tonin at night. Likewise, when PRL levels in intact rats were depressed by treating the animals with bormocriptine, there was no change in either high nighttime NAT activity or melatonin levels. Thus, the earlier observations showing that PRL changes the ultrastructure of the pineal gland [10, 11] suggests that the alterations observed relate to something other than indoleamine metabolism in these cells.

It is possible, however, that hypophysectomy merely phase-shifted the melatonin rhythm and thus, peak melatonin levels occurred earlier or later in the dark phase. Another study in which hypophysectomized rats were used indicates this procedure does not shift the rhythm, it merely dampens it [7]. Thus, this explanation seems untenable. On the other hand, if hypophysectomy merely shifted the NAT and melatonin rhythms in the present study, neither PRL nor GH was able to re-establish the normal phasing of these cycles.

Hypophysectomy, of course, causes a marked reduction in a number of other pituitary-derived hormones as well. Of these, however, neither thyroid stimulating hormone, luteinizing hormone, follicle stimulating hormone, nor adrenocorticotrophin hormone seem to be involved in any substantial way in determining pineal melatonin production. The effect of α-melanocyte stimulating hormone, which derives from the intermediate lobe of the pituitary and which is reduced after hypophysectomy, has not been tested in terms of its ability to influence the conversion of serotonin to melatonin in the pineal gland.

Rather than relying on a single hormone, the nocturnal rise in NAT activity and melatonin production in the pineal gland likely relies on a combination of pituitary and non-pituitary hormones. Furthermore, it would require extensive testing to uncover what this combination of factors may be. The possibility exists that hypothalamic damage as a result of the surgical procedure, rather than the loss of the pituitary gland, accounted for the reduced NAT activity and melatonin level in the pineals of hypophysectomized rats. This explanation seems unlikely considering the diaphragma sellae is quite tough in the rat and numerous studies have shown that this procedure results in no serious damage of the hypothalamus [17]. Finally, had the GH and/or PRL injections been given at some other points thoughout the 24 h, possibly they would have promoted pineal melatonin production.

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Effects of multiple injections of luteinizing hormone-releasing hormone on the induction of pregnancy in androgenized female rats

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Summary. A study of the effect of cyclic (every 4 days) administration of gonadotropin releasing hormone on reproductive performance of the androgenized female rat was carried out. The responses measured were indirect indices of increased gonadotropin output; ovulation rate, uterine decidualization, mating and implantation.
Key words. Androgen-sterilized rat; luteinizing hormone-releasing hormone (LHRH); ovulation; uterine decidualization; mating; implantation.

Administration of androgen to neonatal female rats results in infertility accompanied by persistent vaginal cornification in adulthood. This syndrome may result from disorders of the hypothalamus and the subsequent alteration of the cyclic release of luteinizing hormone (LH) from the pituitary gland [1, 2]. Although ovulation in such androgenized female rats is readily brought about by a single injection of luteinizing hormone-releasing hormone (LHRH) [3, 4]. LH [5–7] or human chorionic gonadotropin (hCG) [5, 8], the rats rarely become pregnant. Hahn and McGuire [4] have suggested that the failure of embryos to become implanted results from a deficit of progesterone from the induced corpora lutea. Implantation failure in androgenized female rats has also been explained by the lowered decidual response in the uterus [9]. However, the reasons for the failure of androgenized female rats to become pregnant are still unknown. In a previous report it was suggested that the uterine sensitivity to blastocyst implantation of androgenized immature rats may be normal [10]. The endocrine environment in androgenized female rats following injections of LH every 4 days is similar to that of normal rats [11, 12]. The pituitary glands of androgenized female rats can release LH in response to LHRH stim-

ulation[7]. The endocrine environment resulting from an endogenous LH surge is more natural than that produced by an exogenous LH surge. Here, the effects of multiple injections of LHRH on the induction of pregnancy in androgenized female rats is studied.

Materials and methods. Animals. Sprague-Dawley rats bred in this laboratory were used. They were kept at 24 ± 1 °C under a 14-h photoperiod (lights were on from 05:00 to 19:00 h), and maintained on water and commercial standard pellet food. Androgen sterilization was brought about by an s.c. injection of 1 mg testosterone propionate (Sigma Chemical Co., St. Louis, MO) in 0.02 ml sesame oil when the rats were 5 days old[9]. This produces almost 100% sterility and vaginal opening occurs around the 32nd day after birth. Rats that were 25 or 37 days old were given multiple s.c. injections of 400 ng synthetic LHRH (Sankyo Pharmaceutical Co., Tokyo) in 0.2 ml physiological saline (9 g NaCl/l). The injections were given at 14:00 h every 4 days until the rats were 53 days old. Groups of rats that received injections of physiological saline served as the controls.

