EFFECTS OF TESTOSTERONE ON THE METABOLISM OF THE ISOLATED LEVATOR ANI MUSCLE OF THE RAT

By

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ABSTRACT

The effects of testosterone were studied on the rate of intracellular accumulation of D-xylose-14C, AIB-14C, glycine-3H, valine-14C and leucine-14C in the isolated levator ani muscle of immature male rats. The effects of testosterone were also investigated on the incorporation of glycine-3H and leucine-14C into the muscle protein as well as on the incorporation of adenine-14C in muscle RNA. The testosterone stimulated AIB transport is described by the formulation of Michaelis-Menten and approximate values of K_m and V_max are calculated for this transport in the levator ani muscle. Testosterone stimulated the intracellular accumulation of D-xylose-14C, AIB-14C and glycine-3H, but only when injected to the rats at least six hours before the in vitro experiment. No effect could be observed when testosterone was added to the incubation media. Testosterone also stimulated the incorporation of glycine-3H and leucine-14C into the muscle protein. When puromycin was added to the medium no radioactivity was found in the protein fraction. Stimulating effects during the incubation period, of previously injected testosterone, were nevertheless observed on the intracellular accumulation of AIB-14C, glycine-3H and valine-14C. Testosterone also stimulated the incorporation of adenine-14C into the muscle RNA.

No effects on these parameters were ever observed in the diaphragm muscles of the same rats.

Protein anabolic effects of androgens have long since been established. Such metabolic effects were observed at first in studies on nitrogen balance in whole animal experiments (for ref. see Kochakian 1964). It was soon recognized, however, that this effect of androgens only concerned specific target organs; predominantly the male secondary sex organs, including some specific muscles, different in different species (Papanicolaou & Falk 1938; Kochakian et al. 1956; Kochakian et al. 1964; Costa et al. 1962; Scow 1952). One of these muscles is the levator ani muscle in
the rat (Wainman & Shipounoff 1941). This muscle was proposed as a general indicator of anabolic steroids (Eisenberg et al. 1949; Eisenberg & Gordan 1950), but the use of this muscle for that purpose has been seriously criticized (Scow 1952; Nimmi & Geiger 1957; Leibetseder & Steininger 1965).

From experiments on the seminal vesicles, prostate, liver and kidney, it has been suggested that the mechanism of action of testosterone should be to increase the ribosomal incorporation of amino acids mainly by increasing the available amount of RNA (Wilson 1962; Wicks & Kenney 1964; Williams-Ashman 1965). It has in addition been proposed that this was the only primary effect of testosterone on the protein metabolism and other effects, e.g. on the amino acid transport were only secondary to the increased protein synthesis (Frieden & Cohen 1958; Bernelli-Zazzera et al. 1958; Wilson 1962). Experiments have been reported with model amino acids, however, which also suggest effects of testosterone on the membrane transport (Metcalf & Broich 1961; Riggs & Walker 1963; Riggs & Wegrzyn 1966).

Conflicting results also exist concerning the effects of testosterone on carbohydrate metabolism and oxygen consumption (Norman & Hiestand 1960; Rudolph & Samuels 1949; Kowalewski & Bekesi 1961) as well as on the question whether testosterone has any effects in vitro or not (Farnsworth & Brown 1961; Loring et al. 1961).

Evidence indicates that similar effects of androgens can also be observed in the levator ani muscle (Saunders et al. 1962; Nimmi & Bavetta 1961; Metcalf & Broich 1961; Pellegrino & Pollera 1967; Bergamini et al. 1965; Kataja & Staehelin 1962).

In the present investigation, an attempt has been made to further elucidate some of the above-mentioned metabolic effects of testosterone. In these studies the previously described intact preparation of the levator ani muscle from immature male rats has been used (Arvill & Ahrén 1965; 1966).

