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Effects of Androgens on Isoproterenol-Sensitive Adenylate Cyclase System of the Rat Prostate

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SUMMARY

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Adrenergic stimulation of the cyclic AMP system of the prostate from intact, sham-operated, and castrated rats has been investigated. Castration markedly reduced isoproterenol activation of adenylate cyclase associated with a fall in β-adrenergic receptor sites in the prostatic membrane. These results imply that a loss in the number of adrenergic receptors might be a major mechanism responsible for the decreased responsiveness of the prostatic adenylate cyclase to isoproterenol induced by castration. In addition to the decreased receptors, 5′-guanylyl-imidodiphosphate (Gpp(NH)p) was without effect on the cylcase activity and the receptor–ligand affinity in the prostate of castrated rats, suggesting some defects in GTP-regulating mechanisms on the receptor–adenylate cyclase complex. Supplementation of testosterone propionate to castrated rats restored the response to isoproterenol of the enzyme activity and the concentration of adrenergic receptors to levels at precastration. These findings suggest that endogenous androgens are involved in maintenance of β-adrenergic receptors linked to the adenylate cyclase system of the prostatic membrane for the full expression of isoproterenol stimulation.

INTRODUCTION

β-adrenergic stimulation in cyclic AMP accumulation and adenylate cyclase activity of the ventral prostate has been reported *in vitro* (1, 2) and *in vivo* (3). Previous studies (4–6) have demonstrated an adrenergic receptor of the β-2 subtype coupled to adenylate cyclase system in the rat prostatic membrane. Possible involvement of cyclic AMP in the actions of androgens on the prostate gland has been reported by several investigators (7, 8). Most other studies (9–12), however have proposed no correlation between cyclic AMP and androgen action in this tissue. Zepp and Thomas (2) have recently reported a decreased cyclic AMP response to isoproterenol in the castrated prostate of mice. The present study was performed to investigate the role of androgens in affecting adrenergic responses of the adenylate cyclase system in the rat prostate.

MATERIALS AND METHODS

Male rats weighing 250 to 300 g of Donryu strains (Nippon rats Co., Ltd., Tokyo) were castrated through the scrotal route under ether anesthesia. Rats, with an incision of the scrotal skin are referred to as "sham" operated. Daily injections of saline or testosterone propionate (5 mg/kg/day) to castrated rats were carried out for 7 days from the first day of operation. The prostate tissue was incubated in Krebs–Ringer bicarbonate buffer solution (pH 7.4) containing 2 mg/ml glucose and 1 mg/ml bovine serum albumin, gassed with 95% O_2–5% CO_2 at 37°C with or without isoproterenol for 15 min. Cyclic AMP was estimated by a competitive binding assay (13) using a protein purified from rabbit skeletal muscle. The assay of adenylate cyclase activity was made by incubation of the 10,000*g* particulate fraction (5, 6, 10, 11) in a total volume of 0.6 ml 40 mM Tris–HCl buffer (pH 7.4) containing 4 mM $MgCl_2$, 10 mM theophylline, 2 mM ATP, 100 μg/ml pyruvate kinase, and 5 mM phosphoenol pyruvate for 10 min. Concentrations of protein and DNA were measured by the methods of Lowry *et al.* (14) and Burton (15). Aliquots of membrane preparation (0.5 to 1.0 mg protein) were assayed for [^{3}H]dihydroalprenolol binding as described previously (4–6). Membranes were incubated with [^{3}H]dihydroalprenolol (2 to 60 nM, 57.9 Ci/mmol, New England Nuclear, Boston, Mass.) in buffer (50 mM Tris–HCl, pH 7.5, and 10 mM $MgCl_2$) in a total volume of 0.2 ml for 10 min at 37°C.

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TABLE 1

Effects of castration on isoproterenol-stimulated cyclic AMP accumulation in the prostate gland

Tissue slices (50 to 100 mg) were incubated in Krebs-Ringer bicarbonate buffer solution for 10 min, then transferred to fresh medium for a final incubation with or without 10 μM isoproterenol. Each result represents the mean ± SE of four to five separate incubations.

