Relationship between testicular inhibin content and serum FSH concentrations in rats after bilateral efferent duct ligation*

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Summary. The testicular inhibin content showed an initial increase in the first 2–3 days after bilateral ligation of the efferent ducts of rats, followed by a subsequent decline to levels significantly below normal by 14 days, and reached 25% of control values at 42 days. Serum concentrations of FSH and LH were significantly increased at Day 6–7 after treatment and were still elevated after 42 days. The decline in testicular inhibin content at times associated with elevated FSH concentrations is consistent with the hypothesis of inhibin being involved in the feedback control of FSH secretion.

Introduction

Ligation of the efferent ducts of the mammalian testis results in an initial accumulation of fluid and secretory products in the seminiferous tubules, and a subsequent decline in testicular weight and atrophy of the seminiferous epithelium (Smith, 1962; Setchell, 1970; Anton, 1979). The latter changes are thought to be the result of pressure damage caused by fluid accumulation in the seminiferous tubule. Before the seminiferous epithelium completely degenerates, serum FSH concentrations are already significantly elevated despite normal serum testosterone concentrations and no changes in the weight of accessory sex glands (Davies, Main & Setchell, 1978; Main, Davies & Setchell, 1978; Collins, Collins, McNeilly & Tsang, 1978; Morris, 1979). These findings suggest a reduced feedback inhibition of pituitary FSH secretion by testicular inhibin (Setchell, Davies & Main, 1977; Main *et al.*, 1978; Collins *et al.*, 1978; Morris, 1979; Le Lannou, Chambon & Le Calve, 1979). At present, the assay systems for inhibin are too insensitive to measure levels in male rat plasma (Au, Robertson & de Kretser, 1984), but the presence of inhibin in both testicular interstitial fluid and rete testis fluid suggests that, under normal conditions, inhibin release from the Sertoli cells is bidirectional (Setchell & Sirinathsinghji, 1972; Au *et al.*, 1984). It has been proposed that, after efferent duct ligation, either the route of inhibin passage to the excurrent duct system where it is reabsorbed and gains entry into the general circulation is blocked (Franchimont, Chari, Hazee-Hagelstein, Debruche & Duraiswami, 1977; Le Lannou *et al.*, 1979), or the direct release of inhibin into the testicular lymphatics and/or venous drainage is reduced following a decline in inhibin production by the Sertoli cells of the degenerating seminiferous epithelium (Davies *et al.*, 1978; Morris, 1979). In the present study, testicular inhibin content in rats after bilateral efferent duct ligation was measured by an in-vitro inhibin bioassay and related to the corresponding changes in serum gonadotrophins.

Materials and Methods

Bilateral efferent duct ligation or sham-operation was performed on 90-day-old rats according to the procedure of Smith (1962). In Exp. 1, 7 animals from each group were killed at intervals of 3, 7,

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14, 28 and 42 days after ligation. In Exp. 2, the effects of bilateral efferent duct ligation were examined every 24 h in the first week after treatment. High-speed supernatants of homogenates of the paired testes from individual animals were charcoal-extracted and then bioassayed for inhibin using rat pituitary cells in culture as previously described (Au *et al.*, 1983). The activity was expressed in terms of the reference standard, an ovine testicular lymph preparation with an assigned potency of 1 U/mg (Eddie, Baker, Higginson & Hudson, 1979). The sensitivity of the inhibin bioassay was 10 units/testis. Serum FSH and LH concentrations were measured by specific radioimmunoassays whose characteristics have been previously described (Au *et al.*, 1983). FSH levels are expressed in terms of NIAMDD-rat-FSH-RP1 and LH levels in terms of NIAMDD-rat-LH-I₅. All samples were measured in a single assay and the intra-assay coefficients of variation were 5 and 6% for FSH and LH, respectively. The sensitivities of the FSH and LH radioimmunoassays were 75 and 0.10 ng/ml respectively. Testicular weight and inhibin content and serum gonadotrophin concentrations in the treated rats were compared to those of the corresponding controls by Student's *t* test.