**MATERIALS AND METHODS**

*Animals:* Male rats from a closed colony of the Sprague-Dawley strain were used. The average body weights were 50 g at the time of the *in vitro* experiment. All rats were given a semisynthetic diet (Gustavsson 1959; Ahrén 1959) and tap water *ad libitum*. Before the experiments the rats were starved for 24 hours. No testicular stimulation on the secondary sex organs could be demonstrated in these prepubertal rats at three weeks of age. In order to confirm this, experiments were carried out where castrated rats were compared with uncastrated rats. The castrations were done at the age of three weeks and the experiments were carried out one to two weeks later. This operation did not change either the weight of the levator ani muscle or the reactions of this muscle to administered testosterone during this time. Because of this, uncastrated rats were used in the following investigation.

*Dissection and incubation of muscles:* The rats were killed by cervical fractures
and the muscles were dissected out as described in a previous paper (Arvill & Ahrén 1966). In short, the levator ani muscles were incubated in connection with the bulbocavernosus muscles in 1 ml medium in 10 ml flasks. The diaphragms were dissected according to Kipnis & Cori (1957) and incubated in 10 ml medium in 50 ml flasks. The diaphragms were preincubated in 10 ml medium for 10 min. Krebs-Ringer bicarbonate buffer, pH 7.4, containing 2.5 mg glucose/ml (if not otherwise noted) was used. The medium was gassed with 95 % O₂ — 5 % CO₂ for one hour and the incubation flasks were gassed immediately before the incubation for 30 sec. with the same gas mixture. The flasks were sealed with tightly fitted rubber stoppers. The incubation flasks were always regassed for 30 sec. when the incubation time exceeded two hours.

The flasks were kept in a gyratory shaking bath at 37°C between one and six hours. After incubation, the muscles were rapidly washed, blotted and dissected free from the surrounding tissue, weighed and homogenized in 1—2 ml of 10 % trichloroacetic acid (TCA).

Chemicals and administration of hormones: The labelled substances were obtained from the Radiochemical Centre, Amersham, England, and from the New England Nuclear Corp., Boston, USA. The substances were used with the following specific activities: a-aminoisobutyric acid-1-¹⁴C (AIB-¹⁴C), 3.0 µc/µmole, L-valine-U-¹⁴C, 2.5 µc/µmole, glycine-2-³H, 100 µc/µmole, L-leucine-U-¹⁴C, 25 µc/µmole, adenine-8-¹⁴C, 5 µc/µmole and D-xylose-U-¹⁴C, 0.62 µc/µmole. The isotopes were added to the medium to make a molarity of 0.1 mM for AIB, L-valine and glycine, 0.01 mM for L-leucine, 0.4 mM for adenine and 0.5 mM for D-xylose.

Crystalline testosterone (British Drug House) and testosterone propionate (TP) in arachis oil (Neohombreol Pharmacia Ltd., 50 mg/ml) were used. The crystalline testosterone was dissolved first in a small amount of ethanol, 1,2-propanediol or Tween 80, diluted with buffer to desired concentrations and used intravenously or subcutaneously. The TP was diluted with arachis oil to appropriate concentration and used intramuscularly. The injected amounts were always kept at 0.1 ml and given in a tuberculin syringe. The control groups were given a corresponding injection of solvent or arachis oil.

In a few experiments, the AIB-¹⁴C accumulation was measured in the levator ani muscle of rats which had not been injected at all and compared with rats which had been injected with arachis oil (0.1 ml, 24 hours before the experiment). The distribution of AIB-¹⁴C was 1.77 ± 0.07 (10 rats) in the not injected group and 2.18 ± 0.17 (10 rats) in rats injected with arachis oil. The reason for this slight increase (p<0.05) is not clear.

The intravenous injections were given under light ether anaesthesia.

Puromycin was used as puromycin dihydrochloride obtained from Nutritional Biochem. Corp., Cleveland, USA and was added to the incubation medium to make a concentration of 500 µg/ml medium.
**Determination of the accumulation of radioactivity in the cell water:** The radioactivity of the incubation media and of the protein-free TCA extracts was measured in a Packard Tri-Carb liquid scintillation spectrometer as described in a previous paper (Arvill & Abrén 1966).

