A Study of 3':5'-Cyclic Mononucleotide-Dependent Protein Kinase from Canine Prostate Glands

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1. An adenosine 3':5'-cyclic monophosphate (cyclic AMP)-dependent protein kinase, located predominantly in the cytosol, was studied in canine prostate. 2. The enzyme exhibited cyclic AMP-binding activity, and could be isolated by chromatography on diethylaminoethyl cellulose. 3. The enzyme was maximally stimulated (fourfold) by 1 μ M-cyclic AMP, and half-maximal activation of the enzyme was observed in presence of 50 nM-cyclic AMP. 4. Equilibrium studies at pH 5.0 indicated the presence of one major class of binding site for cyclic AMP, with an association constant of approx. 10^8 M⁻¹. 5. Stimulation of the enzyme was also observed with the 3':5'-cyclic monophosphate derivatives of cytidine, inosine, guanosine and uridine as well as with dibutyryl cyclic AMP, but higher concentrations of these cyclic nucleotides were required to provide the same degree of activation as that seen with cyclic AMP. 6. Comparing α -casein, protamine and different histone subfractions as substrates, highest cyclic AMP stimulation was demonstrated with histones. 7. Although maximum velocity of the enzyme was enhanced approximately fivefold in presence of cyclic AMP, kinetic studies indicated that the apparent K_m for histone (0.5 mg/ml) remained the same whether determined in the presence or absence of the cyclic nucleotide. 8. In addition, cyclic AMP did not significantly change the apparent K_m for ATP (1.2×10^{-5} M). 9. The purified enzyme showed an absolute requirement for bivalent metal ion. Substitution of Mn²⁺ for Mg²⁺ decreased basal protein kinase activity as well as the stimulation noted with cyclic AMP. Similarly, the basal activity was lowered when Mg²⁺ was replaced by Ca²⁺ and cyclic AMP produced only little stimulation of the prostatic enzyme.

There is a growing body of information which indicates that sex steroids, like peptide hormones and catecholamines, may also exert their diverse metabolic effects on target cells through stimulation of the cyclic AMP-adenylate cyclase system. Although the activity of adenylylase and the endogenous cyclic AMP concentrations in the rat prostate decreased significantly after gonadectomy (Singhal & Sutherland, 1974; Mangan *et al.*, 1973), intravenous administration of testosterone, particularly of dihydrotestosterone, produced significant stimulation of the prostatic enzyme in androgen-deprived rats as rapidly as 1 h after injection (Sutherland & Singhal, 1974). Investigations on the mechanism by which cyclic AMP exerts its diverse effects have concentrated on its ability to stimulate protein kinase activity (Reimann *et al.*, 1971; Brostrom *et al.*, 1970; Langan, 1969; Corbin *et al.*, 1972a; Garren *et al.*, 1971; Sanborn *et al.*, 1973a; Yamashita & Field, 1972; Shlatz & Marinetti, 1971). It has been suggested that changes in the intracellular concentration of cyclic AMP may affect the transcription of DNA, glycogen, lipid or protein metabolism, through the stimulation of cyclic AMP-dependent protein kinases in hormone-responsive cells (Langan,

1969, 1970; Garren *et al.*, 1971; Soderling *et al.*, 1970, 1973). The present paper reports the preparation, subcellular distribution and general characterization of a cyclic AMP-dependent protein kinase isolated from canine prostate.

Materials and Methods

Chemicals

[γ -³²P]ATP (25.5 Ci/mmol) and Omnifluor were obtained from New England Nuclear Corp., Boston, Mass., U.S.A. and cyclic [³H]AMP (28 Ci/mmol) was purchased from Schwarz/Mann Chemical Co., Orangeburg, N.Y., U.S.A. α -Casein, protamine, histone mixture (type IIA), DEAE-cellulose and all unlabelled nucleotides were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A., and histone subfractions were products of Worthington Biochemical Corp., Freehold, N.J., U.S.A.

