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EFFECT OF ANDROGENS ON ISOPROTERENOL-INDUCED INCREASES IN MOUSE ACCESSORY SEX ORGAN CYCLIC AMP *IN VITRO**

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Abstract—Varying concentrations of isoproterenol caused marked increases in cyclic adenosine monophosphate (cAMP) in accessory sex organs (viz prostate and seminal vesicles) obtained from normal (i.e. non-castrate) mice. Such increases were abolished or attenuated when these accessory sex organs were obtained from castrate (7-day) mice. Some differences in isoproterenol-induced responsiveness of cAMP were noted between the prostate gland and the seminal vesicles. Injections of testosterone propionate (5 mg/kg, s.c. daily × 5) to orchidectomized mice were effective in restoring the cAMP levels *in vitro* to pre-castration activity. These findings indicate that normal levels of endogenous androgen are necessary for the full expression of isoproterenol-induced increases in cAMP levels in accessory sex organs.

The involvement of cyclic adenosine monophosphate (cAMP) in the mediation of beta-adrenoceptor stimulation has received considerable attention since the early findings that epinephrine enhanced the formation of this cyclic nucleotide in the liver and in cardiac muscle [1]. Subsequent investigations indicated that this effect could be prevented by the concurrent incubation with the beta-adrenergic receptor blocking agent dichloroisoproterenol [2].

The possible role of cAMP in the modulation of male accessory sex organs was reported by Schultz and Hardman [3] who observed that norepinephrine was effective in elevating the levels of this cyclic nucleotide in isolated rat ductus deferens. Such an elevation could be abolished by the concurrent incubation with propranolol. Earlier investigations from this laboratory [4, 5] indicated that rodent accessory sex organ cAMP was markedly increased by isoproterenol *in vitro*, and that this elevation could be blocked by pre-incubation with propranolol. The responsiveness of cAMP in these tissues was affected by the hormonal status of the animal [5]. More recent studies have reported that the injection of isoproterenol also results in a rapid elevation of adenylate cyclase activity in the rat ventral prostate gland [6].

The purpose of the present studies was to more fully investigate the role of androgens in affecting beta-adrenergic induced changes in cAMP levels in mouse accessory sex organs. Earlier studies [5] were not only more limited in scope, but they employed a narrower dose range of beta-agonist and their blockers. Further, the present studies have more thoroughly examined the role of endogenous androgens and testosterone replacement in the castrate mouse with respect to cAMP responsiveness.

MATERIALS AND METHODS

Mature (35–45 g) male Swiss–Webster mice were used in these investigations. Animals were castrated via the abdominal route while under the influence of pentobarbital anesthesia (65 mg/kg) and their accessory sex organs allowed to regress for a period of 7 days prior to the initiation of testosterone replacement. In order to determine the effects of castration upon the responsiveness *in vitro* of the accessory sex organs to isoproterenol in either intact (i.e. non-castrate) or 7-day castrate mice, tissues were incubated with varying concentrations of the beta-agonist. Animals were killed by cervical dislocation and the prostate gland (anterior lobe) and the seminal vesicles rapidly removed. In those studies designed to examine the effect of androgen replacement in castrate mice on the responsiveness *in vitro* of accessory sex organs to isoproterenol, testosterone propionate (Sigma Chemical Co., St. Louis, MO) (5 mg/kg in corn oil) was injected subcutaneously beginning on day 7 of post-castration and continued daily for a period of 5 days. In preliminary studies, this treatment regimen has been found sufficient to restore both protein levels and basal cAMP content to the levels found in accessory sex organs from uncastrated animals. Mice were sacrificed about 24 hr after the final injection of testosterone propionate, and the accessory sex organs rapidly removed and prepared for the incubation experiments *in vitro*.

Tissues were removed, rinsed in isotonic sodium chloride, blotted and placed in incubation vials containing freshly oxygenated Krebs–Ringer bicarbonate buffer (pH 7.4). The buffer system also contained 5 mM aminophylline. Tissues were incubated at 37° with a 10-min period of pre-incubation being

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employed throughout the experiments. Preliminary experiments found that this pre-incubation step was necessary in order to reduce the variability of responses among individual animals. After this pre-incubation period, the tissues were transferred to clean incubation vials containing fresh Krebs–Ringer bicarbonate buffer. Isoproterenol was added and the tissues were further incubated at 37°. All incubations were carried 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 cAMP and protein assay.

