INHIBITION OF OVULATION BY INTRACRANIAL IMPLANTATION OF PROGESTERONE IN THE 4-DAY CYCLIC RAT

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Summary. Crystalline progesterone or cholesterol (50 µg/rat) was implanted stereotaxically into the anterior pituitary gland or median eminence region on the day before pro-oestrus in the 4-day cyclic rat, and the effects of these steroids on ovulation were studied by examining tubal ova at autopsy on the expected day of oestrus. Progesterone (at 1/10 the effective subcutaneous dose) implanted at either location proved significantly more active in interfering with ovulation than cholesterol ($P < 0.01$). The data provide evidence that progesterone acts at both the hypothalamic and pituitary levels to interfere with ovulation.

Earlier studies have demonstrated that progesterone acts at both ovarian (Redmond, 1968; Stevens, Spies, Hilliard & Sawyer, 1970; Labhsetwar, 1971) and pituitary (Stevens *et al.*, 1970; Labhsetwar, 1971) levels to interfere with ovulation, but whether or not its targets include the central nervous system has not been unequivocally demonstrated. A relatively large implant (400 µg) of progesterone placed in the hypothalamus delayed puberty and the formation of corpora lutea, but a similar implant in the anterior pituitary proved ineffective (Smith, Weick & Davidson, 1969). Because the evidence for the involvement of the central nervous system is circumstantial and often contradictory, we have stereotaxically implanted progesterone into the median eminence region or anterior pituitary gland and studied its effects on ovulation by examining tubal ova at autopsy on the day of expected oestrus.

Adult, sexually mature rats of Alderley Park strain I, which were Wistar-derived and bred randomly for several generations under pathogen-free conditions, were used. They were housed under controlled conditions of light (from 06.00 to 20.00 hours) and temperature (22° C), and allowed free access to a standard diet and tap water. Rats were selected for a close body weight range (160 to 180 g) and regular oestrous cycles of 4 days. Each rat used had at least two consecutive 4-day oestrous cycles before use. They were anaesthetized with pentobarbitone (30 mg/kg, i.p.) supplemented with halothane on the

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morning of the day before pro-oestrus. This dose of barbiturate has been found to be virtually ineffective in interfering with ovulation when given at this time in the cycle (Labhsetwar, unpublished observations; Brown-Grant, 1969). The rats were then placed in a stereotaxic instrument and a needle bearing a mixture of cocoa butter and progesterone was lowered to a desired location, i.e. the median eminence region or anterior pituitary gland, by the technique described by Bainbridge & Labhsetwar (1971). The presence of a hole in the median eminence region or on the dorsal surface of the pituitary gland at autopsy confirmed the original positioning of the tip of the needle. The 'pellet', estimated to contain 1.7 mg cocoa butter mixed with 50 μ g steroid, was ejected and the needle was then withdrawn. Control implants consisted of cocoa butter mixed with 50 μ g cholesterol. The cholesterol was obtained commercially and recrystallized before use to remove impurities.

TABLE 1

EFFECTS OF GRADED DOSES OF PROGESTERONE ADMINISTERED SUBCUTANEOUSLY AT 17.00 HOURS ON THE DAY OF DIOESTRUS ON OVULATION IN THE 4-DAY CYCLIC RAT

<i>Dose (mg/ animal</i>	<i>Body wt† (g)</i>	<i>No. rats ovulating/ No. treated</i>	<i>Ova/ ovulating rat†</i>	<i>Ovarian wt (mg)†</i>	<i>Uterine wt (mg)†</i>	<i>No. of rats with cornified smears/ Total no.</i>
0	216 ± 6	5/5	12.2 ± 0.7	94 ± 5	371 ± 33	5/5
0.25	207 ± 6	4/5	10.0 ± 0.7	94 ± 7	346 ± 5	5/5
0.5	200 ± 6	0/10	0	82 ± 4	345 ± 22	5/5
1.0	193 ± 7*	0/5	0	76 ± 4*	326 ± 28	8/10
2.0	202 ± 4	0/4	0	75 ± 5*	289 ± 11	0/4
4.0	214 ± 4	0/9	0	66 ± 8*	240 ± 14*	0/9

* $P < 0.05$.

† Values expressed as Means ± S.E.