Protein kinase assay

Protein kinase was assayed by measuring the incorporation of ³²P into histone mixture (type IIA) by modification of the method of Sanborn *et al.*

(1973a). The assay medium (final volume 0.13ml, pH 6.5) contained 46mM-sodium acetate, 1.5mM-NaF, 3.1mM-theophylline, 0.24mg of histone mixture, 17mM-MgCl₂, 85 μ M-ATP, 55 μ g of enzyme preparation (peak II) after DEAE-cellulose step and 4.6 μ M-cyclic AMP (for measuring the activity of the cyclic AMP-dependent enzyme). Immediately after the addition of the enzyme to the incubation mixture, the reaction was initiated by the addition of [γ -³²P]-ATP-MgCl₂ (200000 c.p.m.), and the subsequent incubation at 30°C for 7 min was terminated by the addition of 5 ml of 20% trichloroacetic acid. The protein precipitate was then separated by filtering on a Millipore filter (HAWP, 0.45 μ m) and the radioactivity counted in 10 ml of scintillation 'cocktail' containing 7 parts of Omnifluor solution (4g of Omnifluor per litre of scintillation-grade toluene) to 3 parts of ethylene glycol monoethyl ether. Appropriate blanks without histone and enzyme preparation were run simultaneously with the experimental samples. In the substrate-specificity study on protein kinase, α -casein was dissolved by the method of Reimann *et al.* (1971).

Cyclic AMP-binding assay

This was done in 0.05M-sodium acetate-1mM-EDTA, pH 5.0, in a final volume of 250 μ l as modified from the method of Sanborn *et al.* (1973b). Unless stated otherwise, 100 μ g of the enzyme preparation (peak II) after DEAE-cellulose purification was used with 2.4nM-cyclic [³H]AMP. When crude samples were studied, 5mM-theophylline was also added. After an incubation at 0°C for 3 h, the assay mixture was passed through a Millipore filter (HAWP, 0.45 μ m) which was then counted for radioactivity in 10 ml of the scintillation 'cocktail' described for protein kinase assay. Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as the standard.

Preparation of protein kinase

Cyclic AMP-dependent protein kinase was partially purified by modification of reported procedures (Reimann *et al.*, 1971; Sanborn *et al.*, 1973a; Walton & Garren, 1970). A dog (14–18kg body weight) was killed by an intravenous injection of sodium pentobarbital, and the prostate gland was quickly removed and trimmed of all extraneous tissue. The gland was then homogenized in 3 vol. of buffer [0.05M-Tris-HCl (pH 7.4) – 0.25M-sucrose – 0.05M-KCl – 5mM-MgCl₂] and the homogenate was centrifuged at 650g for 30 min. The supernatant was decanted and centrifuged at 130000g for 90 min. The supernatant containing the soluble cyclic AMP-dependent protein kinase was then subjected to precipitation at pH 5.5, and the resulting supernatant was readjusted to pH 7.4 and subjected to (NH₄)₂SO₄ precipitation (0.32g/ml). The protein precipitate was then dissolved in a volume of buffer (0.01M-Tris-HCl-1mM-2-mercaptoethanol, pH 7.4), equivalent to approximately one-fifth of that used for homogenization of the tissue, and dialysed overnight against 3 vol. (6 litres each) of the same buffer. The solution was then centrifuged at 10000g for 10 min to remove any precipitate and was applied to a DEAE-cellulose column previously equilibrated with the same buffer just before loading. The column, after it has been washed with the same buffer again (10 $\times$ bed volume of column), was subsequently eluted with a linear gradient of 0.05–0.35M-NaCl containing 0.01M-Tris-HCl-1mM-2-mercaptoethanol, pH 7.4. Fractions comprising both peaks containing cyclic AMP-binding and cyclic AMP-dependent kinase activities were pooled, dialysed against the column buffer without NaCl and stored in small portions in liquid N₂. Unless indicated otherwise, data presented in this paper describe the characterization of the prostatic protein kinase activity eluted at 0.17M-NaCl during purification on DEAE-cellulose (fraction II).

