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STUDIES ON THE MECHANISM OF TESTOSTERONE ACTION ON GLUCOSE METABOLISM IN THE RAT VENTRAL PROSTATE

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SUMMARY

Experiments were performed to elucidate the mechanism of testosterone action on glucose metabolism. A single injection of testosterone to castrated rats was shown to increase the *in vitro* utilization of glucose by the ventral prostate. The production of $^{14}CO_2$ from ^{14}C-labeled glucose and pyruvate molecules and the incorporation of radioactivity from ^{14}C-labeled glucose into RNA and proteins were also increased by testosterone. The experiments with the nonutilizable analogue of glucose, 2-deoxyglucose—revealed that testosterone increased the assimilation of this compound. Simultaneous changes were seen in the hexose-phosphorylating capacity of the soluble fraction of the prostatic homogenate. From these experiments it was concluded that testosterone is acting to increase the uptake and/or phosphorylation of glucose. The timing of the changes and the necessity of exogenous glucose for the hormonal stimulation of RNA and protein synthesis emphasize the primary importance of the alterations in the uptake and/or phosphorylation of glucose for functional maintanence of the tissue after hormone treatment. The possibility that testosterone simultaneously causes a shift in metabolism to favor the oxidative breakdown of glucose is discussed.

The experiments involving an inhibitor of RNA synthesis indicated that the major part of testosterone effects on RNA synthesis is superimposed upon, and perhaps emanating from the hormonal effects on protein synthesis and/or glucose metabolism. They could not exclude the possibility, however, that testosterone induces as its primary action the synthesis of a few specific species of RNA which direct the production of critical proteins involved in the hormonal activation of glucose metabolism.

INTRODUCTION

Androgens have activatory effects on a number of metabolic functions in the male sex accessory glands, including incorporation of precursors into macromolecules[1–6], nuclear aggregate enzyme activity[7–8], transport phenomena[9] and carbohydrate metabolism[10–12]. Although the hormone effects on these processes are conceivably related, there have been no conclusive reports on this subject. We have recently suggested that the effect of testosterone on RNA synthesis represents predominantly a growth response superimposed upon, and perhaps emanating from the effect of the hormone on protein synthesis and/or glucose metabolism[13]. The possibility was entertained that the activation of glucose metabolism which was dependent on the continuous synthesis of proteins is the primary action of testosterone and the major part of the changes in macromolecular synthesis is related to an increased availability of nucleoside triphosphates as a consequence of the increased metabolism of glucose. The pulse labeling technique employed in our experiments was not able to exclude the possibility, however, that a few selected species of RNA are first synthesized as the primary response to testosterone. The activation of glucose metabolism would thus depend on the synthesis of these specific RNA species.

The present study was undertaken to elucidate the site and mechanism of testosterone action on glucose metabolism. Further resolution of the activation mechanism is demanded before any definitive conclusions can be drawn regarding the causal relationship between testosterone stimulation of RNA and protein synthesis and the stimulated glucose metabolism. A preliminary report of this work has been presented[14].

EXPERIMENTAL

Adult male Sprague–Dawley rats weighing from 200 to 300 g were used. The animals were maintained on standard laboratory chow diet, and were castrated by the trans-scrotal route under ether anesthesia. Other rats were sham-operated to serve as controls. Testosterone (Organon, The Netherlands) was ground in an all-glass homogenizer in 0·9% saline solution and injected subcutaneously in a dose of 2 mg of free testosterone in 0·2 ml of saline. Control rats received the same dose of saline.

Incorporation of ^{14}C-glucose (U) into glycogen. Small pieces of ventral prostate were incubated in 3 ml of Krebs–Ringer phosphate medium, pH 7·4, supplemented with 1 mM ^{14}C-glucose (U) (0·1 μCi/μmol) (New England Nuclear) for 60 min in air. The incorporation of glucose into glycogen was linear with time at least for 60 min in these experimental conditions. The preparation of tissue pieces used in these experiments has been

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described earlier[13]. The amount of tissue per incubation vial was 30–70 mg. After incubation 0·1 ml of glycogen solution (10 mg/ml of water) as carrier and 3 ml of 60% KOH were added quickly to incubation vials, and the samples subjected to a 30-min heat treatment in boiling water bath. Glycogen was precipitated by adding first 0·1 ml of saturated Na_2SO_4-solution and then 95% ethanol (9 ml) at 5°C. After centrifugation the precipitate was dissolved into 1 ml of water and the radioactivity in glycogen was assayed according to the method of Thomas *et al.*[15]. Two samples (0·1 ml) were applied onto the filter papers (Whatman 3 mm). The filter papers were then washed twice with cold, 66% ethanol for 20 min, and a third time with acetone (5 min) and then dried under a heat lamp until the odor of acetone was no longer apparent. Filter papers were placed in scintillation vials containing 10 ml of 0·4% (w/v) PPO and 0·004% (w/v) POPOP in toluene. The activity is expressed as c.p.m./100 mg of wet weight.

