Pharmacology Biochemistry & Behavior, Vol. 12, pp. 755–759. Printed in the U.S.A.

Copulation-Induced Abbreviation of Estrus in the Female Guinea Pig: Block by Clonidine[2]

SHARON M. IRVING[1], ROBERT W. GOY AND GARY A. DAVIS

Wisconsin Regional Primate Research Center, University of Wisconsin, Madison, WI 53706

Received 16 November 1979

IRVING, S. M., R. W. GOY AND G. A. DAVIS. *Copulation-induced abbreviation of estrus in the famale guinea pig: Block by clonidine*. PHARMAC. BIOCHEM. BEHAV. 12(5) 755–759, 1980.—In the female guinea pig, copulation produces a rapid inhibition of subsequent sexual receptivity. Injection of the alpha adrenergic agonist clonidine before mating blocked the early inhibitory effects of coital stimulation, while the serotonergic antagonist methysergide, and the dopaminergic antagonist pimozide were without effect. The effects of clonidine were prevented by pretreatment with the alpha adrenergic antagonist yohimbine. These results suggest that an adrenergic system is involved in the copulation-induced inhibition of receptivity and provide further evidence that such a system has an important facilitative role in the control of female sexual behavior in the guinea pig.

Copulation Estrus Clonidine

IN the female guinea pig, copulation normally leads to a rapid reduction in individual lordosis duration and a significant shortening of the receptive period [15]. This species is particularly sensitive to the inhibitory effects of coital stimulation, but attenuation of the receptive period after copulation has also been found in a number of other animals, including the grasshopper, fruit fly, rat, hamster, chinchilla, sheep, and *Anolis carolinensis* [3, 4, 7, 8, 17, 19, 25].

Pharmacological studies with several species have indicated that the monoamine neurotransmitters may be important in the regulation of female sexual behavior [6, 9, 14, 16]. In addition, changes in the turnover of monoamines in brain have been reported to occur as a result of vaginocervical stimulation [2,11]. Consequently, we were interested in investigating whether changes in monoamine activity could be involved in the behavioral changes triggered by coitus in the guinea pig.

Of the three major monoamines, dopamine appears to be inhibitory to lordosis in the guinea pig [9] as it is in a number of other species. Serotonin is also generally inhibitory to female sexual behavior in most species studied [6, 14, 16], but its role in the guinea pig is still questionable [9,12]. Norepinephrine, on the other hand, seems clearly to have an important facilitatory role in controlling lordosis in the guinea pig. Interfering with adrenergic activity, either through a synthesis inhibitor or an alpha-antagonist, blocks estrogen-progestrone induced estrous behavior [9,22]. Conversely, potentiating alpha-adrenergic activity with the agonist clonidine increases lordosis durations in guinea pigs brought into heat with estrogen and progestrone [9] and can induce lordosis in guinea pigs given either estrogen alone [10] or a norepinephrine synthesis inhibitor to block a normal estrogen-progestrone heat [22]. In other species, particularly rat, any facilitatory effects of an alpha-adrenergic mechanism in lordosis appear to be very modest in comparison with those in the guinea pig [13]. This difference in the neurochemical mediation of lordosis in the two species may be related to behavioral differences between them. Of particular interest is the fact that the inhibitory effects of copulation on estrus are strong in the guinea pig but weak in the rat.

In the present studies, female guinea pigs were given monoaminergic drugs prior to copulation in an effort to block the abbreviation of estrus. Clonidine was used to elevate alpha adrenergic activity while the receptor antagonists pimozide and methysergide were used to suppress the activity of dopaminergic and serotonergic circuits, respectively.

EXPERIMENT 1: ATTENTUATION OF THE ABBREVIATION OF ESTRUS BY PRETREATMENT WITH CLONIDINE

METHOD

The experimental subjects were female guinea pigs, at least 3 months of age, from a genetically heterogeneous ("Topeka") stock maintained at the Wisconsin Regional Primate Research Center. They were ovariectomized under Penthrane anesthesia (Abbott Laboratories, methoxyfluorane) at least two weeks prior to testing and were housed in groups of 7 to 10 in 120×60×30 cm cages on wood chip bedding. Food (Purina Guinea Pig Chow) and water were freely available, supplemented with lettuce twice weekly. Lights were on from 0200 to 1400 hr, and the temperature averaged 20°C.

