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# EFFECT OF ADRENERGIC STIMULATION ON ADENYLATE CYCLASE ACTIVITY IN RAT PROSTATE

SUMIO SHIMA *, YOSHIKO KAWASHIMA, MASANAO HIRAI and MIKIO ASAKURA **

with the technical assistance of HIROSHI KOUYAMA

*Departments of Pharmacology and Neuropsychiatry, St. Marianna University School of Medicine, Kawasaki City, Kanagawa 213 (Japan)*

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## Summary

Adrenergic stimulation of the cyclic AMP system of the prostate gland of the rat has been investigated. The observed order of potency for adrenergic agonists in stimulating prostatic adenylate cyclase activity was isoproterenol > epinephrine ≃ salbutamol > norepinephrine, indicating properties characteristic of beta-2-adrenergic receptor-sensitive adenylate cyclase systems. Dopaminergic stimulation of the enzyme was exclusively inhibited by a dopamine antagonist, haloperidol, suggesting the presence of dopamine-sensitive receptor in the prostate gland. An initial incubation of the gland with isoproterenol or dopamine resulted in a decrease in maximal enzyme activation by catecholamine, either isoproterenol or dopamine, with no change in hormone affinity. The findings that refractoriness of beta-adrenergic and dopaminergic receptor-adenylate cyclase systems was induced by both receptor-agonists suggest an interaction of an agonist-induced desensitization with another receptor or receptor-enzyme complex in the prostate gland.

## Introduction

A possible involvement of the cyclic AMP in the action of androgens on the prostate gland has been reported by several investigators [1—3]. Recent studies,

* To whom reprint requests should be addressed at: Department of Pharmacology, St. Marianna University School of Medicine, 2095 Sugao, Takatsuku, Kawasaki-shi, Kanagawa 213, Japan.

however demonstrated little or no evidence of androgens stimulating adenylate cyclase activity of prostates [4—7]. On the other hand, the possible role of the cyclic AMP in the modulation of male accessory sex organ was observed by Schultz et al. [8] reporting norepinephrine-elevated cyclic AMP levels in isolated rat ductus deferens. Similarly, the ventral prostate treated with isoproterenol in vitro [9,10] and in vivo [11] showed a considerable stimulation in the cyclic AMP accumulation and adenylate cyclase activity. The present study was to more fully investigate the adrenergic stimulation of this cyclic nucleotide and adenylate cyclase activity of rat ventral prostate using various adrenergic agonists. Catecholamines appear to play an important role in regulating the function of their own receptors for mammalian systems. It has been initially demonstrated that the pretreatment of frog erythrocytes with catecholamines in vivo [12,13] and in vitro [14,15] led to a time-dependent decrease in beta-adrenergic receptor number and catecholamine-sensitive adenylate cyclase in the plasma membranes. These studies also demonstrated catecholamine regulation on the catecholamine-sensitive adenylate cyclase system in the prostate gland.

## Materials and Methods

Prostate tissue from male rats weighing 250 to 300 g of Donryu strain (Nippon Rats Co., Ltd., Tokyo) was incubated in buffer A (Krebs-Ringer bicarbonate buffer solution, pH 7.4, 2 mg/ml glucose, 1 mg/ml bovine serum albumin), gassed with 95% $O_2$/5% $CO_2$ at 37°C, with various concentrations of adrenergic agonists for 10 min. Cyclic AMP levels in the tissue were estimated by a competitive binding assay [16], using a protein purified from rabbit skeletal muscle. Preincubation of prostate tissue with catecholamines ($10^{-4}$ M isoproterenol or dopamine) or without catecholamines ($10^{-4}$ M isoproterenol or dopamine) was carried out in buffer A for 15—60 min. These tissues were then washed three times with the same buffer. The assay of adenylate cyclase activity was made by incubation of 10 000 × *g* particles [5,6] in a total volume of 0.6 ml 40 mM Tris-HCl buffer, pH 7.4, 4 mM $MgCl_2$, 10 mM theophylline, 2 mM ATP, 100 μg/ml pyruvate kinase, 5 mM phospho*enol*pyruvate for 10 min at 37°C [17,18]. Protein concentration was measured by the method of Lowry et al. [19]. The following drugs were generously donated: l-isoproterenol (Sumitomo Chemicals, Osaka), salbutamol (Sankyo Pharmaceutical Co., Inc., Osaka), l-propranolol (Japan ICI Pharma, Osaka), phentolamine (Japan Ciba Geigy, Tokyo) and atenolol (Japan ICI Pharma, Osaka). Haloperidol and L-epinephrine were obtained from Dainippon Seiyaku, Tokyo, and Sigma Chemical St. Louis, MO. L-Norepinephrine and dopamine were from Wako Chemicals, Tokyo.

