Effect of testicular damage induced by cryptorchidism on insulin-like growth factor I receptors in rat Sertoli cells

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The distribution and density of functional insulin-like growth factor I (IGF-I) receptors in cryptorchid and scrotal rat testes and epididymides during gonadal development were studied. Cryptorchidism was induced by unilateral gubernaculectomy in 4-day-old animals, and organs were studied at 15, 30, 60 and 90 days of age. Tissue membranes were assayed for ^{125}I -labelled IGF-I binding. Characterization and specificity of binding sites showed that both normal and contralateral undescended testes and epididymides exhibited typical type 1 IGF receptors. In normal testes, IGF-I receptor density was 20.6 nmol g^{-1} wet mass at day 15, and decreased to 12.8 nmol g^{-1} wet mass at adult age (day 90). Cryptorchid testes showed IGF-I receptor concentrations similar to normal testes at day 15 and day 30, but in postpubertal stages displayed a divergent pattern, with a continuous increase at day 60 and day 90, reaching a higher density than those found for immature ages (62 nmol g^{-1} wet mass). Both normal and cryptorchid epididymides had a similar concentration and a comparable decrease in IGF-I receptors throughout development. In studies with immunohistochemical techniques ($\alpha\text{IR-3}$ antibody), IGF-I receptors were found in primary spermatocytes, Sertoli cells and Leydig cells. Cryptorchid tubules showed a lack of germinal epithelium and a marked increase of immunoreactive IGF-I receptors in Sertoli cells, compared with normal tubules from scrotal testes. Intense immunoreactivity for IGF-I receptors was present in the principal cells of epididymal tubules in both normal and cryptorchid organs. These results suggest that tubular damage induced by cryptorchidism in rats is associated with changes in the local regulation of IGF-I receptors.

Introduction

The complex process of germ cell differentiation in males involves, beyond the action of gonadotrophins and androgens, the concurrence of a number of growth and regulatory substances. These substances establish a network of paracrine interactions within the testis, between the interstitial compartment, the Sertoli cells and the germinal cells, in a unique cytoarchitectural microenvironment (Bardin *et al.*, 1990; Skinner, 1991).

Insulin-like growth factor I (IGF-I) is a firm candidate for participation in the local regulation of spermatogenesis in mammals. A complete intratesticular system of IGFs, composed of peptides, receptors and binding proteins, exists in the testis (Ritzen, 1983; Skinner, 1991). Immunoreactive IGF-I has been identified in testicular extracts of rats (D'Ercole *et al.*, 1984; Handelsman *et al.*, 1985). Sertoli cells and Leydig cells from different species can secrete IGF-I *in vitro* (Tres *et al.*, 1986; Smith *et al.*, 1987; Vannelli *et al.*, 1988; Forti *et al.*, 1989; Hansson *et al.*, 1989), and this secretion is further stimulated by gonadotrophins (Closset *et al.*, 1989; Cailleau *et al.*, 1990;

Naville *et al.*, 1990; Spiteri-Grech *et al.*, 1991) and growth hormone (Closset *et al.*, 1989; Spiteri-Grech *et al.*, 1991). Localization of immunoreactive IGF-I indicates that the peptide is present in Sertoli cells, primary pachytene spermatocytes, and some Leydig cells (Tres *et al.*, 1986; Vannelli *et al.*, 1988; Forti *et al.*, 1989), although *in situ* hybridization experiments in humans suggest that mRNA encoding IGF-I may not be produced in seminiferous tubules (Zhou and Bondy, 1993a). Specific receptors for IGF-I are expressed in Sertoli cells (Borland *et al.*, 1984; Oonk and Grootegeod, 1988), and in Leydig cells from rats (Handelsman *et al.*, 1985; Lin *et al.*, 1986a) and humans (Vannelli *et al.*, 1988; Forti *et al.*, 1989). Germinal cells may also be a source of immunoreactive IGF-I receptor and mRNA encoding the IGF-I receptor (Zhou and Bondy, 1993a).

