Effect of Uni- and Bilateral Cryptorchidism on Testicular Inhibin and Testosterone Secretion in Rats

REIKO DEMURA, TOMOHARU SUZUKI, SAEKO NAKAMURA, HIROMI KOMATSU, KAZUKO JIBIKI, EMI ODAGIRI, HIROSHI DEMURA AND KAZUO SHIZUME

Radioassay Center and Dept. of Internal Medicine
Tokyo Women’s Medical College
8-1 Kawadacho, Shinjuku-ku, Tokyo, Japan 162

Abstract

The effect of uni- and bilateral cryptorchidism on testicular inhibin and testosterone secretion and their relationships to gonadotropins were studied in rats. Mature Wistar male rats weighing approximately 300 g were made either uni- or bilaterally cryptorchid. Testicular inhibin and testosterone content and plasma levels of LH and FSH were examined 2 weeks later. A similar remarkable decrease in testicular inhibin content was found in uni- and bilaterally cryptorchid testes. On the other hand, the testicular testosterone content was significantly decreased only in unilaterally cryptorchid testis with an inverse increase in the contralateral testis. Plasma testosterone levels were normal and plasma LH and FSH increased significantly in both of the cryptorchid groups. These results showed that cryptorchidism impairs both Sertoli and Leydig cell functions. While testosterone production was compensated by increased LH for 2 weeks, neither inhibin secretion nor storage changed in cryptorchid or contralateral testes during the same period.

Altered Leydig cell function along with impaired Sertoli cell function in cryptorchidism has been reported by Gomes and Jain (1976), Jones et al. (1977), Kerr et al. (1979, a, b), de Kretser et al. (1979), Keel and Abney (1980), and Risbridger et al. (1981, a, b), mainly based on histological or endocrinological studies measuring testosterone or gonadotropins. Recently, Le Gac-Jegou and de Kretser (1980), Seethalakshmi and Steinberger (1983), and Au et al. (1983), reported decreased testicular inhibin content in bilaterally cryptorchid rats using bioassay. As cryptorchidism is a proper model to use in examining the reciprocal relationship between testicular functions and gonadotropins, we have examined testicular testosterone and inhibin secretion in relation to plasma gonadotropin levels in uni- and bilaterally cryptorchid rats.

Materials and Methods

Mature Wistar male rats weighing approximately 300 g were made cryptorchid either uni- or bilaterally by placing the testis in the peritoneal cavity through the inguinal canal, which was then ligated. Inguinal ligation was performed as a sham operation in the control animals. Care was taken to avoid damage to the sper-
matic duct. All the animals were decapitated 2 weeks later. The testes were removed to assess weight and inhibin and testosterone content and for histological studies. The testes were fixed in 20% formalin solution and 3-4 μm thick sections were stained with hematoxylin-eosin and examined by light microscopy.

Preparation of the testes for inhibin assay have been described earlier (Demura et al., 1987). Briefly, testes were individually homogenized in 2 vol. of Eagle’s Minimum Essential Medium (MEM) in a teflon homogenizer. The homogenates were centrifuged at 20,000 g for 30 min. and 1/4 vol. of 1% dextran and 10% charcoal solution was added to the supernatant. After keeping at 4°C overnight, the supernatant was diluted to contain 10^{-4} testis/50 μl of MEM. This was further diluted 4 times altogether in 3 fold dilutions.

Inhibin bioassay was performed by assessing FSH inhibitory activity using dispersed rat anterior pituitary cells according to Kimura et al. (1983) using a 4 dose parallel assay. The cells were plated to a culture plate with 12×8 dishes so that each dish contain 7-8×10^4 cells in 0.2 ml of MEM. The cells were incubated for 5 days at 37°C in 5% CO₂ and 95% air incubator. The medium was changed on the 3rd day and 50 μl of serially diluted samples were added to dishes and incubated further for 2 days. Each sample was plated quadruplicately to 4 dishes. Inhibin activity was expressed as the FSH level in the culture media or % of normal control.

FSH and LH were measured by radioimmunoassay using r-FSH and r-LH kits kindly supplied by NIADDK, Maryland, USA. As standards RpII were used for both r-LH and r-FSH. Testosterone was measured with a radioimmunoassay kit marketed by Eiken ICL, Tokyo, Japan. Coefficience of variation (C. V.) for in- and interassay for these methods were all less than 10%.

Statistical analyses were made using Student’s t-test.

