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THE INFLUENCE OF TESTOSTERONE ON PENETRATION OF α-AMINOISOBUTYRIC ACID AND 2-DEOXYGLUCOSE IN MALE RAT SEX ACCESSORY TISSUES

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SUMMARY

Cellular penetration of α-aminoisobutyric acid and of 2-deoxyglucose was measured in sex accessory tissues of the castrated male rat 3 to 18 h after a single intravenous injection of testosterone. The hormone had no significant effect on water content or extracellular (inulin) volumes over 18 h in ventral prostate, seminal vesicle and levator ani muscle. α-Aminoisobutyric acid distribution in prostate and seminal vesicle was increased significantly 6 (but not 3) h after testosterone injection and the tissues were concentrating α-aminoisobutyric acid against a concentration gradient by the 18th h; levator ani was not affected until the 12th h. The hormone did not stimulate uptake of 2-deoxyglucose *in vivo* except in levator ani at h 18. Pretreatment with testosterone for 12 h caused significant increases in α-aminoisobutyric acid uptake in all tissues over a 60-min incubation period; 2-deoxyglucose *in vitro* also was taken up in larger amounts, and against a gradient, in all hormone-treated tissues except levator ani. In each tissue *in vitro*, the increased α-aminoisobutyric acid distribution due to testosterone was reduced to or below controls by low temperature (20°; Q_{10} of 1.9 to 2.0), anoxia or the absence of Na, and by the presence of dinitrophenol, ouabain or alanine. The accumulation of 2-deoxyglucose *in vitro* after hormone pretreatment was depressed below untreated controls in the presence of glucose or phlorizin.

From these experiments it may be concluded that within 6 or 12 h of injection, testosterone increased cellular penetration of a model amino acid and a hexose sugar in male sex accessory organs. The process requires energy, is sodium dependent and apparently utilizes carrier systems in common with natural substrates. The temporal and causal relationships between these events and hormone-stimulated metabolism are discussed.

INTRODUCTION

The action of estrogenic hormones on transport of several natural and model substrates in uterus is receiving extensive study[1-4]. However, similar investigations of androgen effects on male sex accessory tissues are fragmentary; experimental

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design varies widely and measurements have not been made earlier than numbers of hours or days after testosterone administration, intervals now known to be well beyond the time of onset of metabolic events stimulated by the hormone. Thus, increased phosphorus accumulation by seminal vesicles was reported to occur 83 h after a single hormone injection[5]; a daily dose of testosterone for 1 to 2 weeks increased uptake of the non-utilizable amino acids, α-aminoisobutyric acid in muscle[6] and cycloleucine in kidney[7]; α-aminoisobutyric acid uptake after a single hormone injection was elevated by the 39th h in levator ani muscle[8]. On the other hand, it has more recently been shown that RNA metabolism of seminal vesicles[9] and prostatic RNA polymerase activity[10] are apparently increased within 60 min, and that protein synthesis in seminal vesicle is elevated at the 12th h[11], after hormone administration.

In the present experiments, the nature of the permeability changes and their timing relative to stimulated metabolic events are examined. The time course of penetration of non-utilizable model substances, α-aminoisobutyric acid and 2-deoxyglucose, are measured in male sex accessory tissues after a single intravenous injection of testosterone. The findings demonstrate an increased cellular penetration of tracer molecules within 6 to 12 h after hormone administration, the time of onset varying with the tissue and tracer studied. Stimulated uptakes of α-aminoisobutyric acid and 2-deoxyglucose are energy dependent and are inhibited in the presence of natural substrates.

MATERIALS AND METHODS

Male Holtzman rats, 250 to 450 g and 2 to 4 months old, were used throughout and were orchidectomized 12 to 16 days prior to use. The animals were maintained under 14 h light and 10 h darkness with standard rat chow and water available *ad libitum*. Ether anesthesia was used throughout.

