

Testicular compensatory hypertrophy in the unilaterally adrenalectomized lizard *Mabuya carinata* (Schn.)

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Abstract. Unilateral adrenalectomy in recrudescence and breeding phases of the reproductive cycle caused atrophic changes in the ipsilateral testis, shown by inhibition of spermatogenic and steroidogenic activity of the ipsilateral testis. The contralateral testis showed compensatory hypertrophy. Unilateral adrenalectomy in regressing and quiescent phases of the reproductive cycle did not induce compensatory hypertrophy in the contralateral testis although there were atrophic changes in the ipsilateral testis.

Key words. Adrenal; testis; unilateral adrenalectomy; compensatory hypertrophy.

An adrenal-gonad relationship has long been recognised in mammals, and adrenalectomy affects various aspects of reproduction¹. Studies on the influence of the adrenal gland on testicular activity of reptiles are rare. Recently, it was shown that bilateral adrenalectomy results in atrophic changes in the testis of the lizard *M. carinata*², thereby indicating an influence of the adrenal gland on the testicular activity in reptiles. It is not known whether unilateral adrenalectomy elicits any change in the testicular activity and, if so, whether the effect is similar in ipsilateral and contralateral testis or not. The present paper deals with the effects of unilateral adrenalectomy on spermatogenic and steroidogenic activity of the testis in different phases of the reproductive cycle in *M. carinata*.

Material and methods

The experiment was carried out during recrudescence (August–September), breeding (November–December), regressing (February–March) and quiescent (June–July) phases of the testicular cycle. Adult male *M. carinata* were collected from areas around Tirupati (longitude 79° 35' N; latitude 13° 58' E) during each phase of the reproductive cycle and brought to Mysore. They were acclimatized to laboratory conditions for 10 days prior to the start of the experiment. The experimental design, which is described below, was the same for all the phases.

The lizards were divided into two groups, experimental and control. Lizards in the control group were sham-operated while those in the experimental group were subjected to unilateral adrenalectomy. The left side adrenal of each lizard in the experimental group was surgically removed. The method of surgery was similar to that described earlier². The lizards were maintained under normal photothermal conditions and provided with food (live silkworms) and saline ad libitum for the duration of the experiment (30 days).

The lizards were autopsied on the 31st day. At autopsy the weights of the body, the right side testis and the left side testis were recorded and later converted into the relative weight of the left testis and the right testis. The right and the left testis of control and experimental animals were separately fixed in Bouin's fluid and processed for histological and histometric studies². A 5% homogenate of the testis was obtained and processed for the biochemical estimation of activity levels of glucose-6-phosphate dehydrogenase (G-6-PDH)³ and Δ^5 -3 β -hydroxysteroid dehydrogenase (Δ^5 -3 β -HSDH)⁴. The enzyme activity was expressed as units/mg protein/min. Statistical analysis was carried out using Student's t-test.

Results and discussion

In general, it was found that the feeding and drinking habits of the adrenalectomized lizards were normal. The testes of controls were well developed and demonstrated all stages of spermatogenesis in the breeding and recrudescence phases of the reproductive cycle. During regressing and quiescent phases of the cycle the spermatogonia, primary spermatocytes and secondary spermatocytes were less numerous and spermatids were absent in the testes of controls. The Leydig cell nuclei were vesicular and hypochromatic in breeding and recrudescence phases and were hyperchromatic and irregular in outline during regressing and quiescent phases. There was no significant difference in the relative weight, mean number of germ cells belonging to different stages of spermatogenesis and activity levels of Δ^5 -3 β -HSDH and G-6-PDH of the right and left side testis of controls in any phase of the cycle. Therefore data on the left and right side testis of controls were pooled to obtain mean values for the purpose of comparison with values of experimental group.

Surgical removal of the left side adrenal gland resulted in a significant decrease in the relative weight, mean

Table 1. Effect of unilateral adrenalectomy on the relative weight of the testis (mg/100 gm body weight \pm SE) in *M. carinata*

Groups	Relative weight of:	Phase of the reproductive cycle			
		Breeding	Regressing	Quiescent	Recrudescent
Sham-operated controls	Right side testis (R ₁)	535.36 \pm 26.22	282.25 \pm 4.48	271.90 \pm 6.49	494.20 \pm 66.30
	Left side testis (L ₁)	499.72 \pm 11.79	277.26 \pm 20.05	268.90 \pm 5.36	458.13 \pm 41.68
Unilaterally adrenalectomized lizards	Right side testis (R ₂)	729.20 \pm 47.83	238.19 \pm 62.60	267.60 \pm 8.20	698.16 \pm 47.31
	Left side testis (L ₂)	215.56 \pm 16.46	43.08 \pm 4.38	61.03 \pm 7.60	50.14 \pm 5.83
		R ₁ Vs. R ₂ - S	R ₁ Vs. R ₂ - NS	R ₁ Vs. R ₂ - NS	R ₁ Vs. R ₂ - NS
		L ₁ Vs. L ₂ - S	L ₁ Vs. L ₂ - S	L ₁ Vs. L ₂ - S	L ₁ Vs. L ₂ - S

S - Significant ($p < 0.05$); NS - insignificant.

