at least a 2¼ log concentration increase of ACh was required to produce 50% of the second relaxation response. Rubbing of the tissue to remove the endothelium was accomplished in all arteries without significantly altering the contractile response to H. Incubation with atropine (10^{-5} M) blocked ACh-induced relaxation indicating that the response is mediated through muscarinic receptors. Explanations for the resistant portion of the vasodilation response to ACh after mechanical removal of the endothelial layer in the TB are that the tissues were 'inadequately' rubbed (still retained some endothelial cells) and/or the smooth muscle cells of the TB possess muscarinic receptors mediating vasodilation[5].

Our studies represent the first direct evidence that endothelium mediated dilation occurs in resistance vessels. It would seem reasonable that endothelial-based vasodilation should become greater as vessels become smaller, the internal elastic lamina thinner and more fenestrated and the intimal/media mass ratio and myoendothelial junction density greater[6]. Also the two smaller arteries studied possess intrinsic tone[2], and this would provide an appropriate background for dilation.

In conclusion, the determination that acetylcholine can cause dilation in vessels as small as 75 µm (ULD) and that this effect is proportionately greater in smaller vessels suggests that if there is a physiological role for endothelium-based relaxation, this would be more profound in smaller compared with larger arteries. The exact role of this mechanism awaits determination.

* Acknowledgments. We thank Christine Quinn for her careful technical assistance in the use of the myographs and Daniel Netto for construction of the small vessel myographs. The tungsten wire used for mounting the vessels was given by Westinghouse Corporation Lamp Division. This work was supported by American Heart Association Fellowship 608 and National Heart, Lung, and Blood Institute Grant HL-32985.

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0014-4754/85/081057-02$1.50 + 0.20/0

Effects of gonadotropin-releasing hormone (LH-RH) on the pars distalis and testis of the Skipper frog, *Rana cyanophlyctis* (Schn.)[1]

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Summary. Administration of LH-RH to adult male Skipper frogs resulted in marked hypertrophy and degranulation of basophils-2 (B2) in the pars distalis of the pituitary and a significant increase in their nuclear and cellular area. Concomitantly, there is a significant increase in the relative weight of the testes, in the number of cell nests containing secondary spermatogonia and primary spermatocytes, and in the nuclear diameter of the Leydig cells. There is also an increase in the Δ^5-3β-hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase activities in the Leydig cells. The results indicate that the B2 cells are gonadotrops and the hormone(s) secreted by B2 cells regulate the spermatogenetic and steroidogenic activity of the testis in *R. cyanophlyctis*.

Key words. LH-RH; frog testis; pituitary cytology; gonadotrops.

Administration of LH-RH affects the cytomorphology of gonadotrops and elevates the plasma levels of FSH and LH in mammals[2,3]. It has been shown that the pars distalis of the pituitary gland of the frog. *Rana catesbeiana* secretes two types of gonadotropins similar to mammalian FSH and LH, and administration of LH-RH causes an increase in the plasma levels of FSH and LH[4-6]. However, to the best of our knowledge, the effects of LH-RH on the cytomorphology of gonadotrops in amphibians have not been investigated. Though it has been assumed that the amphibian pituitary contains two types of gonadotrops similar to those of mammals[7] experimental studies have not yet convincingly proved this, and it is generally accepted that there is only one type of gonadotrop[8,9]. Since LH-RH specifically affects the release of gonadotropins, studies on the cytomorphological changes in the pituitary cell types following administration of LH-RH may help to find out whether there are two types of gonadotrops or not. Hence, the present study aims at investigating the effects of LH-RH on the cell types of the pars distalis with particular reference to gonadotrop(s) in the frog, *Rana cyanophlyctis*. Further, since the administration

Table 1. Effects of LH-RH on the mean nuclear diameter and length of different cell types in the pars distalis of *R. cyanophlyctis*