In experiments *in vivo*, rats were given a single intravenous dose of testosterone (1 mg/100 g body wt.) 3, 6, 12, 18, or 24 h before sacrifice. The hormone solution (5 mg/ml) was prepared by suspending finely ground testosterone propionate (Nutritional Biochemical) in saline with the aid of a drop of Tween 80 (1:4 dilution). Controls received only saline with Tween 80. Twenty min before sacrifice, the renal veins were ligated through a midventral abdominal incision and the incision closed. Fifteen min prior to sacrifice, the appropriate ^{14}C-labeled compound, 8 to 15 μC in 0.1 ml of saline, was injected into the saphenous vein. The labeled substances utilized included: [*carboxy*-^{14}C]inulin (New England Nuclear), 2-deoxy-D-[1-^{14}C]glucose (New England Nuclear) and α-amino[1-^{14}C]isobutyric acid (Tracerlab and Calbiochem). Animals were sacrificed by exsanguination through the dorsal aorta. The ventral prostate, seminal vesicles and levator ani were quickly removed, freed of extraneous fat, blotted and weighed to the nearest 0.2 mg; blotting was performed to remove only surface fluid except in seminal vesicles where vigorous blotting was required to remove seminal secretions. Tissues were analyzed for isotope distribution according to the procedure outlined further below. Water contents of all tissues were determined by wet–dry weight difference in separate groups of rats not receiving isotope but which otherwise were treated as described.

In experiments *in vitro*, ventral prostate, seminal vesicles, levator ani and coagulating gland were removed from exsanguinated animals 3, 6, or 12 h after treatment

with control saline or testosterone propionate (1 mg/100 g body wt.) in saline. Seminal vesicles were freed of adhering coagulating gland and slit longitudinally prior to incubation; prostate and coagulating gland were incubated intact. The levator ani was removed together with a small piece (1 mm) of bulbocavernosus attached to each end, which allowed removal of the levator ani without cutting fibers of that muscle; the extraneous muscle was removed and discarded after incubation and prior to analysis of the samples. Tissues were temporarily stored in ice-cold Krebs–Ringer bicarbonate buffer, then incubated in the buffer (pH 7.4) for 30 to 90 min in sealed 25-ml erlenmeyer flasks containing 0.1 μC/ml of the appropriate labeled compound; the flasks were gassed with 95 % O_2:5 % CO_2 for 20 sec before sealing and then placed in a Dubnoff metabolic shaker (116 excursions/min) at 36°. Concentrations of test materials included: α-aminoisobutyric acid (0.5 mmole/ml), 2-deoxyglucose ($9.4 \cdot 10^{-6}$ mmole/ml) and inulin (0.028 mg/ml). In some experiments the following were added to medium containing 0.5 mmole/ml α-aminoisobutyric acid: alanine ($2 \cdot 10^{-2}$ mole/ml) or the inhibitors, dinitrophenol ($5 \cdot 10^{-4}$ mole/ml) or ouabain ($1 \cdot 10^{-3}$ mole/ml); incubations lasted 60 min in these cases. In another experiment, tissues were preincubated for 15 min in 0.05 M Tris buffer prior to addition of enough α-aminoisobutyric acid to bring the final concentration to 0.5 mmole/ml. A gas phase of 95 % N_2:5 % CO_2 was used in a subsequent anaerobic experiment. The uptake of 2-deoxyglucose was measured in Krebs–Ringer bicarbonate buffer containing glucose (10 mmole/ml) and phlorizin (10^{-4} or 10^{-3} mmole/ml). Following incubation, tissues were rinsed for 6 sec in saline, blotted, weighed and rendered as outlined below.

Tissues were analyzed according to a modification of the technique of SPAZIANI AND GUTMAN[2]. Samples obtained directly from the animals (experiments *in vivo*) were placed in 3 ml of 0.75 M NaOH and heated in a boiling water bath until completely dissolved. In the experiments *in vivo* with 2-deoxyglucose and in all experiments *in vitro*, the tissues were finely minced in 3 ml of distilled water and boiled in a bath for 35 min. Both extraction procedures yielded quantitative recovery of radioactivity. Samples were cooled, neutralized where necessary with 1 M HCl, diluted to 10 ml with water, centrifuged and 1-ml aliquots plated in tared steel planchets. Either 0.1 ml of plasma or 0.1 ml of incubation medium was also treated as above and plated. Counts were obtained in an ultra-thin window gas-flow counter (Nuclear-Chicago) and corrected to infinite thinness.