The formation of $^{14}CO_2$ from ^{14}C-glucose and ^{14}C-pyruvate. Small pieces of ventral prostate were placed into 50-ml Erlenmeyer flasks which had a 10-mm glass center well. The incubation media used (3 ml/flask) was Krebs–Ringer phosphate, pH 7·4, with 1 mM glucose (or pyruvate) with the S.A. of 0·1 μCi/μmol (radiochemicals were purchased from New England Nuclear). Tissues (20 to 40 mg per flask) were incubated in air for 60 min at 37°C in flasks closed with a rubber cap. At the end of incubation 0·4 ml of 4 M H_2SO_4 was injected through the rubber cap into the incubation medium and 0·5 ml of 1M Hyamine Hydroxide in methanol (Nuclear Chicago) into the central well. The $^{14}CO_2$ was trapped by allowing the flasks to stand for 60 min at 37°C. The hyamine solution was taken into a counting vial, the central well was rinsed twice with 0·5 ml of the counting solution, and the washing solutions were combined with hyamine solution. 10 ml of the counting solution were added into vials and the radioactivity was counted. Results are expressed as c.p.m./100 mg of wet weight.

Incorporation of ^{14}C-glucose into RNA and protein. Small pieces of ventral prostate were incubated in 1 ml of Krebs–Ringer phosphate buffer, pH 7·4, containing 1 mM ^{14}C-glucose (U) (0·1 μCi/μmol). After a 60-min incubation the radioactivity in RNA and protein was measured as explained earlier[13]. The tissue pieces were first homogenized in cold water and samples (0·1 ml) of the homogenate were pipetted onto filter paper disks (Whatman No. 3 mm chromatography paper, 2·3 cm. dia.). The radioactivity in filter paper disks was first measured omitting the hot trichloroacetic acid treatments. After the first counting ("counts in RNA and protein") the disks were removed and washed and subjected to heat treatment as explained earlier[13]. After subsequent alcohol–ether, and ether washes the disks were replaced in their original vials and recounted to give "counts in proteins". These were subtracted from the first counts for getting "counts in RNA". The results are expressed as c.p.m./100 μg of RNA or c.p.m./mg of protein. The total RNA content of the tissue was measured according to Munro and Fleck[16] and the protein content according to Lowry *et al.*[17].

Incorporation of ^{3}H-uridine into RNA and ^{14}C-labeled amino acids into protein. The incubation conditions were identical with those for measuring the incorporation of ^{14}C-glucose into RNA and protein, except that 5 μCi of ^{3}H-uridine-5 (27 Ci/mmol, The Radiochemical Centre, Amersham) or 1 μCi of uniformly ^{14}C-labeled amino acid mixture (New England Nuclear) in 0·1 ml of 0·9% saline solution was added instead of ^{14}C-glucose. After homogenizing the tissue pieces in water two samples of 0·1 ml were pipetted onto filter paper disks, and the radioactivity in RNA and proteins was measured as explained earlier[13].

Determination of the radioactivity of the acid-soluble fraction. The acid-soluble fraction was prepared and the total acid-soluble radioactivity determined as explained earlier[13].

Utilization of glucose. The total glucose utilization by the ventral prostate *in vitro* was measured by direct analysis of glucose content in the incubation medium after incubation. Small pieces of prostate were incubated in 1 ml of Krebs–Ringer phosphate medium, pH 7·4, supplemented with 1 mM glucose, and after incubation 1 ml of 6% PCA was added into the incubation medium. The tissue was rapidly homogenized in the acidified incubation medium, and after centrifugation glucose content of the supernatant was analyzed by a standard enzymatic procedure (GOD method, Boehringer Mannheim GMBH). The *in vitro* utilization of glucose by prostatic tissue was shown to be linear with respect to time (for at least up to 60 min), the amount of tissue and the glucose concentration (from 0·1 mM to 1 mM).

Uptake and phosphorylation of 2-deoxyglucose. Small pieces of the ventral prostate were incubated in 1 ml of Krebs–Ringer phosphate buffer, pH 7·4, with 0·1 mM ^{14}C- or ^{3}H-labeled 2-deoxyglucose (New England Nuclear, ^{14}C-2-deoxyglucose with the S.A. of 54·6 mCi/mmol, ^{3}H-2-deoxyglucose with the S.A. of 7·2 Ci/mmol) in air. The incubation time was 20 to 60 min at 37°C in a shaking water bath. Where indicated, additional amounts of unlabeled 2-deoxyglucose (Sigma) or D-glucose were added into the incubation medium. After incubation, tissue pieces were immediately washed with the Krebs–Ringer phosphate medium, blotted and homogenized in ice-cold 5% trichloroacetic acid. Carrier 2-deoxyglucose and 2-deoxyglucose-6-phosphate (2-deoxyglucose-6-P) (Sigma) were added before the homogenization of the tissue. Extraction and chromatographic separation of ^{14}C-2-deoxyglucose and its phosphorylated metabolite were performed essentially according to

