Suppression by LH-releasing hormone (LHRH) of the augmenting effect of estradiol on the secretion of LH and FSH by the rat pituitary gland

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Summary. Estradiol sensitizes the pituitary gland for the gonadotropin-releasing activity of LHRH. LHRH desensitizes the pituitary gland and does so in a dose-dependent manner. Moreover, LHRH dose-dependently suppresses the sensitizing effect of EB. In rats with an LHRH concentration in the plasma of about 90 pg/ml, the sensitizing effect of estradiol is absent.
Key words. LHRH; estradiol; LH/FSH secretion.

Using long-term ovariectomized (OVX) rats, Schuiling et al.[1,2] demonstrated that the effect of estradiol on the LHRH-responsiveness of the pituitary gland varies with the concentration of LHRH present in the plasma before and during estradiol treatment: estradiol increased the LH responses of the pituitary gland to acute increments of LHRH (augmenting or sensitizing effect of estradiol) in OVX rats not pretreated with LHRH, while in such rats pretreated with 250 ng LHRH/h for 6 days, estradiol decreased such responses. These data suggest that the effect of estradiol on the pituitary gland is under the control of LHRH. This suggestion was further investigated in the present study.

Materials and methods. Female Wistar rats, bred at the Groningen Central Animal Laboratory and kept in animal quarters with the lights turned on at 06.00 h and off at 18.00 h, were ovariectomized at the age of 12–14 weeks. At that moment they weighed about 200 g. Ovariectomy was performed in order to prevent interference of ovarian hormones with the response of the pituitary gland to exogenous hormones such as LHRH and estradiol.

All operations (cannulation; insertion of osmotic minipumps and silastic implants) were performed under light ether anesthesia. Rats were killed by decapitation.

In some of the rats, 14 days after ovariectomy, on day 0 at 09.00 h, an Alzet® osmotic minipump (model 2001, Alza Corp., Palo Alto, California, USA) was subcutaneously implanted. The pumps released LHRH at the rate of either 25, 75, 250 or 750 ng/h for 5 days. Control rats received a silastic implant (Dow Corning, Midland, Michigan, USA) with the dimensions of a minipump. Estradiol benzoate (EB), dissolved in arachis oil, was injected s.c. (3 µg/injection of 0.2 ml) at 17.00 h on days 3 and 4.

Blood samples for assay of LH and FSH were taken via a cannula inserted into the right carotid artery. For induction of LH and FSH responses by LHRH the right jugular vein (together with the right carotid artery) was cannulated on the morning of day 5. LHRH, at the maximally stimulating rate of 1 µg/h, was infused throught the intra-jugular cannula[3]. Infusions started at 12.00 h and lasted for 21 h. Maximally stimulating concentrations of LHRH were used because they were considered to overrule the various (sub-maximal) concentrations of LHRH with which rats had been pretreated. In this way measurable and comparable LH and FSH responses could be induced.

LH and FSH were measured by double-antibody radioimmunoassay with anti-ovine LH or FSH as antiserum and rat LH or FSH as tracer[4]. NIAMDDK-rat LH-RP-1 and FSH-RP-1 were used as reference preparations. LHRH was measured by double-antibody solid-phase radioimmunoassay using an antibody produced and characterized as described by Koch et al.[5]. The sensitivity of this assay is 3–5 pg. This assay did not detect immunoreactive LHRH in the peripheral plasma of otherwise untreated rats. The inter- and intra-assay variations are less than 15%. The assays were done by Dr J. de Koning of Leiden University, The Netherlands.

Data are expressed as means ± SEM. The LH and FSH responses induced by LHRH were statistically judged according to 2 parameters: 1) the mean maximal height (MMH) of the plasma LH and FSH concentrations and 2) the quantity of LH and FSH secreted during LHRH infusion, calculated as the area-under-the-curve or integrated LH/FSH release (AUC; 'area units').

Results. The plasma concentrations of LHRH (pg/ml), established by s.c. infusion of LHRH during days 0–5, at the moment of decapitation were as follows; for rats given 25 ng/h: below detectable level; 75 ng/h: 60.5 ± 17.7 (n = 5); 250 ng/h: 105.8 ± 67.0 (n = 4) and 750 ng/h: 318.0 ± 92.4 (n = 5).

