Pharmacology Biochemistry & Behavior, Vol. 27, pp. 265–271. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Evidence for the Reestablishment of Copulatory Behavior in Castrated Male Rats With a Brain-Enhanced Estradiol-Chemical Delivery System[1]

W. R. ANDERSON,*‡ J. W. SIMPKINS,*‡
M. E. BREWSTER,‡§ AND N. BODOR†‡[2]

Department of Pharmacodynamics and Medicinal Chemistry†*
College of Pharmacy and the Center for Drug Design and Delivery‡
University of Florida, Gainesville, FL 32610
and Pharmatec, Inc.,§ Alachua, FL 32615

Received 16 December 1986

ANDERSON, W. R., J. W. SIMPKINS, M. E. BREWSTER AND N. BODOR. *Evidence for the reestablishment of copulatory behavior in castrated male rats with a brain-enhanced estradiol-chemical delivery system.* PHARMACOL BIOCHEM BEHAV 27(2) 265–271, 1987.—We have developed a redox-chemical system for brain-enhanced drug delivery of estradiol based on an interconvertible dihydropyridine ⇌ pyridinium salt carrier. Estradiol, when combined with the carrier, readily crosses the blood-brain barrier and upon oxidation of the carrier is "locked" in the brain. The aim of this study was to evaluate the effects of an estradiol-chemical delivery system (E2-CDS) versus an equimolar dose of estradiol-17-valerate (E2-VAL) on copulatory behavior in orchidectomized rats. The data revealed that a single dose of E2-CDS was more efficacious than E2-VAL in stimulating mounting behavior (percent responding) and the effect was 100% through 5 weeks. E2-CDS increased intromission behavior more than E2-VAL through 28 days. Mount and intromission latencies were reduced by E2-CDS to a greater extent and for a longer time (28 days) than E2-VAL. Neither form of estradiol restored ejaculation parameters or penile reflexes. These data suggest that E2-CDS causes a potent and long-acting stimulation of proceptive and consummatory components of male sexual behavior, presumably acting through the local brain-release of estradiol.

Estradiol Delivery system Blood-brain barrier Copulatory behavior Castration Penile reflexes

MALE sexual behavior is composed of two distinct components: proception and consummation. The proceptive component is composed of the awareness and pursuit of a receptive female and mounting of the female to allow intromission. Penile erection, intromission and eventual ejaculation are the consummatory components of male sexual behavior. Normally, masculine behavior requires secretion of testosterone by the Leydig cells of the testes. In sexually experienced male rats, orchidectomy results in a gradual diminution and the eventual termination of sexual behavior and a syndrome for which testosterone replacement restores sexual behavior in male rats [10,24].

It is believed that expression of the proceptive components of male sexual behavior is dependent upon the aromatization of testosterone to estradiol in the brain; particularly the regions of the preoptic area of the hypothalamus and the amygdala [3, 9, 21, 23]. Two studies have evaluated the effect of estradiol implanted into the brain on male sexual behavior. In one study [3], castrated male rats were implanted bilaterally with cannulae into the preoptic area of the hypothalamus and 10 μg of estradiol was delivered through each cannulae every 3 days for 12 days. Estradiol was reported to be more effective than testosterone in inducing mounts and intromissions. Lisk and Greenwald [20] report that implantation of estradiol benzoate into the preoptic area stimulated mounting, but not intromissions, to levels ob-

[1]This work was presented at the 16th Annual Meeting of the Society for Neuroscience in Washington, DC (November 9–14, 1986).

[2]Requests for reprints should be addressed to N. Bodor, Center for Drug Design and Delivery, College of Pharmacy, Box J-497, J. Hillis Miller Health Center, University of Florida, Gainesville, FL 32610.

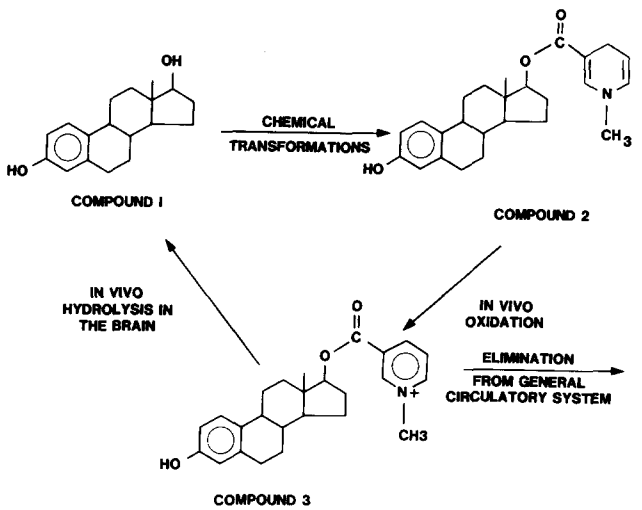

FIG. 1. The brain-enhanced delivery of estradiol (Compound 1). Compound 2 is the chemical delivery system (E2-CDS), and Compound 3 is the oxidized form (quaternary salt) locked into the brain and quickly eliminated from the rest of the body. The trigonelline (N-methylnicotinic acid) formed upon the hydrolysis of Compound 3 is nontoxic [29] and is easily cleared from the brain.

