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STUDIES ON THE INHIBITION OF TESTOSTERONE ACTION BY CYCLOHEXIMIDE: EVIDENCE FOR A PROTEIN ACTIVATOR OF GLUCOSE METABOLISM IN THE VENTRAL PROSTATE OF THE RAT

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SUMMARY

Experiments involving cycloheximide, an inhibitor of protein synthesis, indicate that continued protein synthesis is required in the ventral prostate of the rat for the emergence of the testosterone action in glucose metabolism. RNA synthesis is also required during the initial phase of hormone action, but once initiated, significant activating effects of testosterone are evident for at least 6 h after treatment with actinomycin D. In contrast, administration of cycloheximide at different time intervals after testosterone results in immediate reversal (within 1 h) of the effects of this steroid on glucose oxidation as reflected in the formation of $^{14}CO_2$ from [^{14}C]-glucose by tissue pieces *in vitro*. This is followed by changes in RNA synthesis and other parameters of glucose metabolism such as glucose utilization, the uptake and phosphorylation of the nonutilizable analogue of glucose, 2-deoxyglucose, and the soluble hexokinase activity. Cycloheximide has no effects on RNA synthesis or glucose metabolism in the ventral prostate of castrated rats within a 4-h treatment period. There are indications that the inhibition of glucose oxidation by cycloheximide is not simply due to the lowered energy requirements of the organ and that cycloheximide does not act by inhibiting the transformation of testosterone into dihydrotestosterone.

It is suggested that the sequence of events leading to the amplification of the hormone action e.g. the major changes in RNA and protein synthesis and eventually in the growth and function of the organ, is initiated by the formation of activatory protein(s) with a rapid turnover. On the basis of the data reported in this communication, supplemented with our earlier findings, it is proposed that the newly synthesized protein(s) exert an activatory influence on glucose metabolism causing a shift to a more oxidative phase. The rapid inhibition of glucose oxidation seen after castration supports this conclusion. It is not possible to exclude definitely, that this effect of testosterone is not dependent on new nuclear RNA synthesis.

INTRODUCTION

Testosterone has been shown to activate glucose metabolism in the target tissues measured as glucose utilization, uptake of nonutilizable glucose analogues such as 2-deoxyglucose, the incorporation of radioactivity from [^{14}C]-labeled glucose into RNA and proteins, or the formation of $^{14}CO_2$ from [^{14}C]-labeled glucose[1–3]. Simultaneously changes are seen in the activity of glucose metabolizing enzymes including hexokinase[4, 5]. The time course of the changes in glucose metabolism relative to hormone-induced increases in RNA and protein synthesis, and the necessity of exogenous glucose for the hormonal stimulation of RNA and protein synthesis[3] point to the primary importance of the alterations in glucose metabolism for the hormone action. The primary site and the molecular mechanism of the testosterone effect on glucose metabolism and the causal relationship between the testosterone stimulation of RNA (and protein) synthesis and glucose metabolism are, however, far from clear.

There are indications that androgens, like other steroid hormones, act by binding to specific receptor proteins and that the formed complexes migrate into prostate nuclei, where they regulate nuclear RNA synthesis[6–8]. According to this concept the causal relationship between the activation of RNA (and protein) synthesis and glucose metabolism can be explained by assuming that the new testosterone-induced RNA species direct the production of protein(s) involved in the activation of glucose metabolism. The suppression of the testosterone action on glucose metabolism by previous administration of RNA or protein synthesis is consistent with this interpretation[9].

In the present work we attempted to get evidence for the presence of the up till now hypothetical proteins which activate glucose metabolism in the ventral prostate of testosterone-treated rats. In order to get more information about the time of their synthesis, their turnover and possibly the locus of their action, inhibitors of RNA and protein synthesis were administered into castrated rats before or at different times after testosterone. Because it was not possible to measure directly the amount of these proteins, the assumption was made, that their amounts are

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expressed by the measured parameters known to be stimulated in glucose metabolism in the early phase of hormone action.

EXPERIMENTAL

Adult male Sprague–Dawley rats weighing 250–300 g were used. The animals were maintained on a standard laboratory chow diet, and were castrated 48 h before sacrifice by the transscrotal route under ether anesthesia. Other rats were sham operated to serve as normal controls. Testosterone (17β-hydroxy-4-androsten-3-one, Organon, The Netherlands) or 5α-dihydrotestosterone (5α-androstan-17β-hydroxy-3-one, Sigma) was ground in all-glass homogenizer in 0.9% saline solution and injected into castrated rats subcutaneously in a dose of 2 mg of free steroid in 0.2 ml of saline. Control rats received the same dose of saline solution. The metabolic inhibitors, cycloheximide (Sigma), puromycin dihydrochloride (crystalline, Sigma) and actinomycin D (Lyovac Cosmegen, MSD) were dissolved in 0.9% saline solution and injected intraperitoneally in doses indicated in connexion with "Results".

