Effects of Cyproterone Acetate on Uterine Motility in Rats

ELVERA R. ABBATIELLO ˣ and JOSEPH CATUOGNO

Abstract □ The effect of cyproterone acetate, an antiandrogen, on uterine contractility when the compound is administered to rats during midpregnancy was investigated. Pregnant Wistar rats were given cyproterone acetate subcutaneously on Days 8–11 of gestation. The results indicate that cyproterone acetate, either directly or indirectly, inhibits uterine contractility, an effect that may explain the prolongation of the gestational period observed in all treated animals.

Keyphrases □ Cyproterone acetate—effect on uterine motility, rats □ Uterine motility—effect of cyproterone acetate, rats □ Antiandrogens—effect of cyproterone acetate on uterine motility, rats

An antiandrogen is a substance that prevents the testicular hormone from exerting its normal effects on the organism without interfering with the secretion of the hormone (1). Therefore, an antiandrogen should not cause testicular atrophy. This appears to be true of the progestational steroid cyproterone acetate (6-chloro-1β,2β-dihydro-17-hydroxy-3'*H*-cyclopropa[1,2]pregna-1,4,6-triene-3,20-dione acetate). Its toxicity is low, the LD_{50} being greater than 2 g/kg orally in the mouse.

Preliminary studies have shown that cyproterone acetate is: *(a)* strongly progestational (being 250 times more active than progesterone), *(b)* weakly estrogenic (being less than $1/1000$ as active as estradiol[1]), and *(c)* weakly androgenic (being less than $1/64$ as active as testoterone propionate) (2).

When given to pregnant rats, cyproterone acetate antagonizes fetal testicular androgens, leading to feminization of the male offspring. Changes produced during sexual differentiation are always irreversible, in contrast to changes induced in the adult animal (3).

The extensive literature relative to the effects of cyproterone acetate on the developing offspring is incomplete since little, if any, work has been done to determine the effects of this compound on the pregnant mother. Recent investigations in this laboratory have shown the following:

1. The duration of gestation increases in cyproterone acetate-treated rats.
2. At the time of parturition, rats treated during gestation with cyproterone acetate cannibalized their offspring with much greater frequency than untreated rats. This effect has also been demonstrated in mice treated with cyproterone acetate (4, 5).

The present investigation was undertaken to study the effect of cyproterone acetate treatment during pregnancy on uterine contractions at the terminal stage of gestation.

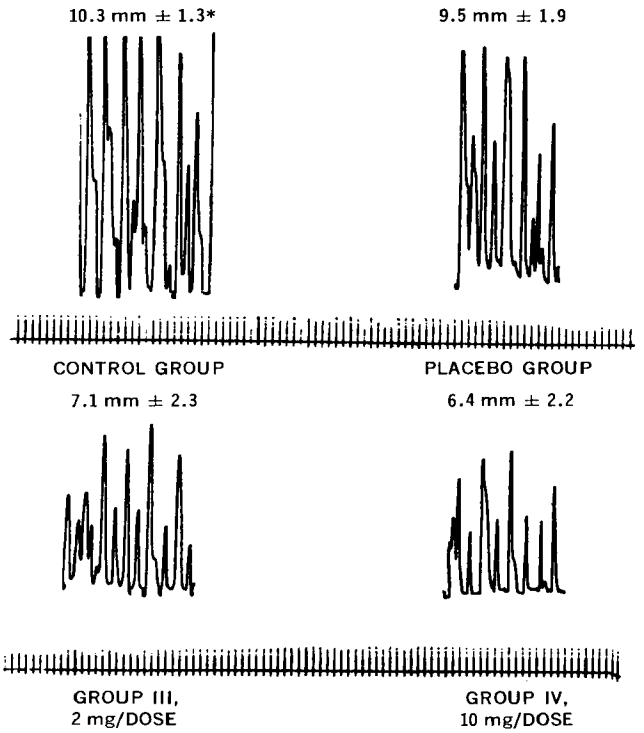

Figure 1—*Spontaneous contractions: paper speed = 0.0005 cm/sec; time marker = 30 sec; calibration = 5 mm of graph = 1 mm of contraction. *Height of contraction: mean ± SD.*

EXPERIMENTAL

Forty Wistar virgin female rats, average weight 295 g, were mated in a 2:1 ratio with 20 Wistar male rats[2]. Vaginal washings were performed to detect the presence of sperm or vaginal plugs, and positive washings were designated as Day 1 of pregnancy. The animals were then randomly divided into four groups of 10 animals.

Group I—The control group received no treatment.

Group II—The placebo group received 0.2 ml of a solution of benzyl benzoate[3] and peanut oil (50:50) subcutaneously on Days 8–11 of gestation.

