Biochem. J. (1972) **128**, 725–727
Printed in Great Britain

An Effect of Prolactin on Prostatic Adenylate Cyclase Activity

By MERIEL P. GOLDER,* A. R. BOYNS,† MAUREEN E. HARPER* and K. GRIFFITHS*
**Tenovus Institute for Cancer Research and †Department of Chemical Pathology, The Welsh National School of Medicine, Heath, Cardiff CF4 4XX, U.K.*

(*Received* 13 *April* 1972)

There is increasing evidence that prostatic growth can be directly affected by pituitary hormones. Grayhack & Lebowitz (1967) showed that pituitary prolactin and testosterone stimulate the growth of the lateral lobe of the prostate in hypophysectomized orchidectomized rats.

The recent isolation of human pituitary prolactin (Lewis *et al.*, 1971) emphasizes the need to examine the role of this hormone in regulating prostatic metabolism. In the present study the effects of prolactin and other hormones on prostatic adenylate cyclase activity were examined. Adenylate cyclase activity was determined by measuring the conversion of [α-^{32}P]ATP or [8-^{14}C]ATP into cyclic [^{32}P]AMP (adenosine 3′:5′-cyclic [^{32}P]monophosphate) or cyclic [8-^{14}C]AMP (Krishna *et al.*, 1968). DEAE-Sephadex was used to separate cyclic AMP from other nucleotides (Rosett *et al.*, 1970), and this allowed sample processing by a batch procedure.

Materials and methods

Cyclic AMP (acid form), AMP, ADP and ATP were obtained from Sigma (London) Chemical Co. Ltd. (London S.W.6, U.K.). [α-^{32}P]ATP and [8-^{14}C]ATP (The Radiochemical Centre, Amersham, Bucks., U.K.) were diluted in distilled water and stored at −30°C before use. Cyclic [8-^{3}H]AMP (ammonium salt) (The Radiochemical Centre) was stored at 4°C and diluted in distilled water immediately before use. DEAE-Sephadex A-25 [Pharmacia (G.B.) Ltd., London W.5, U.K.] was washed with 0.40M-NH_4HCO_3 until the washings were free of chloride (tested with $AgNO_3$), then washed with distilled water. Finally it was equilibrated with 2mM-NH_4HCO_3. Sheep prolactin (NIH-P-S8; potency 28i.u./mg), human luteinizing hormone [IRC-2 (10.12.64); potency 5×NIH-LH-S1], human growth hormone (MRC Research Standard A62/6) and human chorionic somatomammotrophin (MRC human placental lactogen 70/194) were dissolved in 21mM-tris–HCl buffer, pH7.6, before addition to incubation medium. Testosterone [Sigma (London) Chemical Co. Ltd.] was dissolved in ethanol.

Prostatic tissue was taken from 14-week-old male rats (Sprague–Dawley). Fat was removed and the tissue minced finely. A 10% (w/v) homogenate was prepared in 0.25M-sucrose by using a Philpot Stainer homogenizer (12 strokes). The homogenate was filtered through cheesecloth before assay. Homogenate (20μl) was mixed with 10μl of hormone solution for 1min at 0°C. Then 50μl of incubation medium was added (at room temperature) so that the final concentrations of reactants were as follows: $MgCl_2$ (3.1mM); ATP (2.5mM); cyclic AMP (1mM); bovine serum albumin (0.5mg/ml); [α-^{32}P]ATP (25μCi/ml) or [8-^{14}C]ATP (2.5μCi/ml); tris–HCl buffer, pH7.6 (21mM). The tubes were incubated at 37°C for 15min before the addition of 70μl of a solution containing ATP (8.7μM), cyclic AMP (3.5μM) and cyclic [8-^{3}H]AMP (0.29μCi/ml). After the tubes had been heated in a boiling-water bath for 3min they were centrifuged at 1500*g* for 5min, and 100μl of the supernatant was mixed with 1.5ml of a 1:20 (w/v) suspension of DEAE-Sephadex A-25 in 2mM-NH_4HCO_3, The suspension was mixed for 1h at 4°C and then centrifuged at 2000*g* for 5min at 4°C. The supernatant was discarded and the sediment washed twice with distilled water before elution of the adsorbed cyclic AMP with two 1ml volumes of 64mM-NH_4HCO_3. Then 0.2ml of 0.17M-$ZnSO_4$ was added to the combined eluates followed by 0.2ml of 0.15M-$Ba(OH)_2$ (final pH7.5–8.0) to remove traces of AMP. After centrifuging, the $ZnSO_4$–$Ba(OH)_2$ treatment was repeated, and 0.5ml of the supernatant was mixed with toluene scintillant containing solubilizer and its radioactivity counted. The homogeneity of the final supernatant after $ZnSO_4$–$Ba(OH)_2$ treatment was checked by descending chromatography on Whatman 3MM paper (Bär & Hechter, 1969) in the solvent system propan-2-ol–aq. NH_3 (sp.gr. 0.88)–water (7:1:2, by vol.). The chromatogram was developed for 15h at 23°C, and the spots were localized under u.v. light, cut out and placed in counting vials. Water (0.5ml) was added to elute radioactive material before its radioactivity was counted in scintillator as before. Enzyme activity was expressed as nmol of cyclic AMP formed/15min per mg of protein, after subtraction of the value of the blank (boiled tissue).

