Cyclic Adenosine Monophosphate: Andromimetic Action on Seminal Vesicular Enzymes

Abstract. *Administration of adenosine 3′,5′-monophosphate with theophylline produced testosterone-like induction of hexokinase, phosphofructokinase, pyruvate kinase, and glucose-6-phosphate dehydrogenase in the seminal vesicles of both orchidectomized and immature rats. The* N^6-$O^{2'}$*-dibutyryl analog of this cyclic nucleotide produced greater increases in vesicular enzyme activities than those induced by the parent compound. The observed enhancement of the key glycolytic enzymes and of hexose monophosphate shunt dehydrogenase was significantly inhibited by actinomycin D and cycloheximide. The evidence indicates that cyclic adenosine monophosphate may be involved as an intermediary in the action of androgenic hormones on male accessory sex organs.*

Adenosine 3′,5′-monophosphate (cyclic AMP) may be involved in the action of a variety of hormones and certain biogenic amines (*1*). Although different hormones affect a variety of cellular processes, there is evidence that many of them generate cyclic AMP selectively in responsive cells of the target organ by activating the enzyme adenyl cyclase. Once cyclic AMP is generated, it mimics many of the actions ascribed to the hormone which stimulated its synthesis (*1*). Much attention has been paid to the role of this nucleotide in many metabolic states such as gluconeogenesis, steroidogenesis, ketogenesis, lipolysis, and antidiuresis (*2*). To our knowledge, cyclic AMP has not yet been implicated in the mechanism of action of androgens on male secondary sexual tissues although it has been reported to be an intermediary in the action of estrogens on the uterus (*3–5*). The administration of testosterone to orchidectomized rats induced new synthesis of hexokinase (E.C. 2.7.1.1), phosphofructokinase (E.C. 2.7.1.11), and glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) in the prostate and seminal vesicles, which was blocked effectively by actinomycin and cycloheximide (*6*). The androgenic induction of these prostatic and vesicular enzymes was dependent on time and dose. We now report the ability of exogenous cyclic AMP to produce testosterone-like induction of hexokinase, phosphofructokinase, and pyruvate kinase (E.C. 2.7.1.40) (the three key, rate-limiting enzymes of the glycolytic pathway) and of glucose-6-phosphate dehydrogenase in the seminal vesicles of both castrated and immature rats.

Young male Wistar rats (weighing approximately 150 g at the time of surgery) were used 2 weeks after orchidectomy. Seminal vesicles were excised immediately and cleaned of extraneous tissue, and 5 percent homogenates and supernatants were prepared (*6*). The activities of hexokinase (*7*), phosphofructokinase (*8*), pyruvate kinase (*9*), and glucose-6-phosphate dehydrogenase (*10*) were assayed in the supernatant. All enzyme activities were estimated under strictly linear kinetic conditions at 340 nm in a constant recording Unicam spectrophotometer model SP 800, thermostated at 37°C, and calculated as the number of micromoles of substrate metabolized per gram of tissue per hour times the weight of the organ (*6–8*). The data were analyzed statistically, and the significant differences between the means were calculated as *P* values.

Adenosine 5′-monophosphate exerted no significant effect either on seminal vesicular weights or on the activities of any of the enzymes investigated in castrated rats (Table 1). Cyclic AMP, however, resulted in minor, yet statistically significant increases in the enzymes. Greater increases in vesicular wet weights and enzyme activities were observed when cyclic AMP was injected simultaneously with theophylline, an inhibitor of the phosphodiesterase that inactivates cyclic AMP (*1*). The increases produced by theophylline itself (Table 1) suggest that endogenous cyclic AMP may be active as a stimulator of these enzymes in seminal vesicles. When the N^6-$O^{2'}$-dibutyryl analog of cyclic AMP was injected with theophylline, it produced increases in vesicular enzymes that were greater than those observed after treatment with cyclic AMP and theophylline. This analog of cyclic AMP is more soluble in lipid solvents, penetrates cells more readily, and is more resistant to enzymatic degradation than the parent compound (*1*). The increases in vesicular enzymes after the administration of the cyclic nucleotide and theophylline are similar to those obtained with rather high doses (5 to 10 mg per 100 g of body weight) of testosterone propionate (*6*).

We investigated the question of whether cyclic AMP could also enhance the activities of the three key glycolytic enzymes and of glucose-6-phosphate dehydrogenase in seminal vesicles of immature rats. Cyclic AMP (10 mg per rat) and theophylline (10 mg per rat) were injected concurrently by the intraperitoneal route in two equally divided doses at 12-hour intervals to a group of 22 rats (20 days old) weighing approximately 50 to 60 g. After 24 hours, the seminal vesicular weights and the activities of glucose-6-phosphate dehydrogenase and the three glycolytic enzymes increased (Table 2).

