EFFECTS OF BARBITURATE ADMINISTRATION ON OVULATION AND MATING IN THE LABORATORY RAT

Barbara Shirley

Department of Life Sciences, University of Tulsa, Tulsa, Oklahoma

Induction of ovulation by mating was studied in groups of rats injected with dosages of barbiturates which will block ovulation in non-mated rats. Ovulation was induced by mating more readily in barbital-treated animals running 5-day estrous cycles than in those with 4-day cycles. Difference in responsiveness between the two groups was attributed to their unequal rates of estrogen secretion at the time of, or shortly following, barbiturate administration. Barbiturate-treated females, which were not placed with males until metestrus, exhibited delayed ovulation. Delayed ovulation was accompanied by a delay in sexual receptivity in a significant number of cases.

The estrous cycle of the laboratory rat is characterized by several changes which occur at precise times. An increase in uterine wet and dry weight and a distention or "ballooning" of the uterus with intraluminal fluid occur at proestrus (1). Between 2:00 and 4:00 p.m., an interval designated as the critical period, the surge of luteinizing hormone (LH) necessary for ovulation is released from the pituitary (2). Sexual receptivity also begins in the late afternoon of proestrus (3). On the day of estrus ova are found in the oviducts (4) and, if the animal has not mated, only cornified cells are observed in the vaginal smear (2).

All these occurrences of the estrous cycle are dependent to some degree on steroids produced by the ovary and can be inhibited by ovariectomy at diestrus (5, 6) or by the administration at diestrus of the anti-estrogenic compound 1-(*p*-2-Diethylaminoethoxyphenyl)-1-phenyl-2-*p*-methoxyphenylethanol (MER-25) (7).

Administration of a barbiturate just prior to the critical period for LH release on proestrus can block the LH surge and thereby block ovulation even though estrogen secretion earlier in the cycle was normal (8). However, some animals given barbiturate injections will mate at the expected time in the cycle and in these cases mating can result in ovulation although the rat is usually a spontaneous rather than an induced ovulator (9). When ovulation is blocked with barbiturates, and not induced by mating, it often occurs at metestrus (8). Because of the different rates at which rats with 4-day and 5-day estrous cycles secrete estrogen and a somewhat more prolonged critical period for LH release in the 5-day cyclic rat, 4-day and 5-day cyclers do not exhibit the same responsiveness in some experimental situations, e.g., they respond differently to administration of anti-estrogenic compounds (7) and to barbiturates (10, 11, 12).

The purposes of these experiments were: (a) to determine whether the difference in the rate at which 4-day and 5-day cycling rats secrete estrogen would cause a difference in their capacities for induction of ovulation following administration of a barbiturate and (b) to determine whether a delay in ovulation, and its occurrence at metestrus, would be accompanied by a similar delay in the sexual receptivity of barbiturate-treated animals.

Although the effects of barbiturates on the occurrence of mating and of ovulation were of primary interest, uterine and vaginal changes were also recorded since these can serve as useful indicators of relative rates of estrogen secretion.

MATERIALS AND METHODS

Sixty-day old virgin female rats of the Sprague-Dawley strain, obtained from Sprague-Dawley, Inc., were placed singly in cages and subjected to a controlled regimen of lighting with lights on for 14 hours (5:00 a.m. to 7:00 p.m.) during each 24 hour period. Purina laboratory chow and water were provided *ad libitum*. Vaginal smears were taken daily before 10:00 a.m. and animals were used in experiments only after two consecutive 4-day or 5-day cycles had been completed.

On the day of proestrus rats were injected intraperitoneally at 2:00 p.m. with pentobarbital (3 mg/100 g body weight) or at 1:00 p.m. with barbital (30 mg/100 g body weight). Injection times were prior to the critical period for LH release. Control animals were injected intraperitoneally with distilled water (0.3 ml/100 g body weight) but otherwise received treatment identical to that of barbiturate-treated animals.