Treatment	Addition	Cyclic AMP	
		pmol/mg protein	pmol/mg DNA
Intact	None	9.7 ± 2.0	290 ± 60
	Isoproterenol	127.5 ± 15.0	3810 ± 450
Castrated	None	12.5 ± 1.5	225 ± 27
	Isoproterenol	39.5 ± 5.2	718 ± 94
Castrated + Testosterone	None	10.5 ± 2.0	368 ± 70
	Isoproterenol	89.0 ± 27.1	3115 ± 945

Incubations were terminated by dilution with 3 ml ice-cold incubation buffer, followed by filtration through Whatman GF/C glass fiber filters. Specific binding to receptors, defined as the difference between radioactivity bound in the presence and absence of 50 μM propranolol, was approximately 65 to 80% of total binding.

The following drugs were generously donated: 1-isoproterenol (Nicken Chemicals, Tokyo), 1-propranolol (Japan ICI Pharma, Osaka), and cyclic AMP (Daiichi Seiyaku, Tokyo). 5′-Guanylyl-imidophosphate (Gpp(NH)p) and testosterone propionate were obtained from Japan Boehringer-Mannheim, Tokyo, and Sigma Chemicals Company, St. Louis, Mo.

RESULTS

Cyclic AMP levels of the prostate from intact and sham-operated rats were found to be similar in responding to isoproterenol (Table 1), indicating that the influence of the surgical operation was negligible. Castration markedly reduced isoproterenol-induced accumulation of cyclic AMP (Table 1) and activation of adenylate cyclase in the prostate (Fig. 1). Fluoride stimulation of the enzyme was similar in intact, sham-operated, and castrated groups (Fig. 1). [^{3}H]Dihydroalprenolol binding to the prostatic membrane was also reduced by castration (Fig. 2). Changes in isoproterenol-stimulated adenylate cyclase activities and β-adrenergic receptor sites after castration were characterized by a fall in maximum enzyme velocity and binding capacity with no shift in apparent affinity for hormone and receptor–ligand binding (Figs. 1 and 2). Injections of testosterone propionate to orchidectomized rats restored β-adrenergic receptor sites (Fig. 2) and isoproterenol-stimulated adenylate cyclase activity (Fig. 3) to precastration levels. Figures 3, 4, and 5 showed effects of Gpp(NH)p on isoproterenol-sensitive adenylate cyclase and isoproterenol displacement of the bound radioactive dihydroalprenolol in the prostate membrane from sham-operated, castrated, and testosterone-treated castrated rats. Gpp(NH)p stimulated the basal activity of adenylate cyclase in sham-operated and testosterone-treated castrated rats but not that in castrated animals. Adenylate cyclase activity in sham-operated and testosterone-treated castrated rats was responsive to this guanine nucleotide analog as evidenced by a 10-fold increase in affinity for isoproterenol, while the enzyme from castrated rats was not influenced by Gpp(NH)p (Figs. 3 and 4). The affinity of the β-adrener-

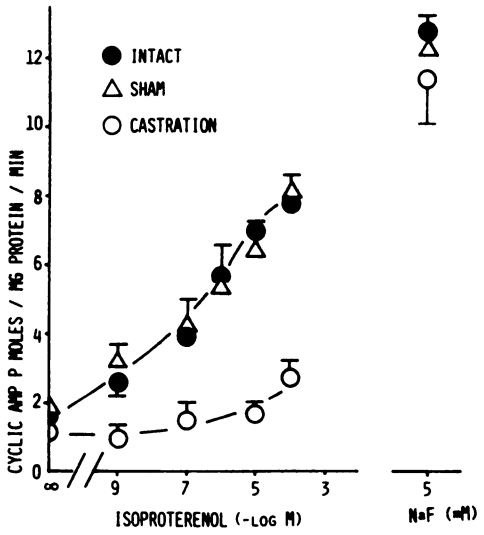

FIG. 1. *Effect of isoproterenol and sodium fluoride on adenylate cyclase activity in prostatic membranes*

Crude membranes from prostates of intact, 7-day castrated, and 7-day sham-operated rats were incubated with various concentrations of isoproterenol or 5 mM NaF for 10 min. Results shown are the mean ± SE (brackets) of three to four incubations.