The utilization of glucose, the uptake and phosphorylation of [^{3}H]-2-deoxyglucose (New England Nuclear, the S.A. of 7.2 Ci/mmol) and the formation of $^{14}CO_2$ from [^{14}C]-glucose (U) (The Radiochemical Centre, Amersham, the S.A. of 3.1 mCi/mmol) by small pieces of the ventral prostate *in vitro* were measured in duplicate or triplicate as described earlier[3]. The details of the determination of the soluble hexokinase (ATP: D-hexose-6-phosphotransferase, EC 2.7.1.1) activity have also been given earlier[5]. The determination of the incorporation of [^{3}H]-uridine (The Radiochemical Centre, Amersham, the S.A. of 27 Ci/mmol) into RNA and uniformly [^{14}C]-labeled amino acid mixture (New England Nuclear) into proteins *in vitro*, the preparation of the acid-soluble fraction and the determination of the total acid-soluble radioactivity were carried out as explained earlier[9]. The *in vivo* protein synthesis was measured by injecting 15 μCi of L-[4,5-^{3}H]-leucine (The Radiochemical Centre, Amersham, the S.A. of 58 Ci/mmol) *per* 300 g of body weight intraperitoneally 1 h before sacrificing the animals. The radioactivity in proteins was measured as in the *in vitro* assays of protein synthesis.

The student's t-test was used to calculate statistical significance. All results are given as a mean $\pm$ S.E.

RESULTS

In order to determine the detailed time course of testosterone action on glucose metabolism in the ventral prostate of the rat, adult male rats were castrated for 48 h and given single injection of testosterone 6–24 h before sacrifice. As shown earlier[3], the formation of $^{14}CO_2$ from [^{14}C]-labeled glucose, the total uptake of [^{3}H]-2-deoxyglucose and the glucose utilization by the tissue pieces of the ventral prostate *in vitro* and the soluble hexokinase activity were all activated by testosterone (Fig. 1). The earliest (detectable at 6 h after testosterone treatment) and most marked changes were seen in the formation of $^{14}CO_2$ from [^{14}C]-glucose and the hexokinase activity. The labeling of proteins *in vivo* with [^{3}H]-leucine and of RNA *in vitro* with [^{3}H]-uridine were also stimulated by testosterone from 6 h onwards (Fig. 1).

The dependence of the activation of glucose metabolism on protein synthesis was investigated by treating the castrated rats 2 h before hormone administration with cycloheximide, a potent inhibitor of eucaryotic protein synthesis. The rather small dose of 60 μg/100 g of body weight, which earlier has been employed succesfully for blocking testosterone action in the male sex accessory glands[4, 5, 9], was chosen. Table 1 shows that cycloheximide caused a rapid, but transient inhibition of protein synthesis in the ventral prostate of the castrated rat. The maximal inhibition was achieved within 2 h, and then the rate of protein synthesis increased gradually and reached the control level at 4–6 h. A stimulated rate of protein synthesis was observed at 14 h. In the presence of cycloheximide, testosterone appeared to stimulate slightly the rate of protein synthesis. This was evident at 4 h after testosterone administration (6 h after cycloheximide administration) e.g. during the recovery of protein

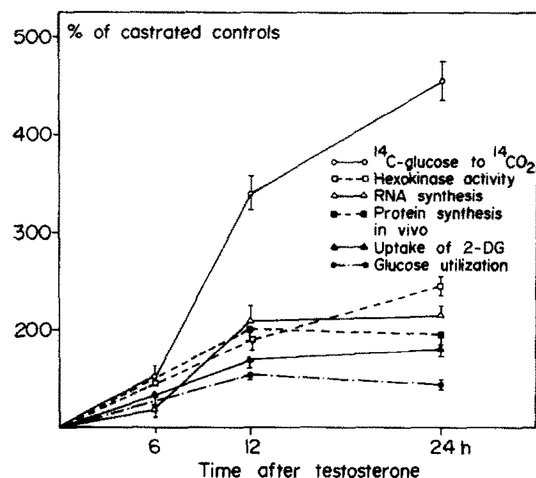

Fig. 1. The response of the ventral prostate of the castrated rat to testosterone. Adult male rats were castrated for 48 h, and treated with 1 testosterone injection (2 mg of free testosterone in 0.2 ml of saline solution subcutaneously) 6–24 h before being killed. Glucose utilization, total uptake of [^{3}H]-2-deoxyglucose (2-DG), the formation of $^{14}CO_2$ from [^{14}C]-glucose, the incorporation of [^{3}H]-uridine into RNA (RNA synthesis) by small tissue pieces of prostate *in vitro*, the *in vivo* incorporation of [^{3}H]-leucine into proteins (Protein synthesis) and the soluble hexokinase activity were measured. RNA and protein synthesis, the $^{14}CO_2$ production and hexokinase activity were significantly ($p < 0.01$, $p < 0.01$, $p < 0.01$ and $p < 0.001$ respectively, student's t-test) activated by testosterone from 6 h and glucose utilization and uptake of 2-DG from 12 h onwards. Every point represents the mean of at least three experiments using three to five rats. The vertical bars show S.E.

