ADENOSINE TRIPHOSPHATASE SYSTEMS IN GENITAL TRACT OF TESTOSTERONE TREATED MALE ADULT MONKEYS

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Summary : Studies on the distribution of Na^+, K^+ -dependent, Mg^{2+} dependent and Ca^{2+} dependent Adenosine Triphosphatase (ATP-ases) in the testes, epididymis, seminal vesicles and prostate glands of mature bonnet monkeys were carried out with and without Testosterone propionate (TP) treatment. Comparatively, the Ca^{2+} dependent ATP-ase was very active in the testes, caput and cauda epididymis and prostate of control animals. However, the Mg^{2+}-dependent ATP-ase activity was predominant in the seminal vesicles, In all the genital tissues the Na^+, K^+ dependent ATP-ase exhibited low activity compared to other ATP-ase systems. On TP treatment at 1 *mg/kg* body wt. dose for 30 consecutive days to the second group of animals, all classes of ATP-ases drastically decreased in the testes, cauda epididymis, seminal vesicles and prostate. While in caput epididymis the Mg^{2+} -dependent ATP-ase was stimulated, the Na^+, K^+ -dependent ATP-ase was decreased both in the caput and corpus epididymis by the hormone treatment. The present study reveals the general inhibitory influence on the ATP-ase systems and thereby ionic transport after long term TP administration.

Key words : Na^+, K^+-ATP-ase Mg^{2+}-ATP-ase Ca^{2+}-ATP-ase   testes
                 epididymis   seminal vesicles   prostate   mature monkey

INTRODUCTION

The adenosine triphosphatases (ATP-ases) in normal mammalian tissues and cells are associated with the intracellular formed elements (8). Na^+, K^+–ATP-ase is an integral part of Na^+, K^+–pump and the splitting of adenosine triphosphate (ATP) provides the energy needed for the active transport of Na^+ and K^+ ions. The Na^+, K^+ gradient in animal cells controls cell volume, renders nerve and muscle cells electrically exicitable, and drives the active transport of sugars and amino acids (14). Similarly, the Ca^{2+}–ATP-ase is an inetgral part of the Ca^{2+} pump, and Ca^{2+} is the intermediary between the nerve impulse and muscle contraction. The transport of Ca^{2+} by the sarcoplasmic reticulum is driven by the hydrolysis of ATP (3). The Mg^{2+} ions in both these systems has a stimulatory effect on the ionic transport. The association of ATP-ases with microsomes, mitochondria and nuclear membranes and the androgenic regulation of these

enzymes in accessory sex organs of rats and monkeys have been documented (1,2).   Further, the enzymatic hydrolysis of ATP by seminal plasma has been reported (7).

In sexually mature animals the accessory sex organs are composed largely of tall columnar epithelial cells that actively secrete fluids which contribute extensively to the seminal plasma (11).   The ATP-ase systems conditioned by Na^+, K^+, Mg^{2+}; Ca^{2+} ions might play an important role in ionic translocation that accompany the secretary functions in these secretory tissues.   Thus, an attempt has been made to delineate the distribution pattern of the three classes of ATP-ases in the testes and accessory sex glands of mature bonnet monkeys so as to understand the type of cationic pump operating in the genital tissues.   In addition the modulatory influence of long term treatment with testosterone propionate (TP) on this enzyme systems in intact animals has been studied as these enzymes are under androgenic control.

MATERIAL AND METHODS

Animals : Mature male bonnet monkeys (Macaca radiata), 4-5 years old and 6-8 *kg* body weight were used in the present investigation.   They were housed in a well ventilated temperature controlled animal house with a constant 14 hrs light and 10 hrs darkness schedule.   Clean water and standard monkey diet (Gold Mohur, Hindustan Lever Ltd., India) were made available to them *ad libitum*.

