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Studies on Testicular Function. II.* Effects of FSH, LH, Testosterone and Dihydrotestosterone on Restoration and Maintenance of Lysosomal Enzyme Activity in the Testis of the Hypophysectomized Rat

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Abstract

Hypophysectomy of the adult male rat depressed the activity of acid phosphatase and acid proteinase, but increased the activity of β-glucuronidase in the testis. Groups of rats were treated with FSH, LH or testosterone alone or in combination after 32 days of post-hypophysectomy regression (restoration experiment) for a further 32 days, or with FSH, testosterone or dihydrotestosterone alone or in combination commencing at 2 days after hypophysectomy (maintenance experiment) for a further 30 days.

The restoration of testicular acid phosphatase activity after post-hypophysectomy regression was partially achieved only by administration of FSH and testosterone in combination. Maintenance of acid phosphatase activity after hypophysectomy was possible with FSH, testosterone or dihydrotestosterone alone or in combination. Total acid proteinase activity was slightly restored by the combination of FSH and LH, but no hormonal combination could maintain the total acid proteinase activity after hypophysectomy. Free acid proteinase activity could be partially maintained by FSH and dihydrotestosterone. β-Glucuronidase activity increased in the testis after hypophysectomy and the most effective hormonal treatment in reducing the free β-glucuronidase activity was FSH. All hormonal treatments were effective in preventing increased activity of β-glucuronidase in the testis after hypophysectomy.

The changes in enzyme activity appear to follow closely the cellular changes in the testis (see Part I of this series). This indicates that the synthesis of acid hydrolytic enzymes in the testis may not be directly controlled by the pituitary gonadotrophins, but that changes in enzyme activity may reflect changes in cellular and cytoplasmic composition of this organ.

Introduction

The lysosome was discovered by tissue fractionation studies (de Duve *et al.* 1955) in which it was shown that some acid hydrolytic enzymes (ribonuclease, deoxyribonuclease, cathepsin and β-glucuronidase) showed an intracellular distribution similar to that of acid phosphatase (Berchet and de Duve 1952). These acid hydrolases were found in the light mitochondrial fraction and were particularly unreactive to their substrates in intact preparations, but when the particles were degraded the enzymes were strongly reactive with their substrates (de Duve *et al.* 1955). The word 'lysosome' was suggested to identify these acid-hydrolase-rich particles. Novikoff *et al.* (1956) published the first electron micrographs of liver lysosomes, which had the morphological features of the pericanalicular dense bodies of hepatocytes.

Lysosomes are found in the testis in both the Leydig cells and the seminiferous tubular epithelium (Dott 1969). Immature rat testis shows a higher β-glucuronidase activity than the mature rat testis (Conchie *et al.* 1956, 1959); the activity starts to

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decrease between 10 and 20 days of age, and reaches its lowest and adult activity at 40 days of age, just at the beginning of the onset of spermiogenesis (Males and Turkington 1971).

Acid phosphatase has been extensively studied in the testis. Very little acid phosphatase is present in the interstitial tissue of the testis (Wislocki 1949; Rollinson 1954; Mathur and Singh 1965). In the seminiferous tubular epithelium acid phosphatase has been shown to be located in some cell types but to be almost absent from others (Wislocki 1949; Rollinson 1954). Spermatids and spermatocytes appear to have the highest acid phosphatase activity (Lake 1962; Mathur and Singh 1965), but ultrastructural studies suggest that acid phosphatase is present in the dense bodies of Sertoli cells and at the surface of spermatids, as well as in the Golgi apparatus of all cell types of the germinal epithelium (Tice and Barnett 1963; Posalaki *et al.* 1968). Males and Turkington (1971) have shown that acid phosphatase activity increases in the immature rat testis between 10 and 40 days after birth, when it reaches a stable adult level.