Examples of the course of the changes in plasma LH and FSH concentrations induced by the 1 µg/h-LHRH infusion on day 5 are depicted in figure 1. EB increased the LH and FSH responses in rats which had been pretreated with 0, 25 or 75 ng LHRH/h. This increase was not observed in the responses induced in rats which had been pretreated with 250 or 750 ng LHRH/h.

Figure 2 shows the mean maximal height and area-under-the-curve of the LH and FSH responses (in part) depicted in figure 1. The figure shows that LHRH dose-dependently decreased the LH and FSH responses. After pretreatment of the rats with 750 ng LHRH/h the LH and FSH responses were maximally decreased. In rats pretreated with both LHRH and EB the responses were already maximally decreased after pretreatment with 250 ng LHRH/h; EB potentiated the desensitizing effect of LHRH.

The figure, however, also shows that the sensitizing effect of EB on the LH and FSH responses became smaller with increasing LHRH infusion rates. Interpolation suggested that the effect of EB would be zero in rats which had been pretreated with about 160 ng LHRH/h. Such an LHRH infusion rate establishes an LHRH concentration of about 90 pg/ml in the plasma. With infusion rates higher than 160 ng/h the effect of EB was either inhibitory or zero.

Discussion. LHRH desensitizes the pituitary gland in a dose-dependent manner. In rats pretreated with only LHRH, the pituitary gland was maximally desensitized after pretreatment with 750 ng LHRH/h. In rats pretreated with both LHRH and EB, however, the gland was already maximally desensitized after 250 ng LHRH/h: EB potentiates the desensitizing effect of LHRH.

EB sensitizes the pituitary gland for the gonadotropin-releasing activity of LHRH (see introduction). However, this sensitizing effect of EB becomes smaller with increasing rates of LHRH-pretreatment: LHRH suppresses the sensitizing effect of estradiol.

This study thus shows that LHRH and estradiol influence each other's effect. The result of this LHRH/estradiol-interaction is that the pituitary LHRH-responsiveness decreases progressively with increasing LHRH concentrations in the blood. At LHRH infusion rates of more than 160 ng/h the pituitary gland of LHRH/EB-pretreated rats is even less re-

