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TESTOSTERONE—STIMULATION OF ADENYL CYCLASE AND CYCLIC 3′,5′-ADENOSINE MONOPHOSPHATE-^{3}H FORMATION IN RAT SEMINAL VESICLES*

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Abstract—Seminal vesicles obtained from normal or castrate rats were capable of converting radioactive adenosine into labeled cyclic 3′,5′-adenosine monophosphate (cAMP-^{3}H). Addition of testosterone (10^{-6} M) *in vitro* caused about a 60 per cent stimulation in the levels of vesicular cAMP-^{3}H. Castration (96 hr) led to a significant decrease in the content of cAMP-^{3}H and adenyl cyclase activity of this accessory organ; it did not significantly decrease the concentration. A single injection of testosterone to castrate rats produced a marked enhancement of vesicular adenyl cyclase (96 hr later). These results support the concept that stimulation of the cAMP–adenyl cyclase system is associated with the mechanism of action of androgens on their target cells.

EVIDENCE that cyclic 3′,5′-adenosine monophosphate (cAMP) may be involved in the mechanism of action of male sex hormone is beginning to emerge. Singhal *et al.*[1] reported that exogenous cAMP caused a significant stimulation of several key glycolytic and hexose monophosphate shunt enzymes in sex accessory organs of castrate rats; theophylline further enhanced these stimulatory events. Endogenous adenyl cyclase may be increased by testosterone in rat sex accessory organs.[2] Smith *et al.*[3] observed increases in mouse prostatic cAMP-^{3}H formation *in vitro* by fluoride and by testosterone. Liao *et al.*[4] reported that dihydrotestosterone caused only about a 10–20 per cent increase in the activity of adenyl cyclase isolated from nuclei of normal rat ventral prostates *in vitro*. Actually, nonsteroidal hormones caused greater increases in prostatic adenyl cyclase than did androgens.[4] Rosenfeld and O'Malley[5] were unable to demonstrate any stimulation of adenyl cyclase in ventral prostates by testosterone regardless of whether the hormone was administered *in vivo* or *in vitro*. Recently, Golder *et al.*[6] reported that testosterone failed to stimulate adenyl cyclase in homogenates of rat prostate *in vitro*. Even though a clear-cut relationship between androgens and cAMP has been difficult to establish, the presence of both adenyl cyclase and this cylcic nucleotide in sex accessory glands and their secretions implicates some degree of interaction between the hormone and the cAMP–adenyl cyclase system. The present report demonstrates that testosterone (*in vitro*) is capable of stimulating

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cAMP-^{3}H formation in seminal vesicles of normal and castrate rats. Furthermore, vesicles from castrated animals injected with testosterone revealed significant increases in cAMP-^{3}H formation from labeled adenosine as well as in their activity of adenyl cyclase.

EXPERIMENTAL

Seminal vesicles obtained from normal or castrate (96 hr) rats (average body wt, 200 g) were used in this investigation. Testosterone (5 mg/100 g body wt) was dissolved in ethanolic 0·9% NaCl and injected i.m. 48 hr prior to sacrifice. The effects of varying concentrations of testosterone *in vitro* were studied on the ability of the vesicles to convert labeled adenosine into cAMP-^{3}H. Vesicular pieces were preincubated at 37°; testosterone was added 30 min later, and the incubation continued for another half-hour. Preliminary studies using a 10-min preincubation followed by a 30-min period in the presence of testosterone failed to convert measurable amounts of cAMP-^{3}H. Vesicles were then immediately frozen in liquid nitrogen, weighed and homogenized in cold perchloric acid. After precipitation and centrifugation, the supernatants were decanted and neutralized with 5 M K_2CO_3. A 10-μl aliquot of the neutralized fraction was spotted on polyethyleneimine (PEI-cellulose) layers which contained fluorescent indicator (Brinkmann Polygram 300). Nonradioactive adenosine, cAMP, 5′-AMP, ADP and ATP were added as a carrier in order to obtain sufficient ultraviolet visualization. The thin-layer plates were developed for 30 min using a solvent system of 95% ethanol and 4 M ammonium acetate (5:2). Spots were visualized with an ultraviolet light (R_f of cAMP = 0·35, R_f of adenosine = 0·63; ATP, ADP and 5′-AMP remained at the origin), cut from the plate, and placed in scintillation counting vials. The PEI-cellulose layer was removed from its plastic backing by sonication and the radioactivity (dis./min/mg) was measured using a suitable scintillation mixture (PPO-POPOP*). The activity of seminal vesicular adenyl cyclase was assayed according to the method of Krishna *et al.*,[7] by measuring the formation of cAMP from ATP as described by Gilman.[8] Enzyme activity was measured for 10 min under strictly linear kinetic conditions and expressed as nanomoles of cAMP formed per hr at 37°.

