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Studies on Testicular Function. III.* Effects of FSH, LH, Testosterone and Dihydrotestosterone on Restoration and Maintenance of Testicular-specific Lactate Dehydrogenase Activity in the Hypophysectomized Rat

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Abstract

Hypophysectomy of the adult male rat depressed the activity of testicular-specific lactate dehydrogenase (LDH-X). Groups of rats were treated with FSH (F), LH (L) or testosterone (T) alone or in combination after 32 days of post-hypophysectomy regression (restoration experiment) for a further 32 days or with F, T or dihydrotestosterone (D) alone or in combination from 2 days after hypophysectomy (maintenance experiment) for 30 days.

LDH-X activity per milligram of protein was restored to normal by the hormonal combinations FT and LT while F, L, or T given alone or the FL and FLT combinations were ineffective. However, the total LDH-X activity per testis was only partially restored by the hormonal combinations FT, LT and FLT.

In the maintenance experiment LDH-X activity per milligram of protein was maintained by all hormonal treatments except D, while the total LDH-X activity per testis was completely maintained by the FT hormonal combination. All other hormonal treatments except F alone maintained the activity of this isoenzyme above the level in the hypophysectomized rat.

The restoration and maintenance of LDH-X activity in the testis of the hypophysectomized rat was successful with those combinations of hormones that were also able to restore or maintain the numbers of late primary spermatocytes and spermatids. Thus, the level of LDH-X activity in the testis is dependent on the presence of late primary spermatocytes and spermatids and hence this enzyme is a good marker for the production of these cells in the rat testis.

Introduction

Lactate dehydrogenase (EC 1.1.1.27) (hereafter designated LDH) is composed of a number of isoenzymes (Markert and Møller 1959) of which one or more are found only in the mature testes and spermatozoa of some animals (Allen 1961; Zinkham *et al.* 1964*a*, 1964*b*; Goldberg and Hawtrey 1967). These unique isoenzymes have been named LDH-X (Blanco and Zinkham 1963). In the rat testis LDH-X appear between 20 and 30 days of age (Blackshaw and Elkington 1970), a time when late primary spermatocytes are being formed in the seminiferous tubular epithelium, and these isoenzymes are probably located in specialized mitochondria found in late primary spermatocytes and spermatozoa (De Domenech *et al.* 1970, 1972).

The possible hormonal control of LDH-X activity in the testis was suggested when Elkington and Blackshaw (1970) found that the depression of LDH-X activity in the testis of the intact rat by oestradiol could be prevented by combinations of testosterone and pregnant mare serum gonadotrophin administered with the oestradiol treatment. Recently Winer and Nikitovitch-Winer (1971) have shown that FSH and LH or testosterone alone will maintain LDH-X activity in the testes of rats hypophy-

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sectomized 2 days before hormonal treatment, and they concluded that the pituitary gonadotrophins may switch on the genes responsible for the production of LDH-X in the testis.

This paper reports two experiments in which LDH-X activity was measured in hypophysectomized rats that were treated before and after testicular regression with pituitary gonadotrophins and androgens to study further the hormonal control of LDH-X activity in the rat testis.

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. The hypophysectomized animals were used in two separate experiments (for details see Elkington and Blackshaw 1974).

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. There were eight groups of treated rats and one group that was not treated or hypophysectomized (C1). The animals were given one of the following hormonal treatments: F, L, T, FT, LT, FL, FLT or no hormone (C2).

Experiment 2

Hypophysectomized rats were allotted to one of six treatment groups at 2 days after hypophysectomy and were treated for 30 days, by intramuscular injection, with F, T or dihydrotestosterone (D) alone or in combination. The groups of treated rats were given one of the following hormonal treatments: F, T, D, FT, FD or no hormone (group C2); there was also one group of normal untreated rats (C1).

Analytical Methods

At the conclusion of the experiments the animals were killed by cervical dislocation, and the testes were removed. The testes were decapsulated and a 10% homogenate (w/v) was prepared in 0·25M sucrose using a motor-driven glass homogenizer fitted with a Teflon pestle. The homogenate was centrifuged at 2500 rev/min in a Christ UJ centrifuge and the supernatant was then centrifuged at 20 000 *g* for 30 min. The supernatant was diluted 10 times and used for total LDH assay (Wilkinson 1962) with sodium pyruvate as substrate. Total protein estimates were made using the Folin-Ciocalteau method (Lowry *et al.* 1951).

