UPTAKE OF THYMIDINE-H^3 BY ACCESSORY SEX ORGANS OF THE IMMATURE MALE RAT: EFFECT OF METHANDROSTENOLONE

Herbert Sheppard, Wen Hui Tsien, Phyllis Mayer and Gisela Seymour

Research Department, CIBA Pharmaceutical Company, Summit, New Jersey

Received April 3, 1964

Testosterone has been examined for its effects on the uptake of labeled amino acids into the proteins of some accessory sex organs of rats by Wilson (1962) and more recently by Silverman et al. (1963). Burkhart (1939, 1942) has shown that testosterone also increases the frequency of mitotic division in these organs. It was therefore, decided to study the changes in uptake of thymidine-H^3 (TdR-H^3) and the content of desoxyribonucleic acid (DNA) in rats treated with methandrostenolone, 17α-methyl-17β hydroxy androsta-1,4-diene-3-one[1], a steroid reported by Desaulles and Krähenbühl (1962) to have strong anabolic activity.

Eighteen immature male rats were castrated and divided into two groups of nine animals each. One group was injected subcutaneously each morning with 4 mg. of methandrostenolone suspended in carboxymethyl cellulose (CMC) while the other received the CMC vehicle only. Twenty-four hours after the second, fourth and seventh injection three animals from each group were injected intraperitoneally with 50 μc of TdR-H^3 (50 μc/μM) per 50 gm. of body weight and sacrificed by decapitation two hours later. The levator ani (LA), seminal vesicles (SV) and ventral prostate (VP) were removed and weighed. Hot perchloric acid fractions were prepared according to a modification of

[1] Dianabol(R), CIBA Pharmaceutical Company

the method of Schneider (1945) and the DNA determinations were carried out according to the method of Burton (1956). Aliquots of the hot perchloric acid extracts were counted in a Tri-Carb Liquid Scintillation Spectrometer with 4 ml. of ethanol and 10 ml. of toluene-DPO-POPOP solution.

TABLE I. Changes in DNA Levels and Thymidine-H^3 Incorporation of Tissues of the Castrated Immature Male Rat With (C + D) and Without (C) Methandrostenolone for 2, 4 and 7 Days.

Tissue	Days	µg of DNA		10^4 DPM		10^5 DPM/mg DNA	
		C	C + D	C	C + D	C	C + D
Levator Ani	2	34.5	33.1	0.11	0.44	0.31	1.33
	4	43.6	62.9	0.11	0.81	0.25	1.29
	7	31.5	68.0	0.11	0.38	0.45	0.56
Seminal Vesicle	2	75.4	125.0	0.54	5.19	0.72	4.15
	4	95.0	289.2	0.00	5.49	0.00	1.88
	7	73.6	381.7	0.11	7.13	0.15	1.86
Ventral Prostate	2	73.6	106.0	0.18	1.72	0.24	1.62
	4	61.7	187.0	0.18	1.07	0.29	0.57
	7	78.5	271.0	0.10	1.65	0.13	0.61

The data in Table I demonstrates that continued treatment with methandrostenolone markedly increases the DNA content of the tissues. The uptake of TdR-H^3 was also stimulated above that of the castrated control during the entire experimental period. It was of interest to note that this was the case with the levator ani on the second day of the experiment although no increase in DNA was chemically measurable. When, however, the tritium was expressed in terms of the weight of DNA, the value was highest following two days of treatment and then decreased toward the values for the castrated controls as the treatment was continued. This could be interpreted as indicating that the fractional rate of cell division was decreasing toward the control level. Such a conclusion agrees with the observations made by Cavazos and Melampy (1954) that the elevated mitotic rate in the SV caused by testosterone actually fell toward control values as treatment was continued. However, other factors could come into play in the whole animal which would either reduce the specific activity of the administered thymidine or make it less available to the tissues in question.

It was, therefore, decided to measure the uptake of TdR-H^3 *in vitro*. Since it had been found in other work that the non-castrated animal responded to methandrostenolone to almost the same extent as the castrated ones, the tissues from non-castrated animals were used for the *in vitro* incubations. Slices of LA, SV and VP were incubated in 1 ml. and of liver in 2 ml. of Krebs-bicarbonate buffer, pH 7.4 for 90 minutes at 37° C. Each flask contained 1 μc of H^3 in 0.005 μM of TdR-H^3. The entire contents of the flask were removed, homogenized and fractionated according to the method described for the *in vivo* experiments. As before, the treated animals received 4.0 mg. of methandrostenolone subcutaneously once each day.

TABLE II. Effect of Methandrostenolone Administration For Various Periods of Time on the *in vitro* Incorporation of Thymidine-H^3 Into the Hot Perchloric Acid Fractions of Various Tissues.

