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EFFECT OF ISOPROTERENOL AND ACETYLCHOLINE TREATMENT *IN VITRO* ON CYCLIC NUCLEOTIDES IN MOUSE SEX ACCESSORY ORGANS*

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Abstract—The effect of treatment *in vitro* with either isoproterenol or acetylcholine on the levels of cyclic AMP and cyclic GMP in male mouse prostate gland and seminal vesicle was studied. In each experiment, tissues were preincubated with aminophylline (5 mM) for 10 min prior to exposure to the autonomic agonist. Treatment with 1×10^{-5} M isoproterenol for 5 min resulted in significant increases in endogenous levels of cyclic AMP in both prostate glands and seminal vesicles obtained from intact mature male mice. This effect of isoproterenol on cyclic AMP could be blocked by pretreatment for 10 min with an equimolar (1×10^{-5} M) concentration of propranolol. On the other hand, isoproterenol (1×10^{-5} M for 5 min) had no effect on sex accessory organ levels of cyclic GMP. If sex accessory organs obtained from intact mature male mice were subjected to acetylcholine (1×10^{-5} M for 5 min), significant increases ($P < 0.05$) in cyclic GMP were noted in the seminal vesicles; this effect could be blocked by atropine pretreatment (1×10^{-5} M for 10 min). Just as isoproterenol had no effect on sex accessory cyclic GMP levels, acetylcholine produced no changes in cyclic AMP in these tissues. Although treatment with neurotransmitters resulted in striking increases in cyclic nucleotides in sex accessory tissues obtained from normal mice, this treatment had little or no effect on cyclic nucleotide levels in tissues obtained from 7-day castrate mice.

Recently, several investigators [1–4] have reported that cyclic AMP might be involved in the actions of the androgens on the male sex accessory organs. In the prostate and seminal vesicles, castration results in decreases in the amount of ^{3}H-cyclic AMP formed from [^{3}H]adenosine. This decrease can be reversed by treatment with testosterone or with dihydrotestosterone. Several androgen-dependent carbohydrate-metabolizing enzymes in the rat ventral prostate gland can be stimulated by the administration of exogenous cyclic AMP [4]. The content of both cyclic AMP and cyclic GMP in mouse sex accessory tissues was reduced after castration [5]. Other workers [6–8] have failed to show any relationship between androgens and cyclic nucleotides in the male sex accessories. Since the role of androgens in the regulation of cyclic nucleotide levels in male sex accessory organs is not firmly established, the present studies were a further attempt to clarify any such interaction.

The autonomic innervation of the male sex accessory organs consists of both sympathetic and parasympathetic components [9–11]. However, the relative influence of these two autonomic divisions in initiating and maintaining the exocrine secretion of the sex accessory glands remains unresolved [12]. Recently, in non-sex accessory tissues, the cyclic nucleotides have been implicated as possible second messengers in the autonomic nervous system [13–16].

The present investigation was undertaken to determine if a relationship exists between the autonomic innervation of these organs and the tissue levels of either cyclic AMP or cyclic GMP. The effects of various autonomic drugs and appropriate blocking agents were investigated in sex accessory tissues obtained from either normal mature male mice or 7-day castrate mice.

MATERIALS AND METHODS

Mature (35–40 g) albino Swiss Webster mice were killed and their sex accessory organs (anterior prostate glands and seminal vesicles) were rapidly removed. The internal secretions of these organs were removed, and the organs were rinsed in isotonic saline, blotted, placed in incubation vials, and preincubated for 10 min in freshly oxygenated Krebs–Ringer bicarbonate buffer (pH 7·4, 37°) containing 5 mM aminophylline. Preliminary experiments have shown this to be necessary in order to decrease the variability of the results due to differential rates of metabolism of the formed cyclic nucleotides between individual animals. When using autonomic blocking agents, this preincubation medium also contained the appropriate antagonist (viz. propranolol or atropine, 1×10^{-5} M). After this preincubation period, the tissues were transferred to clean incubation vials containing fresh Krebs–Ringer bicarbonate buffer. Acetylcholine or isoproterenol was added and the tissues were further incubated at 37° for 5 min. Preliminary work has shown this exposure time to and dose of agonist to be optimal. All incubations were carried

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Table 1. Effect of isoproterenol (1×10^{-5} M) on cyclic nucleotide levels in mouse sex accessory organs *in vitro* in the presence or absence or propranolol (1×10^{-5} M)*

Treatment	Cyclic AMP (pmoles/mg protein)		Cyclic GMP (pmoles/mg protein)	
	Anterior prostate	Seminal vesicle	Anterior prostate	Seminal vesicle
Control	8·91 ± 0·93†	5·42 ± 0·33	8·72 ± 0·67	8·84 ± 0·84
Isoproterenol	17·54 ± 1·53‡§	29·38 ± 6·21‡§	10·43 ± 1·20	9·59 ± 1·46
Isoproterenol + propranolol	9·38 ± 0·93	6·11 ± 0·40		

* Tissues were preincubated in buffer containing aminophylline (5 mM) for 10 min with or without propranolol. Tissues were then placed in fresh buffer containing isoproterenol or buffer alone and further incubated for 5 min.
† Mean ± S.E.M. of six observations/group.
‡ Significantly different from control ($P < 0·05$).
§ Significantly different from isoproterenol + propranolol group ($P < 0·05$).

out under an atmosphere of 95% O_2 and 5% CO_2. After the incubation period the tissues were removed, blotted, and immediately frozen in liquid nitrogen for subsequent cyclic nucleotide and protein assay.