The isoenzymes of LDH were separated by polyacrylamide gel disc electrophoresis (Ornstein and Davis 1962; Blackshaw and Samisoni 1966). Tetrazolium reductase activity was demonstrated, using nitroblue tetrazolium, as purple bands of formazan in the gel (Helm *et al.* 1962), substrates being sodium lactate to demonstrate total LDH and sodium DL-α-hydroxyvalerate for the LDH-X isoenzymes (Allen 1961). The intensity of formazan deposition in each isoenzyme band was estimated using an integrating densitometer (Photovolt Densicord 542) and the relative contributions of the stained areas were calculated. These scanning techniques are semiquantitative only as the slow bands lose activity during electrophoresis and the fast and slow isoenzymes have different substrate affinities. Nevertheless, when used to follow changes in isoenzyme composition of LDH-X, scanning methods are extremely useful.

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, giving degrees of freedom and variance ratios for each source of variation, with the error variance at the base of each variance ratio column. Parameters with significant overall treatment effects were compared using the method of least significant difference (L.S.D.; Steel and Torrie 1960) as there was variable replication within each

group, occasioned by death, or rejection of incompletely hypophysectomized animals. Mean responses for cell types and summaries of the significance of differences are presented in the tables.

Results

Experiment 1 (Restoration)

There were highly significant overall treatment effects of hormones and hypophysectomy on LDH-X activity in the rat testis (analysis of variance, Table 1) and treatment mean responses were compared by the L.S.D. method. Hypophysectomy caused a decrease in the specific activity of LDH-X in the testis and neither FSH, LH nor testosterone given alone were able to return the activity of these isoenzymes to normal (Table 1). The combinations FT and LT were able to return specific

Table 1. Effect of hormonal treatment of rats, hypophysectomized for 32 days, on LDH-X activity in the testis

Values in the table represent mean activities expressed as LDH-X activity (%) × total LDH activity. Treatments were as defined in Materials and Methods; numbers of animals in each treatment group are shown in parentheses

LDH-X activity	C1 (21)	C2 (13)	F (4)	L (10)	T (6)	FT (6)	LT (5)	FL (7)	FLT (6)
I. Per milligram protein	423·6	110·9	281·2	270·2	258·6	353·2	459·7	227·9	212·1
Significance of diff.									
Comparison with C1		**	*	**	*	n.s.	n.s.	**	**
Comparison with C2			*	**	*	**	**	n.s.	n.s.
II. $10^{-3}\times$ activity per testis	16·68	0·98	2·24	2·53	2·93	7·80	7·55	4·26	5·87
Significance of diff.									
Comparison with C1		**	**	**	**	**	**	**	**
Comparison with C2			n.s.	n.s.	n.s.	**	**	n.s.	*

Summary of analyses of variance

Source of variation	Degrees of freedom	Variance ratio (I)[A]	Degrees of freedom	Variance ratio (II)[A]
Treatment	8	8·51**	8	26·79**
Error variance	69	*17714·40*	74	*58·03*

* $P < 0{\cdot}05$. ** $P < 0{\cdot}01$.

[A] For LDH-X activity expressed as C subunits.

LDH-X activity to the control level, but surprisingly FL and FLT did not significantly increase specific LDH-X activity above the level of the untreated hypophysectomized animals. LDH-X activity in the whole organ was calculated and it was found that hypophysectomy decreased this activity. Only the hormonal combinations FT, LT and FLT significantly raised the LDH-X activity per testis above the level found in the hypophysectomized rats (Table 1).

Experiment 2 (Maintenance)

Hormonal treatment of the rats was begun 2 days after hypophysectomy, before LDH-X activity had started to fall (Elkington *et al.* 1973), and was continued for a further 30 days. This experiment was designed to determine if FSH, testosterone or

dihydrotestosterone could, alone or in combination, prevent the fall in LDH-X activity in the testis after hypophysectomy.

There was a significant overall treatment effect of hormones and hypophysectomy on LDH-X activity in the rat testis (analysis of variance, Table 2) and individual results were compared by the L.S.D. method. As expected, hypophysectomy decreased the specific and total testicular activity of LDH-X. Specific activity was maintained by all hormones and combinations of hormones except dihydrotestosterone (Table 2).

