clearance of creatinine (GPR) but also by directly enhancing sodium reabsorption.

e) Inhibition of TXA_2 biosynthesis in glycerol treated rats (table, group 7, 8, 9). The relationships observed between increased urinary excretion of TXB_2 and the development of ARF and sodium excretion were further reinforced when the selective inhibitor of TXA_2 synthesis was used. Thus the administration of OKY-046 in glycerol treated animals significantly diminished urinary TXB_2 excretion. This inhibition of TXA_2 synthesis was associated with a lesser decrease in creatinine clearance and sodium excretion than in untreated animals suggesting a partial protection of the rats against ARF. Since OKY-046 did not significantly change urinary excretion of the vasodilator – natriuretic[3-11] PGE_2 and $6ketoPGF_{1\alpha}$, during the 6 h of the experiment, the protection afforded by OKY-046 must not be related to these PGs but to the decreased release of TXA_2.

The increased fractional excretion of sodium after TXA_2 biosynthesis inhibition (table) could be related to the diminished release of TXA_2 because the urinary excretion of the vasodilator-natriuretic PGs did not change significantly.

In conclusion: These results suggest that 6 h after glycerol injection 1) increased TXA_2 release is accompanied by decreased creatinine clearance (C_{Cr}), sodium excretion ($U_{Na}V$) and fractional excretion of sodium (FE_{NA}%), suggesting the development of the early phase of acute renal failure (ARF), 2) The use of a selective inhibitor of thromboxane A_2-synthetase enhanced sodium excretion and fractional excretion of sodium in normal and saline loaded animals and partially prevented the decrease in creatinine clearance and sodium excretion and significantly increased fractional excretion of sodium in glycerol treated rats suggesting a partial protection against the development of acute renal failure. These relationship between TXA_2 and sodium excretion will be reinforced by further investigation to exclude actions of OKY-046 in sodium excretion unrelated to its inhibitory effect on the enzyme system.

1 Acknowledgment. This work was supported by the Public Benefit Foundation 'Alexander S. Onassis' (G-73). We thank ONO Pharmaceutical Co. Ltd, Kissei Pharmaceutical Co. Ltd, Osaka, Japan, and Dr A Hornych for their generous donations of OKY-046 and anti-6keto-PGF_{1a} antibodies respectively.

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0014-4754/86/060613-03$1.50 + 0.20/0

Pituitary gonadotropin releasing hormone (GnRH) receptor levels in intact and ovariectomized-adrenalectomized female golden hamsters on a short photoperiod

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Summary. Both intact and ovariectomized + adrenalectomized hamsters on a short photoperiod, had a daily surge in plasma LH at approximately 16.00–18.00 h. The number of pituitary GnRH receptors was generally lower in ovariectomized + adrenalectomized hamsters than in intact animals, but both intact and ovariectomized + adrenalectomized hamsters had a decrease in the number of

receptors just prior to the LH surge. These results show that gonadal steroids are not involved in regulating the pre-LH surge fall in the number of GnRH receptors.

Key words. Gonadotropin releasing hormone; GnRH; GnRH receptors; hamsters; photoperiod.

There is a fall in the number of gonadotropin-releasing hormone receptors (GnRH-R) which occurs at about the same time as the pre-ovulatory gonadotropin surge in cycling rats[1,2] and hamsters[3]. More frequent determinations have shown that the fall in GnRH-R actually precedes the gonadotropin surge[4,5]. The cause of the fall is unknown.

Female hamsters maintained on a short photoperiod have daily gonadotropin surges similar to the proestrus gonadotropin surge[6–8]. Furthermore, the daily gonadotropin surge in female hamsters on short photoperiod will occur in the presence or absence of sex steroids in the blood[8–10]. The present study investigated whether there is a fall in the GnRH-R in intact or ovariectomized-adrenalectomized (OVX/ADX) hamsters on a short photoperiod.

Materials and methods. Experiment 1: Eighty female golden hamsters were purchased from Charles River (Wilmington, MA) at 63 days of age. All animals were housed in groups of five or six in polycarbonate cages. They were maintained in a light-tight, artificially illuminated room, with the temperature maintained at 25 ± 1 °C with the lights on from 10.00 to 16.00 h (LD 6:18). After 10 weeks, vaginal washings were examined daily from all animals for three weeks in order to assess estrous cyclicity. Five of the animals had normal estrous cycles at this time and were eliminated from the study. The remaining animals were in constant diestrus. 8–12 of these acyclic hamsters were then decapitated at 08.00, 12.00, 13.00, 14.00, 15.00, 16.00, 17.00, and 21.00 h. The anterior pituitaries were removed, snap frozen, and maintained at below −70 °C until later GnRH receptor assay. Serum was frozen for later RIA of LH.