## Results

Incubation of tissue with various adrenergic agonists, isoproterenol, epinephrine, salbutamol, norepinephrine or dopamine, for 15 min increased cyclic AMP levels in prostate tissue (Table I). The stimulation by these adrenergic agents, except dopamine, of cyclic AMP accumulation was blocked by propanolol but

TABLE I

EFFECT OF ADRENERGIC AGONISTS ON CYCLIC AMP LEVELS IN THE PROSTATE GLAND

50—100 mg tissue slice was incubated in buffer A for 10 min, then transferred to fresh medium for a final incubation with various agents indicated. Each result represents the mean ± S.E. of four to five separate incubations.

| Additions | Cyclic AMP level (pmol/mg protein) |
|---|---|
| None | 9.69 ± 2.00 |
| $10^{-5}$ M isoproterenol | 177.08 ± 7.39 |
| $10^{-5}$ M isoproterenol + $10^{-6}$ M propranolol | 38.62 ± 2.27 |
| $10^{-5}$ M epinephrine | 96.95 ± 4.72 |
| $10^{-5}$ M norepinephrine | 58.62 ± 2.27 |
| $10^{-5}$ M norepinephrine + $10^{-6}$ M propranolol | 5.95 ± 0.31 |
| $10^{-5}$ M norepinephrine + $10^{-4}$ M phentolamine | 69.65 ± 0.32 |
| $10^{-4}$ M dopamine | 52.05 ± 2.11 |
| $10^{-4}$ M dopamine + $10^{-6}$ M propranolol | 49.28 ± 0.66 |
| $10^{-4}$ M dopamine + $10^{-4}$ M haloperidol | 11.20 ± 1.11 |
| $10^{-6}$ M propranolol | 10.85 ± 1.40 |
| $10^{-4}$ M phentolamine | 7.47 ± 0.52 |
| $10^{-6}$ M haloperidol | 6.97 ± 0.08 |

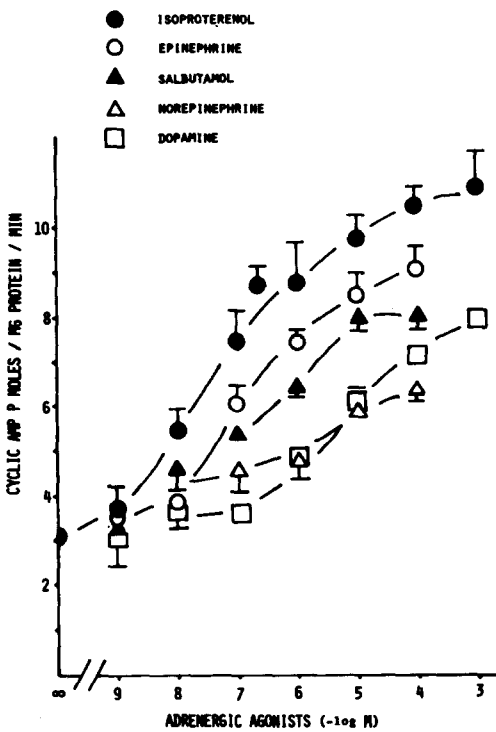


Fig. 1. Effects of adrenergic agonists on adenylate cyclase activity. Prostatic particles were incubated in adenylate cyclase reaction mixtures with the indicated concentrations of adrenergic agonists and adenylate cyclase activity was determined. Each result represents the mean ± S.E. (vertical lines) of five to seven separate incubations.