the method of Smith and Gorski [18]. The radioactivity in the ^{14}C-2-deoxyglucose and ^{14}C-2-deoxyglucose-6-P was determined by cutting a 2·5 cm. strip of the chromatography paper into 0·5 cm.-pieces and placing the pieces separately in 10 ml of toluene counting solution. The chromatographic picture of the TCA-soluble fraction of the ventral prostate of the rat incubated with ^{14}C-2-deoxyglucose indicated that 2-deoxyglucose is not metabolized past 2-deoxyglucose-6-P in this tissue. The unknown compound with slower chromatographic mobility than 2-deoxyglucose-6-P appearing on the radioactive scan is most probably derived from 2-deoxyglucose-6-P during the extraction procedure as indicated earlier [18]. Most of the quantitative assays were performed by using the barium-zinc reagent of Somogyi [19] for the separation of free hexose from phosphorylated one. This method was found to be more convenient for the simultaneous analysis of a large number of samples. Moreover, the results were within a smaller range than with the chromatographic method. In the latter method the tissue was gently homogenized in a small volume of distilled water, and then heated in a boiling water bath until completely dissolved. The extraction procedure has been shown to give a quantitative recovery of radioactivity [9]. The phosphorylated hexose was precipitated with the barium-zinc reagent prepared according to Augustin and Hofmann [20]. Before and after precipitation, samples were centrifuged and aliquot of each supernatant was mixed with dioxane counting solution and counted to get the total radioactivity (in the ^{3}H-2-deoxyglucose and ^{3}H-2-deoxyglucose-6-P) and the radioactivity of ^{3}H-2-deoxyglucose, respectively. The radioactivity in each compound is expressed as c.p.m./100 mg of wet weight.

Assay of hexokinase. The animals were decapitated and the ventral prostates were excised and cleaned of all extraneous tissue. Organs were rapidly weighed and then homogenized in a motor-driven all-glass homogenizer in ten volumes of ice-cold medium containing 50 mM Tris–HCl buffer, pH 7·4, 0·15 M KCl, 5 mM betamercaptoethanol and 1 mM EDTA. The homogenate was centrifuged at 100,000*g* for 45 min. The high speed supernatant fraction or "soluble fraction" was assayed for hexokinase activity. The methodological details of the glucose-6-P dehydrogenase-dependent spectrophotometric method have been published earlier [21]. The assay mixture for hexokinase (^{14}C-2-deoxyglucose as the substrate) was composed of 100 μl of homogenate in Tris–HCl buffer, pH 7·4 (40 mM final concentration), 5 mM ATP, 5 mM $MgCl_2$, 5 mM beta-mercaptoethanol, 1 mM ^{14}C-2-deoxyglucose (0·05 μCi) in a total vol. of 0·5 ml. The assay mixture was incubated at 37°C in a shaking water bath for 5 to 30 min. The reaction was stopped by immersing the tubes in ice. Carrier 2-deoxyglucose (250 μl of 10 mM) was added and the phosphorylated sugar was precipitated with the barium–zinc reagent of Somogyi [19]. Aliquots of the incubation medium after the precipitation of labeled phosphorylated sugar were assessed for their radioactivity. Phosphorylation of both glucose and ^{14}C-2-deoxyglucose was shown to be linear with respect to time and the amount of enzyme solution added, and complete dependence on ATP was evident in both assay systems. The results are expressed as moles of sugar phosphorylated per min per mg of protein.

RESULTS

The effect of testosterone on the glucose utilization by the ventral prostate *in vitro* was investigated first. Small pieces of the ventral prostate were incubated in a glucose-containing medium and the disappearence of glucose from the incubation medium was measured during a 60-min incubation period. Table 1 shows that the glucose utilization by the tissue decreased to 73% at 48 h after castration and testosterone treatment of the castrated animal for 12 h (but not for 6 h) brought it back to the normal level. The increase at 24 h after hormone treatment was of similar magnitude (Table 1). This means that the total glucose utilization by the ventral prostate of the rat is under androgenic influence.

Table 2 demonstrates the effect of testosterone for 24 h on the incorporation over a 60-min incubation period of uniformly labeled ^{14}C-glucose into glycogen, RNA and proteins and the *in vitro* formation of $^{14}CO_2$ by the ventral prostate. No increase in the radioactivity of glycogen isolated from the tissue was demonstrable after hormone treatment. Testosterone treatment, however, increased the incorporation of radioactivity into RNA (1·7-fold), proteins (6-fold) and the formation of $^{14}CO_2$ from ^{14}C-glucose (4·3-fold). Table 3 shows that the formation of $^{14}CO_2$ from ^{14}C-glucose (U) was 40% and 33% of the control value 24 and 48 h after castration, respectively. Following testosterone treatment the formation of $^{14}CO_2$ significantly increased at 12 h (but not at 6 h), and it increased further as a function of the hormone treatment time (Table 3). Also the formation of

Table 1. Effect of castration and testosterone treatment on the glucose utilization by the ventral prostate of the rat *in vitro*

Treatment	Glucose utilization mg/100 mg tissue/h
Normal	54·78 ± 2·32[a]
Castrated 48 h	40·30 ± 1·75
Castrated 48 h, Testosterone 12 h	56·31 ± 2·49[a]
Castrated 48 h, Testosterone 24 h	52·26 ± 1·42[a]
	(Mean ± S.E.)