Results in all experiments are expressed as % space (volume of distribution, ml/100 g tissue) calculated from:

$$\frac{\text{counts/min per g wet wt.} \times 100}{\text{counts/min per ml plasma (or incubation medium)}}$$

In preliminary experiments not involving hormone administration, the ^{14}C-labeled test molecules were injected intravenously and the animals sacrificed 15, 30, or 60 min later. The resulting values for % space were then used in determining the rate of equilibration of the labels between plasma and tissues.

RESULTS

Preliminary studies *in vivo* were conducted in which no hormone was administered and labeled inulin was injected intravenously; this substance ordinarily pene-

TABLE I

INULIN DISTRIBUTION IN MALE SEX ACCESSORY TISSUES OF CASTRATED CONTROL AND TESTOSTERONE-TREATED RATS

Conditions	% *Space (ml/100 g fresh tissue wt.)*		
	Levator ani	*Ventral prostate*	*Seminal vesicle*
A. In vivo			
1. Inulin circulation time (min)			
15	9.5 ± 3.4 (8)*	37.2 ± 9.9 (8)	29.1 ± 2.8 (8)
30	11.9 ± 0.8 (4)	42.2 ± 3.4 (4)	31.7 ± 3.2 (4)
60	11.3 ± 1.2 (6)	37.1 ± 4.7 (6)	29.4 ± 4.0 (6)
2. Time (h) after testosterone injection**			
0	8.6 ± 1.5 (12)	29.5 ± 3.3 (12)	23.9 ± 2.6 (11)
12	8.0 ± 1.6 (5)	34.1 ± 4.3 (5)	27.8 ± 5.0 (5)
24	8.6 ± 1.4 (7)	29.6 ± 5.4 (7)	28.4 ± 4.1 (7)
B. In vitro			
1. Incubation time (min)			
a. Controls			
30	17.9 ± 4.5 (4)	33.5 ± 2.2 (4)	28.5 ± 1.3 (4)
90	20.7 ± 3.3 (5)	43.2 ± 5.2 (5)	29.4 ± 2.9 (5)
b. Testosterone pretreated***			
30	15.7 ± 1.0 (4)	37.7 ± 7.6 (4)	30.6 ± 0.3 (4)
60	19.1 ± 2.5 (4)	48.8 ± 5.3 (4)	29.0 ± 2.5 (4)

* Mean ± S.D.; number of animals in parentheses.
** Inulin circulation time held constant at 15 min.
*** Testosterone injected 12 h before sacrifice.

trates only the interstitial spaces of a tissue and was used to determine the extent of the extracellular compartment. Animals were sacrificed 15, 30 and 60 min after injection. In all tissues, inulin was fully equilibrated between plasma and the extracellular space after 15 min circulation time (Table I, A1). Pretreatment with testosterone for 12 or 24 h failed to alter significantly the extracellular compartment of any tissue investigated (Table I, A2).

Table I, B, summarizes the extracellular (inulin) spaces of male sex accessory tissues measured *in vitro*. Equilibrium with the medium was achieved in levator ani and in seminal vesicles within 30 min of incubation. When inulin spaces measured *in vivo* are compared with those *in vitro* (Table I), it is apparent that incubation significantly altered the extracellular compartment in prostate and levator ani but not in seminal vesicles. Incubation of prostates for 90 min significantly increased the inulin space above that after 30 min of incubation ($P < 0.02$). In common with findings *in vivo*, testosterone pretreatment did not alter the extracellular space of any tissue tested *in vitro*, including the coagulating gland (not shown in the table); inulin space for coagulating gland of controls or 12-h hormone-treated rats was 33 % after 60 min of incubation.

Table II lists the total water content of levator ani, ventral prostate and seminal vesicles measured *in vivo* and *in vitro*. Statistical analysis of variance showed no significant differences between tissues from hormone-treated and control animals.

