Evidence that a 'critical period' is involved in early estrogen-induced LH- and FSH-release during the estrous cycle in the rat[1]

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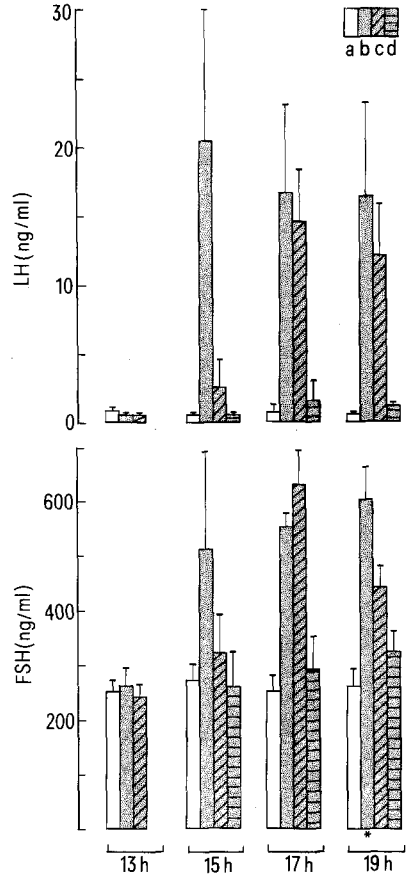
Summary. LH- and FSH-release, which occurred in the afternoon of diestrus 1 following estradiol benzoate administration in the morning of estrus, was completely prevented by pentobarbital, when injected on diestrus 1 at 13.30 h.

In 1949, Everett et al.[3] provided evidence for the existence of a 'critical period' in the afternoon of proestrus during which a neurogenic timing factor controls the ovulatory release of LH in the rat. Later[4] barbiturates were shown to block ovulation when administered on proestrus at 13.00 h, and to shift the critical period by 24 h. Estrogens are able to induce advanced ovulation[5-7] and LH-release[8,9] when injected at early diestrus in 4-day cyclic female rats. Work from our laboratory[6,9] demonstrated that LH-release and ovulation, caused by the injection of estradiol benzoate (EB) in the morning of diestrus 1, could be prevented by pentobarbital (PB) administration on diestrus 2 at 13.00 h, thus suggesting the existence of a critical period at this stage of the estrous cycle. Since Uchida et al.[10] observed LH-release in female rats injected with EB as early as the morning of estrus, we wondered whether a critical period was also involved. The aim of this work was to investigate the timing of an eventual blockade by PB of estrogen-induced LH-release in early EB-injected cyclic female rats.



Action of pentobarbital (PB) on LH- and FSH-release induced by 10 µg estradiol benzoate (EB) during the afternoon of diestrus 1 in 4-day cyclic rats. a, controls; b, EB on estrus at 10.00 h; c, EB + PB on diestrus 1 at 08.00 h; or d, at 13.00 h. (4 or 6* rats per group.)

Material and methods. Adult virgin 3- to 4-month old female Wistar rats (WI strain from our colony) weighing 180 to 220 g were used. They were kept under the normal rhythm of natural lighting at a temperature of 22°–24 °C. They had access to commercial laboratory food and tap water. Estrous rhythm was checked by daily vaginal smears. Only females having experienced 2 or 3 successive 4-day cycles prior to the experiment were used. A 4-day sequence consisted of diestrus 1, diestrus 2, proestrus and estrus.

62 rats were divided into 2 groups. The 46 females of the 1st group all received an injection of 10 µg EB s.c. on estrus at 10.00 h. Some of them were injected with 35 mg PB/kg on diestrus 1 at either 08.00 h (16 rats) or 13.30 h (12 rats). The 2nd group consisted of 16 non-injected rats which served as controls.

All rats were bled by decapitation in the afternoon of diestrus 1 from 13.00 h to 19.00 h. The timing of sacrifice will be presented with the results below. Blood was allowed to clot overnight at 4 °C. Blood samples were centrifuged at 4 °C and sera stored frozen at −20 °C until subsequent use. LH-radioimmunoassay was performed using an antiserum to ovine LH and a laboratory rat preparation (1.2 × NIH LH S_1) as standard and tracer (Kerdelhue et al.)[11]. The labeling procedure was performed according to Kerdelhue et al.[11]. FSH radioimmunoassay was conducted using NIAMDD anti-rat-FSH S_6. The NIAMDD rat FSH RP_1 (2.1 × NIH FSH S_1) was used as standard and a purified laboratory rat preparation was used as tracer. FSH and LH were measured in all samples in duplicate and the results averaged. Statistical analysis was performed by analysis of variance after logarithmic transformation of the data.