The tissues were processed for biochemical analysis by removing them from the liquid nitrogen and immediately homogenizing them (motor-driven Duall glass homogenizer) in 1 ml of cold 5% trichloroacetic acid (TCA). The homogenate was centrifuged (1000 *g*) to separate the protein-containing precipitate from the cyclic nucleotide-containing supernatant fluid. The protein pellet was reconstituted in cold distilled water to a concentration of 4% (w/v) and the amount of protein was determined by the method of Lowry *et al.* [17]. The supernatant fluid containing the cyclic nucleotides was acidified with 0·1 ml of 1 N HCl, extracted five times with 2 vol of diethyl ether to remove the TCA, and the final aqueous phase of the extract subsequently lyophilized. After lyophilization, the cyclic nucleotides were reconstituted in cold sodium acetate buffer (pH 4·0) and the levels of either cyclic AMP or cyclic GMP were determined by competitive protein binding. Kits for the determination of the cyclic nucleotides by competitive protein binding were purchased from Diagnostic Products Corp. (Culver City, Calif.). Preliminary studies indicated that there is good specificity of the binding protein for its respective cyclic nucleotide with little cross-reactivity (less than 1 per cent) for other nucleotides.

Results were statistically analyzed using Student's *t*-test.

RESULTS

Isoproterenol (1×10^{-5} M) significantly ($P < 0·05$) increased the concentration of cyclic AMP in the mouse sex accessory tissues (Table 1). These increases were approximately 95 per cent in the prostate gland, while in the seminal vesicle they exceeded 440 per cent. While isoproterenol produced definite increases in sex accessory cyclic AMP, it was without effect on cyclic GMP in these tissues (Table 1).

The effect of propranolol on the isoproterenol-produced increases in cyclic AMP in the sex accessory tissues was of interest. As can be seen in Table 1, preincubation with propranolol (1×10^{-5} M) for 10 min was completely effective ($P < 0·05$) in blocking the effects of an equimolar concentration of isoproterenol in both the prostate gland and the seminal vesicles. Preliminary experiments have shown propranolol alone to be without effect ($P > 0·05$) on cyclic AMP levels in the sex accessories.

Since isoproterenol would markedly increase sex accessory cyclic AMP without affecting cyclic GMP (Table 1), it was of interest to examine what effect a cholinergic agent might have on these same cyclic nucleotides. Table 2 shows the effects of acetylcholine (1×10^{-5} M) on cyclic nucleotide levels in mouse sex accessory organs. Acetylcholine produced a 210 per

Table 2. Effect of acetylcholine (1×10^{-5} M) on cyclic nucleotide levels in mouse sex accessory organs *in vitro* in the presence or absence of atropine (1×10^{-5} M)*

Treatment	Cyclic AMP (pmoles/mg protein)		Cyclic GMP (pmoles/mg protein)
	Anterior prostate	Seminal vesicle	Seminal vesicle
Control	12·50 ± 0·52†	10·27 ± 0·74	7·95 ± 1·11
Acetylcholine	11·34 ± 0·82	8·92 ± 0·76	24·72 ± 1·74‡§
Acetylcholine + atropine			10·01 ± 1·88

* Tissues were preincubated in buffer containing aminophylline (5 mM) for 10 min with or without atropine. Tissues were then placed in fresh buffer containing acetylcholine or buffer alone and further incubated for 5 min.
† Mean ± S.E.M. of at least four observations/group.
‡ Significantly different from control ($P < 0·05$).
§ Significantly different from acetylcholine + atropine group ($P < 0·05$).

Table 3. Effect of isoproterenol (1×10^{-5} M) on cyclic AMP or acetylcholine (1×10^{-5} M) on cyclic GMP in sex accessory organs obtained from castrate mice *in vitro**

Treatment	Cyclic AMP (pmoles/mg protein)		Cyclic GMP (pmoles/mg protein)	
	Anterior prostate	Seminal vesicle	Anterior prostate	Seminal vesicle
Control	9·80 ± 0·87†	11·77 ± 1·65	21·46 ± 2·89	15·99 ± 1·64
Isoproterenol	11·73 ± 0·55	12·95 ± 0·48		
Acetylcholine			23·26 ± 2·95	22·31 ± 1·67‡

* Tissues from 7-day castrate mice were preincubated in aminophylline (5 mM) for 10 min then removed from the aminophylline and further incubated for 5 min in buffer with or without the appropriate neurotransmitter.