Table 1. The effect of cycloheximide pretreatment on the response to testosterone of the ventral prostate of castrated rats. Cycloheximide was injected intraperitoneally 2 h before testosterone administration in a dose of 60 μg/100 g of body weight. All animals were castrated for 48 h before kill

	Time before killing	Incorporation of [^{3}H]-uridine into RNA (*in vitro*)	Incorporation of [^{3}H]-leu into protein (*in vivo*)
		Acid-insoluble radioactivity c.p.m./100 mg of wet weight/h (Mean ± S.E.)	
Castrated	48 h	25560 ± 1620 (10)*	2000 ± 160 (18)
Castrated	48 h		
Cycloheximide	2 h	26470 ± 1490 (11)	740 ± 40 (16)
Castrated	48 h		
Cycloheximide	4 h	22130 ± 1360 (10)	1920 ± 120 (16)
Castrated	48 h		
Cycloheximide	4 h		
Testosterone	2 h		2230 ± 200 (16)
Castrated	48 h		
Cycloheximide	6 h	20590 ± 1810 (9)	1980 ± 160 (16)
Castrated	48 h		
Cycloheximide	6 h		
Testosterone	4 h		3420 ± 280† (18)
Castrated	48 h		
Testosterone	12 h	53924 ± 4108 (12)	3882 ± 227 (18)
Castrated	48 h		
Cycloheximide	14 h	8740 ± 450 (8)	2940 ± 190 (15)
Castrated	48 h		
Cycloheximide	14 h		
Testosterone	12 h	12730 ± 530 (8)	4090 ± 650 (11)

* number of rats.
† significantly different from cycloheximide-treated castrate ($P < 0.01$), student's *t*-test.

synthesis. The hormonal activation of protein synthesis seemed, however, to subside and was not any more significant at 12 h (Table 1). During the studied period (up to 14 h after inhibitor administration) cycloheximide inhibited only slightly the formation of $^{14}CO_2$ from [^{14}C]-glucose, total uptake of [^{3}H]-2-deoxyglucose, and glucose utilization and had no effect on hexokinase activity (Table 2) in the ventral prostate of the castrated animal. However, cycloheximide greatly reduced (or delayed) the hormonal activation of glucose metabolism (Table 2). There was no significant effect demonstrable in glucose metabolism up to 12 h after testosterone administration in the presence of cycloheximide.

Under the same experimental conditions cycloheximide prevented the response of RNA synthesis to testosterone (Table 1). These results confirm and supplement the earlier findings that the testosterone action is dependent upon the continuous synthesis of proteins. They further suggest that the critical period for

Table 2. The effect of cycloheximide pretreatment on the response to testosterone of the ventral prostate of castrated rats. Cycloheximide was injected intraperitoneally 2 h before testosterone administration in a dose of 60 μg/100 g of body weight

	Time before killing	Glucose utilization mg/100 mg of wet weight/h (Mean ± S.E.)	Uptake of 2-DG c.p.m./100 mg of wet weight/h (Mean ± S.E.)	Hexokinase activity nmol/min/mg prot. (Mean ± S.E.)	Formation of $^{14}CO_2$ from [^{14}C]-glucose c.p.m./100 mg of wet weight/h (Mean ± S.E.)
Castrated	48 h	36.8 ± 4.0 (12)*	62290 ± 2530 (18)	34.7 ± 0.9 (20)	2935 ± 210 (20)
Castrated	48 h,				
Testosterone	12 h	56.3 ± 2.5 (12)†	105430 ± 5610 (12)†	65.7 ± 3.6 (12)†	10147 ± 609 (16)†
Castrated	48 h,				
Cycloheximide	14 h	31.8 ± 2.6 (8)	62391 ± 3040 (10)	36.5 ± 1.5 (8)	2410 ± 275 (16)
Castrated	48 h,				
Cycloheximide	14 h,				
Testosterone	12 h	33.8 ± 2.2 (8)	63297 ± 3928 (10)	41.0 ± 3.4 (8)	2850 ± 490 (12)

* number of rats.
† significantly different from castrates ($P < 0.001$), student's *t*-test.

Table 3. The effect of cycloheximide (60 μg/100 g of body weight) on RNA and protein synthesis and the formation of $^{14}CO_2$ from [^{14}C]-glucose in the ventral prostate of testosterone-treated, castrated rats. Cycloheximide was injected 3, 6 or 9 h after hormone administration and the animal were killed 12 h after hormone administration