Table 1. *Purification of cyclic AMP-binding and cyclic AMP-dependent protein kinase activities from canine prostate*
Standard conditions, as described in the text, were used for assaying the activities of cyclic AMP binding and cyclic AMP-dependent protein kinase.

Step	Procedure	Cyclic [³ H]AMP-binding activity		Protein kinase activity	
		Sp. activity (pmol bound/mg of protein)	Yield (%)	Sp. activity (nmol of ³² P incorporated/mg of protein)	Yield (%)
I	Centrifugation at 130000g (supernatant)	1.03	100	2.02	100
II	Precipitation at pH 5.5 (supernatant)	1.34	119	2.17	98
III	(NH ₄) ₂ SO ₄ precipitation (precipitate)	3.13	74	8.91	107
IV	DEAE-cellulose chromatography				
	Fraction I (minor peak)	1.31	3	6.72	8
	Fraction II (major peak)	6.92	34	11.87	20

Results

Preparation of cyclic AMP-dependent protein kinase

Partial purification of the cyclic AMP-dependent protein kinase activity on diethylaminoethyl cellulose of the soluble fraction of canine prostate glands yielded two peaks (fractions I and II), both containing catalytic as well as cyclic AMP-binding activities (Table 1). The activity of the major peak (fraction II), eluted at 0.17M-NaCl, was stimulated fourfold by 5μM-cyclic AMP and the minor peak (fraction I) stimulated two- to three-fold by the cyclic nucleotide, was eluted at a lower salt concentration (Fig. 1). Occasionally, cyclic AMP-independent protein kinase activity was also observed. Although both kinase and cyclic AMP-binding activities were stable during storage for 6 months in liquid N₂, over 50% of its cyclic AMP-binding capacity was lost after 4 months when the samples were stored at -20°C.

Differential centrifugation of prostate homogenate

Of the four subcellular fractions examined, the cytosol showed the highest amount of total activity as well as the highest specific enzyme activity (Table 2). Maximum enzyme stimulation by cyclic AMP was also detectable with this high-speed supernatant as well as the nuclear pellet. These results agree with the finding that cyclic AMP-binding activity was found predominantly in the cytosol with significant activity also associated with the 650g pellet. In view of the possibility that the enzyme activities in these particulate fractions might be present in an

Fig. 1. Chromatographic behaviour of canine prostatic protein kinase and cyclic AMP-binding activities

The enzyme (0.3g of protein) was applied to a DEAE-cellulose column (16cm x 30cm) and eluted with a linear gradient of 0.05–0.35M-NaCl solution (200ml of each) after extensive washing with 0.05M-NaCl. The buffer contained 0.01 M-Tris-HCl and 1 mM-2-mercaptoethanol, pH7.4. Units are expressed as c.p.m./sample. ●, ³²P incorporated into histone mixture in the presence of 4.6 μM-cyclic AMP without correction for sample blank; ○, cyclic [³H]AMP bound; —, concentration of NaCl in elution buffer.

Table 2. Subcellular localization of protein kinase and cyclic AMP-binding activity from canine prostate

Prostatic tissue was homogenized in 3 vol. of buffer [0.05 M-Tris-HCl (pH 7.4)–0.25 M-sucrose–0.05 M-KCl–5 mM-MgCl₂] with 12 strokes of a motor-driven Potter-Elvehjem homogenizer. After filtration through four layers of cheesecloth, the homogenate was subjected to differential centrifugation. All fractions were washed twice with the homogenizing buffer and once with the same buffer without sucrose. Protein kinase assay was performed as described in the text, with 100 μg of protein in the absence and presence of 4.6 μM-cyclic AMP. Triton X-100 was added to a final concentration of 0.1%. Specific activity of protein kinase is expressed in nmol of ³²P incorporated into histone mixture (type IIA)/mg of protein whereas the total activity is given in terms of mmol of ³²P/5g wet weight of the canine prostate. The cyclic AMP-binding capacity was assayed in the presence of 0.15 μM-cyclic [³H]AMP, 5 mM-theophylline and 100–150 μg of protein from each fraction. Specific activity for cyclic AMP-binding is expressed as pmol of cyclic AMP bound/g of protein, whereas the total activity is given in terms of pmol of cyclic AMP bound/5g wet weight of the canine prostate. Each value is based on two to three separate determinations of protein kinase or cyclic AMP-binding activity.

