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RENIN-LIKE ACTIVITY IN THE RAT EPIDIDYMIS

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Abstract: 'Whole' cauda epididymal homogenate and individual components of the cauda epididymis viz, cauda epididymal cells, epididymal plasma and sperm cells, as well as the blood plasma of the rat were screened for renin-like activity by *in vivo* method.

Enzyme activity was high in 'whole' cauda epididymal homogenate, but very low in blood plasma. Evaluation of individual components of the epididymis revealed a relatively high enzyme activity in the epididymal cells but low activity in epididymal plasma and epididymal sperm cells. The high activity in epididymal cells was not affected by efferent duct ligation for 8 weeks and bilateral nephrectomy. Like most other epididymal functions, renin-like activity in the epididymis was androgen dependent. It was concluded that cauda epididymis of the rat contains the enzyme renin, whose activities may be predominantly intracellular.

Key words: renin-angiotensin, cauda epididymis, renin-like activity, sperm cells, epididymal plasma, angiotensin I

INTRODUCTION

Besides its absorptive functions, the epididymal epithelium actively secretes small organic molecules such as glyceryphosphorylcholine (GPC:17, 7), sialic acid (16), acid glycogen (11), hydrolytic enzymes (8), and a variety of proteins (9, 10). Till date, the precise function(s) of most of these organic molecules are yet to be ascertained. However, the weight of evidence seem to suggest that they may be related to sperm cell maturation and function.

A number of secretomotor agonists, including angiotensin II (AgII), are known to stimulate fluid secretion by activation β-receptors on the basolateral membranes of the epididymal epithelium. These secretory agonists utilize cyclic AMP, but not Ca^{2+} or inositol phosphates, as intracellular second messengers (4, 18, 19). There is now evidence that AgII is elaborated by the rat epididymis (21). The proteolytic enzyme, renin, catalyzes the formation of angiotension I (AgI) from angiotensinogen and therefore initiates the first reaction in the renin-angiotensin cascade. This study sought to evaluate, by *in vivo* method, the presence of renin in the rat epididymis and its possible role in epididymal function.

METHODS

Experimental animals: Male Sprague-Dawley rats weighing between 400 and 450 g were used for the experiment. They were reared under specific pathogen free conditions with a 14 hr/10 hr dark schedule. Standard rat chow and tap water were supplied *ad libitum.*

Collection of epididymal fluid/sperm cells: Rats were anaesthetised with pentobarbitone sodium (SAGATAL) at a dose of 60 mg/kg by intraperitoneal injection. Cauda epididymal plasma and epididymal sperm cells were collected by microdissection and microperfusion technique based on a method described by Wond and Lee (20).

Preparation of tissue homogenate: After collection of epididymal fluid and epididymal plasma, the posterior vena cava of each rat was cannulated for exsanguination and 0.9% (W/V) physiological saline infused into the rats via aortic catheter connected to Harvard infusion pump (Bard, U.K.). The luminally perfused epididymal duct (about 20 cm) was carefully unravelled and dissected free of connective tissue. The epididymal tissue was chopped into small pieces and homogenised in 500 µl sodium phosphate buffer with polytron (Kinematica, Switzerland) at position 4/5 setting (60 sec, 4°C). The homogenate was centrifuged at 10,000G for 5 min (4°C) and the resulting supernatant stored at –20°C.

Sperm cells: Sperm cells in test tubes were washed three times with 2 ml of assay buffer by centrifugation at 800G. The cells were sonically disrupted with Fisher sonic dismembrator (Artek, U.S.A.) using microtip probe (30 sec, 4°C) in 200 µl assay buffer and centrifuged at 10,000G (60 sec, 4°C). The recovered supernatant was stored at –20°C.

Collection of blood plasma: Blood plasma was collected from the carotid artery of the rat under sodium pentobarbitone anaesthesia (60 mg/kg, ip). EDTA was used as anticoagulant.

Bilateral nephrectomy: A 2-3 cm dorsoventral incision was made on the left/right lateral abdominal wall near the costal border under anaesthesia. As far away from the kidneys as possible, a single ligature was placed on the renal artery, renal vein and ureter of each kidney and then transected. The abdominal incisions were closed with wound clips and the animals allowed to recover. Twentyfour hours later, blood plasma was collected from the rats as described.

Efferent duct ligation and castration: In order to assess the contributions of testicular secretions and androgen on the renin-like activity in the cauda epididymis, efferent duct ligation and bilateral orchidectomy respectively were performed on a group of rats. The materials and methods have been described elsewhere (21).

Renin-like activity determination: To determine the renin activity in the tissues, a product-linked method in which renin-like activity was calculated by measuring the rate of generation of AgI by putative renin during incubation was adopted. For an optimum generation of AgI the incubation conditions and the amount of enzyme substrate pool needed to saturate the tissue enzyme were carefully worked out. When sufficient enzyme substrate to saturate the tissue enzyme is not ensured, the incubation time becomes a critical factor (3). Similarly, the chosen pH profile (6.0) for the reaction was found optimum for AgI generation during incubation.

For the enzyme assay, 5 µl of the diluted tissue extracts (epididymal cells/sperm cells) in assay buffer (pH 6.0) containing captopril (1 mmol: SQ 14, 225); EDTA (5 mmol); PMSF (2 mmol) and neomycin sulfate (3 mmol) was incubated for 1 hr in water bath at 37°C with 95 µl of blood plasma from anephric rats as enzyme substrate pool.

For epididymal fluid and blood plasma, 20 µl of the diluted test sample in assay buffer (pH 6.0) containing captopril (1 mmol) and the above angiotensinase inhibitors was incubated with 80 µl of the enzyme substrate pool.