RESULTS

Seminal vesicles from normal rats were capable of converting radioactive adenosine to cAMP-^{3}H *in vitro* (Table 1). Further, the tissue's ability to form labeled cyclic nucleotide was enhanced by adding testosterone with a maximum stimulatory effect (62 per cent) when incubated *in vitro* in the presence of the hormone (10^{-6} M).

Table 2 reveals the effects of castration (96 hr) on the formation of vesicular cAMP-^{3}H. It is of interest that castration had only a slight effect upon cAMP-^{3}H formation when expressed per milligram wet weight. However, when calculated on a total organ basis, cAMP-^{3}H formation was markedly lowered by castration (58 per cent).

The effect of injected testosterone to stimulate endogenous adenyl cyclase activity of rat seminal vesicles (Table 3) was more pronounced than the action of the hormone *in vitro* to enhance cAMP-^{3}H formation (Table 1). Regardless of the basis of

* PPO = 2,5-diphenyloxazole; POPOP = 1,4-*bis*-2-(4-methyl-5-phenyloxazolyl)benzene.

TABLE 1. FORMATION OF cAMP-^{3}H FROM ADENOSINE-^{3}H BY NORMAL RAT SEMINAL VESICLES INCUBATED *in vitro* WITH VARING AMOUNTS OF TESTOSTERONE*

Testosterone concn (M)	cAMP-^{3}H formed (dis./min/mg × 10^3)	Per cent of control
Control	10·2 ± 1·0	100
10^{-10}	11·7 ± 1·1	113
10^{-8}	14·6 ± 0·7	141†
10^{-6}	16·6 ± 0·9	162†

* Portions of vesicles (20–25 mg) were placed in Krebs–Ringer solution (pH 7·4) containing adenosine-^{3}H (10 μCi/flask), gassed (95% O_2–5% CO_2) and incubated in a Dubnoff metabolic shaker (60 os./min at 37°). cAMP-^{3}H levels (mean ±S. E. of four or more determinations) reflect the organ's ability to biotransform adenosine-^{3}H to cAMP-^{3}H during a 1-hr incubation period.

† Significantly increased above controls ($P \leq 0{\cdot}05$).

TABLE 2. EFFECT OF CASTRATION (96 hr) ON THE FORMATION OF cAMP-^{3}H FROM RADIOACTIVE ADENOSINE BY RAT SEMINAL VESICLES *in vitro**

Group	cAMP-^{3}H formed (dis./min/mg × 10^3)	cAMP-^{3}H formed (dis./min/organ × 10^3)
Normal control	10·2 ± 1·0	2325 ± 235
Castrate (96-hr)	9·8 ± 1·3	985 ± 52†

* Portions of vesicles were incubated for 1 hr at 37° in a Krebs–Ringer solution (pH 7·4). cAMP-^{3}H levels (mean ± S. E. of four or more determinations) were observed to be significantly lowered by castration.

† 42% of control (significant at $P \leq 0{\cdot}05$).

expression, vesicular adenyl cyclase was significantly increased ($P \leq 0{\cdot}05$) after the injection of testosterone to castrate animals (Table 3). On a concentration basis, (per gram wet weight or per milligram of protein), there was about a 2-fold increase, whereas cyclase/organ was enhanced over five times by the hormone when compared to castrate controls.

TABLE 3. STIMULATION OF ADENYL CYCLASE BY TESTOSTERONE IN SEMINAL VESICLES OF 96-hr CASTRATE RATS*

Group	Adenyl cyclase activity (cAMP formed) (nMoles/g)	(% Control)	(nMoles/mg protein)	(% Control)	(nMoles/ organ)	(% Control)
Control	359 ± 26	100	4·8 ± 0·4	100	18·3 ± 0·5	100
Testosterone-treated	855 ± 38†	229	9·0 ± 0·5†	189	100·7 ± 9·5†	561

* Animals received a single injection of testosterone (5 mg/100 g body wt, i.m.) 48 hr prior to sacrifice. Vesicular homogenates (10 mg/ml) were incubated for 10 min at 37° in a final volume (1 ml) containing 50 mM Tris–HCl (pH 7·5), 4 mM ATP, 5 mM $MgCl_2$, 10 mM PEP, 1 mg BSA, 2 μg PK, 7 mM caffeine and 4 mM fluoride. Adenyl cyclase activity (mean ±S. E. of four or more pooled vesicles, each run in duplicate) is expressed as nanomoles of cyclic AMP formed/unit/hr.