	Time before killing	Incorporation of [^{3}H]-uridine into RNA (*in vitro*) Acid-insoluble radioactivity c.p.m./100 mg of wet weight/h (Mean ± S.E.)	Incorporation of [^{14}C]-amino acid into protein (*in vitro*) Acid-insoluble radioactivity c.p.m./100 mg of wet weight/h (Mean ± S.E.)	Formation of $^{14}CO_2$ from [^{14}C]-glucose c.p.m./100 mg of wet weight/h (Mean ± S.E.)
Castrated	48 h	65465 ± 7940† (16)*	66050 ± 3560† (12)	2457 ± 196† (17)
Castrated	48 h			
Testosterone	12 h	119350 ± 8520 (16)	140480 ± 20570 (12)	7451 ± 464 (20)
Castrated	48 h			
Testosterone	12 h			
Cycloheximide	9 h	60790 ± 5450† (13)	113440 ± 8960 (13)	2748 ± 272† (18)
Castrated	48 h			
Testosterone	12 h			
Cycloheximide	6 h	52485 ± 4380† (13)	79150 ± 8090‡ (6)	2814 ± 278† (18)
Castrated	48 h			
Testosterone	12 h			
Cycloheximide	3 h	67180 ± 8330† (12)	102250 ± 7110 (13)	3876 ± 462† (12)

* number of rats.
† significantly different from testosterone treated rats ($P < 0.001$), student's t-test.
‡ significantly different from testosterone treated rats ($P < 0.05$), student's t-test.

triggering of testosterone action on RNA synthesis and glucose metabolism may well be as soon as within 4 h after hormone administration, the period, when the protein synthesis was maximally inhibited by cycloheximide.

In order to determine whether or when the hormone action becomes refractory to the inhibitor, cycloheximide was injected after testosterone administration. Table 3 shows that cycloheximide had inhibitory effects on the formation of $^{14}CO_2$ from [^{14}C]-glucose and the synthesis of RNA irrespective of the time interval between the administration of hormone and cycloheximide or the length of the treatment period. These results are explained by assuming that rapidly turning over proteins are involved in the hormonal activation of RNA synthesis and glucose metabolism.

To get a more accurate estimate of the half lives of the hypothetical proteins involved in the hormonal activation of RNA synthesis and glucose metabolism the detailed time sequence of the changes in RNA synthesis and glucose metabolism were investigated after cycloheximide administration. For convenience normal adult male rats were chosen for these experiments. The most rapid effects were seen in the formation of $^{14}CO_2$ from [^{14}C]-glucose, when normal rats were treated with a small dose of cycloheximide (60 μg/100 g of body weight). The maximal inhibition was achieved already within 1 h (Fig. 2). There was a simultaneous decrease in the rate of protein synthesis and hexokinase activity ($P < 0.05$). As can be seen the cycloheximide inhibition of the formation of $^{14}CO_2$ from [^{14}C]-glucose by the ventral prostate of normal rat was much more rapid and pronounced than that seen in the castrated rat suggesting that proteins with extremely short half-lives acting on glucose metabolism are present only in the ventral prostate exposed to testosterone. As seen earlier the protein synthesis recovered quite rapidly after cycloheximide administration and reached a value close to control within 6 h in the ventral prostate of normal rat. In contrast to this, there was only a slight restoration in the formation of $^{14}CO_2$ from [^{14}C]-glucose. There was no concomitant inhibition of glucose utilization and total uptake of [^{3}H]-2-deoxyglucose (in fact, there was a slight increase) during the 2 h period, when the formation of $^{14}CO_2$ from [^{14}C]-glucose was maximally inhibited (Fig. 2). Thereafter, these processes gradually declined. The synthesis of RNA decreased at $t_{1/2}$ of less than 3 h (Fig. 2).

Comparable results were obtained with puromycin, which, like cycloheximide, caused rapid and parallel inhibition of protein synthesis and $^{14}CO_2$ formation from [^{14}C]-glucose when in a dose of 12 mg/100 g of body weight to normal rats (Fig. 3). These results with puromycin suggest that the inhibition of glucose metabolism by cycloheximide is related to the inhibition of protein synthesis rather than to some unspecific toxic action of cycloheximide. Further evidence was obtained when the dose dependence of the inhibition of protein synthesis and of the $^{14}CO_2$ formation from [^{14}C]-glucose was investigated in normal rat. The effect of cycloheximide was dose-dependent between 5 and 40 μg/100 g of body weight and a remarkable parallellism was found between the inhibition of protein synthesis and the inhibition of the formation of $^{14}CO_2$ from [^{14}C]-glucose (Fig. 4).

