Diminished oestrogen sensitivity and increased ovulation rate in adult female rats after prepubertal treatment with oestrogen or pimozide

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Summary. Immature female rats were implanted with oestradiol benzoate or cholesterol in the medial preoptic area at different ages, and the inhibition of the ovariectomy-induced increase of LH secretion by s.c. injected oestradiol was investigated. Medial preoptic oestrogen implants reduced the inhibition of LH secretion in 4-week-old rats, but not in younger animals. Elevation of the circulating oestrogen concentration or suppression of the central nervous dopamine activity by daily injections of oestradiol and pimozide, respectively, from Day 26 to the day of vaginal opening, i.e. during the time when the mechanism of the oestrogen-induced desensitization of the negative oestrogen feedback matures, resulted in considerable diminution of the LH-inhibiting effect of oestradiol in ovariectomized adult females. In intact cyclic rats, both prepubertal treatments led to a significant increase of the average number of eggs per ovulation that was mainly caused by reduction of the number of animals with a low ovulation rate.

Keywords: negative oestrogen feedback; oestrogen sensitivity; pimozide; female sexual maturation; ovulation rate; rat

Introduction

The negative feedback action of oestrogen is essentially involved in the neurohormonal control of the cyclic ovarian function (Karsch *et al.*, 1978). However, in spite of the very effective inhibition of gonadotrophin secretion by oestrogen, the basal circulating LH concentration increases in the face of rising oestrogen concentrations in the blood both prepubertally (rat: Meijs-Roelofs *et al.*, 1983; Döcke *et al.*, 1984a; Urbanski & Ojeda, 1985; sheep: Foster *et al.*, 1985; cattle: Schams *et al.*, 1981; man: Sizonenko, 1978) and during the preovulatory phase of the ovarian cycle (rat: Fox & Smith, 1985; sheep: Karsch *et al.*, 1983; cattle: Schams *et al.*, 1977; man: Hoff *et al.*, 1983). The increase of tonic LH secretion seems to be required for the completion of follicle maturation, occurrence of ovulation, and adequate corpus luteum function (McNatty *et al.*, 1981; Haresign *et al.*, 1983; McNeilly *et al.*, 1984).

A simultaneous rise of circulating oestrogen and basal LH concentrations can only occur if the gonadotrophin-inhibiting effect of oestrogen decreases. This desensitization of the negative oestrogen feedback has been demonstrated in prepubertal rats (Döcke *et al.*, 1984b), sheep (Foster & Ryan, 1979), pigs (Berardinelli *et al.*, 1984) and heifers (Day *et al.*, 1984) and has also been established in adult rats during the preovulatory phase of the ovarian cycle (Döcke *et al.*, 1984d). Oestrogen itself can induce desensitization to its gonadotrophin-suppressing effect by an action on the medial preoptic area (MPOA): implantation of very low amounts of oestradiol benzoate into the MPOA diminished considerably the inhibition of LH and FSH secretion by s.c. injected oestradiol benzoate in ovariectomized immature (Döcke *et al.*, 1984c) and adult (Döcke *et al.*,

1984d) female rats, whereas removal of similar oestrogen implants from the MPOA resulted in enhanced sensitivity to the inhibitory effect of oestradiol on LH release (Döcke *et al.*, 1985). The oestrogen-induced desensitization of the negative oestrogen feedback is probably mediated by suppression of dopamine activity in the MPOA (Döcke *et al.*, 1987) and, possibly, by inhibition of β -endorphin release from the mediobasal hypothalamus (Stürzebecher *et al.*, 1988).

The present experiments were performed to determine at which stage of post-natal development the mechanism of the oestrogen feedback matures in female rats, and to examine whether alteration of circulating oestrogen concentration or of dopamine activity during this time can influence the sensitivity to oestrogen and ovulation in adulthood.

