Changes in the Expression Pattern of Luteinizing Hormone Receptor mRNA in Rat Testis during Degeneration of Seminiferous Epithelium

Authors: Iizuka, Akiko, Park, Min Kyun, and Mori, Takao

Source: Zoological Science, 15(2) : 255-261

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.15.255

ZOOLOGICAL SCIENCE **15**: 255–261 (1998)

Changes in the Expression Pattern of Luteinizing Hormone Receptor mRNA in Rat Testis during Degeneration of Seminiferous Epithelium

Akiko Iizuka*, Min Kyun Park and Takao Mori

Department of Biological Sciences, Graduate School of Science, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

ABSTRACT—Previously we reported that the experimental unilateral cryptorchidism altered the expression pattern of LH receptor mRNA in abdominal testis. To clarify whether the change was due to exposure of Leydig cells to high temperature in the abdomen, we examined the effect of unilateral efferent duct ligation on the expression. Two weeks after the operation, a relatively decreased expression of 1.8 kb transcript, increased expression of other transcripts, and degeneration of germ cells were observed in the efferent duct-ligated testes. As these changes were similar to those seen in abdominal testes of cryptorchid rats, exposure of Leydig cells to high temperature was not responsible for the changes in expression pattern of LH receptor mRNA. To examine the correlation between the changes in the receptor mRNA expression and the degeneration of seminiferous epithelium, expression of the mRNA was analyzed in unilaterally cryptorchid rats for 28 days after the operation. In scrotal testes which showed no histological changes, a major, 1.8 kb and several minor, 6.5, 2.6, 1.4, 1.1 and 0.9 kb, transcripts were detected throughout the experimental period. In abdominal testes, the expression of 1.8 kb transcript declined rapidly within three days, while that of 6.5 kb and 2.6 kb was increased, reaching a maximum 14 days after the operation. Histological observations of cryptorchid and efferent duct-ligated testes revealed that these changes in the receptor mRNA expression paralleled the successive disappearance of spermatids and spermatocytes. These results suggest that the changes in expression pattern of LH receptor mRNA are closely related with degeneration of germ cells and that intratesticular paracrine factor(s) from seminiferous tubules might be concerned to the phenomenon.

INTRODUCTION

Luteinizing hormone (LH) receptor is expressed in the Leydig cells in the testis and mediates the effects of LH, such as stimulation of steroidogenesis. In 1989, the cDNA for this receptor molecule was cloned from the rat luteal cDNA library (McFarland *et al*., 1989), and thereafter the existence of multiple species of LH receptor mRNA was revealed by means of cloning of cDNA molecules, Northern hybridization and polymerase chain reaction (Aatsinki *et al*., 1992; Koo *et al*., 1994; Wang *et al*., 1991). Previous studies indicated that the expression patterns of the mRNA transcripts depend on sex, age or experimental conditions (Iizuka *et al*., 1996; LaPolt *et al*., 1991; Tena-Sempere *et al*., 1994; Veldhuizen-Tsoerkan *et al*., 1994; Vihko *et al*., 1992; Zhang *et al*., 1994). Ovary and fetal testis express major transcripts of 6.5-7.0 kb and 2.5-2.7 kb, whereas in adult testis an additional 1.8 kb transcript is detected. LaPolt *et al*., (1991) showed that the expression of the 1.8 kb transcript detected in adult testis was not decreased after a hCG injection, while other transcripts were down-regulated. Furthermore, 1.8 kb transcript was expressed persistently after the destruction of mature Leydig cells by an ethylene dimethane sulfonate (EDS) injection (Tena-Sempere *et al*., 1994; Veldhuizen-Tsoerkan *et al*., 1994). However, the regulatory mechanism for the expression patterns have yet to be clarified. Recently we have studied the effects of unilateral cryptorchidism (UCD) on the expression of mRNAs for FSH and LH receptor and found that the expression pattern of LH receptor mRNA transcripts was markedly altered in the abdominal testis 4 weeks after the operation (Iizuka *et al*., 1996) It is possible that an alteration in the paracrine environment of the Leydig cells occurs along with the degeneration of seminiferous epithelium in abdominal testis, leading to changes in the expression pattern of LH receptor mRNA transcripts. However, we could not rule out the possibility that exposure of Leydig cells to abdominal high temperature affected the expression pattern of LH receptor mRNA directly, since the whole testis was exposed to unusual higher temperature in abdomen in cryptorchidism. If high temperature around the Leydig cells is responsible for the changes in the expression pattern, degeneration of seminiferous epithelium without an elevation

* Corresponding author: Tel. +81-3-3812-2111 ext. 4436; FAX. +81-3-3816-1965.

of temperature would not induce such alterations. It is reported that ligation of efferent ducts induces pressure atrophy of the seminiferous epithelium (Smith, 1962). In the present study, therefore, we examined whether efferent duct ligation (EDL) affects the expression pattern of LH receptor mRNA transcripts. In addition, we examined the sequential changes in the expression pattern of the receptor mRNA in the UCD rats during degeneration of the seminiferous epithelium to discuss the relationship between the mRNA expression and the loss of germ cells.