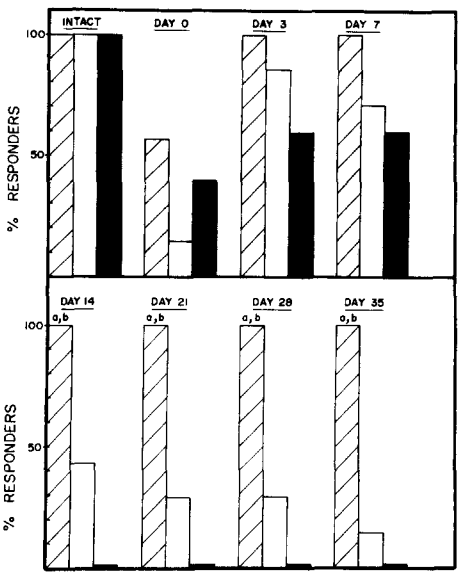

FIG. 2. Effect of E2-CDS (slanted-line columns), E2-VAL (clear columns), and DMSO (solid columns) on the mounting percentage (percent responders) in castrated male rats from day 0 to day 35 after a single IV injection. Groups were analyzed by the Fisher exact test and differences ($p<0.05$) from control are depicted by (a) and from E2-VAL by (b).

served in male golden hamsters. While it appears that estradiol acts centrally to stimulate some components of male sexual behavior, it is not clear to what extent a peripheral action of brain-implanted estradiol is related to the stimulation of masculine sexual behavior.

It appears a combination of estradiol and dihydrotestosterone is required to fully reestablish male sexual behavior. Dihydrotestosterone alone is ineffective in fully reestablishing male behavior in castrated rats and requires estradiol to be completely effective. Several investigators [14, 19, 20] concluded that combination of brain stimulation by estradiol (preoptic area) and peripheral stimulation by dihydrotestosterone is necessary for the reinstatement of a male sexual behavior equivalent to that elicited by testosterone alone.

Steroid hormones are highly lipophilic and readily penetrate the blood-brain barrier when administered peripherally or centrally. This physiologically meaningful brain penetration by peripheral steroid hormones limits the use of these steroids in separating brain from peripheral effects of the steroids. Peripherally applied steroids affect steroid-responsive tissues throughout the body and steroids applied to the brain, even in minute amounts, reach the peripheral circulation. Additionally, this latter method of steroid delivery to the brain requires extensive brain surgery and the amount of drug administered is highly variable. Thus an improved method for the enhanced delivery of steroid hormones to the brain is needed.

An alternative way to determine the components of male sexual behavior which are mediated by estradiol is to selectively deliver the steroid to the brain. Such a method for the brain-enhanced delivery of drugs was developed by Bodor, Farag and Brewster [4]. This method of delivery is based upon the dihydropyridine $\rightleftharpoons$ pyridinium salt redox system which has been demonstrated to accomplish brain-enhanced drug delivery when applied to a number of pharmacologically active species including phenethylamine [5], dopamine [6,26], gamma-aminobutyric acid [1] and testosterone [7].

Recently, we applied this chemical delivery system to enhance the delivery of estradiol to the brain [8, 15, 27]. The delivery system design utilizes lipophilicity to achieve improved entry into the brain and conversion in the brain to a hydrophilic form (due to the oxidation of the carrier) which decreases its rate of exit from the brain. Such a delivery system for estradiol (Fig. 1) is indicated by Compound #2, where the hydroxyl function of estradiol is bound to the dihydropyridine carrier. Brain-enhanced delivery of estradiol requires a series of chemical processes, including oxidation of the dihydropyridine ring to the corresponding quaternary pyridinium salt (Compound #3) which provides the basis of locking the molecule in the brain, hydrolysis of the ester by non-specific esterases at the C-17 position and the release of estradiol (Compound #1).