Results and discussion

DEAE-Sephadex A-25 removed over 95% of the added AMP, ADP, ATP and cyclic AMP from solution (Table 1). Treatment with 64mM-NH_4HCO_3 eluted a considerable fraction of the cyclic AMP, a small proportion of AMP but insignificant quantities

Table 1. *Adsorption of nucleotides on DEAE-Sephadex A-25 and their subsequent elution*

A 1.5ml volume of gel suspension (1:20, w/v) in 2mM-NH_4HCO_3 was mixed with 0.1ml of nucleotide solution (6mM) for 1h at 4°C. After washing with two 1ml volumes of 2mM-NH_4HCO_3, nucleotides were eluted with 64mM-NH_4HCO_3. Concentrations were determined by measuring the absorbance at 270nm or by counting the radioactivity.

	Cyclic AMP	AMP	ADP	ATP	Cyclic [^{3}H]AMP	[^{32}P]ATP
Amount adsorbed by DEAE-Sephadex (% of total)	97.5	96.5	99.7	99.7	96.1	99.9
Amount recovered from DEAE-Sephadex (% of total)	56.7	12.4	1.4	0.3	51.3	0.3

Table 2. *Effects of hormones on adenylate cyclase activity in rat prostate honogenate*

Experimental details are given in the text. Where indicated, testosterone was added in 10μl of ethanol. Results are given as means ± S.D. of quadruplicate (Expt. 1) or triplicate (Expt. 2) determinations. *P* values were obtained by using Student's *t* test (N.S., not significant). Abbreviations: OMtH, sheep prolactin; HGH, human growth hormone; HLH, human luteinizing hormone; HCS, human chorionic somatomammotrophin.

Substrate	Hormone	Concn. (μg/ml)	Cyclic AMP formation (nmol/15min per mg of protein)	*P*
Expt. 1: [8-^{14}C]ATP	None	—	2.354 ± 0.153	
	OMtH	1.5	3.216 ± 0.279	<0.005
	OMtH	60.0	2.399 ± 0.182	N.S.
Expt. 2: [α-^{32}P]ATP	None	—	0.644 ± 0.198	
	OMtH	1.5	1.646 ± 0.340	<0.05
	OMtH	60.0	0.647 ± 0.520	N.S.
	Testosterone	0.5	1.150 ± 0.545	N.S.
	Testosterone	125.0	0.343 ± 0.425	N.S.
	OMtH+ testosterone	1.5 0.5	1.688 ± 0.214	<0.01
	OMtH+ testosterone	60.0 125.0	0.849 ± 0.156	N.S.
	HLH	1.7	2.371 ± 0.533	<0.05
	HGH	1.7	1.984 ± 0.253	<0.01
	HCS	1.7	3.689 ± 1.002	<0.05
	Ethanol	—	1.191 ± 0.399	N.S.

of ADP and ATP. $ZnSO_4$–$Ba(OH)_2$ treatment removed over 99.5% of AMP but less than 2% of cyclic AMP from solutions of the nucleotides (1mM).

Prostate homogenates were incubated with sheep prolactin at two different concentrations of the hormone. The low concentration (1.5μg/ml) enhanced adenylate cyclase activity, but there was no effect at the higher concentration (60μg/ml) (Table 2, Expt. 1). Testosterone had no effect on enzyme activity when added alone or in combination with prolactin. Human luteinizing hormone, chorionic somatomammotrophin and growth hormone stimulated adenylate cyclase activity (Table 2, Expt. 2).