Group III—This test group received 0.2 ml of a solution con-

[1] Vaginal smear method of Allen and Doisy (M. X. Zarrow *et al.*, "Experimental Endocrinology: A Sourcebook of Basic Techniques," Academic, New York, N.Y., 1964).

[2] The animals were obtained from Camm Research Institute, Wayne, N.Y.

[3] Eastman Organic Chemicals, Rochester, N.Y.

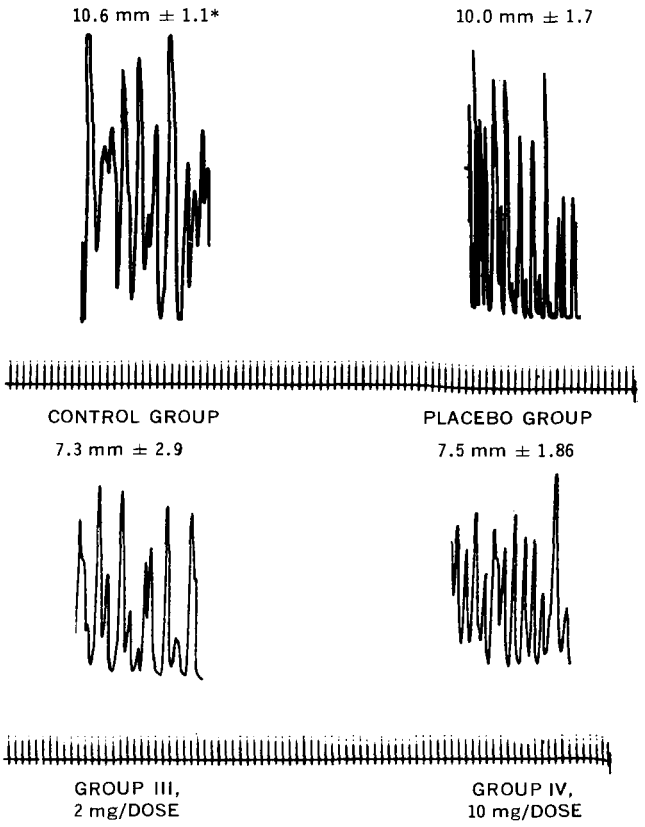

Figure 2—*The effect of 10 μunits of oxytocin on uterine contraction. Paper speed = 0.0005 cm/sec; time marker = 30 sec; calibration = 5 mm of graph = 1 mm of contraction. *Height of contraction: mean ± SD.*

taining 2 mg cyproterone acetate[4] dissolved in benzyl benzoate and 50% peanut oil subcutaneously on Days 8–11 of gestation. This dose was chosen because of its frequency of use in previous studies as the low dose producing an effect.

Group IV—This test group received 0.2 ml of a solution containing 10 mg cyproterone acetate dissolved in benzyl benzoate and 50% peanut oil subcutaneously on Days 8–11 of gestation. This dose was chosen because it was the standard dose used in several reported investigations (3, 7, 8).

On the 21st day of gestation, the animals were sacrificed. The abdominal cavity was opened, the fetuses were removed, and a 10-mm section of uterine muscle was obtained. This section was then cleaned of all fatty tissue and associated blood vessels and set up in a 10-ml isolated organ bath at 37.5°. The composition of the bathing solution was (grams per liter): NaCl, 9; KCl, 0.42; $NaHCO_3$, 0.5; $CaCl_2$, 0.24; $MgCl_2$, 0.44; and glucose, 0.5[5] (8). The bath was aerated with a gas mixture of 95% O_2 and 5% CO_2[6], and the pH was 7.

Mechanical records were obtained on the physiograph[7] through an isotonic myograph.

The spontaneous motility of the isolated preparation was studied for 45–60 min. Between 1 and 3 hr, the activity of the uterus decreases and this is the most convenient phase for the study of drug actions. The effects of 2.5 and 10 μunits of oxytocin[8] on the isolated uterine muscle were studied within this interval. Each dose of oxytocin was injected into the bath as a 0.1-ml solution.

Assay of the oxytocic effect was accomplished by a bracketing procedure (8). It consisted of four doses of oxytocin per comparison: 10 μunits followed by 2.5 μunits, then 2.5 μunits again, and, finally, 10 μunits. Each dose was allowed to act on the muscle for 10 min, followed by a flushing of the fluid and a rest period of 6 min before the next dose. The bath was also flushed 30 min after the initial setup.

[4] Lot 21236, Internal Code 11586B micronized, Schering Corp., Bloomfield, N.J.