Groups and treatments	Mean nuclear diameter (µm ± SE) of Acidophils-1	Acidophils-2	Basophils-1	Basophils-2	Basophils-2
1) Controls (5)	4.95 ± 0.18	4.72 ± 0.09	4.40 ± 0.08	4.40 ± 0.06	4.60 ± 0.03
2) Frogs treated with LH-RH (5)	4.60 ± 0.13	4.61 ± 0.18	4.58 ± 0.09	5.48 ± 0.06	4.68 ± 0.09
	NS	NS	NS	$p < 0.05$	NS
	Mean length (µm ± SE)				
1) Controls	11.50 ± 0.48	9.12 ± 0.30	7.40 ± 0.19	11.28 ± 0.20	9.16 ± 0.20
2) Frogs treated with LH-RH (5)	10.98 ± 0.18	8.62 ± 0.38	7.86 ± 0.12	12.61 ± 0.21	9.41 ± 0.20
	NS	NS	NS	$p < 0.05$	NS

Number in parentheses indicates number of animals used. Means of control and experimental groups are compared using Student's t-test and judged significant if $p < 0.05$. NS = not significant.

of LH-RH and consequent release of pituitary gonadotropins is known to affect the testis function[10,11], the concomitant changes in the spermatogenetic and steroidogenic activity in the testis are also investigated.

Adult male *R. cyanophlyctis*, collected during April, and acclimatized to laboratory conditions for a week, were divided into two groups, 10 in each group. The frogs in the first group received 0.1 ml amphibian Ringer solution/frog. Each frog in the second group was given 40 ng synthetic LH-RH (chloride form, NICHD batch 2, distributed by NIAMDD, NIH, USA) dissolved in 0.1 ml amphibian Ringer solution. Injections (i.m.) were given on alternate days for 29 days and the frogs were autopsied 24 h after the last injection. At autopsy the weight of the body and that of the testes were recorded and the left testis was fixed in Bouin's fluid for histological and histometric studies[12]. The right testis was used for histochemical localization of $\Delta^5$3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) activity as described earlier[13].

The spermatogenetic stages were identified following the description of Oordt[14] as follows: stage 0, primary spermatogonia; stage I, secondary spermatogonia (less than 10 cells in a nest); stage II, secondary spermatogonia (more than 10 cells per nest); stage III, primary permatocytes; stage IV, secondary spermatocytes; stage V, spermatids. The pituitary gland, together with the brain, was fixed in Bouin-Hollande-sublimate, embedded in wax and sectioned at 4 µm. The sagittal sections were stained using three methods; Alcian blue-PAS-Orange-G, Cleveland and Wolfe's trichrome, and lead hematoxylin. The nuclear diameter and maximum length of various types of cells found in the pars distalis were measured with the help of an ocular micrometer. The nuclear and cellular areas of B2 and B3 cells were measured using camera lucida drawings and a planimeter. The means of control and treated groups were compared using Student's t-test.

The observations indicate the presence of three types of basophils (B1, B2 and B3) and two types of acidophils (A1 and A2) in the pars distalis of *R. cyanophlyctis*, as reported earlier[12]. The cytoplasm of basophils-2 (B2) in the control frogs was compact and granulated. Administration of LH-RH resulted in a marked degranulation and vacuolization of B2 cells, whereas the other cell types were not affected. There was a significant ($p < 0.05$) increase in the mean nuclear diameter, nuclear area, length and area of B2 cells in LH-RH treated frogs when compared to those of controls (tables 1 and 2). It has been shown that administration of LH-RH to hypophysectomized newts bearing a pituitary graft preserves B2 cells[10]; however, in this study[10], the cytomorphological changes in the B2 cells following LH-RH administration have not been investigated. Since LH-RH specifically affects the release of gonadotropins in the pars distalis of mammals[2] and amphibians[5,6], the marked cytological changes in the B2 cells following LH-RH administration in *R. cyanophlyctis* indicate that B2 cells are gonadotrops. Similar cytological changes in the B2 cells have been reported after castration in other anurans and it is suggested that the B2 cells are the gonadotrops[15].

However, Kasinathan et al.[7] have reported that castration and administration of sex steroids to castrates affect not only B2 cells but also B3 cells in *Rana hexadactyla* and suggested that both B2 and B3 cells are gonadotrops. In the present study LH-RH treatment did not cause any cytomorphological changes in B3 cells and our observations do not support the view that B3 cells are also gonadotrops. Immunohistochemical studies have shown that B3 cells are corticotrops in amphibians[16]. Therefore it appears that the response of B3 cells to castration and steroid treatment in *R. hexadactyla* may be due to the effect of castration on the pituitary-adrenal system as reported in mammals[17].