TABLE II

WATER CONTENT OF MALE SEX ACCESSORY TISSUES IN CASTRATED CONTROL AND TESTOSTERONE-TREATED RATS

Conditions	% *Water (ml/100 g fresh tissue wt.)*		
	Levator ani	*Ventral prostate*	*Seminal vesicle*
A. In vivo			
1. Time (h) after testosterone injection			
0	76.2 ± 0.5 (14)*	83.1 ± 1.1 (14)	76.4 ± 1.1 (14)
3	76.9 ± 0.2 (4)	82.2 ± 0.8 (4)	76.6 ± 0.7 (4)
6	76.0 ± 0.5 (4)	82.6 ± 0.8 (4)	75.8 ± 1.7 (4)
12	76.4 ± 0.2 (4)	83.6 ± 1.0 (4)	77.5 ± 0.7 (4)
18	76.8 ± 0.4 (4)	83.4 ± 1.0 (4)	77.6 ± 0.7 (4)
B. In vitro			
1. Incubation time (min)			
a. Controls			
30	80.8 ± 1.0 (4)	86.0 ± 0.8 (4)	78.6 ± 0.8 (4)
90	81.0 ± 0.8 (4)	86.1 ± 1.1 (4)	78.8 ± 0.4 (4)
b. Testosterone pretreated**			
30	79.1 ± 0.7 (4)	85.0 ± 0.1 (4)	78.1 ± 1.2 (4)
90	81.2 ± 0.2 (3)	85.8 ± 0.4 (4)	79.1 ± 1.0 (4)

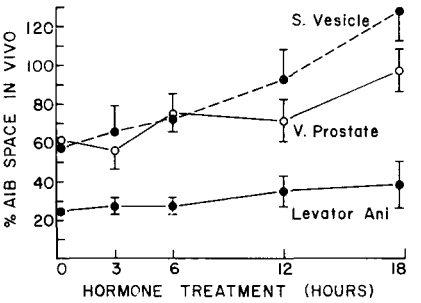
* Mean ± S.D.; number of animals in parentheses.
** Testosterone injected 12 h before sacrifice.

Water content data from the groups were subsequently averaged and the water volumes *in vivo* compared with those *in vitro*; incubation of the tissues resulted in a significant increase in % water over *in vivo* levels ($P < 0.001$ in all cases).

In preliminary uptake measurements not involving hormone treatment, 10 to 15 μC of ^{14}C-labeled α-aminoisobutyric acid was injected into control animals which were sacrificed 15, 30 or 60 min later. Tissue α-aminoisobutyric acid did not reach equilibrium with plasma within 60 min; at the 60-min interval, α-aminoisobutyric acid spaces were 48 ± 6 % for levator ani, 95 ± 12 % for prostate and 93 ± 13 % for seminal vesicles. It is apparent that α-aminoisobutyric acid penetrated tissue compartments greater than the extracellular volume and greater than the total water volume of prostate and seminal vesicles but not of levator ani.

The effects of 3 to 18 h of testosterone pretreatment on α-aminoisobutyric acid uptake *in vivo* are shown in Fig. 1. All animals were sacrificed 15 min after intravenous injection of 10 to 15 μC of ^{14}C-labeled α-aminoisobutyric acid. The α-aminoisobutyric acid space values in seminal vesicles rose from 59 ± 9 % in 27 untreated control rats to 73 ± 7 %, 93 ± 15 % and 129 ± 16 % after 6, 12 and 18 h of testosterone treatment, respectively. Each increase is statistically significant ($P < 0.01$ in each case) and the 18-h value represents cellular accumulation against a gradient. Three-hour treatment with testosterone, however, produced no significant change over controls. α-Aminoisobutyric acid uptake in ventral prostate increased significantly from 60 ± 10 % in controls to 76 ± 10 %, 72 ± 11 % and 98 ± 11 % after 6, 12 and 18 h of hormone pretreatment, respectively ($P < 0.01$ in each case). Again, 3-h treatment

produced no significant change. Levator ani muscle was less responsive to testosterone than the glandular tissues (Fig. 1). Pretreatment for 12 and 18 h gave α-aminoisobutyric acid distribution values of 34 ± 8 % and 38 ± 12 % which were significantly greater than the control value of 25 ± 6 % ($P < 0.01$ in each case). Six-hour treatment was without effect.



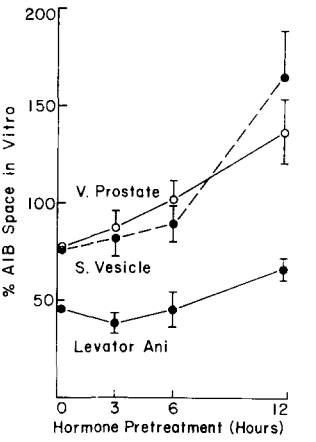


Fig. 1. Distribution *in vivo* of α-amino[^{14}C]isobutyric acid with time after testosterone treatment; α-aminoisobutyric acid (AIB) injected 15 min before sacrifice in each case. Each control point (0 time) is an average of 27 animals; other points, 5 to 6 animals. Standard deviations are shown.