[a] Significantly different from castrate ($P < 0·001$).

Table 2. Incorporation of ^{14}C-Glucose (U) into glycogen, RNA and protein, and formation of $^{14}CO_2$ from ^{14}C-glucose (U) by the ventral prostate of the rat *in vitro*

		GLYCOGEN cpm/100 mg tissue	RNA cpm/mg RNA	PROTEIN cpm/mg protein	$^{14}CO_2$ cpm/100 mg tissue
CASTRATED	48 h	865 ± 18	710 ± 130	59 ± 6	2935 ± 211
CASTRATED TESTOSTERONE	48 h, 24 h	875 ± 84	1220 ± 260	358 ± 17	12603 ± 874

Table 3. Formation of $^{14}CO_2$ from ^{14}C-glucose (U), ^{14}C-glucose-6 and ^{14}C-pyruvate-2 by the ventral prostate *in vitro*

		^{14}C-GLUCOSE (U) cpm/100 mg tissue	^{14}C-GLUCOSE-6 cpm/100 mg tissue	^{14}C-PYRUVATE-2 cpm/100 mg tissue
NORMAL		9022 ± 463	2040 ± 174	16450 ± 2320
CASTRATED	24 h	3635 ± 289	460 ± 224	9450 ± 576
CASTRATED	48 h	2935 ± 211	394 ± 25	7740 ± 580
CASTRATED TESTOSTERONE	48 h, 6 h	2795 ± 459		
CASTRATED TESTOSTERONE	48 h, 12 h	7083 ± 494		
CASTRATED TESTOSTERONE	48 h, 24 h	12603 ± 874	4358 ± 628	19980 ± 4110

(MEAN ± S.E.)

$^{14}CO_2$ from ^{14}C-glucose-6 and ^{14}C-pyruvate-2 which are oxidized to $^{14}CO_2$ largely in the Krebs cycle[22], showed marked changes under the same experimental conditions.

The fact that a number of end points of glucose metabolism showed increased incorporation of radioactivity after hormone treatment suggests that testosterone is influencing some rate-limiting step or steps common to all the end points measured. The uptake and phosphorylation are the first and, in most cases, limiting steps of sugar utilization by the cells. Therefore the study of the influence of testosterone on penetration and phosphorylation of glucose was of interest in explaining hormonal action on glucose metabolism. Studies were made of the penetration of ^{14}C-labeled 2-deoxyglucose, a sugar, whose metabolism stops at the 2-deoxyglucose-6-P level to obtain more accurate data concerning the sugar transport and phosphorylation rates.

Small pieces of the ventral prostate removed from normal rats were incubated with ^{14}C-2-deoxyglucose or ^{3}H-2-deoxyglucose and thereafter the radioactivity appearing with the cells in the 2-deoxyglucose and 2-deoxyglucose-6-P was determined either chromatographically according to the method of Smith and Gorski[18] or by separating free hexose from phosphorylated hexose with the barium-zinc reagent of Somogyi. Most of the radioactivity within cells was found in the phosphorylated sugar. The chromatographic analysis indicated that 2-deoxyglucose is not metabolized past 2-deoxyglucose-6-P in this tissue. As shown in Fig. 1 the appearance of the radioactivity in the 2-deoxyglucose-6-P was, under the experimental conditions, linear with time and with the amount of tissue added. When glucose was added into the medium and uptake and/or phosphorylation of ^{14}C-2-deoxyglucose was markedly reduced (Fig. 2) suggesting the competition between the nonutilizable tracer and its respective natural analogue for the common binding sites and/or carrier systems. Furthermore increasing the concentration of non-radioactive 2-deoxyglucose in the incubation medium from 0·1 mM to 0·5 mM decreased the total radioactivity found in the tissue but did not change the relative amounts of ^{14}C-2-deoxyglucose and ^{14}C-2-deoxyglucose-6-P.

Castration of the rats for 48 h decreased the total uptake of ^{3}H-2-deoxyglucose by the prostatic cells to 70% of the normal value over a 60-min *in vitro* incubation period, but had only small effects on the relative amounts of free and phosphorylated hexose found within the tissue (Table 4). When the castrated rats were treated with testosterone for 12 h before sacrificing them, a significant stimulation of the total uptake of ^{3}H-2-deoxyglucose (as free and phosphorylated form) was demonstrable.