A number of regulatory mechanisms of IGF-I action in testes have been described. The concentration of IGF-I and its receptor is high in immature rat testes and decreases with gonadal development and sexual maturation (Oonk and Grootegeod, 1988; Hansson *et al.*, 1989). Some IGF-binding proteins (IGFBP), which modulate the transport and bioavailability of IGF-I, are under the inhibitory influence of FSH in Sertoli cells (Cailleau *et al.*, 1990; Smith *et al.*, 1990; Smith *et al.*, 1992), whereas LH stimulates IGFBP secretion in Leydig cells

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in culture (Cailleau *et al.*, 1990). LH and hCG increase the number of IGF-I receptors in Leydig cells (Lin *et al.*, 1987, 1988; Perrard-Sapori *et al.*, 1987; Nagpal *et al.*, 1991). Studies *in vivo* show an increase in testicular IGF-I in rats with induced tubular damage and spermatogenic arrest (Bartlett *et al.*, 1990; Spiteri-Grech *et al.*, 1991). It is thus possible that the IGF-I system could participate in local adaptive responses to certain pathophysiological conditions of the testis; however, there are no available studies on IGF-I receptor expression in such circumstances.

Cryptorchidism is a well identified clinical condition which causes male infertility. It constitutes a model of primary testicular damage that can be experimentally reproduced *in vivo* without altering the hormonal milieu (Rajalakshmi and Prasad, 1974; Bergh, 1983; Bergh *et al.*, 1987). In this study, the changes in density and the histological distribution of IGF-I receptors throughout development in testis and epididymis from rats with induced unilateral cryptorchidism were investigated.

Materials and Methods

Experimental cryptorchidism

Cryptorchidism was unilaterally induced in male Wistar rats by microsurgical distal gubernaculectomy at day 4 after birth under anaesthesia with hypothermia plus ethyl ether. Spermatogenesis is initiated in Wistar rats soon after day 15 of age, when testes are still abdominal. Puberty and testicular descent is completed at about day 35, and after 3 months of age animals are fully developed (Bergh and Helander, 1978; Bergh *et al.*, 1978). Gubernaculectomy prevents testicular descent and scrotal development (Bergh *et al.*, 1978). Animals were killed by decapitation at 15, 30, 60 and 90 days of age. Testes were removed, measured and weighed, and then frozen in isopentyl alcohol and kept at -70°C until processing.

Tissue preparation

Testes and epididymides (30 abdominal and 42 contralateral scrotal) were minced with a glass homogenizer in 1 mmol NaHCO_3 l^{-1} with protease inhibitors (0.5 mg Bacitracin ml^{-1} , 1 μg phenylmethyl sulfonyl fluoride (PMSF) ml^{-1} , 1 μg Aprotinin ml^{-1}). A first centrifugation was performed at 600 g for 10 min to eliminate gross tissue remnants and nuclei. The supernatant was centrifuged again at 12 000 g for 30 min and washed. The pellet was resuspended in Hepes buffer (100 mmol Hepes l^{-1} , 120 mmol NaCl l^{-1} , 1.2 mmol $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ l^{-1} , 2.5 mmol KCl l^{-1} , 15 mmol sodium acetate l^{-1} , 10 mmol glucose l^{-1} , 1 mmol EDTA l^{-1}) pH 7.6. Protein concentration was determined by the Bradford method (Bio-Rad Laboratories, Madrid) and adjusted between 0.5 and 1.5 mg ml^{-1} . The average yield of membrane protein was 8 mg g^{-1} wet mass of tissue (range 6.2–9.6), and was similar for control and cryptorchid organs throughout development. Membranes were stored at -70°C until binding experiments were performed.

Binding studies

Membrane suspensions (25–75 μg protein per tube) were incubated with constant amounts of ^{125}I -labelled human recom-