Simpkins *et al.* [27] observed that a single administration of the estradiol-chemical delivery system (E2-CDS) suppressed LH secretion in orchidectomized male rats for greater than 24 days while peripheral E2 levels were only transiently elevated following the injection. On the basis of these observations, we propose that our E2-CDS could be used as an effective tool to dissociate the central from peripheral effects of the steroid. The purpose of the present investigation was to determine the components of male sexual behavior which were influenced by selective delivery of E2 to the brain and to define the duration of the responses to this novel steroid delivery system.

METHOD

Synthesis of E2-CDS

The E2-CDS (3-Hydroxy-17β-[[(1-methyl-1,4-dihydropyridin-3-yl)-carbonyl]oxy]estra-1,3,5(10)-triene (estradiol 17-(1,4-dihydro-trigonellinate) was synthesized as previously described by Bodor *et al.* [8]. Briefly, the 3, 17β-dinicotinate ester of estradiol was made by refluxing 17β-

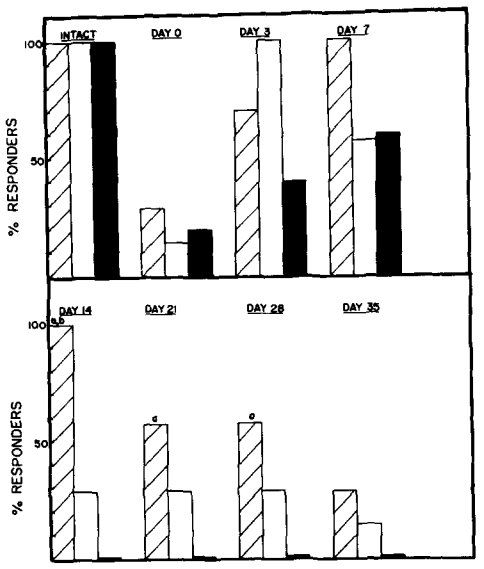

FIG. 3. Effect of E2-CDS (slanted-line columns), E2-VAL (clear columns), and DMSO (solid columns) on the intromission percentage (percent responders) in castrated male rats from day 0 to day 35 after a single IV injection. Groups were analyzed by the Fisher exact test and group differences are indicated by (a) (different from control) and by (b) (different from E2-VAL).

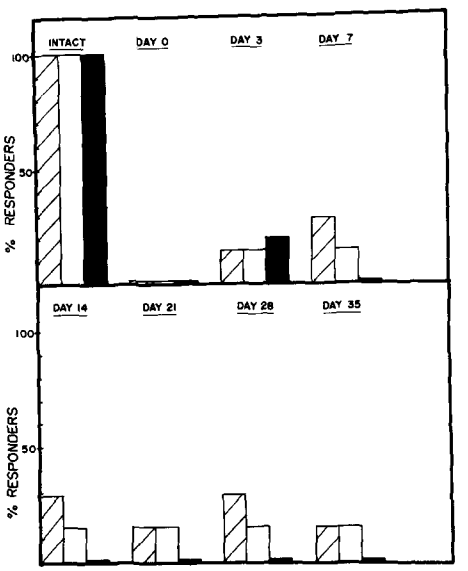

FIG. 4. Effect of E2-CDS (slanted-line columns), E2-VAL (clear columns), and DMSO (solid columns) on the ejaculation percentage (percent responders) in castrated male rats from day 0 to day 35 after a single IV injection. None of the mean values were significantly different among the three groups at any time examined.

estradiol with nicotinoyl chloride or nicotinic anhydride in pyridine. This derivative was selectively hydrolyzed to the 17-monoester of estradiol with potassium bicarbonate in 95% methanol. The monoester of estradiol was then quaternized with methyl iodide. The delivery system (E2-CDS) was then prepared by reduction of the obtained quaternary salt with $Na_2S_2O_4$. The structure of each intermediate and the final product (E2-CDS) was confirmed by nuclear magnetic resonance and elemental analysis [8].

Animals

Adult male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) were used for this study. Animals were maintained singly in wire-bottom cages in a room which was climate-controlled (ambient temperature at 23°C) with a 14 hour light, 10 hour dark cycle of artificial lighting. To accommodate tests of male sexual behavior during the dark phase of the daily light-dark cycle, the time of lights off was set at 1100 hr. Animals were provided with Purina rat chow and tap water ad lib.