Table 1. Effects of cyclic AMP and 5′-AMP on vesicular weights and enzyme activities in castrated rats. Each value is the mean ± S.E. based on three to four determinations of enzyme activity in seminal vesicles pooled from four orchidectomized animals. Cyclic AMP, 5′-AMP, and dibut yry cyclic AMP were injected intraperitoneally at a dose of 10 mg per rat, in two equally divided doses at 12-hour intervals. Theophylline (10 mg per rat) was also administered by the intraperitoneal route in two doses concurrently with cyclic AMP. Control rats were injected with physiological saline. All animals were killed 24 hours after the treatment was begun. The data are also given in percentages (in parentheses), taking the values of control animals as 100 percent. The following abbreviations are used: HK, hexokinase; PFK, phosphofructokinase; PK, pyruvate kinase; and G6-PDH, glucose-6-phosphate dehydrogenase.

Treatment	Organ weight (mg)	HK	PFK	PK	G6-PDH
Control	39 ± 4 (100)	1.3 ± 0.1 (100)	3.6 ± 0.3 (100)	51.6 ± 3.1 (100)	2.9 ± 0.2 (100)
5′-AMP	41 ± 1 (102)	1.6 ± 0.1 (123)	4.1 ± 0.1 (114)	57.4 ± 4.3 (112)	3.5 ± 0.2 (120)
Cyclic AMP	46 ± 2 (118)	1.5 ± 0.0 (115)*	5.6 ± 0.2 (155)*	64.2 ± 1.4 (123)*	4.0 ± 0.2 (139)*
Theophylline	43 ± 2 (110)	1.5 ± 0.1 (115)	5.5 ± 0.5 (153)*	100.2 ± 2.3 (192)*	4.2 ± 0.1 (145)*
Cyclic AMP + theophylline	63 ± 1 (164)*	2.7 ± 0.3 (213)*	9.2 ± 0.1 (256)*	175.1 ± 7.1 (336)*	5.7 ± 0.5 (196)*
Dibutyryl cyclic AMP + theophylline	67 ± 1 (172)*	4.7 ± 0.1 (362)*	11.8 ± 0.0 (328)*	188.5 ± 2.0 (371)*	6.2 ± 0.4 (214)*

* Statistically significant difference as compared to the values of control rats ($P = < .05$).

Table 2. Stimulation by cyclic AMP and theophylline of seminal vesicular enzymes in immature rats. Each value is the mean ± S.E. based on four determinations of enzyme activity in seminal vesicles pooled from five to six immature rats. Cyclic AMP (10 mg per rat) and theophylline (10 mg per rat) were administered concurrently by the intraperitoneal route in two equally divided doses at 12-hour intervals. All animals were killed 24 hours after treatment was begun. The data are also given in percentages (in parentheses), taking the values of control rats as 100 percent.

Treatment	Organ weight (mg)	HK	PFK	PK	G6-PDH
Control	14 ± 1 (100)	0.8 ± 0.1 (100)	1.3 ± 0.1 (100)	27.2 ± 2.1 (100)	0.8 ± 0.0 (100)
Cyclic AMP + theophylline	20 ± 1 (143)*	1.4 ± 0.1 (175)*	3.4 ± 0.1 (261)*	46.9 ± 0.1 (174)*	1.6 ± 0.1 (200)*

* Statistically significant difference as compared to the values of control rats ($P = < .05$).

The androgenic induction of prostatic and vesicular hexokinase, phosphofructokinase, and glucose-6-phosphate dehydrogenase may entail enzyme synthesis de novo involving an early stimulation of the synthesis of certain RNA species (*6*). We investigated the nature of the increases in enzyme activities stimulated by cyclic AMP and theophylline by using two compounds known to inhibit RNA and protein synthesis. Four groups, each consisting of ten castrated rats, were employed: control animals; animals treated with cyclic AMP and theophylline; and animals injected with cyclic AMP and theophylline and then given either actinomycin or cycloheximide. Rats from all groups were killed 24 hours after treatment with cyclic AMP was begun. Actinomycin D, which binds to DNA and blocks DNA-directed synthesis of nuclear RNA (*11*), inhibited the nucleotide-induced enzyme increases in the seminal vesicles (Fig 1). Cycloheximide, which inhibits protein synthesis by blocking the transfer of amino acids from tRNA to protein (*12*), also prevented the cyclic AMP-induced increases in glucose-6-phosphate dehydrogenase and in the three key glycolytic enzymes suggesting that both new RNA and protein synthesis may be involved in the observed stimulation of the vesicular enzymes. Furthermore, the ability of actinomycin D to inhibit the nucleotide-induced increases in enzyme activities indicates that, like testosterone, the cyclic AMP–stimulated changes in vesicular enzymes may also involve participation of messenger RNA synthesis at the gene locus (*5*, *6*). Actinomycin D can inhibit the formation of rat liver serine dehydratase induced by the dibutyryl analog of cyclic AMP (*13*).

