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A POSSIBLE MECHANISM OF REGULATION OF RAT PROSTATE FUNCTION BY TESTOSTERONE*

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SUMMARY

1. The hypothesis that testosterone stimulates cation transport in the rat ventral prostate was tested.

2. Uptake of K^+ by minced prostate tissue was significantly stimulated by testosterone (10^{-7} M) and inhibited by ouabain (0.5 mM). The androgen was not effective on liver mince or kidney cortex slices.

3. Brief (15 min) pretreatment of prostate microsomes with various concentrations of testosterone (10^{-8}–10^{-6} M) showed that the steroid maximally stimulated ($Na^+ + K^+$)-dependent, ouabain-sensitive ATPase, but not Mg^{2+}-dependent, ($Na^+ + K^+$)-independent ATPase. Analogues of testosterone including 19-nortestosterone, 17β-hydroxy-5α- and 5β-androstan-3-one and 3α-hydroxy-5α- and 5β-androstan-17-one were ineffective to slightly inhibitory.

4. It is suggested that the elements of the plasma membrane which, on hormonal stimulation, cause accelerated sodium pump activity may be the hypothetical receptors regulating prostatic growth and function; that this regulation is attributable to the metabolic pace-setting action of the Na^+-pump apparatus.

INTRODUCTION

It was shown recently that testosterone (10^{-7} M) significantly increased the production and outflow of citrate from rat prostate minces[1] incubated with malate *plus* [^{14}C]acetate. On finding that almost none of the added ^{14}C of the treated group appeared as $^{14}CO_2$ it was suggested that the radioactive citrate was being selectively removed from the cell through either passive diffusion or active transport. If the anion, citrate, is indeed being removed by an active process, there must be either a compensatory influx of some other anion or an equal efflux of a cation to achieve electrical neutrality across the plasma membrane. In addition, since citrate efflux is increased by the presence of testosterone, it follows that the compensatory anion influx or cation efflux may also be affected by the steroid. This study was undertaken to test the effect of testosterone on cation movements, *i.e.* on the so-called Na^+-pump apparatus.

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MATERIAL

Ventral prostate, kidney cortex and liver of 300–350 g Wistar rats were used. The animals were anesthetized by intraperitoneal administration of sodium pentobarbital (20 mg in 1 ml of 0.9 % NaCl solution), injected with 300 units heparin *via* tail or renal vein, decapitated and thoroughly bled. Liver, kidney and ventral prostate were excised and stored in ice-cold Ringer solution to deplete $[K^+]$ and increase $[Na^+]$ within the cells[2]. Liver and prostate were finely minced with scissors at 0°. Slices of kidney cortex were prepared in the cold room with a Stadie-Riggs microtome.

METHODS

Incubations were made of 400–500 mg prostate minces, 500 mg kidney cortex slices or 800 mg liver mince in 6 ml of Ringer phosphate buffer (pH 7.4) containing 1 mg glucose and 1.5 μmoles $MgCl_2$ per ml, and, where indicated, steroid (10^{-7} M), for 30 min at 37° in a Dubnoff metabolic incubator continuously gassed with O_2 (4 l/min).

Following incubation, the tissue was immediately centrifuged for 10 min in the cold at 1000 × *g* and the supernatant medium decanted as completely as possible. The tissue pellet was spread on clean paper and carefully blotted to remove as much as possible of the adhering extracellular fluid. To disrupt the cells and thus release the intracellular contents, the blotted pellets were suspended in 1.5 ml distilled water and held in an ice bath during sonic disruption. Of the homogeneous suspension, 0.5 ml was transferred to a separate vessel for determination of DNA by the procedure of STUMPF[3]. The remaining 1 ml of suspension was frozen, thawed and centrifuged at 1600 × *g* for 10 min to yield a clear supernatant fraction upon which K^+ concentration was determined by flame photometry. The mequiv K^+ per g dry weight of tissue was calculated using the separately determined information that 1 mg dry weight of tissue is equivalent to 16 μg DNA.

ATPase assay. Ventral prostatic microsomes were isolated in 0.25 M sucrose *plus* 0.05 M Tris–HCl (pH 7.5) by the procedure of SCHNEIDER AND HOGEBOOM[4] with the one exception that the centrifugal force employed to sediment mitochondria was increased from 15000 × *g* to 18000 × *g*. The microsomes were taken up in a quantity of 75 mM Tris–HCl (pH 7.4) containing, in 0.5 ml, either $MgCl_2$ (2.5 μmoles), NaCl (100 μmoles) and KCl (25 μmoles) (Soln. A) or $MgCl_2$ (2.5 μmoles) and ouabain (0.5 mmole) (Soln. B) such that 1 ml contained the equivalent of 44 mg wet weight of tissue (88 μg microsomal protein). 0.5-ml aliquots of each preparation were dispensed into tubes prepared by adding ethanol or an ethanolic solution of steroid and evaporating off the solvent under N_2.

