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The role of fat body in testicular spermatogenesis and steroidogenesis in *Rana hexadactyla* Lesson

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Abstract. The fat body appears to contribute cholesterol for testicular steroidogenesis. It also appears to provide prostaglandins and cyclic AMP for testicular steroidogenesis since fatectomy impairs this process which is corrected by the addition of prostaglandins and cyclic AMP. Of the two, prostaglandins have a more important role in spermatogenesis and cyclic AMP functions in steroidogenesis These functions of the fat body suggests that it constitutes a link in the hypothalamo-hypophysial-gonadal axis.

Keywords. Fat body; spermatogenesis; steroidogenesis; prostaglandins; cyclic AMP; fatectomy.

Introduction

The fat body is an yellowish organ consisting of finger-shaped processes attached to the caput end of each testis. The spermatogenesis was impaired in *Rana hexadactyla* on removal of the fat body while maintaining conditions for normal spermatogenesis (Kasinathan *et al.*, 1978). This suggested a possible role for the fat body in spermatogenesis and a function as an intermediary in the action of trophic hormones and sex hormones required for testicular function. A similar role of fat bodies in the spermatogenesis has been established in *Rana esculenta* (Chieffi *et al.*, 1975).

In this paper, we report the biological and biochemical role of fat body in *Rana hexadactyla* Lesson with a special emphasis on cholesterol, required for steroidogenesis, and on prostaglandins and cyclic AMP reported to influence spermatogenesis (Marsh and Savard, 1966; Marsh, 1968; Butcher *et al.*, 1968; Bartke and Koerner, 1974; Tso and Lacy, 1975). Histological studies have also been made under different experimental conditions.

Materials and methods

Adult male frogs, *Rana hexadactyla* averaging 55 g body weight and approximately 80 mm snout to vent length (which represents sexual maturity) were selected for investigation. They were fed *ad lib* on live earthworms provided in a

wiremesh box and left in a corner of the aquarium on a stone slab. Fatectomy was done as described earlier (Kasinathan *et al.*, 1978) and the operated frogs were used after 21 days. The animals were grouped into 12 groups of 5 animals each as detailed below, viz., (1) control, (2) prostaglandin F_2 α (PGF_2 α)-treated, (3) PGE_2-treated, (4) cyclic AMP-treated, (5) cAMP + PGF_2 α-treated, (6) cAMP + PGE_2-treated, (7) fatectomised, (8) fatectomised + PGF_2α, (9) fatectomised + PGE_2 and (10) fatectomised + cAMP, (11) fatectomised + cAMP + PGF_2α, (12) fatectomised + cAMP + PGE_2. The fatectomised animals when used for studies on the effect of drugs were shamoperated frogs, kept in the laboratory and injected with buffer for the entire duration of the experiment. The concerned groups were treated with PGs and cAMP as described below:

PGF_2 α and PGE_2 solutions (adjusted to pH 7·4), 100 *μg*/0·1 ml, were prepared (Rosemann and Talkowsky, 1973). The prostaglandins (300 *μg)* were administered in six equally divided doses at 10–12 h intervals into the dorsal lymph sacs of frogs and 12 h after the last injection. The prostaglandins were similarly administered to fatectomised frogs.

Six doses of dibutyryl cAMP 50 g each, spread over a period of 12 h between injections, were administered to normal, PG-treated and fatectomised frogs.

Buffer solution used as vehicle for injections was administered into dorsal lymph sacs of control frogs which were sacrificed along with the experimental groups.

Histology

Testes were collected and fixed in Bouin, sectioned at 7*μ* and stained with Haemalum eosin. Quantitative assessment of spermatogenetic stages was done adopting Van Oordt's (1956) classification. The presence and nature of spermatogonia, sertoli cells, and interstitium have also been noted.

Biochemical studies

Estimation of cholesterol : All estimations were done on individual animals belonging to each group. Testes and fat bodies were extracted in a minimum volume of Falsch mixture (Folsch *et al.*, 1957). Cholesterol was estimated by Leibermann-Burchard reaction (Varley, 1976) after precipitation with digitonin before and after saponification (Venugopala Rao and Ramakrishnan, 1973). Phospholipids were estimated by thin layer chromatographic method (Parker and Peterson, 1965). Triacyl glycerol was estimated by the method of Van Handel and Smith (1957).

