INDUCTION OF OVULATION IN URETHANE-TREATED RATS

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Subcutaneous injection of urethane (1 g/kg body weight) on the morning of proestrus blocked spontaneous ovulation of rats. When rats injected with urethane were paired with males, however, a significant number of the treated animals ovulated regardless of whether or not they copulated. In cases in which the females did not mate, induction of ovulation was attributed to some type of physical contact with the males or to their presence. When ovulation was induced, a full complement of ova was shed. Results of urethane administration were compared to results that have been reported when barbiturates were used to block ovulation.

INTRODUCTION

Spontaneous ovulation of rats can be blocked by any one of several pharmacological agents, including the barbiturates (1) and urethane (2), if the drug is injected prior to release of the surge of pituitary luteinizing hormone (LH) that occurs between 14.00 and 16.00 h on proestrus, a period known as the critical period. The barbiturates block ovulation by inhibiting the surge of LH from the pituitary gland (1). Urethane also inhibits LH release but does so indirectly by inhibiting release of luteinizing hormone-releasing-hormone (LH-RH) from the hypothalamus, thus interfering with hypothalamic stimulation of the pituitary (2). Urethane at a dosage sufficient to block ovulation also differs in action from the barbiturates in that it causes a much longer period of anesthesia (3).

Injection of LH can cause both barbiturate-blocked and urethane-blocked rats to ovulate. However, the dosage of exogenous LH that is required to override the ovulation-blocking effect of urethane has been reported by some investigators (3) to be lower than that required to cause ovulation in barbiturate-blocked rats, but reported by others (2) to be greater than the dosage required to override barbiturate blockade.

Although barbiturates block pituitary LH release, and thereby block spontaneous ovulation, stimulation of the hypothalamus of a barbiturate-blocked rat causes pituitary LH to be released and consequently induces ovulation. Several experiments have shown that electrical stimulation of the hypothalami of pentobarbital-blocked rats causes the animals to exhibit elevated plasma LH levels and to ovulate (4, 5). In addition it has been shown that stimulation of the hypothalami of barbiturate-blocked rats by mating causes the animals to ovulate (6, 7). Since urethane inhibits function of the hypothalamus, the present study was done to determine whether mating of urethane-blocked rats could induce them to ovulate, as it does barbiturate-blocked rats, or whether the inhibitory influence of urethane on hypothalamic LH-RH release would prevent the induction of ovulation.

A dosage of barbital that will block ovulation, if administered at proestrus, causes an increase in uterine weight at estrus and in addition prolongs the ballooning of the uterus that is characteristic of proestrus (7). Therefore, the effects of urethane on the uterus were examined as a part of the present investigation.

MATERIALS AND METHODS

Virgin, female rats (200 to 300 g) of the Sprague-Dawley strain were kept on a rigidly controlled lighting schedule with lights on from 05.00 to 19.00 hr. Other environmental conditions in the animal colony have been previously described (8). Purina laboratory chow and water were provided *ad libitum.* Vaginal smears were taken from all animals each morning to monitor their estrous cycles and rats were assigned to experimental groups only after completing a minimum of two consecutive estrous cycles of the same length. Rats with both four-day and five-day estrous cycles were included in the study.

Urethane (Sigma), dissolved in physiological saline in a concentration of 0.33 g/m*l*, was injected subcutaneously in a dos-

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age of 1.0 g/kg body weight. (Pilot studies had revealed that this dosage provided an effective block of ovulation.) Control rats were injected with physiological saline in the same ratio of volume to body weight (3 m*l*/kg). All injections were given between 08.00 and 10.00 hr on proestrus.

Some urethane-treated rats were placed in the cages of proven male breeders at a time between 14.00 and 16.00 hr on proestrus and left with the males until 10.00 hr the following day. (Males were used as breeders no more frequently than one day in five to increase the likelihood of their mating.) Each female was checked on the day of estrus and the presence of one or more vaginal plugs or the presence of sperm in the vaginal smear or in the uterus was used as evidence that an animal had mated. One group of urethane-injected females was not placed with males.

Autopsies of female rats were performed between 15.00 and 18.00 hr on estrus. Urethane causes prolonged anesthesia and it was, therefore, expected that rats paired with males might not mate until shortly before being removed from the cages of the males. Since ovulation has been shown to occur 9 to 11 hr after hypophyseal activation (9), autopsies were postponed until late in the afternoon on estrus to allow time for ovulation to occur even if mating should induce pituitary LH release as late as the morning of estrus.

At autopsy the oviducts of each rat were excised, placed between microscope slides, and examined at a magnification of 100× for the presence of ova (oocytes). Any ova that were found were counted. The uterus from each rat was checked for accumulation of intraluminal fluid (ballooning) and removed. After any fluid that the uterus contained was expressed, the uterine tissue was weighed, then dried to a constant weight and reweighed.