Estrus was induced by giving each animal a subcutaneous

[1]Curent address: Sharon M. Irving, 387 Tropicana Way, Union City, California.

[2]This research was supported by grants to Dr. Gary Davis, HD11515 and Dr. Robert Goy, MH 21312. Publication number 19-017 of The Wisconsin Regional Primate Center.

injection of 5 μg/kg estradiol benzoate dissolved in corn oil, followed 36 hr later by 0.4 mg/animal progesterone injected subcutaneously in corn oil. The progesterone injections were made at either 0600 or 0800 hr on the day of testing. The lights were left on in the testing room throughout the testing period.

Immediately before the progesterone injection, and hourly thereafter, each animal was tested for lordosis using a manual stimulation technique ("fingering") [30]. At every testing, each female was fingered three times, and lordosis durations, to the nearest sec, were recorded. All statistical analyses were based on the mean of these three lordosis durations.

When the mean lordosis duration of a female reached 5 sec, she was assigned to a treatment condition. The assignments were alternated among treatment groups, so that the number of subjects in each group, and the mean latency to the first occurrence of lordosis of those subjects, would be comparable for all groups. There were three treatment groups: vehicle unmated, vehicle mated, and clonidine mated. At the time of assignment, clonidine HCl (0.2 mg/kg in 1 ml/kg saline, IP) or saline was injected. Five min later, females were either mated or placed alone in an empty isolation cage for a period of time comparable to that of mating. Matings were carried out in 50×50×35 cm cages in which a sexually experienced male had been adapted for at least 1 hr. The number of intromissions, and the latencies in minutes to intromission and ejaculation were recorded. After ejaculation, the female was removed from the male's cage, and returned to the group cage. Each animal was fingered every 30 min for the first 2 hr after treatment, and then hourly until no response could be elicited from any animal on two consecutive hourly tests. An individual female was considered to be out of heat when she did not show lordosis for two consecutive hours.

Data Analysis

Two behavioral measures were obtained in this study: lordosis duration in seconds at each hourly testing, and duration of estrus in hours. Duration of estrus data were analyzed by Kruskal-Wallis analysis of variance, followed by Mann-Whitney U Tests. Lordosis durations were analyzed by a 2-way analysis of variance for repeated measures, followed by tests for simple main effects.

TABLE 1

EFFECTS OF CLONIDINE ON THE ABBREVIATION OF ESTRUS

Treatment	n	Duration of estrus (hr)	Percent in heat 2 hr after mating
Saline-unmated	8	6.4 ± 0.9	100
Clonidine-mated	8	4.6 ± 1.1*	88
Saline-mated	8	0.4 ± 0.2†	0

All data presented as mean ± SEM.
* vs † - $p<0.001$.
The two values with the * superscipt do not differ statistically.

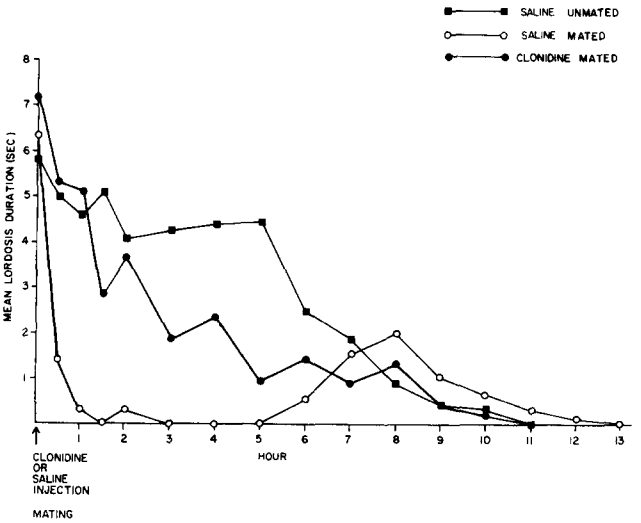

FIG. 1. Lordosis durations after mating: effect of clonidine. Ovariectomized guinea pigs were brought into heat with estradiol benzoate and progesterone and treated for lordosis by fingering. When each female displayed a criterion lordosis duration of at least 5 sec (4–7 hr after progesterone), she was assigned to one of the three groups above. Clonidine or saline was injected immediately, and mating was carried out 5 min later.