MATERIALS AND METHODS

Animals and treatments

Sprague-Dawley rats were purchased from Japan Clea Co. (Tokyo, Japan) and maintained under controlled conditions of light (12 hr light, 12 hr darkness; lights on at 06:00) and temperature (25 ± 0.5°C) with free access to pelleted food (CE-7; Japan Clea) and tap water. All experimental procedures were approved by the Animal Care and Use Committee of the Graduate School of Science, University of Tokyo, and all experiments conformed to the regulations described in the NIH Guide to the Care and Use of Laboratory Animals.

In the study of the effects of EDL, four 3.5-month-old male rats were anesthetized with pentobarbital (50 mg/kg body weight) and efferent ducts of the right testes were ligated using the technique described by Smith (1962). Fourteen days after the operation, rats were killed by decapitation. Two 4-month-old male rats were used as untreated controls.

For the study of the effects of UCD, 17 adult male rats were operated. The right testes were relocated into the abdomen as previously described (Kerr and Donachie, 1986). The rats (n = 3–5/group) were killed 3, 7, 14 and 28 days after the operation when they were at 5 months of age. Three 5-month-old rats were used as untreated controls.

At autopsy, testes were excised, weighed and cut into two. One piece was fixed in Bouin's solution (EDL rats) or 4% paraformaldehyde (UCD rats) and examined histologically. Total RNA was immediately extracted from the other piece using the acid guanidinium-phenol-chloroform method as previously described (Chomczynski and Sacchi, 1987).

Northern hybridization

Poly $(A)^+$ RNA was purified with oligo (dT)-latex (oligotex-dT30 Super, Takara Shuzo, Shiga, Japan) from total RNA and used in Northern hybridization analyses. In EDL study, samples of poly $(A)^+$ RNA (1 testis/sample) were prepared and analyzed separately. In UCD study, sample preparation and Northern hybridization was performed for three times separately. The first and second Northern hybridizations were performed on RNA samples each prepared from one testis. To obtain stronger signals, 1–3 testes were pooled in each experimental group in the following third sample preparation and Northern hybridization. The samples were electrophoresed on 1% formaldehyde - agarose gels and then transferred onto nylon membranes (Zeta-Probe, Bio-Rad Laboratories, Hercules, CA, USA). The blotted membranes were hybridized with α-^{32}P labeled cDNA probe corresponding to nucleotide 78–852 of rat LH receptor cDNA clone and washed as previously described (Iizuka *et al.*, 1996). Sizes of mRNA transcripts were determined using RNA markers (Boehringer-Mannheim, Indianapolis, IN, USA). The membranes were exposed to Kodak X-OMAT film (Eastman Kodak, Rochester, NY, USA) at –70°C with an intensifying screen. Autoradiographs were scanned on an image scanner (GT-9000, Seiko Epson, Nagano, Japan) and band intensity was analyzed by NIH image.

RESULTS

Effects of efferent duct ligation

Fourteen days after unilateral ligation of efferent ducts, the weight of efferent duct-ligated testis was significantly decreased as compared to that of contralateral intact testis in the same rat (Fig. 1). As shown in Fig. 2, few spermatozoa and spermatids were observed in the ligated testis. Some tubules were lined only by Sertoli cells and spermatogonia while other tubules contained thin layers of spermatocytes. The recovery of poly $(A)^+$ RNA exraction per tissue weight was lower in the ligated than intact testis (data not shown). Figure 3 shows the result of Northern hybridization. In control testis, a major transcript of 1.8 kb and other transcripts of 6.5, 2.6 and 1.4 kb were detected. The expression of LH receptor mRNA transcripts was markedly altered 14 days after EDL operation: the intensity of the 1.8 kb transcript was relatively decreased below the other four species. This band pattern was similar to that seen in abdominal testis in the cryptorchid rats in our previous study (Iizuka *et al.*, 1996). In contralateral intact testis, there were no changes in either histology or expression of mRNA relative to control testis.