Recently, Vanha-Perttula (1971*a*, 1971*b*, 1971*c*, 1971*d*) studied the biochemical nature of rat testicular acid phosphatase, and described four different enzymes which may be located at different sites in the testis. Enzyme I may be in the interstitial tissue lysosomes, enzyme II may be membrane-bound in the interstitial and tubular tissue, enzyme III may be bound in the seminiferous tubules, and enzyme IV may be specifically located within the seminiferous tubules, and may have a special function in spermatogenesis (Vanha-Perttula and Nikkanen 1973).

This paper describes experiments designed to investigate the hormonal control of hydrolytic enzyme activity in the rat testis, and to relate these activities to changes in germinal cell populations. The enzymes studied were acid phosphatase (E.C. 3.1.3.2), soluble acid proteinase (E.C. 3.4.4.23) and β-glucuronidase (E.C. 3.2.1.31).

Materials and Methods

Young adult male crossbred rats (the first cross of Wistar and Manchester hooded) were hypophysectomized by the parapharyngeal route, housed at 25°C, and fed on standard rat pellets and an aqueous suspension of 5% whole milk powder and 5% glucose. Ten control animals were not hypophysectomized but were maintained under similar conditions (group C1). The hypophysectomized animals were used in two separate experiments (see Elkington and Blackshaw 1974 for details).

Experiment 1

Hypophysectomized rats were treated by intramuscular injection at 32 days after the operation, for a further 32 days, with FSH (F), LH (L) or testosterone propionate (T) alone or in combination, to investigate hormonal effects on restoration of testicular hydrolase activities towards control levels. There were eight groups of treated rats and one group of normal animals (C1). The hypophysectomized animals were given one of the following hormonal treatments: F, L, T, FT, LT, FL, FLT or no treatment (group C2).

Experiment 2

Hypophysectomized animals were allotted to one of six groups at 2 days after hypophysectomy and were treated for 30 days, by intramuscular injection, with FSH (F), testosterone (T) or dihydrotestosterone (D) alone or in combination, to investigate hormonal effects on maintenance of testicular hydrolase activities at control levels. The hypophysectomized animals were given one of the following hormonal treatments: F, T, D, FT, FD or no treatment (C2); there was also one group of normal animals (C1).

Analytical Methods and Analysis of Data

At the end of the experiments the animals were killed by cervical dislocation and the testes removed and weighed. The testes were homogenized (10% w/v) in ice-cold 0·25M sucrose with a motor-driven glass–Teflon homogenizer. The homogenate was immediately centrifuged at 2500 rev/min in a Christ UJ 15 centrifuge for 5 min (4°C). Aliquots (4·5 ml) of the crude supernatant were diluted with 0·5 ml of either 0·25M sucrose or 1% Triton X-100 for the determination of free and total enzyme activities respectively. [The Triton X-100 disrupts lysosomes and releases bound enzymes into the medium (Wattiaux and de Duve 1956).] After standing for 10 min at 4°C, the preparations were centrifuged for 30 min at 20 000 *g* and the supernatants used for the enzyme determinations. A supernatant was diluted 25 times for acid phosphatase (Torriani 1960), 10 times for β-glucuronidase (Plaice 1961) and left undiluted for acid proteinase measurements (Barrett 1967). The substrates for these reactions were 0·05M *p*-nitrophenyl phosphate (acid phosphatase), 0·025M phenolphthalein glucuronide (β-glucuronidase) and 4% denatured sheep haemoglobin (acid proteinase). All these methods were found to give a linear response over the times of incubation and the substrate levels were optimal for the reaction. The incubation times for acid phosphatase and β-glucuronidase were 30 min and 18 h respectively at 37°C, while acid proteinase was incubated at 45°C for 1 h. Enzyme activity was expressed as the change in optical density per testis at a wavelength of 404 nm for acid phosphatase, 280 nm for acid proteinase and 550 nm for β-glucuronidase.