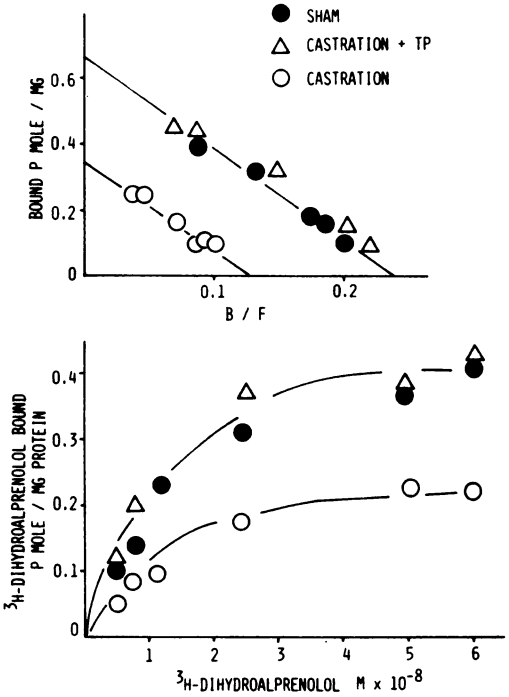

FIG. 2. *Specific [^{3}H]dihydroalprenolol binding and Scatchard analysis in prostatic membranes as a function of the radioligand concentration*

Crude membranes from sham-operated, castrated, and castrated plus testosterone-treated rats were incubated with 2 to 60 nM [^{3}H]-dihydroalprenolol for 10 min. Values shown represent the mean of triplicate determinations. TP, testosterone propionate treated for 7 days.

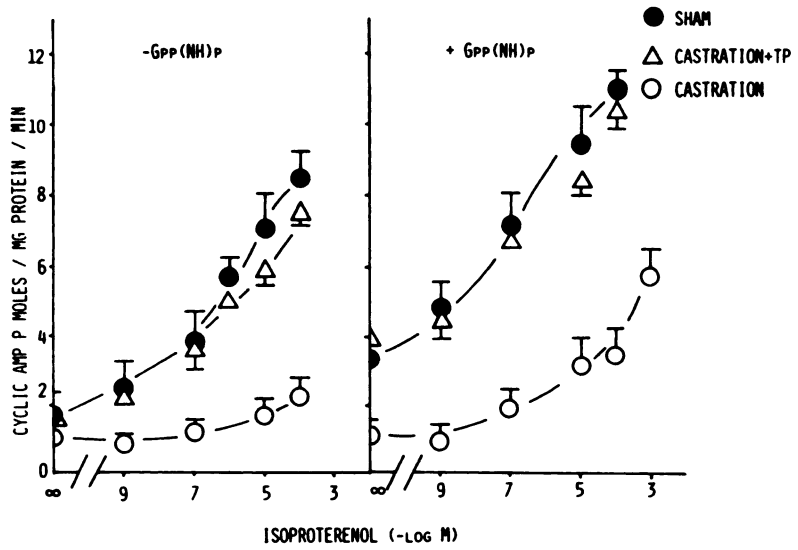

FIG. 3. *Effect of isoproterenol on adenylate cyclase activity in prostatic membranes*

Crude membranes from sham-operated, castrated, and castrated plus testosterone-treated rats were incubated with various concentrations of isoproterenol for 10 min in the presence (right) or absence (left) of 50 μM Gpp(NH)p. Results shown are the mean ± SE (brackets) of three incubations.

gic receptor of noncastrated rats for isoproterenol was decreased by Gpp(NH)p one order of magnitude (Fig. 5). On the other hand, Gpp(NH)p was without effect on binding to membranes from castrated rats.

DISCUSSION

Direct stimulation by androgen treatment of cyclic AMP systems in prostates from castrated rats has been reported (7, 8). Most other studies (9–12), however, have demonstrated little or no evidence of androgens stimulating adenylate cyclase activity of the prostate. The present study also showed no change in basal and fluoride-stimulated adenylate cyclase activity after castration. The response of the prostate to isoproterenol markedly decreased after castration, confirming those reports (1, 2) with regard to effects of castration on isoproterenol-induced increase in cyclic AMP levels. Zepp and Thomas (2) postulated that androgens may be acting to restore the responsiveness of cyclic AMP systems to isoproterenol in the prostate of castrated rats, at least in part, by blocking extraneuronal uptake, thus increasing the concentration of isoproterenol at the receptor. In the present experiment, reduction in the activity of isoproterenol-sensitive adenylate cyclase by castration was associated with a decreased number of β-adrenergic receptors. These results suggested tht deprivation of endogenous androgens may account for a loss of adrenergic receptors linked to the catalytic unit which remains integral even after castration. Furthermore, injections of testosterone propionate to castrated animals completely returned the decreased enzyme activity and receptor sites to precastration levels, suggesting that androgens induce or maintain β-adrenergic receptors coupled to the cyclase. It has been well established that androgens stimulate RNA and