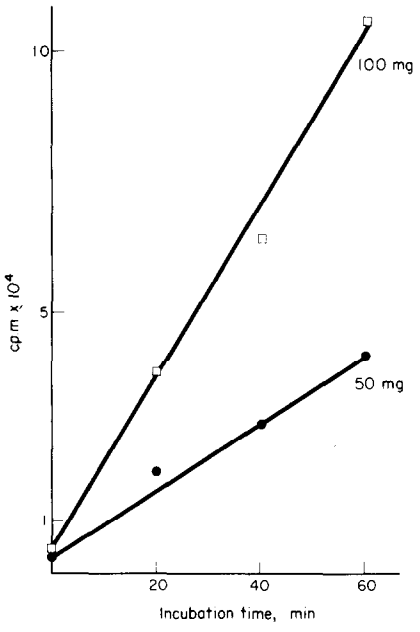

Fig. 1. The effect of incubation time and the amount of tissue of the formation of ^{14}C-2-deoxyglucose-6-P by the ventral prostate *in vitro*. Small pieces of the ventral prostate from normal rats were incubated with 0·1 mM ^{14}C-2-deoxyglucose for 20 to 60 min. After the incubation the tissue was analyzed for the ^{14}C-2-deoxyglucose content according to the method of Smith and Gorski[18].

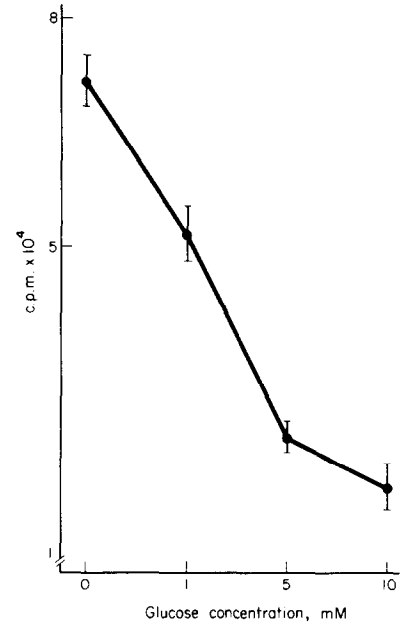

Fig. 2. The effect of glucose on the formation of ^{14}C-2-deoxyglucose-6-P by the ventral prostate from normal rats *in vitro*. Small pieces of the ventral prostate were incubated with 0·1 mM ^{14}C-2-deoxyglucose for 60 min. Different amounts of non-radioactive glucose were added into the incubation medium. After the incubation the tissue was analyzed for the ^{14}C-2-deoxyglucose-6-P according to the method of Smith and Gorski[18]. The results are expressed as c.p.m./100 mg of wet weight.

The total uptake was further increased by testosterone at 24 h (Table 4). Only marginal changes were seen in the ratio of ^{3}H-2-deoxyglucose to ^{3}H-2-deoxyglucose-6-P at 12 h and 24 h following testosterone treatment. It was further shown that the incubation of tissue samples from testosterone-treated and castrated rats at a higher concentration of 2-deoxyglucose (0·5 mM) revealed equivalent difference between these two groups (unpublished results).

Because it is possible that the amount of 2-deoxyglucose-6-P was a measure of the rate of penetration rather than that of phosphorylation, capacity studies were undertaken to determine the hexokinase activity of the cell-free extract. As shown in Table 5 the exposure to testosterone for 12 or 24 h activated the phosphorylation capacity when glucose or ^{14}C-2-deoxyglucose was used as the substrate. This suggests that increased hexokinase-catalyzed sugar phosphorylation is one of the sites involved in testosterone-induced glucose metabolism. However, it cannot be concluded from these data whether the increased total radioactivity of 2-deoxyglucose found within the tissue resulted from testosterone-increased uptake or phosphorylation of hexose or from both increased uptake and phosphorylation. Earlier findings[13] have indicated and the testosterone-stimulated synthesis of RNA is predominantly limited to that fraction of the total RNA synthesis which is extremely sensitive to actinomycin D. To see whether the testosterone action on glucose metabolism, particularly on uptake and phosphorylation is dependent upon this hormone-inducible, highly actinomycin D-sensitive part of RNA synthesis, the effect of a low dose of actinomycin D (25 μg/100 g) on glucose metabolism was investigated.

Confirming earlier findings,[13] testosterone treatment of castrated rats for 12 h was shown to increase the *in vitro* incorporation of ^{3}H-uridine into RNA (Table 6). The measurements of the acid-soluble radioactivity revealed that the increased labeling of RNA resulted at least partly from the increased precursor uptake. Actinomycin D in a dose of 25 μg/100 g given 2 h before the hormone abolished totally the hormone-sensitive part in the incorporation of ^{3}H-uridine into RNA, but had no effect on the ^{3}H-uridine incorporation into RNA of the ventral prostate of the castrated rat (Table 6). The effect of the same low dose of actinomycin D on glucose metabolism was tested next. Actinomycin D did not prevent the hormonal stimulation of the glucose metabolism (Table 7). The glucose utilization, the total uptake of ^{3}H-2-deoxyglucose and the production of $^{14}CO_2$ from ^{14}C-glucose were all significantly activated by testosterone under the influence of actinomycin D. This means that the activation of glucose metabolism does not result from the demonstrable major changes of RNA synthesis, rather it represents an

Table 4. Effect of castration and testosterone treatment on the uptake and phosphorylation of ^{3}H-2-deoxyglucose by the ventral prostate of rat *in vitro*

	^{3}H-2-dG + ^{3}H-2-dGP cpm/100 mg tissue/h	^{3}H-2-dG cpm/100 mg tissue/h
NORMAL	129642 ± 3436[a]	12720 ± 301[a]
CASTRATED 48 h	90428 ± 1831	9853 ± 300
CASTRATED 48 h, TESTOSTERONE 12 h	115380 ± 5611[a]	11900 ± 540[b]
CASTRATED 48 h, TESTOSTERONE 24 h	134256 ± 4263[a]	15054 ± 569[a]

(MEAN ± S.E.)