Fig. 2. Distribution *in vitro* of α-amino[^{14}C]isobutyric acid after 60 min incubation in Krebs–Ringer bicarbonate medium. Tissues incubated after removal from animals pretreated with testosterone for time intervals shown. Each point is average of 3 to 8 determinations. AIB, α-aminoisobutyric acid.

In a series of experiments *in vitro*, seminal vesicles, prostate and levator ani were removed from rats treated 3 to 12 h previously with testosterone. Fig. 2 depicts the α-aminoisobutyric acid uptake by each tissue over a 60-min incubation period. α-Aminoisobutyric acid spaces rose from control values of 47 ± 9 %, 77 ± 12 % and 77 ± 10 % for levator ani, prostate and seminal vesicles, respectively, to 67 ± 7 %, 138 ± 17 % and 167 ± 23 % after 12-h pretreatment ($P < 0.01$ in each case). When testosterone pretreatment was reduced from 12 h to 6 or 3 h, no significant change over controls was seen in any of the three tissues (Fig. 2).

These results suggest that α-aminoisobutyric acid uptake by sex accessory organs might be an energy-dependent, active process and experiments were designed to test this possibility. Tissues from rats pretreated for 12 h with testosterone were incubated 60 min in the presence or absence of (a) $5 \cdot 10^{-4}$ M dinitrophenol, (b) an anoxic atmosphere (95 N_2:5 % CO_2) or (c) reduced temperature (20°), and compared with tissues from untreated controls (Table III). In each hormone-treated tissue studied, the α-aminoisobutyric acid space after incubation at 20° was significantly less than that of either the experimental controls or the untreated controls incubated at 36° ($P < 0.01$ in each case). Q_{10} values calculated for α-aminoisobutyric acid uptakes at these temperatures were 1.99, 1.82 and 1.90 for levator ani, ventral prostate and seminal vesicle, respectively. Dinitrophenol significantly blocked hormone-induced α-aminoisobutyric acid uptake in all tissues; the levels were reduced essentially to those of the untreated controls (Table III). Similarly, α-aminoisobutyric acid

TABLE III

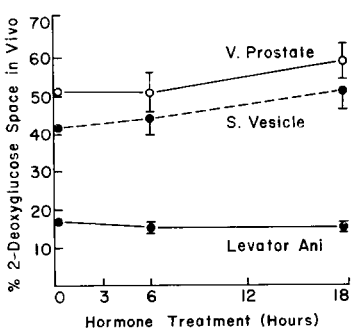
THE EFFECT OF INHIBITORS ON TESTOSTERONE-STIMULATED UPTAKE OF α-AMINOISOBUTYRIC ACID BY CASTRATED-RAT SEX ACCESSORY TISSUES *in vitro*

Treatment	% *α-Aminoisobutyric acid space (ml/100 g fresh tissue wt.)***		
	Levator ani	*Ventral prostate*	*Seminal vesicles*
Control	46.5 ± 9.3 (7)*	77.4 ± 12 (4)*	76.8 ± 9.9 (8)*
12-h testosterone pretreatment	67.4 ± 6.7 (3)	138 ± 17 (4)	167 ± 23 (4)
+ dinitrophenol ($5 \cdot 10^{-4}$ M)	32.7 ± 1.8 (3)*	91.3 ± 14 (4)*	80.4 ± 9.1 (4)*
+ 20° incubation	22.5 ± 1.1 (4)*	53.1 ± 7.4 (4)*	59.6 ± 4.1 (4)*
+ 95% N_2:5% CO_2	53.2 ± 6.1 (4)	100 ± 7.4 (4)*	70.7 ± 3.6 (4)*
+ Tris	35.1 ± 4.9 (4)*	61.7 ± 3.7 (4)*	43.4 ± 5.7 (4)*
+ ouabain (0.8 mM)	33.4 ± 5.2 (4)*	102 ± 12 (4)*	88.8 ± 2.2 (4)*
+ alanine (20 mM)	29.4 ± 2.9 (4)*	51.8 ± 3.7 (4)*	52.5 ± 2.9 (4)*

* $P < 0.05$ when compared with 12-h testosterone pretreatment values.
** Mean ± S.D. with number of animals in parentheses. All tissues were incubated for 60 min and at 36° unless otherwise indicated.

spaces in pretreated prostate and seminal vesicles, after 60-min incubations in the anoxic atmosphere, were reduced to values which compared favorably with untreated controls incubated in oxygen; levator ani did not show a significant change.

