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The Inhibition of Prolactin Secretion Due to Intrahypothalamic Pituitary Grafts Is Reversed by Estradiol Benzoate But Not by Progesterone (42369)

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Abstract. Anterior pituitary (AP) tissue grafted into the hypothalamus inhibited the luteotrophic response to mating and prevented pseudopregnancy (PSP) and pregnancy. All normal rats given 10 μg estradiol benzoate (EB) on estrus became PSP (15 days) while the same treatment caused 10-day PSPs in 20/21 grafted rats. Doses of 30 μg EB or 10 μg EB plus reserpine (1 mg/kg) resulted in 15-day PSP in grafted rats. By contrast progesterone (P; 10 mg on estrus) did not prolong cycles in rats with hypothalamic grafts though it did in 50% of normals. Earlier studies showed that PSP or pregnancy was restored in the grafted rats by blockade of dopamine (DA) secretion. The results above show that EB was similarly effective in restoring PSP while P was not, suggesting that EB both raised prolactin and lowered DA while P was unable to lower DA in rats with AP grafts in the hypothalamus.

Anterior pituitary (AP) grafts in the hypothalamus prevent pseudopregnancy (PSP) and pregnancy in rats (1, 2) by inhibiting the luteotropic surges of prolactin (PRL) which normally follow coitus (3). It was reported recently that the inhibition of the PRL response in these rats could be overcome by blockade of either dopamine (DA) secretion or DA receptors (4).

When estrous rats were treated once with either EB (10 μg) or P (10 mg), twice daily PRL surges were initiated which sustained PSP in 100 and 50% of rats, respectively (5, 6). The present study sought to determine whether EB or P treatment on the day of estrus could similarly initiate luteotropic PRL surges and hence sustain PSP in rats with intrahypothalamic AP grafts.

Materials and Methods. Small (<0.5-mg) pieces of AP tissue from neonatal donors (<13 days) were introduced stereotaxically into the anterior hypothalami of closely related females of the same inbred strain of rats as described previously (1, 4). Rats were housed and managed as before (4). Vaginal smears were examined daily and the presence of spermatozoa in grafted rats, or >95% of cells cornified in unmated normals, was taken as the day of estrus (Day 0). The proestrous smear (day before estrus) usually showed nucleated and cornified epithelial cells in a 50:50 ratio. The duration of PSP was recorded between consecutive estrous smears or between estrus and the next proestrous smear. In treated rats the estrous or proestrous smears for 2–4 days after EB were included in the PSP duration.

Normal and grafted rats were used after two or more 4- or 5-day estrous cycles. The grafted rats had all exhibited at least two cycles where matings with fertile bucks were followed by a 4- or 5-day cycle (1, 2, 4). At the conclusion of the study serial sections of the brains of the grafted rats were examined for the presence, site, and size of the grafts. The data below were derived only from rats in which AP grafts were seen *post mortem.* When grafts failed to "take" the rats became pregnant and were excluded from further study.

Treatments. EB and P were prepared in safflower oil to provide the appropriate doses (10 and 30 μg EB and 10 mg P) in 0.3 ml. Injections (sc) were made before 1000 hr. Reserpine was injected sc at 1 mg/kg in 1% ascorbic acid.

Results. *Estradiol benzoate.* A single injection of 10 μg EB was given sc before 1000 hr on the morning of estrus to 11 normal unmated rats and to 21 mated rats with intrahypothalamic AP grafts. The preceding cycle in all cases was of 4 days duration despite the occurrence of fertile coitus in the grafted rats. A PSP cycle of 9–18 days ($\bar{x}$ = 14.7 days) immediately followed EB in the normals and a PSP of 7–15 days ($\bar{x}$ = 9.8 days) followed in 20 out of 21 rats with grafts. The other grafted rat had a 4-day cycle. The mean duration of PSP in the mated rats with AP grafts in the

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TABLE I. DURATION OF PSEUDOPREGNANCY IN NORMAL RATS AND RATS WITH INTRAHYPOTHALAMIC PITUITARY GRAFTS AFTER ESTRADIOL BENZOATE (EB) TREATMENT ON ESTRUS

Rats	Number of cycles	Mean cycle length (days ± SE)
Normals		
Pretreatment	11	4.00
EB, 10 μg	11	14.73 ± 1.01[a]
Post treatment	7	4.14 ± 0.20
Grafted		
Pretreatment	63	4.20 ± 0.15
EB, 10 μg	21	9.76 ± 0.54[b]
Post treatment	24	3.91 ± 0.21
Pretreatment	20	4.30 ± 0.13
EB, 30 μg	12	15.58 ± 0.68[c]
Post treatment	12	4.82 ± 0.18
Pretreatment	7	4.0
EB, 10 μg + RES	7	14.40 ± 1.23[d]
Post treatment	10	4.60 ± 0.16

Note. Normals never mated, grafted all mated; RES = 1 μg reserpine/kg sc.