The assay tubes were shaken at 15 min intervals during incubation and the reaction terminated after 1 hr by freezing at –20°C. For each sample, a duplicate was prepared but held at 4°C as non-generated (blank) control.

Angiotensin I concentration was determined by radioimmunossay using a standard assay kit (Amersham International plc, Amersham, Buckinghamshire, U.K.).

Fifty µl of diluted radiolabelled peptide was added to each assay tube, followed by 100 ul of the antiserum. The reaction mixture was vortex mixed and incubated at 4°C for 24 hr. Serial dilution of standard AgI was prepared in assay tubes and run in parallel with the test samples. The separation procedure and determination of AgI concentrations were as described previously (21). Mean recovery of exogenous AgI added to

the test sample was 92 ± 2.40%. The antiserum to AgI showed less than 0.5% cross reaction with AgII. The within assay coefficient of variation was 9% with a sensitivity of 5 fmol/assay tube. The results were expressed as ng AgI/hr/mg protein x 10^{-3}. Protein concentration was estimated by the method of Lowry et al (12), using bovine serum albumin (BSA) as standard.

RESULTS

The disposition of renin-like activity in the rat cauda epididymal tissue and blood plasma is presented in Table I. The enzyme activity was high generally in the 'whole' cauda epididymis comprising epididymal cells and luminal contents. However, when the individual components of the cauda epididymis were analysed, activity was found to be very high in epididymal cells but relatively very low in epididymal plasma and sperm cells. The enzyme activity in epididymal cells was significantly ($P<0.01$) higher than that measured in the blood plasma.

TABLE I : Renin-like activity in the cauda epididymal tissue and blood plasma of the rat. Values represent the mean ± SEM with number of animals used in parenthesis. †denotes cauda epididymal cells and the luminal contents.

Sample	*n*	*Renin-like activity (ng AgI/hr/mg prot. x 10^{-3})*
Whole cauda epididymis †	(5)	994.0 ± 25.0
Epididymal plasma	(5)	7.0 ± 1.0
Epididymal cells	(5)	961.0 ± 86.0
Sperm cells	(4)	3.0 ± 0.4
Blood plasma	(4)	16.0 ± 1.0

Effect of efferent duct ligation on renin-like activity: Eight weeks after efferent duct ligation, renin-like activity in the sperm free cauda epididymides of the ligated testis was slightly elevated. However, the increase was not statistically significant (Table II).

TABLE II : Effect of efferent duct ligation on renin-like activity in the cauda epididymis of the rat. Values are the mean ± SEM. Number of animals used are indicated in parenthesis.

Sample	*n*	*Renin-like activity (ng AgI/hr/mg prot. x 10^{-3})*
Contralateral (unligated) testis	(4)	726.1 ± 54.5
Ipsilateral (ligated) testis	(4)	776.4 ± 42.1

Effect of castration and bilateral nephrectomy on renin-like activity: Renin activity was significantly ($P<0.01$) reduced in the castrated group (242±28.12 ng AgI/hr/mg prot. x 10^{-3}, n=8 compared with 726±54.11 ng AgI/hr/mg prot. x 10^{-3}, n=8 for control). The degree of attenuation was 68.7%.. This effect was reversed by testosterone replacement therapy (695±32.14 ng AgI/hr/mg prot. x 10^{-3}, n=8) for 8 weeks.

In contrast, enzyme activity was slightly elevated above control levels inepididymal cells of bilaterally nephrectomised rats, whereas activity was undetectable in the blood plasma of anephric rats (Table III).

TABLE III : Effect of bilateral nephrectomy on the renin-like activity in the cauda epididymal cells and blood plasma of rats. Results are the mean ± SEM, with number of animals shown in parenthesis.

*Renin-like activity in blood plasma of anephric rats was below the detection limit of assay technique.

Sample	*n*	*Renin-like activity (ng AgI/hr/mg prot. x 10^{-3})*
Control	(4)	737.0 ± 11.0
Experimental	(4)	770.0 ± 23.0
Blood plasma	(4)	*Not detectable

DISCUSSION

In this study, renin-like activity was demonstrated in the cauda epididymal tissue as well as the blood plasma of the rat.

In the determination of renin activity, the assay protocol employed satisfied the optimum conditions required for AgI generation at a near neutral pH (6.0), although possible interference by other aspartyl protease enzymes was not excluded since these have been found to exhibit the propensity to generate AgI at acid pH due to their structural similarities with renin.

Enzyme activity was very high in the 'whole' cauda epididymal homogenate (Table I) but this activity was more concentrated in epididymal cells with relatively little activity in epididymal plasma and spermatozoa. This seemingly low activity in sperm cells fits well with the observations of other investigators in differentiating germ cells of human (14) and rat (15) testis. Thus, it is conceivable that renin expression in the epididymis is an intracellular phenomenon in epithelial cells since very low activity was found in the fluid flushed out from the cauda epididymides of the rat. This view has been supported by the experiment on efferent duct ligation in which the testis was functionally separated from the epididymis for eight weeks. As indicated in the results (Table II), renin activity in the ligated testis compared favourably with the activity in the contralateral unligated side. This high activity in the epididymal cells could not have been derived from the blood but synthesised *de novo* by epididymal cells since virtually no activity was found in the blood plasma of rats subjected to both efferent duct ligation and bilateral nephrectomy; whereas activity in the ligated and unligated testis of the rats were again statistically not different.

This study further complements and extends an earlier report of an endogenous renin-angiotensin system in the cauda epididymis of the rat (21) which may function as a paracrine agent in the regulation of epididymal function.

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