses of MPA and TAM were combined to maximize the possibility of producing behavioral suppression.

METHOD

When housed individually, the males were assigned to either MPA + TAM (500 μg + 400 μg, respectively) or oil vehicle only. All remaining procedures were as described previously.

RESULTS

Aggressive behavior exhibited by MPA + TAM males is shown in Fig. 3. A repeated measures analysis of variance showed that the groups differed significantly in the amount of fighting behavior, $F(1,18)=5.47$, $p<0.05$. Interestingly, the effects were opposite those that were anticipated, i.e., the drug treatment led to increased levels of aggression. The remainder of the analysis showed no significant change in fighting over the test period nor was the drug × day interaction significant.

Morphologically, the MPA + TAM treatment did not significantly decrease testis weight ($t_{10}=1.4$, ns) but did lower seminal vesicle weight ($t_{10}=8.6$, $p<0.05$.

GENERAL DISCUSSION

The administration of MPA or TAM, either alone or in combination, failed to suppress fighting behavior in CF-1 mice but did decrease the size of androgen-dependent peripheral tissues. These findings were in accord with expectations with two exceptions. First, it was anticipated that combined antiandrogen-antiestrogen therapy would decrease aggression. As seen in Experiment 3, aggressive behavior was actually increased by the treatment. And second, TAM apparently increased aggression in CF-1 males (Experiment 2). While this latter result may partially explain the failure of the combined treatment to modulate fighting, the finding of behavioral facilitation by TAM suggests that its antagonist properties may be limited to certain physiological responses (e.g., uterine growth, lordotic behavior).

The lack of effect of MPA, an antiandrogen, on fighting behavior in CF-1 males is not surprising in light of numerous studies that have demonstrated that estrogens, as a product of aromatization, can promote aggression in many strains of mice, including CF-1's [10, 14, 30, 32]. If this is a viable explanation for the observed results, however, it is necessary to consider how MPA has successfully lowered aggressiveness in some human males. Two explanations are available. One is that the endocrine contribution to aggressiveness in humans is solely via an androgen-responsive system. If true, MPA would be an effective aggression-suppressing agent. However, aromatization occurs in humans [29] and sexual behavior has been maintained in a castrated male by combined estrogen-progestin treatment [12]. These observations suggest that the existence of *only* an androgen-responsive system in humans is unlikely and, like mice, multiple pathways are probably available.

How then, does MPA produce its desired clinical effect? A review of the literature on a range of conditions shows that a positive response to MPA, i.e., a decrease in violence, violent fantasies, or deviant behavior, is seen when MPA decreases testosterone levels to less than 250 ng/dl, a hypogonadal state [11, 16, 22, 23, 26]. This observation, when combined with the likelihood that estrogens contribute to the maintenance of masculine behavior, suggests that the primary mechanism mediating the ability of MPA to modulate certain behaviors may not be its antiandrogenic effect in neural tissues but rather its ability to inhibit T synthesis coupled with its antigonadotrophic action.

Tamoxifen treatment resulted in enhanced aggression when given alone or in conjunction with an antiandrogen. Had fighting behavior continued at control levels when TAM was given alone, the results could have been attributed to the function of an androgen-responsive pathway in CF-1 mice [32]. However, the increased aggression seen with or without MPA suggests that TAM was acting as an agonist in the mouse CNS. Interestingly, other investigators [18,19] have characterized TAM as an agonist in mice based on its uterotrophic actions. However, Pavlik and co-workers [28] recently showed that TAM antagonized uterine growth in CF-1 mice when given in low doses and in conjunction with estradiol. At higher doses (greater than 10 μg/day), a significantly lesser degree of antagonism was observed. Because the dosages in the present study were derived from behavioral work with rats [15] and were substantially higher than those used by Pavlik *et al.*, the potentiation of aggression may have been related to this factor. Studies are planned to assess whether TAM is strictly an agonist in neural tissues in mice or whether antagonist effects also can be produced. Should the former occur, it would suggest that antagonist properties of TAM may be limited to specific behavioral systems, e.g., lordosis. In addition, these investigations may also provide information concerning the potentiation of aggression by TAM beyond that exhibited by intact or antiandrogen-treated males. A possible explanation for this finding is a synergistic effect of TAM with endogenous estrogens, although this concept is highly speculative at present.