[a] Significantly different from castrate ($p < 0.001$)

[b] Significantly different from castrate ($p < 0.01$)

Table 5. The effects of testosterone and actinomycin D (25 μg/100 g/100 g of body weight) treatments on soluble hexokinase activity in the ventral prostate of the castrated rat

	HEXOKINASE ACTIVITY nmoles / min / mg protein	
	D - GLUCOSE	^{14}C - 2 - DEOXYGLUCOSE
CASTRATED 48 h	30.02 ± 1.24	29 ± 3
CASTRATED 48 h, TESTOSTERONE 12 h	65.69 ± 3.58 [a]	73 ± 15 [a]
CASTRATED 48 h, TESTOSTERONE 24 h	86.06 ± 5.90 [a]	
CASTRATED 48 h, ACTINOMYCIN D 14 h	27.28 ± 3.38	
CASTRATED 48 h, ACTINOMYCIN D 14 h, TESTOSTERONE 12 h	36.54 ± 2.67 [b]	
CASTRATED 48 h, ACTINOMYCIN D 26 h	26.34 ± 1.94	
CASTRATED 48 h, ACTINOMYCIN D 26 h, TESTOSTERONE 24 h	44.15 ± 3.47 [c]	

(MEAN ± S.E.)

[a] Significantly different from castrate ($p < 0.001$)

[b] Significantly different from actinomycin D - treated (14 h) castrate ($p < 0.05$)

[c] Significantly different from actinomycin D - treated (26 h) castrate ($p < 0.001$)

independent hormonal action. It is notable that actinomycin D by itself lowered the uptake and/or phosphorylation of 2-deoxyglucose and the glucose utilization in the ventral prostate of the castrated animal, but had no effect on the hormonal stimulation of these processes. The inhibitor prevented the major part of the increase in hexokinase activity (Table 5) suggesting that the increase in hexokinase activity is associated with the hormone-dependent portion of RNA synthesis. However, a definitive stimulation of hexokinase by testosterone was still present after actinomycin D pretreatment (Table 5). Starch-gel electrophoresis pattern of hexokinase revealed that the changes in hexokinase II account for those seen in the soluble fraction of the ventral prostate after actinomycin D and testosterone treatments (unpublished observations).

To elucidate further the interrelationship between glucose metabolism and the synthesis of RNA and proteins, the significance of exogenous glucose for precursor incorporation into RNA and proteins was investigated. The stimulation by testosterone of the labeling of RNA and proteins

Table 6. The effect of actinomycin D (25 μg/100 g) and the composition of the incubation medium on the incorporation of ^{3}H-uridine into RNA and ^{14}C-labeled aminoacids into proteins by the ventral prostate of castrated and testosterone-treated castrated rats *in vitro*

		INCUBATION MEDIUM	RNA		PROTEIN	
			acid-insoluble cpm/100 mg tissue	acid-soluble	acid-insoluble cpm/100 mg tissue	acid-soluble
CASTRATED	48 h	glucose	32119 ± 3160	499417 ± 18783	83534 ± 9458	172034 ± 7634
CASTRATED	48 h	none	31337 ± 1859	516372 ± 16887	75481 ± 6207	196175 ± 18190
CASTRATED ACTINOMYCIN D	48 h, 14 h	glucose	21301 ± 3160	413610 ± 51772	72741 ± 6753	171803 ± 3702
CASTRATED ACTINOMYCIN D	48 h, 14 h	none	21455 ± 2785	519185 ± 23667	78500 ± 5360	177685 ± 4442
CASTRATED TESTOSTERONE	48 h, 12 h	glucose	55070 ± 2515[a]	566820 ± 16504[c]	138523 ± 12500[a]	181449 ± 7796
CASTRATED TESTOSTERONE	48 h, 12 h	none	36437 ± 1488[b]	481290 ± 14229	88225 ± 6440	195181 ± 6734
CASTRATED ACTINOMYCIN D TESTOSTERONE	48 h, 14 h, 12 h	glucose	21878 ± 2593	530122 ± 22800	96588 ± 8574[b]	168098 ± 5137
CASTRATED ACTINOMYCIN D TESTOSTERONE	48 h, 14 h 12 h	none	20314 ± 780	483436 ± 24543	75692 ± 747	179041 ± 6244

(MEAN ± S.E.)

