PROTEIN KINASE ACTIVITIES IN RAT EPIDIDYMIS. EFFECTS OF MATURATION AND TESTOSTERONE TREATMENT [a]

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SUMMARY

Cyclic AMP dependent and independent forms of protein kinases were found in rat epididymis. With histone as substrate cAMP activation of the enzyme was greater than with casein. No activation was seen with protamine. Specific activities of protein kinase were higher in mature than in immature rats.

Testosterone treatment of prepuberal rats increases the activities in caput epididymis.

In mature hemicastrated rats, the caput epididymis from the operated side have lower enzymatic levels than those of the intact side.

In recent years several papers have been published on the study of cAMP-dependent protein kinases. In these publications, the possibility has been envisaged that those enzymes act as mediators of hormonal actions in their target tissues. (1, 2, 3, 4, 5).

Furthermore the specificity of some actions of cAMP has been asigned to the special nature of dependent protein kinases or to the presence of specific substrates in the tissues (5).

The epididymis of the rat is an androgen dependent organ with several, so far, poorly understood physiological aspects.

To our knowledge in this organ the activity of protein kinases and their responsiveness to cAMP has not been study. Moreover, the possibility that these enzymes play a part as mediator for an androgen effect is unknown.

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The present work describes an study of protein kinase activity and its dependence on cAMP in cauda and caput epididymis of the rat. The effects of sexual maturation, castration and testosterone treatment on these activities are also analyzed.

MATERIALS AND METHODS

Mature (90 days) and immature (18 days) Wistar male rats were used. They were killed by a blow on the head and the epididymides quickly removed. The organs were divided into three portions: cauda, caput and corpus. For the present work, only the first two were used.

Treatment of the animals: hemicastration: three rats were orchidectomized on their right side, avoiding damage of the epididymis.

Testosterone administration: testosterone propionate (2 mg) dissolved in sesame oil was injected subcutaneously to four 18 day-old rats. Animals were sacrificed 3 days latter. Three littermate controls were injected with the vehicle.

The caput and cauda epididymides were homogeinized in 4 mM EDTA (pH 7.0), the homogenate centrifuged (3000 x g, 15 min) and the sediment discarded.

Protein kinase assay: protein kinase catalytic activity was determined by the incorporation of phosphate from (γ - ^{32}P) ATP into casein (1 mg) (Fisher Co.) protamine (400 μg) (Free base, Sigma) or histone (200 μg) (Calf thymus, type II, Sigma). The standard assay contained in a final volume of 0.15 ml: 6 μ moles of Na glycerophosphate (pH 6.4), 1.2 μ moles of Na F, 0.045 μ moles of EGTA, 0.3 μ moles of theophyline, 2 μ moles of Mg acetate and 0.3 μ moles of (γ - ^{32}P) ATP with or without 2 mμ moles of cAMP (Sigma). The mixture was incubated at 32°C for 10 min and the reaction was stopped by addition of 2 ml of 5% trichloroacetic acid, 25 μl of 5% bovine serum albumin was added as carrier protein.

The samples were centrifuged and the precipitate dissolved in 0.25 ml of 1 M sodium ammonium phosphate solution. The protein in the samples was then precipitated, washed again and dissolved in 0.1 ml of a 1 M NaOH solution. Finally, the radioactivity was counted in a Packard model 3320 liquid scintillator spectrometer using Bray's solution (6). ATP ^{32}(γ labeled) was prepared by the method of Glynn and Chappell (7)

as modified by Walsh et al (8), and casein as described by Reimann (3).

Protein content was determined according to Lowry (9). Systems lacking the enzyme preparation served as blanks in all assays. Incorporation of ^{32}P into the endogenous protein of the enzyme preparation was insignificant.

RESULTS

1) Protein kinase activity in caput and cauda epididymis of mature and immature rats.

Protein kinase levels (both cAMP dependent and independent activities) in rat epididymides are shown on table I. The activity was studied using three enzymatic substrates: histone, casein and protamine. Data reported on table I demonstrate that the degree of enzymatic activation with cAMP is substrate dependent. Thus, with histone the activation was frankly greater than with casein. With protamine no activation accured.

Comparing the results from immature and mature animals, it is evident that in the latter, protein kinases have higher specific activities when histone or casein were the substrates. This result was not observed with protamine: in contrast in some cases a significant diminution was observed.

No differences in enzymatic activities appear to exist between cauda and caput with casein or histone. Cauda, showed higher protein kinase activities with protamine as substrate.

2) Effect of hemicastration and testosterone treatment on protein kinase activities in cauda and caput epididymis.

Table II shows the action of hemicastration on protein kinase levels in cauda and caput epididymis.

Employing histone or casein as substrate, the caput from the hemicastrated side (ipsilateral) have less enzymatic activity (cAMP dependent and independent) than those from the contralateral ("normal") side, with protamine no changes were detected. In cauda, hemicastration did not produce any variation.