Experiment 2: Sixty female golden hamsters arrived from Charles River at 63 days of age and were maintained on LD 6:18 as above. After nine weeks, these hamsters were assessed for estrous cyclicity for 3 weeks, and the animals with regular estrous cycles were eliminated from the study. All of the remaining hamsters were then bilaterally ovariectomized and adrenalectomized in order to eliminate most sex steroids from the blood. One week later, groups of the animals were decapitated at 08.00, 13.00, 16.00, 18.00, and 21.00 h. Pituitaries and serum were then saved as described above.

For use in the binding assay, pituitaries were thawed on ice, and two pooled pituitaries were homogenized by hand in 2.0 ml of a 0.1 M sucrose solution as previously described[11]. The homogenate was centrifuged at 10,800 × g for 15 min, and the pellet was resuspended in Tris-HCl buffer (10 mM) to a concentration of approximately 50 µg protein/200 µl.

The number of GnRH receptors was determined, as previously described[11], using D-Ala[6], desGly[10], GnRH ethylamide (D-Ala) as both labeled and unlabeled hormone. The [^{125}I]D-Ala tracer was prepared as previously described[12], and the specific activity was 900–1300 µCi/µg. All determinations of binding capacity were performed by saturation analysis since numerous previous studies in rats and hamsters have not shown changes in receptor affinity in various physiological situations[1–4,11,13,14] similar to those described in this paper. Saturation analysis involved adding a near saturating amount of D-Ala (800 pg D-Ala and 300 pg ^{125}I D-Ala) to 200 µl pituitary homogenate in a total volume of 500 µl (assay buffer: 10 mM Tris-HCl containing 1 mM dithiothreitol, and 0.1% BSA). After incubation for 1 h at 4 °C, bound hormone was separated by centrifugation at 27,000 × g for 15 min at 4 °C. Nonspecific binding (2–3%) was assessed in tubes containing 20 ng unlabeled D-Ala analog. This method has been previously validated in more detail elsewhere[13].

Serum LH was determined by double antibody RIA using ^{125}I ovine LH, Niswender No. 15 anti-ovine LH antibody, and rat LH RP-1 as standard[15]. This assay has been previously validated for measurement of hamster LH[16]. The intra- and inter-assay coefficients of variation were 3% and 8.5% respectively.

The sensitivity of the assay was 2 ng/tube. Differences between groups were determined by one-way analysis of variance with post hoc testing with Duncan's multiple range test.

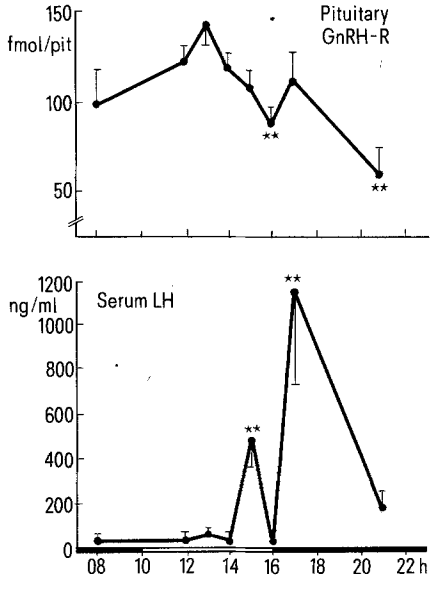

Figure 1. Pituitary GnRH receptor (GnRH-R) and serum LH levels in intact hamsters maintained on an LD 6:18 photoperiod. The abscissa represents the time of day and the solid bar along the abscissa indicates the time of lights off. The points represent the $\bar{x} \pm$ SEM of 4–6 determinations. In the top panel, ** indicates $p < 0.01$ compared to the 13.00 h value. In the bottom panel, ** indicates $p < 0.01$ compared to 08.00 h LH levels.

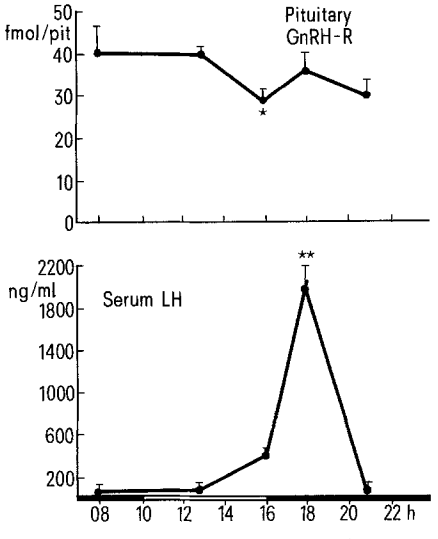

Figure 2. Pituitary GnRH receptor (GnRH-R) and serum LH levels in ovariectomized-adrenalectomized hamsters on an LD 6:18 photoperiod. The points represent the $\bar{x} \pm$ SEM of 4–6 determinations. In the top panel, the 16.00 h value (*) was significantly different than the 13.00 h point at $p = 0.04$. No other GnRH-R values were significantly different. In the bottom panel, ** indicates $p < 0.01$ compared to the 08.00 h levels. See figure 1 for further details.