Fractions	Protein kinase activity (Triton-X-100-treated)				Cyclic AMP-binding activity			
	Sp. activity		Total activity		Non-treated		Triton-X-100-treated	
	-Cyclic AMP	+Cyclic AMP	-Cyclic AMP	+Cyclic AMP	Sp. activity	Total activity	Sp. activity	Total activity
650g Pellet	0.25	0.61	12.6	30.3	0.41	20.1	0.66	32.7
20200g Pellet	0.06	0.10	2.9	5.5	0.15	8.0	0.28	14.8
100000g Pellet	0.39	0.60	9.4	14.3	0.31	7.4	0.62	14.9
100000g Supernatant	0.62	1.85	111.8	398.2	1.93	416.4	1.86	407.1

occluded form, the effect of Triton X-100 on both kinase and cyclic AMP-binding activities of the subcellular fractions was investigated. A comparison of the enzyme activities in the untreated fractions with those in fractions treated with Triton X-100 revealed that there were latent activities in all three untreated particulate fractions that could be unmasked by addition of the detergent. It is noteworthy that Triton X-100 (0.1%) not only failed to enhance enzyme activities in the high-speed supernatant, but rather caused a slight decrease in specific, as well as total, catalytic and cyclic AMP-binding activities.

Effect of various incubation conditions on protein kinase activity

Although enzyme activity at 30°C in the absence of cyclic AMP was proportional to the reaction time up to 20min, maximal cyclic AMP stimulation and linearity in enzyme activity with time were observed for only 7min when protein kinase was assayed in the presence of the nucleotide. In studying the effect of various amounts of enzyme on prostatic protein kinase activity, the incorporation of ^{32}P into the histone mixture was linear up to approx. 60 μg of protein of the enzyme preparation both in the presence and absence of cyclic AMP.

Effect of various concentrations of cyclic AMP

The relationship between protein kinase activity and cyclic AMP concentration was also studied.

Fig. 2. Scatchard plot describing the interaction at equilibrium between prostatic cyclic AMP-dependent protein kinase and cyclic AMP

Cyclic AMP-binding activity was assayed in an incubation medium (final volume 0.25ml, pH 5.0) containing 2.4nM-cyclic [^3H]AMP, 0–72nM-unlabelled cyclic AMP, 0.05M-sodium acetate, 1mM-EDTA and 100 μg of protein of fraction II enzyme preparation after DEAE-cellulose chromatography. Incubation was carried out at 0°C for 10h and protein-bound cyclic AMP was separated by Millipore filtration as described in the text. $K_{\text{assoc.}}$ is $7.8 \times 10^7 \text{M}^{-1}$ and the concentration of binding sites is 18pmol/mg of protein.

Although the concentration of cyclic AMP necessary for half-maximal velocity was about 50nM, maximum stimulation of the enzyme was observed with 1 μM -cyclic AMP. Further, higher concentrations of cyclic AMP were found to cause progressively lower stimulation of the prostatic enzyme activity.

Equilibrium studies on cyclic AMP-binding

To understand further the molecular interaction of cyclic AMP with its dependent protein kinase, association of the nucleotide with the enzyme was studied at equilibrium. Scatchard-plot analysis (Scatchard, 1949) of the binding data demonstrated non-co-operativity and one order of cyclic AMP-binding sites (Fig. 2). The association constant ($K_{\text{assoc.}}$) at 0°C and pH 5.0 was about $7.8 \times 10^7 \text{M}^{-1}$, and the concentration of binding sites was 18pmol/mg of protein of the enzyme preparation.