A significant increase in the mean relative weight of the testes, tubule diameter, Leydig cell nuclear diameter, the number of cell nests containing secondary spermatogonia (stage II) and primary spermatocytes, and an increase in the Leydig cell Δ^5-3β-HSDH and G-6-PDH activities (tables 3 and 4) in LH-RH treated frogs when compared with the corresponding parameters for controls, suggests the stimulation of spermatogenetic and steroidogenic activity of the testis. Since the proliferation of spermatogonia and the stimulation of the Leydig cells are known to be gonadotropin-dependent in anurans[18], it is suggested that there is an enhanced release of gonadotropin(s) from B2 cells in LH-FSH-RH treated frogs and this results in the stimulation of testicular activity in *R. cyanophlyctis*.

Table 2. Effects of LH-RH on the cellular and nuclear area of basophils-2 and basophils-3 in the pars distalis of *Rana cyanophlyctis*

Groups and treatments	Basophils-2 Nuclear area (µm² ± SE)	Cellular area (µm² ± SE)	Basophils-3 Nuclear area (µm² ± SE)	Cellular area (µm² ± SE)
1) Controls (5)	12.47 ± 1.04	69.68 ± 6.80	18.69 ± 1.31	59.90 ± 6.10
2) Frogs treated with LH-RH (5)	22.68 ± 2.13 $p < 0.05$	123.45 ± 2.40 $p < 0.01$	15.68 ± 1.31 NS	56.73 ± 5.92 NS

Number in parentheses indicates number of animals used. Means of control and experimental groups are compared using Student's t-test and judged significant if $p < 0.05$. NS = not significant.

Table 3. Effects of LH-RH on the testis of *R. cyanophlyctis*

Groups	Mean testis wt (mg/100 g b.wt)	Mean seminiferous tubule diameter (µm ± SE)	Mean Leydig cell nuclear diameter (µm ± SE)	Δ^5-3β-HSDH activity*	G-6-PDH activity*
1) Controls (5)	240.05 ± 17.43	230.72 ± 5.01	4.99 ± 0.09	+ + +	+ + + +
2) Frogs treated with LH-RH (5)	372.75 ± 4.99 $p < 0.01$	292.71 ± 8.93 $p < 0.01$	5.48 ± 0.07 $p < 0.05$	+ + + +	+ + + + +

Number in parentheses indicates number of animals used. *Intensity of enzyme activity is visually graded, + + + + +, denotes maximum activity and +, denotes weak activity. Means of control and experimental groups are compared using Student's t-test and judged significant if $p < 0.05$.

Table 4. Effects of LH-RH on the spermatogenesis of *R. cyanophlyctis*

Groups	Number of cell nests/tubule cross section/stage Stage 0	Stage I	Stage II	Stage III	Stage IV	Stage V
1) Controls	3.93 ± 0.40	3.93 ± 0.22	4.23 ± 0.41	2.98 ± 0.34	2.15 ± 0.13	1.40 ± 0.15
2) Frogs treated with LH-RH (5)	3.75 ± 0.14 NS	4.50 ± 0.24 NS	5.60 ± 0.35 $p < 0.05$	4.28 ± 0.30 $p < 0.05$	2.12 ± 0.27 NS	1.48 ± 0.19 NS

Number in parentheses indicates number of animals used. Means of control and experimental groups are compared using Student's t-test and judged significant if $p < 0.05$. NS = not significant.

It is interesting to note that in the present study LH-RH treatment resulted in cytomorphological changes in only one cell type whereas in mammals it affects the cytomorphology of two cell types namely LH and FSH gonadotrops[3]. Therefore, it is suggested that the pars distalis of *R. cyanophlyctis* contains one type of gonadotrop represented by the B2 cell type and the hormone(s) secreted by B2 cells regulate the spermatogenetic and steroidogenic activity of the testis in *R. cyanophlyctis*.