Behavioral Tests

To establish baseline behavior for each animal (n=30), tests of male sexual behavior were conducted prior to orchidectomy. Each male was tested every 5 days until four successive and consistent behavioral patterns were achieved. Rats were then bilaterally orchidectomized via a single mid-ventral incision and animals were rehoused for 28 days with no further behavioral testing. At 28 days after orchidectomy, rats were again tested for sexual behavior and were randomly divided among 3 experimental groups. These three groups received either the E2-CDS (3.0 mg/kg; n=7), estradiol valerate (E2-VAL at 2.7 mg/kg; n=7) at a dose equimolar to that of the E2-CDS, or the vehicle, dimethyl sulfoxide (DMSO at 0.5 ml/kg; n=5) via a single tail vein injection. Estradiol valerate was chosen as a drug control since it, like E2-CDS, is a long-acting 17-substituted ester of estradiol. The dose for E2-CDS (3.0 mg/kg) was chosen on the basis of its ability to suppress LH secretion for 24 days or longer [15,27] and this dose is 20 times lower than doses used in other studies in which no toxicity was observed [8]. Tests of male sexual behavior were conducted at 3, 7, 14, 21, 28, 35 and 42 days after drug administration.

All behavioral tests were conducted in rectangular glass arenas (31×30×36 cm) in a dimly lit room (7 watt bulb) between 1300 and 1700 hours (lights off at 1100 hours). The male was placed into an arena for 5 minutes prior to the introduction of a stimulus female via the top of the chamber. The stimulus female (bilaterally ovariectomized) was rendered sexually receptive by a SC injection of 100 μg estradiol benzoate and 500 μg progesterone in 0.1 ml corn oil 48 and 4 hours, respectively, prior to testing.

Behavioral events and their times of occurrence were recorded on a Lafayette Instrument event recorder (model 56041) which enables the manual recording of the observed events and the automatic recording of the time of the event. Testing was performed as described above and the following parameters of male copulatory behavior were calculated from the records of the test: Mount latency (ML), the time from the introduction of the female to the initial mount or intromission; intromission latency (IL), the time from introduction of the female to the first intromission; ejaculatory latency (EL), time from first intromission to ejaculation; and post-ejaculatory interval (PEI), time for ejaculation to the first intromission of the next copulatory series. Tests were considered negative if IL exceeded 15 minutes, EL exceeded 30 minutes or PEI exceeded 15 minutes.

At 24 hours before each copulatory test, male rats were evaluated for spontaneous penile erection reflexes between

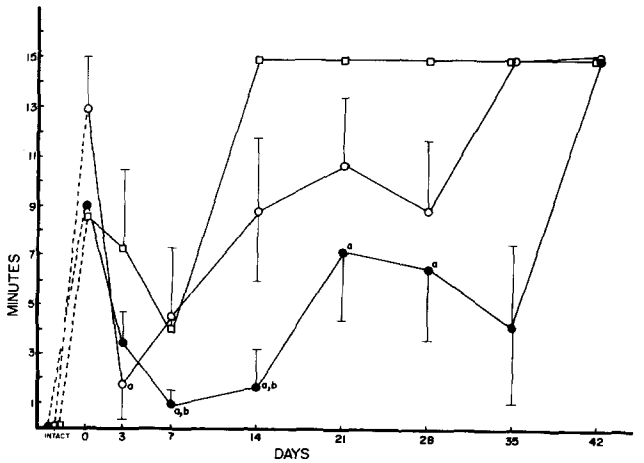

FIG. 5. Effect of E2-CDS (●), E2-VAL (○), and DMSO (□) on the mounting latency in castrated male rats from day 0 to day 42 after a single IV injection. Each point represents the group mean±1 SEM. The symbol (a) denotes differences from DMSO-treated animals and symbol (b) indicates differences from E2-VAL treated rats as analyzed by ANOVA and SNK statistics.

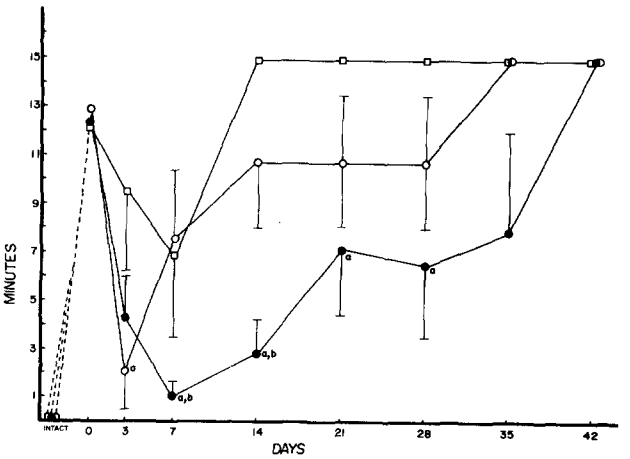

FIG. 6. Effect of E2-CDS (●), E2-VAL (○), and DMSO (□) on the intromission latency in castrated male rats from day 0 to day 42 after a single IV injection. Each point represents the group mean±1 SEM. The symbol (a) denotes differences from DMSO-treated animals and symbol (b) indicates differences from E2-VAL treated rats as analyzed by ANOVA and SNK statistics.