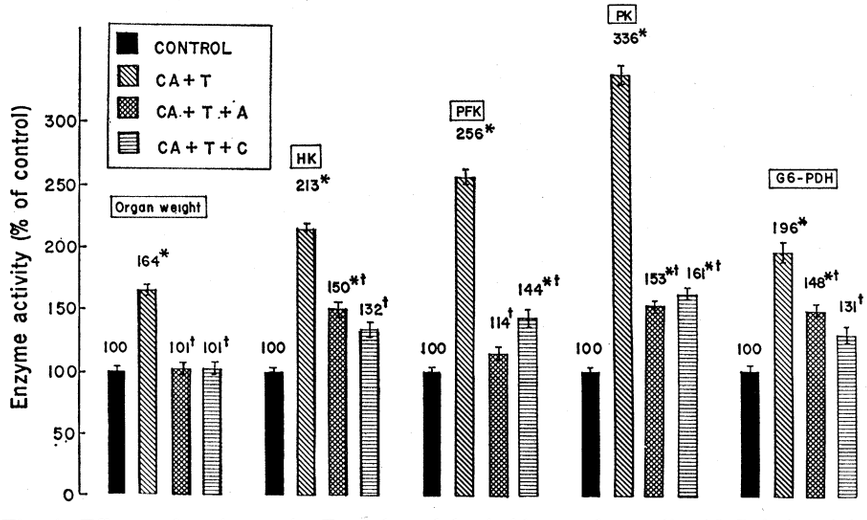

Fig. 1. Effects of actinomycin D and cycloheximide on the cyclic AMP–induced increases in vesicular wet weights and enzyme activities. Castrated rats were injected with cyclic AMP (10 mg per rat) and theophylline (10 mg per rat) concurrently by the intraperitoneal route in two equally divided doses at 12-hour intervals. Actinomycin (25 μg/100 g) or cycloheximide (70 μg/100 g) were also given intraperitoneally twice at 12-hour intervals. All rats were killed 24 hours after treatment was begun. Each bar represents the mean and S.E. of three determinations of enzyme activity in seminal vesicles pooled from three to four animals. Data are given in percentages, taking the control values as 100 percent. Abbreviations are: CA, cyclic AMP; T, theophylline; A, actinomycin; and C, cycloheximide. *Statistically significant difference as compared to the values of control rats ($P = < .05$). †Statistically significant difference as compared to the values of animals treated with cyclic AMP and theophylline without the administration of actinomycin or cycloheximide ($P = < .05$).

Although cyclic AMP was discovered initially as the intracellular mediator of the glycogenolytic effects of epinephrine and glucagon in the liver, it has since been recognized to be a second messenger mediating a variety of hormonal effects (*1*). According to the "two-messenger" hypothesis of hormone action, the first messenger, the hormones, travel from the sites of release to the cells of their target tissues to cause an alteration in the intracellular concentration of a second messenger which in many cases has now been recognized to be cyclic AMP (*1*). Hormones known to exert some of their effects by changing the concentration of cyclic AMP in cells of their target tissues include adrenocorticotropin, insulin, glucagon, vasopressin, epinephrine, norepinephrine, serotonin, and histamine (*1*, *14*). Szego *et al.* have demonstrated that the cyclic AMP content in the intact uterus is depleted after ovariectomy; the concentration of uterine cyclic AMP is almost doubled within 15 seconds after an intravenous injection of 17β-estradiol to ovariectomized rats (*3*, *4*). They also found that the estrogen-induced changes in uterine concentrations of cyclic AMP were prevented by β-adrenergic blocking agents and pointed out the need to find out whether compounds like propranolol would influence the characteristic effects of estrogens upon the uterus. Hechter *et al.* demonstrated that, in addition to cyclic AMP, other substances such as glucose, adenosine, and guanosine monophosphate produced "estrogen-like" effects on uterine RNA and protein synthesis in vitro. Whereas their studies did not permit any definite conclusion concerning the role of cyclic AMP in estradiol action, it was suggested that a set of specific nucleotides generated by estrogen action may provide intracellular regulatory signals in the uterine anabolic response (*5*). Although cyclic AMP is believed to mediate a variety of hormonal effects, evidence exists that the response of certain tissues to hormones may be independent of the adenyl cyclase–cyclic AMP system. Granner *et al.* (*15*) demonstrated the ability of dexamethasone to induce tyrosine aminotransferase in HTC cells which were apparently devoid of adenyl cyclase and cyclic AMP. Additionally, Wicks (*16*) has shown that a combina-