[a] significantly different from castrate ($p < 0.001$)

[b] significantly different from actinomycin D-treated castrate ($p < 0.01$)

[c] significantly different from castrate ($p < 0.01$)

Table 7. The effect of actinomycin D (25 μg/100 g) on the glucose utilization, the uptake and phosphorylation of 2-deoxyglucose and the formation of $^{14}CO_2$ from ^{14}C-glucose (U) by the ventral prostate of castrated and testosterone-treated castrated rat *in vitro*

		GLUCOSE UTILIZATION	UPTAKE AND PHOSPHORYLATION OF ^{3}H-2-dG		FORMATION OF $^{14}CO_2$ FROM ^{14}C-GLUCOSE (U)
			^{3}H-2-dG + ^{3}H-2-dGP	^{3}H-2-dG	
		mg/100 mg/h	cpm/100 mg/h	cpm/100 mg/h	cpm/100 mg/h
CASTRATED ACTINOMYCIN D	48 h, 14 h	36.23 ± 1.00	77509 ± 3530	9561 ± 253	1306 ± 115
CASTRATED ACTINOMYCIN D TESTOSTERONE	48 h, 14 h, 12 h	43.81 ± 2.36[a]	106160 ± 9528[b]	9823 ± 910	3729 ± 297[c]
CASTRATED ACTINOMYCIN D	48 h, 26 h	31.70 ± 1.56	78435 ± 3888	9595 ± 540	2319 ± 476
CASTRATED ACTINOMYCIN D TESTOSTERONE	48 h, 26 h, 24 h	41.35 ± 2.46[b]	105619 ± 4974[b]	12611 ± 465[c]	6910 ± 663[c]

(MEAN ± S.E.)

[a] significantly different from actinomycin D-treated castrate ($p < 0.05$)

[b] significantly different from actinomycin D-treated castrate ($p < 0.01$)

[c] significantly different from actinomycin D-treated castrate ($p < 0.001$)

emerged only in the presence of glucose in the incubation medium (Table 6). Furthermore, the hormonal stimulation was only on the glucose-dependent portion of RNA and protein labeling. The addition of glucose into the incubation medium did not appreciably influence the labeling of RNA and proteins of the ventral prostate of the castrated rats during a 60-min labeling period *in vitro*.

As shown earlier [13] the increased labeling of proteins after hormone treatment was not prevented by a low dose of actinomycin D (25 μg/100 g). Furthermore, this hormone-dependent, actinomycin D-resistant labeling of proteins emerged only in the presence of glucose in the incubation medium, as expected (Table 7). These results indicated that the hormone-induced activation of glucose metabolism is an essential and independent part in the response of the ventral

prostate to testosterone and may account for major macromolecular effects of testosterone on the tissue.

DISCUSSION

The data reported in the present communication suggest that testosterone influences the glucose metabolism in the ventral prostate of the rat by increasing the uptake and/or phosphorylation of glucose and possibly by causing a shift in metabolism to favor the oxidative breakdown of glucose. These two phenomena would explain the stimulation of the glucose utilization, the uptake of 2-deoxyglucose, the production of $^{14}CO_2$ from ^{14}C-glucose and the incorporation of ^{14}C-glucose into RNA and proteins seen in the present experiments.

The labeled glucose analogue, 2-deoxyglucose, which is not metabolized past 2-deoxyglucose-6-P in the ventral prostate of the rat, was used for the analysis of the first two steps in glucose metabolism. The use of 2-deoxyglucose is based on the generally approved assumption that these two sugars, glucose and 2-deoxyglucose, are associated with the same mechanism during transit and phosphorylation. The inhibition of 2-deoxyglucose-6-P production by glucose in the incubation medium serves as an indication of this in the ventral prostate of the rat. Our studies on 2-deoxyglucose-6-P production imply that testosterone is controlling the transport and/or phosphorylation of glucose. Testosterone treatment of castrated rats caused significant increases in the *in vitro* assimilation of 2-deoxyglucose by the ventral prostate confirming earlier findings of Mills and Spaziani[9] and Thomas *et al.*[24]. There is good evidence that testosterone does this without altering the volume of the extracellular space or the total water compartment. Whether the rate-limiting and testosterone-controlled step in the assimilation was the penetration of 2-deoxyglucose into the cells or the phosphorylation of 2-deoxyglucose cannot be distinguished from these experiments.

The determination of the glucose and 2-deoxyglucose phosphorylating enzymes of the soluble fraction of the ventral prostate revealed that hexokinase activity was increased by testosterone within a few hours (6 h, unpublished results) after hormone administration. This confirms earlier findings of Santti and Villee[21]. On the basis of data presented in this communication, supplemented with data provided by previous studies it may be concluded that the changes in hexokinase activity (particularly in type II)[21] parallel those in glucose metabolism as judged by measuring the glucose utilization, the uptake and phosphorylation of 2-deoxyglucose and the formation of $^{14}CO_2$ from ^{14}C-glucose.