0800 hr and 1000 hr. The rat was gently placed on its back in a Plexiglas cylinder with the penile sheath retracted and held in place with a wooden applicator. The test was considered positive if erection occurred within 15 minutes after sheath retraction. Penile responses (number of erections, cups and flips) were manually recorded for 20 minutes after the first erection [11].

Statistical Treatment

The significance of differences among mean values for the events recorded was determined by analysis of variance and Student-Newman-Keuls (SNK) tests [30]. The significance of differences among groups for animals responding to a treatment was analyzed by the Fisher exact test [30]. The level of probability for all tests was $p<0.05$.

RESULTS

Prior to orchidectomy, each gonadally-intact rat was evaluated for copulatory parameters to determine baseline responses. Individual scores for ejaculation latency (EL) and post-ejaculatory interval (PEI) were deemed consistent if the standard error of 4 consecutive EL and PEI values was less than 15% of the mean. All animals met these criteria after 5 trials.

Four weeks after orchidectomy, rats were distributed among experimental drug groups pending their copulatory behavioral performance. Only those animals displaying ejaculation latencies greater than 15 minutes were included in the remainder of the study. Results of this pre-treatment copulatory behavior study revealed 75% of castrate males lost the capacity to ejaculate. These non-ejaculators were then randomly distributed among all treatment groups with 7 rats in the two estradiol-treated groups and 5 rats receiving the vehicle, DMSO.

Orchidectomy was more effective in reducing intromission response (Fig. 3) than mounting response (Fig. 2) when compared to intact levels (total mounting percent of 42% versus 21% of rats eliciting intromissions after castration). The effect of castration on mount latency (Fig. 5) was less dramatic (intact ML<0.1 min vs. castrate ML of 9–13 min) than the effect upon intromission latency (Fig. 6; EL<0.1 min with intact versus 12–13 minutes after orchidectomy). The number of mounts were not strongly reduced 28 days after orchidectomy (Fig. 7), however there was a 75% decrease observed in the number of intromissions due to bilateral gonadectomy (Fig. 8).

As shown in Fig. 2, E2-CDS restored mounting behavior in 100% of the animals by day 3 and this response to E2-CDS was greater than DMSO controls from 2 through 5 weeks. At no time after its administration did E2-VAL fully restore mounting behavior with only 2 rats responding by 14 days.

E2-VAL fully restored intromission behavior by three days but the response waned by 1 week, and at no time did it differ from control levels (Fig. 3). On the other hand, all E2-CDS treated rats intromitted by 7 days and to a greater extent than either E2-VAL or DMSO-treated animals by 2 weeks (Fig. 3). More than 50% of rats receiving the E2-CDS displayed intromission as long as 21 days and they returned to control levels by 5 weeks (Fig. 3).

Ejaculation responses, which were abolished by 4 weeks post-castration, were not restored to a significant degree by either form of estradiol and no more than 2 rats in any of the experimental groups ejaculated (Fig. 4).

ML (Fig. 5) was sharply reduced by 3 days after administration of either the E2-CDS or E2-VAL. ML of E2-CDS treated rats continued to be less than control through 28 days and less than E2-VAL through 2 weeks. In comparison, the ML of animals treated with E2-VAL returned to control levels by 7 days. IL (Fig. 6) were affected in a manner similar to ML. E2-CDS and E2-VAL reduced IL by 3 days. However, E2-VAL lost its effectiveness by 1 week while E2-CDS continued to reduce IL to levels less than observed in controls through 4 weeks.

MF (Fig. 7) appeared to be increased by E2-VAL and E2-CDS by 7 and 3 days, respectively, but returned to castrate levels by 2 weeks. Only at 14 days were significant differences observed such that the MF of E2-CDS rats were greater than that of either E2-VAL and DMSO-treated animals.

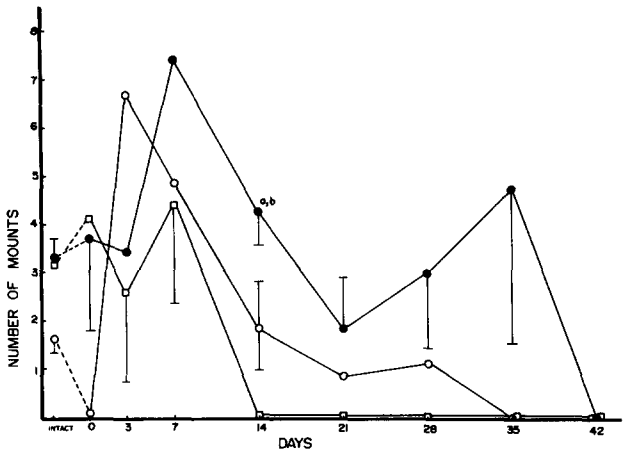

FIG. 7. Effect of E2-CDS (●), E2-VAL (○), and DMSO (□) on the mounting frequency in castrated male rats from day 0 to day 42 after a single IV injection. Each point represents the group mean±1 SEM. The symbol (a) denotes differences from DMSO-treated animals and symbol (b) indicates differences from E2-VAL treated rats as analyzed by ANOVA and SNK statistics.