tion of corticosteroids and cyclic AMP produced additive maximum effects on tyrosine α-ketoglutarate aminotransferase in explants of fetal rat liver maintained in organ culture. Our results demonstrate that cyclic AMP, when injected with theophylline to orchidectomized or immature rats, is capable of producing androgen-like induction of several carbohydrate-metabolizing enzymes in the seminal vesicles. These data suggest that cyclic AMP may play the role of a "second messenger" in the action of androgens on rat seminal vesicles. However, since adrenalcortical hormones may exert androgen-like effects on accessory sexual tissues (*17*), it is not possible, at present, to rule out the involvement of adrenal cortex in the observed stimulation of various seminal vesicular enzymes induced by cyclic AMP. Additional support for the involvement of this cyclic nucleotide in testosterone action must await the demonstration that male sex hormones are capable of producing an increase in the intracellular concentration of cyclic AMP in secondary sexual tissues.

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References and Notes

1. E. W. Sutherland, I. Øye, R. W. Butcher, *Recent Progr. Hormone Res.* **21**, 623 (1965); E. W. Sutherland and G. A. Robison, *Pharmacol. Rev.* **18**, 145 (1966); G. A. Robison, R. W. Butcher, E. W. Sutherland, *Ann. N.Y. Acad. Sci.* **139**, 703 (1967); R. W. Butcher, G. A. Robison, J. G. Hardman, E. W. Sutherland, *Advan. Enzyme Regul. Proc. Symp. Regul. Enzyme Activ. Syn. Norm. Neoplast. Tissues* **6**, 357 (1968); E. W. Sutherland, G. A. Robison, R. W. Butcher, *Circulation* **37**, 279 (1968).
2. J. Berthet, *Proc. Int. Congr. Biochem., 4th, Vienna* (1960), p. 107; H. Imura, S. Matsukura, H. Matsuyama, T. Setsuda, T. Miyake, *Endocrinology* **76**, 933 (1965); R. W. Butcher, R. J. Ho, H. C. Meng, E. W. Sutherland, *J. Biol. Chem.* **240**, 4515 (1965); J. H. Exton and C. R. Park, *Pharmacol. Rev.* **18**, 181 (1966); J. Orloff and J. S. Handler, *Amer. J. Med.* **42**, 757 (1967).
3. C. M. Szego, *Fed. Proc.* **24**, 1343 (1965); ——— and J. S. Davis, *Proc. Int. Congr. Biochem., 7th, Tokyo* (1967), p. 863.
→ C. M. Szego and J. S. Davis, *Proc. Nat. Acad. Sci. U.S.* **58**, 1711 (1967); *Mol. Pharmacol.* **5**, 470 (1969).
5. O. Hechter, K. Yoshinaga, I. D. K. Halkerston, K. Birchall, *Arch. Biochem. Biophys.* **122**, 449 (1967).
6. R. L. Singhal and J. R. E. Valadares, *Biochem. J.* **110**, 704 (1968); R. L. Singhal and G. M. Ling, *Can. J. Physiol. Pharmacol.* **47**, 233 (1969); R. L. Singhal, D. Wang, G. M. Ling, *Proc. Can. Fed. Biol. Soc.* **11**, 146 (1968); R. L. Singhal and R. Vijayvargiya, in preparation.
7. J. R. E. Valadares, R. L. Singhal, M. R. Parulekar, *Science* **159**, 990 (1968).
8. R. L. Singhal, J. R. E. Valadares, G. M. Ling, *J. Biol. Chem.* **242**, 2593 (1967); *Amer. J. Physiol.* **217**, 793 (1969).
9. R. Vijayvargiya, W. S. Schwark, R. L. Singhal, *Can. J. Biochem.* **47**, 895 (1969); R. L. Singhal and J. R. E. Valadares, *Amer. J. Physiol.* **218**, 321 (1970).
10. G. E. Glock and P. McLean, *Biochem. J.* **55**, 400 (1953).
11. E. Reich, R. M. Franklin, A. J. Shatkin, E. L. Tatum, *Science* **134**, 556 (1961); T. Tamaoki and G. C. Mueller, *Biochem. Biophys. Res. Commun.* **9**, 451 (1962).
12. H. L. Ennis and M. Lubin, *Fed. Proc.* **23**, 269 (1964).
13. J. Jost, A. W. Hsie, H. V. Rickenberg, *Biochem. Biophys. Res. Commun.* **34**, 748 (1969).
14. R. C. Haynes, Jr., E. W. Sutherland, T. W. Rall, *Recent Progr. Hormone Res.* **16**, 121 (1960); E. W. Sutherland and T. W. Rall, *Pharmacol. Rev.* **12**, 265 (1960); T. E. Mansour, E. W. Sutherland, T. W. Rall, E. J. Bueding, *J. Biol. Chem.* **235**, 466 (1960); J. Orloff and J. S. Handler, *Biochem. Biophys. Res. Commun.* **5**, 63 (1961); R. W. Butcher, J. G. T. Sneyd, C. R. Park, E. W. Sutherland, *J. Biol. Chem.* **241**, 1651 (1966); T. W. Rall and S. Kakiuchi, in *Molecular Basis of Some Aspects of Mental Activity*, O. Walaas, Ed. (Academic Press, New York, 1966), vol. 1, p. 417.
15. D. Granner, L. R. Chase, G. W. Aurbach, G. M. Tomkins, *Science* **162**, 1018 (1968).
→ W. D. Wicks, *ibid.* **160**, 997 (1968).
17. D. Price and H. G. Williams-Ashman, in *Sex and Internal Secretions*, W. C. Young, Ed. (Williams and Wilkins, New York, 1961), vol. 1, p. 423.
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Satellite DNA in Constitutive Heterochromatin of the Guinea Pig

