Biochemical Pharmacology, Vol. 27, pp. 1831–1832.

0006-2952/78/0715-1831$02.00/0

EFFECT OF SOME STEROIDS ON BOVINE PANCREATIC RIBONUCLEASE ACTIVITY *IN VITRO*

S. M. EL-SEWEDY, E. A. EL-BASSIOUNI and S. T. ASSAR

Department of Applied Medical Chemistry, Department of Pharmacology, Medical Research Institute, Alexandria University; and Department of Biochemistry, Zagazig University, Egypt

(*Received* 22 *December* 1977; *accepted* 11 *April* 1978)

Abstract—The effect of different groups of steroidal compounds on bovine pancreatic RNase was studied *in vitro*. Cortisone and its derivatives as well as oestrogens and diethylstilbesterol acted as activators. Compounds with androgenic properties were effective inhibitors of the enzyme. It is possible that the studied compounds exert their effect, at least in part, by direct action on bovine pancreatic RNase.

Steroid hormones and related substances are known to affect several enzymatic reactions, including RNA metabolism [1–3]. The mechanism through which such effects take place has not been fully illucidated. Whether these compounds exert their action on RNase *in vivo*, directly on the enzyme or by affecting its *de novo* synthesis is not clear. Besides, some published reports have suggested a possible relationship between hormones, the growth of some tumours and RNase activity [4–6].

Therefore, this study was undertaken to investigate the *in vitro* effect of some natural and synthetic hormonal materials on the activity of bovine pancreatic RNase (EC 2.7.7.16).

MATERIALS AND METHODS

A highly polymerized yeast RNA and bovine pancreatic RNase were obtained from Sigma Chemical Co., U.S.A. The hormonal materials tested were products of Chemo Puro Manufacturing Corp., and Sigma Chemical Co., U.S.A.

The RNase activity was determined according to the method Schucher and Hokin [7], as modified by Venetianer and Straub [8]. The incubation medium contained: 4 mg RNA, 0.025 μg RNase, different concentrations of the hormonal material studied in a final vol. of 1 ml of 0.04 M phosphate buffer, pH 7.4. The hormonal materials were dissolved in ethanol–water mixture. The reaction was started by the addition of the substrate and the tubes were incubated at 37° for 10 min. The reaction was terminated by the addition of 4 ml cold-ethanol–acetic acid mixture (15:1, v/v). Blanks were obtained by zero-time incubation. The tubes were then vortexed, stored for one hour at refrigerator temperature and centrifuged. The absorption readings of the clear supernatants were determined spectrophotometrically at 260 nm. The activity of RNase was calculated according to Sigulem *et al.* [9]. The enzyme activity was arbitrarily defined as:

(Sample absorption—zero time absorption) × 1000.

The data were ultimately expressed in units/ml as the mean value of four experiments ± S.D.

RESULTS AND DISCUSSION

The results obtained on the effect of the studied hormones on the activity of RNase are summarized in Table 1. With increasing concentrations of cortisone, the activation of the enzyme decreased and at the highest concentration used (10^{-4} M) a 10% inhibition was observed. The related compounds: prednisolone, dexamethazone and its 21-phosphate ester exhibited a positive relationship between their concentrations and the activation of RNase in the range tested.

Prednisone and 9-α-fluoroprednisolone have been reported to induce an increase in RNase activity *in vivo* [5, 10]. Also, in partially hepatectomized and cortisol treated rats an increase in RNA-polymerase and nuclease activities was detected [11].

Oestradiol-17-β and its derivatives; oestradiol dipropionate, ethinylestradiol and mestranol also activated RNase. Over the range of concentrations tested, while there was a slight decrease in enzyme activation by oestradiol-17-β, both oestradiol dipropionate and ethinylestradiol exhibited peak activation of 20% over control at 10^{-6} M. The activation of RNase, however, increased from 13 to 23 per cent with increasing concentration of mestranol. The pharmacologically related compound diethylstilbesterol, also acted as activator of the enzyme (Table 1). Goldberg *et al.* [12], detected higher levels of RNase *in vivo* under conditions of oestrogen secretion.