This parallelism was also seen in the actinomycin D- treated rats. The injection of testosterone into these rats caused the ^{14}C-2-deoxyglucose-6-P level to increase, and small, but significant changes were simultaneously seen in the soluble hexokinase activity. These results suggest the direct causal relationship between the soluble hexokinase activity (particularly type II) and the assimilation of hexoses by the ventral prostate. As a summary, the evidence presented in this communication supports the theory that testosterone controls step(s) located at or prior to the formation of glucose-6-P.

An increase in the formation of $^{14}CO_2$ from ^{14}C-glucose-6 and ^{14}C-2-pyruvate which substrates are largely oxidized to $^{14}CO_2$ in the Krebs cycle indicates that this pathway is involved in activation of glucose metabolism by testosterone. An accelerated cycling of ^{14}C from ^{14}C-glucose through the Krebs cycle could account for the increased formation of $^{14}CO_2$ in the testosterone-treated group. Such a mechanism would be consistent with the increased oxygen uptake previously found by many authors[1, 10–12] to occur after testosterone treatment in the sex accessory glands. Rudolph and Samuels[11] have observed that while the Q_{O_2} of the rat seminal vesicle decreased following castration and was restored by androgen administration, the rate of anaerobic glycolysis was independent of these conditions. Barron and Huggins[10] have also found that castration results in a decline in the respiration (but not the glycolysis) of dog prostate tissue slices. Ritter has concluded from these studies and his own[23] that within the first few hours after administration of androgen to castrated animals there occurs a redirection of prostatic energy metabolism toward a more efficient production of ATP as a result of activation of respiratory rather than glycolytic processes. In that case the difference in the hexokinase activity between castrated and testosterone-treated castrated rats would be related to differences in respiration and availability of energy. Since hexokinase is an important consumer of ATP and a source of ADP, it would provide an important point of interaction between oxidation and glucose utilization. However, the tentative nature of these conclusions must be emphasized. Much more work is needed before the early action of androgen on the oxidative processes and energy production in the mitochondria is thoroughly elucidated.

The experiments involving an inhibitor of RNA synthesis, actinomycin D, preclude the direct causal relationship between RNA synthesis and glucose metabolism. A small dose of actinomycin D blocked hormonal stimulation of the labeling of RNA, while the activation of the glucose utilization, the uptake and phosphorylation of 2-deoxyglucose and the formation of $^{14}CO_2$ from ^{14}C-glucose remained practically unaffected. Furthermore, testosterone stimulated significantly the soluble hexokinase activity in the presence of actinomycin D. From these results we concluded that the major part of the testosterone effects on RNA synthesis is superimposed upon, and perhaps

emanating from the hormonal effects on protein synthesis and/or glucose metabolism.

The timing of the uptake effects relative to hormone-stimulated increases in RNA and protein synthesis and the necessity of exogenous glucose for the stimulation of macromolecular synthesis suggest that the hormonal activation of the tissue is dependent particularly upon alterations in the uptake and/or phosphorylation of glucose. However, definitive conclusions cannot yet be drawn regarding the causal relationship between testosterone stimulation of the synthesis of RNA and proteins and the glucose metabolism (including uptake and phosphorylation). The latter effects may depend upon the synthesis of specific RNA species which direct production of one or more critical proteins e.g. membrane carrier proteins, hexokinase or proteins directly involved in energy production. The total inhibition of the testosterone action with a high dose of actinomycin D supports such an argument[13]. Moreover, there remains the possibility that testosterone acts directly on more than one process, on the synthesis of RNA species and independently to stimulate the transport of important substrates and/or generation of energy.

The effect of a low dose of actinomycin D on the labeling of different RNA species has recently been analysed by our research group[25]. The total RNA isolated from the ventral prostate of the rat was separated into tRNA, ribosomal RNA and two DNA-like RNA fractions by chromatography on methylated albumin kieselguhr (MAK) columns. The treatment of the castrated rat with testosterone increased markedly the production of ribosomal RNA, and the bulk of the testosterone effect on the total RNA labeling was found in this fraction. Furthermore, it seemed likely that testosterone also stimulated both the synthesis and processing of DNA-like RNA. When antinomycin D was given 2 h before hormone administration in a dose of 25 μg *per* 100 g of body weight, there was no noticeable increase in the labeling of any fraction above the level seen in the actinomycin D-treated, castrated rat. Earlier results of the prostatic polymerases are consistent with these results[26]. Most of the enhancement of prostatic RNA polymerase activity, which results from the injection of testosterone to castrated rats, can be suppressed by injecting a small dose (25 μg/100 g) of actinomycin D. If the testosterone action is based on the synthesis of new RNA species, as generally assumed, the amount of labeling in these species must be very small and not detectable by the methods currently used for the qualitative analysis of RNA. Mainwaring *et al.* have very recently described a specific poly(A)-enriched 6-15 S RNA fraction from the ventral prostate[27]. This fraction was stringently controlled by androgens and was relatively refractory to the administration of actinomycin D (25 μg/100 g). We have recently confirmed this finding (unpublished results). It is tempting to speculate that the testosterone action on glucose metabolism is mediated by RNA species located in this fraction.

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