Vaginal smears were taken daily until autopsy on the morning of the expected day of oestrus, i.e. approximately 48 hr after surgery. To study the extent of diffusion of steroid and cocoa butter, the experiment was repeated using two rats/group with the difference that the steroid in the pellet was replaced with Sudan Black. After autopsy, the brains of these rats were serially sectioned, and photographed. Diffusion of dye could be easily studied in the photographs. From implants placed into the median eminence region, dye had diffused into the third ventricle and had also migrated along the path of the needle. No diffusion to the hypophysial region could be seen in either rat. When implants were placed into the anterior pituitary, the dye diffused into the space between the hypophysis and the ventral surface of the hypothalamus. It had also penetrated the brain along the needle path in the mammillary region. Thus, while our study permits differentiation of effects of implants between the hypothalamus and anterior pituitary, it does not permit further resolution with respect to specific hypothalamic nuclear groups. At autopsy, uterine and ovarian weights were recorded and the number of tubal ova/rat determined. In a few groups of rats, progesterone was administered subcutaneously to determine the minimal effective dose by the systemic route. All data were

TABLE 2
EFFECTS OF INTRACRANIAL IMPLANTATION OF CHOLESTEROL OR OF TWO DIFFERENT PREPARATIONS OF PROGESTERONE ON OVULATION IN 4-DAY CYCLIC RATS

Implant group	Nature of implant	Location of implant	Body wt (g)†	No. rats ovulating/ No. treated	% Inhibition of ovulation	Oval/ovulating rat†	No. rats with cornified smear/ No. treated	Ovarian wt (mg)†	Uterine wt (mg)†
A	Cocoa butter alone	Median eminence ^c	185 ± 3	10/14 ^d	(20)	11.0 ± 0.6	14/14	80 ± 3	325 ± 6
B	Cocoa butter alone	Pituitary ^c	226 ± 3	6/6	(0)	13.8 ± 0.5	6/6	87 ± 2	358 ± 12
C	Progesterone* in oil vehicle*	Median eminence	180 ± 4	3/19	(84)	9.0 ± 0.6	19/19	73 ± 2	327 ± 19
D	Oil vehicle* alone	Median eminence	163 ± 5	3/8	(63)	11.0 ± 0.6	8/8	73 ± 3	288 ± 13
E	Crystalline progesterone ^b	Median eminence	189 ± 3	2/11	(82)	12.0 ± 2.0	8/11	65 ± 2	350 ± 17
F	Cholesterol ^b alone	Median eminence	183 ± 3	7/9 ^d	(22)	11.7 ± 0.7	9/9	76 ± 5	302 ± 7
G	Crystalline progesterone ^b	Pituitary	173 ± 4	0/9	(100)	0	8/9	67 ± 2	319 ± 11
H	Cholesterol ^b alone	Pituitary	203 ± 3	9/14 ^d	(36)	10.2 ± 1.3	14/14	85 ± 3	345 ± 15

** $P < 0.01$; *** $P < 0.002$.

* Vehicle was supplied by Organon Co. and contained, in addition, benzyl alcohol, and cresol. (See text).

† Values expressed as Means ± S.E.

^a Dose was either 17 or 40 µg/implant.

^b 50 µg/implant in each case.

^c Part data from an earlier study (Bainbridge & Labhsetwar, 1971).

^d Not significantly different from each other by Fisher's test.

analysed for statistical significance by a computerized *t* test or by Fisher's exact probability test using Finney's tables (Siegel, 1956).

In rats given progesterone subcutaneously in oil late in dioestrus, i.e. at 17.00 hours, and killed on the morning of expected oestrus, the minimum dose effective in blocking ovulation was found to be 0.5 mg (Table 1). At this dose, inhibition of ovulation was not accompanied by any interference with vaginal cornification or uterine weight increase (Table 1). However, both of these responses were blocked or modified by doses of progesterone in excess of 2 mg and ovarian weights were reduced.

A tenth of the smallest systemically active dose of progesterone was selected for intracranial implantation. In the initial experiment, progesterone dissolved in oil and other organic solvents as supplied by Organon Laboratories Ltd was used and proved highly effective in interfering with ovulation when implanted into the median eminence region (Group C). However, similar effects were exerted by the vehicle alone (Group D). According to the supplier, the vehicle was composed of arachis oil mixed with 10% benzyl alcohol and 0.3% cresol. It is apparent from these results that non-specific substances can also interfere with ovulation.

The entire experiment was then repeated with crystalline progesterone (B.D.H., Poole) and recrystallized cholesterol made up in cocoa butter which was known to be only marginally effective by itself in interfering with ovulation (Table 2, Group A). A dose of 50 μ g progesterone implanted either into the median eminence region or anterior pituitary gland proved significantly more active than a similar dose of cholesterol in inhibiting ovulation ($P < 0.01$ in each case, Groups E to H) and no interference with vaginal cornification or uterine weight increase was observed (Table 2). This selective action of progesterone suggests that its effect was not exerted at the peripheral level. Our data thus suggest that intracranially implanted progesterone can act both at the hypothalamic and pituitary level to inhibit ovulation. This, however, does not mean that during pregnancy or pseudopregnancy endogenously produced progesterone acts selectively at those sites. If it is assumed that the daily output in rats exceeds 2.0 mg/day, it may well act, in addition, directly on the ovaries, as demonstrated earlier (Stevens *et al.*, 1970; Labhsetwar, 1971).

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