† Mean ± S.E.M. of at least four observations/group.

‡ Significantly different from control ($P < 0·05$).

cent increase in cyclic GMP levels in the seminal vesicle, while pretreatment with an equimolar concentration of atropine (1×10^{-5} M) for 10 min abolished this response. The cyclic GMP response of the prostate gland to acetylcholine (not shown) was inconsistent in several experiments, hence was considered too variable to allow any interpretation. As can be seen also in Table 2, acetylcholine had no effect ($P > 0·05$) on sex accessory levels of cyclic AMP.

It was next of interest to determine if the hormonal state (i.e. castration) of the animal could modify the effects of the autonomic agents on the levels of cyclic nucleotides in the sex accessory organs. While isoproterenol provoked significant increases in cyclic AMP in organs obtained from normal mice (Table 1), castration abolished the response to this adrenergic agent (Table 3). Similarly, although acetylcholine markedly increased cyclic GMP levels in seminal vesicles from normal mice (Table 2), the response of this cyclic nucleotide to acetylcholine in vesicles from castrate animals was considerably less than that in tissues from intact animals (28 vs 210 per cent, respectively) (Table 3). Also, acetylcholine was ineffective in elevating cyclic GMP in prostates from castrate mice (Table 3).

DISCUSSION

The present studies reveal that isoproterenol can elevate endogenous levels of cyclic AMP in sex accessory organs of the mouse. Similar findings have been reported to occur in the rat uterus [18] and in the rat prostate gland [19]. The present findings reveal that castration abolishes the stimulatory effects of isoproterenol in androgen-dependent tissues like the prostate and the seminal vesicles.

Just as castration can abolish the stimulatory effects of isoproterenol upon cyclic AMP levels, so too can the addition of propranolol lead to a blocking of the effects of this adrenergic agent. Blockade by propranolol suggests that beta-adrenergic innervation is somehow involved in the regulation of cyclic AMP levels in mouse sex accessory organs, but such an interpretation does not explain the loss of effect of isoproterenol in the sex accessory organs obtained from castrate animals. It is well known that castration can reduce many biochemical parameters in sex accessory organs, but less is known about the autonomic regulation of these androgen-dependent tissues. Denervation studies reveal a several-fold increase in sensitivity of vas deferens smooth muscle to autonomic neurotransmitters [20]. Other workers have reported the stimulation of canine prostate gland secretions by various cholinomimetic agents [21] and, to a lesser extent, sympathomimetic amines [22]. In other steroid-dependent tissues such as the uterus, epinephrine reportedly stimulates adenylate cyclase activity [23]. Epinephrine also produces a relaxation in the smooth musculature of the rat uterus [23]. In the present studies, due presumably to the greater proportion of smooth musculature in the seminal vesicles, the cyclic AMP response of the prostate to isoproterenol was not as marked.

Even though acetylcholine can stimulate the secretory activity of sex accessory tissues [12], a 10^{-5} M concentration of this cholinergic agent failed to affect cyclic AMP in either the mouse prostate gland or seminal vesicles. While a wider range of acetylcholine doses would reinforce the idea that cholinergic agents do not affect sex accessory cyclic AMP levels, the present dose of 10^{-5} M was nevertheless effective in influencing another cyclic nucleotide (*viz.* cyclic GMP).

Cyclic GMP has been found to be involved with cholinergic activity in a variety of tissues including the brain [24], heart [25] and submaxillary gland [26], as well as in the ductus deferens [26,27] and the uterus [28]. Changes in uterine cyclic GMP were associated with cholinergically induced contractions of the smooth muscles [28]. Likewise, the present studies reveal that the smooth muscle-rich seminal vesicles exhibit a greater stimulatory cyclic GMP response to acetylcholine. The fact that atropine blocked the response of cyclic GMP to acetylcholine supports the notion that cholinergic activity is associated with the modulation of cyclic GMP, possibly via a muscarinic mechanism. As was the case with isoproterenol and cyclic AMP, castration reduced the response of cyclic GMP to acetylcholine in the seminal vesicle. That castration did not completely abolish the increase in cyclic GMP suggests that this cyclic nucleotide is more closely associated with the smooth muscle of the seminal vesicle than is cyclic AMP and is affected somewhat less by the androgenic state of the animal. Either cyclic nucleotide appears to be altered by autonomic agents with isoproterenol apparently more apt to affect cyclic AMP, while acetylcholine is more inclined toward changing levels of cyclic GMP.

The present studies reveal that both hormone and autonomic factors can affect levels of cyclic nucleotides in androgen-dependent tissues. Further studies will more clearly establish the role that the adrenergic mediators play in the regulation of cyclic AMP and the role that cholinergic mediators play in the modulation of cyclic GMP. The present findings suggest somewhat of an antagonistic action between the two divisions of the autonomic nervous system with regard to sex accessory levels of cyclic nucleotides. Further, the hormonal milieu appears to be important for the biochemical responses of the sex accessory tissues to autonomic stimulation.

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