In all instances the results were tested by the analysis of variance (Steel and Torrie 1960). Mean squares for treatment effects were isolated and tested for significance using the residual variance as error; the analyses are presented in summary form in the tables. Parameters with significant overall treatment effects were compared using the least significant difference (Steel and Torrie 1960), as there was variable replication within each group occasioned by death or rejection of incompletely hypophysectomized animals. Mean enzyme responses and summaries of the significance calculations are presented in the tables, but means were not compared when the treatment effect was not significant (Snedecor and Cochran 1967).

Results

Experiment 1

A significant fall in free and total acid phosphatase activity per testis after hypophysectomy (group C2 cf. C1, Table 1) was returned towards normal by the hormonal combinations FT, LT, FL and FLT, with FT and FLT being the most effective (Table 1). There was no significant change in free acid proteinase activity per testis after hypophysectomy and hormonal treatment, but the total acid proteinase activity per testis decreased significantly after the operation (Table 1). No hormonal combination except FL improved the response, and in this case the response was poor.

There was an increase in free β-glucuronidase activity per testis, but total β-glucuronidase activity per testis changed little after the operation (Table 1). FSH alone was the most effective hormonal treatment in restoring free β-glucuronidase activity towards normal, while treatments L, T, FL and FLT were ineffective. FSH alone reduced total β-glucuronidase activity per testis below the control level but, surprisingly, the combination FLT and to a lesser extent testosterone alone significantly raised the enzyme activity above the control levels (Table 1).

Experiment 2

Both free and total acid phosphatase activity per testis decreased significantly after hypophysectomy (C2 cf. C1, Table 2). All hormonal treatments were able to partially prevent this fall in activity, but the most effective treatments were testosterone alone and the FT and FD combinations. There was a significant decrease in both free and total acid proteinase activity per testis after hypophysectomy (Table 2). The FD

combination could partially prevent the decrease in free acid proteinase activity, but no hormonal combination could maintain total acid proteinase activity.

There was a significant increase in free β-glucuronidase activity per testis after hypophysectomy (Table 2), which was effectively prevented by all of the hormonal combinations, FSH and testosterone alone being the least effective treatments. There

Table 1. Effect of hormonal treatment of rats, hypophysectomized for 32 days, on acid phosphatase, acid proteinase and β-glucuronidase activity in the testis (experiment 1)

Values represent mean enzyme activities (changes in optical density) per testis for the numbers of animals shown in parentheses. For details of treatments and analytical procedures, see Methods section. L.S.D. comparisons were made only for those parameters with significant overall treatment effects (see analyses of variance)

Enzyme	C1 (21)	C2 (13)	F (4)	L (10)	T (6)	FT (6)	LT (5)	FL (7)	FLT (6)
I. Acid phosphatase									
(a) Free	289·2	29·4	29·9	54·1	78·8	206·3	136·4	100·4	215·4
Significance:[A]									
Comparison with C1		**	**	**	**	**	**	**	**
Comparison with C2			n.s.	n.s.	n.s.	**	**	*	**
(b) Total	517·1	48·4	61·1	96·5	101·2	293·4	168·9	147·6	309·1
Significance:									
Comparison with C1		**	**	**	**	**	**	**	**
Comparison with C2			n.s.	n.s.	n.s.	**	**	*	**
II. Acid proteinase[B]									
(a) Free	3·3	1·6	1·7	1·8	1·3	1·9	1·5	3·6	1·7
(b) Total	12·4	5·0	6·4	4·9	4·7	8·0	7·6	8·4	7·0
Significance:									
Comparison with C1		**	**	**	**	**	**	**	**
Comparison with C2			n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.
III. β-glucuronidase									
(a) Free	17·0	64·6	14·6	42·9	48·5	35·6	35·4	79·1	59·2
Significance:									
Comparison with C1		**	n.s.	**	**	n.s.	n.s.	**	**
Comparison with C2			**	*	n.s.	**	*	n.s.	n.s.
(b) Total	126·8	165·3	52·3	151·3	176·0	154·4	159·1	124·3	235·4
Significance:									
Comparison with C1		*	*	n.s.	*	n.s.	n.s.	n.s.	**
Comparison with C2			**	n.s.	n.s.	n.s.	n.s.	n.s.	*