[5] All chemicals except glucose were supplied by J. T. Baker Chemical Co., Phillipsburg, N.J. Glucose was supplied by Sigma Chemical Chemical Co., St. Louis, Mo.

[6] Matheson Co., East Rutherford, N.J.

[7] Desk model type DMP-4A, Narco-Bio-Systems, Inc., Houston, Tex.

[8] Pitocin, Parke-Davis, Detroit, Mich.

Table I—Statistical Analysis of Uterine Contractions

Spontaneous Contractions	*p*-Values[a]
Control *versus* placebo	N.S.
Control *versus* Group III	0.0025
Control *versus* Group IV	0.0005
Placebo *versus* Group III	0.0125
Placebo *versus* Group IV	0.0025
Group III *versus* Group IV	N.S.
10 μunits Oxytocin	
Control *versus* placebo	N.S.
Control *versus* Group III	0.0005
Control *versus* Group IV	0.0005
Placebo *versus* Group III	0.0005
Placebo *versus* Group IV	0.0005
Group III *versus* Group IV	N.S.
2.5 μunits Oxytocin	
Control *versus* placebo	N.S.
Control *versus* Group III	0.025
Control *versus* Group IV	N.S.
Placebo *versus* Group III	N.S.
Group III *versus* Group IV	N.S.

[a] *t*-Test of significance. Degrees of freedom = 18/test. N.S. = not significant.

RESULTS

The control group uterine sections exhibited strong, uniform, and continuous contractions, with similar results manifested by the sections obtained from placebo animals (Fig. 1). The mean strengths of contraction (*i.e.*, measurement of the strongest contraction per strip) were: control, 10.3 ± 1.3 mm; and placebo, 9.5 ± 1.9 mm.

The animals in Group III showed a variety of spontaneous activity. The range of contractions in the spontaneous activity portion was 3–10.5 mm, and the mean strength of contractions was 7.1 ± 2.3 mm. Group IV, except for three samples, exhibited weak but continuous contractions, with a mean strength of contraction of 6.4 ± 2.2 mm.

The decreased spontaneous contractions in the strips taken from the cyproterone acetate-treated animals suggests that estrogen dominance in the myometrium is not as great as in the control and placebo groups. The effects of 2.5 μunits of oxytocin on the isolated uterine sections are shown in Fig. 3. The mean strengths of contraction were: control, 7.4 ± 3.7 mm; placebo, 5.5 ± 4.0 mm; Group III, 4.7 ± 3.7 mm; and Group IV, 5.6 ± 1.8 mm.

The data obtained during this investigation indicate that no significant differences in the strengths of contractions exist between the control and placebo groups in any of the three parameters measured (Table I). Moreover, the strengths of contractions in Groups III and IV are significantly lower than those of the control and placebo groups when comparing spontaneous contractions and following the addition of 10 μunits of oxytocin to the tissue bath. Furthermore, the strengths of contractions in all groups following the addition of 2.5 μunits of oxytocin are lower but tend to return to levels comparable to those obtained spontaneously and subsequent to the addition of 10 μunits of oxytocin to the tissue bath.

DISCUSSION

In the rat, the data obtained during this investigation indicate that cyproterone acetate has an inhibiting effect on uterine contractions. This inhibitory effect is apparent in spontaneous contractions of the uterine segments as well as in oxytocin-induced contractions.

The ovulatory cycle in the rat is considered to be 5–6 days. The animal will mate only when in estrus, a period of high estrogen concentration. Once conception occurs, progesterone synthesis increases to a higher level than estrogen in an effort to maintain pregnancy. This high level of progesterone continues until approximately midpregnancy, at which time estrogen levels slowly begin to increase. Estrogen dominance occurs late in the last week

of pregnancy and is needed to initiate uterine excitability and induce contractions at a force capable of expelling the fetuses.

It appears from this study that the administration of cyproterone acetate, an antiandrogenic, progestational compound, at midpregnancy prolongs the progesterone dominance. That the action of this compound is prolonged is in agreement with the findings of Bridge and Scott (2) who observed that prostatic secretion in dogs treated with cyproterone acetate was abolished for a period greater than 47 days after the termination of drug treatment.

The duration of action of this compound would also explain the increase in the gestational period of rats treated at midpregnancy with cyproterone acetate and the inability of these animals to deliver at term. Since the undelivered fetuses continue to grow, forceful and painful expulsion might account for the high percentage of cannibalization observed in these animals (4).

Since it was determined that the force of contractions is reduced following the administration of cyproterone acetate, investigations are presently in progress to assay the levels of estrogens and progesterone in the treated animals. The results obtained from these studies are important in elucidating the mechanism of action of this compound on uterine contractility.