Following preincubation of the four sets of tubes at 37° for 15 min, Tris–ATP (2.5 μmoles in 0.5 ml Tris–HCl) was added to all tubes and incubation was continued at 37° for 20 min. The reaction was stopped by the addition of 2.0 ml of 10 % trichloroacetic acid containing 5 g acid-washed charcoal (Norit) per 100 ml. Following centrifugation, the P_i of an aliquot of the supernatant fluid was determined by the procedure of FISKE AND SUBBAROW[5]. The activity of the $(Na^+ + K^+)$-dependent ATPase was calculated from the difference in P_i liberated in Solns. A and B per mg microsomal protein per h.

RESULTS

Effect of testosterone on potassium uptake. Table I shows that the intracellular K^+ concentration of tissue incubated in the presence of testosterone (10^{-7} M) is significantly greater than in the tissue concurrently incubated without the steroid. The increase in potassium from the depleted concentration achieved during storage at 0° is nearly 30% greater in treated than in untreated tissue. That the uptake of K^+ involves the Na^+ pump is clearly suggested by the marked fall in K^+ concentration found when ouabain is present during the incubation.

Tissue specificity. In order to confirm this finding and determine if the response to the steroid was peculiar to prostatic tissue or was a non-specific effect, a second series was performed in which responses of prostate, liver and kidney were concurrently tested. It is apparent from Table II that of the tissues examined only the prostate exhibits a significant response to the steroid.

Testosterone on microsomal ATPase. In all tissues in which this type of system has been studied, it has been found that the transport process is coupled to a microsomal, $(Na^+ + K^+)$-dependent, ouabain-sensitive ATPase[6].

When prostatic microsomes were isolated and tested for this enzyme, a dose-related inhibition by ouabain was found (Fig. 1). Over 150 trials showed that approx. 20% of the total microsomal ATPase is dependent upon activation by Na^+ and K^+.

It appeared that the greater uptake of K^+ by minces incubated with testo-

TABLE I

EFFECT OF TESTOSTERONE AND OUABAIN ON INTRACELLULAR K^+

K^+ concentration was depleted by storage of tissue in Ringer solution at 0° for 1 h. Tissue was then incubated 30 min at 37° in Ringer phosphate buffer (pH 7.4) containing glucose (0.1%) *plus*, where indicated, testosterone (10^{-7} M) or ouabain (0.5 mM).

Group	*Treatment*	*Trials*	$[K^+]$ (*mequiv/g*)	*Difference between groups*			*P*
				Groups	*mequiv/g*	%	
1	Depleted	22	97.70 ± 13.08				
2	Control	32	125.00 ± 1.97	2 *vs.* 1	+27.3	+27.9	< 0.01
3	Testosterone	31	133.00 ± 2.31	3 *vs.* 1	+35.3	+36.1	< 0.01
				3 *vs.* 2	+ 8.0	+29.3	< 0.02
4	Ouabain	14	39.10 ± 9.84	4 *vs.* 1	−58.6	−60.0	< 0.01

TABLE II

TISSUE SPECIFICITY OF TESTOSTERONE ON K^+ UPTAKE

Conditions as in Table I. N.S.: not significant by inspection. Figures in parentheses are number of trials.

Tissue	$[K^+]$ (*mequiv/g*)			*t*	*P*
	Control	*Treated*	*Difference*		
Prostate	110 (16)	136 (19)	+26	1.96	< 0.05
Liver	102 (18)	82 (18)	−20	1.29	> 0.20
Kidney	98 (15)	104 (15)	+ 4		N.S.

sterone might be due to an action on the cation-dependent ATPase system. Table III shows that this is indeed the case, there being nearly 25 % greater activation of the enzyme in steroid-treated than in control microsomes. Extensive analyses have revealed no effect of testosterone on $(Na^+ + K^+)$-independent, Mg^{2+}-dependent ATPase.

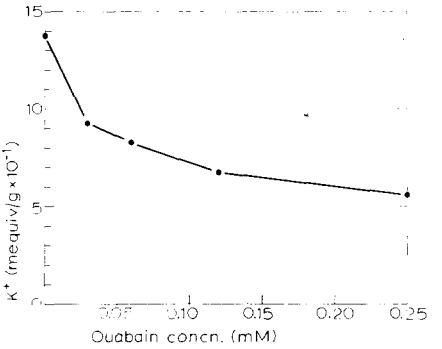


Fig. 1. Effect of ouabain concentration on intracellular K^+ concentration. Following storage for 1 h at 0° in Ringer phosphate buffer (pH 7.4), portions of the tissue were incubated 30 min at 37° in fresh Ringer buffer containing glucose (0.1 %) and the indicated concentrations of ouabain.

TABLE III

EFFECT OF TESTOSTERONE ON $(Na^+ + K^+)$-DEPENDENT ATPase OF PROSTATIC MICROSOMES

Assay of $(Na^+ + K^+)$-dependent ATPase is described under METHODS. Per cent activation is [(P_i of tube A − P_i of tube B)/P_i of tube B] × 100 %.