Results

1) Testicular weight

The weights of cryptorchid testes were markedly decreased in both the uni- and bilaterally cryptorchid groups. The weight of the non-cryptorchid testis in the unilaterally cryptorchid group was normal (Fig. 1).

2) Histological studies

The seminiferous tubules of the cryptorchid testis obtained from the unilateral group were smaller than normal while their lumina were enlarged and spermatogonia, spermatocytes and spermatids were decreased in number as in bilaterally cryptorchid testes. The intertubular spaces were enlarged and the Leydig cells were slightly hypertrophied. No abnormalities were seen in the non-cryptorchid testis of the unilaterally cryptorchid group (Fig. 2).

3) Plasma and testicular testosterone

There was observed no significant difference between plasma testosterone levels in normal and uni- or bilaterally cryptorchid groups, though the mean value in the bilaterally cryptorchid group was lower (Fig. 3).

![Graph showing testicular weight comparison between normal and cryptorchid testes.](https://via.placeholder.com/150)

Fig. 1. Testicular weights in normal and cryptorchid rats (n=5 in each group).
3) The testicular testosterone content was significantly decreased only in the cryptorchid testis of the unilateral group with an inverse increase in the contralateral testis. The bilateral group had a lower testosterone content but it did not significantly differ from normal controls (Fig. 4).

4) Testicular inhibin content

The testicular inhibin content was markedly decreased in cryptorchid testes. The decrease in inhibin activity measured by FSH levels was almost identical in uni- and bilaterally cryptorchid groups (Fig. 5). The inhibin content using 1/9 diluted samples obtained from different groups is shown in Fig. 6 as % of normal control group.

5) Plasma LH and FSH levels

Plasma LH levels increased significantly in both uni- (p<0.05) and bilaterally cryptorchid rats (p<0.01) (Fig. 7). Plasma FSH levels increased significantly only in the bilaterally cryptorchid group (p<0.01) (Fig. 8).

Discussion

The present studies demonstrated that testicular inhibin content decreased in unilaterally cryptorchid testis to the same extent as in bilaterally cryptorchid testes. The values were 1/3 of those in normal and contralateral testis of unilaterally cryptorchid rats. As histological studies demonstrated similar degenerative changes in Sertoli cells in both uni- and bilaterally cryptorchid testes, it is suggested that these changes may cause decreased inhibin secretion or storage in cryptorchid testes. Previous studies, especially those by Au et al. (1983), demonstrated decreased inhibin content in bilaterally cryptorchid testes with increased plasma levels of LH and FSH by 6 weeks and supported the hypothesis of a feedback regulation of
Fig. 3. Plasma testosterone levels in normal and cryptorchid rats (n=5 in each group).

Fig. 4. Testicular testosterone content in normal and cryptorchid rats (n=5 in each group).

Fig. 5. Testicular inhibin activity as assayed by FSH levels in culture media of serially diluted testicular extracts obtained from control and cryptorchid rats (each point represents the mean of 5 samples x 4 wells).
FSH by inhibin. In the present study, plasma FSH levels also increased in cryptorchid rats, though the difference between normal and unilaterally cryptorchid rats was not significant. These findings were compatible with the idea that inhibin was involved in the feedback regulation of FSH, supposing that the decrease in the plasma inhibin concentration was greater in the bilateral cryptorchid group than in the unilateral group. In spite of a negative feedback regulation of FSH by inhibin, there seemed to be no reciprocal effects of increased FSH on inhibin secretion in the cryptorchid state. There was observed no difference between inhibin content in the cryptorchid and contralateral testis in the unilateral group and also in the unilaterally cryptorchid testes.

On the other hand, a reciprocal relationship between LH and testosterone secretions was observed in the present study. De-
creased testicular testosterone content in cryptorchid testis with an inverse increase in contralateral testis in unilateral cryptorchidism or normal plasma testosterone levels with increased plasma LH levels in both uni- and bilaterally cryptorchid rats suggested a reciprocal feedback regulation between LH and testosterone. Thus Leydig cell failure seemed to be compensated in cryptorchidism by 2 weeks after surgery.

Franchimont et al. (1980) reported that inhibin secretion by Sertoli cells increased following the administration of gonadotropins, especially FSH. However our previous studies (Demura et al., 1987) failed to demonstrate any increase in testicular inhibin content following the administration of 10 IU of HMG to normal rats for 5 days.

To investigate the effect of FSH on inhibin secretion under cryptorchid states, sequential changes in plasma levels of FSH and inhibin need to be studied. At the same time, the effect of testosterone on FSH and/or inhibin secretion must be studied.

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