Fig. 3. Effect of various 3':5'-cyclic mononucleotides on prostatic protein kinase activities

Kinetic activity is expressed as a percentage of that observed in the presence of 1 μM -cyclic AMP. Assays were carried out under standard conditions as described in the text, except for the variation in the concentration of the indicated cyclic nucleotide. The various 3':5'-cyclic mononucleotides tested were cyclic AMP ($\bullet$), cyclic TMP ($\square$), cyclic CMP ($\blacktriangle$), cyclic IMP ($\circ$), cyclic GMP ($\blacksquare$), 6-N,2'-O-dibutyryl cyclic AMP ($\diamond$) and cyclic UMP ($\triangle$). Protein kinase activity is expressed as the percentage of ^{32}P incorporated/mg of protein in the presence of 1 μM -cyclic AMP.

Nucleotide specificity of protein kinase

The effect of several 3':5'-cyclic mononucleotides on the activity of prostatic protein kinase is shown in Fig. 3. At low concentrations, cyclic AMP was far more effective than any of the other nucleotides in activating the enzyme. Although kinase activity was at its maximum with 1 μ M-cyclic AMP, cyclic IMP, the most potent activator among the analogues tested, produced only 30% of the stimulation elicited by cyclic AMP at equimolar concentration. Nevertheless, various cyclic mononucleotides including those of guanosine, inosine, cytosine, uridine as well as the 6-*N*,2-*O*-dibutyryl derivative of cyclic AMP, at the highest concentration examined, were also capable of stimulating enzyme activity to near-maximal values. In contrast, the effect of cyclic dTMP on kinase activity was only slightly stimulatory.

Ability of various proteins to serve as substrate

Cyclic AMP (5 μ M) markedly enhanced 32 P transfer to calf thymus histones, especially fractions F1 and F3, as classified by the terminology of Johns (1964) (Fig. 4). Although both basal and cyclic AMP-stimulated activities of the enzyme was highest with F2b histones, the cyclic AMP stimulation of the enzyme in this fraction was considerably lower.

Fig. 4. Substrate specificity of soluble prostatic cyclic AMP-dependent protein kinase

Standard assay conditions were used except for the addition of other protein substrates (240 μ g). α -Casein was dissolved by the method of Reimann *et al.* (1971). Values in parentheses represent percentage stimulation of kinase activity in the presence of 4.6 μ M-cyclic AMP (■), taking the control (□) as 100%. F1, F2a, F2b and F3 are histones.

Although basal enzyme activity was measurable with both protamine and α -casein, little or no stimulation could be demonstrated with cyclic AMP (Fig. 4).

Apparent K_m for histones and ATP

The effects of various histone and ATP concentrations on protein kinase activity were examined by Lineweaver-Burk plots. Although cyclic AMP did not significantly change the apparent K_m for histone (0.5 mg/ml), the maximum velocity was increased approximately fivefold by the nucleotide. Values of 1.1 and 5.0 nmol of 32 P incorporated/7 min per mg of protein were obtained in the absence and presence of cyclic AMP respectively. As seen in the case of histones, the concentration of ATP needed for half-maximal activity was not affected significantly by the addition of cyclic AMP, whereas maximal activity was greatly increased. The apparent K_m for ATP was 1.2×10^{-5} M in the presence and absence of the nucleotide. Addition of cyclic AMP, however, again increased maximal velocity from 0.57 to 1.5 nmol 32 P incorporated/7 min per mg of protein.

Bivalent metal ions

Studies on the effect of certain bivalent metal ions on protein kinase activity demonstrated an absolute requirement of the enzyme for a bivalent metal. Although minimal basal activity was observed in the absence of any bivalent cations, little or no stimulation by cyclic AMP could be detected. However, in the presence of 1.7 mM- Mg^{2+} , kinase activity was markedly increased and could be further enhanced 3.5-fold in the presence of the cyclic nucleotide

Table 3. Effect of certain bivalent metal ions on cyclic AMP-dependent protein kinase activity of the canine prostate

Protein kinase activity was assayed under standard conditions as described in the text both in the absence and presence of 4.6 μ M-cyclic AMP. Enzyme activity is expressed as nmol of 32 P incorporated into histone (type IIA) per mg of protein of the enzyme preparation after DEAE-cellulose purification. Each value is based on two to three separate determinations of enzyme activity.