The animals were divided into two groups of five each,   One group was given daily injections of testosterone propionate (1 *mg/kg*, body wt) in propane-1,2-diol for a period of 30 days intramuscularly.   The control group received the vehicle only.   All the animals were sacrificed 24 hrs after the last injection by anesthetizing with an overdose of 30 *mg/kg*, body wt of sodium pentabarbitone in water injected intraperitoneally.   The testes epididymis, seminal vesicles and prostate gland were dissected out, cleared off from adhering connective tissue and blood vessels, washed in saline, blotted and weighed accurately on torsion balance to the nearest 0.01 *mg* and stored below -20°C until enzymatic assays were carried out.

The tissues were homogenised in 10 volume of 0.1M Tris-HCl buffer (pH 7.5) using a Teflon homogenizer and then centrifuged at 10,000 x g at 4°C for 15 min and the supernatant was taken for the assays.

The method of Shiosaka *et al.* (13) was used to assay the Na^+, K^+, Ca^{2+}, and Mg^{2+} dependent adenosine triphosphatases (E.C. 3.6.1.3). The final incubation mixture consisted of 0.2 *ml* Tris-HCl buffer (pH 7.5); 0.1 *ml* of enzyme (tissue extracts); 0.1 *ml* ATP solution (11.023 *mg* in 1 *ml* water) and 100 mM/0.1 *ml* of $MgCl_3$/NaCl/KCl/Ca Cl_2 as in

the case of specific assay. The blank contained distilled water instead of enzyme extract. The reaction mixture was incubated at 37°C for 15 min and the reaction terminated with 2 ml of 5% TCA. The tubes were kept in cold (4°C) for 30 min and centrifuged at 3000 rpm at room temperature for 5 min. The inorganic phosphorus present in the supernant was determined colorimetrically by the method of Fiske and Subba Row (4).

The unit of enzyme activity is defined as micrograms of inorganic phosphorus (Pi) formed/min/gm tissue. The data were analysed statistically using Students 't' test (12).

RESULTS

The distribution pattern of ATP-ases in the testes and epididymis are shown in Table I. In control animals, the Ca^{2+}-dependent ATP-ase was very active in the testes, caput and cauda epididymis. Mg^{2+}-dependent ATP-ase exhibited medium activity. The Na^+, K^+-dependent ATP-ase showed the least activity in all the tissues studied. However, in the corpus epididymis, the activity of Mg^{2+}-dependent ATP-ase was higher

TABLE I : Distribution of Na^+, K^+-ATP-ase, Mg^{2+}-ATP-ase and Ca^{2+} ATP-ase
in the reproductive tissues of mature male monkeys.@

Organs	Group	Adenosine triphosphatases (μg Pi formed/min/gm tissue)		
		$Na^+ K^+$-ATP-ase	Mg^{2+}-ATP-ase	Ca^{2+}-ATP-ase
Testes	Control	181.76±11.46	336.50±42.40	352.57±24.59
	Exp.	46.58±5.34•••	88.82±7.63•••	176.28±14.51•••
Epididymis	Control	100.41±9.45	15.64±0.91	115.06±10.11
Caput	Exp.	29.15±1.96•••	27.45±3.10•••	110.15±7.63
Cauda	Control	145.63±11.47	157.44±13.58	233.67±21.68
	Exp.	114.63±8.89•	120.11±9.14•	105.81±9.79•••
Corpus	Control	115.06±10.24	122.60±7.65	118.76±15.47
	Exp.	32.57±12.46•••	105.74±12.46	137.81±11.52
Seminal	Control	397.94±26.59	605.87±55.55	589.51±40.14
vesicles	Exp.	97.21±7.66•••	99.34±5.84•••	135.40±15.01•••
Prostate	Control	369.21±34.58	514.13±22.14	560.68±15.58
gland	Exp.	68.91±5.85•••	55.68±3.68•••	40.71±0.01•••

@Each value is Mean ± S.E.M. of 5 experiments.
Exp = TP administration at 1 mg/kg. body wt/day/30 days.
•P<0.05;   •••P<0.001   vs control group

than the Ca^{2+}-dependent ATP-ase, Testosterone propionate administration brought about drastic decrease ($P<0.001$) in the activities of all classes of ATP-ases in the testes and cauda epididymis.   However, in the caput epididymis, the Mg^{2+}-dependent ATP-ase was markedly activated ($P<0.001$), while the Na^+ K+-dependent ATP-ase activity was significantly inhibited ($P<0.001$).   In the corpus epididymis the Na+, K+-dependent ATP-ase was lowered by the hormone treatment whereas the activities of other classes of ATP-ases were not altered.