Ovulation. Twenty-four hours after the last injection of LHRH, the animals were killed and their oviducts and ovaries were examined under a dissecting microscope for the presence of ova.

Mating and implantation. To determine whether the rats were capable of mating and implantation, adult male rats previously proven to be fertile were placed in cages with androgenized female rats treated with LHRH. The presence of spermatozoa in the vaginal smear the following morning was defined as Day 1 of pregnancy. In some androgenized female rats, on Day 1, a pituitary gland was transplanted from a normal rat into the kidney capsule under ether anesthesia to maintain functional corpora lutea[4, 8]. All of these animals were killed on Day 10 and the number of animals with implantations was recorded.

Uterine decidualization. Attempts were made to cause uterine decidualization in androgenized female rats after ovulation had been brought about by LHRH. To induce functional corpora lutea, transplantation of a pituitary gland into the renal capsule was used in this experiment also. The transplantation was done on the day after the last injection of LHRH. On Day 5, the endometrium of the right uterine horn was traumatized with a bent needle by the technique of De Feo[13]. The contralateral horn served as the control. On Day 10, the stimulated and intact horns were excised, cleaned of fat and adhering tissue, and weighed separately.

Statistics. Fisher's exact probability test was used to compare the percentages of ovulation, mating, and conception in different groups. Significant differences between groups in the number of eggs recovered and the uterine weights were evaluated by Student's t-test and Duncan's new multiple range test, respectively. Differences were considered significant at the 5% level.

Results. Induction of ovulation, mating, and implantation. One injection of LHRH every 4 days induced a regular 4-day estrous cycle from approximately 40 days of age. The saline-treated control groups had persistent estrous smears. Eggs were observed in all rats within 24 h after the last injection of LHRH (table 1, Groups 3 and 4), but the control rats did not ovulate (table 1, Groups 1 and 2). The number of eggs recovered was not significantly different for rats in which the cyclic administration of LHRH was begun before and after vaginal opening (table 1, Group 3 vs 4). The mating rate of the rats that received cyclic administration of LHRH was 100% (table 2, Groups 7 and 8); the saline-treated control rats did not mate (table 2, Groups 5 and 6). When the mated animals were autopsied on Day 10, no pregnancies had occurred (table 2, Groups 7 and 8). However, pregnancies were found in some of the rats that had received a pituitary transplant after the cyclic administration of LHRH (table 2, Groups 9 and 10).

Table 1. Ovulation in androgenized female rats given multiple s.c. injections of physiological saline (Groups 1–2) or 400 ng LHRH (Groups 3–4) at 14:00 h every 4 days, starting before (Day 25) or after (Day 37) vaginal opening, until 53 days of age.

Group	Beginning of LHRH treatment	No. of rats ovulating (%)	Mean ± SEM No. of eggs recovered
1	Day 25	0/5 (0)	0
2	Day 37	0/5 (0)	0
3	Day 25	5/5 (100)	10 ± 0.6
4	Day 37	5/5 (100)	8 ± 0.2

Induction of deciduomata. The traumatized horn of rats in the groups that received cyclic administration of LHRH starting before or after vaginal opening (table 3, Groups 13 and 14) formed deciduomata after a pituitary gland had been transplanted into the renal capsule, but the uterine trauma caused no response in the saline-treated control groups (table 3, Groups 11 and 12). The response was greater in the group treated with LHRH starting before vaginal opening than in those treated starting after (table 3, Group 13 vs 14). The mean uterine weight of rats treated before vaginal opening was almost the same as that of normal pseudopregnant rats (table 3, Group 13 vs 15).

Discussion. The pituitary glands of androgenized female rats can release LH in response to LHRH stimulation[7]. In the present study, because the cyclic preovulatory LH surge is absent in androgenized rats[1, 2], a classical approach was taken to circumvent the probable deficit in cyclic release of LH by an s.c. injection of LHRH given every 4 days. The full effective dose of LHRH that can induce an estrous cycle following ovulation was used[4, 7, 10]. The cyclic injection of LHRH, begun either before or after vaginal opening, induced a regular 4-day estrous cycle from approximately 40 days of age. The ovulation and mating rates in androgenized rats following the cyclic injection of LHRH were normal with either timing. However, when mated animals were autopsied on Day 10, no pregnancies were found. In androge-

Table 2. Mating and conception in androgenized female rats given multiple s.c. injections of physiological saline (Groups 5–6) or 400 ng LHRH (Groups 7–10) at 14:00 h every 4 days, starting before (Day 25) or after (Day 37) vaginal opening, until 53 days of age.