† Significantly increased over castrate controls ($P \leq 0{\cdot}05$).

DISCUSSION

While the relationship between several other hormones and cAMP metabolism may be more conclusive, evidence suggests that androgens are also capable of affecting the adenyl cyclase–cAMP system in the cells of their target organs. Of course it would be presumptuous to state that the sole mechanism of action of androgens is mediated exclusively via the adenyl cyclase–cAMP system. Endogenous cAMP levels are enhanced in secondary sexual tissues of castrate rats at 8 and 24 hr after the administration of testosterone.[2] Liao *et al.*[4] found that 5′α-dihydrotestosterone, *in vitro*, caused only a slight increase in nuclear adenyl cyclase activity of the ventral prostate gland. On the other hand, testosterone (*in vivo* or *in vitro*) failed to stimulate adenyl cyclase activity of a membrane fraction of ventral prostates obtained from hypophysectomized rats.[5] It should be noted that neither of these groups of investigators[4,5] examined the influence of androgens on adenyl cyclase activity of the seminal vesicles. Golder *et al.*[6] did study adenyl cyclase activity in homogenates of rat prostate (lobe not specified), but were unable to demonstrate any significant stimulation of this enzyme by testosterone. These same investigators[6] have suggested that testosterone stimulation of the prostate gland does not depend on adenyl cyclase *per se*, but its action may be potentiated by an effect of prolactin on the enzyme. It is possible that the prostate gland might be less responsive to changes in cAMP metabolism than seminal vesicles when stimulated by testosterone. Indeed, this androgen was found to produce a greater increase in vesicular cAMP than in the prostate gland.[1] Testosterone *in vitro* more readily enhances cAMP–^{3}H levels in mouse seminal vesicles than in the prostate gland.[3] It also has been reported that cAMP in ejaculates of either humans or dogs is largely of vesicular origin and is not derived from the prostate.[9] Dog prostates, however, can convert labeled adenosine to cAMP–^{3}H *in vitro*.[10]

Castration (96 hr) resulted in a significant decrease in both total cAMP–^{3}H levels and adenyl cyclase activity of seminal vesicles. Similar findings were reported in mouse seminal vesicles 24 hr after castration.[3] Liao *et al.*[4] found no change in nuclear prostatic adenyl cyclase after castration, whereas Singhal *et al.*[2] reported that the total endogenous levels of sex accessory gland cAMP were decreased by about 80 per cent in castrate rats.

In the present studies, ATP–^{3}H levels were not measured. It is possible that the enhancement of cAMP–^{3}H levels in sex accessory organs is partly due to elevations in the pool size of ATP–^{3}H. Testosterone reportedly increases sex accessory gland ATP levels, but such increases occur at 2–3 hr after the administration *in vitro* of androgen.[11]

Aside from the direct effects of testosterone upon sex accessory gland adenyl cyclase–cAMP systems, the cyclic nucleotide itself has been found to mimic certain metabolic effects of androgens. Such an andromimetic action is evidenced by the ability of intraperitoneally administered cAMP to stimulate several key glycolytic and hexose monophosphate shunt enzymes in the prostate and seminal vesicles of both castrated and immature rats.[1] Similarly, Hoskins and Stephens[12] found that cAMP was a potent activator of spermatozoal phosphofructokinase, indicating that enzymes found in other male reproductive organs whose activities were influenced by androgens are likewise affected by exogenous cAMP.

The present results together with other recent findings[1–3,10] reinforce the role of cAMP as a mediator in at least some of the actions of male sex hormones. The

majority of criteria outlined by Sutherland *et al.*[13] which must be fulfilled before the role of cAMP can be firmly established in the action of a given hormone now seem to have been met for androgens as well. The relationship between androgens and cAMP is still not as conspicuous as this interaction between other hormones and this cyclic nucleotide. While Liao *et al.*[4] and Golder *et al.*[6] did find slight increases in adenyl cyclase activity, testosterone was unable to produce statistically significant elevations. The negative findings of Rosenfeld and O'Malley[5] are difficult to reconcile, but their results were obtained from hypophysectomized rats. Golder *et al.*[6] have suggested that pituitary hormones may influence the interaction of testosterone and cAMP in the prostate gland. That exogenous cAMP can mimic the action of testosterone on sex accessory organs and that phosphodiesterase inhibitors (e.g. theophylline) can enhance the metabolic effects of a submaximal dose of this androgen fulfill some of the criteria outlined by Sutherland *et al.*[13] Furthermore, the present observations that both endogenous adenyl cyclase and cAMP–^{3}H formation are enhanced by testosterone provide compelling evidence in favor of the involvement of cAMP in an androgen's action upon rat sex accessory organs.

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