Angiotensin converting enzyme in human seminal plasma is synthesized by the testis, epididymis and prostate

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Summary

The activity of angiotensin converting enzyme (ACE) was assessed in human body fluids (serum, seminal plasma, prostatic secretions), in tissue extracts of the testis, epididymis, prostate and skeletal muscle, in split ejaculates and in seminal plasma obtained from patients before and after vasectomy. To ensure the specificity of the results the dependence of ACE activity on specific inhibitors was evaluated. Enzyme activity found in tissues of the male genital tract was considerably higher than that in serum and other tissues. ACE in human seminal plasma is synthesized by the testis, epididymis and prostate in different amounts.

Keywords: ACE in seminal plasma, ACE activity in tissues of the male genital tract, origin of ACE activity.

Introduction

Angiotensin converting enzyme (E.C.3.4.15.1), a membrane-bound glycoprotein, is detectable in all tissues and body fluids of mammals (Soffer, 1976). The enzyme plays a key role in the renin-angiotensin-aldosterone system (Peach, 1977). Several tissues have been found to contain multiple forms of the enzyme (van Sande, Scharpe & Neels, 1985a). A 140 kD form of ACE has been purified from human lung, kidney and testis. In addition, the testis contains a 90 kD form of ACE (Lanzillo *et al.*, 1985), while from seminal plasma, the isolation of a 330 kD enzyme has been reported (Depierre, Bargetzi & Roth, 1978). Pulmonary ACE splits off the C-terminal dipeptide from the dekapeptide angiotensin I (A-I) to give the vasoconstrictor angiotensin II (A-II). The enzyme is able to cleave a variety of other peptides not structurally related to A-I (Ondetti & Cushman, 1982). ACE involvement in bradykinin inactivation has been demonstrated (Erdös & Yang, 1966; Ng & Vane, 1968; Kaneko & Moriwaki, 1981). Several natural and synthetic peptides have been identified as ACE inhibitors (Depierre, Bargetzi & Roth, 1979; Ondetti & Cushman, 1984). The release of C-terminal tripeptides by ACE has also been reported (Scharpe *et al.*, 1986).

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The discovery that ACE inhibitors are effective anti-hypertensive drugs (Ondetti, Rubin & Cushman, 1977) led to the assumption that this enzyme is involved in the regulation of blood pressure. The primary cellular localization of ACE is in vascular endothelial cells near the luminal surface, juxtaposed to the circulation (Caldwell *et al.*, 1976; Vivet, Callard & Gamoudi, 1987). In several tissues, ACE is associated with enkephalinase (E.C.3.4.24.11), a neutral metallo-endopeptidase with similar catalytic properties (Erdös *et al.*, 1985).

The highest ACE activities in man are detectable in prostatic secretions (Yokoyama *et al.*, 1980) and seminal plasma (Krassnigg *et al.*, 1984; van Sande *et al.*, 1985b) (Table 1). However, its role in fertilization, if any, is unknown.

Table 1. ACE activity in human body fluids (means $\pm$ SD). Numbers in parentheses indicate the number of samples assayed

Body fluid		ACE activity (mU/ml)
Seminal plasma (normozoospermic men)	(40)	1980.0 ± 640.7
Seminal plasma (chronic inflammation of the male genital tract)	(72)[*]	1690.0 ± 864.4
Prostatic secretions (message via the rectum)	(15)	1498.0 ± 19.0
Serum (males)	(36)	37.5 ± 9.0
Serum (pulmonary sarcoidosis)	(34)[†]	69.0 ± 26.0

[*] Significantly different from seminal plasma of normozoospermic men ($P < 0.05$) by Mann–Whitney *U*-test.
[†] Significantly different from values in normal serum (Alberts, Van der Schoot & van Daatselaar, 1983).

To identify the tissues responsible for synthesis and to estimate their contribution to ACE activity in seminal plasma, extracts of testis, epididymis and prostate were investigated. The results were compared with ACE activity in skeletal muscle, serum, seminal plasma, split ejaculates, prostatic secretions and seminal plasma obtained from patients before and after vasectomy.