To investigate the transport system's dependence on sodium ion, 12-h testosterone-pretreated sex accessory tissues were incubated in Na-free Tris buffer containing labeled α-aminoisobutyric acid. α-Aminoisobutyric acid uptakes were reduced to levels equal to, or less than, control tissues incubated in Krebs–Ringer bicarbonate medium (Table III). Also, α-aminoisobutyric acid penetration was significantly decreased in accessory tissues from 12-h pretreated rats incubated in the bicarbonate medium in the presence of 0.8 mmole/ml ouabain (Table III). 20 mM alanine also markedly reduced α-aminoisobutyric acid penetration over the 60-min incubation period (Table III). Thus, α-aminoisobutyric acid uptake *in vitro* by male sex accessory



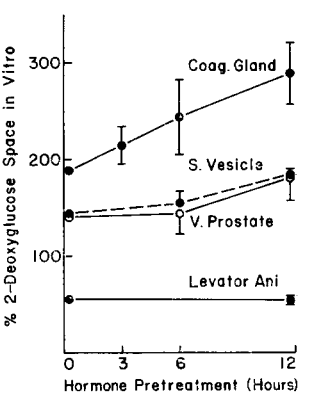


Fig. 3. Distribution *in vivo* of 2-deoxy[^{14}C]glucose with time after testosterone treatment; deoxyglucose injected 15 min before sacrifice in each case. Each point is average of 4 animals.

Fig. 4. Distribution *in vitro* of 2-deoxy[^{14}C]glucose after 60 min incubation. Tissues incubated after removal from animals pretreated with testosterone for periods indicated. Each point is average of 3 to 8 determinations.

tissues appears to depend upon energy metabolism and Na^+ and apparently utilizes the same transport site as the natural amino acid, alanine.

In other experiments, the effects of testosterone on the uptake *in vivo* of the non-utilizable glucose analogue, 2-deoxyglucose were studied (Fig. 3). Control values for deoxyglucose space were 17 ± 1 %, 51 ± 2 % and 42 ± 8 % for levator ani, prostate and seminal vesicles, respectively. After 18 h of testosterone treatment, only prostate showed a statistically significant increase over controls ($P < 0.05$) *in vivo*. Deoxyglucose was rapidly accumulated *in vitro* by all tissues investigated and was distributed into volumes well in excess of the extracellular spaces after only 15 min of incubation. The coagulating gland, prostate and seminal vesicles accumulated deoxyglucose against a concentration gradient to volumes considerably greater than the total water in these tissues within 60 min of incubation (Fig. 4). Testosterone pretreatment for 12 h prior to 60 min incubation significantly increased accumulation of the sugar in coagulating gland, prostate and seminal vesicles but not in levator ani (Fig. 4). Pretreatment for 6 h produced a significant increase over controls only in the coagulating gland (space: 248 ± 40 %; $P < 0.05$); the coagulating gland did not respond to 3-h pretreatment.

TABLE IV

THE EFFECT OF INHIBITORS ON TESTOSTERONE-STIMULATED UPTAKE OF 2-DEOXYGLUCOSE BY CASTRATED-RAT SEX ACCESSORY TISSUES *in vitro*

Treatment	% *Deoxyglucose space (ml/100 g fresh tissue wt.)* *		
	Coagulating gland	*Ventral prostate*	*Seminal vesicles*
Control	192 ± 23 (8)	143 ± 14 (4)	144 ± 10 (4)
12-h testosterone pretreatment	293 ± 33 (4)**	185 ± 25 (4)**	186 ± 4.5 (3)**
+ glucose (10^{-2} M)	89.9 ± 9.1 (4)**	79.3 ± 7.2 (4)**	93.0 ± 10 (4)**
+ phlorizin (10^{-4} M)	271 ± 33 (4)**	179 ± 11 (4)**	184 ± 17 (4)**
+ phlorizin (10^{-3} M)	160 ± 12 (4)***	129 ± 15 (4)***	123 ± 4.4 (4)**,***

* Mean ± S.D. with number of animals in parentheses.
** $P < 0.05$ compared with untreated controls.
*** $P < 0.05$ compared with the testosterone-treated group.