Summary of analyses of variance

Source of variation	D.F.	Ia	Ib	D.F.	IIa	D.F.	IIb	D.F.	IIIa	IIIb
		Variance ratios:								
Treatment	8	35·88**	53·79**	8	1·16	8	8·73**	8	8·59**	8·25**
Error variance	79	*3299·70*	*7086·50*	61	*5·40*	78	*12·76*	79	*537·50*	*2846·00*

* $P < 0·05$. ** $P < 0·01$.

[A] Significance of differences between treatments.

[B] The number of animals in groups C1, C2, L and T was 21, 6, 8 and 7 respectively for free acid proteinase and 24, 13, 13 and 9 respectively for total acid proteinase activity determinations.

was also a significant increase in total β-glucuronidase activity after hypophysectomy, and all hormonal combinations prevented this rise.

Discussion

Males and Turkington (1971) demonstrated that β-glucuronidase activity decreased and acid phosphatase activity increased in the testis at a time when spermatocytes and spermatids were being produced in the immature rat testis. They also showed that

Table 2. Effect of hormonal treatment of rats, hypophysectomized for 2 days, on acid phosphatase, acid proteinase and β-glucuronidase activity in the testis (experiment 2)

Values represent mean enzyme activities (changes in optical density) per testis for the numbers of animals shown in parentheses. Other details as for Table 1

Enzyme	C1 (13)	C2 (7)	F (5)	T (5)	D (4)	FT (5)	FD (6)
I. Acid phosphatase							
(a) Free	322	65	190	277	180	280	275
Significance of differences:							
Comparison with C1		**	**	**	**	*	**
Comparison with C2			**	**	**	**	**
(b) Total	539	85	269	381	232	449	379
Significance of differences:							
Comparison with C1		**	**	**	**	**	**
Comparison with C2			**	**	**	**	**
II. Acid proteinase							
(a) Free	3·1	2·1	2·3	1·3	2·1	1·7	2·8
Significance of differences:							
Comparison with C1		**	**	**	**	**	n.s.
Comparison with C2			n.s.	n.s.	n.s.	n.s.	*
(b) Total	14·2	8·2	11·0	8·0	9·2	7·7	8·9
Significance of differences:							
Comparison with C1		**	**	**	**	**	**
Comparison with C2			n.s.	n.s.	n.s.	n.s.	n.s.
III. β-glucuronidase							
(a) Free	16	84	49	29	28	20	23
Significance of differences:							
Comparison with C1		**	**	*	n.s.	n.s.	n.s.
Comparison with C2			**	**	**	**	**
(b) Total	125	221	119	152	148	136	123
Significance of differences:							
Comparison with C1		**	n.s.	*	n.s.	n.s.	n.s.
Comparison with C2			**	**	**	**	**

Summary of analyses of variance

Source of variation	D.F.	Variance ratios: Ia	Ib	IIa	IIb	IIIa	IIIb
Treatment	6	21·00**	33·18**	2·67*	7·19**	9·63**	4·63**
Error variance	38	*2809*	*566·3*	*1·02*	*7·11*	*429*	*1882*

* $P < 0·05$. ** $P < 0·01$.

testosterone alone or FSH and LH together could reverse the decrease in acid phosphatase and the increase in β-glucuronidase activity that occured after hypophysectomy, but did not comment that this was possibly due to changes in cell populations or to volume changes in the testis. In another study (Elkington *et al.* 1973), β-glucuronidase activity increased slightly, while acid proteinase and acid phosphatase activity decreased at a time when the nuclear indices of primary spermatocytes and spermatids were falling. This indicates that primary spermatocyte and spermatid populations are closely associated with acid phophatase and acid proteinase. In contrast, much of the β-glucuronidase was present in the cells remaining after hypophysectomy, which are Sertoli cells and spermatogonia, particularly the former (Elkington *et al.* 1973).

