DIFFERENT TIME-LAGS IN TESTOSTERONE ACTION ON SUGAR METABOLISM IN RAT LEVATOR ANI MUSCLE

Ettore BERGAMINI and Roberto PAGNI
Istituto di Patologia Generale, University of Siena, Siena, Italy

Received 5 July 1969

1. Introduction

Rat levator ani muscle is a target organ for androgens: normally present only in the male rat [1] it undergoes a marked atrophy following castration [2] and after testosterone injection a rapid recovery occurs [2] with an increase in the protein content [3,4] the aminoacid uptake [5] and the incorporation of labeled precursors both in RNA and proteins [5].

As regards testosterone effect on sugar metabolism in this muscle, an increase in glycogen content has been demonstrated 12 hr after the male hormone administration [3]. This has been attributed to a virtually contemporaneous enhancement of both sugar uptake and phosphorylation and of glycogen-synthetase I (UDPG:α-1,4-glucan-4-glucosyl-transferase, EC 2.4.1.11) activity.

Yet experiments with metabolic inhibitors indicate that whereas testosterone-enhanced glycogen synthesis is blocked by actinomycin D [3,6] increased sugar uptake and phosphorylation are not [6]. This fact points to the existence of at least two different actions of the male hormone on sugar metabolism, one dependent and one independent from RNA synthesis.

On this basis, it was thought that the time-lags of the testosterone effects on sugar uptake, sugar phosphorylation, and ^{14}C-glucose incorporation into glycogen should differ, and if so, this could help in the clarification of the mechanism of action of androgen in glycogen synthesis.

2. Materials and methods

2.1. *Animals*

Randomized groups of immature male albino rats of Wistar-Glaxo strain (50 g body weight) received testosterone both intravenously (1 mg in 0.075 ml ethanolsaline 1:1 v/v) and intramuscularly (5 mg in 0.1 ml arachis oil) or vehicle only. At various intervals (from 0 to 15 hr) muscles were removed under nembutal anaesthesia (5 mg/100 g body weight). An intact levator ani preparation was used [7].

2.2. *In vitro incubation*

Muscles were incubated at 37° in Dubnoff apparatus with shaking in 2 ml Krebs-Ringer bicarbonate pH 7.4, gas O_2/CO_2 (95/5%). Medium contained pyruvate 10 mM, inulin 7.5 mg/ml and ^{14}C-xylose 0.5 μC/ml (S.A. 0.05 mC/mmole) or 2-deoxyglucose (2-dGlc) 10 mM respectively in sugar penetration or phosphorylation studies. In glucose into glycogen incorporation experiments only inulin (7.5 mg/ml) and ^{14}C-glucose (1μC/ml, S.A. 0.10 mC/mmole) were added.

2.3. *Xylose uptake*

Incubation lasted 40 min. At the end levator ani muscle was rapidly rinsed with saline and freed from bulbo-cavernosus, blotted on filter paper and weighed. Sugar was then extracted in water at 100° for 10 min. Aliquots of the extract were spread on filter paper

disks, dried and counted to an error less than 1% in a liquid scintillation counter (Nuclear Chicago Mod. 725). On other aliquots colorimetrical determination of inulin content was performed [8] to evaluate extracellular space. Results are expressed as sugar distribution ratio between intracellular water and incubation medium as described in ref. [3].

2.4. *2-dGlc phosphorylation*

Incubation lasted 1 hr. Muscles were rapidly frozen in liquid nitrogen and sugar extracted as described (see 2.3). Total 2-dGlc and non-phosphorylated 2-dGlc were determined colorimetrically [9] respectively before and after treating the extract according to the Somogy procedure [10]. From these values and inulin distribution space 2-dGlc-6-P and free 2-dGlc content in intracellular water were calculated (see ref. [3]).