RESULTS

Pretreatment with the alpha adrenergic agonist clonidine blocked the early inhibitory effects of coital stimulation on estrous behavior in the guinea pig, and the duration of estrus following copulation was significantly longer in clonidine-treated females than in their saline controls (Table 1). While saline-mated females were out of heat an average of 0.44 hr after exposure to the males, animals treated with clonidine stayed in heat for an average of 4.63 hr ($p<0.001$). This value was not statistically different from the 6.41 hr duration displayed by saline-treated unmated females. There were no differences between the two mated groups in the copulatory stimulation received from the male as measured by intromission frequency and mount, intromission, and ejaculation latencies.

Lordosis durations after mating were also affected by clonidine pretreatment (Fig. 1). Those saline-treated mated females that were still in heat 0.5 hr after mating displayed markedly reduced lordosis duration at that time. The average duration for the saline-treated mated group was significantly smaller than that for the saline-treated unmated group ($p<0.01$). The mated females receiving clonidine, on the other hand, were not different from the saline-unmated animals at Hr 0, 0.5, 1 or 2 (Fig. 1). However, at Hr 1.5, 3, 4 and 5, the lordosis duration of the clonidine group were significantly depressed below the saline-unmated group ($p<0.05$). This later depression in lordosis duration in the clonidine-treated mated females may be due to the short-lived action of the drug [9].

Beginning at Hr 6, there was a marked increase in the mean lordosis duration of the saline-mated females after mating (Fig. 1). This was due to the fact that two of the eight females in that group showed long-duration lordosis responses after periods of 6 and 8 hr of failure to display lordosis. Young, Dempsey and Myers [29] also noted recovery in a small percentage of mated females. In the present work the average recovery rate for saline-mated females across all studies was less than 10%.

TABLE 2

PREVENTION OF THE EFFECT OF CLONIDINE BY PRETREATMENT WITH YOHIMBINE

Treatment	n	Duration of estrus (hr)	Percent in heat 2 hr after mating
Saline-unmated	8	7.1 ± 1.2*	88
Yohimbine-unmated	8	5.0 ± 0.8†	100
Yohimbine-clonidine-mated	8	1.1 ± 0.5‡	25
Saline-mated	8	0.9 ± 0.6§	25

* vs † - $p<0.052$.
* vs ‡ - $p<0.001$.
† vs ‡ - $p<0.001$.
‡ vs § - ns.

EXPERIMENT 2: BLOCKING THE EFFECT OF CLONIDINE WITH YOHIMBINE

The preceding study suggests that stimulation of alpha adrenergic receptors with clonidine can markedly attenuate the inhibitory effects of copulation. To further test for the specificity of this effect, the alpha adrenergic receptor antagonist, yohimbine, was administered prior to clonidine.

METHOD

The general procedures were as in Experiment 1. When each animal reached a 5 sec mean lordosis duration, it was assigned to one of four treatment groups: vehicle-unmated, yohimbine-unmated, yohimbine-clonidine-mated, or vehicle-mated. At that time, yohimbine HCl (2 mg/kg in 1 ml/kg saline, IP) or saline was administered. One hr later, clonidine HCl (0.2 mg/kg, IP) or its saline was injected. Five min later, mating began.

RESULTS

Table 2 and Fig. 2 demonstrate that pretreament with the alpha adrenergic antagonist yohimbine, in a dose which by itself was only slightly inhibitory, completely blocked the actions of clonidine in the mated female.