Restoration of acid phosphatase appears to be related to total germ cell numbers (Elkington and Blackshaw 1974) and it is evident that FSH and testosterone interact to effect these changes. In the case of maintenance of acid phosphatase activity after hypophysectomy, the requirements were a little less demanding; testosterone alone proved reasonably satisfactory for enzyme maintenance, but only maintained the numbers of early primary spermatocytes and round spermatids (Elkington and Blackshaw 1974). The best response for maintenance of acid phosphatase activity and germ cell numbers was again observed after administration of a gonadotrophin and testosterone.

Vanha-Perttula (1971*a*, 1971*b*, 1971*c*, 1971*d*) reported that most of the lysosomal acid phosphatase in the rat testis was in Leydig cells. In the experiments reported here, about 50% of the acid phosphatase activity in the testis was bound within the lysosomes. If acid phosphatase activity is demonstrated histochemically in the testis, using sodium α-naphthyl phosphate as substrate, then it would be expected that acid phosphatase II (Vanha-Perrula 1971*d*) would be detected, mainly in the interstitial lysosomes (Vanha-Perttula 1971*d*). However, in other histochemical studies acid phosphatase that will hydrolyse sodium α-naphthyl phosphate has been found mainly in seminiferous tubular epithelium of the rat testis (Elkington and Blackshaw 1971; Elkington *et al.* 1973). According to Vanha-Perttula (1971*d*) the tubular acid phosphatases (III and IV) do not hydrolyse α-naphthyl phosphate and should not be detected by this technique. Testicular degeneration induced by cryptorchidism reduced the activities of the tubular enzymes (III and IV) and increased that of the interstitial enzyme (II) (Vanha-Perttula and Nikkanen 1973), but from histochemical studies, changes in acid phosphatase levels are limited to the seminiferous tubule (Blackshaw, unpublished data). It appears that further work is necessary to establish the location of the testicular acid phosphatases.

The relative independence of acid proteinase levels to the exogenous hormones used after hypophysectomy suggests that other hormones of the pituitary may be important in controlling its activity. However, the significance of acid proteinase in the testicular economy is not clear; the substrate used for its detection (denatured haemoglobin) is not ideal, and the development of specific substrates is desirable to properly characterize proteolytic enzymes and their location in the testis.

β-Glucuronidase has been detected and measured using specific substrates; the evidence is good that it does not occur in the later germ cells to any great extent (Males and Turkington 1971) and it is probably associated with Sertoli and other somatic cells. In general, β-glucuronidase rose after hypophysectomy and there was an increased free : total ratio of this enzyme, indicating a rise in the fragility of lysosomes, which is

in line with the concept of lysosomes as part of the phagocytic and lytic mechanisms of tissues (Cahn and Fedorko 1969).

Following post-hypophysectomy regression the increased level of β-glucuronidase was unaffected by androgen, either directly from exogenous testosterone or by the action of LH on androgen secretion, although these hormones antagonized the depressive actions of FSH. These alterations in total enzyme content of the testis may be related to interactions between improved Sertoli cell function and increased numbers of germ cells. β-Glucuronidase maintenance requirements were similar to those of acid phosphatase, although less demanding, and reflect the reduction in testicular damage and requirements for phagocytosis of cells and debris.

The results presented here suggest that acid hydrolytic enzyme synthesis in the testis is not controlled directly by the pituitary gonadotrophins, but that changes in enzyme activity reflect changes in the cellular composition of the testis during degeneration and regeneration following hormonal treatment, and also show that it may be useful to refer changes in enzyme activity in the testis to quantitative evaluations of spermatogenesis.

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