Effects of Testosterone on Folate Coenzymes in the Rat (36420)

CARLA BOVINA, BRUNELLA TOLOMELLI, CARLOTTA ROVINETTI, AND MARIO MARCHETTI

Istituto di Chimica Biologica e di Biochimica Applicata dell'Università di Bologna, 40126 Bologna, Italy

Testosterone, frequently regarded as a male sex hormone, is a growth and development hormone. It achieves its effects by stimulating the synthesis of specific enzymes and structural proteins (1). It has been shown that administration of testosterone to castrated male rats increases the incorporation of labeled amino acids in proteins of accessory reproductive glands (2) and other tissues (3). Hormone administration markedly increases RNA synthesis, the first and most dramatically altered process among those observed in castrated rats. For these reasons, the synthesis of new RNA has been considered a prerequisite for all the other metabolic changes (4–6), especially protein synthesis, which depends on it.

Since folate coenzymes are essential as 1-C donors in the synthesis of purine and pyrimidine nucleotides (7, 8), their storage can markedly influence nucleic acid synthesis.

Therefore, we studied the influence of testosterone on the content of folate coenzymes,[1] to have more detailed information on the mechanism of hormone action in this metabolic area.

In the present paper the content of various folate coenzymes in ventral prostate, in seminal vesicles as well as in liver of male castrated rats, and castrated and testosterone-treated rats has been examined.

Materials and Methods. Male Wistar rats (250–400 g), which were 15-weeks-old, were divided into 4 groups. The animals of groups 3 and 4 were orchiectomized via the scrotal route under ether anesthesia. Four weeks after gonadectomy the rats of groups 2 and 4 were injected sc with 5 doses of testosterone propionate (1 mg in 0.2 ml of sesame oil/100 g body wt) every other day for 10 days. The rats of groups 1 and 3 were injected with the same volume of vehicle. The animals were fed on a stock diet with no restrictions on their food intake throughout the experiment. The rats were killed by cervical fracture 48 hr after the last injection, and the liver, ventral prostate and seminal vesicles were quickly removed and placed in ice cold water.

To determine the folate coenzymes part of the tissues was homogenized with 9 vol of 1% (w/v) potassium ascorbate pH 6.0, another part was homogenized with 9 vol of water. The homogenates were placed in a 95° water bath for 5 min, quickly cooled in iced water and centrifuged. To the extracts were added hog kidney conjugase prepared according to Eigen and Shockman (9), 0.1 *M* acetate buffer pH 4.7, 1% (w/v) potassium ascorbate pH 4.7; the latter was omitted in the enzyme treatment of the water extracts. The samples were incubated 4 hr at 37° and saved at —20° for the determination of the folate derivatives. The microbiological, "aseptic" and "autoclaved" assays, were performed by the method described by Bird *et al.* (10). The amount of each component with folate activity was calculated using the procedure recommended by Bird *et al.* (10).

Results and Discussion. The data (Table I) concerning the liver show a significant decrease of total folate activities in castrated rats when compared with the control animals ($p < .05$). The decrease was due to differences in the reduced forms of folate ($p < .05$) and, in particular, to 10-HCO-H_4folate ($p < .05$) and H_4folate ($p < .001$).

[1] Abbreviations used are: H_4folate, tetrahydrofolate; 5-CH_3-H_4folate, 5-methyltetrahydrofolate; 5 (or 10)-HCO-H_4folate, 5 (or 10)-formyltetrahydrofolate.

5-CH_3-H_4folate increased ($p < .01$). Testosterone treatment did not cause any significant changes in normal rats while it restored the normal levels of coenzymes in castrated animals.

The data (Table I) concerning the prostate show a decrease, in castrated rats when compared with control animals, of folate activities, especially 5-HCO-H_4folate, 10-HCO-H_4folate and H_4folate each with $p < .001$. Hormone treatment of castrated animals returns to normal values the folate content of this tissue.

From the data (Table I) concerning seminal vesicles it appears that castration does not change the total folate coenzymes; slight differences were seen in 5-HCO-H_4folate ($p < .01$) and H_4folate ($p < .05$).

These results demonstrate clearly that testosterone can affect the tissue storage of folate coenzymes. In fact, castration generally leads to a decrease in the content of folate derivatives. In target organs the decrease, much more evident in the prostate than in seminal vesicles, appears in all folate coenzymes; on the contrary in the liver there is a slowing down in only a few coenzymatic forms. Testosterone treatment of castrated rats is able to reverse these modifications.

Summary. In the liver of castrated rats a significant decrease of total folate coenzymes and, in particular, of 10-HCO-H_4folate and H_4folate was observed. Treatment with testosterone normalizes the folate content of these structures. The decrease in folate coenzymes is evident in the prostate but here too, hormone administration restores the normal folate content. Castration does not significantly alter the content or distribution of folate coenzymes in seminal vesicles.

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TABLE I. Effect of Castration on the Folate Coenzyme Levels in Rat Liver, Prostate, Seminal Vesicles.

Compounds	Folate coenzymes (ng/g of tissue)[a]					
	Liver		Prostate		Seminal vesicles	
	Normal rats	Castrated rats	Normal rats	Castrated rats	Normal rats	Castrated rats
All folate forms	13,300 ± 850	10,350 ± 650[c]	1,265 ± 89	665 ± 78[c]	805 ± 102	690 ± 93[b]
All non reduced forms	610 ± 52	690 ± 88[b]	115 ± 10	105 ± 7[b]	45 ± 8	70 ± 16[b]
All tetrahydro forms	12,690 ± 1028	9,660 ± 790[c]	1,150 ± 92	560 ± 68[c]	760 ± 103	620 ± 94[b]
5-CH_3-H_4folate	1,960 ± 255	3,950 ± 586[d]	325 ± 35	235 ± 19[c]	210 ± 19	165 ± 26[b]
10-HCO-H_4folate	4,205 ± 350	3,010 ± 216[c]	360 ± 27	95 ± 13[c]	175 ± 12	145 ± 7[b]
5-HCO-H_4folate	1,890 ± 205	1,610 ± 195[b]	60 ± 7	20 ± 6[c]	40 ± 5	20 ± 4[a]
H_4folate	4,630 ± 390	1,100 ± 106[c]	410 ± 33	200 ± 23[c]	343 ± 22	280 ± 14[c]

[a] Values represent the mean of six determinations on different animals ± SEM.

[b-e] Significance of differences from values for normal rats is designated as follows: [b] not significant ($p > .05$); [c] $p < .05$; [d] $p < .01$; [e] $p < .001$.

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