Effects of unilateral cryptorchidism

Figure 4 summarizes the changes in testicular weight after the UCD operation. Weight of abdominal testis was decreased, while that of scrotal testis showed no changes. Figure 5 shows the histological features after UCD operation. Scrotal testis did not show any changes (Fig. 5A-D), while

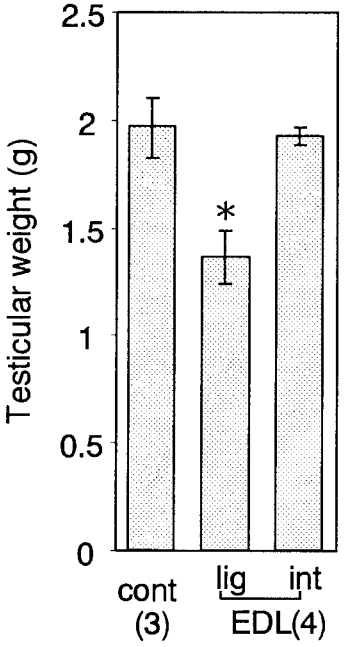


Fig. 1. Testicular weight of control and unilateral EDL rats. Values are given as the means ± SEM and the numbers of animals are shown in parentheses. cont: testis of control rat, lig: testis with efferent duct ligation, and int: contralateral intact testis of the EDL rat. *$P < 0.01$ compared to intact testes of EDL rat (Student's *t*-test).

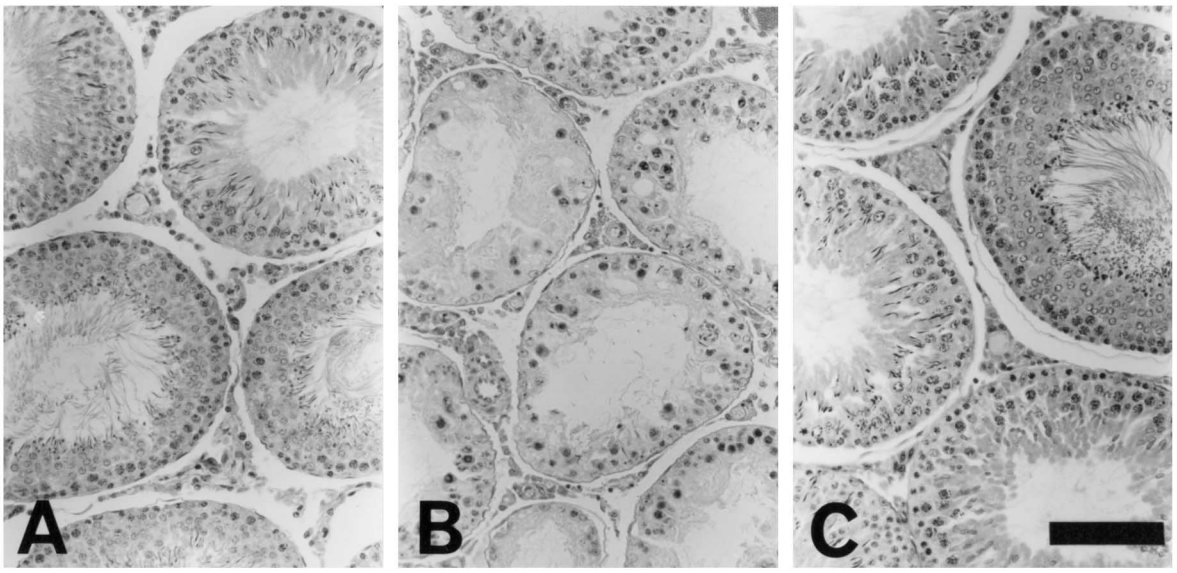
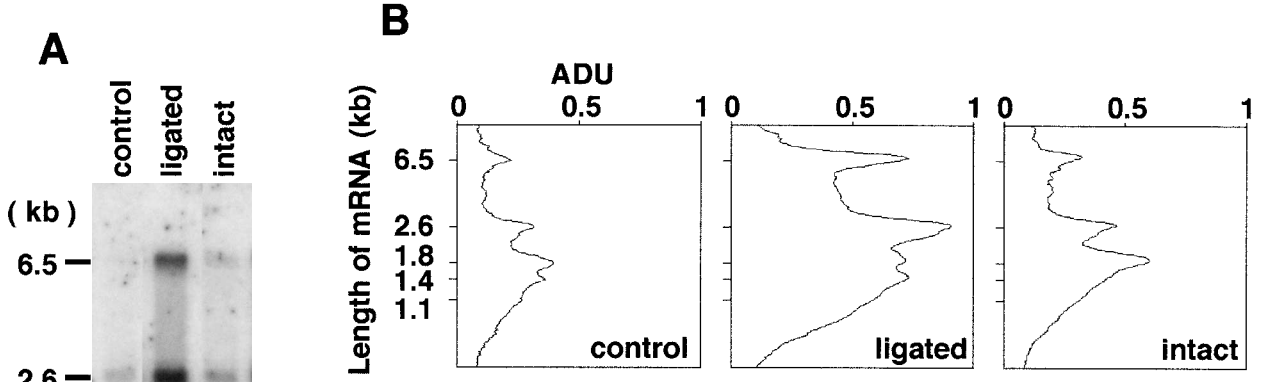


Fig. 2. Histology of control testis (**A**), and efferent duct-ligated testis (**B**) and contralateral intact testis (**C**) of EDL rat. The control testis and intact testis of EDL rat showed active spermatogenic features. On the other hand, spermatozoa and spermatids were rare, and thin layers of spermatocytes, spermatogonia and Sertoli cells were observed in seminiferous epithelia in the efferent duct-ligated testis. Bar = 100 μm.