Group	Beginning of LHRH treatment	No. of rats mating (%)	Pituitary implant*		No. of rats with implants on Day 10 after mating (%)	
5	Day 25	0/5	(0)	Without	0/5	(0)
6	Day 37	0/5	(0)	Without	0/5	(0)
7	Day 25	8/8	(100)	Without	0/8	(0)
8	Day 37	8/8	(100)	Without	0/8	(0)
9	Day 25	10/10	(100)	With	6/10	(60)
10	Day 37	8/8	(100)	With	3/8	(38)

* Pituitary gland was transplanted from a normal rat into the kidney capsule of the androgenized female rat on Day 1 after mating.

Table 3. Uterine decidualization in androgenized female rats given multiple s.c. injections of physiological saline (Groups 11–12) or 400 ng LHRH (Groups 13–14) at 14:00 h every 4 days, starting before (Day 25) or after (Day 37) vaginal opening, until 53 days of age.

Group	Beginning of LHRH treatment	No. of rats	Uterine weight (mg)* Traumatized horn	Control horn
11	Day 25	5	279 ± 84^a	225 ± 21^a
12	Day 37	5	250 ± 20^a	243 ± 17^a
13	Day 25	7	1340 ± 90^c	203 ± 17^a
14	Day 37	7	675 ± 50^b	240 ± 26^a
15#	–	6	1350 ± 106^c	210 ± 20^a

* Values are mean ± SEM. Values with different alphabetical superscripts in any one column differ at a level of $p < 0.05$ (Duncan's new multiple range test). # Normal pseudopregnant rats: pseudopregnancy was induced by transplantation of a pituitary under the renal capsule.

nized female rats, vaginal cervical stimulation does not induce functional corpora lutea,[14] but reserpine treatment or isotransplants of pituitaries beneath the kidney capsule do[8,14]. Barraclough and Fajer[5] have reported that progesterone secretion by the corpora lutea of androgenized rats decreases and that administration of prolactin results in increased secretion of progesterone by these corpora lutea. When a pituitary gland was transplanted from a normal rat into the kidney capsule of an androgenized rat to maintain functional corpora lutea, implantation occurred in some of the rats. These findings suggest that failure of embryos to implant may result from a deficit of progesterone from the induced corpora lutea.

The vaginal opening in androgenized female rats occurs at approximately 32 days of age. The uterine sensitivity to the decidual reaction, i.e. endometrial scratching, elicited a better response ($p < 0.05$) in the rats that received multiple LHRH injections before vaginal opening. In fact, the response was the normal decidual response such as is observed in pseudopregnant rats. These results indicate that the cyclic LH surges before vaginal opening may be necessary for the induction of pregnancy in androgenized rats.

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Antagonism of V_2-receptor effect of antidiuretic hormone by atrial natriuretic peptide in man

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Summary. Human α-atrial natriuretic peptide (h-αANP) makes the urine of dehydrated volunteers hypotonic to plasma despite high circulating concentrations of antidiuretic hormone. Urinary dilution with h-αANP also occurs in subjects receiving indomethacin. Therefore, h-αANP antagonises effects of antidiuretic hormone on distal tubular V_2-receptors in man, probably without involving prostaglandins.
Key words. Atrial natriuretic peptide; antidiuretic hormone; hydraulic conductivity; prostaglandins.

Atrial natriuretic peptides may be important hormones for the control of the extracellular fluid volume, and they have many intrarenal actions that could increase the excretion of sodium and water. They increase the rate of glomerular filtration, but they may also reduce sodium and water reabsorption from proximal and medullary tubules[1,2]. Moreover, atrial peptides can antagonise the stimulation of water flow caused by arginine vasopressin (AVP) across the toad bladder[3], and, recently, it has been shown that the synthetic rat atrial natriuretic peptide atriopeptin III can also inhibit the increase of hydraulic conductivity caused by AVP in isolated perfused rabbit collecting ducts[4]. These observations add another possible explanation for the diuretic effect of atrial peptides in the intact animal[4]. So far, however, the complexity of the intact animal and the number of potential intrarenal mechanisms available has not allowed specific mechanisms to be assigned unambiguously to either the natriuretic or water-diuretic effects of atrial peptides in vivo. Despite this, we report prima facie evidence that h-αANP actually does antagonise the effect of endogenous antidiuretic hormone on the hydraulic conductivity of the distal tubular epithelium of normal volunteers and that this mechanism