The reductions in the durations of estrus and lordosis produced by the yohimbine in unmated animals (Table 2, Fig. 2) were not significant, but pilot work indicated that higher doses (4 mg/kg, 6 mg/kg) had more marked inhibitory actions.

EXPERIMENT 3: INABILITY OF CLONIDINE TO RESTORE LORDOSIS WHEN GIVEN AFTER MATING

METHOD

This experiment was carried out as described for Experiment 1, except that clonidine HCl (0.2 mg/kg, IP) or saline was not given until 2 hr after mating.

RESULTS

Mating inhibited lordosis in all subjects in both groups. No animal in either treatment group displayed lordosis when fingered immediately before the clonidine or saline injection. No overall significant increase in the lordosis response was seen after clonidine injection. Two of the six clonidine treated females showed lordosis after the injection, but the duration of lordosis was very short (mean maximum duration

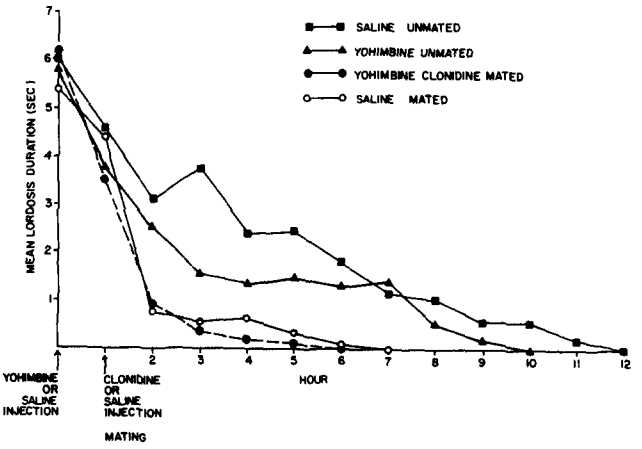

FIG. 2. Lordosis durations after mating: effect of clonidine and yohimbine. Animals were treated as in Fig. 1, except that yohimbine or saline was given at the time of criterion, while clonidine injections and mating were carried out 1 hr later.

was 1.35 sec), it was not repeatedly elicitable, and the animals were responsive on only one or two of the hourly tests. This was not significantly different from the behavior of the saline-treated group in which one of the six animals responded weakly and briefly after injection of saline.

High doses of clonidine (0.4, 0.6, and 1.2 mg/kg) were also tested in this paradigm, but they produced a marked debilitation which obscured any possible actions on sexual behavior. No debilitating effects of clonidine were seen at the 0.2 mg/kg dose in Experiments 1, 2 and 3.

EXPERIMENT 4: LACK OF EFFECT OF PIMOZIDE OR METHYSERGIDE PRETREATMENT

METHOD

General testing procedures were as previously described. At the first hour that lordosis occurred, the female was assigned to one of three groups: vehicle unmated, vehicle mated or drug mated, and the drug or vehicle injections were administered. Pimozide was given at a dose of 1.5 mg/kg (in 0.4 ml/kg 50% dimethylsulfoxide [DMSO], IP) and animals were mated 2 hr later. Methysergide maleate was administered at a dose of 10 mg/kg (in 1 ml/kg saline, IP) and animals

were mated 3 hr later. Separate groups of concurrent vehicle control animals were run for methysergide (saline) and pimozide (DMSO).

RESULTS

Methysergide pretreatment did not block the inhibitory effects of copulation (Table 3). Methysergide-treated mated females displayed a mean estrous duration of 1.1 hr, which was not statistically different from the 0.2-hr duration of the saline-mated animals and was significantly shorter than the 5.4-hr duration of the saline-unmated controls ($p<0.02$). The 10 mg/kg dose used in the study had only minor inhibitory effects in the unmated female. This dose of methysergide has been shown to be behaviorally effective in Hartley guinea pigs [9], and would be expected to produce a substantial decrease in serotonergic activity. Higher doses of methysergide (20 mg/kg, 30 mg/kg) were tested in the present study, but they inhibited receptivity to the extent that mating was prevented.