2.5. *In vitro glycogen synthesis from* ^{14}C-*glucose*

Incubation lasted 30 min after 30 min preincubation. Glycogen was purified as described [3] and dissolved in water. Aliquots were spread on filter paper disks, dried and counted to an error less than 3% (see 2.3). On other aliquots glycogen recoveries were colorimetrically checked [11]. Results are given as μmoles of glucose incorporated into glycogen/g wet weight/30 min.

2.6. *Materials*

Testosterone propionate was a gift of Vister (Casatenovo Brianza). Inulin, pyruvate and 2-dGlc were purchased from Sigma Chem. Co. (St. Louis, MO); uniformly labeled ^{14}C-xylose (S.A. 3 mC/mmole) from the Radiochemical Centre (Amersham). All products used were of analytical grade.

3. Results and discussion

Testosterone administration increases xylose penetration in levator ani muscle (fig. 1) but a time-lag of 6.5 hr is apparent, which is longer than that reported by Arvill for AIB uptake enhancement [5]. However in this case, as in that of insulin, anoxia and direct electrical stimulation, the increase in sugar uptake depends on activation of the transport mechanism (see ref. [12]) which is completed in a short time, up

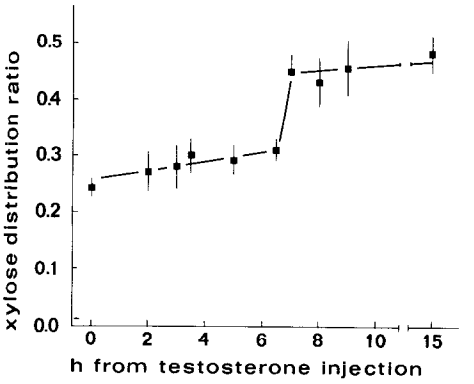

Fig. 1. Xylose uptake by rat levator ani muscle incubated *in vitro* (see text) as a function of the duration of testosterone treatment *in vivo*. Values are expressed as distribution ratio of xylose between intracellular water and incubation medium (see text). Each point is the mean of at least 5 cases. Vertical bars represent S.E.M.

to 30 min (fig. 1). The lag period before activation could mean that testosterone effect on transport is not a primary one, and that some earlier modifications have to occur either in the target organs or in other tissues. In this respect it is to be remembered that no effect on AIB uptake was shown by the male hormone *in vitro* [5] and that acute denervation completely blocks testosterone enhancement of sugar transport [12]. From fig. 1 it can be seen also that expositions longer than 7 hr to the male hormone do not further increase xylose uptake.

Also 2-dGlc *in vitro* phosphorylation is increased by testosterone given *in vivo*. Sugar phosphorylation increase shows, however, a time-lag longer than that of sugar uptake (fig. 2) as it is apparent only after 8.5 hr. This is in line with previous suggestions [3] that this phenomenon is not dependent on increased substrate availability, but on the increase found in hexokinase activity. The reasons of this greater time-lag are not clear as the conditions we found to inhibit the stimulation of sugar phosphorylation by testosterone (denervation and cycloheximide administration, unpublished) block sugar uptake increases also.

^{14}C-glucose incorporation into glycogen sharply rises after 8.5 hr of hormonal treatment showing a time-lag which is at least as long as that of sugar phosphorylation (fig. 3); values are still rising after 13.5 hr.

In line with previous suggestions [3] our data con-

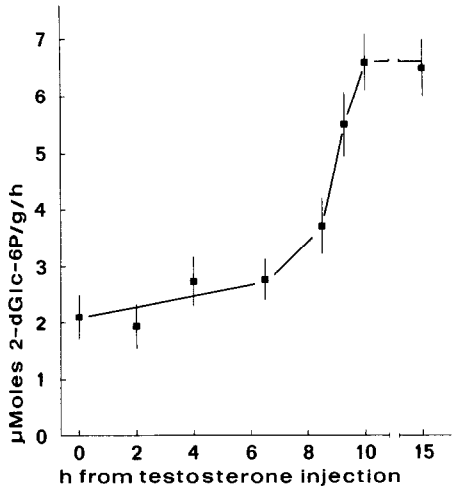

Fig. 2. 2-Deoxyglucose phosphorylation *in vitro* (see text) by rat levator ani muscle as a function of the duration of testosterone treatment *in vivo*. Values are expressed as µmoles of phosphorylated sugar/g wet weight/hr. Each point is the mean of at least 6 cases. Vertical bars represent S.E.M.

