SPERMINE AND SPERMIDINE OF THE PROSTATE GLAND OF ORCHIECTOMIZED RATS AND THEIR EFFECT ON RNA POLYMERASE ACTIVITY

C.M. Caldarera, M.S. Moruzzi, B. Barbiroli and G. Moruzzi

Istituti di Chimica Biologica delle Università di Bologna e Parma

Received September 5, 1968

The dependence of nucleic acid metabolism in the prostate gland upon the blood levels of androgenic steroids is well established (Williams-Ashman et al., 1964). More recently it has also been shown (Liao, 1965) that prostatic ribosomes from orchiectomized rats exhibit a decreased ability to incorporate amino acids into proteins and that the administration of testosterone induces a rapid increase in protein synthesis. The hormone seems to be responsible for the increase of the template RNA's levels and it may help us to understand that the nucleic acid biosynthesis is under the testosterone control (Liao et al., 1966). Recent investigations in vivo show the effect of testosterone on DNA-dependent RNA polymerase activity in the prostate gland of orchiectomized rats (Hancock et al., 1962; Liao et al., 1965).

In the present paper we report the behaviour of spermine and spermidine in the ventral prostate gland of orchiectomized and testosterone-treated rats. According to our previous findings (Caldarera et al., 1965) together with the reports of other investigators (Dykstra

and Herbst, 1965; Cohen and Raina, 1967), these compounds are correlated to the nucleic acid metabolism. Both polyamines cause an increase in the incorporation rate of labelled precursors into RNA and DNA in chick embryo when the polyamine content is experimentally increased (Moruzzi et al., 1968). Furthermore we report the effect in vitro of spermine and spermidine on DNA-dependent RNA polymerase activity in purified nuclei of rat ventral prostate.

MATERIALS AND METHODS

Four months old male albino rats of the Wistar strain were orchiectomized via the scrotal route. The animals were divided into two groups. Group 1 received a daily injection of testosterone propionate (1 mg/100 g body wt.) in oil; group 2 (control) was injected with the same volume of oil. The animals were killed by cervical dislocation two, four, and six days after surgery and the ventral prostate quickly removed. The pooled prostates were homogenized in a glass-potter homogenizer in 3% w/v $HClO_4$ and polyamines assayed according to Raina and Cohen (1966).

The two DNA-dependent RNA polymerase reactions were determined in purified rat prostate nuclei (Widnell and Tata, 1964) by using the conditions described by Widnell and Tata (1966).

The reactions were stopped by the addition of 5 ml of ice-cold 0.5 N-$HClO_4$ and 400 µg of RNA carrier.

The precipitates were washed twice with 5 ml of ethanol. The residues were dissolved in 0.5 ml of formic acid, 5 ml of ethylene glycol monomethyl ether were added followed by 10 ml of scintillator fluid (Bray, 1960). Under these conditions the counting efficiency for all

samples was about 50%. DNA was determined by the method of Fleck and Munro (1962).

RESULTS AND DISCUSSION

Table 1 reports the spermine and spermidine contents in the rat prostate following the orchiectomy in testosterone treated and untreated rats. The results show high levels of spermine and spermidine content in the rat ventral prostate as compared with other organs (Rhodes and Williams-Ashman, 1964).

Table 1

Effect of testosterone on spermine and spermidine contents in the ventral prostate gland of orchiectomized rats.

Days after surgery	orchiectomized animals		orchiectomized and testosterone-treated animals	
	spermine	spermidine	spermine	spermidine
	(μmoles/g wet tissue)			
2	6.4*	5.6	5.3	4.8
4	3.4	3.3	5.4	5.6
6	0.8	0.6	5.1	4.8

*Results are the average values of three determinations of pooled prostates (2 prostates in each pool).

The polyamines content decreases progressively in castrated animals reaching its lowest level (-90%) six day after surgery, whereas no changes were noted in the control. Besides one observes a close relationship between these compounds and the testicular hormone.

The effect of spermine and spermidine on DNA-dependent RNA polymerase reaction with Mg^{++} activated or $Mn^{++}/(NH_4)_2SO_4$ in the prostate gland nuclei of orchiectomized rats from five days are shown in Fig. 1 and 2, respectively.

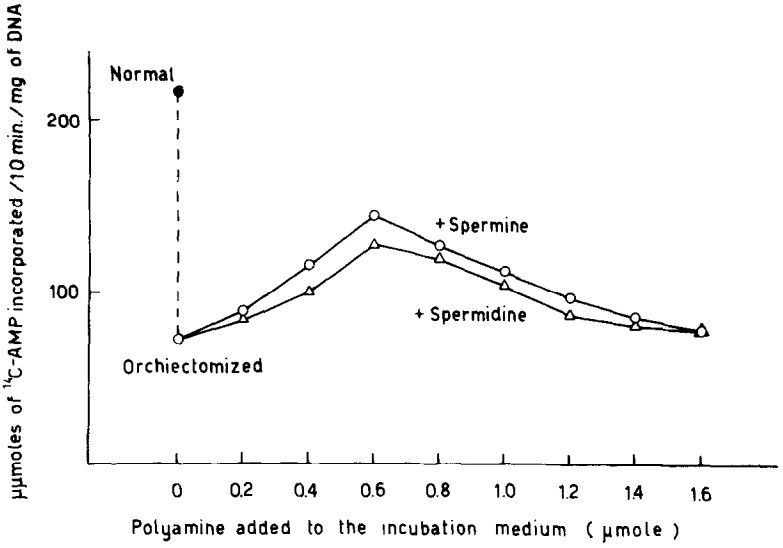

Figure 1

Dose-response curve for the effect of spermine and spermidine on DNA-dependent Mg^{++}-activated RNA polymerase activity in isolated prostate nuclei from orchiectomized rats. The curves were plotted using average values from three determinations of pooled prostates.