C Intensities of hybridization signals

	control	ligated	intact
6.5 kb	0.14	0.65	0.25
2.6 kb	0.24	0.83	0.39
1.8 kb	0.32	0.64	0.52
1.4 kb	0.28	0.65	0.33

Fig. 3. Northern hybridization of poly (A)$^+$ RNA (4 μg / lane) isolated from testis of a control rat, and efferent duct-ligated testis and contralateral intact testis of an EDL rat. Samples were run on a 1% denaturing agarose gel, transferred to nylon membrane, and hybridized to the cDNA probe corresponding to the extracellular domain of the LH receptor. Hybridizations were performed on individual samples prepared from three (control) or four (EDL) rats. Representative results are shown in **A**. The membrane was exposed to X-ray film for 6 days. Band intensities of all samples were optically measured by NIH image, and the mean profile of each experimental group is indicated in **B**. ADU, arbitrary densitometric units. The intensities of major signals subtracted with background levels are indicated in table **C**.

abdominal testis showed remarkable changes. Three days after the operation, the seminiferous epithelium was disorganized and multinucleated cells containing spermatids were present in some sections of seminiferous tubules (Fig. 5E). A small number of spermatozoa and spermatids was observed at day 7 (Fig. 5F), and only a few spermatocytes were observed at day 14 (Fig. 5G). There were no significant changes in histology between day 14 and 28 (Fig. 5H). The recovery of

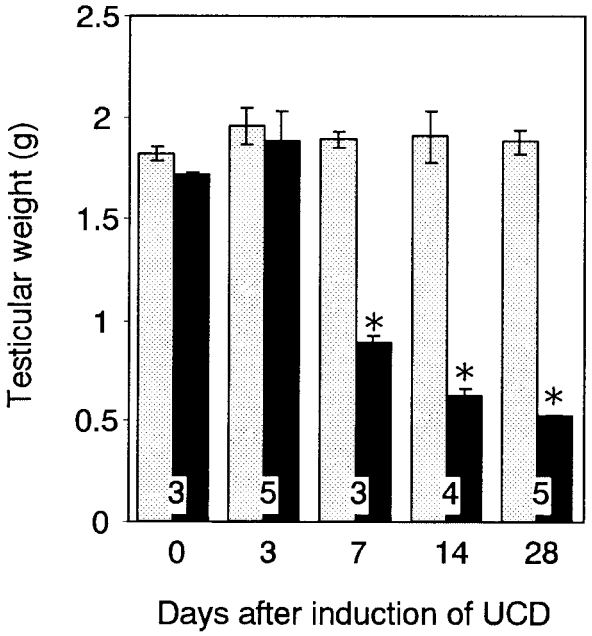


Fig. 4. Testicular weight of UCD rats. Stippled bars represent the scrotal testes and solid bars the abdominal testes. Values are given as the means ± SEM and the numbers in the column represent rats examined. *$P < 0.001$ compared to scrotal testes (Student's *t*-test).

poly $(A)^+$ RNA extraction was always lower in abdominal testis than in scrotal or control testis (data not shown). The band patterns of LH receptor mRNA transcripts in abdominal testes gradually changed, while the patterns in scrotal testes did not show any changes (Fig. 6A). Densitometric analyses of the band patterns showed that 1.8 kb transcript was the most abundant and shorter transcripts exceeded longer ones in expression amount before the operation (Fig. 6B). The first change after the operation was a relative decrease of intensity of 1.8 kb transcript, which occurred within 3 days. In addition, the intensity of other transcripts, in particular those of 6.5 kb and 2.6 kb, increased gradually for 14 days.