Enzymes : Aspartate aminotransferase (E.C. 2·6·1·1) and alanine aminotransferase (E.C. 2·6·1·2) were estimated as described by Chyne (1964). Alkaline phosphatase (E.C.3·1·3·1), acid phosphatase (E.C. 3.1.3.2) and lactate dehydrogenase (E.C. 1·1·1·27) were estimated according to Varley (1976) and King (1965).

Vitamin C : L-ascorbic acid was estimated by the method of Roe *et al.* (1948).

Results

The histological changes in the different groups are depicted as a flow diagram in figure 1.

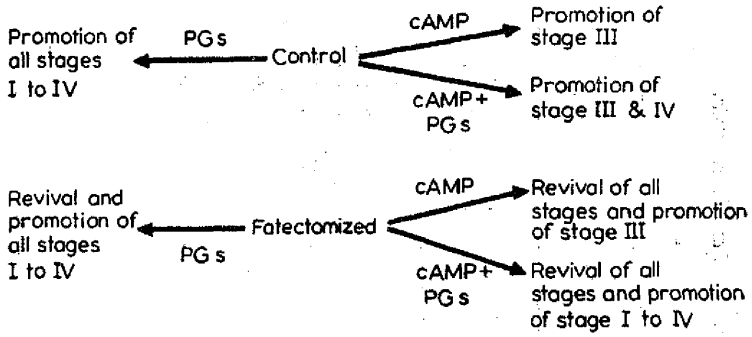

Figure. 1. Effect of PGs and cAMP on spermatogenesis.

Administration of prostaglandins and cAMP caused an increase in weight of the testis and the diameter of the seminiferous tubule (table 1).

Total cholesterol levels in the testis of frogs treated with $PGF_2\alpha$ and PGE_2 were decreased by 31% ($P < 0{\cdot}05$) and 40% ($P < 0{\cdot}001$) (table 2). But fatectomy followed by PG administration exhibited a decrease of 63% ($P < 0{\cdot}001$) and 55% ($P < 0{\cdot}05$). Treatment with cAMP to control animals resulted in a 78% decline ($P < 0{\cdot}001$); a further decrease to 83% ($P < 0{\cdot}001$) was observed in these frogs treated with cAMP and PGs. Fatectomised frogs receiving cAMP alone showed a very significant reduction of 87% ($P < 0{\cdot}05$) whereas in fatectomised frogs receiving both cAMP and PGs, cholesterol content was so low that it could not be estimated.

The total cholesterol in the fat body increased significantly when $PGF_2\alpha$ ($P < 0{\cdot}001$) and PGE_2 ($P < 0{\cdot}05$) were injected to normal frogs. The total cholesterol in the fat body in control frogs treated with cAMP with or without PG showed a marked reduction compared to normal frogs (table 2).

No significant variations were observed in the testicular triacyl glycerol and phospholipids contents under any of the experimental conditions (data not given).

Activities of lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase and acid phosphatase were increased in the fatectomised testes (table 3), while acid phosphatase was undetectable in the fat body.

The level of L-ascorbic acid in the testes decreased in animals injected with either PGs, cAMP or in fatectomy, while vitamin C could not be detected in the fat body. Vitamin C content of testis of normal frog, i.e., $41{\cdot}73 \pm 1{\cdot}2$ (S.E.) was reduced to a range of $30{\cdot}66 \pm 1{\cdot}3$ to $37{\cdot}99 \pm 1{\cdot}7$ ($P < 0{\cdot}05$) in various experimental conditions.

Discussion

Role of fat body in spermatogenesis

These studies have shown that both PGs and cAMP had a profound influence on the spermatogenesis in *Rana hexadactyla* and on the weight of the testes. While cAMP promoted only stage III of spermatogenesis, PGs promoted all the four stages and thus appear to be more potent for testicular spermatogenesis (figure 1).

Table 1. Effect of prostaglandins and cAMP on the spermatogenesis of normal and fatectomised *Rana hexadactyla*.