1 The free gift of LH-RH from NIH is gratefully acknowledged. Authors are grateful to CSIR, New Delhi, for the award of a SRF to the first author and to Karnatak University, Dharwad for providing laboratory facilities.
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0014-4754/85/081058-03$1.50 + 0.20/0

Clomiphene citrate can mimic the augmentative (positive) but not the depressing (negative) effect of estradiol on the LHRH-stimulated release of LH and FSH by the pituitary gland of the long-term ovariectomized rat

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Summary. In the long-term ovariectomized rat, both estradiol benzoate (EB) and clomiphene citrate enhance the release of LH induced by luteinizing hormone-releasing hormone (LHRH). EB also enhances the release of FSH. In rats pretreated with LHRH, EB strongly depresses the LHRH-induced LH/FSH release, but clomiphene enhances this release, regardless of the presence of EB.
Key words. Ovariectomized rat; LHRH; estradiol benzoate; clomiphene citrate; LH release.

Clomiphene citrate is a nonsteroidal agent which is frequently and successfully used for induction of ovulation in anovulatory women[3,19]. Despite its widespread use, the drug's mode of action has not been fully clarified, e.g. data obtained with pituitary cell cultures indicate the drug to have both estrogen-agonistic and estrogen-antagonistic properties[9-11,20,28]. It is, however, generally believed that clomiphene acts as a competitive inhibitor of estradiol at hypothalamic and/or pituitary receptor sites[1,12,14].

When estradiol benzoate (EB) is administered to long-term ovariectomized (OVX) rats, the elevated, pulsatile secretion of LH of such animals decreases almost instantaneously [3]. This acute effect of estrogen is not due to suppression of the hypothalamic LHRH pulses, but to decreased pituitary LHRH-responsiveness[24]. However, about 9 h after administration of EB the pituitary LHRH-responsiveness begins to increase markedly, a development which coincides with the EB-induced suppression of the hypothalamic LHRH secretion[22,23]. This might suggest that suppression of the hypothalamic LHRH secretion is involved in the estrogen-induced sensitization of the pituitary gland. In this study we tested this hypothesis and re-investigated the mode of action of clomiphene on the pituitary gland in 2-week OVX rats. One series of rats was pretreated with exogenous LHRH, delivered by s.c. implanted Alzet osmotic minipumps, in order to prevent lowering of LHRH exposure of the pituitary gland due to EB-induced suppression of the hypothalamic LHRH secretion. The LHRH-releasing minipumps were therefore implanted before administration of EB. Another series of OVX rats was not pretreated with LHRH.

Materials and methods. Wistar rats were ovariectomized at the age of 3 months and used for experiments 2 weeks later. Ovariectomy was performed to eliminate the influence of ovarian hormones. The general arrangement of the experiments was as follows: some of the rats received LHRH for 6 days at the rate of 250 ng/h (released by Alzet® osmotic minipumps, model 2001, s.c. implanted at 09.00 h on day 1); other rats received a 'sham-pump', i.e. a piece of silastic with the dimensions of a minipump. In all rats responses of LH and FSH were induced on day 6 by continuous infusion (through an intra-jugular cannula) of LHRH at the rate of 1 µg/h for 21 h (the infusions started at 12.00 h). The high dose of LHRH was chosen for methodological reasons: after the described LHRH pretreatment significant LH/FSH responses could only be induced in the rats still bearing the minipumps with a strong stimulus.

Both in the case of the LHRH pretreatment and of the sham-procedure four different pretreatments preceded the LHRH infusions. I: clomiphene (100 µg by s.c. injection) was given 75, 27 and 0 h before the LHRH infusion. II: estradiol benzoate (3 µg by s.c. injection) was given 75 and 27 h before the infusion. III: treatments I and II were combined. IV: control rats received solvent.

Blood samples were taken via an intra-carotid cannula (cannulation was performed at least 2 h before LHRH infusion). Operations were carried out under appropriate ether anesthesia. Plasma LH and FSH concentrations were measured by double antibody radioimmunoassay with NIAMDDK rat LH- and FSH-RP-1 as reference preparations. LH/FSH responses were judged