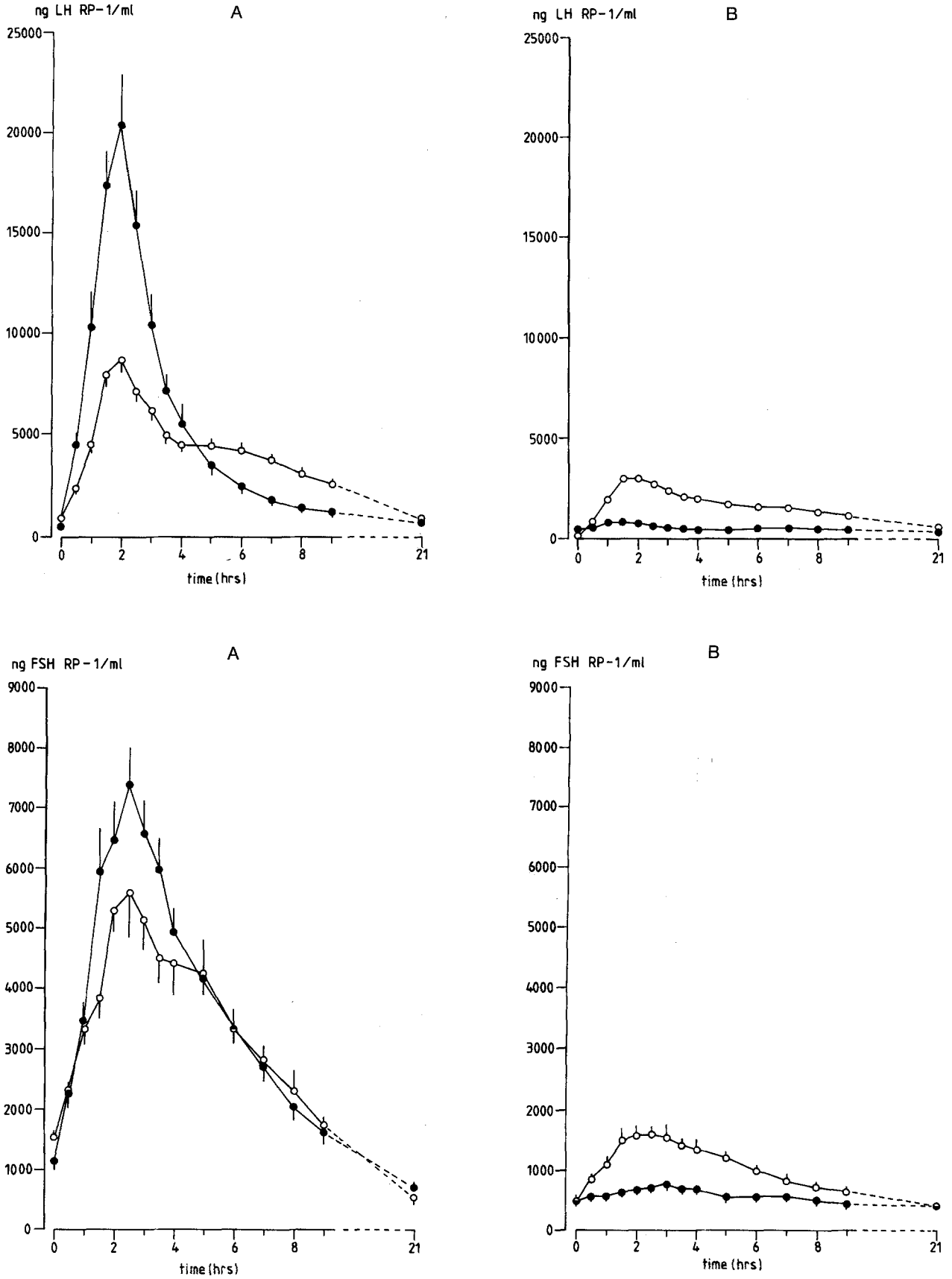

Figure 1. Examples of the course of change in plasma LH (upper panels) and FSH (lower panels) concentrations (mean ± SEM) during 21 h of constant rate infusion with LHRH (1 µg/h) in ovariectomized rats previously treated for 5 days with 25 (A) or 250 (B) ng LHRH/h. Rats were injected with either estradiol benzoate (●–●) or oil (○–○). No. of rats: see legend to figure 2.

