Effects of progesterone implants in the mesencephalon of ovariectomized rats on LH and FSH release triggered by exogenous gonadal steroids

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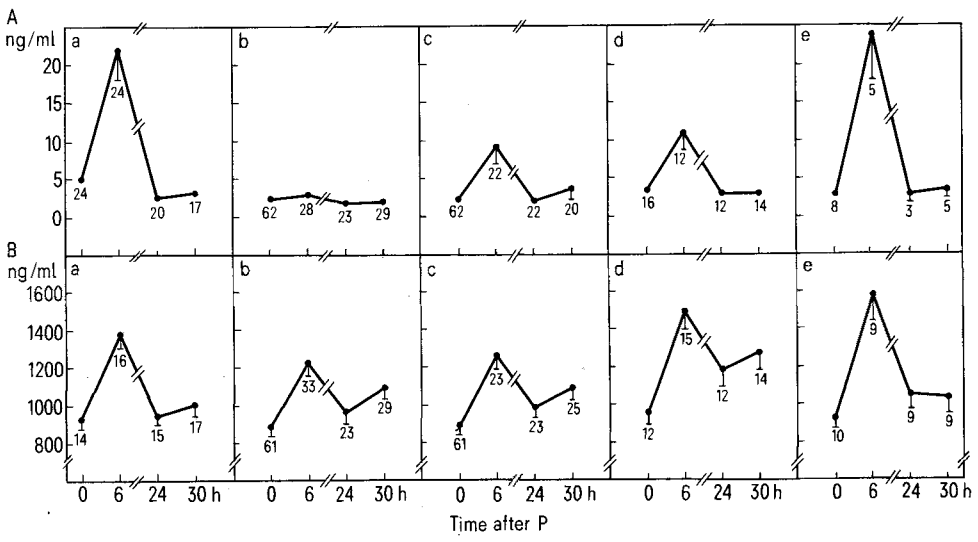
Summary. Unilateral implantation of crystalline progesterone into the caudal mesencephalic reticular formation (MRF) of chronically ovariectomized adult rats prevents the triggering effect of exogenous gonadal steroids on LH release and does not affect the release of FSH in the same animal.

Stimulatory and inhibitory effects of progesterone (P) on ovulation and pituitary gonadotropin secretion are well recognized. The hormone exerts an ovulation-blocking effect acting at the level of the ovary[1], the pituitary[1,2] and the hypothalamus[2]. P is also effective in stimulating the release of LH[3] and FSH[4]. The main sites of the stimulatory feedback action of P on LH release appear to be located in the diagonal band of Broca, the preoptic suprachiasmatic area and the anterior hypothalamic area[5], whereas the preoptic area seems to be indispensable for the stimulatory effect of P on FSH release[6]. Among various extrahypothalamic structures which participate in the control of reproductive processes, the mesencephalon is recognized as one that contains P-sensitive cells, which may be involved in the control of the pituitary, of neural and behavioral activation and of sexual behavior[7]. Particularly sensitive to P was found to be the mesencephalic reticular formation (MRF)[8]. These findings are consistent with the demonstration that local implants of crystalline P into the caudal part of the MRF of estrogen (E)-P primed female rats lead to the display of lordosis[9]. This paper shows that, on the other hand, P crystals implanted unilaterally into the caudal MRF are effective in inhibiting the LH surge in the circulation of E-P primed female rats, whereas FSH levels remain unaffected.

Materials and methods. Wistar female rats from a local random bred colony were used. They were housed under conditions of controlled temperature (24±2 °C) and illumination (lights on from 05.00 h to 19.00 h) and given free access to water and laboratory chow. When 160–190 g in weight, the animals were bilaterally ovariectomized under ether anesthesia and thereafter divided into 5 groups. 1 group of animals (group a) was saved to serve as E-P primed controls and the remaining 4 subjected, on day 21 after ovariectomy, to the following treatments: experimental animals (group b) received the outer cannula to be used for intracranial implantation of P; control-1 animals (group c) also received the outer cannula, but were later spared from the implantation of the steroid; control-2 animals (group d) received the outer cannula, which was immediately thereafter withdrawn from the brain tissue; and sham-operated animals (group e) were subjected to the same operation procedure as former ones, but only to the point of rupture of the brain membranes, after which they were released from the stereotaxic instrument. On day 25 following ovariectomy, animals from all the 5 groups were treated as follows: at 10.00 h the animals were primed with estradiol benzoate (EB, 5 µg in 0.2 ml olive oil, s.c.) and 48 h later (zero time) injected with P (2 mg in 0.2 ml olive oil, s.c.) to trigger LH and FSH surges in the circulation. Both steroids were purchased from Sigma Chemical Co., St. Louis, Mo. Concomitantly with the systemic injection of P, animals from the experimental group received, in addition, implants of P crystals into the caudal MRF. The site of the implantation was defined by the following coordinates[10]: A, +1.4 mm; H, −2.0 mm; L, 1.5 mm (left hemisphere). The implantation was performed with the aid of a double cannula. A stainless steel tube (0.48 mm OD), as an outer cannula, was stereotaxically oriented into the MRF and fixed to the skull with dental cement. The operation was performed using tribromoethanol as anaesthetic (TBE, 2.5% solution in 0.9% NaCl, 10 ml/kg b.wt, i.p., Winthrop Laboratories, New York, N.Y.). At the time of implantation, crystalline P was tapped into one end of the inner cannula (0.35 mm OD), which was thereafter inserted through the outer cannula into the brain. The P-loaded insert was left in the animals for the next 30 h. Blood samples for hormone assays were taken from the EB primed rats just before the treatment with P as well as 6, 24 and 30 h later. Blood was collected by cardiac puncture from animals under ether anesthesia. The plasma was separated by centrifugation of heparinized blood and