Materials and methods

Semen samples and tissue pretreatment
Semen samples were obtained from patients of the Andrological Outpatient Service of the Department of Dermatology, University of Munich, after 4–5 days of sexual abstinence (Schirren, 1982). Two hours after liquefaction the samples were centrifuged ($600 \times g$, 30 min, $4°C$). Seminal plasma was stored until use at $-20°C$. Split ejaculates (Schirren, 1982) and semen obtained from patients before and after vasectomy were pretreated identically. Serum was collected from andrological patients. Tissue specimens were obtained by open urological surgery. Prostatic fluid was obtained by digital massage via the rectum. All tissues were frozen in liquid nitrogen immediately after receipt. Homogenization of the minced tissues was carried out at $0–4°C$ for 1 min in 0.9% NaCl (2.0 ml/1.0 g of tissue). Debris was removed by centrifugation ($1500 \times g$, 60 min, $4°C$) and the supernatants were analysed for protein concentration and ACE activity.

Determination of ACE activity

ACE activity was measured according to Cushman & Cheung (1971b). To ensure the specifity of the results the dependence of the enzyme activity on proteinase inhibitors and the specific ACE inhibitor captopril was evaluated (Table 2). In all ACE activity determinations, hippuryl-L-His-L-Leu (HHL) was used as substrate. Non-specific proteolytic activity which could interfere in the assay was suppressed by addition of n-butanol (2.5%; v.v) (Hara, Fukuyama & Epstein, 1971). ACE activity has been expressed in mU/ml of body fluid (1 U being the amount of enzyme that produces 1 µmol hippuric acid/min) or in mU/mg protein in tissue extracts.

Table 2. Effect of inhibitors on ACE activity in male serum, seminal plasma and in extracts of male genital organs

	Serum (males)	Seminal plasma	Tissue extracts		
			Testis	Epididymis	Prostate
EDTA (10^{-4} mole/1)	0.9	0.6	0.2	0.1	0.5
Gossypol (10^{-6} mole/1)	1.1	0.7	0.7	1.3	2.7
Captopril (10^{-9} mole/1)	8.4	4.6	1.9	5.5	0.9
Bradykinin potentiator B (10^{-3} mole/1)	0.4	1.1	3.8	2.0	1.9

Data are expressed as the concentration required to inhibit ACT activity by 50%.

All chemicals used in the assays and for pretreatment of the samples were of analytical grade. The synthetic ACE substrate HHL was obtained from Bachem (Switzerland). Gossypol acetic acid (equimolar crystalline complex) and bradykinin potentiator B were purchased from Sigma (USA). Captopril was kindly supplied by Squibb-Heyden (Germany).

Determination of protein concentration

Protein concentration was estimated according to Lowry *et al.* (1951) using bovine serum albumin as standard.

Statistical methods

Data on ACE activity were analysed by the Mann–Whitney *U*-test.

Results

ACE activity was estimated in identically pretreated tissue extracts from epididymis, testis, prostate and skeletal muscle, and in seminal plasma, prostatic secretions and serum from males. The ACE values determined were regarded as specific when more than 90% of the enzyme activity could be inhibited by 10 µmol/l captopril. In human body fluids great differences in ACE activity were detectable (Table 1). The catalytic properties of ACE in body fluids and in male genital organs were in good agreement (Table 2). The highest enzyme level in organs from the male genital tract was found in prostatic extracts. Epididymis and testis also

contained raised levels of ACE activity in comparison to the values detectable in extracts of skeletal muscle (Table 3).

Data obtained from split ejaculates demonstrated that the prostate contributes a large amount to the ACE activity in seminal plasma. Thus, the first fraction of the split ejaculate, which is derived largely from prostate secretions, contained the highest ACE activity (Table 4).

ACE activity was measured in seminal plasma from vasectomized patients before and after surgery. After vasectomy, ACE values decreased significantly, suggesting that $43.1 \pm 4.7\%$ of the enzyme activity originates from the epididymis and testis (Table 5). The estimated ACE activity in prostate secretions obtained from urological patients was 1489.0 ± 19.0 mU/ml. In comparison, seminal plasma from normozoospermic men contained 2152.0 ± 182.0 mU/ml ACE activity (Table 6).