Exp. No.	Tissue	Time	10^4 DPM/mg DNA		μg of DNA	
			Normal	Normal + Drug	Normal	Normal + Drug
1	Ventral Prostate	2 day	6.89	16.42	98.8	113.8
		7 day	7.09	7.28	171.3	269.0
	Levator Ani	2 day	4.81	11.18	41.4	51.9
		7 day	5.16	5.01	43.3	82.5
	Seminal Vesicle	2 day	4.45	32.55	87.4	136.7
		7 day	9.26	24.0	91.4	386.0
	Liver	2 day	7.25	6.35	363.0	336
		7 day	9.99	7.35	317.0	309
2	Seminal Vesicle	2 hr.	5.27	5.05	71.7	75.8
		4 hr.	4.34	5.57	66.7	78.4
		6 hr.	3.31	4.93	74.5	67.9
		24 hr.	11.47	65.50	79.1	74.6

In Table II, Experiment 1, it is seen that as observed *in vivo*, the uptake of tritium when expressed on a per mg. of DNA basis was greatest on the second day and at or near to control levels on the seventh day of treatment At the same time the incorporation of TdR-H^3 by liver slices was not affected at all. It was soon found that stimulation was quite marked as early as 24 hours after one injection. In experiment 2 it can be seen that no significant stimulation of TdR-H^3 uptake was evident in the seminal vesicle up to six

hours after the subcutaneous injection of 4.0 mg. of methandrostenolone, whereas after 24 hours the stimulation was almost six times that of the controls. It is of interest that this marked stimulation occurred when no significant change in DNA levels was measurable. Similar results have been observed using thymidine-2-C^{14} thus eliminating H^3-exchange as a factor. Since the rate of absorption of the drug from the injection site and its accumulation in the seminal vesicle is not known, no correlation can be made between activity and drug concentration.

Inasmuch as Wilson (1962) had demonstrated an increased uptake of amino acids into the protein of the seminal vesicle 24 hours after the administration of testosterone it was decided to compare the stimulation of the uptake of l-leucine-U-C^{14} into protein with that of TdR-H^3 into DNA fraction. Each flask contained both 0.1 μc of l-leucine-U-C^{14} (0.009 μM) and 1.0 μc of TdR-H^3 (0.005 μM).

TABLE III. Effect of Methandrostenolone on the in vitro Incorporation of l-leucine-U-C^{14} and Thymidine-H^3 Into Protein and DNA of the Levator Ani, Seminal Vesicle and Ventral Prostate of the Normal Immature Male Rat.

Tissue	Treatment	μg of DNA	H^3-CPM/ mg DNA	mg Prot.	C^{14}-CPM/ mg Prot.
Levator Ani	Normal	41.6	1,000	1.58	145
	Normal + Drug	42.8	4,500	1.65	210
Seminal Vesicle	Normal	85.1	2,000	0.66	2210
	Normal + Drug	90.2	19,500	1.15	1270
Ventral Prostate	Normal	102.0	1,230	1.56	900
	Normal + Drug	128.0	20,000	1.64	805

It is seen in Table III that 24 hours after the administration of 4.0 mg. of methandrostenolone no significant change in the incorporation of C^{14} into the hot acid insoluble protein (HAIP) fraction of the SV, LA or VP was noticed. The lack of stimulation of amino acid incorporation into the HAIP of the VP agrees with the findings of Wilson (1962) for

testosterone but the results with the SV do not. In contrast these same tissues showed a marked stimulation of the incorporation of TdR-H^3 into the hot acid soluble fraction. It is apparent, therefore, that the uptake of TdR-H^3 under the conditions of these experiments is a more sensitive measure of the stimulating properties of methandrostenolone than is the uptake of C^{14} into protein. In fact, it may be possible to use the enhancement of TdR incorporation as a rapid assay for anabolic or androgenic activity.

The mechanism by which methandrostenolone stimulates DNA replication and cell division is one which for the moment must remain obscure. Any description of the phenomenon must be able to explain why certain tissues respond and others do not. Further investigation of this problem is underway to try to contribute to an understanding of the mechanisms controlling and regulating mammalian cell growth and division.

REFERENCES

1) Burkhart, E. Z.: Proc. Soc. Exptl. Biol. Med. 40: 137 (1939).

2) Burkhart, E. Z.: J. Exptl. Zool. 89: 135 (1942).

3) Burton, K.: Biochem. J. 62: 315 (1956).

4) Cavazos, L. F. and R. M. Melampy: Endo. 54: 640 (1954).

5) Desaulles, P. A. and C. Krähenbühl: Protein Metabolism, P. 170 (1962). Edited by F. Gross, published by Springer Verlag, Berlin-Gottingen-Heidelberg.

6) Schneider, W. C.: J. Biol. Chem. 161: 293 (1945).

7) Silverman, D. A., S. Lias and H. G. Williams-Ashman: Nature 199: 808 (1963).

8) Wilson, J. D.: J. Clin. Invest. 41: 153 (1962).