Incubations of coagulating gland, seminal vesicle and prostate for 60 min in bicarbonate buffer medium containing 10^{-2} M glucose depressed hormone-stimulated deoxyglucose uptake in all tissues to levels significantly below untreated control values ($P < 0.05$, Table IV). Incubation of testosterone-pretreated tissues in medium containing 10^{-3} M (but not 10^{-4} M) phlorizin resulted in a marked depression of deoxyglucose space in all tissue to levels below either control group ($P < 0.05$ Table IV).

DISCUSSION

The extracellular (inulin) volume of the levator ani, found to be 9.5 % in the present experiments, agrees well with values reported for other rat muscles: 10 % for gastrocnemius[12], 11 % for general skeletal muscle (inulin, thiocyanate)[13] and 9 %

for psoas majora (inulin, sucrose)[2]. The extracellular space reported here is, however, less than the sucrose values of 13 % (*in vivo*) and 24 % (*in vitro*) found for levator ani by ARVIN AND AHREN[14]; the difference may derive from the latter authors' use of immature animals whereas the present work utilized 70 to 120-day-old rats. The literature affords little information on the apparent extracellular compartment of male sex accessory tissues. RUDOLPH AND STARNES[15] reported a sodium space of 37 % for seminal vesicles and 32 % for ventral prostate from rats castrated 28 days previously, in fair agreement with our inulin values of 29 % and 37 % for these tissues, respectively. The vigorous blotting required to remove secretions in the seminal vesicles undoubtedly introduces some error in the measurement of the extracellular compartment of this organ.

Unlike estrogenic hormones which cause early hydration of uterine tissue[2], testosterone in the present study did not significantly alter water content of male sex accessory tissues within the first 18 h after injection. However, RUDOLPH AND SAMUELS[16] found a significant hydration of seminal vesicles within 10 h of a single subcutaneous injection; the discrepancy might be due to differences in the degree to which vesicular secretions are removed. The increase in total wet weight of seminal vesicles at 18 h reported by WICKS AND VILLEE[17] may be ascribed to an increased tissue mass since water content was not determined. It should be noted that WILSON[11] saw no change in seminal vesicle wet weight at the 12-h interval.

WILSON[11] demonstrated a stimulating effect of testosterone on incorporation of radioactive tyrosine into protein of slices of seminal vesicle pretreated 12 h with the hormone; no measurable change in specific activity of the tissue's tyrosine pool occurred, leading the author to conclude protein synthesis had been increased without a change in amino acid transport. Our studies, however, clearly show increased uptake of non-utilizable α-aminoisobutyric acid as early as 6 h after hormone injection, an effect markedly inhibited by the natural amino acid, alanine. These results indicate testosterone does stimulate amino acid transport into cells, findings which are consistent with WILSON's data if, for example, the increased rate of tyrosine influx just balanced its incorporation into protein. The question might be resolved definitively by measuring transport in the presence of inhibitors of protein synthesis.

The assumption was made that testosterone-stimulated penetration of α-aminoisobutyric acid into sex accessory organs was an active process if experimental conditions which disrupted energy-yielding cellular mechanisms also reduced cellular α-aminoisobutyric acid accumulation. Incubation of 12-h testosterone-treated seminal vesicles, prostate or levator ani in medium containing dinitrophenol inhibited α-aminoisobutyric acid uptake to levels equal to or less than levels shown for untreated tissues. Comparable levels of dinitrophenol have been shown to block α-aminoisobutyric acid transport in rat intestine[18] but not in kidney slices[19]. It may be assumed that the reduction in cellular α-aminoisobutyric acid accumulation in the present experiments resulted from a dinitrophenol-related reduction in tissue ATP content; KOSTYO AND SCHMIDT[20] reported an 80 % decrease in diaphragm muscle ATP after incubation in $5 \cdot 10^{-4}$ M dinitrophenol. The α-aminoisobutyric acid space in 12-h testosterone-treated accessory tissues was also markedly decreased by incubation at reduced temperature. Values for Q_{10} calculated from α-aminoisobutyric acid spaces of hormone-treated tissues incubated at 20° and 36° were 1.8 to 2.0 suggesting an energy-dependent process[21,22]. On the basis of these collective observations, it is

concluded that increased cellular penetration of α-aminoisobutyric acid as affected by testosterone in male sex accessory organs is an energy-dependent process.