The testosterone derivatives used in this study were found to exert a marked inhibitory effect on RNase. The ethinyl derivatives of this group of androgens were slightly more effective inhibitors of RNase than the testosterone esters tested especially at high concentrations. A large decrease in serum RNase accompanied the administration of the anabolic hormone oxandrolone [10].

From the data obtained, it seems that the compounds used in this study can exert their

Table 1. *In vitro* effect of some steroidal hormones on bovine pancreatic RNase activity

Hormones	RNase activity (units/ml) ± S.D.				
	Hormone concentration (M)				
	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
Control	(310 ± 6.25)*				
Cortisone acetate	370 ± 8.55	365 ± 5.25	340 ± 6.82	315 ± 6.72	280 ± 8.25
Prednisolone	312 ± 5.22	325 ± 4.85	335 ± 4.85	348 ± 6.28	352 ± 2.33
Dexamethazone	315 ± 3.85	325 ± 4.22	338 ± 3.52	345 ± 5.22	358 ± 6.55
Dexamethazone-21-Phosphate	312 ± 3.55	320 ± 4.66	335 ± 3.65	348 ± 4.85	358 ± 6.33
Oestradiol-17-β	380 ± 6.35	374 ± 2.36	368 ± 3.94	362 ± 5.25	362 ± 6.22
Oestradiol dipropionate	359 ± 6.55	366 ± 3.65	372 ± 4.82	360 ± 5.36	341 ± 5.63
Ethinyl-Oestradiol	372 ± 6.76	366 ± 5.28	372 ± 3.65	353 ± 4.56	335 ± 6.25
Mestranol	356 ± 5.76	362 ± 6.66	372 ± 7.86	387 ± 8.68	387 ± 5.85
Diethyl-stillbsterol	372 ± 3.88	369 ± 5.63	381 ± 5.86	378 ± 5.68	387 ± 6.66
Testosterone Valerianate	260 ± 3.68	255 ± 5.45	255 ± 3.85	270 ± 3.85	260 ± 5.35
Testosterone Cypionate	295 ± 6.53	295 ± 5.35	282 ± 4.53	260 ± 6.38	243 ± 6.36
Ethisterone	298 ± 5.22	280 ± 5.62	250 ± 6.85	222 ± 8.22	208 ± 6.52
Norethynodrel	295 ± 3.88	290 ± 5.64	290 ± 3.63	250 ± 6.65	205 ± 7.68

*This value represents the mean of 13 experiments ± S.D. Incubation medium contained all materials of enzymatic assay and solvent used except the hormonal material.

activation or inhibition, at least in part, by direct action on the bovine pancreatic RNase, especially since the interaction between hormones and RNA polynucleotides is considered unlikely [3].

REFERENCES

1. G. M. Tomkins and E. S. Maxwell, *Ann. Rev. Biochem.* **32**, 677 (1963).
2. H. G. Williams-Ashman, *Cancer Res.* **25**, 1096 (1965).
3. G. M. Grodsky, in *Review of Physiological Chemistry* (Ed. H. A. Harper), p. 446, 15th edn. Lang Medical Publications, California, U.S.A. (1975).
4. A. Graffi and W. Arnold, *Acta biol. med. germ.* **30**, K 15 (1973).
5. R. M. MacLeod, C. E. King and V. P. Hollander, *Cancer Res.* **23**, 1045 (1963).
6. F. Rosen, N. Kaiser, R. J. Milholland and S. D. Gailani, *Abstract XI th. International Cancer Congress*, **1**, 225 (1974).
7. R. Schucher and L. E. Hokin, *J. biol. Chem.* **210**, 551 (1954).
8. P. Venetianer and F. B. Straub, *Acta physiol. Hung.* **27**, 303 (1965).
9. R. M. Sigulem, J. A. Brasel, E. G. Velasco, P. Rosso, and M. Winick, *Am. J. clin. Nutr.* **26**, 793 (1973).
10. A. A. Albanese, E. J. Lorenze, L. A. Orto, E. H. Wein, D. N. Zavattaro and R. De Carlo, *N.Y. J. Med.*, June 15, 1595 (1972).
11. W. Schmid, *Abstract* 9th. FEBS Meeting, p. 141 (1974).
12. D. M. Goldberg, C. Watts and D. M. Hart, *Br. Med. J.* **1**, 424 (1968).