The chi square test, with Yates' correction for continuity, and Student's t-test were used to analyze the data.

TABLE 1. *Effect of urethane on ovulation and on the uterus in mated and unmated rats.*

Treatment	*N*	Number ovulating/ no. in group	Ova/ ovulation ± SEM	Uterine wet wt. (mg) ± SEM	Uterine dry wt. (mg) ± SEM	No. ballooned uteri/ no. in group	Initial[a] body wt. (g) ± SEM	Final[b] body wt. (g) ± SEM
Saline	6	6/6	8.5±1.4	475.1±24.5	91.8±5.2	0/6	246±10	246±10
Urethane[c]	10	1/10	2.0[e]	478.3±24.8	94.7±5.2	6/10	256±9	244±8
Urethane, placed with males[d]	26	15/26	5.9±1.0	460.1±13.9	88.7±2.7	6/26	251±6	234±5
Urethane, mated	8	6/8	7.7±2.0	426.6±16.5	85.5±3.6	1/8	262±11	244±10

[a]Body weight at time of injection
[b]Body weight at autopsy
[c]Rats not placed with males
[d]Rats that did not mate when placed with males
[e]Only one rat ovulated; no SEM to report

RESULTS

Urethane in the dosage used provided a deep and long-lasting anesthesia. Urethane-injected female rats were completely unresponsive when they were placed in the cages of male rats. At this time, the males became sexually aroused and attempted to mount the females, but no copulations were witnessed during the short time that the rats were observed after being paired. Urethane-treated rats were in many cases

TABLE 2. *Statistical comparisons[a] of numbers of rats ovulating at estrus following urethane treatment at proestrus.*

Treatment		Urethane[b]	Urethane, placed with males[c]	Urethane, mated
	Number ovulating/ no. in group	1/10	15/26	6/8
Saline	6/6	$P < 0.001$	$0.10 > P > 0.05$	$0.50 > P > 0.30$
Urethane[b]	1/10		$0.02 > P > 0.01$	$0.01 > P > 0.001$
Urethane, placed with males[c]	15/26			$0.70 > P > 0.50$

[a]Any probability above 0.05 was not considered significant
[b]Rats not placed with males
[c]Rats that did not mate when placed with males

TABLE 3. *Statistical comparisons[a] of numbers of rats exhibiting uterine ballooning at estrus following urethane treatment at proestrus.*

Treatment		Urethane[b]	Urethane, placed with males[c]	Urethane, mated
	Number with ballooned uterus/ no. in group	6/10	6/26	1/8
Saline	0/6	$0.10 > P > 0.05$	$0.50 > P > 0.30$	$P > 0.99$
Urethane[b]	6/10		$0.05 > P > 0.02$	$0.10 > P > 0.05$
Urethane, placed with males[c]	6/26			$P > 0.99$

See footnotes for Table 2

still deeply sedated at the time that they were removed from the cages of the males on the morning of estrus. This prolonged effect of urethane was expected to cause a low incidence of mating. Our expectations were confirmed as only 8 of 34 rats left overnight with males were found to have mated when examined at estrus (Table 1).

Rats injected with urethane were classified in three groups: (a) those that were not placed with males; (b) those that were placed with males but did not mate; and (c) those that mated. The drug was effective in blocking ovulation in rats not placed with males (Tables 1 and 2). However, the number of rats that ovulated in each of the urethane-treated groups placed with males was significantly greater (Table 2) than the number that ovulated in the urethane-treated group not placed with males. Ovulation in rats placed with males was not dependent on the rats having mated. The numbers of rats that ovulated in the two groups given urethane and later placed with males did not differ significantly from one another or from the number that ovulated in the control group. Similarly, the two groups of urethane-treated rats that were placed with males also failed to differ significantly from one another ($p > 0.05$) in the numbers of ova shed (Table 1). In neither of these groups was the number of ova shed per ovulation significantly different from the number of ova shed by rats injected with saline ($p > 0.05$). Since only one rat ovulated in the group which was given urethane but not placed with males, the number of ova shed per ovulation following that treatment could not be compared statistically to the numbers of ova shed by rats subjected to other experimental conditions. The single rat that ovulated in that group shed only two ova; this was considered to be a partial ovulation.

Although the wet and dry weights of uteri from those urethane-treated rats that mated were somewhat lower than the weights of uteri from rats in other treatment groups (Table 1), no statistically significant differences between the uterine weights of rats in any groups were found ($p > 0.05$). In addition there were no significant differences between body weights (Table 1) of rats in the four groups studied, either at the time at which the rats were injected ($p > 0.05$) or the time at which they were autopsied ($p > 0.05$).