The Mg^{++}-activated reaction appears markedly decreased in the nuclei prostate of orchiectomized rats and the addition of 0.6 µmole of spermine to the incubation medium causes an increased activity by 70%. Spermidine induces a similar but not so evident effect.

The Mn^{++}-activated reaction too is depressed in the nuclei prostate of orchiectomized animals and the addition of 0.8 µmole of spermine to the incubation medium promotes the maximal increase (+120%). Spermidine has no relevant effect.

These results are of particular interest considering that the product of the $Mn^{++}/(NH_4)_2SO_4$-activated reaction (in term of base composition and nearest-neighbor frequency) represents a more DNA-like RNA, ac-

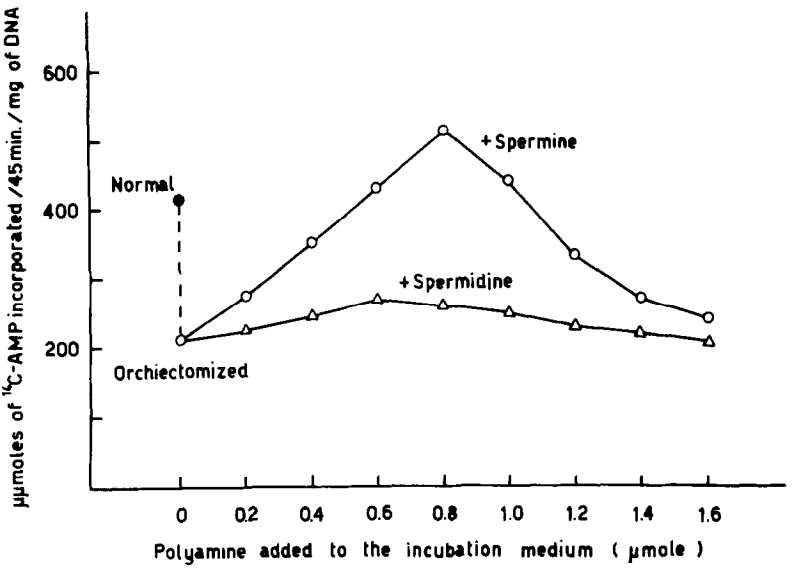

Figure 2

Dose-response curve for the effect of spermine and spermidine on DNA-dependent $Mn^{++}/(NH_4)_2SO_4$-activated RNA polymerase activity in isolated prostate nuclei from orchiectomized rats. Spermine or spermidine was added after 15' of preincubation. The curves were plotted using average values from three determinations of pooled prostates.

cording to Widnell and Tata (1962), while that of the Mg^{++}-activated reaction is a ribosomal type of RNA.

Our results show a close relationship between testosterone and prostate polyamine levels. Besides spermine and spermidine are able to increase the RNA polymerase activity which control the RNA biosynthesis.

As Doly et al. (1965) could not show a direct effect of testosterone on soluble RNA polymerase preparation, the results we have obtained lead us to see the testosterone effects to be mediated by spermine or spermidine.

REFERENCES

Bray, G.A., Analyt. Biochem. 1, 279 (1960).

Caldarera, C.M., Barbiroli, B. and Moruzzi, G., Bioch. J. 97, 84 (1965).
Cohen, S.S. and Raina, A., in Organizational Biosynthesis, Ed. by Vogel, H.J., Lampen, J.O. and Bryson, p. 157, New York, Academic Press Inc., 1967.
Doly, J., Ramuz M., Mandel, P. and Chambon P., Life Science, 4, 1961 (1965).
Dykstra, W.G. and Herbst, E.J., Science, 149, 428 (1965).
Fleck, A. and Munro, H.N., Biochim. Biophys. Acta, 55, 571 (1962).
Hancock, R.L., Zelis, R.F., Shaw, M. and Williams-Asham, H.G., Biochim. Biophys. Acta, 55, 257 (1962).
Liao, S., J. Biol. Chem. 240, 1236 (1965).
Liao, S., Leininger, K.R., Sagher and Barton R.W., Endocrinology, 77, 763 (1965).
Liao, S., Liu, A.H. and Barton, R.W., J. Biol. Chem. 241, 3869 (1966).
Moruzzi, G., Barbiroli, G. and Caldarera, C.M., Bioch. J. 107, 609 (1968).
Raina, A. and Cohen, S.S., Proc. Natl. Acad. Sci. U.S., 55, 1587 (1966).
Rhodes, J.B. and Williams-Ashman, H.G., Med. exp. 10, 281 (1964).
Widnell, C.C. and Tata, J.R., Bioch. J. 92, 313 (1964).
Widnell, C.C. and Tata, J.R., Biochim. Biophys. Acta, 123, 478 (1966).
Williams-Ashman, H.G., Liao, S., Hancock, R.L., Jurkorwitz, L. and Silverman, D.A., Recent Progress in Hormone Research, 20, 247 (1964).