binant IGF-I (10 000 c.p.m. per tube, 170 pg ml^{-1} , Amersham, Madrid), for 16 h at 4°C in a total volume of 150 μl in Hepes buffer plus 1% (w/v) BSA, and 0.5 mg Bacitracin, pH 7.8. Nonspecific binding was determined by incubation with 250 ng unlabelled IGF-I ml^{-1} (Bachem, H-5555, Bubendorf). The percentage of specific binding was corrected according to the protein concentration of each sample. The relationship between ^{125}I -labelled IGF-I specific binding and membrane protein concentration within the range 0.25–2 mg ml^{-1} was linear ($r = 0.998$). This allowed normalization of specific binding to 1 mg ml^{-1} (50 μg per tube) membrane protein in each case. After incubation, membranes were centrifuged at 12 000 g for 10 min at 4°C . The pellet was washed with 250 μl cold PBS and centrifuged again at 12 000 g for 3 min. After a final aspiration of the supernatant and drying, the tips of the tubes were cut and placed in counting vials, and the radioactivity associated with membranes was determined. The initial characterization showed maximum binding at pH 7.8–8.0, which decreased at lower pH (data not shown). Degradation was determined using trichloroacetic acid (final concentration, 5% w/v) to precipitate intact peptide. Total degradation of ^{125}I -labelled IGF-I during incubation conditions (16 h at 4°C) was 12%. Specific degradation induced by membranes in the binding assay averaged 3.5%, and was not influenced by the protein concentration of tissue preparations. Control binding studies were carried out using membranes previously incubated and washed (24 h at 4°C) with Hepes assay buffer. This method eliminates most endogenous IGF-I and binding proteins present in the membrane preparation (Grizzard *et al.*, 1984). Aliquots of the same batch in the same experiment were used to compare the percentage of labelled IGF-I as well as the displacement of saturating concentrations of IGF-I and insulin. No differences were observed between washed and unwashed membrane preparations (data not shown). This finding suggests that IGF-I was mainly bound to a true type 1 specific receptor, and that endogenous contaminants were unlikely to be present in the preparations used for analysis.

Experimental variation was minimized by measuring IGF-I binding to scrotal and cryptorchid testes throughout development in a single assay using the same batch of ^{125}I -labelled IGF-I. Simultaneous study of membranes from scrotal and cryptorchid organs in the same assay allowed appropriate comparison of the binding characteristics of different tissues. Specificity studies and subsequent Scatchard analyses were performed by incubating labelled IGF-I with different concentrations of unlabelled IGF-I, insulin or a monoclonal anti-IGF-I receptor antibody ($\alpha\text{IR-3}$ clone, Oncogene Science Inc., Uniondale, NY). This antibody specifically inhibits IGF-I binding to its own receptor, and does not compete for insulin binding (Jacobs *et al.*, 1986).

Immunohistochemistry

Testes and epididymides from normal ($n = 4$) and cryptorchid ($n = 5$) animals were used for immunohistochemical localization of IGF-I receptors. Frozen testicular and epididymal tissue fragments were fixed in Bouin's solution and embedded in paraffin wax. Sections, 5 μm thick, were stained using the immunoperoxidase technique. Paraffin wax was removed from

Table 1. Testicular mass of control ($n = 42$) and cryptorchid ($n = 30$) rat testes during development

Treatment		Age (days)			
		15	30	60	90
Control	n	8	14	9	11
	Mass (g)	0.19 ± 0.03	1.09 ± 0.18	2.47 ± 0.31	3.16 ± 0.55
Cryptorchid	n	9	9	6	6
	Mass (g)	0.11 ± 0.06	0.70 ± 0.22	1.06 ± 0.34	1.29 ± 0.55

Values are means $\pm$ SEM; n = number of testes.

the sections and they were preincubated with normal horse serum and incubated with anti-human IGF-I receptor monoclonal antibody α IR-3 ($5 \mu\text{g ml}^{-1}$), in PBS, 2% BSA (w/v) for 18 h at room temperature as described by Vanelli *et al.* (1988) and Forti *et al.* (1989). Thereafter, the sections were incubated with biotinylated horse antimouse IgG (H + L) ($< 1\%$ cross-reactivity with rat IgG), produced in horse (Vector, Burlingame, CA), at 1:100 titre for 45 min. Antigen-antibody complexes were identified using the avidin-biotin-peroxidase complex, with a Vectastatin ABC kit (Vector). Peroxidase activity was demonstrated with a solution of 0.07% (w/v) 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma Chemical Co., St Louis, MO) in PBS containing 0.08% (v/v) hydrogen peroxide. Sections were counterstained with haematoxylin. The specificity of immunohistochemical reactions was verified by using the following procedures: (i) incubation of sections with non-immune mouse serum instead of primary antibody; (ii) omission of primary or secondary antibodies; and (iii) incubation with different dilutions of monoclonal antibody (5, 3 and $0.5 \mu\text{g ml}^{-1}$). Sections from control and treated organs were always processed in the same experiment.