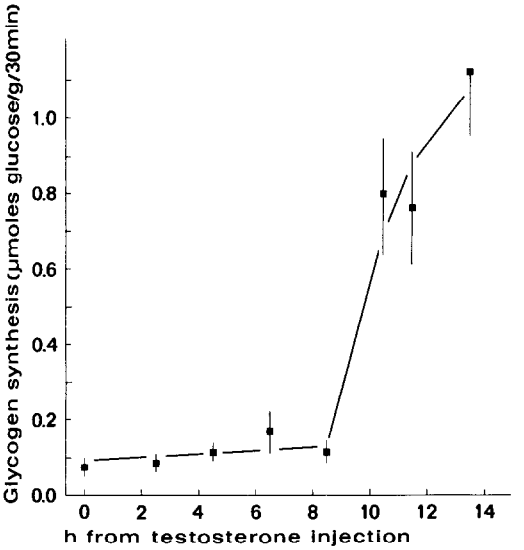

Fig. 3. Glycogen synthesis from glucose during *in vitro* incubation (see text) as a function of duration of testosterone treatment *in vivo*. Results are expressed as µmoles ^{14}C-glucose incorporated into glycogen/g muscle wet weight during 30 min incubation. Each point is the mean of at least 8 cases. Vertical bars represent S.E.M.

firm that testosterone effect on glycogen synthesis is not dependent on increased sugar penetration: no differences in glycogen synthesis are apparent till 8.5, that is 2 hr after the rise in sugar penetration appears. On the contrary the rates of sugar phosphorylation and of glycogen synthesis rise nearly at the same time: phosphorylation may therefore push glycogen synthesis and partly explain the observed increase in glycogen content. But sugar phosphorylation shows only a 3-fold increase which is completed after 10 hr whereas glycogen synthesis *in vitro* (and also *in vivo*, unpublished) shows at least a 6-fold rise after 10.5 hr and a 10-fold increase after 13.5 hr. Therefore these results, which can quantitatively explain the observed accumulation of glycogen (see ref. [3]), confirm the importance of the increase of glycogen-synthetase I activity caused by testosterone [3].

Acknowledgement

This work was supported by grants from CNR and Ministero della Pubblica Istruzione.

References

[1] E.Gutmann, V.Hanzlikova and R.Cihak, Experientia 23 (1967) 852.
[2] P.Wainman and G.C.Shipounoff, Endocrinology 29 (1941) 975.
[3] E.Bergamini, G.Bombara and C.Pellegrino, Biochim. Biophys. Acta 177 (1969) 220.
[4] R.Pagni, in preparation.
[5] A.Arvill, Acta Endocrinol. 56 suppl. 122 (1967). 1.
[6] E.Bergamini and R.Pagni, in preparation.
[7] A.Arvill and K.Ahrén, Acta Endocrinol. 52 (1966) 325.
[8] J.H.Roe, J.H.Epstein and N.P.Goldstein, J. Biol. Chem. 178 (1949) 839.
[9] G.Ashwell, in: Methods in Enzymology, eds. E.F.Neufeld and V.Ginsburg vol. 8 (Academic Press, New York and London, 1966) pp. 85–95.
[10] M.Somogyi, J. Biol. Chem. 160 (1945) 69.
[11] W.Z.Hassid and S.Abraham, in: Methods in Enzymology eds. S.P.Colowick and N.O.Kaplan vol. 3 (Academic Press, New York, 1957) pp. 35–36.
[12] E.Bergamini, Biochim. Biophys. Acta (1969) in press.