[a-d] Comparisons: *a*, *c*, and *d* > *b*; all $P < 0.01$; *a* vs *c* or *d*, N.S.

hypothalamus was significantly shorter ($P < 0.01$ by t test) than that in normals. These data are shown in Table I.

Rats with intrahypothalamic AP grafts were then given either 30 μg EB on estrus or 10 μg EB plus reserpine (1 mg/kg) and mean PSP durations of 15.6 and 14.4 days, respectively, followed, i.e., not different from that of the group of unmated normals. None of the rats with grafts became pregnant even though their vaginal cycles had been significantly prolonged. Cornified cells were present and leucocytes absent in vaginal smears for 2 days in 20 rats with grafts, for 3 days in 13, and for 4 and 5 days in 2 other rats each. Spermatozoa were seen in most of these smears, and with leucocytes in some others, i.e., for 2 days (21 rats), 3 days (16 rats), 4 days (6 rats), and 5 days (2 rats). The duration of PSP after 10 μg EB was unrelated to the duration of the non-leucocytic smears after treatment.

Except for two 3-day and one 6-day cycle, the first post-treatment cycles after EB-induced PSPs were of normal duration (4–5 days).

Progesterone. Progesterone (10 mg sc) was given to 25 normal unmated rats before 1000 hr on estrus following two 4-day cycles. PSP cycles of 11–17 days ($\bar{x}$ = 14.2 days) followed in 13 normal rats while 5- or 6-day cycles ($\bar{x}$ = 5.2 days) followed in 12 others. The same treatment, given on the day of spermatozoa in the smear to 14 rats with AP grafts in the hypothalamus, was followed in only 1 rat by a 15-day PSP and in the remaining 13 by 4- to 6-day cycles ($\bar{x}$ = 4.9 days).

The proportion of rats which responded to 10 mg P with PSP was significantly greater for normal rats (13/25) than for rats with intrahypothalamic grafts (1/14) by χ^2 ($0.05 < P(2) > 0.01$). In most of the grafted rats the cycle was prolonged only by 1 day (see Table II).

In another 13 rats with AP grafts in the hypothalamus 10 mg of P was given on the day of estrus and again 48 hr later. The double treatment prolonged cycles in 12 rats to 6–8 days ($\bar{x}$ = 7.33 days) while the other rat became pregnant. The mean cycle length after the two treatments with P 48 hr apart (7.33 days) was significantly greater ($P < 0.01$, t = 9.26) than the mean following a single P treatment (i.e., 4.92 days) but was not further increased by a third P injection given 48 hr after the second (cycle = 6.0 ± 0.55 days) in another 5 rats.

Discussion. Stimulation of the uterine cervix at coitus is the physiological input signal for twice daily surges of PRL which provide the initial luteotropic stimulus in the rat (7). Coitus does not induce PSP or pregnancy in rats bearing grafts of anterior pituitary tissue in the hypothalamus (1, 2) and the luteotropic surges of PRL are absent (3). It was reported recently that the blockade of DA synthesis or of DA receptors for 3–4 days by reserpine, α-methyl-tyrosine, or haloperidol, respectively, resulted in prolongation of cycles and the support of PSP or pregnancy in the grafted rats (4). Each of these treatments elevates PRL in the short term (8–11) but, in view of the vaginal smear data and the induction of PSP or pregnancy in the grafted rats, PRL must have been elevated over a period of several days probably as daily or twice daily surges (12, 13). It seems most likely that a single dose of any of these three drugs given to normal rats on diestrus Day 1 initiates the luteotropic pattern of PRL secretion also as they regularly induce PSP (4, 10).

Estradiol benzoate and progesterone given once on the day of estrus also established PSP

TABLE II. EFFECT OF PROGESTERONE (10 mg) ADMINISTERED ON THE DAY OF ESTRUS ON CYCLE LENGTHS IN NORMAL RATS AND RATS WITH INTRAHYPOTHALAMIC PITUITARY GRAFTS

Rats	Treatment	Number of cycles	Mean cycle length (days ± SE)
Normal	Pretreatment	25	4.0
	P, Day 0	12	5.25 ± 0.13[b]
	P, Day 0	13	14.23 ± 0.62[c]
	Post treatment	23	4.00 ± 0.09
Grafted	Pretreatment	30	4.00
	(i) P, Day 0	13[a]	4.92 ± 0.18[d]
	(ii) P, Day 0 + D2	12[a]	7.33 ± 0.19[e]
	(iii) P, Day 0 + D2 + D4	5	6.00 ± 0.55[f]
	Post treatment		
	(i)	42	4.21 ± 0.08
	(ii)	27	4.15 ± 0.13
	(iii)	10	4.00

Note. P = progesterone 10 mg sc before 1000 hr. Day 0 = estrus, D2 = diestrus day 2, D4 = 4th day after estrus.
[a] Excludes 1 rat which became pregnant.
[b-f] Comparisons: $c > b$, $e > d$, $P < 0.01$; b vs d or f, N.S.