DISCUSSION

Recently we found that UCD operation induced marked alterations in the expression pattern of mRNA transcripts for LH receptor (Iizuka *et al.*, 1996). To understand the biological meaning of the heterogeneity of LH receptor mRNA, we aimed to clarify the cause for the alterations in the expression pattern. We hypothesized that changes in the expression pattern of the receptor mRNA were related to degeneration of germ cells. However, we could not rule out the possibility that the exposure of Leydig cells to unusual high temperature influenced the mRNA expression. In the present study, therefore, we examined the effects of EDL on the mRNA expression, in order to clarify whether the expression pattern might be altered when germ cells were degenerated in ordinary scrotal temperature. The treatment resulted in a degeneration of seminiferous epithelium, which is in agreement with a previous study by Smith (1962). Northern hybridization revealed increased expression of 6.5 and 2.6 kb transcripts and relatively poor expression of 1.8 kb transcript which is the most abundant in intact testis. In these changes, increases of 6.5 and 2.6 kb transcripts may be explained as previously reported (Iizuka *et al.*, 1996). Namely, Leydig cell component is relatively increased in the efferent duct-ligated testis because of loss of germ cells, which results in increase of LH receptor mRNA amount per poly $(A)^+$ RNA weight. Therefore, the changes in expression pattern of LH receptor mRNA in the ligated testis are similar to those observed in the abdominal testis of UCD rats. This result demonstrates that an elevation of temperature was not necessary to the changes in the expression pattern of LH receptor mRNA, but a treatment which degenerates seminiferous epithelium could induce the expression changes.

To correlate the degeneration of seminiferous epithelium with the expression pattern of LH receptor mRNA, changes in both the histology and the mRNA expression were examined 3, 7, 14, and 28 days after UCD operation. In abdominal testis, spermatids were severely degenerated within 3 days and subsequently spermatocytes disappeared, which is consistent with previous results (Clegg, 1963; Iizuka *et al.*, 1996). The changes in composition of the transcripts in abdominal testis started by day 3, proceeded throughout the experimental period, and resulted in a general shift from the short to the long form of the transcripts by day 28. Although thin layers of spermatocytes were often retained in the seminiferous tubules 14 days after EDL operation, the tubules containing spermatocytes were much fewer in the abdominal testis 14 days after UCD operation. In EDL testis, the signal of the 2.6 kb transcript predominated over the 6.5, 1.8 and 1.4 kb signals bearing lower intensity. This band pattern seems to be intermediate between those of day 7 and 14 of abdominal testes in UCD rats, suggesting that the expression pattern of LH receptor mRNA is closely related to the process of germ cell degeneration. Since the expression pattern was not altered in the contralateral intact testis in EDL or UCD rats, the effects of the operation on the expression seems to occur within a testis and is independent of the circulating factors. Thus, these changes seems to be induced by some paracrine factor(s) secreted from seminiferous tubule to interstitium. Although there are many reports that paracrine factors affect the proliferation and functions of Leydig cells *in vitro* (for reviews see Saez, 1994; Skinner, 1991), it is unclear whether these factors have significant roles *in vivo*. The present results suggest that paracrine factor(s) involved in the loss of germ cells may participate in the regulation of LH receptor mRNA expression *in vivo*. Further analyses are needed to identify these paracrine factor(s) and to determine their source.

The present results on the expression of multiple species of LH receptor mRNA in the intact testis agree with previous studies (Iizuka *et al.*, 1996; LaPolt *et al.*, 1991; Wang *et al.*, 1991). The abundant expression of 1.8 kb transcript is the most prominent feature of the adult testis, as this transcript is a minor species in ovary and fetal testis (LaPolt *et al.*, 1991;