Ballooning of the uterus at estrus was seen in a significantly greater number of rats in the group that was given urethane but not placed with males than in the group of urethane-treated rats that was placed with males but which failed to mate (Tables 1 and 3). No other groups differed significantly from one another ($p > 0.05$) in the numbers of rats exhibiting uterine ballooning at estrus (Table 3).

DISCUSSION

Placing of urethane-injected female rats with males induced ovulation in a significant number of cases. This result was similar to the result that is seen when barbiturate-treated rats are placed with males but differed in at least one significant way: The presence of male rats without mating does not induce ovulation in barbiturate-blocked rats (7) but the rats may be induced to ovulate by mating (6, 7). Yet, a significantly greater number of rats was found to ovulate in a group that was given urethane and placed with males, even though they did not mate, than was found to ovulate in a group given urethane and not placed with males.

It has been reported that mounts by a male rat without intromission can induce a female to ovulate (10). Results of some experiments have indicated, however, that the stimulation of coitus is necessary to induce ovulation in the rat, e.g., when chlorpromazine-blocked females were placed with "spent" males (ones that had recently mated with receptive females), the blocked females failed to ovulate (11). In the chlorpromazine study (11), as in the present study, female rats were left with males overnight and evidences of ejaculation (presence of vaginal plugs or vaginal sperm) were sought the following day but females were not watched throughout the night to verify that no intromissions occurred. In our study the females that failed to mate were still so deeply sedated at the time of their removal from the males' cages on the morning of estrus that any responsiveness to the males would have been passive. Consequently, urethane-treated females that ovulated without having mated were considered to have been induced to ovulate by the presence of the males and/or physical contact with the males exclusive of copulation e.g., by the males nudging the females or sniffing the genitalia of the females. Our data, therefore, are in agreement with the report that male rats can in some instances induce females to ovulate without the stimulus of copulation (10).

It has been found that urethane may only reduce and delay release of LH in the rat, rather than completely blocking LH release, and that the amount of exogenous LH necessary to elicit ovulation in a urethane-blocked rat is lower than that required to overcome barbiturate blockade (3). These findings suggest that a stimulus sufficient to induce ovulation in a urethane-blocked rat needs to stimulate only release of the remainder of the LH complement necessary for ovulation rather than release of the total requirement. It is probable, therefore, that the presence of a male rat without copulation can cause release of enough LH to meet the requirement for ovulation in a urethane-blocked rat but that the amount of LH released is less than that which would be required to overcome barbiturate blockade.

It has been reported that a partial ovulation can follow urethane injection but that the effect of a barbiturate on ovulation is "all or none" (3). In the present study the single rat that ovulated in a group that was injected with urethane but not paired with males had only a partial ovulation. The number of ova shed by each of the other groups of urethane-treated rats was not significantly less ($p > 0.05$) than the number of ova shed by the group of saline-injected controls. This indicated that when ovulation was induced by mating or some other interaction with a male rat, a full complement of ova was shed.

Increased uterine weight in rats given barbital as an ovulation-blocking agent has

been attributed to a concomitant increase in estrogen secretion (7), since uterine weights can be correlated with estrogen titers (12). Urethane caused no significant change in uterine weights of test animals and it was, therefore, concluded that this drug neither stimulates nor prolongs estrogen secretion to the extent that certain barbiturates do while blocking ovulation.

Uterine ballooning occurs characteristically at proestrus (13) and in the normal cycle the uterus is no longer ballooned on the day of estrus. Barbiturates differ in the degree to which they cause uterine ballooning to persist; barbital causes uterine ballooning in some rats to be prolonged until estrus but pentobarbital does not (7). Administration of urethane to rats that were not subsequently placed with males caused a significantly greater number of these rats to have ballooned uteri at estrus than was seen in the group of urethane-treated rats that was placed with males but which failed to mate, a group in which many rats ovulated. Since progesterone secretion late in the afternoon of proestrus is necessary if loss of luminal fluid from the uterus is to occur by the day of estrus (14), our data indicate that the amount of progester-one secreted on proestrus by the urethane-treated rats that were not placed with males, a group in which ovulation was blocked, was less that that secreted by rats in a group in which ovulation was induced.

In conclusion our experiments indicated that, although urethane will act to block spontaneous ovulation as the barbiturates will, rats that are placed with males following treatment with urethane exhibit some responses that are unlike responses seen in barbiturate-treated females placed with males. These differences suggest a need for further examination and comparison of the effects of different types of ovulation-blocking agents on pituitary LH release and on ovarian hormone activity.

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