Membrane transport of certain amino acids has been shown to require sodium ion[23]; accumulation of glycine[24] and of α-aminoisobutyric acid[20] is greatly reduced in sodium-free choline buffer. GUROFF AND UDENFRIEND[25] were able to demonstrate that tyrosine penetration of diaphragm, reported to be a passive process, was independent of sodium. In the present experiments, the increased transport of α-aminoisobutyric acid due to testosterone was markedly reduced by 60-min incubations in sodium-free Tris buffer. Further evidence for sodium dependence in this system was obtained with ouabain, which blocks sodium fluxes and the transport of several amino acids into a variety of tissues[26,27]. A concentration of $8 \cdot 10^{-4}$ M inhibited testosterone-induced α-aminoisobutyric acid penetration into cells of the sex accessories. However, CSAKY[28] pointed out that at 10^{-6} M, ouabain blocks sugar and amino acid transport pumps while at 10^{-3} M, the drug acts to uncouple oxidative phosphorylation in a manner similar to dinitrophenol. Thus, ouabain inhibition of α-aminoisobutyric acid uptake reported here may well be due to reduced energy production rather than to inhibition of membrane ATPase or blockage of sodium fluxes as have been suggested[26]. Incubation of sex accessory tissues in the presence of alanine resulted in complete suppression of ^{14}C-labeled α-aminoisobutyric acid accumulation as stimulated by testosterone. Similarly, glucose in the medium reduced uptake of ^{14}C-labeled deoxyglucose to below control levels. These results suggest competition between the non-utilizable tracers and their respective natural analogues for common binding sites and carrier systems.

The studies in this report attempt to characterize the uptake effects and their timing relative to hormone-stimulated increases in metabolism. The possibility is entertained that transport has a role in the hormone's mechanism of action in the sense that functional maintenance and growth in male sex accessory tissues may depend upon alterations in the rate of entry of substrate molecules. The results indicate that a single injection of testosterone does increase accumulation of α-aminoisobutyric acid and 2-deoxyglucose in cells of levator ani, seminal vesicles, prostate and coagulating gland while not altering the volume of the extracellular space or the total water compartment of these tissues. Several of the changes were first observed 6 h after hormone administration, though others were not augmented until the 12th or 18th h. Thus, many of these permeability changes occur well before the onset of cell division and associated DNA synthesis which occur 40–50 h after hormone administration[9,29]. The alterations in transport also precede hormonal stimulation of tyrosine and valine incorporation into seminal vesicle protein[11], and increased fructose synthesis and respiration in seminal vesicles 10 h after hormone injection[16]. Testosterone-stimulated α-aminoisobutyric acid transport occurs at least simultaneously with the increased leucine incorporation into seminal vesicle proteins 6 h after treatment reported by BUTENANDT, GUNTHER AND TURBA[30]. However, studies have appeared which described metabolic changes in less than 6 h after hormone administration. Radioactive phosphorus incorporation into RNA of seminal vesicles is stimulated in 20 min[9] while prostatic RNA polymerase activity is increased within 1 h of injection[10]. Increased synthesis of two classes of RNA appears to occur within 90 min of treatment[31,32]. Taken together with experiments involving longer-term treatment, these studies are the basis for the view that the direct and primary action of testosterone is on messenger RNA synthesis[33].

Definitive conclusions cannot yet be drawn regarding the causal relationship between testosterone stimulation of RNA synthesis and the increased cellular permeability which follows shortly thereafter. Transport changes could depend on the synthesis of specific RNA species which direct production of one or more critical proteins, *e.g.*, membrane carrier molecules, enzymes directly involved in energy production or nucleotide triphosphatases; the cleavage of ATP apparently is central to the active transport process[26], and it is of interest in this connection that FARNSWORTH[34] has recently reported an *in vitro* testosterone stimulation of ATPase activity in prostatic minces. There remains the possibility, however, that testosterone acts directly on more than one process, on the synthesis of RNA species and independently to stimulate the transport of important substrates.

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