Cation	Concn. of cation (mM)	Protein kinase activity	
		-Cyclic AMP	+Cyclic AMP
None		0.19	0.20
Mg^{2+}	1.69	0.61	2.18
	16.9	1.46	5.43
Mn^{2+}	1.69	0.32	0.97
	16.9	0.18	0.56
Ca^{2+}	1.69	0.17	0.33
	16.9	0.06	0.04

(Table 3). Although increasing the Mg^{2+} concentration to 17mM further augmented basal and cyclic AMP-stimulated kinase activity, the percentage stimulation remained relatively unaltered. When equimolar concentrations of Mn^{2+} were tested instead of Mg^{2+} , enzyme activities were somewhat decreased. Though cyclic AMP caused a slight increase in the activity of the enzyme in the presence of 1.7mM- Ca^{2+} , higher concentration of the metal (17mM) inhibited both the basal and the cyclic AMP-stimulated prostatic protein kinase activities (Table 3).

Discussion

The properties of cyclic AMP-stimulated protein kinases have now been studied in a variety of tissues. Although the assay conditions used were not identical with those used in the present study, it is of interest to compare some of their characteristics with our findings for the canine prostate-gland enzyme. In general, the kinetic and physical properties of the prostatic cyclic AMP-dependent protein kinase seem to resemble those described for the enzymes from other mammalian tissues (Reimann *et al.*, 1971; Brostrom *et al.*, 1970; Langan, 1969; Corbin *et al.*, 1972a; Garren *et al.*, 1971; Sanborn *et al.*, 1973a; Majumder & Turkington, 1971; Chen & Walsh, 1971; Maeno *et al.*, 1971). In lactating-rat mammary glands (Majumder & Turkington, 1971), subcellular distribution studies have demonstrated that as much as 98% of the total kinase activity was recoverable in the soluble fraction. Similar findings have also been reported for protein kinases from bovine endometrium (Sandborn *et al.*, 1973a) and rat liver (Chen & Walsh, 1971). Although no cyclic AMP-binding protein devoid of kinase activity was found, a small peak of cyclic AMP-dependent kinase was occasionally observed. Only the major fraction was used in the studies reported in this paper. Although the similarities and differences in kinetic and physical properties of the two activity peaks remain to be determined, it is of interest that multiple forms of cyclic AMP-dependent protein kinase have been reported in skeletal muscle (Corbin *et al.*, 1972b), liver (Chen & Walsh, 1971; Kumon *et al.*, 1972), cardiac tissue (Rubin *et al.*, 1972) and adrenals (Gill & Garren, 1971). The results on the ability of various cyclic nucleotides to stimulate the prostatic enzyme are also in line with those reported earlier for protein kinases from several other tissues (Reimann *et al.*, 1971; Brostrom *et al.*, 1970; Miyamoto *et al.*, 1969; Corbin & Krebs, 1969; Kuo & Greengard, 1969). In general, cyclic IMP has been shown to be most effective at low concentrations. This is not surprising considering the high degree of structural similarity between cyclic IMP and cyclic AMP.

Cyclic GMP also stimulates protein kinases although much higher concentrations are needed than those for cyclic AMP. An exception to this, however, was demonstrated with the cyclic GMP-stimulated protein kinase from lobster muscle (Kuo & Greengard, 1970). This enzyme had a lower apparent K_m for cyclic GMP than for cyclic AMP. Although dibutyryl cyclic AMP, the lipid-soluble analogue of cyclic AMP, widely used for facilitating passage of cyclic AMP across cell membranes, was much less active than cyclic AMP, it did activate the enzyme fully at high concentrations. The possibility cannot be excluded, however, that some hydrolysis of the dibutyryl derivative to free cyclic AMP occurs during the incubation period of the assay.