In closing, the results indicate that efforts to modulate the expression of testosterone-dependent behaviors through the administration of steroid antagonists may not yield positive results. The limitations of this type of intervention may be tied to metabolic factors and/or an unexpected effect of a compound (e.g., TAM) due to an incomplete understanding of its mechanism of action. Based on these observations, it appears that the clinical success of antagonist therapy with MPA is due primarily to a sustained decrease in T production. If this is the case, alternative pharmacological treatments that are more specific than MPA (e.g., antigonadotrophins) may prove even more useful in the management of aggressive and/or deviant behaviors.

ACKNOWLEDGEMENT

The authors thank John Nyby for his comments.

REFERENCES

1. Babieri, R. L.; Ryan, K. J. Direct effects of medroxyprogesterone acetate (MPA) and megestrol acetate (MGA) on rat testicular steroidogenesis. Acta Endocrinol. 94:419–425; 1980.
2. Beatty, W. W. Gonadal hormones and nonreproductive behaviors in rodents: Organizational and activational influences. Horm. Behav. 12:112–163; 1979.
3. Belis, J. A.; Blume, C. D.; Mawhinney, M. G. Androgen and estrogen binding in male guinea pig accessory sex organs. Endocrinology 101:726–740; 1977.
4. Berlin, F. S.; Krout, E. Pedophilia: Diagnostic concepts, treatment, and ethical considerations. Am. J. Forensic Psychiatry 7:13–30; 1986.
5. Birke, L. I. A.; Holzhausen, C.; Murphy, S.; Sadler, D. Effects of neonatal medroxyprogesterone acetate on postnatal sexual differentiation of female rats. J. Reprod. Fertil. 71:309–314; 1984.
6. Birke, L. I. A.; Sadler, D. Progestin-induced changes in play behaviour of the prepubertal rat. Physiol. Behav. 30:341–347; 1983.
7. Blumer, D.; Migeon, C. Hormone and hormonal agents in the treatment of aggression. J. Nerv. Ment. Dis. 160:127–137; 1975.
8. Bradford, J. M. W. Research on sex offenders. Psychiatr. Clin. North Am. 6:715–731; 1983.
9. Brain, P. F. Pituitary-gonadal influences on social aggression. In: Svare, B. B., ed. Hormones and aggressive behavior. New York: Plenum Press; 1983:3–26.
10. Clark, C. R.; Nowell, N. W. The effect of the antiestrogen CI-628 on androgen-induced aggressive behavior in castrated male mice. Horm. Behav. 12:205–210; 1979.
11. Cordoba, O. A.; Chapel, J. L. Medroxyprogesterone acetate antiandrogen treatment of hypersexuality in a pedophiliac sex offender. Am. J. Psychiatry 140:1036–1039; 1983.
12. Davidson, J. M.; Camargo, C.; Smith, E. R.; Kwan, M. Maintenance of sexual function in a castrated man treated with ovarian steroids. Arch. Sex. Behav. 12:263–274; 1983.
13. Denenberg, V. H.; Gaulin-Kremer, E.; Gandelman, R.; Zarrow, M. X. The development of standard stimulus animals for mouse *(Mus musculus)* aggression testing. Anim. Behav. 21:590–598; 1973.
14. Edwards, D. A.; Burge, K. G. Estrogenic arousal of aggressive behavior and masculine sexual behavior in male and female mice. Horm. Behav. 2:239–245; 1971.
15. Etgen, A. M. Antiestrogens: Effects of tamoxifen, nafoxidine, and CI-628 on sexual behavior, cytoplasmic receptors, and nuclear binding of estrogen. Horm. Behav. 13:97–112; 1979.