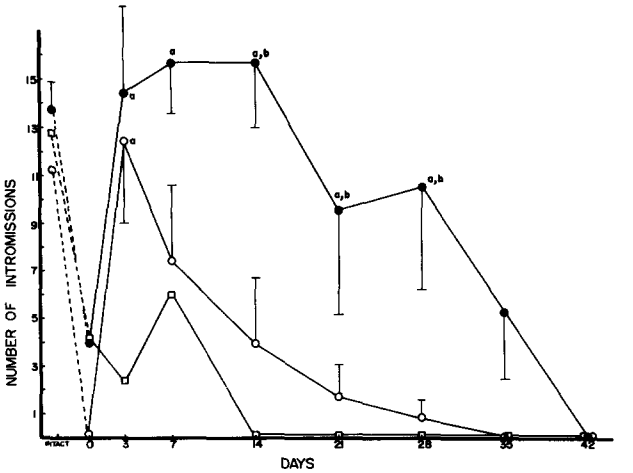

FIG. 8. Effect of E2-CDS (●), E2-VAL (○), and DMSO (□) on the intromission frequency in castrated male rats from day 0 to day 42 after a single IV injection. Each point represents the group mean±1 SEM. The symbol (a) denotes differences from DMSO-treated animals and symbol (b) indicates differences from E2-VAL treated rats as analyzed by ANOVA and SNK statistics.

IF (Fig. 8) was profoundly affected by the E2-CDS from 3 days through 28 days. In E2-CDS rats, there was nearly a 4-fold increase over castrate levels by 3 days and this elevation continued through 28 days. An increase in IF was observed in E2-VAL treated rats by 3 days but IF returned to control levels by 1 week.

The capacity of both the E2-CDS and the E2-VAL to return male copulatory behavior to levels observed prior to orchidectomy were also evaluated. Mount latencies were not returned to intact levels by either form of estradiol, however we did observe that intromission latencies (in rats administered E2-VAL) were equivalent to those latencies observed in gonadally-intact rats (Fig. 6). It is interesting to note that mount frequencies were not appreciably altered by either estradiol. Mount frequencies did increase over intact MF at days 3 and 7 when animals were given either the E2-VAL or E2-CDS. The most dramatic effect elicited by the E2-CDS was on IF which returned to intact levels through 28 days. IF was similarly affected by E2-VAL through 7 days then diminished to castrate levels.

Spontaneous penile erection reflexes were observed in 100% of gonadally-intact rats. Orchidectomy completely abolished penile reflexes by 28 days and neither form of estradiol was able to induce spontaneous penile erections.

DISCUSSION

The results of the present study indicate that an estradiol-chemical delivery system can increase proceptive as well as consummatory (intromission) components of male sexual behavior (in castrate rats) more effectively and for longer periods of time than the other 17-substituted ester of estradiol, E2-VAL. Our findings are supported by previous studies. Pfaff [25] gave estradiol benzoate (10 μg/day) to castrated male rats for 9 to 11 days and observed increased mounting and intromissions and reduced mounting latency to levels comparable to those observed following administration of testosterone propionate (200 μg/day). Similar results were reported by Sodersten [28] after either estradiol benzoate (100 μg/day) or testosterone propionate (100 μg/day) was given for 24 to 28 days to 6 week post-orchidectomized rats. The number of ejaculations were less affected by estradiol than testosterone. Gray, Smith and Davidson [16] observed that systemically administered estradiol was less effective than testosterone in reestablishing consummatory behavioral components. They implanted Silastic capsules with estradiol or testosterone and assessed sexual behavior 7 days later and found estradiol less effective in reinstating intromissions and ejaculations. These studies suggest that the proceptive component is preferably affected by estradiol.