The mean values of right and left side testis of experimental group were compared with testis of respective side of controls using Student's t-test.

number of spermatogonia and activity levels of G-6-PDH and Δ^5 -3 β -HSDH of the left side testis when compared with those of controls (tables 1, 2 and 3) in all the phases of the reproductive cycle. The secondary spermatocytes and spermatids were absent in the left testis of unilaterally adrenalectomized lizards in all

phases. The Leydic cell nuclei were shrunken and it was not possible to measure their nuclear diameter in the left side testis of unilaterally adrenalectomized lizards.

These regressive changes in the left testis were accompanied by an activation of spermatogenesis in the right testis as evidenced by a significant increase in the mean

Table 2. Effect of unilateral adrenalectomy on spermatogenesis in the testis of *M. carinata*

Phase of the reproductive cycle	Groups	Mean number of germ cells/stage/tubule cross section \pm SE			
		Spermatogonia	Primary spermatocytes	Secondary spermatocytes	Spermatids
Breeding	Controls	18.85 \pm 1.35	56.80 \pm 5.40	95.45 \pm 15.85	36.45 \pm 7.85
	Unilaterally adrenalectomized				
	Right side testis (adrenal intact)	45.95 \pm 2.55 ^{a,*}	155.85 \pm 4.35 ^{a,*}	227.05 \pm 12.80 ^{a,*}	39.40 \pm 1.79 ^{a,NS}
Regressing	Left side testis (adrenalectomized)	7.50 \pm 2.50 ^{a,b,*}	7.00 \pm 1.00 ^{a,b,*}	-	-
	Controls	20.70 \pm 1.30	23.95 \pm 11.05	3.55 \pm 1.65	0.40 \pm 0.01
	Unilaterally adrenalectomized				
Quiescent	Right side testis (adrenal intact)	23.60 \pm 0.40 ^{a,NS}	25.40 \pm 1.40 ^{a,NS}	24.20 \pm 1.80 ^{a,*}	-
	Left side testis (adrenalectomized)	8.20 \pm 0.60 ^{a,b,*}	12.00 \pm 3.00 ^{a,NS;b,*}	-	-
	Controls	18.05 \pm 6.50	11.15 \pm 4.30	2.20 \pm 0.20	-
Recrudescent	Unilaterally adrenalectomized				
	Right side testis (adrenal intact)	15.13 \pm 0.98 ^{a,NS}	10.37 \pm 0.77 ^{a,NS}	-	-
	Left side testis (adrenalectomized)	-	-	-	-
Recrudescent	Controls	17.15 \pm 2.19	84.75 \pm 5.12	74.19 \pm 8.51	8.23 \pm 1.50
	Unilaterally adrenalectomized				
	Right side testis (adrenal intact)	37.73 \pm 12.1 ^{a,NS}	109.30 \pm 13.9 ^{a,NS}	174.30 \pm 15.35 ^{a,*}	18.10 \pm 1.79 ^{a,*}
Recrudescent	Left side testis (adrenalectomized)	1.80 \pm 0.40 ^{a,b,*}	2.50 \pm 0.05 ^{a,b,*}	-	-

-Indicates nil.

The means of control and experimental groups were compared using Student's t-test and judged significant if * $p < 0.05$; NS-Insignificant.

^aCompared with control; ^bCompared with right side testis of unilaterally adrenalectomized lizards.

Table 3. Effect of unilateral adrenalectomy on the activity levels of G-6-PDH and Δ^5 - 3β -HSDH in the testis of *M. carinata*

Phase of the reproductive cycle	Enzyme studied	Controls	Right side testis (adrenal intact)	Left side testis (adrenalectomized side)
Breeding	G-6-PDH	32.69 \pm 1.72	22.92 \pm 3.68 ^{a,NS}	17.07 \pm 1.05 ^{a,*,b,NS}
	Δ^5 - 3β -HSDH	64.98 \pm 15.36	33.33 \pm 0.91 ^{a,NS}	22.43 \pm 2.09 ^{a,NS;b,*}
Regressing	G-6-PDH	25.81 \pm 1.21	19.64 \pm 1.67 ^{a,*}	0.85 \pm 0.06 ^{a,b,*}
	Δ^5 - 3β -HSDH	18.40 \pm 3.39	14.63 \pm 0.64 ^{a,NS}	8.66 \pm 1.30 ^{a,NS;b,*}
Quiescent	G-6-PDH	16.90 \pm 1.32	10.29 \pm 1.18 ^{a,*}	0.39 \pm 0.09 ^{a,b,*}
	Δ^5 - 3β -HSDH	14.48 \pm 1.32	8.60 \pm 1.43 ^{a,*}	5.84 \pm 1.36 ^{a,*,b,NS}
Recrudescence	G-6-PDH	23.10 \pm 6.90	38.67 \pm 9.69 ^{a,NS}	7.90 \pm 1.22 ^{a,NS;b,*}
	Δ^5 - 3β -HSDH	22.81 \pm 4.23	25.31 \pm 4.30 ^{a,NS}	12.43 \pm 2.71 ^{a,b,NS}