Statistical analyses

Analysis of differences between control and treated organs used the Kruskal Wallis test. Comparisons within age-matched groups were performed with the Mann-Whitney U test.

Results

Effects of cryptorchidism on testis and epididymis

Cryptorchid testes showed a progressive delay in testicular growth compared with control organs (Table 1). The reduction in mass was 42% at day 15, and reached 59% at day 90. Undescended epididymides had only slight, nonsignificant changes in mass.

Examination of adult cryptorchid testes under the microscope showed a marked impairment of spermatogenesis. Virtually all the tubules showed a decrease in diameter, and an absence of germinal epithelium. Interstitial spaces remained unchanged under examination by light microscopy examination. Epididymides showed histological changes consisting of a decrease in tubular diameter, an increase in the height of principal cells and vacuolation.

Fig. 1. Specificity of binding of insulin-like growth factor I (IGF-I) to membrane receptors in (a) scrotal testis and (b) epididymis from 30- and 90-day-old rats. Triplicate samples of membranes were incubated with ^{125}I -labelled IGF-I, in the presence or absence of indicated amounts of unlabelled IGF-I (●), insulin (○) or anti-IGF-I receptor antibody (α IR-3) (△). Each point represents the mean of two experiments.

Binding studies in membrane suspensions

Binding studies of IGF-I were performed in membranes prepared from control and cryptorchid rat testes and epididymides at 15, 30, 60 and 90 days of age. Labelled IGF-I appeared to bind to a type 1 IGF receptor in testes and epididymides of rats. Traditional competition studies, using

Table 2. Analysis of developmental changes in insulin-like growth factor I (IGF-I) receptors in scrotal or cryptorchid rat testes

Treatment	IGF-I receptor	Age (days)			
		15	30	60	90
Scrotal	R_o (pmol mg^{-1} protein)	2385	1819	939	1606
	R_o (nmol g^{-1} mass)	20.63	16.8	7.48	12.79
Cryptorchid	R_o (pmol mg^{-1} protein)	2506	1934	1488	9354
	R_o (nmol g^{-1} mass)	24.13	14.4	9.28	61.97

Data correspond to an experiment using membranes from two to five organs. Linear correlation of Scatchard plots was always higher than 0.93. R_o , total binding capacity.

membranes from adult animals (90 days), showed that the concentration of unlabelled IGF-I competing for 50% of the specific binding (IC_{50}) was 10 ng ml^{-1} in both tissues. Insulin was 1000 times less potent in competition for labelled IGF-I (Fig. 1). Scatchard analysis was applied to calculate the dissociation constant (K_d) and the total binding capacity (R_o) in different tissues and ages (Table 2). The effect of coinubating labelled IGF-I and increasing concentrations of the monoclonal antibody, $\alpha\text{IR-3}$, which specifically binds to the IGF-I receptor (Kull *et al.*, 1983) is shown (Fig. 1a). At high concentrations ($15 \mu\text{g ml}^{-1}$), $\alpha\text{IR-3}$ could completely abolish specific binding of IGF-I to testicular membranes. These data indirectly confirm that in normal testis, IGF-I was mainly bound to specific IGF-I receptors and not to IGF-binding proteins or to other structurally related receptors (for example insulin and IGF-II).

The specific binding was studied in membranes from 72 testes (42 normal and 30 cryptorchid). There was a decrease in IGF-I specific binding in normal testes throughout postnatal development ($P < 0.001$, Fig. 2a). Scatchard analysis (Table 2) confirmed that the decrease in binding corresponded to a reduction in receptor binding capacity from 20.6 nmol (day 15) to 12.8 nmol (day 90) g^{-1} wet mass tissue, while the K_d value remained relatively constant ($1.0\text{--}1.7 \text{ nmol l}^{-1}$).