The uptake of amino acids was calculated and expressed as the distribution between the radioactivity in the intracellular water and the radioactivity in the medium (= CPM per ml intracellular water : CPM per ml medium). The uptake of D-xylose was expressed as the percentage distribution in intracellular water (100 × CPM per ml of intracellular water : CPM per ml medium).

The total and extracellular tissue water contents were determined as described previously (Arvill & Abrén 1966). Testosterone did not influence these compartments in either the levator ani muscle or the diaphragm.

**Chromatography:** Protein-free TCA extracts of muscles which were incubated with AIB-14C for two hours and samples of the stock solution of AIB-14C were examined by paper chromatography as described previously (Arvill & Abrén 1967a). These chromatograms of the TCA-extracts of the muscle preparations showed radioactivity only in the spot corresponding to AIB. This was also the case when the rats were pretreated with testosterone.

**Determination of incorporated radioactivity in muscle protein and muscle RNA:** The method for determining the incorporation of labelled amino acids into the muscle protein was a slight modification of the method described by Manchester & Young (1958) and has been described in detail in a previous paper (Arvill & Abrén 1967b).

The extraction of RNA was performed by the phenol-extraction procedure (Kirby 1956) in a modification used by Wool & Munro (1963). This method has also been described in detail previously (Arvill & Abrén 1967b).

The radioactivity of samples of the extracted protein and RNA were determined in the scintillation spectrometer first without standard and then after addition of a known internal standard. From the recovered disintegrations per minute (DPM) the incorporated amount of radioactivity was calculated in the protein fractions and expressed as µmoles of the amino acid from which it originated. The incorporated amount of adenine-14C was expressed as DPM/100 µg RNA. The RNA content of the samples was calculated from the extinction at 260 mÅ by using an extinction coefficient of 34.2 mg/ml/cm (Scott et al. 1956).

**Statistical analysis:** Mean values are given ± the standard error of the mean. Comparison between different groups was performed according to Student's t-test. A difference which gave p<0.05 was considered significant in this study. Calculations of regression line, correlation coefficient and test of linearity were performed according to Brownlee (1965).
RESULTS

The effects on the distribution of AIB-\(^{14}\)C.

The in vitro distribution of AIB-\(^{14}\)C was noticeably increased in the levator ani muscle when testosterone was injected to the rats prior to the in vitro experiment (see Table 1).

Table 1.
The effect of testosterone on the accumulation of AIB-\(^{14}\)C, glycine-\(^{3}\)H, valine-\(^{14}\)C and leucine-\(^{14}\)C in the levator ani muscle.\(^1\)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Control(^2)</th>
<th>Testosterone(^2)</th>
<th>Control (\pm) (^8) puromycin</th>
<th>Testosterone(^2) (\pm) puromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIB</td>
<td>1.38 ± 0.08 (4) 5.09 ± 0.38 (4)</td>
<td>0.95 ± 0.06 (4) 3.21 ± 0.36 (4)</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Glycine</td>
<td>3.50 ± 0.13 (9) 9.11 ± 0.23 (9)</td>
<td>5.04 ± 0.40 (4) 8.18 ± 0.13 (4)</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Valine</td>
<td>1.01 ± 0.07 (3) 0.94 ± 0.13 (3)</td>
<td>1.75 ± 0.07 (3) 1.95 ± 0.03 (3)</td>
<td>p&lt;0.05</td>
<td>n. s.</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.95 ± 0.05 (9) 0.31 ± 0.06 (8)</td>
<td>2.02 ± 0.08 (3) 2.00 ± 0.15 (3)</td>
<td>p&lt;0.001</td>
<td>n. s.</td>
</tr>
</tbody>
</table>

\(^1\) The testosterone was given as testosterone propionate 5 mg i. m. 24 hours before the experiment. The amino acid accumulations are expressed as CPM/ml intracellular water: CPM/ml medium after two hours incubation.