Enzyme activity is expressed in units/mg protein.

The means of control and experimental groups were compared using Student's t-test and judged significant if *p < 0.05.

NS-Insignificant.

^aCompared with control; ^bCompared with right side testis of unilaterally adrenalectomized lizard.

number of germ cells belonging to all the stages of spermatogenesis in breeding and recrudescence phases when compared with those of the controls (table 2). These parameters did not show significant difference from controls in regressing and quiescent phases (table 2), except for a significant increase in secondary spermatocytes in regressing phase. The activity levels of Δ^5 - 3β -HSDH and G-6-PDH of the right testis did not show an increase in any phase of the reproductive cycle when compared with those of controls. These results indicate inhibition of spermatogenic and steroidogenic activity of the left testis following surgical removal of the left adrenal gland. The regressive changes were accompanied by activation of the spermatogenic activity and an increase in the relative weight of the right side testis in breeding and recrudescence phases of the testicular cycle. Compensatory hypertrophy of the contralateral testis has been observed in unilaterally castrated *M. carinata*⁵ and in another lizard, *Calotes versicolor*⁶. It is suggested that decreased androgenic feedback results in the augmented secretion of gonadotropins and leads to the compensatory increases in the activity of the contralateral testis in mammals⁷. It appears that functional inactivation of the left testis due to the absence of the adrenal gland on that side leads to compensatory hypertrophy of the contralateral (right side) testis in *M. carinata* during recrudescence and breeding phases of the cycle. It is interesting to note that the compensatory hypertrophy of the contralateral testis was not observed in regressing and quiescent phases of the cycle in the present study. The fact that compensatory hypertrophy of the contralateral testis is observed only in recrudescence and breeding phases but not in regressed phase in unilaterally castrated reptiles⁶ supports our view that functional inactivation of the left side testis lead to the compensatory hypertrophy of the right testis in *M. carinata*.

Bilateral adrenalectomy suppresses spermatogenic and steroidogenic activity of the testis on both sides in all the phases of the reproductive cycle in *M. carinata*². In the

present study the regressive changes observed in the left testis following surgical removal of the left adrenal gland are similar to those found in the testis following bilateral adrenalectomy. It is reported that testicular degeneration following adrenalectomy is due to impairment of steroidogenic activity in the testis in mammals⁸⁻¹⁰. It is suggested that adrenal glucocorticoid is essential for the maintenance of spermatogenesis and testicular growth, which appears to be regulated through steroidogenic activity of the testis¹⁰. In *M. carinata* arrest of spermatogenesis following either bilateral or unilateral adrenalectomy was accompanied by a decrease in the activity levels of G-6-PDH and Δ^5 - 3β -HSDH. Hence, it appears that the decreased steroidogenic potential of the testis following adrenalectomy leads to suppression of spermatogenesis in *M. carinata*. The decreased steroidogenic potential following adrenalectomy in *M. carinata* might be due to a lack of influence of corticosteroids, as found in mammals^{9,10}.

It is interesting to note that regressive changes were found only in the left testis following surgical removal of the left adrenal gland. It appears that the adrenal gland exerts a local action on the testis of the same side (ipsilateral). In contrast to other vertebrates, the adrenal gland of reptiles lacks any clear anatomical relationship with the kidney, being more closely related to gonads and gonoducts¹¹. The adrenal gland is situated over the epididymis in *M. carinata* and there is a common artery branching to the testis and to the adrenal gland. The testicular branches pass over the surface of the adrenal. Hence, adrenal secretions might locally influence the testis. However, further studies are needed on the circulation between the adrenal gland and testis to confirm this view.

The adrenalectomy might cause some damage to blood vessels and disrupt the blood circulation to the testis. In the present study care was taken to avoid major damage to the circulation during surgery, and adequate blood supply to the testis was confirmed at autopsy. In our earlier study it was found that bilateral adrenalectomy

causes inhibition of testicular activity and replacement therapy with dexamethasone normalises testicular activity in *M. carinata*¹². Therefore, regressive changes in the ipsilateral testis following unilateral adrenalectomy in *M. carinata* are not due to disruption of blood circulation.

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