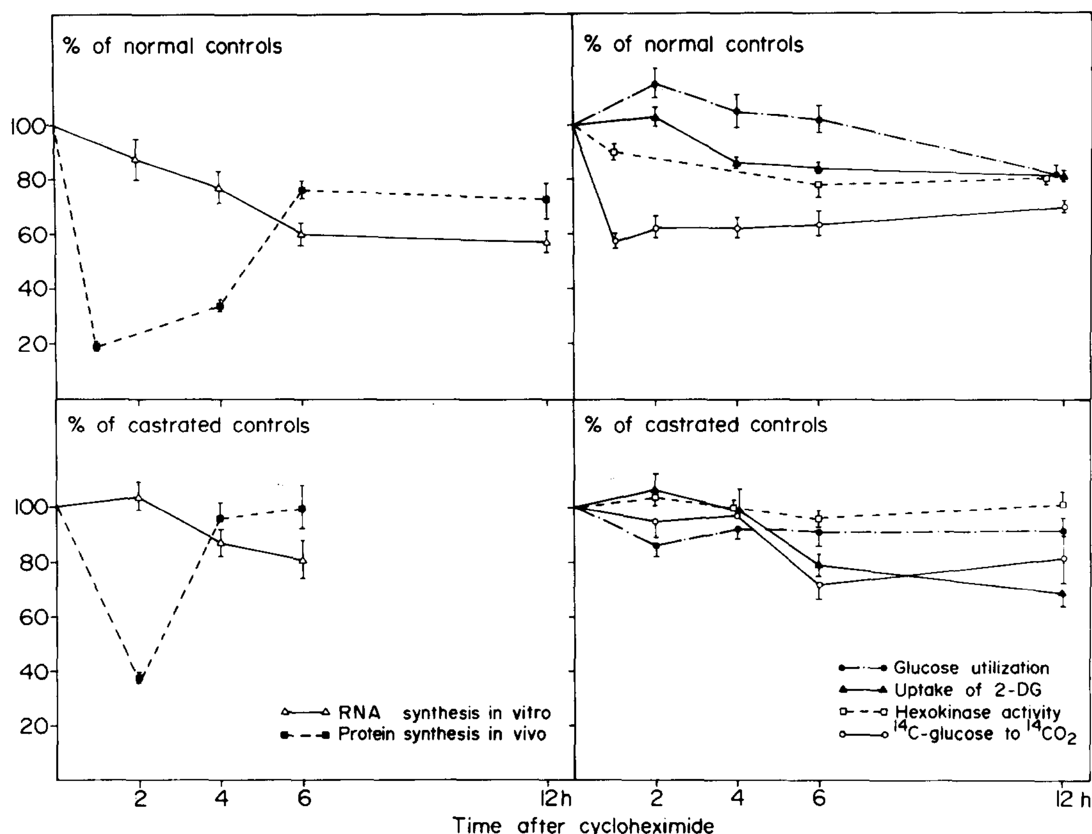

Fig. 2. The effect of cycloheximide on the ventral prostate of normal and castrated rats. Cycloheximide was injected intraperitoneally in a dose of 60 μg/100 g of body weight and the animals were killed at the indicated times after treatment. Glucose utilization, total uptake of [^{3}H]-2-deoxyglucose (2-DG), the formation of $^{14}CO_2$ from [^{14}C]-glucose, the incorporation of [^{3}H]-uridine into RNA (RNA synthesis) by small tissue pieces of prostate *in vitro*, the *in vivo* incorporation of [^{3}H]-leucine into proteins (Protein synthesis) and the soluble hexokinase activity were measured. The results are expressed as % of normal or castrated control. Every point represents the mean of at least three experiments using three to five rats. Different experiments yielded similar time courses. The vertical bars show S.E.

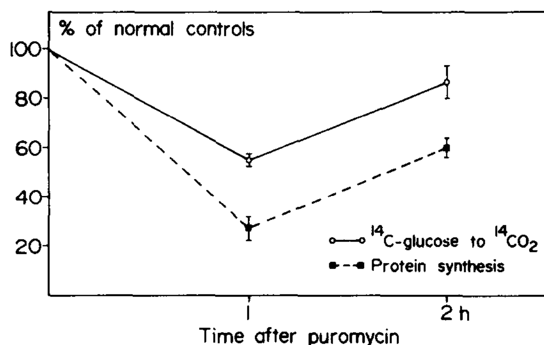

Fig. 3. The effect of puromycin dihydrochloride (12 mg/100 g of body weight intraperitoneally into normal rats) on the *in vivo* incorporation of [^{3}H]-leucine into prostatic proteins (Protein synthesis) and the formation of $^{14}CO_2$ from [^{14}C]-glucose by small tissue pieces of prostate *in vitro*. The animals were killed at the stated time after treatment. Each point represents the mean of two experiments using five to six rats. The vertical bars show S.E.

The possibility was considered that cycloheximide blocked the testosterone action by inhibiting its transformation into dihydrotestosterone known to be an intracellularly active androgen[10–12]. Our results indicate that cycloheximide prevents the action of dihydrotestosterone on the formation of $^{14}CO_2$ from [^{14}C]-glucose as well as that of testosterone (Table 4).

Actinomycin D was next administered to castrated rats at different time intervals after testosterone to establish the critical period of the RNA synthesis in the hormone action on glucose metabolism. The experiments employed the dose, which previously has been shown to prevent the hormonal activation of glucose oxidation[3]. Table 5 shows that the continuous synthesis of RNA at least up to 6 h is essential for hormone action on glucose oxidation.