The tissues were processed for biochemical analysis as previously described [5] except that after lyophilization the cAMP was reconstituted with ice-cold 50 mM Tris–HCl buffer (pH 7.5), and the levels of cAMP were determined using the competitive protein binding assay of Gilman [7] as modified by Tovey *et al.* [8]. Commercially available binding protein was used in these experiments (Diagnostic Products, Los Angeles, CA). Preliminary studies established good specificity of the binding protein for cAMP with little cross-reactivity (less than 1 per cent) for other nucleotides. Proteins were determined by the method of Lowry *et al.* [9].

RESULTS

Figure 1 shows the dose–response relationship between various concentrations of isoproterenol and cAMP levels in prostate glands obtained from intact and 7-day castrate mice after 5 min of exposure to the agonist *in vitro*. It is evident in tissues obtained from both the intact and castrate animals that the threshold of response of cAMP was approximately 1×10^{-6} M. In the prostate obtained from intact animals, the cAMP response to isoproterenol continued to increase through a concentration of isoproterenol of 1×10^{-4}M, at which point the cAMP levels had increased 7-fold compared to that of 1×10^{-8} M isoproterenol. In contrast, the prostates obtained from castrate animals were markedly less sensitive to isoproterenol with cAMP levels being increased only about 3-fold by 1×10^{-4} M isoproterenol relative to the levels after exposure to 1×10^{-8} M isoproterenol.

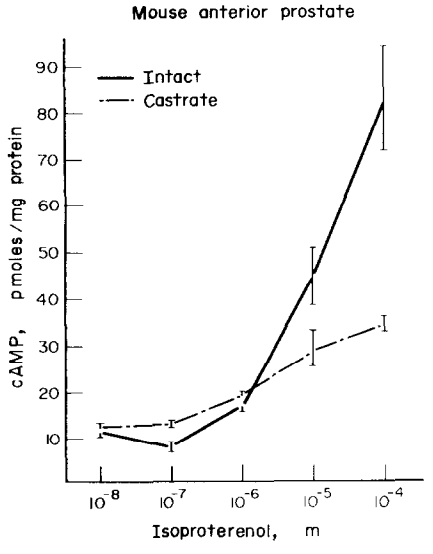

Fig. 1. Concentration-response *in vitro* of anterior prostate gland cAMP to isoproterenol in intact vs 7-day castrate mice. The tissues were pre-incubated for 10 min in aminophylline (5 mM) followed by exposure to isoproterenol for 5 min. Each point represents the mean ± S. E. M. of the response of tissues obtained from five animals.

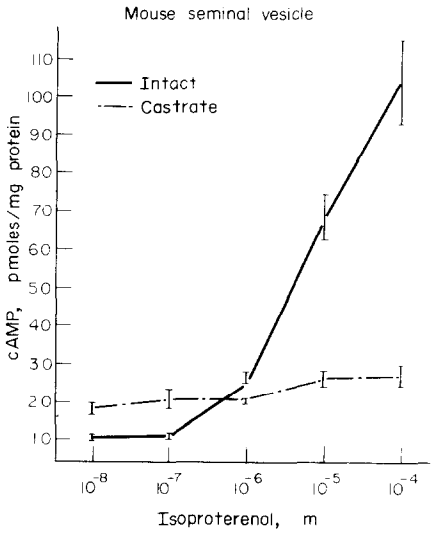

Fig. 2. Concentration-response *in vitro* of seminal vesicle cAMP to isoproterenol in intact vs 7-day castrate mice. The tissues were pre-incubated for 10 min in aminophylline (5 mM) followed by exposure to isoproterenol for 5 min. Each point represents the mean ± S. E. M. of the response of tissues obtained from five animals.

In the seminal vesicles (Fig. 2), as in the prostate gland (Fig. 1), the threshold of cAMP in those tissues obtained from intact animals occurred at a concentration of approximately 1×10^{-6} M. In these tissues, the levels of cAMP continued to increase through a concentration of 1×10^{-4} M at which point the increase in cAMP over those levels achieved after incubation at 1×10^{-8} M isoproterenol was 10-fold. However, in those tissues obtained from castrate animals, the sensitivity of the cAMP system to isoproterenol was dramatically depressed. In those tissues obtained from castrate animals, 1×10^{-4} M isoproterenol was only able to increase cAMP levels 1.5-fold relative to those levels observed in tissues exposed to 1×10^{-8} M isoproterenol.

In order to ascertain whether castration was reducing the apparent sensitivity of the accessory sex organ cAMP system to isoproterenol by altering the time-response pattern, the responsiveness to cAMP to various durations of isoproterenol incubations (1×10^{-5} M) was compared *in vitro* (Fig. 3). In both the prostate glands and the seminal vesicles, isoproterenol produced its maximum effect within the first 5 min of incubation when the tissues were obtained from intact animals. Tissues obtained from castrate animals underwent only minimal changes in cAMP. Longer periods of incubation were ineffective in further increasing the levels of cAMP in tissues obtained from non-castrate animals. Again it was seen that the sensitivity of these tissues to isoproterenol was markedly reduced after castration.