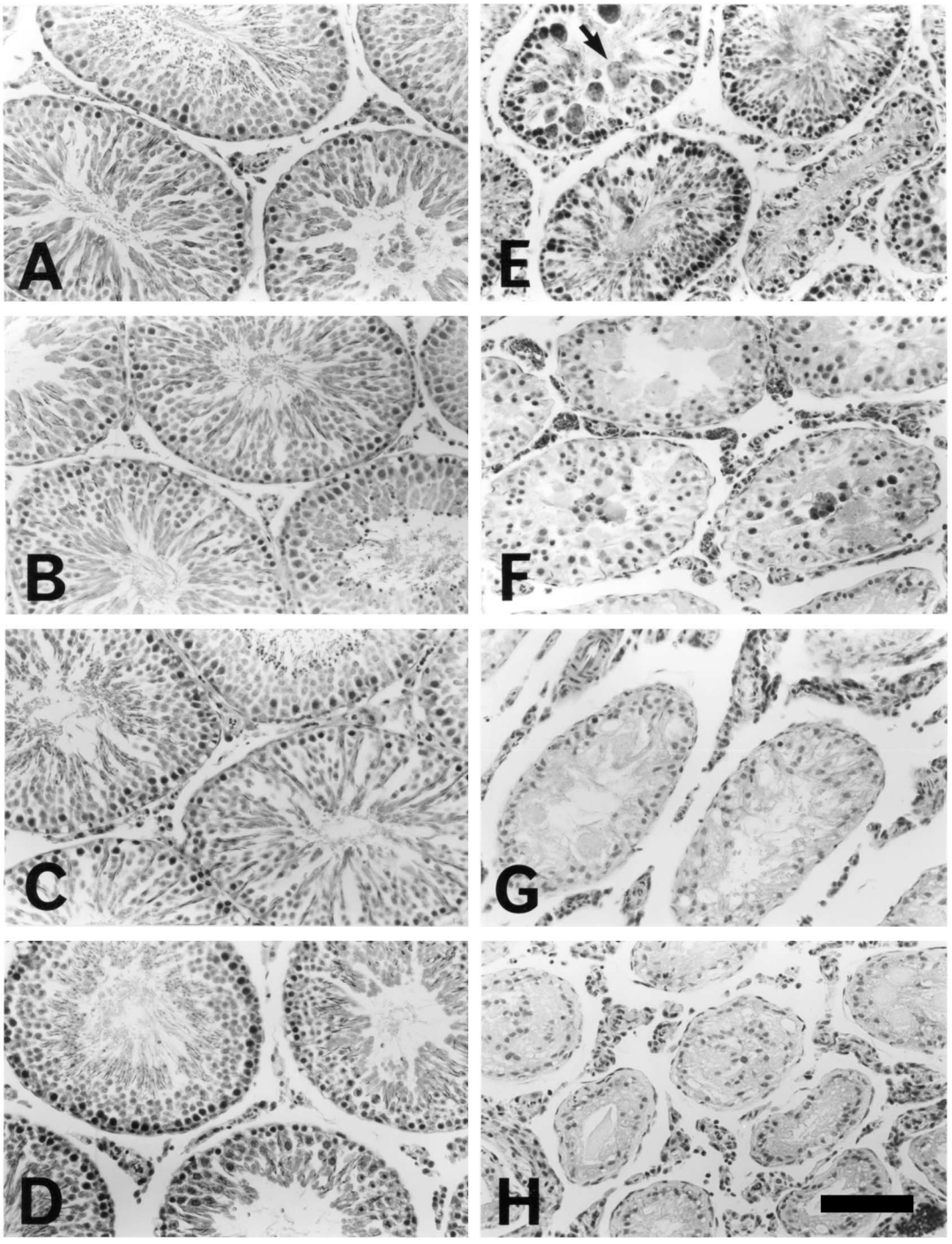
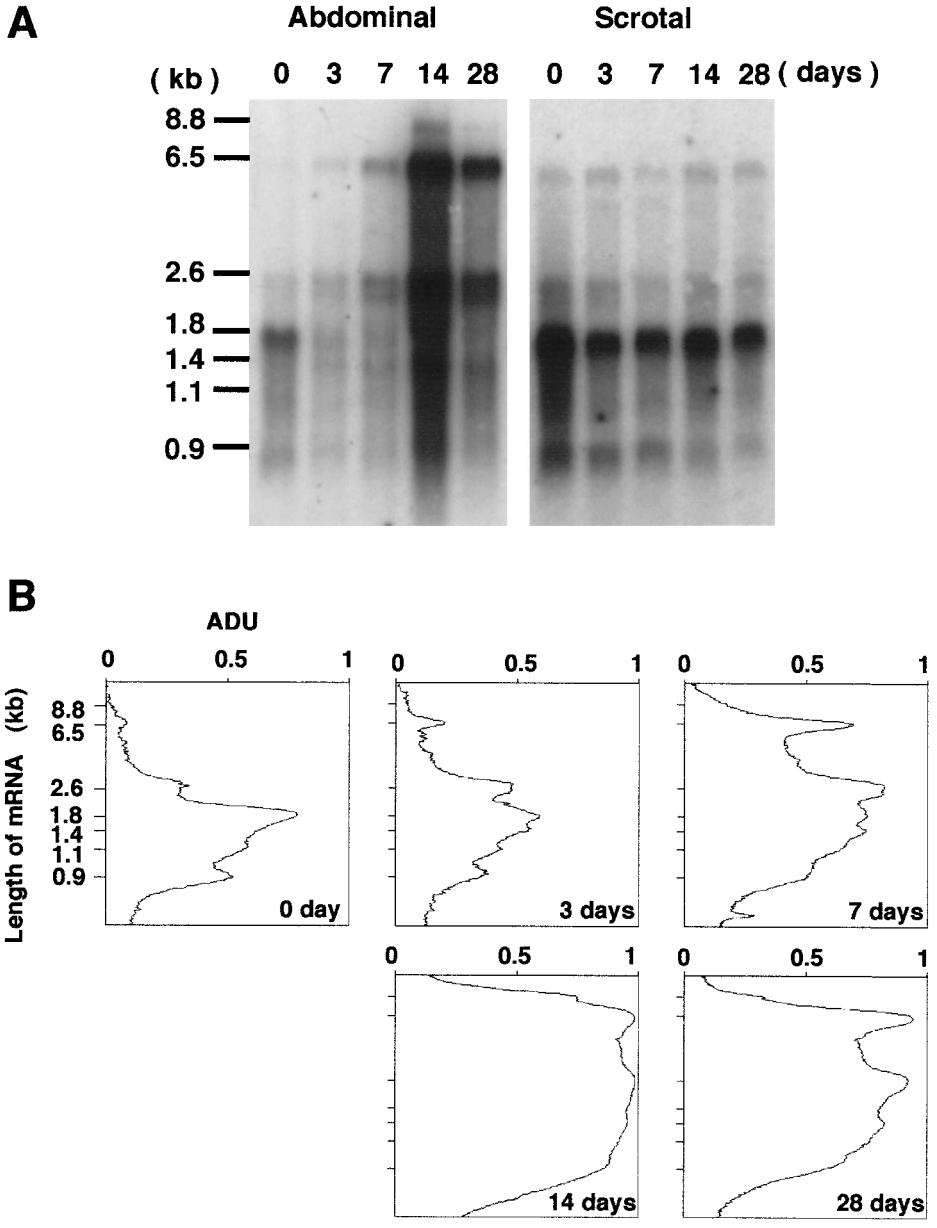