Results. The serum LH levels in the intact hamsters increased at 15.00 h, fell back to a low level at 16.00 h, then increased sharply at 17.00 h, but were low again at 21.00 h (fig. 1). The GnRH-R was maximal at 13.00 h, and decreased to a low level at 16.00 and 21.00 h (fig. 1).

In the OVX/ADX hamsters, LH levels were maximal at 18.00 h and much lower at the other sampling times (fig. 2). The GnRH-R was generally lower in the OVX/ADX hamsters than in the intact animals, but there was a decrease in the GnRH-R between 12.00 and 16.00 h in the OVX/ADX hamsters similar to the fall in the intact animals (fig. 2).

Discussion. Although none of the animals in the present experiments were maintained on a long photoperiod, the number of GnRH receptors in intact hamsters on LD 6:18 was generally comparable to that reported for normally cycling rats[1,2]. The cause of the fall in the number of pituitary GnRH receptors preceding the pre-ovulatory gonadotropin surge is perplexing. The decrease is apparently not due to in vivo or in vitro occupation of the receptors by GnRH[4], and a fall in binding occurs in ovariectomized rats implanted with estrogen[5] suggesting that changes in serum estrogen levels are not involved. However, there may be time of day changes in estrogen and progesterone levels in ovariectomized, estrogen implanted animals due to adrenal secretion of progesterone and due to rhythms in the metabolism of estrogens.

The present study shows that there is a fall in the number of GnRH receptors preceding the LH surge in intact or ovariectomized-adrenalectomized female hamsters on short photoperiod. These results indicate that changes in the levels of gonadal steroids are not the cause of the fall in the number of receptors and that elevated estrogen levels are not necessary for the decline to take place.

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0014-4754/86/060615-03$1.50 + 0.20/0

β-Adrenergic stimulation of androgen production by fetal mouse Leydig cells in primary culture

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Summary. The responsiveness of fetal mouse Leydig cells to catecholamines (epinephrine, norepinephrine), a β-agonist agent (L-isoproterenol) and hCG was investigated in vitro. Fetal Leydig cells when freshly isolated were unable to respond to L-isoproterenol (10^{-5} M). However, L-isoproterenol, epinephrine and norepinephrine significantly stimulated androgen production by fetal Leydig cells after 24 h of primary culture. Androgen production was increased in both conditions and to a greater extent by hCG. Propranolol blocked the stimulatory effect of L-isoproterenol and epinephrine. It is concluded that catecholamines can regulate fetal testosterone biosynthesis.

Key words. Fetus; Leydig cells; androgen; catecholamines; primary culture.

It is well established that gonadotropins (LH/hCG) are the main hormones which regulate testicular testosterone biosynthesis. In the course of studies of factors influencing the steroidogenic activity of the testis, it was recently reported that catecholamines can exert a stimulatory effect on androgen production by adult interstitial cells in primary culture[2-3], via specific β-adrenergic receptors[4]. The presence of higher concentrations of norepinephrine in the neonatal testis as compared with prepubertal and adult testes[5] has raised the question of whether there is a direct effect of catecholamines on androgen production during fetal development. Recently we have established an in vitro primary culture of fetal mouse Leydig cells that maintains steroidogenic responsiveness to gonadotropin[6]. In this system the direct effect of L-isoproterenol, epinephrine and norepinephrine upon androgen production was examined.

Material and methods. Fetal Leydig cells were isolated from 18-day-old fetal mouse testes. The complete protocol has been described elsewhere[6]. Briefly, fetal testicular cells were obtained by mechanical dissection and collagenase treatment. Aliquots of the cell suspension were incubated for 2 h in Falcon culture dishes. Medium 199 supplemented with 15 mM Hepes (Eurobio, Paris, France), 0.1% BSA (ICN Pharmaceutical Inc.), glucose, 1% fetal calf serum (Difco Laboratoires, Detroit, USA), 100 U penicillin/ml and 100 µg streptomycin/ml (Difco Laboratories) was used. At the end of this period, floating cells were removed and firmly attached cells were washed 3 times. About 70% of the firmly attached cells stained positively for 3β-hydroxysteroid dehydrogenase as described previously[6]. Viability of these cells exceeded 90% estimated by trypan blue staining. Fetal Leydig cells, freshly isolated or cultured for 24 h before any treatment in medium 199 containing 15 mM Hepes, 0.1% glucose/1, 100 U penicillin/ml and 100 µg/ml streptomycin, were then incubated for 3 h at 37 °C in supplemented medium, which contained 0.1 mM 3-isobutyl-1-methylxantine (IBMX), a phosphodiesterase inhibitor which increases endogenous levels of cAMP and 0.1 mM ascorbic acid, to reduce breakdown of catecholamines.