Finally, the time sequence of the changes after castration was determined. The most rapid and marked changes were seen in the formation of $^{14}CO_2$ from

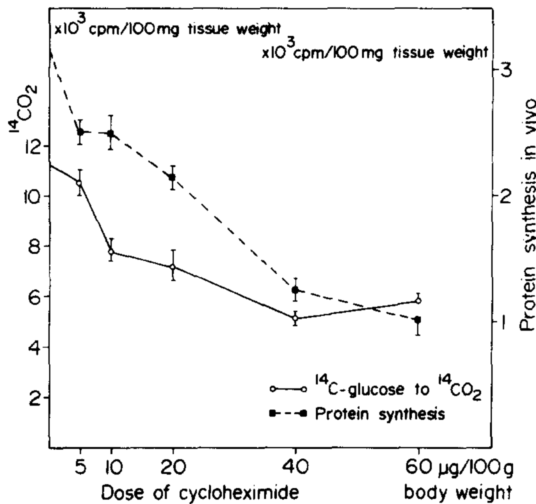

Fig. 4. The dose response toward cycloheximide of the *in vivo* incorporation of [^{3}H]-leucine into prostatic proteins (Protein synthesis) and the formation of $^{14}CO_2$ from [^{14}C]-glucose by small tissue pieces of prostate *in vitro*. Cycloheximide was injected intraperitoneally into normal rats in indicated doses. The incorporation of [^{3}H]-leucine into proteins and the formation of $^{14}CO_2$ from [^{14}C]-glucose were recorded during the first and second hour after cycloheximide administration, respectively. Each point represents the mean of three experiments using four to six rats. Different experiments yielded similar dose responses. The vertical bars show S.E.

[^{14}C]-glucose. It was significantly inhibited already at 4 h after castration, which is well before any demonstrable decrease in the general synthesis of RNA and proteins or any other measured parameter of glucose metabolism (Fig. 5).

Table 4. The effects of cycloheximide pretreatment on the response to 5α-dihydrotestosterone (5α-DHT) of the ventral prostate of castrated rats. Cycloheximide was injected intraperitoneally 2 h before hormone administration (2 mg of free steroid in 0.2 ml of saline solution subcutaneously) in a dose of 60 µg/100 g of body weight

	Time before killing	Formation of $^{14}CO_2$ from [^{14}C]-glucose c.p.m./100 mg of wet weight/h (Mean ± S.E.)
Castrated	48 h	2935 ± 210 (20)*
Castrated 5α-DHT	48 h, 12 h	13470 ± 875 (10)
Castrated Cycloheximide	48 h, 14 h	2410 ± 275 (10)
Castrated Cycloheximide 5α-DHT	48 h, 14 h, 12 h	2780 ± 185 (10)

* number of rats.

DISCUSSION

The present study together with earlier results indicates that the testosterone action in the rat ventral prostate is suppressed by a low dose (60 to 70 µg/100 g of body weight) of cycloheximide. This inhibitory action of cycloheximide is seen with the hormonal activation of RNA synthesis and glucose metabolism as well as the hormone-induced increases in enzyme activities and the size of the gland[2,5,7]. Only one exception is known to the cycloheximide sensitivity of the testosterone action. Liang and Liao[13] have recently shown that steroids, including androgen, enhance significantly the activity of the fac-

Table 5. The effects of actinomycin D (100 µg/100 g of body weight) on RNA and protein synthesis and the formation of $^{14}CO_2$ from [^{14}C]-glucose in the ventral prostate of testosterone treated, castrated rats. Actinomycin D was injected 3, 6 or 9 h after hormone administration and the animals were sacrificed 12 h or 15 h after hormone administration

	Time before killing	Incorporation of [^{3}H]-uridine into RNA (*in vitro*) Acid-insoluble radioactivity c.p.m./100 mg of wet weight/h (Mean ± S.E.)	Incorporation of [^{14}C]-amino acid into protein (*in vitro*) Acid-insoluble radioactivity c.p.m./100 mg of wet weight/h (Mean ± S.E.)	Formation of [^{14}C]O_2 from [^{14}C]-glucose c.p.m./100 mg of wet weight/h (Mean ± S.E.)
Castrated	48 h	65460 ± 7940† (20)*	66050 ± 3560† (12)	2457 ± 196 (17)
Castrated Testosterone	48 h, 12 h	119350 ± 8520 (16)	140480 ± 20570 (12)	7451 ± 464 (20)
Castrated Testosterone Act. D	48 h, 12 h, 9 h	17610 ± 2340† (8)	79680 ± 10860† (13)	3279 ± 420† (14)
Castrated Testosterone Act. D	48 h, 12 h, 6 h	18780 ± 2230† (12)	91200 ± 6570† (12)	5003 ± 533† (14)
Castrated Testosterone Act. D	48 h, 12 h, 3 h	25320 ± 4380† (14)	99230 ± 8390† (14)	6034 ± 600 (13)
Castrated Testosterone	48 h, 15 h		127940 ± 16310 (12)	8841 ± 761 (12)
Castrated Testosterone Act. D	48 h, 15 h, 6 h		92300 ± 6640 (20)	7619 ± 606 (20)

* number of rats.
† significantly different from testosterone treated rats ($P < 0.001$), student's t-test