Mean plasma concentrations of LH (A) and FSH (B) in chronically ovariectomized EB-primed rats at the time of treatment with P (zero time) and 6, 24 and 30 h later; a, ovariectomized EB-primed controls; b, animals with implants of P crystals in the MRF; c, control-1 group; d, control-2 group; e, sham-operated animals. Figures below each experimental point indicate numbers of corresponding samples. Vertical lines give SEM.

stored at −20 °C until assayed. The plasma LH and FSH concentrations were determined by the double antibody RIA method, using the NIAMDD kits[11] and following the directions supplied with the kits with minor modifications. Results were expressed in terms of the NIH-LH-S1 reference preparation for LH and the NIAMD-Rat FSH-RP-1 reference preparation for FSH. Statistical evaluation of the differences between means was performed using Student's t-test.

Results and discussion. As can be seen from the figure, which summarizes the results obtained in this work, mean plasma concentrations of LH and FSH 2 days following EB injection (i.e., at zero time) were similar among all 4 groups of chronically ovariectomized rats that were subjected to stereotaxic manipulation, ranging from 2.3±0.16 to 3.3±0.30 ng/ml for LH and from 883±29.5 to 948±49.9 ng/ml for FSH. The values did not differ significantly from those found in ovariectomized controls (4.9±0.35 ng/ml for LH and 930±45.4 ng/ml for FSH). As should be expected from earlier work on the stimulatory action of gonadal steroids on gonadotropin release[12], the systemic injection of P at zero time induced 6 h later in the spayed EB primed rats a profound elevation of plasma LH (22.2±3.85 ng/ml) and FSH (1393±76.2 ng/ml). The values were again low by the next morning, and remained low (LH) or increased slightly (FSH) in the afternoon. Similar triggering effects of P on LH and FSH release were observed in sham-operated animals, in which respective values for LH and FSH, 6 h later, were 24.2±6.04 ng/ml and 1583±132.1 ng/ml. On the other hand, LH surge was completely blocked (3.0±0.42 ng/ml) by crystals of P implanted into the MRF, and partially but significantly inhibited by inserting the tip of the cannula alone into this brain structure (9.1±1.96 ng/ml and 11.1±2.19 ng/ml for groups c and d, respectively). The latter effect is attributable to non-specific damages to the brain tissue, produced by the penetration of the cannula. Contrary to the observed effects on LH release, however, neither P crystals nor cannula alone, when inserted into the MRF, were significantly effective in altering FSH release, as compared to that in ovariectomized EB-P-treated controls. Thus, 6 h following P treatment, the FSH levels in the circulation of animals from groups b, c, d and e were, respectively, 1227±65.0, 1273±69.8, 1494±92.3 and 1583±132.1 ng/ml, as compared to 1393±76.2 ng/ml for group a. These findings suggest that the investigated area of the MRF is a site of the inhibitory feedback action of P in the control of LH release, but not of FSH release. The dissociation of LH from FSH release, observed in this work as well as in other laboratories and under different experimental conditions[6, 13–14], which cannot be explained by direct action of gonadal steroids on the anterior pituitary to change its responsiveness, keeps the question of the existance of 1 or 2 different releasing hormones still open.

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A dramatic role of terpenoids in increasing rice production

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Summary. Earlier work has established the role of terpenoids and their derivatives as a new group of plant growth regulators. Some of these terpenoids have now been tested in field trials and have been found to increase rice production significantly.

A major breakthrough in the rice improvement program has been achieved by the introduction of semi-dwarf, tropical indica plant type varieties. These semi-dwarf varieties had a much higher yield than the conventional tall varieties because of their improved plant type, and increased response to heavy doses of nitrogen. The improved production technology worked out for these varieties further made it possible to realize the potential yield of these varieties (10 t/ha) particularly in Punjab (India).

The rice breeders are continuing their global efforts towards further genetic improvement of the yield potential of rice varieties by incorporating disease and insect-pest resistance. Considerable success has been achieved in this, but no variety has so far been released which could potentially outyield the high yielding varieties like IR 8 and PR 106. The agronomists and physiologists, on the other hand, are trying hard to raise the yield level of the present high yielding varieties by innovating new production technology. To raise the yield levels beyond the present varietal potential of 10 t/ha is a real challenge for the rice scientists in future. The productivity can be increased either by breeding more high yielding varieties, which is a time-consuming method or by the use of new growth regulators.

The biological potentialities of terpenoids[3] in general and