Pentobarbital was administered to two groups of 4-day cycler animals to allow a later comparison of experimental results with those reported by other investigators. Barbital, a barbiturate that is more effective than pentobarbital in inhibiting ovulation by 5-day cyclers, was administered to other groups of 4-day and 5-day cyclers so that the responsiveness of animals with different cycle lengths could be compared without the added variable of different barbiturates.

Female rats were placed in the cages of proven male rats between 3:40 and 4:00 p.m. on proestrus or estrus to test their sexual receptivity. The females remained with the males until after 10:00 a.m. on the day of autopsy (either the day of estrus or metestrus). The criteria used for determining that mating had occurred were the presence of sperm in the vaginal smear and/or the presence of one or more vaginal plugs on the floor of the cage. When an animal was not autopsied until metestrus, a laparotomy was performed in the morning on the day of estrus to check uterine size and to check for swelling and translucence of the oviducts. These changes in oviducts were used as the criteria that ovulation had occurred.

At autopsy, the oviducts were checked for evidence of ovulation and ovaries were removed, trimmed, and weighed. The uterus was examined for ballooning and then removed. Fluid was expressed from the lumen of the uterus and the uterus was subsequently trimmed, weighed, and then placed in a drying oven in preparation for a determination of its dry weight three days later.

Data dealing with positive or negative responses, i.e., the occurrence (or failure to occur) of mating, ovulation, vaginal cornification, and uterine ballooning, were analyzed by means of chi square tests (13) or tables for use with binomial samples (14). Analyses of variance and Student's *t*-test were used to test the significance of data on organ weights (13).

RESULTS

Estrous autopsies. Pentobarbital did not significantly block ovulation ($p>0.05$) of females which were paired with males in the interval between drug administration on proestrus and autopsy on estrus (Table 1); it was effective, however, in inhibiting ovulation at estrus if females were not placed with males ($p<0.05$), as indicated by data from females which were laparotomized on the morning of estrus without having been paired with males the previous evening (Table 2). These data suggest that ovulation at estrus was induced by mating following pentobarbital treatment, as reported by others (9), since pentobarbital caused no significant decrease ($p>0.05$) in the number of animals which mated at the expected time in the cycle. Two animals in the group autopsied at estrus had ovulated, however, without the stimulatory influence of coitus. Barbital also failed to inhibit mating significantly ($p>0.05$) in

TABLE 1. *Comparison of the reproductive systems of control and barbiturate-treated rats autopsied at estrus*

Treatment	N	Ovulated	Mated	Mean ovarian weight (mg)	Uterine ballooning	Mean uterine weight (mg) Wet	Dry	Vaginal cornification
4-day cyclers								
Water	5	5/5	4/5	54.7	0/5	428	78	1/5
Pentobarbital	8	6/8	5/8	55.2	0/8	439	80	1/8
Barbital	9	1/9[a]	6/9	49.7	9/9[a]	518[a]	95[a]	2/9
5-day cyclers								
Water	5	5/5	5/5	51.6	0/5	479[b]	89[b]	0/5
Barbital	11	3/11[a]	4/11	50.7	2/11[b]	481[b]	92[b]	0/11

[a] Significantly different from control group ($p<0.05$).
[b] Significantly different from 4-day cyclers receiving the same treatment ($p<0.05$).

TABLE 2. *Comparison of the reproductive systems of control and barbiturate-treated rats laparotomized at estrus and autopsied at metestrus*

Treatment	N	Ovulated Estrus	Ovulated Metest.	Mated	Mean ovarian weight (mg)	Uterine ballooning Estrus	Uterine ballooning Metest.	Mean uterine weight (mg) Wet	Mean uterine weight (mg) Dry	Vaginal cornification Estrus	Vaginal cornification Metest.
4-day cyclers											
Water	5	5/5	0/5	0/5	45.7	0/5	0/5	322	61	4/5	0/5
Pentobarbital	10	2/10[a]	7/10[a]	4/10	50.4[a]	2/10	0/10	366	69	5/10	1/10
5-day cyclers											
Water	5	5/5	0/5	0/5	47.6	0/5	0/5	337	63	5/5	0/5
Barbital	7	1/7[a]	5/7	5/7	53.4	5/7	0/7	450[a,b]	84[a,b]	1/7	2/7