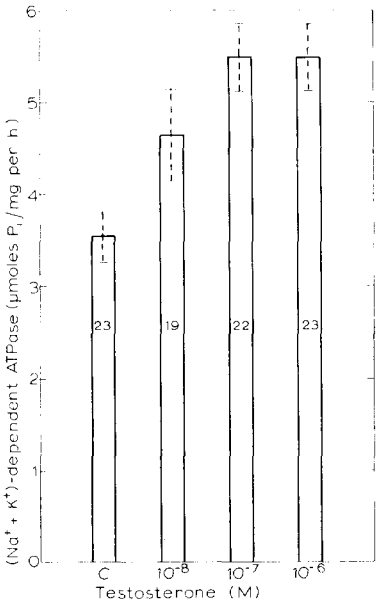
Group	*Number of trials*		*Activation by $Na^+ + K^+$ (%)*
Control	13		7.9
Treated*	21		9.9
		Difference	2.0
		% Difference	25.3
		S.E.	1.095
		$P \approx 0.02$	

* Testosterone (10^{-7} M).

The level of $(Na^+ + K^+)$-dependent ATPase activity is of the same order of magnitude as is found in cerebral microsomes[7]. To determine if the testosterone concentration (10^{-7} M) was optimal for activation, a second series of assays was performed to compare activation by 10^{-8} M, 10^{-7} M and 10^{-6} M. Fig. 2 shows again that the 10^{-7} M concentration significantly increases the cation-dependent ATPase activity, that an increase to 10^{-6} M is no more effective than 10^{-7} M while a decrease to 10^{-8} M produces a hydrolytic rate intermediate between that of the control and the 10^{-7} M group although not significantly different from either of them ($0.2 > P > 0.1$). Therefore, 10^{-7} M testosterone does effect maximal activation.

It was important to determine if the stimulatory effect of testosterone on the microsomal enzyme was specific or was duplicable by related but biologically less active steroids. The effects of testosterone, 19-nortestosterone, 17β-hydroxy-5α- and

5β-androstan-3-one and 3α-hydroxy-5α- and 5β-androstan-17-one as stimuli of membrane ATPase are compared in Fig. 3. Of these compounds only testosterone is an effective stimulus. The other compounds tend to inhibit the reaction. This finding has been confirmed.



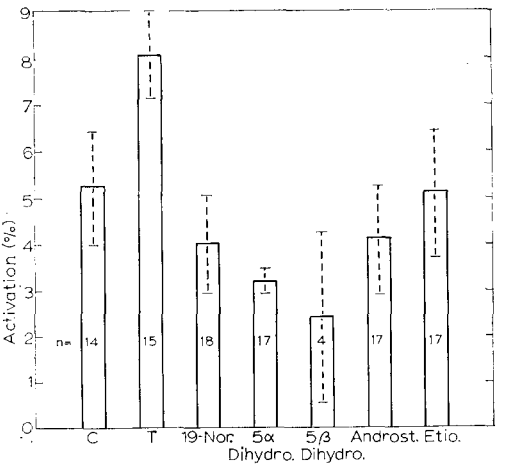


Fig. 2. Response of $(Na^+ + K^+)$-dependent ATPase to change in testosterone concentration. Microsomes, pretreated with the indicated concentrations of testosterone were assayed for $(Na^+ + K^+)$-dependent ATPase.

Fig. 3. Steroid specificity of prostatic $(Na^+ + K^+)$-dependent ATPase. Prostatic microsomes, were assayed for $(Na^+ + K^+)$-dependent ATPase following pretreatment with the following steroids at 10^{-7} M: C, control; T, testosterone; 19-Nor., 19-nortestosterone; 5α-Dihydro., 17β-hydroxy-5α-androstan-3-one; 5β-Dihydro., 17β-hydroxy-5β-androstan-3-one; Androst., 3α-hydroxy-androstan-17-one; Etio., 3α-hydroxy-androstan-17-one. Vertical bars indicate range of 1 S.E. Figures on bars are number of trials. Activation is expressed as in Table III.

DISCUSSION

These studies show that testosterone does accelerate those activities of the rat prostate which are characteristic of the sodium pump as described by SKOU[9]. If one accepts the argument presented in the INTRODUCTION, a coupling of the movement of anions to the transport of cations may explain the increased evolution of citrate in testosterone-treated prostatic minces.

Of considerably greater significance is the demonstration that the cation-dependent, ouabain-sensitive ATPase activity of prostatic microsomes is increased by treatment of these particulates *in vitro*. Since, by definition, microsomes are fragments of the plasma membrane and the endoplasmic reticulum, it appears that one or both of these are quite directly responsive to hormonal stimulation. This conclusion is borne out by the hormone-evoked increase in K^+ uptake.

It is tempting to speculate that the hormone-responsive elements of the plasma

membrane may be the long-sought receptors[10] through which androgen effects regulation of prostatic growth and function. While far more work is needed to determine if this is true, the studies of BLOND AND WHITTAM[11] and WHITTAM AND AGER[12] showing that the metabolic pace of erythrocytes and kidney cortex cells is set by the rate of cation transport are compatible with this interpretation and suggest the type of investigation which is required.

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