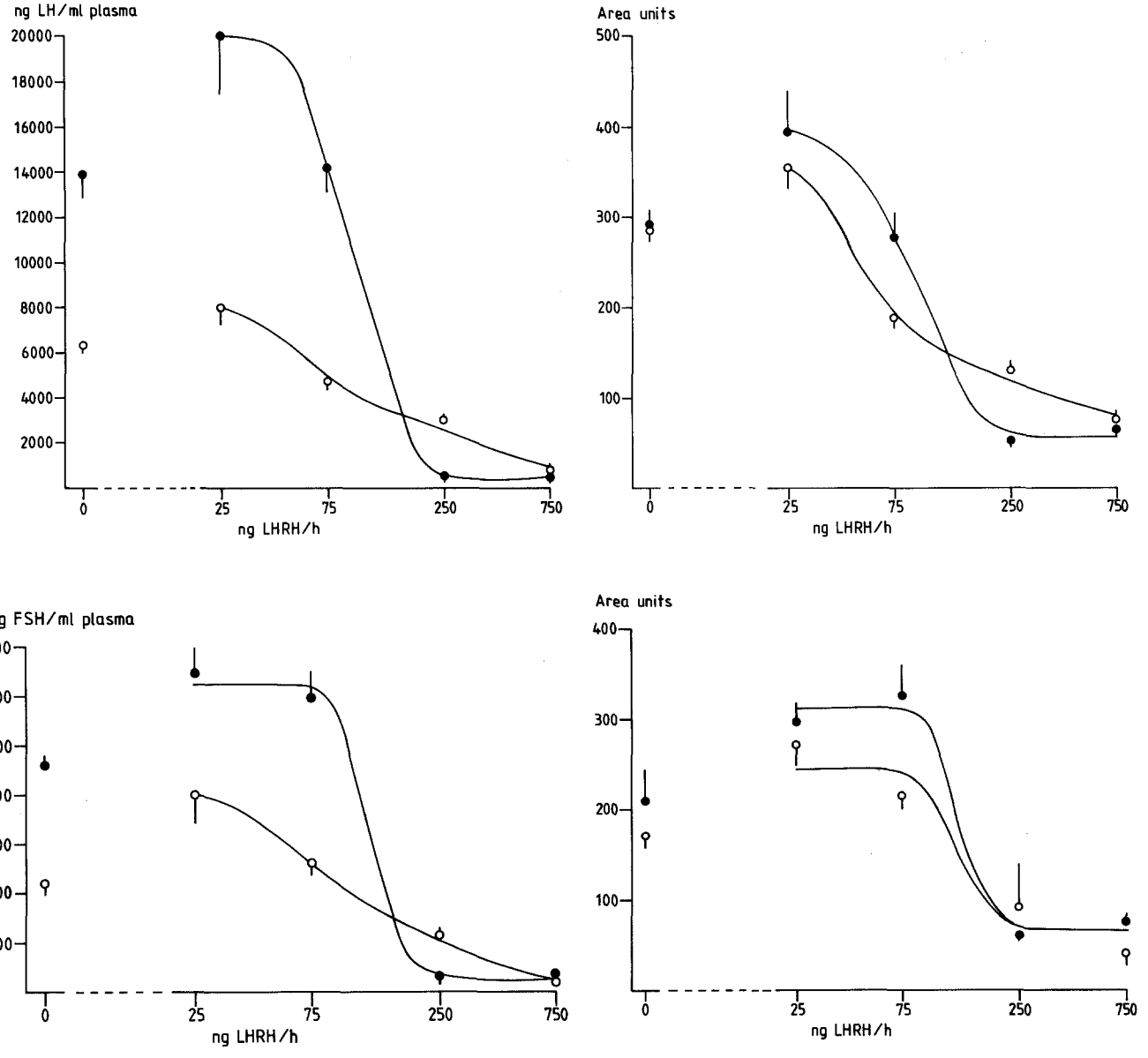

Figure 2. Mean maximal height (left panels) and area-under-the-curve (right panels of the LH and FSH curves (in part) depicted in figure 1. Rats had been pretreated with different doses of LHRH for 5 days (25, 75, 250, or 750 ng/h), delivered by s.c. implanted osmotic minipumps. Control rats received a silicone elastomer sham pump. Rats were injected with either estradiol benzoate (•–•) or oil (o–o).

LHRH-treatment (ng/h)	No. of rats: oil	EB
–	7	7
25	9	9
75	9	7
250	6	6
750	5	7

sponsive than that of LHRH/oil-pretreated rats. The infusion rate of 160 ng/h causes a plasma LHRH concentration of about 90 pg/ml.

The method of LHRH administration used here is definitely unphysiological; physiologically, LHRH is secreted in pulses and not at a constant rate [5, 6]. One might, however, argue that delivery of exogenous LHRH in a more or less physiological way [7] is impossible anyhow, simply because physiologically, LHRH is secreted into the hypophyseal portal vessels, whereas exogenous LHRH is delivered into the systemic circulation. This route of LHRH delivery introduces pharmacokinetic elements (elimination; volume of distribution) which do not play a role under physiological circumstances [8]. However, the plasma LHRH concentration of 90 pg/ml (which we assume was also present in the portal vessel blood), is within the physiological range; in plasma prepared from pituitary stalk blood of pro-estrous rats at the time of the pre-ovulatory LH-surge, Sarkar et al. [9] measured about 150 pg/ml, and in that of OVX rats, Sarkar and Fink [10] found LHRH concentrations of up to 400 pg/ml. As the plasma estradiol concentrations established by the present EB-treatment were also within the physiological range [1], it is

tempting to suggest that the interaction between LHRH and estradiol reported in this paper does play a role in the regulation of the secretion of LH and FSH by the hypothalamus-pituitary system during the ovulatory cycle.