Materials and Methods

Animals. Female Wistar rats of a colony strain were reared in the laboratory. They were housed in an air-conditioned room with lights on from 05:00 to 19:00 h. Standard dry pellets and tap water were available *ad libitum*. Vaginal opening was recorded for some of the rats, and vaginal smears were taken daily from the day of vaginal opening. Ovariectomy was performed under ether anaesthesia between 08:30 and 09:00 h. For s.c. injections, oestradiol benzoate (VEB Jenapharm, Jena, G.D.R.) was dissolved in peanut oil, and the dopamine receptor blocker pimozide (VEB Arzneimittelwerk Dresden, Dresden, G.D.R.) was micro-suspended in a 0.1 M solution of tartaric acid.

The number of ovulated eggs was determined by killing the rats in the morning of the day after oestrus, determined from the vaginal smear patterns. The oviducts were removed, squeezed between two slides and the ova counted under a dissecting microscope.

Implantation of oestradiol benzoate. Implants containing one part oestradiol benzoate to 360 parts cholesterol were prepared by thoroughly mixing both substances for 20 min, heating the powder to just above the melting point and, with continuous stirring, aspirating the mixture into calibrated glass capillary tubes with an inner diameter of 0.2 mm and an outer diameter of approximately 0.35 mm. After coagulation, the outer surface of the tubes was cleaned with ether under microscopic control to ensure that the substances were limited to the interior of the tubing. One set of implants prepared simultaneously was used throughout the experiment. Similarly prepared cholesterol implants were used for controls.

The rats were anaesthetized with ether and placed in a stereotaxic instrument. After bilateral introduction in the MPOA the capillary tubes were fixed to the skull with dental cement and removed at autopsy.

Assay of serum LH concentrations. Trunk blood was collected at autopsy, which was performed between 13:30 and 14:00 h. Serum LH was measured by an homologous double-antibody radioimmunoassay. NIAMDD Rat LH-I-5 (NIAMDD Rat Pituitary Hormone Distribution Program, NIH, Bethesda, MD, U.S.A.) was radioiodinated with ^{125}I (IMS 30; Amersham International plc, Bucks, U.K.) using the chloramine-T method (Greenwood *et al.*, 1963). The labelled hormone was purified by chromatography on a Sephadex-G 50 column and by disc electrophoresis. NIAMDD-A Rat LH-S-5 was used as antibody. The sensitivity of the assay was 1.2 ng/ml, and the intra- and inter-assay coefficients of variation were 5.4% and 11.1%. All samples were run in duplicate. Results were expressed as ng NIAMDD LH-RP-1/ml.

Evaluation of implantations. The brains of the implanted rats were removed at autopsy and fixed in 10% formalin. Frozen serial sections (15 μm) were cut transversely and stained with haematoxylin and eosin to determine the tracks of the capillary tubes. Rats were only included in the evaluation if the implants were located in the ventral part of the medial preoptic nucleus between A 7020 and A 6670 μm by the co-ordinates of König & Klippel (1963).

Statistical analysis. Results are presented as means $\pm$ s.e.m. (serum LH concentrations) or means $\pm$ s.d. (numbers of eggs). Groups of data were compared using Student's *t* test for unpaired samples after application of the Fisher test and the χ^2 test as a test of goodness of fit.

Results

Maturation of the mechanism for desensitization of the negative oestrogen feedback

Immature female rats were bilaterally implanted with oestradiol benzoate or cholesterol in the MPOA at 18, 24 or 31 days of age and ovariectomized in the morning of the 3rd day after implantation. Females of the two younger groups were injected s.c. with 0.05 μg oestradiol benzoate/100 g body weight at 13:00 h on this day. In this strain of rats, spontaneous prepubertal desensitization of the negative oestrogen feedback begins after the 4th week of life (Döcke *et al.*, 1984b), and so

Fig. 1. Maturation of the oestrogen-induced desensitization of the negative oestrogen feedback in immature female rats. The rats were implanted with oestradiol benzoate (OB) or cholesterol (Chol) in the MPOA at different ages and treated as outlined in the figure. Ov = ovariectomy. Values are means $\pm$ s.e.m. The numbers of rats per group are indicated above each bar. **** $P < 0.01$** compared with the corresponding controls implanted with cholesterol.