(14–17) and initated twice daily surges of PRL in all rats given 10 μg EB and in 50% of those given 10 mg P (5, 6). When these treatments were applied above to rats with anterior pituitary grafts in the hypothalamus it was found that the 10-μg dose of EB regulary induced a 10-day PSP compared to a 15-day PSP in normals. At 30 μg EB or when 10 μg was given together with reserpine (a dose found ineffective by itself on estrus; unpublished data), a longer (15-day) PSP followed. While 10 μg EB alone caused shorter PSPs in grafted than in normal rats, this dose was equally effective in terms of PSP incidence as only 1 of 21 grafted rats failed to respond. It has been reported that EB can reverse the DA-induced inhibition over PRL secretion *in vitro* (18), that estrogen can inhibit DA release into portal blood (19) though perhaps indirectly through raising PRL (20, 21), or it may decrease the entry of DA into anterior pituitary cells (22). Whichever of these mechanisms was involved, a single injection of 10 μg EB overcame DA inhibition and raised serum PRL for 72 hr and, if given on the day of estrus, twice daily PRL surges continued thereafter (5).

EB induced PSP in rats with intrahypothalamic pituitary grafts, as did blockade of DA or its receptors (4). By contrast, P was unable to establish PSP in grafted rats while it did so in about 50% of normals. The success of 10 mg P given on estrus in inducing PSP in normal rats was reported to be 72% in 4-day cycle rats and to fall to 40% if they were isolated and not subjected to vaginal smears (16), indicating neural reinforcement of the hormonal events. De Greef and Zeilmaker (5) found no PRL surges in 50% of rats which did not become PSP after 10 mg P, the same proportion which failed to show PSP above. Their suggestion that a second 10 mg P 2 days after that given on estrus could lead to PSP in rats refractory to one injection (5) was tried but found unsuccessful in rats with hypothalamic pituitary grafts. In view of the fact that DA blocking drugs induced pseudopregnancy in these rats (4) while P could not, it would seem unlikely that the induction of twice daily PRL surges by P (5, 6) involves inhibition of DA secretion. In fact it has been suggested that P may have a negative feedback on PRL release by increasing dopaminergic activity within the tuberoinfundibular system (23).

A positive feedback of P on PRL has been suggested as a means whereby P induces PSP (15, 16). P rises within 24–30 hr of the elevation of PRL (7), augments the nocturnal PRL surges (24), can reinforce suboptimal coital stimuli (25), and prolongs the time of occurrence of PRL surges (26). In normal rats P, as well as causing an initial rise in PRL, may depress the inhibitory action of the medial preoptic area over the suprachiasmatic nucleus (27, 28) so that the PRL surges become asso-

ciated with the circadian light cycle. Because no rats with AP grafts in the hypothalamus became PSP after P it seems likely that DA was not depressed (4) or PRL did not rise or both.

While the mechanism whereby P initially elevates PRL remains uncertain, it seems clear from the above that if correctly timed with respect to the cycle, sustained high serum PRL will subsequently induce the twice daily peaks of PSP in normal rats. In rats with hypothalamic pituitary grafts generation of these surges require the additional removal of DA inhibition which prevents the release of PRL.

By what means elevation of PRL may lead to subsequent PRL surges of PSP is not clear. After 10 μg EB, the uteri were ballooned for 44 hr and nonleucocytic smears persisted for up to 72 hr (5). These observations were confirmed above and behavioral estrus sometimes lasted 5 days. Thus E_2 levels or their physiological and behavioral effects were prolonged after 10 μg EB and PRL remained above 200 ng/ml into the third day after this treatment (5). Except through this elevation of PRL EB itself may be unimportant in the induction of PSP and the PRL surges, as these surges can be brought about by DA blockade (4), by P (5, 6), or by sterile coitus (28). The evidence points to the duration of PRL elevation as a key to the induction of the PSP surges. The shorter acting E or EB in 1- or 5-μg doses (5, 17, 29) all caused a shorter duration of elevation of PRL and were all less effective than 10 μg EB in establishing PSP. Likewise injections of PRL for 1 or 2 days were unable to induce PSP while injections for 4 days did so (30) and prolonging the duration of exposure to sustained PRL from AP autografts from 2 to 4 days significantly increased the proportion of rats responding with PSP (26). The onset of twice daily PRL surges after removal of the isografts (26) suggests that the surge pattern might be induced by rebound from short loop feedback inhibition of the *in situ* pituitary, with a PRL-induced rise in DA in portal blood (21) being followed by enhanced PRL release as DA concentrations subside (31). It would be of interest to know whether the hormone-induced PSP pattern of PRL surges requires the presence of ovarian progesterone to establish appropriate neural links in the hypothalamus (27, 28).

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