\(^2\) Mean±S. E. Number of rats in parentheses.

In order to investigate the effect of different methods of administration of testosterone, experiments were carried out where the AIB-\(^{14}\)C distribution ratios after two hours of incubation were compared in levator ani preparations from rats injected with testosterone in three different ways. The results of these experiments are shown in Fig. 1. Testosterone in aqueous solution (0.5 mg) injected intravenously, stimulated the distribution of AIB-\(^{14}\)C in the muscles after six hours (p<0.005). This stimulation decreased to a value not significantly above the control level after 18 hours. When the same amount of aqueous testosterone (0.5 mg) was administered subcutaneously, the effect of testosterone was postponed with the maximal effect occurring after 18 hours (p<0.002). When 0.5 mg of TP was given intramuscularly in oil solution, the total effect was more pronounced with a significant effect after 10—18 hours and a persisting effect for more than 24 hours. In fact, the same stimulation of testosterone propionate was still found after 48 hours (p<0.001, not shown in Fig. 1).

The effect of three different concentrations of TP (0.05, 0.5 and 5 mg) on the AIB-\(^{14}\)C distribution after two hours incubation was investigated 24 hours after
Effect of the *in vivo* mode of administrations of testosterone on the *in vitro* distribution ratios of AIB-\(^{14}\)C in the levator ani muscle. For subcutaneous and intravenous administrations, 0.5 mg testosterone in aqueous solution was injected and for intramuscular injections, 0.5 mg TP in arachis oil was used. The muscles were incubated for two hours in bicarbonate-buffer and the results are expressed as per cent increase of AIB-\(^{14}\)C distribution ratio (CPM/ml intracellular water : CPM/ml medium) above the control level. Significant effects are obtained after six and ten hours respectively (p<0.005) intravenously, 18 and 24 hours (p<0.002) subcutaneously, 18 and 24 hours (p<0.001) intramuscularly. The values are the mean of between 5 and 20 observations and the S. E. is indicated by vertical lines.

The results are shown in Fig. 2. The effect of 0.05 mg TP was not significant above the control level while 0.5 and 5 mg TP gave about the same increase of AIB-\(^{14}\)C accumulation in the levator ani muscle (p<0.001).

Since the most pronounced effect of testosterone on the AIB-\(^{14}\)C distribution was obtained when 5 mg TP was injected intramuscularly 24 hours prior to the *in vitro* experiment, this procedure was used as routine in most of the following experiments.

As can be seen from Table 1 the levator ani muscles of testosterone injected rats still showed stimulation of the AIB-\(^{14}\)C uptake when puromycin was added to the incubation media in a concentration which completely blocked the incorporation of radioactivity in the muscle protein (500 \(\mu\)g/ml).

When glucose was omitted from the incubation medium, a slight but significant (p<0.05) decrease of the stimulation of testosterone on AIB-\(^{14}\)C accumulation was
The effect of different concentrations of TP on the AIB-14C distribution ratio in the isolated levator ani muscle. The muscles were incubated for two hours in Krebs bicarbonate buffer. Injections of 0.5 and 5 mg TP, significantly increased (p<0.001) the distribution of AIB-14C (CPM/ml intracellular water : CPM/ml medium). The values are the mean of five observations and the S. E. is indicated by vertical lines on top of the bars.

found while the control level was the same as when glucose was present in a concentration of 2.5 mg/ml.