Results and discussion. The data presented in the figure indicate that estradiol benzoate caused LH- as well as FSH-release from 15.00 h to 19.00 h on diestrus 1 when injected in the morning of estrus. Statistical analysis confirmed these results (controls vs EB-treated rats: LH at 15.00 h, $p < 0.05$; at 17.00 h, $p > 0.05$[12]; at 19.00 h, $p < 0.01$; FSH at 15.00 h, $p < 0.05$; at 17.00 h, $p < 0.05$; at 19.00 h, $p < 0.01$). They also show that LH- and FSH-release failed to occur following PB injection on diestrus 1 at 13.30 h (EB vs controls: LH and FSH at 15.00 h, 17.00 h, 19.00 h, NS) and was delayed by 2 h after PB injection at 08.00 h (EB-treated vs controls: LH and FSH at 15.00 h, NS). This blockade of estrogen-induced LH- and FSH-release by PB allows the assumption that there is, in the afternoon of diestrus 1, a daily normal signal for LH- and FSH-surges, which may be observed under early estradiol stimulus in intact cycling rats. Daily LH-surges in the afternoon have been observed in either ovariectomized[13-15] or so-called 'pseudointact' short term ovariectomized[16] rats. Taking into account that the existence of a critical period for LH-release has been previously observed on diestrus 2[9], proestrus and estrus[3,4] we can conclude that the neurogenic mechanisms which control the LH-surge occur repeatedly on each day of the normal estrous cycle.

1 This investigation was partially financed by the CNRS (ERA 566).

2 Acknowledgments. We are grateful to Dr S. Plas-Roser for her expert assistance and advice on radioimmunoassays. We are indebted to Mrs Ch. Lazarus for her technical assistance. We wish to express our gratitude to Dr B. Kerdelhue and Dr A.F. Parlow for providing iodinated hormones and reagents for the determination of LH and FSH.

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Binding of iproniazid to the polymeric forms of iodide peroxidase

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Summary. ^{14}C labelled iproniazid binds to iodide peroxidase more effectively in low ionic strength buffer than in high ionic strength buffer, suggesting preferential binding to the monomeric form of iodide peroxidase. During column chromatography, under conditions that separate iodide peroxidase into multiple forms, iproniazid is bound selectively to the monomeric form. Thus, this antithyroid agent appears to bind preferentially to the monomeric enzyme form, or possibly to cause dissociation of the polymeric to the monomeric form.

Although there are many inhibitors of thyroid peroxidase, the details of their inhibitory mechanisms have not been elucidated.We have investigated the action of iproniazid, an inactivator of iodide peroxidase, in relation to the structure of the enzyme.

Iproniazid(isonicotinyl isopropyl hydrazine), an irreversible inhibitor of monoamine oxidase, was observed to inhibit horse radish peroxidase (HRP)[2]. Iproniazid binds to the nonheme portion of HRP irreversibly and stoichiometrically[2]. As with HRP, iproniazid binds to purified iodide peroxidase and inhibits the enzyme[3]. Spectral changes produced by iproniazid in iodide peroxidase were similar to those produced with HRP[4]. The binding is believed to be covalent.

Recently, iodide peroxidase was reported by Nagasaka and DeGroot to exist in polymeric forms[5]. We, in this communication, demonstrate differences in the sensitivity of these polymeric forms of enzyme to iproniazid.

Methods. Calf thyroids were obtained from an abattoir and kept on ice until dissected. Solubilization and purification of the iodide peroxidase enzyme were described in a previous paper[6]. Polymeric forms of iodide peroxidase were prepared using sephadex G-200 chromatography. Details of the method of preparation were described in a previous paper[5].

Enzyme activity was determined by measuring ^{131}I incorporation into tyrosine. Details of the assay were reported previously[6]. All chemicals were obtained from commercial sources unless otherwise stated.

^{14}C iproniazid was prepared by Dr H. Kaegi and Dr W. Burger at Hoffmann-La Roche and 1–5 μCi was used after dilution with unlabelled iproniazid. Preincubations of the enzyme with iproniazid ($1\text{–}3 \times 10^{-3}$ M) were carried out at 37 °C for 2 h in varying concentrations of phosphate buffer (from 0.005 M to 0.2 M, pH 7.0). Following incubation of the enzyme with iproniazid (in 0.5 ml) for the periods of time shown in the legends, 0.1 ml of this solution was diluted 5-fold to measure the residual enzyme activity and 10% TCA was added to the residual reaction mixture to stop the reaction and the solution was dialyzed against water (1000 vol.) overnight. After dialysis, precipitated protein was collected, dissolved in NCS (Packard) solution, and the radioactivity bound to the protein was measured in Bray's solution by liquid scintillation counting.

To prepare iproniazid-bound iodide peroxidase for column chromatography, solubilized iodinating enzyme[6] was precipitated by 40% saturated ammonium sulfate solution,

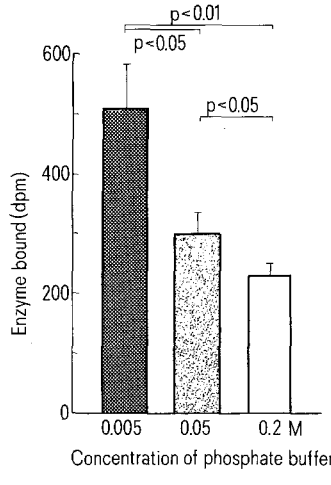

Fig. 1. Effect of ionic strength on iproniazid binding to iodide peroxidase. The ^{14}C radioactivity of iproniazid (1 μCi ^{14}C iproniazid and 1 μmole stable iproniazid per ml) bound to iodide peroxidase after 1 h incubation in 0.005 M (▩), 0.05 M (▨), and 0.2 M (□), pH 7.0, phosphate buffer is graphed. Each bar represents mean ± SE of 5 experiments. Student's t-test was applied for statistical analysis.