REFERENCES

(1) F. A. Beach and W. H. Westbrook, *Endocrinology*, **42**, 379(1968).

(2) R. W. Bridge and W. W. Scott, *Invest. Urol.*, **2**, 99(1964).

(3) F. Neumann and W. Elger, *Proc. Symp. Steroid Horm., 2nd*, **1965**, 168.

(4) E. R. Abbatiello, *J. Pharm. Sci.*, **63**, 152(1974).

(5) H. Steinbeck and W. Elger, *Acta Endocrinol., Suppl.*, **119**, 63(1967).

(6) R. E. Whalen and D. A. Edwards, *Endocrinology*, **84**, 155(1969).

(7) R. A. Turner, "Screening Methods in Pharmacology," Academic, New York, N.Y., 1965, pp. 118–126.

(8) A. Cohen, R. A. Hall, B. Heath-Brown, M. W. Parks, and A. H. Rees, *Brit. J. Pharmacol.*, **12**, 194(1957).

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[x] To whom inquiries should be directed.

Anticonvulsant Activity of *N,N'*-Bis[3-(3-substituted urea)propyl]piperazines

ARVIND K. CHATURVEDI [*], JAYANTI P. BARTHWAL [*], SURENDRA S. PARMAR [*‡x], and VIRGIL I. STENBERG [‡]

Abstract □ Several *N,N'*-bis[3-(3-substituted urea)propyl]piperazines were synthesized and characterized by their sharp melting points, elemental analyses, and IR spectra. All substituted piperazines were found to possess anticonvulsant activity, which was reflected by 20–70% protection observed against pentylenetetrazol-induced seizures in mice. Some of these compounds inhibited oxidation of pyruvic acid by rat brain homogenate. No correlation could be observed between the anticonvulsant activity possessed by these substituted piperazines and their ability to inhibit the oxidation of pyruvic acid.

Keyphrases □ *N,N'*-Bis[3-(3-substituted urea)propyl]piperazines—synthesis, anticonvulsant activity and relationship to inhibition of pyruvic acid oxidation □ Structure–activity relationships—*N,N'*-bis[3-(3-substituted urea)propyl]piperazines, anticonvulsant activity, rats, inhibition of pyruvic acid oxidation □ Oxidation, pyruvic acid—effect of piperazinoureas, relationship to anticonvulsant activity □ Piperazines, *N,N'*-bis[3-(3-substituted urea)propyl]—synthesis, anticonvulsant activity and relationship to inhibition of pyruvic acid oxidation

Anticonvulsant, antireserpine, and central nervous system (CNS) depressant properties exhibited by substituted piperazines (1, 2) and the inhibition of the oxidation of pyruvic acid by *N,N'*-bis[3-(3-substituted thiourea)propyl]piperazines (3) possessing anticonvulsant activity (4) led to the synthesis of some *N,N'*-bis[3-(3-substituted urea)propyl]piperazines (piperazinoureas). In the present study, attempts were made to correlate the anticonvulsant activity possessed by these piperazinoureas with their enzyme inhibitory effectiveness.

EXPERIMENTAL[1]

***N,N'*-Bis[3-(3-substituted urea)propyl]piperazines (I–XIII)**—Compounds I–XIII were prepared by refluxing a mixture of *N,N'*-bis(3-aminopropyl)piperazine[2] (0.01 mole) and the appropriate isocyanate (0.02 mole) in dry benzene on a steam bath for 2 hr. Excess benzene was removed by distillation under reduced pressure. The solid mass which separated out was collected by filtration and recrystallized from suitable solvents. These substituted piperazinoureas were characterized by their sharp melting points, elemental analyses[3], and IR spectra (Table I).

Determination of Anticonvulsant Activity—Anticonvulsant activity was determined in mice of either sex weighing 25–30 g. The mice were divided into groups of 10, keeping the group weights as near the same as possible. Each piperazinourea was suspended in 5% aqueous gum acacia to give a concentration of 0.25% (w/v). The test compound was injected in a group of 10 animals at a dose of 100 mg/kg ip.

Four hours after the administration of the piperazinourea, the mice were injected with pentylenetetrazol (90 mg/kg sc). This dose

[1] All compounds were analyzed for their carbon, hydrogen, and nitrogen content. Melting points were taken in open capillary tubes and are corrected. IR spectra were obtained with Perkin-Elmer Infracord spectrophotometer model 137 equipped with NaCl optics in KBr films in the range of 700–3500 cm^{-1}.

[2] Aldrich Chemical Co., Milwaukee, Wis.

[3] Central Drug Research Institute, Lucknow, India.