The accumulation of AIB, stimulated by testosterone, was determined with five different concentrations of AIB in the medium (1, 2, 10, 20 and 50 mM). The rate of accumulation was assumed to consist of two parts; one saturable and one not saturable within the concentrations used. These two components of AIB accumulation were then calculated for a period of incubation between 30 and 90 min for reasons discussed in more detail in a previous paper (Arvill & Ahren 1967 b). The diffusion constant of this AIB uptake was calculated to 0.36 in this period in a way similar to that used by Akedo & Christensen (1962) in the diaphragm. The calculated values of the saturable transport (V) were then plotted in a Lineweaver-Burk diagram (Fig. 3). The regression line of this plotting was proved linear with a correlation coefficient of 0.83 (p<0.001) and the equation \( \bar{Yx} = 0.22 + 0.28 (x - 0.33) \). From this equation approximate values of the constants \( K_m \) and \( V_{max} \) of Michaelis-Menten were calculated to 1.69 \( \mu \)moles/ml and 7.69 \( \mu \)moles per ml intracellular water \( \times \) hour, respectively.

Testosterone did not have any effect on the levator ani muscle, if added to the incubation medium, when this was tested on the accumulation of AIB-14C. This was tested with several experimental modifications. The concentration of testosterone in the medium was varied in steps between \( 10^{-5} \) and \( 10^{-1} \) mg/ml (i.e. between \( 35 \times 10^{-9} \) and \( 35 \times 10^{-5} \) M) and the incubation time was extended to more than six hours.
The reciprocal of the calculated value of \( V \) (\( \mu \text{moles/ml cellular water in one hour} \)) plotted against the reciprocal of the concentration of amino acid in the medium (5 mM) in levator ani muscle of testosterone injected rats (5 mg TP, 24 hours prior to the *in vitro* experiment).

No effects of testosterone were seen in the *in vitro* distribution of AIB-\(^{14}\text{C} \) in the diaphragm neither when injected to the rats nor when added to the incubation medium.

*The effects on glycine-\(^{3}\text{H}, \text{valine-}^{14}\text{C and leucine-}^{14}\text{C}.*

Testosterone (5 mg TP i. m. 24 hours prior to the incubation) stimulated the distribution ratio of glycine-\(^{3}\text{H} \), but not of valine-\(^{14}\text{C} \) or leucine-\(^{14}\text{C} \) in the levator ani muscle. When puromycin was added to the incubation medium (500 \( \mu \text{g/ml} \)) the levator ani muscles from the testosterone injected rats showed stimulation of the distribution of glycine-\(^{3}\text{H} \) and valine-\(^{14}\text{C} \), but not of leucine-\(^{14}\text{C} \). The results are given in Table 1.

The effects of testosterone on the incorporation into the protein of glycine-\(^{3}\text{H} \) and leucine-\(^{14}\text{C} \) are shown in Table 2. As can be seen from this table, testosterone significantly stimulated the incorporation of the two amino acids examined.
Table 2.
The effect of testosterone on the incorporation of glycine-3H and leucine-14C into the protein and of adenine-14C into the RNA of the levator ani muscle.¹)

<table>
<thead>
<tr>
<th>Labelled substance</th>
<th>Control²)</th>
<th>Testosterone²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>0.071 ± 0.004 (3)</td>
<td>0.092 ± 0.006 (3)</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.23 ± 0.02 (3)</td>
<td>0.52 ± 0.03 (3)</td>
</tr>
<tr>
<td>Adenine</td>
<td>1100 ± 110 (4)</td>
<td>2160 ± 60 (4)</td>
</tr>
</tbody>
</table>

¹) The testosterone was given as testosterone propionate 5 mg i. m. 24 hours before the experiment. The incorporation of glycine-3H and leucine-14C are expressed as μmoles/g protein and the incorporation of adenine-14C is expressed as DPM/100 μg RNA. The incubation time was two hours.

²) Mean ± S. E. Number of rats in parentheses.