Abstract. *Total DNA and DNA from the heterochromatin and euchromatin fractions of male guinea pig liver nuclei were analyzed by cesium sulfate–silver density-gradient centrifugation. Total DNA is composed of three components: a heavy satellite DNA, a main DNA of intermediate density, and a light satellite DNA. Heterochromatin DNA shows a fourfold enrichment in the satellite components while euchromatin DNA is relatively devoid of them. The strands of both satellite DNA's are separable by centrifugation in alkaline cesium chloride. Base analyses on the separate strands demonstrate that the two satellite DNA's represent different species.*

The heterochromatin of mammalian somatic cells is generally divided into two classes: (i) facultative heterochromatin, which is mostly inactive and results from the heterochromatization of one of the two X chromosomes in females, and (ii) constitutive heterochromatin, which resides in homologous chromosomes and is of unknown function (*1*). Because of its variable expression in adult somatic tissues as well as its failure to be observed during early embryogenesis in lower animals, it has been suggested that constitutive heterochromatin is not a specific substance but a variable state of chromatin (*1*). We found evidence to the contrary when we showed that mammalian constitutive heterochromatin is present in specific chromosomes throughout development (*2*). Also with the use of a modification of Frenster's technique (*3*), liver and brain nuclei from male mice were subjected to sonication, and the total chromatin was fractionated by differential centrifugation yielding three fractions: a heavy fraction which could be shown cytologically to be composed mainly of masses of heterochromatin, a lighter fraction of euchromatin contaminated with masses of heterochromatin, and a very light fraction of euchromatin. After DNA extraction and analysis in neutral CsCl, the DNA of the heterochromatin fraction was primarily composed of a unique type of DNA, termed satellite DNA, while that of the euchromatin fraction was composed of bulk DNA (*4*). We used the same technique to study guinea pig chromatin. The DNA of the heterochromatin fraction was rich in two types of satellite DNA, providing further evidence that mammalian constitutive heterochromatin may be composed of DNA of unique properties.

Nuclei from 10 to 20 livers of male guinea pigs (1 week old) were isolated in a solution of 2.2*M* sucrose, 3 m*M* $MgCl_2$, and 0.5 m*M* $CaCl_2$ and disrupted with ultrasound (15 seconds at 7 amp and 20,000 cycles per second) (*4*). The resulting chromatin suspension was composed of three fractions: (i) a fraction of heterochromatin associated with nucleoli, which was isolated by sedimentation at 3500*g* for 20 minutes; (ii) an intermediate fraction of euchromatin, heterochromatin, and nucleoli isolated by sedimentation at 12,000*g* for 60 minutes; and (iii) a euchromatin fraction isolated by precipitation with two volumes of ethanol. In five separate experiments, the relative amounts of the three fractions represented about 25, 30, and 55 percent, respectively, of the total amount of chromatin DNA. All fractions were monitored by light