As anticipated from its cyclic-nucleotide dependence, the prostatic enzyme exhibited high affinity for cyclic AMP ($K_{assoc.} = 0.78 \times 10^{-8} M^{-1}$, pH 5.0, 0°C). It is noteworthy, under the same experimental conditions, cyclic AMP-dependent protein kinase from bovine endometrium also demonstrated a single class of cyclic AMP-binding sites with $K_{assoc.}$ of 0.6×10^8 – $1.0 \times 10^8 M^{-1}$. Although $K_{assoc.}$ decreased slightly to $0.4 \times 10^8 M^{-1}$ at 20°C, the number of binding sites per mg of protein remained unaltered for the uterine enzyme (Sanborn *et al.*, 1973a). In addition, two cyclic AMP-dependent protein kinases exhibiting a slight difference in the affinity for cyclic AMP at pH 7.5 have also been reported in rat hepatic tissue ($K_{assoc.} = 0.25 \times 10^8 M^{-1}$, $1 \times 10^8 M^{-1}$) (Chen & Walsh, 1971).

With regard to the protein substrate specificity, like the enzymes from heart (Brostrom *et al.*, 1970), adipose tissue (Corbin *et al.*, 1972a), liver (Langan, 1969), brain (Miyamoto *et al.*, 1969), trout testis (Jergil & Dixon, 1970), skeletal muscle (Reimann *et al.*, 1971) and bovine thyroid (Yamashita & Field, 1972), the prostatic protein kinase was found to phosphorylate histones much more readily than casein and protamine. Of several proteins tested as substrates for the adipose-tissue enzyme, muscle glycogen synthetase, muscle phosphorylase and histone F2b incorporated phosphate at a much higher rate than the other proteins. Histone F1, which has been shown to be phosphorylated *in vitro* by this enzyme, was also phosphorylated *in vitro* and *in vivo* by the liver protein kinase (Langan, 1969, 1970). An exception to this generality, however, has been observed for cyclic AMP-stimulated protein kinases from certain lower species such as lobster, fish and roundworm. These enzymes phosphorylated casein more readily than histone (Kuo & Greengard, 1969).

Although the functional importance of cyclic AMP-dependent protein kinase in certain hormone-mediated responses has been well documented, its physiological role in the mechanism of action of androgen on male accessory sexual tissues remain to be elucidated. Smith (1972) reported significant

increases in prostatic cyclic [^3H]AMP synthesis from [^3H]adenosine in the presence of testosterone or dihydrotestosterone, and administration of either of these androgenic hormones was shown to produce rapid increases in adenylate cyclase activity of the rat prostate gland (Singhal & Sutherland, 1974). Further, the administration of exogenous cyclic AMP has been found to produce testosterone-like stimulation of prostatic size and the induction of several rate-limiting enzymes involved in carbohydrate metabolism (Singhal *et al.*, 1971). Although the enzyme was found predominantly in the cytosol, small amounts of activity were consistently associated with the nuclear fraction. This is notable, particularly because a small but considerable amount of adenylate cyclase activity has been found to be associated with the nuclear fraction purified from rat prostate; enzyme activity decreased significantly after gonadectomy and was restored to normal range within 1 h of the androgen injection (Mangan *et al.*, 1973). In this connexion, evidence has been obtained to show that whereas castration caused a significant elevation in the activity of both prostatic cyclic AMP-dependent and -independent protein kinase, administration of testosterone to androgen-deprived rats was capable of reversing these effects (Ichii *et al.*, 1973). However, Ahmed & Ishida (1971) reported that the incorporation of ^{32}P into nuclear phosphoproteins of ventral prostates of orchidectomized rats was greatly decreased when compared with nuclei from orchidectomized rats treated with testosterone or from normal intact animals. It is thus postulated that the 'andromimetic' action of cyclic AMP on the induction of certain prostatic enzymes involved in carbohydrate metabolism may be mediated through a cyclic AMP-dependent phosphorylation step catalysed by protein kinase, in a manner proposed by Langan (1969, 1970) for the induction of hepatic protein biosynthesis. Detailed studies on the influence of androgens on the activity of prostatic protein kinase in various subcellular fractions are essential to gain deeper insight into the involvement of the cyclic AMP-adenylate cyclase-protein kinase system in the overall action of male gonadal steroids on the growth and differentiation of androgen-dependent organs.

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