16. Gagne, P. Treatment of sex offenders with medroxyprogesterone acetate. Am. J. Psychiatry 138:644–646; 1981.
17. Hall, N. R.; Luttge, W. G. Maintenance of sexual behavior in castrated male SW mice using the antiandrogen, cyproterone acetate. Pharmacol. Biochem. Behav. 3:551–555; 1975.
18. Jordan, V. C.; Dix, C. J.; Naylor, K. E.; Prestwich, G.; Rowsby, L. Nonsteroidal antiestrogens: Their biological effects and potential mechanisms of action. J. Toxicol. Environ. Health 4:363–390; 1978.
19. Jordan, V. C.; Rowsby, L.; Dix, C. J.; Prestwich, G. Dose-related effects of non-steroidal antioestrogens and oestrogens on the measurement of cytoplasmic oestrogen receptors in rat and mouse uterus. J. Endocrinol. 78:71–81; 1978.
20. Luttge, W. G. Anti-estrogen inhibition of testosterone-stimulated aggression in mice. Experientia 35:273–274; 1978.
21. Manni, A.; Arafah, B. M. Tamoxifen-induced remission in breast cancer by escalating the dose to 40 mg daily after progression on 20 mg daily. Cancer 48:873–875; 1981.
22. Meyer, W. J.; Walker, P. A.; Emory, L. E.; Smith, E. R. Physical, metabolic, and hormonal effects on men of long-term therapy with medroxyprogesterone acetate. Fertil. Steril. 43:102–109; 1985.
23. Money, J.; Wiedeking, C.; Walker, P.; Migeon, C.; Meyer, W.; Borgaonkar, D. 47, XYY and 46, XY males with antisocial and/or sex-offending behavior: Antiandrogen therapy plus counseling. Psychoneuroendocrinology 1:165–178; 1975.
24. Neubauer, B. L.; Mawlinney, M. G. Actions of androgen and estrogen on guinea pig seminal vesicle epithelium and muscle. Endocrinology 108:680–687; 1981.
25. Neumann, F.; von Berswordt-Wallrake, W.; Elger, W.; Steinbeck, H.; Hahn, J. D.; Dramer, M. Aspects of androgen-dependent events as studied by antiandrogens. Rec. Prog. Horm. Res. 26:337–410; 1970.
26. Novak, E.; Hendrix, J. W.; Szeckman, C. E.; Kaiser, D. G.; Stoll, R. G. Endogenous serum testosterone in males after different doses and routes of administration of medroxyprogesterone acetate. J. Reprod. Med. 25:157–160; 1980.
27. O'Connor, M.; Baker, H. W. G. Depo-medroxy progesterone acetate as an adjunctive treatment in three aggressive schizophrenic patients. Acta Psychiatr. Scand. 67:399–403; 1983.
28. Pavlik, E. J.; van Nagell, J. R.; Nelson, K.; Gallion, H.; Donaldson, E. S.; Kenady, D. E.; Baranowska-Kortylewicz, J. Antagonism to estradiol in the mouse: Reduced entry of receptors complexed with 4-hyroxytamoxifen into a Mg^{2+}-soluble chromatin fraction. Endocrinology 118:1924–1934; 1986.
29. Ryan, K. J.; Naffolin, F.; Reddy, V.; Flores, F.; Petro, Z. Estrogen formation in the brain. Am. J. Obstet. Gynecol. 114:454–460; 1972.
30. Simon, N. G.; Gandelman, R. The aggression-promoting and aggression-eliciting properties of estrogen in male mice. Physiol. Behav. 21:161–164; 1978.
31. Simon, N. G.; Gray, J.; Gandelman, R. An empirically derived scoring system for intermale aggression in mice. Aggress. Behav. 9:157–166; 1983.
32. Simon, N. G.; Whalen, R. E. Hormonal regulation of aggression: Evidence for a relationship between genotype, receptor binding, and behavioral sensitivity to androgen and estrogen. Aggress. Behav. 12:245–247; 1986.
33. Tagnon, H. Antiestrogens in treatment of breast cancer. Cancer 39:2959–2964; 1977.