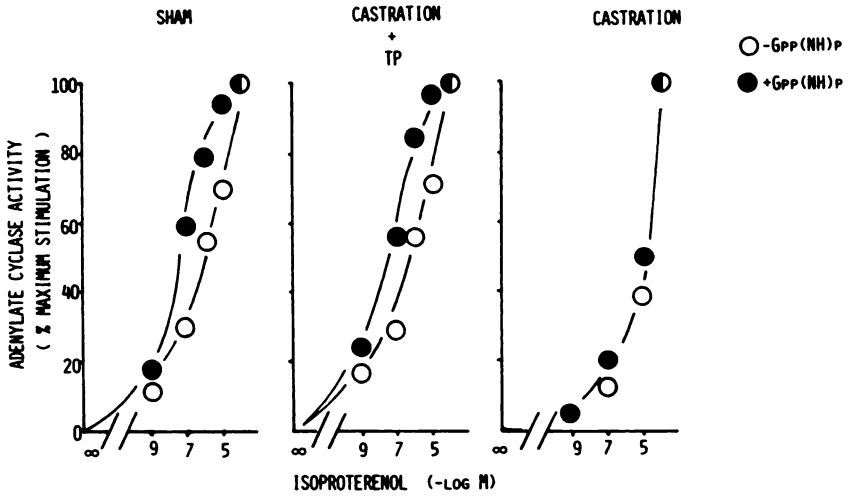

FIG. 4. *Effect of Gpp(NH)p on isoproterenol stimulation of adenylate cyclase activity in prostatic membranes*

Results of maximum stimulation of the enzyme by 0.1 mM isoproterenol, obtained from Fig. 3, are set at 100%. Concentration of Gpp(NH)p was 50 μM. TP, testosterone propionate treated for 7 days.

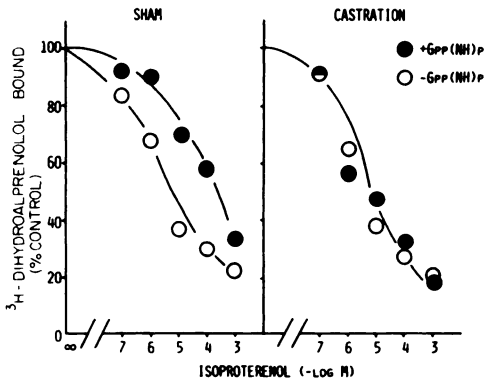

FIG. 5. *Isoproterenol competition for [^{3}H]dihydroalprenolol binding sites*

Crude membranes from sham-operated and castrated rats were assayed for 50 nM [^{3}H]dihydroalprenolol in the presence of the indicated concentrations of isoproterenol, with or without 50 μM Gpp(NH)p. Maximum radioligand binding amounted to 0.41 ± 0.15 pmol/mg protein in sham-operated rats and 45 to 60% less in castrated animals, which were taken as 100%. Values shown represent the mean of triplicate determinations.

protein synthesis in the prostate (12, 16–19). Induction or *de novo* synthesis of protein components corresponding to the receptor moiety for catecholamines might be involved in androgenic action on the prostate.

Bilezikian and Gammon (20) have reported that a decrease in β-receptors and adenylate cyclase activity may be related to a decrease in cell size during maturation of rat erythrocytes. Involution of the prostate after castration was accompanied by a great decrease in the cytoplasmic volume of the epithelial cells (10). A fall in the number of receptors after castration might be related to a decrease in cell size of prostatic cells.

Guanine nucleotides have been shown to augment many different hormone-sensitive adenylate cyclase systems, including the β-adrenergically regulated enzyme. They act by modulating the receptor binding function (21–24) and/or the hypothetic coupling mechanism between the receptor site and the catalytic moiety of the enzyme complex (25–30). The prostatic membrane from noncastrated rats was responsive to Gpp(NH)p in decreasing the receptor–ligand affinity and increasing the hormone affinity of the cyclase. In the prostate of castrated rats, the GTP analog was without effect in modulating the receptor binding and the cylase activation, suggesting some defects in the guanine nucleotide-regulating mechanisms due to androgen deficiency.

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