The E2-CDS of E2-VAL did not appreciably restore ejaculation response presumably because of lack of stimulation of sensory components of peripheral target tissues. It has been shown that castrate rats receiving estradiol only do not redevelop penile papillae and require more intromissions and a longer time to ejaculate [2]. Therefore, it is not surprising that either form of estradiol failed to elicit ejaculation response. Failure of our E2-CDS to elicit spontaneous penile reflexes in castrated rats is supported by Gray *et al.* [16]. They found that treatment with estradiol alone did not reinstate penile reflexes unless combined with testosterone. However, estradiol administered alone is capable of maintaining or restoring mounting and intromission behavior but has little effect on peripheral androgen-sensitive tissues such as the penis, seminal vesicles and prostate gland [11, 25, 28].

Previous studies of the loci (brain vs. periphery) which mediate the effects of estradiol on male copulatory behavior have used routes of administration which deliver the hormone to both the central nervous system and the periphery. Brain estradiol implants in microgram concentrations likely deliver estradiol to peripheral tissues at physiological levels [13]. Therefore it is not certain from these studies to what extent a central versus a peripheral action of estradiol is related to the stimulation of masculine sexual behavior.

E2-CDS is effective in chronically increasing the proceptive and consummatory components of male sexual behavior in orchidectomized rats presumably through a local action in the central nervous system. We have shown recently that a single 3.0 mg/kg IV dose of E2-CDS suppresses serum LH levels for at least 24 days in orchidectomized rats [27] while

estradiol (equimolar; 2.7 mg/kg) suppressed serum LH for only 4 days. Serum levels of estradiol were not elevated over orchidectomized controls after 4–8 days, suggesting a local brain release of estradiol accounting for the suppression of LH [15] and the presently observed stimulation of male copulatory behavior. Additional pharmacokinetic studies revealed that the quaternary form (locked-in) of the delivery system persists in brain (half-life of 23 hours) but is rapidly cleared from the liver, lung, and kidney (by 0.77, 5.5 and 7.0 hours, respectively) [27]. Since the half-life of the lipophilic dihydropyridine in the brain is only 29.2 min [8], and this agent probably does not bind to steroid receptors, it is unlikely that the parent compound is responsible for the behavioral effects noted. Although we cannot be certain of the brain site of action of E2-CDS on copulatory behavior, the localization of aromatase enzymes which convert testosterone to estradiol [17, 18, 22] suggest that release of estradiol from the E2-CDS in the preoptic area and/or the amygdala, could account for its effects on copulatory behavior.

The "lock-in" phenomenon of the quaternary salt of the E2-CDS and the consequent sustained release of free estradiol from the quaternary salt may be useful in the treatment of sexual dysfunction in cases of psychological impotence not complicated by deficits in peripheral androgen-responsive tissues. Studies combining E2-CDS with androgens (i.e., testosterone and/or dihydrotestosterone) need to be attempted to more fully address psychological impotence coupled with deficiencies of peripheral androgens.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. John T. Clark for his advice for testing protocol, Joe Meert for graphics and Mrs. Lisa King for typing this manuscript. Supported in part by NIH Grants AG02021 (J.W.S.), GM27167 (N.B.) and a grant from Pharmatec, Inc. and Gynex, Inc.