Conditions	Spermatogenetic stages						Testis weight mg	Lumen diameter μ
	0	1	2	3	4	5		
Initial value	1·24±0·16	2·01±0·24	2·12±0·78	4·04±0·55	2·02±0·62	1·34±0·18	0·984±0·12	321·30±0·94
Control	1·10±0·04	1·64±0·18	1·96±0·12	3·52±0·62	1·86±0·21	0·98±0·52	0·892±0·02	326·00±0·24
$PGF_2\alpha$	1·20±0·12	2·04±0·02	2·98±0·23	4·82±0·04	3·65±0·17	1·20±0·14	1·024±0·14	330·00±0·88
PGE_2	1·19±0·06	2·14±0·08	2·84±0·16	4·75±0·14	3·24±0·01	1·10±0·11	0·998±0·28	326·42±1·26
cAMP	0·92±0·18	1·12±0·62	1·10±0·18	6·10±0·34	2·32±0·14	0·12±0·06	1·124±0·16	312·00±0·18
cAMP + $PGF_2\alpha$	0·84±0·16	0·92±0·22	1·84+0·22	5·84±0·71	4·01±0·52	0·64±0·24	1·261±0·19	326·00±0·42
cAMP + PGE_2	0·98±0·24	1·84±0·52	2·14±0·12	5·12±0·68	4·16±0·19	0·72±0·21	1·082±0·24	330·00±0·14
Fatectomised	0·60±0·12	0·40±0·12	1·02±0·26	2·04±0·18	1·54±0·08	2·05±0·14	0·684±0·92	279·62±0·74
Fatectomy + $PGF_2\alpha$	1·12±0·21	1·84±0·12	2·01±0·12	3·86±0·11	1·94±0·04	1·21±0·02	1·010±0·84	300·00±1·12
Fatectomy + PGE_2	1·18±0·14	1·92±0·04	1·90±0·11	3·62±0·42	1·79±0·16	1·11±0·10	1·092±0·64	312·04±0·57
Fatectomy + cAMP	1·02±0·18	1·58±0·24	2·01±0·14	3·68±0·28	2·14±0·12	1·02±0·14	0·994±0·16	310·00±0·12
Fatectomy + cAMP + $PGF_2\alpha$	1·10±0·29	2·64±0·58	3·56±0·58	4·09±0·58	3·89±0·16	1·14±0·68	1·140±0·68	325·00±0·16
Fatectomy + cAMP + PGE_2	0·98±0·21	1·86±0·18	2·34±0·42	4·21±0·12	3·82±0·64	1·12±0·04	1·021±0·12	318·00±0·42

Values are average of 5 estimations in separate animals ± S. E.

Initial values are from normal animals; Controls are vehicle-treated animals.

Table 2. Total cholesterol content in testes and fat body of *Rana hexadactyla.*

Group	Conditions	Total cholesterol (mg/100 mg tissue)	
		Testes	Fat body
1	Control	2·40±0·10	0·197±0·01
2	+ $PGF_2\alpha$	1·65±0·17**	0·245±0·004**
3	+ PGE_2	1·45±0·09*	0·226±0·002**
4	+ cAMP	0·53±0·06*	0·148±0·001*
5	+ cAMP + $PGF_2\alpha$	0·42±0·07*	0·134±0·002*
6	+ cAMP + PGE_2	0·41±0·09*	0·116±0·001
7	Fatectomy	0·67±0·04*	
8	+ $PGF_2\alpha$	0·91±0·03*	
9	+ PGE_2	1·09±0·10**	
10	+ cAMP	0·30±0·09	
11	+ cAMP + $PGF_2\alpha$	Not detectable	
12	+ cAMP + PGE_2	,,	

Average of six estimations in separate animals ± S.E.

* P < 0·001 **** P < 0·05.

Table 3. Activities[1] of some enzymes in the testes and the fat body of normal and fatectomised *Rana hexadactyla.*

	Lactate dehydrogenase (I.U.)	Aspartate aminotransferase (I.U.)	Alanine aminotransferase (I.U.)	Acid phosphatase (K.A. units)	Alkaline phosphatase (K.A. units)
Normal testes	800	168	Negligible	Negligible	30
Testes from fatectomised	1350	530	305	12	6
Normal fat body	350	418	98	Negligible	26

[1]Activities are expressed per 100 mg tissue.
I.U, International unit; K.A. unit, King-Armstrong unit.