Table 2. Effect of hormonal treatment of rats, hypophysectomized for 2 days, on LDH-X activity in the testis

Values in the table represent mean activities expressed as LDH-X activity (%) × total LDH activity. Details of treatments are given in Materials and Methods; numbers of animals in each treatment group are shown in parentheses

LDH-X activity	C1 (14)	C2 (7)	F (5)	T (5)	D (4)	FT (5)	FD (9)
I. Per milligram protein	415	223	472	408	225	481	385
Significance of differences							
Comparison with C1		**	n.s.	n.s.	**	n.s.	n.s.
Comparison with C2			**	**	n.s.	**	**
II. 10^{-3} × activity per testis	16·54	2·38	5·19	13·11	9·76	16·03	12·72
Significance of differences							
Comparison with C1		**	**	*	**	n.s.	**
Comparison with C2			n.s.	**	**	**	**

Summary of analyses of variance

Source of variation	Degrees of freedom	Variance ratios:[A] I	II
Treatment	6	8·77**	17·18**
Error variance	42	*7674·69*	*10·61*

* $P < 0{\cdot}05$. ** $P < 0{\cdot}01$.

[A] For LDH-X activity (units).

When the total testicular content of LDH-X was examined, only the FT hormonal combination provided complete maintenance, but all hormones and combinations except FSH alone maintained activity significantly above the level of the hypophysectomized animal (Table 2).

Discussion

Hypophysectomy of the rat causes a depression of spermatogenesis (Smith 1927, 1930) and produces a testis, at 32 days post-hypophysectomy, that contains mainly early primary spermatocytes, spermatogonia and Sertoli cells (Elkington *et al.* 1973; Elkington and Blackshaw 1974). After hypophysectomy there was a fall in total LDH-X activity at 8 days, which coincided with the initial depression in primary spermatocyte and spermatid numbers. The specific activity of LDH-X depends on the enzyme content of the later germ cells and the contribution of other cells and fluids to the total soluble protein. Thus the maintenance of specific activity until 16 days after hypophysectomy may reflect a loss of cytoplasm from both germ and

non-germ cell sources. Later losses of germ cells would then only reduce specific enzyme activity.

The fall in LDH-X activity appears to be related directly to a reduction in the numbers of late primary spermatocytes and spermatids. If the enzyme activity is related to the nuclear indices of these cells there is no change in the LDH-X content of the remaining spermatocytes and spermatids during degeneration, and the effects of gonadotrophins on LDH-X activity are probably secondary to their effects on cellular maturation and division (Elkington and Blackshaw 1974).

Examination of the repair experiment using FSH, LH and testosterone after 32 days post-hypophysectomy regression shows unequivocally that none of the hormones used alone had significant effects on total LDH-X activity or the production of late primary spermatocytes and spermatids (Elkington and Blackshaw 1974). The combined action of FSH or LH and testosterone was necessary for significant restoration of both total LDH-X activity and late primary spermatocyte and spermatid nuclear indices. Only the FT and LT hormonal combinations were able to restore the specific activity of LDH-X back to the control level as well as partially restore the nuclear indices of late primary spermatocytes and spermatids, and this may reflect the reported effects of FSH on protein (Means and Hall 1967, 1968, 1969, 1971), cyclic-AMP (Murad *et al.* 1969; Kuehl *et al.* 1970) and steroid synthesis in the testis (Lacy and Lofts 1965).

Hormonal combinations of gonadotrophins and testosterone were most effective in the partial restoration of total LDH-X activity and of the more mature germ cells, further linking LDH-X synthesis with the later primary spermatocytes. The requirements for maintenance and repair were not the same: testosterone could not reinitiate significant synthesis after regression but could almost maintain LDH-X synthesis after hypophysectomy. FSH, despite the claim of Winer and Nikitovitch-Winer (1971), had little capacity under either situation but acted synergistically with testosterone to maintain cell numbers and enzyme synthesis. The enzyme carnitine acetyltransferase (EC 2.3.1.7) is a similar marker enzyme to LDH-X (Vernon *et al.* 1971) associated with the later germ cells. Go *et al.* (1971) have shown that the activity of carnitine acetyltransferase can be restored after post-hypophysectomy regression by FSH and LH or testosterone combinations.

These experiments show that, provided spermatogenesis is maintained in the testis, the LDH-X content of this organ will also be maintained and this activity is a good indication of late primary spermatocyte and spermatid production by the testis. The results also show that in an organ such as the testis, with a heterogeneous cell population, where there may be specific cellular locations of enzymes, the measurement of enzyme activity must be related to both organ size and the proportions of the cell types.

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