Fig. 5. Histological changes after the UCD operation. **A**–**D** show scrotal and **E**–**H** show contralateral abdominal testes of UCD rats 3 (**A**, **E**), 7 (**B**, **F**), 14 (**C**, **G**) and 28 (**D**, **H**) days after the operation. In abdominal testis at day 3 (**E**), multinucleate cells containing spermatids were observed (arrow). Staining of the multinucleate cells were faint at day 7 (**F**). Most seminiferous tubules contain only Sertoli cells and spermatogonia at day 14 (**G**) and 28 (**H**). Scrotal testes did not show any histological changes. Bar = 100 μm.

Tena-Sempere *et al*., 1994; Segaloff *et al*., 1990; Wang *et al*., 1991; Zhang *et al*., 1994). The 1.8 kb transcript is not thought to code for the membrane-bound receptor protein, since LaPolt *et al*. (1991) have revealed by Northern hybridization that the 1.8 kb transcript does not hybridize to cRNA probe corresponding to the transmembrane region and Koo *et al*. (1994) have detected transcripts of 1.8 kb probably polyadenylated in introns 3, 4, and 10 of LH receptor gene. In testis which did not contain mature Leydig cells after EDS treatment, expression of 1.8 kb transcript was maintained without any decrease while expression of the other transcripts was severely suppressed (Tena-Sempere *et al*., 1994; Veldhuizen-Tsoerkan *et al*., 1994). The expression of longer transcripts which are thought to code for the full length of the receptor protein was restored



C **Intensities of hybridization signals**

	0 day	3 days	7 days	14 days	28 days
6.5 kb	0.075	0.19	0.68	0.97	0.93
2.6 kb	0.29	0.44	0.78	0.95	0.89
1.8 kb	0.74	0.54	0.70	0.90	0.75

Fig. 6. Northern hybridization of poly $(A)^+$ RNA samples extracted from the testes of control rats (0 day) and abdominal or scrotal testes at different times following the induction of UCD. Samples were run on a 1% denaturing agarose gel, transferred to nylon membrane, and hybridized to the LH receptor cDNA probe. Sample preparation and hybridization were repeated three times, and similar results obtained each time. A representative result is shown in **A**. Six micrograms poly $(A)^+$ RNA was loaded per well. The exposure time was 10 days (abdominal testes) or 4 weeks (scrotal testes). Intensities of signals from abdominal testes are indicated in **B**. The intensities of major signals subtracted with background levels are indicated in table **C**.

from 20 days after the EDS treatment when mature Leydig cells were observed. Thus, it was concluded that the Leydig cell precursors express only the 1.8 kb truncated form of LH receptor mRNA, and the longer, functional receptor mRNA appears as Leydig cells mature. Recent report that translational efficiency of the 1.8 kb transcript is lower than that of 6.5 kb supports this concept (Tena-Sempere *et al*., 1997). Kerr and Donachie (1986) studied the effects of UCD operation after EDS treatment and found that the regeneration of a new population of Leydig cells occurred more rapidly in the abdominal testis than in the scrotal testis of the same animal. Considering these previous data, our results on the expression pattern of LH receptor mRNA transcripts can be explained as follows. In intact adult testis, there were a number of Leydig cell precursors as well as mature Leydig cells, the former expressing the 1.8 kb transcript for LH receptor abundantly. When germ cells were damaged, paracrine environment of the Leydig cell precursors changed, so that the precursors ceased expressing 1.8 kb transcript and started to differentiate to mature cells which could express the longer transcripts. Consequently, the amount of 6.5 and 2.6 kb transcripts increased relative to that of 1.8 kb transcript. To evaluate this hypothesis, it is necessary to determine the source of each species of LH receptor mRNA in the testis. In addition, quantitative analyses on the expression changes of LH receptor mRNA per testis are needed to consider the effect of these changes to testicular functions, as the present study focuses the changes per poly $(A)^+$ RNA weight.