Cryptorchid testes showed an initial decrease in specific binding, similar to that observed in normal testes during pubertal development (day 15 and day 30). However, at day 60 and day 90, the specific binding of cryptorchid testes increased and was significantly higher than that of normal testes (Fig. 2a) owing to a rise in the number of receptors of IGF-I (Table 2). Cryptorchid testes at 90 days had 62 nmol receptor g^{-1} wet mass, which is five times higher than the density of scrotal receptors. Total IGF-I receptor capacity per testis (90 days) was 34.5 nmol in normal organs versus 74.4 nmol in undescended testes. The slopes of the Scatchard plots obtained from scrotal and cryptorchid testes at day 90 were similar (1.5 and 1.6 nmol l^{-1} , respectively) (Fig. 3). This finding confirmed that the rise in specific binding was produced by an increase in the number of receptors and not by a change in their affinity.

Binding studies were performed using membrane suspensions from the epididymis. Control epididymides showed a decrease in IGF-I-specific binding during growth (Fig. 2b) from $2130 \text{ pmol mg}^{-1}$ membrane protein at day 15 to $1264 \text{ pmol mg}^{-1}$ protein at day 90. In general, specific binding and receptor content were similar in both normal and cryptorchid epididymides throughout development. The affinity of the

binding sites was 1.2 nmol l^{-1} , and showed no changes between control and undescended epididymides.

Immunohistochemical localization of IGF-I receptors in adult (day 90) testis and epididymis

High levels of immunoreactivity for IGF-I receptors were detected in the seminiferous tubule. Within the tubules, the strongest signal was found in the cytoplasm of large round cells, which were interpreted as being primary spermatocytes, whereas lower positivity was detected in spermatogonia, early spermatids and in the cytoplasm of Sertoli cells (Fig. 4e). Late spermatids and mature spermatozoa were clearly negative for immunoreactive IGF-I receptor. In the interstitium, most of the Leydig cells showed strong positivity in their cytoplasm (Fig. 4g). Peritubular cells, as well as endothelial cells of interstitial vessels, were negative.

In cryptorchid testes, a clear increase in immunoreactive IGF-I receptors was evident in Sertoli cells compared with contralateral scrotal testes with normal spermatogenesis (Fig. 4a, c). No changes were seen in the interstitial space, either in the distribution or intensity of immunoreactive IGF-I receptors between control and cryptorchid testes.

In epididymides, immunoreactive IGF-I receptors were identified in the membranes and in the cytoplasm of principal cells of the epididymal tubules (Fig. 5). The apical brush border, which contains tall microvilli, also showed IGF-I receptors. Other cell types, such as basal and clear cells, did not express IGF-I receptors. The pattern of IGF-I receptor distribution and the intensity of immunostaining were similar in cryptorchid and scrotal epididymides.

Discussion

Developmental regulation of IGF-I receptor expression has been described in different tissues and species (Bassas *et al.*, 1988; Zhou and Bondy, 1993b). The results of the study reported here confirm and extend those of Oonk and Grootegeod (1988), which showed that IGF-I receptors are abundant both in the testes and epididymides of rats, and that the density of IGF-I receptors decreases significantly throughout normal postnatal development of those organs. Characterization of IGF-I binding, by competition with insulin or with a monoclonal anti-IGF-I receptor antibody, indicates that the

Fig. 3. Scatchard plot of ^{125}I -labelled insulin-like growth factor I (IGF-I) binding to testicular membranes from (●) normal and (○) cryptorchid adult rat testes. Specific binding was expressed as the ratio bound:free hormone (B:F) and plotted as function of bound hormone. The means of two separate determinations with triplicate incubations are shown. The slope (k_a) was similar in scrotal and undescended testes. The correlation coefficients of the linear plot were 0.93 and 0.98 for normal and cryptorchid testes, respectively.

binding sites correspond to typical IGF-I receptors, displaying features similar to those reported by Handelsman *et al.* (1985) and Oonk and Grootegoed (1988). The changes in IGF-I binding are due to changes in receptor concentration rather than to modifications of receptor affinity. Immunoreactive IGF-I receptors are distributed in the Leydig cells and in the tubular compartment of the testes. Sertoli cells, as well as primary spermatocytes, contain IGF-I receptors.