34-day-old females were injected with $0.1 \mu\text{g}$ oestradiol benzoate/100 g body weight. All rats were autopsied exactly 24 h after the injection, and the serum LH concentration was estimated.

Figure 1 demonstrates that oestradiol injected on Day 21 suppressed the ovariectomy-induced increase of LH secretion to a similar degree in females implanted in the MPOA with oestradiol benzoate or with cholesterol at 18 days of age. In contrast, implantation of oestradiol on Day 24 resulted in a significant reduction of the LH-inhibiting effect of oestrogen 3 days later as compared to the controls implanted with cholesterol. A desensitizing effect of medial preoptic oestradiol implants was again not demonstrable in the oldest group of rats.

Effect of pretreatment with oestradiol or pimozide on the oestrogen sensitivity in adulthood

Female rats were injected daily with $0.05 \mu\text{g}$ oestradiol benzoate/100 g body weight, 0.126 mg pimozide/100 g body weight or with oil from the 26th day of age to the day of vaginal opening. Daily vaginal smears were taken after vaginal opening had occurred. The rats were ovariectomized on the day after the first or 10th vaginal oestrus and injected s.c. with $3 \mu\text{g}$ oestradiol benzoate/100 g body weight or oil at 13:00 h on the 3rd day after ovariectomy (Fig. 2). Evaluation of the serum LH concentrations 24 h after the injection revealed that rats treated prepubertally with oestrogen or pimozide did not respond to the administration of oestradiol benzoate, whereas significant inhibition of LH secretion by oestradiol was recorded in females pretreated with oil.

Fig. 2. Effect of prepubertal treatment with oestradiol benzoate (OB) or pimozide on the sensitivity to the LH-inhibiting effect of oestradiol benzoate in adult female rats. VO = vaginal opening. For details of treatment see text. Values are means $\pm$ s.e.m., and the numbers of rats per group are indicated above each bar. ** $P < 0.01$ compared with the corresponding controls injected with oil in adulthood.

Effect of pretreatment with oestradiol or pimozide on ovulation

Groups of rats pretreated prepubertally as in the experiment described above were autopsied on the morning of the day of metoestrus after completion of the 10th vaginal cycle, and the number of ovulated eggs was determined. There were (mean $\pm$ s.d.) 8.7 ± 1.98 , 10.3 ± 1.94 and 9.8 ± 1.60 eggs in 29 females pretreated with oil, 38 rats injected with oestradiol benzoate and 22 rats pretreated with pimozide, respectively ($P < 0.01$ and $P < 0.05$ as compared with the controls pretreated with oil). An additional group of 19 females injected prepubertally with oestradiol benzoate was killed after the 5th vaginal cycle. In these rats 10.4 ± 1.71 eggs were found.

The frequency distributions of the numbers of eggs per ovulation in rats autopsied after the 10th vaginal cycle are summarized in Fig. 3. The high frequency of ovulations yielding less than 10 ova as recorded in the controls was clearly reduced after pretreatment with oestradiol or with pimozide. Moreover, although the distribution of ovulations in the control group differed significant from a normal distribution ($P < 0.001$), this was not the case in either experimental group ($P > 0.10$).

Discussion

Döcke *et al.* (1984c) have shown that immature female rats implanted in the MPOA with small amounts of oestradiol benzoate at 24 days of age exhibited precocious prepubertal desensitization to the LH- and FSH-inhibiting effects of oestrogen around Day 30. In accordance with this finding, the present results suggest that the mechanism of the oestrogen-induced desensitization of the negative oestrogen feedback begins to mature in female rats during the 4th week of post-natal life.

Fig. 3. Effect of prepubertal treatment with oestradiol benzoate (OB) or pimozide from Day 26 to the day of vaginal opening on the frequency distribution of the numbers of eggs per rat recorded at the 10th post-pubertal ovulation. The differences between the controls injected prepubertally with oil and the rats pretreated with oestradiol or with pimozide are significant ($P < 0.001$; test of goodness of fit).