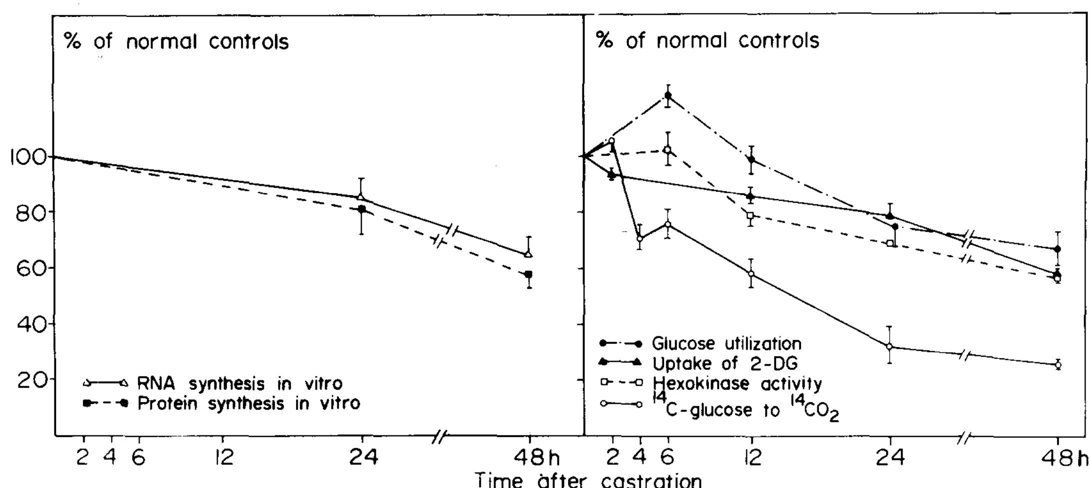

Fig. 5. The effect of castration on the ventral prostate of normal rats. Glucose utilization, total uptake of [^{3}H]-2-deoxyglucose (2-DG), the formation of $^{14}CO_2$ from [^{14}C]-glucose, the incorporation of [^{3}H]-uridine into RNA (RNA synthesis) and of [^{14}C]-amino acids into proteins (Protein synthesis) by small tissue pieces of prostate *in vitro* and the hexokinase activity were measured at the indicated times after castration. The results are expressed as % of normal control. Each point represents the mean of at least three experiments using three to five rats. Different experiments yielded similar time courses. The vertical bars show S.E.

tor involved in the very first step of protein synthesis in the target cells; this effect is not suppressed by cycloheximide. Whether the hormonal activation of protein synthesis in the presence of cycloheximide seen in the present study (Table 1) is due to this rapid effect of androgen is an intriguing possibility and needs to be corroborated.

It seems likely that cycloheximide prevents the testosterone action in glucose metabolism by inhibiting the synthesis of proteins. 1. The toxicity of cycloheximide has generally been related to the inhibition of protein synthesis on the 80 *s* ribosomes[14–16]. In accordance with this, cycloheximide administration caused a rapid and pronounced inhibition of the general protein synthesis in the ventral prostate of normal and testosterone-treated and nontreated, castrated rats. 2. A direct correlation was demonstrated between the inhibition of protein synthesis and the reduction in glucose oxidation measured as the formation of $^{14}CO_2$ from [^{14}C]-glucose in the ventral prostate of normal animals. Both processes gave an identical dose response curve with cycloheximide as well. 3. The possibility was excluded that cycloheximide acts through interfering with the formation of dihydrotestosterone, an active intracellular androgen. 4. The results with adrenalectomized male rats[9] suggest that adrenocortical hyperactivity is not involved in the suppressive effect of systemic cycloheximide on prostatic stimulation of testosterone. 5. Equivalent results were obtained with puromycin dihydrochloride, an inhibitor of protein synthesis known to act by a different mechanism.

Several possibilities should be considered when attempting to explain the molecular mechanism involved in the critical relationship between protein synthesis and the hormonal activation of glucose metabolism and RNA synthesis. On the basis of the results obtained in this study we are inclined to argue that testosterone induces the synthesis of proteins with extremely short half-lives (with a $t_{1/2}$ considerably less than 1 h). These proteins would be responsible for the activation of glucose metabolism and presumably of RNA synthesis, too. This conclusion is based mainly on the results showing that cycloheximide reduced rapidly the formation of $^{14}CO_2$ from [^{14}C]-glucose by the ventral prostate of normal and testosterone-treated, castrated rats, while the effect on that in nontreated, castrated rats emerged slowly. The presence of rapidly turning over proteins in the testosterone activated glucose metabolism was further supported by the observations that the earliest detectable and most marked changes after castration were seen in the formation of $^{14}CO_2$ from [^{14}C]-glucose. In fact, the decline (50% in 4–6 h) in the formation of $^{14}CO_2$ from [^{14}C]-glucose is comparable to the rate of disappearance of androgens from the gland after castration[17] suggesting that these events are closely inter-related.

After a single intraperitoneal injection of cycloheximide the maximal inhibition of protein synthesis was achieved within 1–2 h. Thereafter, protein synthesis recovered rapidly and reached a value close to the control at 4–6 h after cycloheximide administration. Because the response to testosterone of the ventral prostate of castrated rats as recorded 12 h after hormone administration was blocked by cycloheximide, it was concluded that the critical period for triggering of the testosterone action may well be as soon as within 4 h after hormone administration. During this period testosterone would induce the synthesis of the proteins responsible for the activation of glucose metabolism.