If indeed the estrogen-induced increase of the pituitary LHRH-responsiveness, which occurs during the ovulatory cycle [11] can only occur in the absence of significant concentrations of LHRH in the portal vessel blood, then adequate suppression of the hypothalamic LHRH secretion – by estrogen – is a prerequisite for normal gonadal function. This would imply that LH and FSH are only secreted in aphysiological manner if the effects of estradiol on the hypothalamus and on the pituitary gland are well-tuned to each other, and that any perturbation in the negative feedback of estradiol on the secretion of LHRH by the hypothalamus will also affect the effect of estradiol on the pituitary gland.

In summary, this study shows that 1) LHRH desensitizes the pituitary gland for its own action and that this effect of LHRH is dose-dependent; 2) estradiol potentiates this desensitizing effect of LHRH; 3) estradiol sensitizes the pituitary gland for the LH/FSH-releasing activity of LHRH; 4) LHRH suppresses the sensitizing effect of estradiol.

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Preliminary observations on the co-existence of regulatory peptides in cells of the baboon endocrine pancreas

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Summary. Immunocytochemical procedures at ultrastructural and light microscopy level revealed, in the Chacma baboon endocrine pancreas, cells which were immunoreactive for glucagon and pancreatic polypeptide (PP). Some D cells were observed to contain secretory granules with both the appearance and immunoreactivity of A cell secretory granules.
Key words. Dual immunoreactivity; baboon; endocrine pancreas; immunolabelling; glucagon; pancreatic polypeptide; somatostatin.

It was proposed over fifty years ago that D cells might represent modified A cells [1]. The advent of ultrastructural studies revealed an intermediate cell type, containing both A and D type secretory granules but with the development of immunolabelling procedures, there was no further mention of intermediate cell types.

More recently, cells containing both glucagon and PP were localized in the pancreas of the frog [2], rat [3, 4] and mouse [5] as well as in human colorectal mucosa [6] and in cat intestine [7]. In the adult primate pancreas they have only been shown to occur separately in distinct cell types.

Materials and methods. Pancreas biopsies from 10 Chacma baboons used for transplantation studies were processed as previously described [8, 9]. Semithin sections on slides were labelled, using the Avidin-Biotin technique, in a particular sequence so that any dual immunoreactivity (DIR) could be observed in adjacent sections of the same cell layer. The sequence of primary antisera was as follows: anti-PYY; anti-PP; PP absorbed antiglucagon; glucagon absorbed antisomatostatin and somatostatin absorbed antiglucagon. Antisera absorbed with their homologous antigens and method controls were applied on subsequent slides. The antisera absorbed with heterologous antigens were used to eliminate labelling due to cross reactivity. Antisera were absorbed with homologous and heterologous antigens by incubating antisera for 24 h at 4 °C with 10 nmol of antigen per ml of antiserum diluted optimally for ultrastructural localization or 30 nmol per ml of antiserum diluted for localization at light microscopy. Areas found to contain cells displaying DIR were trimmed and gold sections cut and picked up on gold or nickel grids for immunolabelling with colloidal gold for electron microscopy using higher dilutions of the same antisera [9].

One of the baboons was found to be pregnant and the foetal pancreas was included in the study.

Results. Cells which displayed DIR for glucagon and PP (fig. 1 a, b) and for glucagon and somatostatin (fig. 2 a, b) were observed in both the adult and foetal baboon endocrine pancreas.

Most of the areas of endocrine cells seen in the foetal baboon pancreas contained cells which were immunoreactive for glucagon and PP. In the adult baboon a maximum of 10% of islets in an LM section contained such cells which appeared to be confined to a small number of islets in which they then occurred quite commonly (fig. 1). Ultrastructural labelling revealed that the degree of overlap of glucagon and PP immunoreactivity appears to vary from just one or two granules labelling for PP in a cell with the morphological appearance of an A cell (fig. 3) to a more even content of glucagon and PP. Preliminary evidence suggests that electron dense A-type granules, immunoreactive for glucagon, appear to co-exist with less electron dense granules im-