[a] Significantly different from control group ($p<0.05$).
[b] Significantly different from the corresponding group of 4-day cyclers ($p<0.05$).

both 4-day and 5-day cyclers (Table 1). Unlike pentobarbital, it inhibited ovulation at estrus ($p<0.05$) despite mating having occurred.

Within each group of rats the number of animals which ovulated was compared to the number which mated. This was done to determine whether these two cyclic events had such similarities in their controls that a blockage of mating would be accompanied by a blockage of ovulation in that cycle or whether the two events were not so closely linked and one could be inhibited without an appreciable inhibition of the other. Only in the group of 4-day cyclers treated with barbital was a significant difference seen ($p<0.05$) between the number of animals which mated and the number which ovulated (Table 1). Only one of the six barbital-treated 4-day cyclers which mated also ovulated indicating that coital stimulation did not provide a sufficient stimulus to overcome the blockade of ovulation by barbital. Fewer of the barbital-treated 5-day cyclers mated (Table 1) but, of the four which did mate, two ovulated. In addition another animal ovulated which had not mated. The number of 5-day cyclers which mated was not significantly different ($p>0.05$) from the number which ovulated following the barbital treatment.

The ovarian weights of barbital-treated animals did not differ from those of the controls ($p>0.05$) nor did the ovarian weights of 4-day and 5-day cyclers differ ($p>0.05$) despite there being some differences in the numbers of animals ovulating in the various treatment groups (Table 1). Ovarian weights following ovulation are in some cases greater than before, due to corpora lutea being heavier than the follicles which preceded them.

The only significant increase in the incidence of uterine ballooning at estrus was seen in 4-day barbital-injected rats, the same group of rats which failed to exhibit induced ovulation at estrus (Table 1). With regard to uterine ballooning, this group differed from its control group ($p<0.01$), from barbital-treated 5-day cyclers ($p<0.01$), and from 4-day cyclers injected with pentobarbital ($p<0.01$). The uterine ballooning on the day of expected estrus in the 4-day cyclers was strongly suggestive that barbiturate injection had prolonged the cycle of these animals to the extent that they were still exhibiting proestrous characteristics on the day following their expected occurrence. Some swelling of the uterus was seen in other barbiturate-treated animals as well but the uterus was not considered ballooned unless distended with intraluminal fluid.

Both the mean wet weight and the mean dry weight of uteri removed from barbital-treated 4-day cyclers were greater than those of controls ($p<0.05$) and greater than the uterine weights of 5-day cyclers given barbital ($p<0.05$) (Table 1) indicating that barbital elevated the rate of estrogen secretion by the rats with 4-day cycles. Pentobarbital was not effective, however, in increasing the uterine weight of 4-day cyclers ($p>0.05$). Because of their characteristically higher estrogen levels, 5-day cyclers in the control group had greater uterine weights, wet and dry, at estrus than controls with 4-day cycles ($p<0.05$).

A failure of rats to exhibit vaginal cornification at estrus reflected to some extent the incidence of mating, i.e., animals which mated, whether experimental or control animals, generally failed to have a vaginal smear consisting of only cornified cells on

the day of estrus (Table 1). Some decrease in the number of cornified cells observed and the presence of some leukocytes in vaginal smears often follows mating and was not considered atypical. However, some of the barbiturate-treated rats which did not mate did not have the vaginal cornification characteristic of estrus; half of the pentobarbital-treated 4-day cyclers which were not paired with males prior to examination of their vaginal smears on estrus failed to exhibit completely cornified vaginal smears (Table 2). Five-day cyclers given a barbital injection at proestrus and not placed with males until several hours after examination of their vaginal smears at estrus had a significant reduction ($p<0.05$) in the occurrence of estrous vaginal cornification (Table 2) despite their having had no opportunity for mating.

