Comparison of the effects of antiprogestins RU38486, ZK98299 and ORG31710 on periovulatory hypophysial, ovarian and adrenal hormone secretion in the rat

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ABSTRACT. The antiprogestin (AP) RU38486 (RU) blocks progesterone (P) and glucocorticoid (G) actions. Administration of 4 mg RU on proestrous morning to cyclic rats dissociates LH and FSH secretion on proestrous afternoon, early estrus and on estrous afternoon. In order to ascertain which action blocked by RU is predominant in the control of periovulatory LH and FSH secretion, a study was made on the effects of: a) 1 or 4 mg of ZK98299 (ZK) (type I P antagonist; Schering), b) 2 or 8 mg of Org31710 (OR) (type II P antagonist lacking anti-G actions; Organon) or c) 1 or 4 mg of RU (type II P antagonist; Exelgyn) to 4-day cyclic rats on proestrous morning on serum concentrations of LH, FSH, inhibin-α (I), estradiol-17β (E), progesterone (P) and corticosterone (B) at 18:30 h on proestrus and at 02:00 and 18:30 h on estrus. Controls, receiving 0.2 ml oil, had elevated serum concentrations of all six hormones on proestrous afternoon; at early estrus, only serum concentrations of I, E, P and B were unaffected. At early estrus, basal serum concentrations of LH and E increased while FSH secretion was abolished. Serum levels of I, P and B did not differ from controls. AP treatments increased basal LH concentration, hyperstimulated FSH secretion and reduced serum I concentration on the afternoon of estrus. E, P and B serum levels did not differ from controls at this stage. Treatment with 1 mg ZK was less effective in reducing serum FSH on proestrous afternoon and at early estrus, and had no effect on serum concentrations of any hormone on estrous afternoon. These results indicate that blockade of P receptor activation by P is, predominantly, the mechanism of AP action on periovulatory gonadotropin secretion in rats. (J. Endocrinol. Invest. 23: 151-157, 2000)

INTRODUCTION

It is generally accepted that luteinizing hormone (LH) secretion is controlled primarily by hypothalamic LH-releasing hormone (LHRH). On the other hand, regulation of follicle-stimulating hormone (FSH) secretion is not yet fully understood. Thus, to some extent, control of LH can be separated from control of FSH (1). This differential control of LH and FSH results in dissociation of serum LH and FSH levels in several physiological and experimental conditions (2, 3).

Over the past five years experimental work has shown that administration of the antisteroid RU38486, an antiprogestagen (AP) at the receptor (R) (4), to cyclic rats in proestrus differentially affects LH and FSH secretion: a) abolishes the primary surge of FSH but only decreases the magnitude of that of LH on proestrus afternoon (5-8); b) blocks the secondary surge of FSH and increases basal secretion of LH at early estrus (9, 10); and c) induces a selective hypersecretion of FSH on estrous afternoon (11).

Also, when injected on proestrus, RU38486 induces
a 1-day shortening of the estrous cycle (12). The antisteroid RU38486 is a type II AP (13) with high binding affinity for both PR and GR (14). In addition, on the basis of its mechanism of action (15) it has been suggested that RU38486 may also block ligand-independent activation of PR (16, 17). Because P (18), B (19), as well as other ligands (20), may be involved in periovulatory secretion of LH and FSH, the properties of RU38486 make it difficult to ascertain precisely which action is predominant in the regulation of periovulatory gonadotropin secretion.

The use of APs with different mechanisms of action and affinities for GR seems to offer a suitable approach for investigating the dissociation of LH and FSH secretion after injection of RU38486.

The present study aimed to determine the effects of APs: RU38486 (type II with anti-G activity) (21), ZK98299 (type I with anti-G activity) (22) and Org31710 (type II lacking anti-G actions) (23) on periovulatory LH and FSH secretion.

MATERIALS AND METHODS

Animals

Virgin female Wistar rats weighing 185-210 g were used. The rats were housed under a 14 h light, 10 h dark schedule, with lights on at 05:00 h and at 21-23 C room temperature. Vaginal smears were taken daily and only rats showing consistent 4-day cycles were used in these experiments.

Drugs and treatments

APs RU38486 (RU) (11β-[4-dimethyl-aminophenyl]-17β-hydroxy-17α-[prop-1-inyl]-estra-4, 9-diene-3-one) (Exelgyn, Paris, France) and ZK98299 (ZK) (11β-[4-dimethylaminopropyl]-17α-hydroxy-17β-[3-hydroxypropyl]-13α-methyl-4, 9 gonadien-3-one) (Schering, Berlin, Germany) were suspended in olive oil at 5 and 11 mg/ml. AP Org31710 (OR) (6β, 11β, 17β)-11-(4-dimethylaminophenyl)-6-methyl-4', 5’-dihydro [estra-4, 9-diene-17, 2'(3H')-furan]-3-one (Organon, Oss, The Netherlands), was suspended in olive oil at 10 and 40 mg/ml.

Experiments

Six groups of rats were given sc injections of each AP solution at 09:00 h on proestrus. One group of rats was injected with 0.2 ml olive oil and served as control. At 18:30 h on proestrus and at 02:00 and 18:30 h on estrus, less than 1 ml blood was taken by direct jugular venipuncture while rats were under light ether anesthesia. Blood was allowed to clot at room temperature, then centrifuged at 4 C for 10 minutes, and serum was separated and stored frozen at -20 C until assayed by RIA for LH, FSH, inhibin-α (I), estradiol-17β (E), progesterone (P) and corticosterone (B). Rats were then left undisturbed until the next day of vaginal estrus when they were killed with an overdose of ether between 10:00 and 11:00. The ampullary region of the fallopian tubes was examined for the presence of eggs.

Determination of LH and FSH

Serum concentrations of LH and FSH were measured in duplicate in 50 μl samples using the double-antibody RIA method with RIA kits supplied by the NIH (Bethesda, MD) and according to the method described previously (9). Rat LH-I-9 and FSH-I-8 were labeled with 125I by the chloramine T method (24). Concentrations of LH and FSH were expressed as ng/ml of serum of the reference preparation LH-rat-RP-3 and FSH-rat-RP-2. The intraassay coefficients of variation were 7% and 8% for LH and FSH, respectively. The interassay coefficients of variation were 9% and 13% for LH and FSH, respectively. The sensitivity of the assay was 7.5 and 20 pg/tube for LH and FSH, respectively.

Determination of steroids

Serum P and E concentrations were determined using commercially-obtained kits (Diagnosis Products Corporation, Los Angeles, CA). Assay sensitivities were 10 pg/tube and 1 pg/tube for P and E, respectively. The intraassay coefficients of variation were 6 and 7%, respectively.

Serum B concentrations were measured using a commercially-obtained kit (ICN Biomedicals, Costa Mesa, CA). Assay sensitivity was 2.5 ng/tube and the intraassay coefficient of variation was 4%.

Determination of inhibin-α

Serum inhibin-like immunoreactivity was estimated according to the method described in (25), using a bovine follicular fluid preparation with an arbitrary potency of 1 U/μg protein as standard (26). Antibody was raised against 32-kDa bovine follicular inhibin and cross-reacted with free α subunits. These materials were purchased from the Institute for Reproduction and Development, Monash University (Clayton, Victoria, Australia). All samples were run in the same assay, and the intraassay coefficient of variation was 8.2%.

Statistical evaluation

Results are expressed as mean±SE. Data were evaluated for statistically significant differences by one- or two-way ANOVA followed by Newman-Keuls multiple range test. When appropriate, the Fisher’s non parametric exact probability test was used. A difference was considered to be statistically significant if p<0.05.