Fig. 4.
The effect of testosterone (5 mg TP injected i. m. 24 hours before the in vitro experiment) on the accumulation of D-xylose-14C in the levator ani muscle. The muscles were incubated in Krebs-bicarbonate buffer without glucose in the medium. The accumulation of D-xylose-14C is expressed as the percentage distribution in intracellular water (100 × CPM/ml intracellular water : CPM/ml medium). The values represent the mean of five observations and the S. E. is indicated by vertical lines. When no such lines are given, the S. E. is too small to be indicated.
The effects on the incorporation of adenine-\textsuperscript{14}C into muscle RNA.

As can be seen from Table 2, testosterone (5 mg TP i.m. 24 hours prior to the incubation) caused a significant increase in the incorporation of adenine-\textsuperscript{14}C into the RNA fraction (extracted as by Wool & Munro 1963) of the levator ani muscle with about 100\%\% (p<0.001). The muscles were incubated for two hours in a medium containing glucose (2.5 mg/ml medium).

Effect on D-xylose-\textsuperscript{14}C accumulation.

The effect of testosterone (5 mg TP i.m. 24 hours prior to the incubation) was investigated on the \textit{in vitro} accumulation of D-xylose-\textsuperscript{14}C in the levator ani muscle with and without glucose in the medium. Testosterone stimulated this accumulation extensively. In Fig. 4, the effect is shown without glucose in the medium. Glucose in the medium (2.5 mg/ml) was found to reduce both the control level and the effect of testosterone with about 30\%\% (p<0.02) after two hours incubation time.

No effect of testosterone could be found on the accumulation of D-xylose-\textsuperscript{14}C in the diaphragm.

DISCUSSION

It has been concluded from different experiments that androgens act only in specific muscles of the body and do not stimulate muscle tissue in general. Among these specific muscles are counted the masseter and temporalis muscles of the guinea pig and the levator ani muscle of the rat (for ref. see “Introduction”). This assumption is strongly supported by the results of the present investigation where no effects at all could be detected in the rat diaphragm while several metabolic events were stimulated by testosterone in the levator ani muscle from the same rats. This fact further strengthens the criticism against the widespread use of the levator ani muscle as an indicator of general anabolic agents but demonstrates that this muscle is very useful as a test object in experiments on the action of testosterone.

From experiments on the prostate, Farnsworth & Brown (1961) reported that testosterone \textit{in vitro} stimulated the incorporation of \textsuperscript{35}S-cystine and \textsuperscript{14}C-leucine in the protein. In muscle, testosterone has been reported to decrease the oxygen consumption and enzyme formation \textit{in vitro} (Loring et al. 1961; Eisenberg et al. 1949).

In the present investigation, testosterone had no stimulatory or inhibitive effect if added only to the incubation media when this was tested on the accumulation of AIB-\textsuperscript{14}C or D-xylose-\textsuperscript{14}C. In addition, testosterone had to act \textit{in vivo} for some time (at least six hours) before any stimulation could be found on the above-mentioned parameters. This finding is in agreement with experiments of
others e.g. Jakubovic & Cekan (1966) who compared different effects of testosterone on the time relations in the levator ani muscle. In their experiments, the hormone had to be given in vivo at least 12 hours prior to the sacrifice of the rats before a stimulation could be observed on the incorporation of formate-\(^{14}\)C-Na into the protein of this muscle. In other systems a shorter latency might be obtained with testosterone. Wicks & Kenney (1964) reported an effect of testosterone after 20 min. on the RNA synthesis in the rat seminal vesicles. The time relationship for testosterone on the RNA synthesis in the levator ani muscle was not investigated in the present experiments.

The fact that testosterone has to act in vivo at least six hours before any effect can be seen on the in vitro accumulation of AIB-\(^ {14}\)C, however, does not seem to be the only explanation of the failure of testosterone to be active when added in vitro to this system, as no effect could be seen in vitro even after about the same time as was sufficient when testosterone was administered in vivo. The explanation might therefore be either that testosterone has to be metabolized in vivo or that some other factor has to be present in combination with this hormone, or a combination of these possibilities. A preliminary attempt was made to further analyse which factor could be missing, by the addition of gonadotrophins (LH 25 \(\mu\)g/ml, 38.5 IU/ml and FSH 50 \(\mu\)g/ml, 1.35 IU/ml) and growth hormone (25 \(\mu\)g/ml) to the incubation media in different combinations with testosterone. This did not, however, change the results mentioned above.