Cryptorchidism produces a marked and distinct effect on the developmental pattern of testicular IGF-I receptor density. Before sexual maturation is completed (day 15 and day 30 of age) there are no differences in the number of IGF-I receptors between cryptorchid and control organs. However, in the postpubertal period, the IGF-I receptor concentration in undescended testes is five times higher than normal. The almost complete loss of germ cells seen in cryptorchid testes could theoretically account for an apparent increase in IGF-I receptor density, as mature germ cells express few receptors while contributing significantly to the membrane contents of the preparations tested. Nevertheless, this methodological objection does not invalidate the conclusion because: (1) the total IGF-I receptor capacity per testis showed a two-fold increase in cryptorchid

Fig. 2. Changes in binding of insulin-like growth factor I (IGF-I) to (a) control (■) and cryptorchid (□) testes and (b) control (□) and cryptorchid (■) epididymides during development of rats. Values represent means $\pm$ SEM of ^{125}I -labelled IGF-I specific binding of membranes (1 mg ml^{-1} protein) from organs (n) tested in triplicate in the same experiment. Differences between cryptorchid and control testes were significant at 60 days (* $P < 0.01$) and 90 days (** $P < 0.05$).

Fig. 4. Immunohistochemistry of insulin-like growth factor I (IGF-I) receptors in normal and cryptorchid adult (day 90) rat testes. Sections from (a) scrotal and (c) cryptorchid testes were incubated with anti-IGF-I receptor antibody α IR-3 ($5 \mu\text{g ml}^{-1}$). Tubules from cryptorchid testes (c) show smaller diameter and absence of germ cells, and intense immunoreactivity for IGF-I receptors in the cytoplasm of Sertoli cells. A more detailed view of normal seminiferous tubule (e) shows positive staining in Sertoli cells and in primary spermatocytes (arrows). The interstitial space (g) shows Leydig cells with clear positivity for IGF-I receptor (arrows) and negative capillary cells (*). Negative controls (b, d, f and h) were adjacent sections processed in the same way, but primary antibody was substituted by a similar concentration of non-immune mouse serum. All sections were counterstained with haematoxylin. Scale bars represent $25 \mu\text{m}$.

Fig. 5. Localization of insulin-like growth factor I (IGF-I) receptor in normal and cryptorchid epididymal tubules. Sections from (a) scrotal caput epididymis and (c) cryptorchid corpus epididymis show strong immunoreactivity in the membranes and the cytoplasm of principal cells of epididymal tubules. The apical brush border showed intense IGF-I receptor expression (e, f and g). Other cell types showed no IGF-I receptors (arrow, g). IGF-I receptor intensity was unchanged between epididymides corresponding to scrotal and cryptorchid rats. Adjacent control sections (b and d) were incubated with non-immune mouse serum instead of primary antibody. All sections were counterstained with haematoxylin, except for (f) in which only 3,3'-diaminobenzidine tetrahydrochloride was used. Scale bars represents 25 μ m.

versus scrotal testes at adult age; (2) IGF-I receptor concentration in cryptorchid adult testes (90 days) was three times higher than in prepubertal organs (15 days), even though differentiated germ cells were absent in both cases; and (3) immunolocalization of IGF-I receptors, using sections of similar thickness, stained in the same experiment, showed an increased expression of the receptor in Sertoli cells but not in other cell types of cryptorchid testis.

Some additional caveats can challenge this simple interpretation. For this reason, significant contamination of tissue preparations by IGFBP was excluded. Moreover, the relative yield in the preparation of membranes was similar both in normal and cryptorchid organs throughout development.

Undescended testes suffer a progressive impairment of spermatogenesis during gonadal development. The first