Pimozide treatment was also unable to attenuate the inhibitory effects of coital stimulation (Table 4). Pimozide-treated mated females showed a rapid decrease in lordosis duration after copulation, and were out of heat an average of 0.4 hr after mating with a male. This estrous duration was not statistically different from the 1.1-hr duration displayed by DMSO-treated mated animals, and both were significantly shorter than the 4.8-hr duration of DMSO-treated unmated females ($p<0.05$).

TABLE 3

EFFECTS OF METHYSERGIDE ON THE ABBREVIATION OF ESTRUS

Treatment	n	Duration of estrus (hr)	Percent in heat at 1.5 hr
Saline-unmated	6	5.4 ± 1.2*	83
Methysergide-unmated	6	6.8 ± 0.6*	100
Methysergide-mated	6	1.1 ± 1.0†	17
Saline-mated	6	0.2 ± 0.1†	0

* vs † - $p<0.05$.
Values with the same superscript do not differ statistically.

TABLE 4

EFFECTS OF PIMOZIDE ON THE ABBREVIATION OF ESTRUS

Treatment	n	Duration of estrus (hr)	Percent of heating 1.5 hr after mating
DMSO-unmated	6	4.8 ± 0.4*	100
Pimozide-mated	6	0.4 ± 0.3†	17
DMSO-mated	6	1.1 ± 1.0†	17

* vs † - $p<0.05$.
Values with the same superscript do not differ statistically.

DISCUSSION

The alpha adrenergic agonist clonidine significantly increased the duration of estrus displayed after copulation, and blocked the early inhibitory effects of coital stimulation on lordosis duration. These effects of clonidine were prevented by pretreatment with the alpha adrenergic antagonist yohimbine, suggesting that the clonidine was working through alpha receptors. Neither the serotonergic antagonist methysergide nor the dopaminergic antagonist pimozide was able to block the inhibitory effects of copulation. We have no direct evidence that these drugs affected receptors in our animals, but their ineffectiveness in rather high doses, coupled with the effectiveness of clonidine in a moderate dose would suggest that noradrenergic changes may be relatively more important for the abbreviation of estrus. Previous studies in the guinea pig have demonstrated that the expression of estrogen-progestrone induced receptivity can be blocked by inhibiting norepinephrine synthesis or by blocking alpha adrenergic receptors [9,21]. All together, these data suggest that noradrenergic activity may play a particularly important facilitative role in the control of sexual behavior in the female guinea pig, and that copulation may inhibit receptivity, at least in part, by interfering with noradrenergic activity.

Before any such conclusion can be drawn, it is important to consider the pharmacological and physiological specificity of the actions of clonidine. While clonidine is generally thought to be a selective alpha adrenergic agonist, it has been suggested that some of the physiological effects of clonidine may be mediated through histamine receptors [18]. However, the findings that the effect of clonidine was blocked by an alpha adrenergic antagonist in the present study and that clonidine can promote lordosis in guinea pigs rendered unresponsive to estrogen-progesterone treatment by the inhibition of NE synthesis [22], would indicate that clonidine is acting on lordosis through an alpha adrenergic system. It cannot be ruled out that clonidine might be acting through epinephrine, rather than norepinephrine receptors, and in fact, the alpha antagonist used in this study, yohimbine, has been proposed to act selectively at epinephrine synapses [5]. There is no direct evidence for this suggestion, but there is evidence that there are two kinds of alpha receptor in the brain, that one of these may occur at, among other sites, epinephrine synapses, and that yohimbine may be specific for that receptor [13]. Whichever alpha receptor this antagonist may act on the guinea pig, it does appear to be one that is important in the control of lordosis, since yohimbine itself produced some suppression of estrous behavior.