REFERENCES

1. Anderson, W. R., J. W. Simpkins, P. A. Woodard, D. Winwood, W. C. Stern and N. Bodor. Anxiolytic activity of a brain delivery system for GABA. *Psychopharmacology (Berlin),* in press.
2. Baum, M. J. and J. T. M. Vreeburg. Copulation in castrated male rats following combined treatment with estradiol and dihydrotestosterone. *Science* **182:** 283–285, 1973.
3. Beyer, C., G. Morali, F. Naftolin, K. Larsson and G. Perez-Palacios. Effect of some antiestrogens and aromatase inhibitors on androgen induced sexual behavior in castrated male rats. *Horm Behav* **7:** 353–362, 1976.
4. Bodor, N., H. Farag and M. E. Brewster. Site-specific, sustained release of drugs to the brain. *Science* **214:** 1370–1372, 1981.
5. Bodor, N. and H. H. Farag. Improved delivery through biological membranes: II. A redox chemical drug-delivery system and its use for brain-specific delivery of phenylethylamine. *J Med Chem* **26:** 313–318, 1983.
6. Bodor, N. and J. W. Simpkins. Redox delivery system for brain-specific sustained release of dopamine. *Science* **221:** 65–67, 1983.
7. Bodor, N. and H. H. Farag. Improved delivery through biological membranes: XIV. Brain-specific, sustained delivery of testosterone using a redox chemical delivery system. *J Pharm Sci* **73:** 385–389, 1984.
8. Bodor, N., J. McCornack and M. E. Brewster. Improved delivery through biological membranes: XXII. Synthesis and distribution of brain-selective estrogen delivery system. *Int J Pharmaceut,* in press.
9. Christensen, L. W. and L. G. Clemens. Intrahypothalamic implants of testosterone or estradiol and resumption of masculine sexual behavior in long-term castrated male rats. *Endocrinology* **95:** 984–990, 1974.
10. Davidson, J. M. Hormones and reproductive behavior. In: *Hormones and Behavior,* edited by S. Levine. New York: Academic Press, 1972, pp. 63–103.
11. Davidson, J. M., P. Johnson, G. J. Bloch, E. R. Smith and R. J. Weick. Comparative responses to androgen of anatomical behavioral, and other parameters. *Excerpta Med Int Congr Series* **219:** 727–730, 1972.
12. Davidson, J. M., M. L. Stefanick, B. J. Sachs and E. R. Smith. Role of androgen in sexual reflexes of the male rat. *Physiol Behav* **21:** 141–146, 1978.
13. Davis, P. G. and R. J. Barfield. Activation of feminine sexual behavior in castrated male rats by intrahypothalamic implants of estradiol benzoate. *Neuroendocrinology* **28:** 228–233, 1979.
14. DeBold, J. F. and L. G. Clemens. Aromatization and the induction of male sexual behavior in male, female and androgenized female hamster. *Horm Behav* **11:** 401–413, 1978.
15. Estes, K. S., M. E. Brewster, J. W. Simpkins and N. Bodor. A novel redox system for CNS-directed delivery of estradiol causes sustained LH-suppression in castrate rats. *Life Sci,* in press.
16. Gray, G. D., E. R. Smith and J. D. Davidson. Hormonal regulation of penile erection in castrated male rats. *Physiol Behav* **24:** 463–468, 1980.
17. Jones, K. J., B. S. McEwen and D. W. Pfaff. Regional specificity in estradiol effects on [^{3}H]-uridine incorporation in rat brain. *Mol Cell Endocrinol* **45:** 57–63, 1986.
18. Krey, L. C., F. Kamel and B. S. McEwen. Parameters of neuroendocrine aromatization and estrogen receptor occupation in the male rat. *Brain Res* **193:** 277–283, 1980.
19. Larsson, K. and P. Sodersten. Sexual behavior in male rats treated with estrogen in combination with dihydrotestosterone. *Horm Behav* **4:** 289–299, 1973.
20. Lisk, R. D. and D. P. Greenwald. Central plus peripheral stimulation by androgen is necessary for complete restoration of copulatory behavior in the male hamster. *Neuroendocrinology* **36:** 211–217, 1983.
21. McEwen, B. S. Neural gonadal steroid actions. *Science* **211:** 1303–1311, 1981.
22. McEwen, B. S. and L. C. Krey. Properties of estrogen-sensitive neurons: aromatization, progestin receptor induction and neuroendocrine effects. In: *Metabolism of Hormonal Steroids in the Neuroendocrine Structures,* edited by F. Celotti, F. Naftolin and L. Martini. New York: Raven Press, 1984, pp. 117–128.
23. MacLusky, N. J., A. Philip, C. Hurlburt and F. Naftolin. Estrogen metabolism in neuroendocrine structures. In: *Metabolism of Hormonal Steroids in the Neuroendocrine Structures,* edited by F. Celotti, F. Naftolin and L. Martini. New York: Raven Press, 1984, pp. 103–116.
24. Malmnas, C-O. Testosterone-activated copulatory behavior in the castrated rat. *Acta Physiol Scand* Suppl **395:** 9–46, 1973.
25. Pfaff, D. Nature of sex hormone effects on rat sex behavior: specificity of effects and individual patterns of response. *J Comp Physiol Psychol* **73:** 349–358, 1970.

26. Simpkins, J. W., N. Bodor and A. Enz. Direct evidence for brain-specific release of dopamine from a redox delivery system. *J Pharm Sci* **74**: 1033–1036, 1985.
27. Simpkins, J. W., J. McCornack, K. S. Estes, M. E. Brewster, E. Shek and N. Bodor. Sustained brain-specific delivery of estradiol causes long-term suppression of LH secretion. *J Med Chem* **29**: 1809–1812, 1986.
28. Sodersten, P. Estrogen-activated sexual behavior in male rats. *Horm Behav* **4**: 247–256, 1973.
29. Windholz, M., S. Budaveri, R. F. Blumetti and E. S. Otterbein (eds.). *The Merck Index*, tenth edition. Rahway, NJ: Merck and Company, 1983.
30. Zar, J. H. *Biostatistical Analysis*. Englewood Cliffs, NJ: Prentice Hall Inc., 1974.