The inhibition of LH secretion by oestradiol injected on Day 21 was not reduced by implants of oestradiol benzoate placed in the MPOA on Day 18, but implantation on Day 24 resulted in significant diminution of the oestrogen-induced suppression of LH release on Day 28.

During the 5th week of life, the animals again did not respond to the medial preoptic implantation of oestradiol benzoate. This was probably due to the spontaneous prepubertal desensitization of the negative oestrogen feedback that occurs in the strain of rats used at this time (Döcke *et al.*, 1984b). This assumption is supported by findings which showed that bilateral lesions of the MPOA lead to considerable diminution of the oestrogen sensitivity in 29-day-old females (Döcke *et al.*, 1984c), but not in rats approaching the onset of puberty (unpublished data).

Further experiments demonstrated that elevation of the circulating oestrogen concentration or inhibition of the central nervous dopamine activity from Day 26 to the day of vaginal opening, i.e. during maturation of the oestrogen-induced desensitization of the negative oestrogen feedback, reduced markedly the oestrogen sensitivity in adulthood. The LH response to an injection of oestradiol benzoate or oil was examined 3 days after ovariectomy of adult cyclic rats because, under these conditions, the influence of ovarian oestrogen secretion on the efficacy of the negative oestrogen feedback has apparently declined and constantly high oestrogen sensitivity is recorded throughout the ovarian cycle (Döcke *et al.*, 1985).

Long-lasting diminution of the gonadotrophin-inhibiting effect of oestrogen following elevation of the prepubertal circulating oestrogen concentration may also occur in humans. Apter & Vihko (1985) recorded higher prepubertal serum oestradiol concentrations in girls with early menarche than in those exhibiting menarche at later ages. In the former girls, who also showed

earlier onset of ovarian cycles, significantly increased circulating oestrogen concentrations, suggesting desensitization of the negative oestrogen feedback, were found up to at least 17–25 years of age (Vihko & Apter, 1984).

Döcke *et al.* (1987) have concluded that oestrogen may induce desensitization to its negative feedback action in prepubertal and adult female rats by suppressing dopamine activity in the MPOA which belongs to the incerto-hypothalamic dopamine system (Björklund *et al.*, 1975). The equivalent responses obtained in the present study after prepubertal treatment with oestradiol benzoate or with pimoziide, which inhibits the dopamine neurotransmission by blocking the dopamine receptors (Seeman, 1981), support this assumption.

Reduced sensitivity of the negative oestrogen feedback should result in improvement of the ovulation rate, because more ovarian follicles can develop and the serum oestrogen concentration can increase to a higher level without suppressing the tonic gonadotrophin secretion and arresting the final maturation of ovarian follicles. This hypothesis could be confirmed. A significant increase of the mean number of eggs per ovulation was found after prepubertal administration of both oestradiol benzoate and pimoziide. This effect appeared to depend primarily upon reduction of the number of ovulations yielding fewer than 10 eggs and not on further augmentation of the ovulatory reaction in rats with a spontaneously high ovulation rate (Fig. 3). In this way, normal distribution of the numbers of eggs was achieved with a maximum at 10 ova per rat.

Numerous studies have been performed to increase the ovulation rate in farm animals. Promising results have been reported after active immunization of ewes against androstenedione (Scaramuzzi *et al.*, 1977; Cox *et al.*, 1982), testosterone (Scaramuzzi *et al.*, 1981; Cox *et al.*, 1982) or bovine follicular fluid (Cummins *et al.*, 1986), which are probably also due to diminution of the inhibition of tonic gonadotrophin secretion (Martensz & Scaramuzzi, 1979; Cummins *et al.*, 1986). Attempts to induce similar effects in heifers have been only partly successful (Wise & Schanbacher, 1983; Price *et al.*, 1987a, b). The present findings point to the possibility that increased ovulation rates may be obtained by prepubertal treatment with oestrogen or dopamine antagonists. They also suggest that this method may be suitable to increase the average number of ovulations in polyovulatory species such as the pig by improving the ovulation rate in animals with low prolificacy.

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