Clonidine, apparently through its action on alpha receptors, has been demonstrated to decrease serotonin and dopamine turnover [1], though the dopamine effect may be confined to adrenergic neurons [26]. Decreased activity of either of these neurotransmitters might be expected to facilitate the expression of estrous behavior, though it is questionable for serotonin in the guinea pig [9,12]. Since direct antagonism of dopamine and serotonin receptors with drugs, did not alter the coitally-induced abbreviation of estrus it seems unlikely that either of these neurotransmitters could play a substantial role in the action of clonidine on lordosis.

Stimulation of alpha receptors by clonidine has also been reported to produce significant analgesia [23], but this probably cannot account for clonidine's ability to counteract the abbreviation of estrus. Slimp [28] found that extensive genital analgesia produced by section of the pelvic, pudendal, and genitofemoral nerves did not block the abbreviation of estrus in the guinea pig. The dose of clonidine used in the present study was relatively low in comparison with the doses producing significant analgesic effects in rats and mice

[23, 24, 27], and it is unlikely that it could have produced as severe an analgesia as genital denervation.

It would appear, then, that clonidine acts directly on an alpha adrenergic circuit involved in the facilitation of sexual behavior in the female guinea pig, and that coitus may abbreviate estrus by suppressing activity in that circuit. Though clonidine can block the inhibitory effects of mating, it cannot restore lordosis once these effects have been established. Some non-adrenergic mechanism could be responsible for this persistent inhibition, but this finding could also be explained if the inhibition occurred at the level of the alpha adrenergic receptor and was not readily reversible. The report of Slimp [28] that three major pelvic afferents were unnecessary for the abbreviation of estrus suggests that the inhibition could be due to some chemical factor produced peripherally in response to copulation. Clonidine, if it were present before mating, could block the access of this factor to the alpha adrenergic receptor, but once the factor were bound, the drug might not be able to reactivate the receptor. We are presently examining the possibility that a prostaglandin may serve as such a factor.

ACKNOWLEDGEMENTS

We would like to thank the following companies for their kind gifts of drugs: Boehringer Ingelheim (clonidine); Janssen Pharmaceutica (pimozide); Sandoz (methysergide).