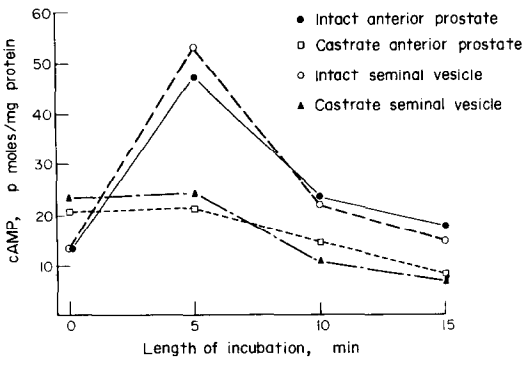

Fig. 3. Time-response *in vitro* of anterior prostate gland and seminal vesicle cAMP to isoproterenol in intact vs 7-day castrate mice. The tissues were pre-incubated for 10 min in aminophylline (5 mM) followed by exposure to isoproterenol (1×10^{-5} M) for varying lengths of time. Each point represents the mean ± S. E. M. of the response of tissues obtained from at least three animals.

With the finding that castration markedly reduced the sensitivity of accessory sex organ cAMP to isoproterenol, it was next of interest to determine if this effect could be restored by testosterone injections in the castrate animal. Figure 4 (prostate) and Fig. 5 (seminal vesicles) show the effects of incubation *in vitro* with or without isoproterenol (1×10^{-5} M) on accessory sex organs obtained from testosterone-treated castrate mice. Somewhat expectedly, the responsiveness of the cAMP system to isoproterenol in accessory sex organs obtained from castrate mice treated only with corn oil vehicle was greatly attenuated relative to the responsiveness in these same tissues obtained from intact or non-castrate

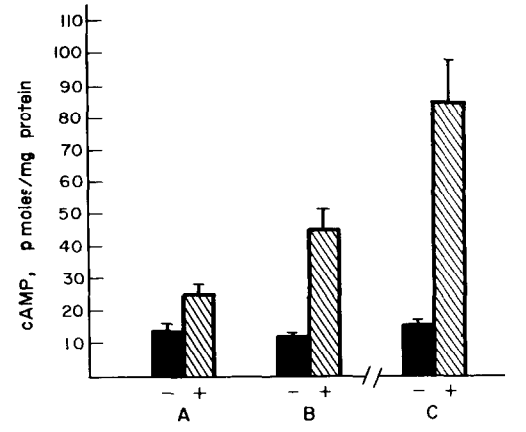

Fig. 4. Effect of testosterone propionate on the responsiveness *in vitro* of the prostate gland cAMP to isoproterenol in castrate mice. Group A: castrate + vehicle-injected animals; group B: castrate + testosterone propionate (5 mg/kg daily × 5); and group C: intact (or non-castrate) animals. Testosterone or corn oil vehicle injections began on day 7 of post-castration. Tissues were pre-incubated for 10 min in a buffer containing aminophylline (5 mM) and then transferred to fresh Krebs–Ringer buffer containing isoproterenol (1×10^{-5} M) (stripped bars) or without beta-agonist (solid bars) for an additional 5-min period of incubation. Each point represents the mean ± S. E. M. of the responses of appropriate tissues obtained from at least five animals.

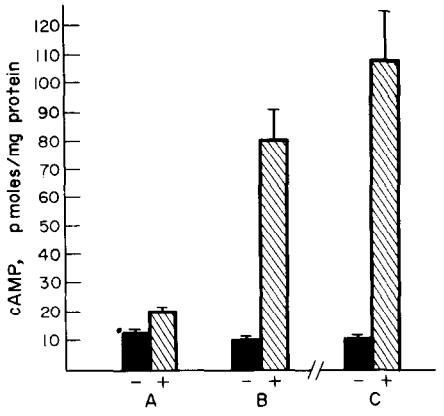

Fig. 5. Effect of testosterone propionate on the responsiveness *in vitro* of the seminal vesicle cAMP to isoproterenol in castrate mice. Group A: castrate + vehicle-injected animals; group B: castrate + testosterone propionate (5 mg/kg daily × 5); and group C: intact (or non-castrate) animals. Testosterone or corn oil vehicle injections began on day 7 of post-castration. Tissues were pre-incubated for 10 min in a buffer containing aminophylline (5 mM) and then transferred to fresh Krebs–Ringer buffer containing isoproterenol (1×10^{-5} M) (stripped bars) or without beta-agonist (solid bars) for an additional 5-min period of incubation. Each point represents the mean ± S. E. M. of the responses of appropriate tissues obtained from at least five animals.

mice. When testosterone was injected into castrate animals, the responsiveness *in vitro* of the accessory sex organ cAMP was enhanced to a level approaching that in tissues obtained from intact or non-castrate mice.