Fatectomy caused impairment of spermatogenesis. As the impairment of all spermatogenetic stages in fatectomised animals was corrected by exogenous administration of PGs and cAMP or an extract of the fat body, it appears that fat body supplies or supplements these substances for spermatogenesis and steroidogenesis. In fatectomised animals, the defective spermatogenesis may be

due to non-availability of adequate PGs and probably cAPM for completion of the process.

Analysis of the data reveals (figure 1) that while PG-like substances from the fat body are necessary for the maintenance of spermatogenesis in Amphibia, cAPM can only help in the proliferation of the spermatocyclic cell-nests of stage III. It has also been noticed that cAMP can act synergistically with PG for the maintenance of the spermatogenesis during the latter half of sperm maturation.

Studies on cholesterol level of testis and fat body have revealed (as summarized in figure 2) that there appears to be an equilibrium between the cholesterol content of the two organs and the fat body seems to contribute cholesterol for possible steroidogenesis. The equilibrium between the two is indicated by the administration of $PGF_2\alpha$ and PGE_2 to control animals when the cholesterol content of fat body is significantly increased (table 2). This could be due to the mobilisation of testicular cholesterol to the fat body, $PGF_2\alpha$ having a greater effect that PGE_2.

The observation that cholesterol from the fat body probably contributes to steroidogenesis is shown by its decrease on the administration of cAMP with or without PGs. This decrease is not due to mobilization of cholesterol from the fat body to the testes, as under these conditions, testicular cholesterol is also decreased significantly (table 2). It appears, therefore, that both testicular cholesterol and fat body cholesterol contribute to steroidogenesis in these tissues. If fat body provides only prostaglandins and cAMP, removal of fat body should cause depleetion to PG and cAMP. In such a condition, i.e., fatectomy, for want of these compounds, testicular cholesterol might not be metabolised to steroids in the usual way and hence there should be an increase in the testicular cholesterol. The drastic decrease from 2·4 to 0·67g/100 g in testicular cholesterol on fatectomy shows that along with cAMP and prostaglandin, the cholesterol of fat body which is probably used for testicular steroidogenesis has been removed. This additional amount of cholesterol has to be contributed by the testes in addition to mobilizing its cholesterol. This would explain decrease of testicular cholesterol in fatectomy.

Though it appears that steroidogenesis is augmented in fatectomy, there is an impairment of spermatogenesis. This shows that increased catabolism of cholesterol alone in the testes cannot maintain normal spermatogenesis and that some aditional factors may be required.

cAMP causes greater utilisation of testicular cholesterol than prostaglandin. A combination of prostaglandins and cAMP has additive effect in depleting most of the testicular cholesterol in experimental animals. As the depletion is aggravated by the combination of prostaglandins and cAMP, their action is synergistic.

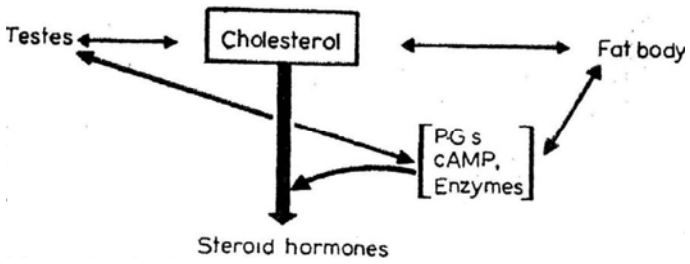

Figure. 2. Biochemical link between testes and fat body steroidogenesis

This is also confirmed by histological findings. While cAMP promotes stage III, both cAMP and prostaglandins promote stages III and IV. As exogenous prostaglandins and cAMP correct the impairment of spermatogenesis in the testes of fatectomised animals, it is suggested that there could be an exchange of prostaglandins and cAMP between the testes and fat body schematically shown in figure 2.

Vitamin C is not detectable in fat body. Hence, fat body could not contribute the same to testicular steroidogenesis. However, conditions under which testicular cholesterol was decreased (i.e., fatectomy, administration of prostaglandins and cAMP) were also accompanied by a decrease of vitamin C in the testes.

The enzyme systems of testes are affected in fatectomy showing increased metabolism in general. Lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase and acid phosphatase are increased in fatectomised testes, while alkaline phosphatase is decreased. Fat body thus seems to help maintain the levels of these enzymes in the testes (figure 2).

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