morphological changes appear in immature Sertoli cells, and are soon followed by degeneration of spermatocytes (Bergh, 1983). Leydig cells are unaffected initially but will eventually decrease in size, and show impairment of steroidogenic efficiency in adult life (Bergh and Damber, 1978; Bergh *et al.*, 1985; Kerr *et al.*, 1988). The higher temperature present in abdominal testes triggers a sequence of functional and structural events leading to irreversible tubular damage and a rise in gonadotrophin concentrations (Kormano, 1967; Bergh, 1983; Kerr *et al.*, 1988). Altered Sertoli cell function produces a marked decrease in FSH receptors in abdominal testes at 30 days of age (Bergh *et al.*, 1985, 1987). LH receptors are also reduced at this age. Leydig cells are not sensitive to the abdominal temperature, but abnormal paracrine influences from the altered seminiferous tubules could reduce LH receptors and steroidogenesis (Risbridger *et al.*, 1981; Bergh *et al.*, 1987). The data shown here provide the first evidence that primary testicular damage *in vivo*, in the absence of other endocrine alterations, is associated with an increase in the local expression of IGF-I receptors. It is possible that cell-to-cell contacts between Sertoli cells and stage-specific differentiating germ cells could modulate the expression of IGF-I receptors in Sertoli cells (Galdieri *et al.*, 1984). Alternatively, an increase in gonadotrophin concentration could stimulate the synthesis of IGF-I receptors. However, two considerations militate against this hypothesis: (1) the changes in IGF-I receptors affect only undescended testes, whereas scrotal testes from the same animals are unaffected; and (2) high LH and FSH concentrations (Kerr *et al.*, 1988) may have little effect on Sertoli cells when the rise in IGF-I receptors is detected, since LH and FSH receptors are markedly reduced at that time (Bergh *et al.*, 1987).

Specific IGF-I receptors are present in the membranes of rat epididymides throughout development. As for testes, specific binding is higher in immature animals and declines progressively until adulthood. Immunohistochemical localization of IGF-I receptors shows that they are limited to the principal cells of the epididymal epithelium. Typical histological changes were found at 90 days of age in cryptorchid epididymides, in agreement with the study of Rajalakshmi and Prasad (1974). Nevertheless (in contrast to testes), epididymides from cryptorchid rats did not show changes in the number or in the distribution of IGF-I receptors, compared with scrotal organs. IGF-I receptors have not been described previously in the epididymis, although IGF-I is present in rat epididymides (Hansson *et al.*, 1988; Leheup and Grignon, 1993). The peptide is located in the apical cytoplasmic border of the epithelial cells from the epididymis in immature rats. These findings suggest that IGF-I produced in the epididymis or present in the epididymal fluid binds to specific cellular receptors of the epididymal epithelium. The physiological function of IGF-I in the epididymis is unknown, but it may have a role in supporting division and differentiation (Leheup *et al.*, 1989).

IGF-I has been found in peritubular cells, pachytene spermatocytes and Sertoli cells in rats (Tres *et al.*, 1986). A possible physiological role of the IGF-I system in the testis *in vivo* is suggested, based on many biological effects studied in experiments *in vitro*. IGF-I potentiates the actions of gonadotrophins on Leydig cells by increasing its sensitivity to LH (Bernier *et al.*, 1986; Perrard-Sapori *et al.*, 1987; Chatelain *et al.*, 1991) and the production of testosterone (Bernier *et al.*, 1986; Lin *et al.*,

1986a, b; Kasson and Hsueh, 1987; Perrard-Sapori *et al.*, 1987; Chatelain *et al.*, 1991; Gelber *et al.*, 1992). IGF-I increases the transport of glucose (Mita *et al.*, 1985; Oonk *et al.*, 1989) and the production of lactate (Oonk *et al.*, 1989) in cultured rat Sertoli cells. Moreover, IGF-I stimulates the synthesis of DNA and the proliferation of pig Sertoli cells *in vitro* (Jaillard *et al.*, 1987). IGF-I increases the production of premitotic DNA in spermatogonia, and maintains the synthesis of premeiotic DNA in germ cells from rats (Söder *et al.*, 1992). The presence of IGF-I receptors in primary spermatocytes suggests that IGF-I could act through autocrine and paracrine mechanisms as a regulator of spermatogenesis under normal physiological conditions (Spiteri-Grech *et al.*, 1993).

One of the possible mechanisms by which testicular effects of IGF-I can be regulated is the modification of the density of its receptors. This may result in amplification of the biological responses to local concentrations of IGF-I. The results of this study show that damage to seminiferous tubules induced by cryptorchidism is followed by a specific increase in IGF-I receptors in Sertoli cells *in vivo*. Other clinical conditions producing testicular injury and germ cell loss could share a similar compensatory response (Antich *et al.*, 1993). Further studies will be necessary to elucidate whether this upregulatory mechanism also includes changes in the testicular production of IGF-I, or in the synthesis of some of its binding proteins.

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