The changes in the ATP-ase systems in the prostate and seminal vesicles of normal and TP treated animals are illsutrated in Table I.   While Mg^{2+} -dependent ATP-ase was the most active enzyme in the seminal vesicle of control monkeys, the Ca^{2+}-dependent ATP-ase was the predominant enzyme in the prostate of these animals.   The lowest activity was exhibited by the Na^+, K+-dependent ATP-ase in both these tissues.   TP adminsitration induced a significant ($P<0.001$) decrease in all three enzyme systems in both seminal vesicle and prostate similar to that observed in the testes and caudal segment of the epididymis.

DISCUSSION

The distribution pattern of ATP-ase enzyme systems in the genital tissues of control monkeys reveal the active involvement of Ca^{2+} pump probably with the muscular contraction and secretory activities of the accessory sex organs except the seminal vesicles and corpus epididymis.   The activation of this system is known to be accompanied by increased calcium uptake in the cells and phosphoryl transfer which requires both Ca^{2+} and Mg^{2+} ions (6).   Comparatively greater Ca^{2+}-ATP-ase   activity has been observed by Ahmed and Williams-Ashman (1) in their in vitro studies on rat prostatic preparations.   Arunakaran et al. (2) have also demonstrated very high concentration of Ca^{2+}-dependent ATP-ase in nuclear and mitochondrial fractions of prostate compared to the seminal vesicles of mature monkeys.   In immature monkeys however, the Ca^{2+}-pump has been shown to be very active in the seminal vesicles rather than the prostatic gland (9).   Sexual maturation of the animal probably alters the tissue specificity to a particular ATP-ase system.

The preferable activation of the Mg^{2+}-pump in the seminal vesicle of control animals probably suggests the derivation of Mg^{2+}-stimulated ATP-ase activity from a contractile (myosin-type) ATP-ase originating from the fibromuscular cells of this glandular tissue (1).   The specific impact of the cationic pump in the seminal vesicles, prostate and corpus epididymis is well seen by the high concentrations of all classes of ATP-ases in these genital tissues (15) which also will reflect the activation of the ionic transport associated with secretions of these glandular tissues.

Castration in rats causes a decrease in the specific activity of Na^+, K^+-dependent ATP-ase in the prostate and seminal vesicles and these changes are prevented by androgen replacement in these castrated animals (5). Further, addition of Mg^{2+} and either K^+ and Na^+ ions alone to prostatic microsomal membrane preparations obtained from castrated adult rats have restored the Na^+, K^+–ATP-ase activity (1). Thus, androgen deprival has been shown to result in a virtual obliteration of extra liberation of inorganic phosphorus induced by Na^+, K^+, and Mg^{2+} ions.

In contrast to these observations, our study with non-castrated intact monkeys shows that long term androgen administration to these animals causes inhibition not only of Na^+, K^+–pump in all the genital tract tissues studied but also inhibits Mg^{2+} and Ca^{2+} pumps with the exception of the caput and corpus epididymis. This observation suggests the probable adverse effect of the androgen on the biosynthesis of the above enzyme systems. In agreement with our report is the study of Ahmed and Williams-Ashman (1), who have observed a decrease in the Na^+, K -dependent ATP-ase activity in ventral prostate of intact rat after testosterone administration. Similarly, Kumaran et al. (9) have shown a significant decrease in Ca^{2+} dependent ATP-ase in the seminal vesicles of testosterone treated non-castrated monkeys.

The activation of magnesium pump in the caput with no great impact on other cationic pumps by testosterone administration probably indicates the stimulation of Mg^{2+}-dependent ionic transport associated with the secretory activity of this segment.

Thus, the present study reveals not only the differential response of the various genital tissue ATP-ase systems to TP treatment but also indicates the general inhibitory effect of the hormone on the enzyme systems if given for a long term to intact monkeys.

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