The administration of testosterone propionate (2 mg S.C.) to immature rats produces a significant increase of protein kinase activities in caput epididymis (Table III).

Table I

Protein kinase activity in caput and cauda epididymis of mature and immature rats (in p moles of ^{32}P incorporated/mg protein/min) (a)

Group	cAMP	CAPUT EPIDIDYMIS			CAUDA EPIDIDYMIS		
		Histone	Casein	Protamine	Histone	Casein	Protamine
Immature rats (18 day-old)	-	135 ± 9	245±22	387 ± 57	138 ± 27	253±11	638 ± 66 (c)
	+	305 ±15	313±32	435 ± 40 (c)	336 ± 41	348±14	598 ± 67
	Increase with cAMP in %	126	28	12	143	37	- 6
Mature (90 day-old)	-	252±24	348±17	328±60.	263 ± 15	288±21	463 ± 21 (c)
	+	487±19	457±21	302±18 (c)	662 ± 29	435±11	476 ± 17
	Increase with cAMP in %	93	31	- 8	152	51	3
Increase with maturation P<	(b)	0.001	0.02	——	0.01	0.05	——

(a) Experimental conditions as described in Materials and Methods.
(b) P values are obtained by "t" test comparing immature v.s. mature animals.
(c) Statistical significant ($p < 0.05$) dimimution in mature with respect to immature.

Table II

Effect of hemicastration on protein kinase activities in cauda and caput epididymis (in p moles of ^{32}P incorporated/mg protein/min) (a)

Group	cAMP	CAPUT EPIDIDYMIS			CAUDA EPIDIDYMIS		
		Histone	Casein	Protamine	Histone	Casein	Protamine
Epididymis from hemicastrated side	-	117 ± 12	197 ± 2	261 ± 23	225 ± 7	282 ± 9	418 ± 28
	+	254 ± 31	253 ± 25	279 ± 37	533 ± 53	433 ± 28	399 ± 19
	Increase with cAMP in %	117	28	7	137	53	- 4
Epididymis from intact side	-	194 ± 8	294 ± 9	254 ± 19	222 ± 13	262 ± 15	328 ± 24
	+	531 ± 20	429 ± 29	309 ± 14	569 ± 19	419 ± 15	382 ± 48
	Increase with cAMP in %	174	46	22	156	60	16
Diminution with hemicastration P< (b)		0.01	0.01	N.S.	N.S.	N.S.	N.S.

(a) Experimental conditions as described in Materials and Methods.

(b) P values are obtained by "t" test comparing "hemicastrated side" v.s. "intact side"

Table III

Effect of testosterone administration on protein kinase activities of caput epididymis from immature rats (in p moles of ^{32}P incorporated/ mg protein/min) (a)

Group	Without cAMP	With cAMP	Increase with cAMP in %
Control	101 ± 6	292 ± 42	190
Testosterone Treated	172 ± 6	390 ± 13	128
Increase with testosterone	(b) $p < 0.001$	(b) $p < 0.05$	---

(a) Experimental conditions as described in Materials and Methods employing histone as substrate.
(b) P values are obtained by "t" test comparing control v.s. treated.

DISCUSSION

The results reported in this paper support the following conclusions:

1) Cyclic AMP activation of rat epididymal protein kinase is higher with histone than with casein as substrates.

2) With both substrates, mature animals have higher protein kinase specific activities in their cauda and caput epididymis than immature rats.

3) In hemicastrated rats the caput epididymis from the operated side has minor enzymatic levels than the one of the intact side.

4) Testosterone administration to immature rats produced a significant increment in protein kinase activities in caput epididymis.

5) Using protamine as substrate the enzymatic activity was not cAMP dependent, immature animals having higher activities than mature rats.

In both cases, cauda epididymis show higher values than caput. In a previous paper Reddi et al (10) reported the presence of enzymes catalyzing the phosphorylation of many different proteins in rat testes by ATP. The authors found that these enzymatic activities were not altered by the

hormonal "status" of the animals. Furthermore, the prostatic phosphorylating capacity was not influenced by orchidectomy nor by androgen administration. On the other hand K.Ahmed (11), employing both nuclei isolated from rat ventral prostate and nuclear phosphoproteins, found that, castration caused a decline in the rate of ^{32}P incorporation into phosphoprotein, this fact having been completely prevented by testosterone administration.

The values obtained in the present work show a dependence of protein kinase activities on the hormonal treatment in the epididymis. Besides the maturation process and the hemicastration have a clear action on these activities.

That different substrates originate a dissimilar dependence on cAMP points to the heterogenous nature of protein kinase. However, further research will be necessary in order to confirm the presence of various protein kinases, having differents specificities and/or different degrees of cAMP dependence.

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