From Fig. 1, it is clearly seen that different ways of administration of testosterone influenced the AIB-\(^ {14}\)C distribution ratio in the levator ani muscle. From these experiments it seems probable that the time of resorption of the hormone is essential for the duration and intensity of the hormonal effect. This is supported by an experiment of van der Vies (1965) in the levator ani muscle and seminal vesicles of the rat. From this experiments van der Vies concluded that the rate at which a drug enters the body and particularly the variations of this rate in the course of time, not only determine the intensity and durations of action but also change its pharmacological pattern’’.

A somewhat surprising observation was the fact, that arachis oil stimulated the AIB-\(^ {14}\)C distribution of the levator ani muscle. As this oil was given in a considerable amount (0.1 ml) in the upper part of the leg, i.e. in relatively close relation to the levator ani muscle, this might induce some unspecific vascular reaction in this whole area, thus explaining the slight increase of the metabolic activity in this muscle. It is also possible that the stitch as such could produce this effect. The stimulation of AIB-\(^ {14}\)C distribution with arachis oil was quite small in comparison with the effects of testosterone injections and in addition the hormonal effects were compared with arachis oil treated controls.

One of the main questions concerning the action of testosterone is the connection between the various effects of the hormone in the muscle tissue. The results
of the present investigation have shown effects of testosterone on several secondary parameters: it stimulates the intracellular accumulation of D-xylose-14C, AIB-14C, glycine-3H but also the incorporation of glycine-3H and leucine-14C in the muscle protein and adenine-14C in the muscle RNA. This pattern of stimulation roughly resembles the findings in the levator ani muscle with insulin (Arvill & Ahrén 1967 a, b), which suggests that these two hormones might act more or less on the same cellular processes with similar mechanisms. The addition of puromycin indicates that a continued protein synthesis is not a prerequisite for an effect of injected testosterone — as for insulin in vitro — on the membrane transport during the incubation period. The long period of testosterone action in vitro, however, does of course make it difficult to exclude the fact that the increase of amino acid transport is a consequence of some other effect in the cell e.g. on the RNA-synthesis. The close resemblance between the approximate calculations of $K_m$ and $V_{\text{max}}$ for the AIB transport in the levator ani muscle with testosterone and insulin is also suggestive (see Arvill & Ahrén 1967 b).

Beside the fact that insulin but not testosterone acts in vitro, there are other differences, which exist between the effects of these hormones. Insulin stimulates the metabolism in the diaphragm, while testosterone, probably dependent on receptor specificity, is ineffective in this muscle. Another difference also seems to exist in the relation between the effects of the hormones on the carbohydrate and amino acid metabolism. When glucose is omitted from the medium the effect of insulin on the AIB-14C distribution is unaffected while the stimulation on the accumulation of the same amino acid obtained in muscles of testosterone injected rats is reduced. Whether also other effects of testosterone is affected by the glucose metabolism is not clearly investigated. Bergamini et al. (1965) have proposed that the stimulation of incorporation of glucose-14C into glycogen in the levator ani muscle is independent of the effects on the protein synthesis which, however, only is based on the differences in time courses between the changes of total glycogen and N-contents of the muscle during castration and testosterone stimulation.

Although impressive informations seem to exist concerning the effect of testosterone on the RNA formation in muscle as the primary mechanism of action (see also Breuer & Florini 1966; Fujii & Ville 1967) the possibility should not be overlooked that other effects might be induced by testosterone which not necessarily are secondary to an increased RNA synthesis. This might e.g. be true for the stimulations of the membrane transport of amino acids and of the carbohydrate utilization. Further experiments are, however, necessary to elucidate this problem.
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