Results

Testicular weight and inhibin content

In Exp. 1, the weight of the ligated testes was significantly higher (132%; $P < 0.005$) at Day 3, then decreased to normal at Day 7, and remained at 31–40% below control values ($P < 0.001$) from Day 14 to Day 42 (Table 1). The inhibin content in the ligated testes showed similar changes (Table 1); an initial increase at Day 3 (296%; $P < 0.001$), was followed by a decline at Day 7 to levels which were still higher than (137%), but not significantly different from those of the controls. At Day 14, there was a significant reduction ($P < 0.001$) in the inhibin content which was only 25% of normal levels at Day 42.

In Exp. 2, fluid and inhibin accumulation in the ligated testes declined earlier than in Exp. 1 (Text-fig. 1). Testicular weight showed an initial increase ($P < 0.001$) at Days 1 and 2 after ligation, and remained in the normal range until Day 7 when a significant reduction ($P < 0.025$) occurred. Similar changes in the inhibin content (Text-fig. 1) were also noted except at Day 3 when normal testicular weight was associated with significantly ($P < 0.025$) elevated inhibin activity in the ligated testes, and at Day 7 when the testicular inhibin content was still not significantly different from that of controls.

Table 1. Effects of bilateral efferent duct ligation (EDL) on testis weight, testicular inhibin content and serum gonadotrophin concentrations in rats after 3–42 days of treatment

		Days after ligation				
Treatment		3	7	14	28	42
Testis weight (g)	Control	2.98 ± 0.06	3.29 ± 0.09	3.43 ± 0.12	3.73 ± 0.11	3.25 ± 0.07
	EDL	3.94 ± 0.21**	3.03 ± 0.19	2.05 ± 0.25***	2.30 ± 0.10***	2.24 ± 0.17***
Testicular inhibin content (U)	Control	228 ± 29	230 ± 16	209 ± 25	254 ± 20	261 ± 17
	EDL	674 ± 43***	316 ± 64	121 ± 18**	111 ± 11***	59 ± 9***
Serum FSH (ng/ml)	Control	341 ± 20	314 ± 29	376 ± 36	310 ± 37	340 ± 43
	EDL	458 ± 103	516 ± 19***	742 ± 27***	599 ± 65**	461 ± 30*
Serum LH (ng/ml)	Control	0.58 ± 0.12	0.81 ± 0.09	0.57 ± 0.09	0.72 ± 0.07	0.76 ± 0.08
	EDL	0.65 ± 0.15	1.24 ± 0.13*	1.52 ± 0.22**	1.42 ± 0.07***	1.54 ± 0.17**

Values are mean ± s.e.m. of 5–7 animals.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; compared to corresponding control value.

Text-fig. 1. Changes in rat testicular weight with time after bilateral efferent duct ligation (EDL); the difference between the control and experimental group represents the ability of the seminiferous tubules to secrete fluid. The changes in the inhibin content with time after ligation are also shown. Values are mean $\pm$ s.e.m. for 7 rats/group. * P < 0.01; ** P < 0.001 compared to control value.

Text-fig. 2. Changes in the concentrations of serum FSH and LH after bilateral efferent duct ligation of rats. Values are mean $\pm$ s.e.m. for 7 rats/group. * P < 0.025; ** P < 0.001 compared to control value.

Serum FSH and LH concentrations

In both experiments (Table 1; Text-fig. 2) serum FSH and LH concentrations were significantly elevated (P < 0.01) from Days 6 and 7 after ligation and for up to 42 days (Table 1). If the data for inhibin, FSH and LH from 7 to 42 days are taken together, FSH and LH concentrations were inversely correlated to inhibin values (FSH *vs* inhibin, $r = -0.35$, P < 0.01; LH *vs* inhibin; $r = -0.46$, P < 0.001; $n = 54$).

Discussion

This study demonstrated that, after bilateral efferent duct ligation in rats, the testicular inhibin content showed an initial increase for 1–3 days followed by a subsequent decline. The decline parallels the ability of the seminiferous tubule to secrete fluid after efferent duct ligation and would be consistent with the view that both products are secreted by the Sertoli cell (Steinberger & Steinberger, 1976; Waites & Gladwell, 1982; Le Gac & de Kretser, 1982; Verhoeven & Franchimont, 1983). Furthermore, the later changes in FSH and inhibin are consistent with a role for inhibin involved in the feedback control of pituitary FSH secretion, since the decrease in testicular inhibin content was accompanied by a rise in serum FSH. The decline in the testicular inhibin content 14 days after efferent duct ligation is consistent with the concept that this procedure results in pressure atrophy of the seminiferous epithelium and impaired Sertoli cell function (Smith, 1962; Risbridger, Kerr, Peake & de Kretser, 1981b).