REFERENCES

1. Andén, N. -E., H. Corrodi, K. Fuxe, B. Hökfelt, C. Rydin and T. Svensson. Evidence for central noradrenaline receptor stimulation by slonidine. *Life Sci.* **9:** 513–523, 1970.
2. Bensch, C., H. Lescure, J. Robert and J. M. A. Faure. Catecholamine histofluorescence in the median eminence of female rabbits activated by mating. *J. Neural Trans.* **36:** 1–18, 1975.
3. Bignami, G. and F. A. Beach. Mating behavior in the chinchilla. *Anim. Behav.* **16:** 45–53, 1968.
4. Blandau, R. J., J. L. Boling and W. C. Young. The length of heat in the albino rat as determined by the copulatory response. *Anat. Rec.* **79:** 453–463, 1941.
5. Bolme, P., H. Corrodi, K. Fuxe, T. Hökfelt, P. Lidbrink and M. Goldstein. Possible involvement of central adrenaline neurons in vasomotor and respiratory control. Studies with clonidine and its interactions with piperoxane and yohimbine. *Eur. J. Pharmac.* **28:** 89–94, 1974.
6. Carter, C. S., J. M. Bahr and V. D. Ramirez. Monoamines, estrogen and female sexual behavior in the golden hamster. *Brain Res.* **144:** 109–121, 1978.
7. Carter, C. S. and M. W. Schein. Sexual receptivity and exhaustion in the female golden hamster. *Hormones Behav.* **2:** 191–200, 1971.
8. Crews, D. Coition-induced inhibition of sexual receptivity in female lizards (*Anolis carolinensis*). *Physiol. Behav.* **11:** 463–468, 1973.
9. Crowley, W. R., H. H. Feder and L. P. Morin. Role of monoamines in sexual behavior of the female guinea pig. *Pharmac. Biochem. Behav.* **4:** 67–71, 1976.
10. Crowley, W. R., B. L. Nock and H. H. Feder. Facilitation of lordosis behavior by clonidine in female guinea pigs. *Pharmac. Biochem. Behav.* **8:** 207–209, 1978.
11. Crowley, W. R., J. F. Rodriguez-Sierra and B. R. Komisaruk. Monoaminergic mediation of the antinociceptive effect of vaginal stimulation in rats. *Brain Res.* **137:** 67–84, 1977.
12. Davis, G. A. and E. English. Monoamines and lordosis in the female guinea pig. *Hormones Behav.* **8:** 88–93, 1977.
13. Davis, G. A. and R. Kohl. The influence of α-receptors on lordosis in the female rat. *Pharmac. Biochem. Behav.* **6:** 47–53, 1977.
14. Everitt, B. J., K. Fuxe, T. Hökfelt and G. Jonsson. Role of monoamines in the control by hormones of sexual receptivity in the female rat. *J. comp. physiol. Psychol.* **89:** 556–572, 1975.
15. Goldfoot, D. A. and R. W. Goy. Abbreviation of behavioral estrus in guinea pigs by coital and vagino-cervical stimulation. *J. comp. physiol. Psychol.* **72:** 426–434, 1970.
16. Gradwell, P. B., B. J. Everitt and J. Herbert. 5-hydroxytryptamine the central nervous system and sexual receptivity of female rhesus monkeys. *Brain Res.* **88:** 281–293, 1975.
17. Haskell, P. T. Stridulation and associated behavior in certain orthoptera. 3. The influence of the gonads. *Anim. Behav.* **8:** 76–81, 1960.
18. Karppanen, H., I. Paakkari, P. Paakkari, R. Huotari and A. -L. Orma. Possible involvement of central histamine H_2-receptors in the hypotensive effect of clonidine. *Nature* **259:** 587–588, 1976.
19. Manning, A. The control of sexual receptivity in female drosophila. *Anim. Behav.* **15:** 239–250, 1967.
20. Marrone, B. L., J. F. Rodriguez-Sierra and H. H. Feder. Contrasting effects of prostaglandins on lordosis behavior in rats and guinea pigs. Paper presented at: *A. Meet. East. Conf. Reprod. Behav.*, Madison, Wisconsin, May 31–June 3, 1978.
21. Nock, B. and H. H. Feder. Studies on the role of norepinephrine in female sexual behavior of the guinea pig. Paper presented at: *A. Meet. East. Conf. Reprod. Behav.*, Madison, Wisconsin, May 31–June 3, 1978.
22. Nock, B. and H. H. Feder. Noradrenergic transmission and female sexual behavior of guinea pigs. *Brain Res.* **166:** 369–380, 1979.
23. Paalzow, L. Analgesia produced by clonidine in mice and rats. *J. Pharm. Pharmac.* **26:** 361–363, 1974.
24. Paalzow, G. and L. Paalzow. Clonidine antinociceptive activity: Effects of drugs influencing central monoaminergic and cholinergic mechanisms in the rat. *Arch. Pharmac.* **292:** 119–126, 1976.
25. Parsons, S. D. and G. L. Hunter. Effect of the ram on duration of oestrus in the ewe. *J. Reprod. Fert.* **14:** 61–70, 1967.
26. Rochette, L. and J. Bralet. Effect of clonidine on the synthesis of cerebral dopamine. *Biochem. Pharmac.* **24:** 303–305, 1974.
27. Schmitt, H., J. C. LeDovarec and N. Petillot. Antinociceptive effects of some α-sympathomimetic agents. *Neuropharmacology* **13:** 289–294, 1974.
28. Slimp, J. C. Reduction of genital sensory input and sexual behavior of female guinea pigs. *Physiol. Behav.* **18:** 1027–1031, 1977.
29. Young, W. C., E. W. Dempsey and H. I. Myers. Cyclic reproductive behavior in the female guinea pig. *J. comp. Psychol.* **19:** 313–335, 1935.
30. Young, W. C., E. W. Dempsey, C. W. Hagquist and J. L. Boling. The determination of heat in the guinea pig. *J. Lab. clin. Med.* **23:** 300–303, 1937.