Metestrous autopsies. Pentobarbital administered to 4-day cyclers caused a significant number of animals to ovulate a day late ($p<0.05$) if they were not mated at estrus (Table 2). Several barbital-treated 5-day cyclers also ovulated a day later than usual but the incidence of late ovulation in this group was not significantly different from that of controls ($p>0.05$). There was no significant increase in the incidence of delayed sexual receptivity ($p>0.05$) revealed by either the pentobarbital or barbital-treated groups of animals when each group was compared to its control group. However, when data from the two barbiturate-treated groups were pooled and the data from the two control groups also combined, it was found that nine of seventeen animals receiving some type of barbiturate treatment mated a day late whereas none of the ten controls mated at metestrus. This comparison of treated vs. control animals indicated a significant increase ($p<0.05$) in the incidence of delayed sexual receptivity due to barbiturate treatment.

The only increase in ovarian weight caused by barbiturate treatment was that observed at metestrus in pentobarbital-treated 4-day cyclers ($p<0.01$) (Table 2). A significant number of these animals had ovulated at metestrus ($p<0.05$) and, as stated earlier, a high incidence of ovulation can increase ovarian weight due to corpora lutea development following ovulation.

Ballooning of the uterus was not seen in any control or experimental animal at metestrus (Table 2) although some swelling of the uterus was seen in rats which had received barbiturates. Both wet weights and dry weights of uteri removed from barbital-treated 5-day cyclers were greater than those of uteri from control animals ($p<0.01$) and from pentobarbital-treated 4-day cyclers ($p<0.05$) even though the uteri were not ballooned. Thus, barbital administration resulted in an increase in estrogen secretion by 5-day cyclers (indicated by their greater uterine weights) which was observed two days after the injection (Table 2), whereas an increase in estrogen secretion had occurred on the day following administration of barbital to animals running 4-day cycles (Table 1).

The incidence of vaginal cornification at metestrus in barbiturate-treated groups of rats was not significantly greater ($p>0.05$) than in control groups (Table 2). The increased estrogen secretion by barbital-treated 5-day cyclers at metestrus, while sufficient to cause uterine weights of these animals to be significantly greater than those of controls ($p<0.01$), was apparently insufficient to cause cornification of the vaginal cells at the time the smears were taken.

DISCUSSION

It was readily apparent that rats with 4-day estrous cycles responded differently to barbital treatment than did 5-day cyclers. A difference in degree of effectiveness might have been expected since previous reports (10, 11, 12) have indicated that animals of different cycle lengths do not respond similarly to pentobarbital treatment. Of particular interest, however, was the fact that the induction of ovulation by mating, a phenomenon reported by others (9, 15), did not occur as readily in 4-day cyclers as in 5-day cyclers receiving the same treatment. This result was similar to the findings of Dominguez and Smith (12) who reported that pentobarbital given in a single injection on proestrus or prior to proestrus would delay or suppress several events of the estrous cycle to a greater degree in 4-day than in 5-day cyclers.

The estrogen titer is higher in 5-day cyclers at proestrus than in 4-day cyclers (6). Therefore, at the time of barbital injection the levels of this hormone would not have been the same in animals of differ-

ent cycle lengths and this could have caused the 4-day cyclers to respond differently than 5-day cyclers with regard to the induction of ovulation by mating. However, barbital stimulated estrogen secretion by 4-day cyclers to the extent that it had surpassed that of barbital-treated 5-day cyclers by the day of estrus as indicated by the significantly greater uterine weights of the treated rats with the shorter cycle. This change in estrogen secretion could also have contributed to the difference seen between 4-day and 5-day cyclers in their capacities for induced ovulation.