In summary, the treatments which degenerate the seminiferous epithelium resulted in remarkable changes in the composition of LH receptor mRNA. These changes parallel the degeneration of germ cells, suggesting the significant role of a local communication mechanism between seminiferous tubule and interstitium, which controls the expression of LH receptor in Leydig cells.

ACKNOWLEDGMENTS

This research was supported by a Sasakawa Scientific Research Grant from the Japan Science Society and a JSPS Research Fellowship for Young Scientists awarded to A. Iizuka, and by Grants-in-Aid for Scientific Research (A) and (B) from the Ministry of Education, Science, Sports and Culture, Japan to T. Mori.

REFERENCES

Aatsinki JT, Pietilä ET, Lakkakorpi JT, Rajaniemi HJ (1992) Expression of the LH/CG receptor gene in rat ovarian tissue is regulated by an extensive alternative splicing of the primary transcript. Mol Cell Endocrinol 84: 127–135

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156–159

Clegg EJ (1963) Studies of artificial cryptorchidism: degenerative and regenerative changes in the germinal epithelium of the rat testis. J Endocrinol 27: 241–251

Iizuka A, Park MK, Mori T (1996) Effects of unilateral cryptorchidism on the exprssion of gonadotropin receptor mRNA. Biochem Biophys Res Commun 221: 290–294

Kerr JB, Donachie K (1986) Regeneration of Leydig cells in unilaterally cryptorchid rats: evidence for stimulation by local testicular factors. Cell Tissue Res 245: 649–655

Koo YB, Ji I, Ji TH (1994) Characterization of different sizes of rat luteinizing hormone/chorionic gonadotropin receptor messenger ribonucleic acids. Endocrinology 134: 19–26

LaPolt PS, Jia X, Sincich A, Hsueh AJW (1991) Ligand-induced down regulation of testicular and ovarian luteinizing hormone (LH) receptor is preceded by tissue-specific inhibition of alternatively processed LH receptor transcripts. Mol Endocrinol 5: 397–403

McFarland KC, Sprengel R, Phillips HS, Kohler M, Rosemblit N, Nikolics K, Segaloff DL, Seeburg PH (1989) Lutropin-choriogonadotropin receptor: an unusual member of the G protein-coupled receptor family. Science 245: 494–499

Saez JM (1994) Leydig cells: endocrine, paracrine, and autocrine regulation. Endocr Rev 15: 574–625

Segaloff DL, Wang H, Richards JS (1990) Hormonal regulation of luteinizing hormone-chorionic gonadotropin receptor mRNA in rat ovarian cells during follicular development and luteinization. Mol Endocrinol 4: 1856–1865

Skinner MK (1991) Cell-cell interactions in the testis. Endocr Rev 12: 45–77

Smith G (1962) The effects of ligation of the vasa efferentia and vasectomy on testicular function in the adult rat. J Endocrinol 23: 385–399

Tena-Sempere M, Zhang F, Huhtaniemi I (1994) Persistent expression of a truncated form of the luteinizing hormone receptor messenger ribonucleic acid in the rat testis after selective Leydig cell destruction by ethylene dimethane sulfonate. Endocrinology 135: 1018–1024

Tena-Sempere M, Rannikko A, Kero J, Zhang FP, Huhtaniemi IT (1997) Molecular mechanisms of reappearance of luteinizing hormone receptor expression and function inrat testis after selective Leydig cell destruction by ethylene dimethane sulfonate. Endocrinology 135: 1018–1024

Veldhuizen-Tsoerkan M, Ivell R, Teerds KJ (1994) hCG-induced changes in LH/CG receptor mRNA transcript levels in the testis of adult hypophysectomized, ethane dimethyl sulphonate-treated rats. Mol Cell Endocrinol 105: 37–44

Vihko KK, Nishimori K, LaPolt PS, Hsueh AJW (1992) Expression of testicular messenger ribonucleic acid for luteinizing hormone receptor in the rat: developmental regulation of multiple transcripts during postnalal life. Biol Reprod 46: 1016–1020

Wang H, Ascoli M, Segaloff DL (1991) Multiple luteinizing hormone/chorionic gonadotropin receptor messenger ribonucleic acid transcripts. Endocrinology 129: 133–138

Zhang F, Hämäläinen T, Kaipia A, Pakarinen P, Huhtaniemi I (1994) Ontogeny of luteinizing hormone receptor gene expression in the rat testis. Endocrinology 134: 2206–2213

(Received November 13, 1997 / Accepted December 25, 1997)