The changes in FSH and inhibin after efferent duct ligation are not always inversely related particularly in the first week. In the first 2 days, significantly elevated concentrations of inhibin were associated with no changes in serum FSH whereas after 6–7 days FSH levels rise although testicular inhibin content remains normal. The increase in inhibin content of the testis for 48 h after efferent duct ligation indicates that the inhibin secreted into the seminiferous tubule lumen is trapped. However, the accumulated inhibin does not appear to influence FSH secretion significantly, probably because of the integrity of the blood–testis barrier (Nykanen & Kormano, 1978; Osman & Ploen, 1978; Osman, 1978) and the failure of the raised intratubular levels to effect an increase in the secretion of inhibin across the base of the Sertoli cell into testicular lymphatics (Au *et al.*, 1984). Nevertheless, others have suggested that the passage of inhibin into rete testis fluid and its subsequent absorption represents an important pathway by which inhibin reaches the circulation (Le Lannou *et al.*, 1979; Lipner & Rush, 1981). The failure of FSH levels to rise immediately after efferent duct ligation would raise some doubt as to the importance of this exit pathway. Similar conclusions were reached by other investigators who noted inconsistent changes in serum FSH levels in hemicastrated rams in which chronic cannulation of the rete testes drained the seminiferous tubule fluid away from the epididymis (Walton, Evins & Waites, 1978; Blanc *et al.*, 1978; Davies *et al.*, 1979).

However, opposite conclusions can be reached from an examination of the data during Days 6–7 of Exp. 2 when FSH values were elevated in the presence of normal testicular inhibin levels. Based on these results it could be argued that efferent duct ligation prevented the absorption of inhibin at an extratesticular site, resulting in the failure of normal testicular inhibin levels to maintain control of FSH. The data and techniques used in the present study are unable to demonstrate conclusively the major pathway by which inhibin gains access to the circulation. Since inhibin activity is present in testicular interstitial fluid and lymph in higher concentrations than in plasma (Baker *et al.*, 1978; Krause, 1978; Au *et al.*, 1984), inhibin must be secreted across the basal aspect of the Sertoli cells as well as their luminal surface. This bidirectional release has also been noted for androgen-binding protein, another Sertoli cell product (Gunsalus, Musto & Bardin, 1978, 1980). Until assays with sufficient sensitivity to measure inhibin levels in blood are available, it will not be possible to follow acute changes in the circulating concentrations of inhibin after efferent duct ligation.

The present study also confirms that serum LH levels rise after bilateral efferent duct ligation in rats (Collins *et al.*, 1978; Risbridger *et al.*, 1981b). It could be argued that this rise in LH is due to the decline in testicular inhibin levels since some investigators have noted the suppressive effect of inhibin-containing extracts on LH secretion, particularly at high doses (Setchell *et al.*, 1977; Franchimont *et al.*, 1977; Lee *et al.*, 1979; de Jong, Smith, & van der Molen, 1979). However, alterations in Leydig cell function have been shown after efferent duct ligation in rats and they consist of hypertrophy, hyperresponsivity to hCG *in vitro* and loss of LH receptors (Risbridger *et al.*, 1981b). These changes have been interpreted as evidence of a local control of Leydig cells by the

seminiferous tubules (Risbridger *et al.*, 1981b; de Kretser, 1982). The elevated serum LH concentrations after bilateral efferent duct ligation are accompanied by normal serum testosterone values and may indicate a state of compensated Leydig cell failure, resembling changes seen in other models of spermatogenic damage such as fetal irradiation, vitamin A deficiency and cryptorchidism (see review by de Kretser, 1982; Rich, Kerr & de Kretser, 1979; Risbridger, Kerr & de Kretser, 1981a). The failure of testosterone secretion to rise in the presence of elevated LH concentrations may result from a combination of the effects of decreased blood flow through the testis and the reduced numbers of LH receptors on the Leydig cells after testicular damage (de Kretser, 1982).

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