DISCUSSION

The present studies indicate that, in contrast to the accessory sex glands obtained from normal (i.e. non-castrate) mice, the cAMP system(s) in those same organs obtained from 7-day castrate animals were far less responsive to stimulation by isoproterenol. Although in tissues obtained from castrate mice the initial response of cAMP to isoproterenol occurred at approximately the same concentration (viz 1×10^{-6} M) as that seen in tissues obtained from intact animals, the cAMP response of these tissues to higher concentrations of isoproterenol in the bathing medium was considerably reduced relative to that in the accessory sex tissues obtained from intact animals. The fact that the time-course of response of the cAMP system in the tissues obtained from castrate animals to isoproterenol was not altered relative to that seen in accessory sex tissues obtained from intact animals indicates that castration was not changing the temporal pattern of cAMP responsiveness to isoproterenol. When testosterone propionate was injected into castrate mice, the responsiveness of the cAMP system in the accessory sex organs was restored to normal levels. The present results are in agreement with those of Chew and Rinard [10] who reported that, in the rat uterus, estrogen pre-treatment significantly enhanced the sensitivity of the cAMP system to epinephrine.

Explanations for the reduced sensitivity of the cAMP of the accessory sex tissues to incubation with isoproterenol in castrate animals relative to tissues obtained from intact animals remain obscure. It is possible that this effect is partly due to the inability of the tissue to adequately form cAMP after castration. Sutherland and Singhal [11] found that rat prostatic adenylate cyclase activity was significantly reduced by castration (6 days). Testosterone injections were able to restore the activity of this enzyme. Thus, it may be that, with reduced adenylate cyclase activity in the accessory sex organs after castration, these tissues are not as capable of producing cAMP in response to isoproterenol as those tissues obtained from intact or testosterone-treated castrate animals. A second mechanism which may be partially responsible for the decreased responsiveness of accessory sex organ cAMP to isoproterenol is the potentially increased activity of the extraneuronal catecholamine uptake system in accessory sex organs from castrate animals. One mechanism for the inactivation of catecholamines may be by uptake and subsequent metabolism of the catecholamines by surrounding extraneuronal structures such as the effector organ itself [12]. Exogenously applied catecholamines might be inactivated by this extraneuronal uptake mechanism and, as a result, would be unavailable for combination with the adrenergic receptor. Isoproterenol has a relatively high affinity for extraneuronal uptake [12]. Several steroids, including testosterone, androsterone, estradiol-17-β and corticosterone, are among the most potent inhibitors of extraneuronal uptake of catecholamines in the rat heart, regardless of the sex of the animal [13]. Although, to the best of the present authors' knowledge, extraneuronal uptake of catecholamines in the anterior prostate gland and seminal vesicle of the mouse has not been studied, it is conceivable that such a mechanism could partially account for the reduced effectiveness of isoproterenol *in vitro* on the cAMP system in the sex accessories from castrate animals. Testosterone may be acting to restore the responsiveness of the cAMP system to isoproterenol in these tissues, at least in part, by blocking extraneuronal uptake and subsequent inactivation of isoproterenol, thus increasing the concentration of this catecholamine at the receptor. This possible mechanism warrants further investigation.

The present findings *in vitro* using mouse prostate glands are similar to those reports using rat prostate glands *in vivo* [6] with regard to the effects of isoproterenol-induced increases in cAMP levels. Tsang and Singhal [6] reported that isoproterenol (1 mg/kg, i.p.) caused a rapid and dramatic increase in cAMP (and adenylate cyclase activity) in the rat ventral prostate gland. Maximal stimulation reportedly occurred 5 min after the injection of isoproterenol [6]. In the present studies, the maximal increases *in vitro* were also observed at 5 min (Fig. 3), although such similar time-course responses might simply be coincidental. It appears that propranolol can block isoproterenol-induced increases in either the rat [6] or the mouse [5]. Such a blockade can occur in either the system *in vitro* or *in vivo*. Propranolol, but not phentolamine, can block this beta-adrenergic agonist effect upon accessory sex organs [6]. Likewise, epinephrine-induced increases in uterine cAMP levels can be blocked by propranolol [14]. Thus, beta-adrenergic mechanisms are involved in modulating cyclic nucleotide levels in tissues of both the male and the female reproductive system.

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