If an increased estrogen secretion, following treatment of 4-day cyclers with barbital, were responsible for blocking ovulation to the extent that mating could not invoke it, then estrogen would have to be considered as capable of suppressing ovulation at a certain time in the cycle despite its necessity on proestrus for the release of the LH required for ovulation. Such apparently conflicting influences by a single hormone have been reported for progesterone as well, e.g., it is capable, depending on the circumstances in which it is administered, either of advancing or of inhibiting ovulation (16, 17). With regard to estrogen's possible influence on mating-induced ovulation, the results seen could have been due either to an altered timing of estrogen secretion in the cycle, to an altered amount of estrogen secreted prior to estrus, or to a combination of these factors. Since induction of ovulation by mating has been attributed to an ovulatory release of LH (9), it must be inferred that the estrogen levels of 4-day cyclers treated with barbital were not conducive to release of pituitary LH sufficient for ovulation even though mating occurred.

A significant number of rats which received a barbiturate treatment and which were autopsied at metestrus was found to have mated in the preceding night, 24 hours later than the usual period of sexual receptivity. A delay in mating by some 4-day cyclic rats following pentobarbital treatment was reported by Everett and Sawyer (8) but they did not indicate whether the delay was observed in a significant number of animals nor did they examine the incidence of delayed receptivity in 5-day cyclers.

Ovulation by barbiturate-treated animals can occur at metestrus without induction by mating (8) and some animals in this study ovulated at metestrus without having mated. Late ovulation in barbiturate-treated animals has been attributed to a delayed surge of LH (8) but there is no evidence that the late LH surge can cause mating as well. If mating were greatly influenced by LH release, a block of the proestrous LH surge by barbiturates would have significantly affected mating during the night between proestrus and estrus but that did not occur.

The sexual receptivity which some barbiturate-treated rats exhibited at metestrus and the delayed ovulation seen at metestrus in these groups of animals did suggest some link of the mechanisms regulating these two events. Continuation of the species requires a synchrony of ovulation and sexual receptivity in the reproductive cycle and an intimate connection between their controls would not be surprising. These experiments did not clarify which of the hormonal and/or neural mechanisms affecting ovulation also affect sexual receptivity but did indicate that the two cyclic occurrences are temporally related and that further examination of the similarities between their controls would be warranted.

In general, animals which mated at estrus or metestrus had vaginal smears consisting of a mixture of cornified cells and leukocytes on the day of autopsy. The presence of relatively fewer cornified cells in the smears following mating has been considered partly due to the loss of cornified cells with plugs. The fact that some vaginal smears, taken from animals which had not mated and which were producing high levels of estrogen, did not contain only cornified cells may have been due to there being a longer time required for estrogen to cause cornification of vaginal cells than is required for some of estrogen's other effects. It should be remembered that in a normal cycle estrogen secretion at proestrus is sufficient to bring about LH release and to cause uterine ballooning but the vaginal smears do not usually consist entirely of cornified cells until the following day.

Many studies of reproductive cycles of the rat have been done using rats of only one cycle length. When both 4-day and 5-day cyclers have been used in studies of barbiturate treatment, pentobarbital has been commonly used as the central nervous sys-

tem depressant for 4-day cyclers while barbital has been administered to 5-day cyclers because it is considered the more effective barbiturate for use with rats having the longer cycle length (10). In this study it was found that, with the dosages used, 4-day cyclers treated with pentobarbital responded differently than those treated with barbital and 4-day cyclers treated with barbital differed in their responses from 5-day cyclers treated with the same substance. Some caution would, therefore, seem warranted should any attempt be made to correlate results of two experiments in which rats were treated with barbiturates if there were a difference in either the cycle lengths of the animals used or a difference in the type of barbiturates used in the two investigations.

ACKNOWLEDGMENT

Partial support for this research was provided by a Tulsa University Faculty Research Grant (R-310-81).

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