Paper chromatography was performed on the 'cyclic AMP' fraction isolated from incubations of prostatic tissue and sheep prolactin. The increased radioactivity in this fraction produced by the addition of prolactin was shown to be due to a component having the mobility of cyclic AMP.

There is good reason to believe that the formation of cyclic AMP from ATP by adenylate cyclase is necessary for the mediation of protein-hormone action (Jost & Rickenberg, 1971). In the present studies sheep prolactin stimulated adenylate cyclase activity when present in doses of 1.5μg/ml (0.042i.u./ml), but was ineffective at a higher concentration of 60μg/ml (1.68i.u./ml). This correlates with the results obtained by Lasnitzki (1970), who reported that prolactin at low doses (0.01–0.05i.u./ml) would sustain the growth of rat prostate *in vitro* whereas

higher doses (0.10i.u./ml) failed to maintain epithelial and secretory activity.

Testosterone did not stimulate adenylate cyclase activity in our homogenates, confirming the findings of Rosenfeld & O'Malley (1970). The steroid supports prostate growth *in vitro*, although this effect can be reversed by high doses of prolactin (Lasnitzki, 1970). Prolactin enhances the uptake of radioactive testosterone by the prostate (Farnsworth, 1970). In our experiments the addition of testosterone to prolactin did not augment the effect of the latter on adenylate cyclase activity. Testosterone stimulation of the prostate does not therefore appear to depend on adenylate cyclase activation *per se*, but its action may be potentiated by an effect of prolactin on the enzyme. In other tissues, e.g. chick oviduct, steroids can stimulate adenylate cyclase systems, at least *in vivo* (Rosenfeld & O'Malley, 1970).

Human chorionic somatomammotrophin stimulated adenylate cyclase activity in the prostate. Prolactin and the chorionic somatomammotrophin have a trophic effect on breast, and the latter hormone can displace prolactin from binding sites on breast tissue whereas human growth hormone is relatively inactive in this respect (Turkington, 1971). Human growth hormone stimulated prostatic adenylate cyclase activity in our experiments, a finding that is at variance with the failure of hormone to promote testosterone uptake by the prostate *in vitro* (Farnsworth, 1970).

Administration of luteinizing hormone *in vivo* stimulates prostatic growth partly by promoting testosterone secretion from the testes (Farnsworth, 1970). The present results suggest the possibility that it may work directly via the adenylate cyclase system.

Steroids have been utilized as the basis of the endocrinological treatment of prostatic neoplasia for many years (Huggins *et al.*, 1941). The role of pituitary hormones, e.g. prolactin (Gala & Reece, 1964), released by these agents must also be considered in investigating their mechanism of action.

We thank Dr. V. C. Stevens, Dr. A. S. Hartree and Dr. P. M. Cotes for gifts of purified hormones. The generous support of the Tenovus Organization made this work possible.

Bär, H. P. & Hechter, O. (1969) *Anal. Biochem.* **29**, 476
Farnsworth, W. E. (1970) in *Some Aspects of the Aetiology and Biochemistry of Prostatic Cancer* (Griffiths, K. & Pierrepoint, C. G., eds.), p. 3, Tenovus Workshop Publications, Cardiff
Gala, R. R. & Reece, R. P. (1964) *Proc. Soc. Exp. Biol. Med.* **118**, 350
Grayhack, J. T. & Lebowitz, J. M. (1967) *Invest. Urol.* **5**, 87
Huggins, C., Scott, W. W. & Hodges, C. V. (1941) *J. Urol.* **46**, 997
Jost, J.-P. & Rickenberg, H. V. (1971) *Annu. Rev. Biochem.* **40**, 741
Krishna, G., Weiss, B. & Brodie, B. B. (1968) *J. Pharmacol. Exp. Ther.* **163**, 379
Lasnitzki, I. (1970) in *Some Aspects of the Aetiology and Biochemistry of Prostatic Cancer* (Griffiths, K. & Pierrepoint, C. G., eds.), p. 68, Tenovus Workshop Publications, Cardiff
Lewis, U. J., Singh, R. N. P. & Seavey, B. K. (1971) *Biochem. Biophys. Res. Commun.* **44**, 1169
Rosenfeld, M. G. & O'Malley, B. W. (1970) *Science* **168**, 253
Rosett, T., Smith, J. G., Matsuo, I., Bailey, P. A., Smith, D. B. & Surakiat, S. (1970) *J. Chromatogr.* **49**, 308
Turkington, R. W. (1971) *J. Clin. Invest.* **50**, 94A