Table 3. ACE activity (mU/mg protein) in tissue extracts of male genital organs and skeletal muscle (means $\pm$ SD)

	Epididymis	Testis	Prostate	Skeletal muscle
ACE activity	16.4 ± 3.1	12.5 ± 1.8	28.0 ± 2.5	3.1 ± 1.1
Protein (mg/ml)	3.7	3.6	3.9	3.8
Number of samples	14	16	5	5

Table 4. ACE activity (mU/l) in split ejaculates (means $\pm$ SD)

	Split ejaculates	
	Fraction I	Fraction II
ACE activity	2490.0 ± 902.3	1640.1 ± 788.7
Range	$890 - 3880.1$	$460.0 - 3331.0$
Number of samples	19	19

Table 5. ACE activity (mU/ml) in seminal plasma obtained from patients before and after vasectomy

Patient no.	Before vasectomy	After vasectomy	Difference	Decrease (%)
1	2156.0	1238.0	918.0	42.6
2	1970.0	490.0	1480.0	75.0
3	2212.1	1352.0	860.1	39.0
4	5170.0	2830.0	2340.0	45.0
5	2100.0	1130.0	970.0	46.0
6	1790.0	1360.0	430.0	24.0
7	2240.0	1195.0	1045.0	47.0
8	2220.0	1420.0	800.0	36.0
9	2184.0	1472.0	712.0	33.0
(mean)	2449.1	1387.4	1061.7	43.1

Table 6. Comparison of ACE activity (mU/ml) in prostatic secretions and in seminal plasma of normozoospermic men (means $\pm$ SD)

	Prostatic secretions	Seminal plasma
ACE activity	1489.0 $\pm$ 19.0	2152 $\pm$ 182.0
Range	1470.0 – 1505.0	1960.0 – 2324.0
Number of samples	15	30

Discussion

Determination of ACE activity in body fluids and tissues is of diagnostic importance in several diseases (Schweisfurth, 1980). Raised levels of ACE in serum have been found in sarcoidosis (Silverstein *et al.*, 1976). In hyperthyroidism the hyperactive thyroid gland is probably not the source of the enhanced ACE values (Cushman & Cheung, 1971a). Increased ACE activity in diabetic patients, especially those with retinopathy, has also been reported (Lieberman & Sastre, 1980). As vascular endothelial cells are regarded as sites for ACE synthesis (Ryan *et al.*, 1975), it is assumed that diffuse vascular damage in diabetic patients could be responsible for release of the enzyme into the circulation. The diagnostic importance of ACE activity determinations in seminal plasma has been demonstrated in the evaluation of patients with chronic inflammation of the male accessory sex glands (Hohlbrugger, Pschorr & Dahlheim, 1984). Also of diagnostic interest are the high ACE levels in benign prostatic hypertrophy (van Sande *et al.*, 1985b).

In animals, the age-dependent occurrence of ACE in the testis and epididymis is well documented (Jaiswal *et al.*, 1984). Precise age-related studies have revealed that immature male animals have no detectable ACE activity in their genital organs. However, the enzyme activity appears when sperm become detectable in the testis and epididymis during the course of sexual maturation (Jaiswal *et al.*, 1983). Other investigators have demonstrated the androgen-dependence of ACE activity (Rohen, 1978). This is supported by data indicating enhancement of ACE activity in the testis of animals by sexual stimulation. This suggests a possible involvement of the enzyme in sperm maturation. However, other groups have been unable to support these findings, since they demonstrated nearly identical levels of ACE activity in the caput and cauda epididymis (Hohlbrugger, Schweisfurth & Dahlheim, 1982). The prostate is also the main source for the kininogenase kallikrein (tissue kallikrein; E.C.3.4.21.35) of the male genital tract (Fink *et al.*, 1985). Kallikrein is involved in the stimulation of sperm motility (Schill & Haberland, 1974). ACE has no comparable effect (Placzek *et al.*, 1986). Otherwise A-I and A-II seem to enhance sperm motility *in vitro* comparable to the action of bradykinin (Mizutani & Schill, 1985).

The method used for ACE determination in the present study is regarded as highly specific as it enabled determination of enzyme activity in the presence of other proteolytic activity. Our results show the highest levels of ACE in prostatic tissue and raised levels in the epididymis compared with levels in the testis. This is in agreement with the results of immunohistochemical studies (Vivet *et al.*, 1987).

The levels of ACE activity in prostatic secretions were reduced in comparison to seminal plasma, indicating possible contamination of the prostatic fluid with secretions from the seminal vesicle. No significant differences in the catalytic properties of ACE from the tissues investigated were detectable. The measured ACE activity in split ejaculates and in seminal plasma obtained from patients before and after vasectomy demonstrates the notable contribution of the testis and epididymis to the total ACE activity in seminal plasma.

Acknowledgment

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