The site of action of the hypothetical, testosterone induced protein remains unknown. Since the changes in the formation of $^{14}CO_2$ from [^{14}C]-glucose seen after castration and cycloheximide treatment of normal rats clearly preceded and were more marked than those in the uptake and phosphorylation of [^3H]-2-deoxyglucose, and the utilization of glucose the function of these proteins could be to channel glucose to a more oxidative metabolism. In the interpretation of the experiments using [^{14}C]-labeled glucose, we have assumed[3], that the hormonal increase in the $^{14}CO_2$ production from [^{14}C]-glucose reflects an accelerated cycling of ^{14}C through the Krebs cycle. The simplest interpretation of our results would thus be that testosterone, through the induction of specific proteins, causes a raised level of pyruvate which is actively oxidized through the Krebs cycle. Taken into account the early effects of cycloheximide on hexokinase activity (seen at 1 h), the possibility, that hexokinase isozyme II[5] or one of its distinct forms[18] is one of the early testosterone induced proteins, deserves full consideration.

An alternative explanation for our results is that the increase in the production of $^{14}CO_2$ from [^{14}C]-glucose is a response to the increased energy requirement resulting from hormonally stimulated RNA and protein synthesis. Several of our findings suggest that the link between the hormonal control of glucose metabolism and protein synthesis is mediated by a more complicated way than simply by changes in the nucleoside triphosphate levels: 1. There was a rapid and marked inhibition of $^{14}CO_2$ production from [^{14}C]-glucose after castration preceding those in general RNA and protein synthesis; 2. The protein synthesis was restored more rapidly than the glucose oxidation after cycloheximide treatment of normal and castrated rats. 3. Cycloheximide inhibited protein synthesis in the ventral prostate of castrated rats and in several other organs of normal rats (unpublished results) without having an effect on glucose oxidation. 4. There are indications that cycloheximide does not affect the actual nucleoside triphosphate levels of the cells[19]. Although our hypothesis on the existence of testosterone-inducible, short-lived proteins that act on glucose metabolism is highly tentative at this time and many details require experimental confirmation, it does adequately explain our results from the studies on the effect of cycloheximide on the testosterone action. In this context it is pertinent to mention that the catabolic action of cortisol on thymocytes has been postulated to be expressed through the induction of protein(s) with short half-lives that rapidly inhibit glucose transport and phosphorylation processes[20, 21].

There have been several studies on the effect of cycloheximide on the rate of RNA synthesis. The *in vivo* administration of cycloheximide has been shown to be associated with a time dependent inhibition of ribosomal RNA synthesis in rat liver nuclei[22–24]. The intraperitoneal administration of cycloheximide as late as 10 min prior to the end of 2 h estrogen exposure or the addition of cycloheximide directly to the incubation medium also prevents the hormone-stimulated increase of nucleolar *in vitro* RNA synthesis of rat uterus[25, 26]. These observations have been interpreted as evidence for short-lived protein(s) in the normal synthesis of ribosomal RNA. The testosterone induced RNA synthesis consists mainly of ribosomal RNA and its precursors in the ventral prostate of the rat[27–32]. The inhibition of RNA synthesis by cycloheximide in our study could thus be explained by assuming that testosterone triggers the synthesis of protein(s) essential for the testosterone provoked rRNA synthesis. However, the inhibition of RNA synthesis after castration and by cycloheximide in normal rats was clearly preceded by the decrease in glucose oxidation. Our results thus leave open the question of whether the lack of a short-lived protein(s) or the possible decrease in the nucleotide triphosphate level due to the inhibition of glucose oxidation is the primary event leading to the inhibition of RNA synthesis.

The administration of actinomycin D just before or immediately after testosterone also abolished the early hormonal activation of glucose metabolism. There are at least two possible interpretations for this finding: 1. Actinomycin D blocks the initial synthesis of a hormone-specific RNA species needed for the development of most, if not all, of the secondary parameters of hormone action, including the activation of glucose metabolism. 2. Actinomycin D blocks off rapidly turning over permissive RNA species implicated in the hormonal activation of glucose metabolism.

In other words, the inhibitor would abolish the hormone action without directly blocking the component increased by testosterone. The use of actinomycin D does not make it possible to distinguish between these two theories. However, the actinomycin D experiments offered further evidence that the triggering of the testosterone action on glucose metabolism takes place in the very early phase of hormone action (within 6 h).

In our interpretation of the results we have used the common practice of equating isotope incorporation into RNA and proteins with the rate of synthesis. Since no further analysis of the acid-soluble fraction was carried out we cannot exclude the possibility that our findings with regard to the incorporation of [^3H]-uridine into RNA and [^{14}C]-labeled amino acids or [^3H]-leucine were influenced by changes in the intracellular pools of these precursors. However, from the determinations of the radioactivity in the acid-soluble fractions (data not shown) we have concluded that the major part of the changes in the labeling of RNA and protein cannot be due to changes in the uptake of precursors.

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