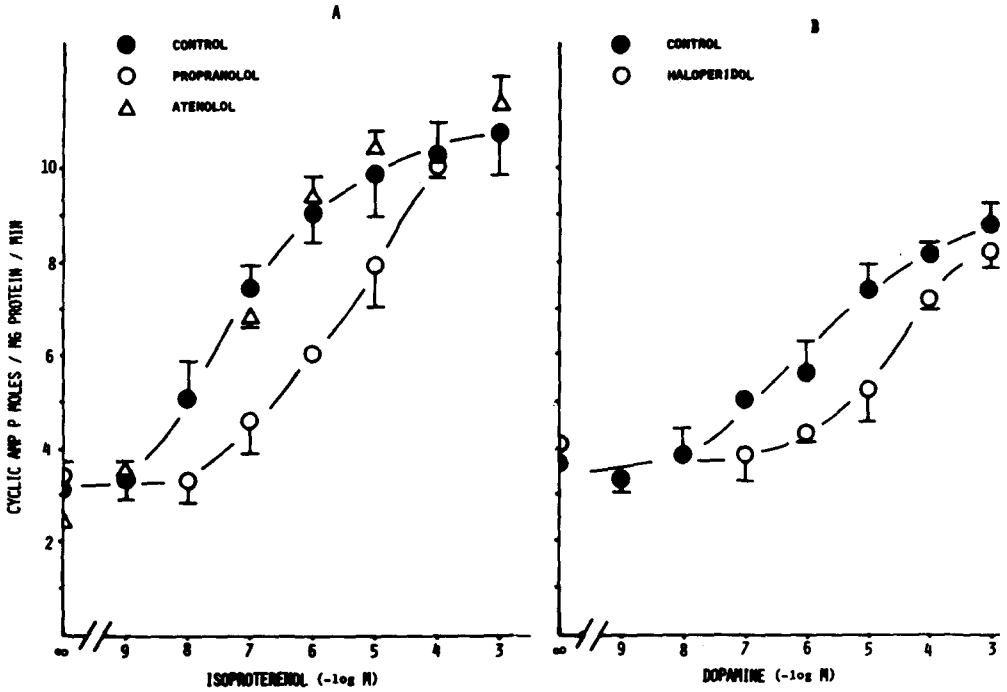


Fig. 2. Effects of antagonists on catecholamine-stimulated adenylate cyclase activity. Prostatic particles were incubated in adenylate cyclase reaction mixtures containing the indicated concentrations of catecholamines with or without antagonists. (A) Isoproterenol stimulation in the presence (open circles, $10^{-6}$ M propranolol; triangles, $10^{-5}$ M atenolol) or absence (closed circles) of antagonists. (B) Dopaminergic stimulation in the presence (open circles) or absence (closed circles) of $10^{-5}$ M haloperidol. Each result represents the mean ± S.E. (vertical lines) of five separate incubations.

TABLE II

EFFECTS OF ADRENERGIC AGENTS ON ADENYLATE CYCLASE ACTIVITY OF THE PROSTATE GLAND

Prostatic particles were incubated in adenylate cyclase reaction mixture with various agents indicated and adenylate cyclase activity was determined. Each result represents the mean ± S.E. of five to seven separate incubations.

| Additions | Adenylate cyclase activity (cyclic AMP formed/pmol/mg protein per min) |
|---|---|
| None | 2.32 ± 0.30 |
| $10^{-5}$ M isoproterenol | 8.94 ± 0.18 |
| $10^{-5}$ M isoproterenol + $10^{-6}$ M propranolol | 5.23 ± 0.62 |
| $10^{-5}$ M isoproterenol + $10^{-4}$ M haloperidol | 7.99 ± 0.13 |
| $10^{-5}$ M salbutamol | 6.89 ± 0.36 |
| $10^{-5}$ M salbutamol + $10^{-6}$ M propranolol | 2.09 ± 0.22 |
| $10^{-4}$ M dopamine | 6.83 ± 0.41 |
| $10^{-4}$ M dopamine + $10^{-6}$ M propranolol | 6.08 ± 0.32 |
| $10^{-4}$ M dopamine + $10^{-4}$ M haloperidol | 2.05 ± 0.17 |
| $10^{-5}$ M isoproterenol + $10^{-4}$ M dopamine | 8.68 ± 0.20 |
| $10^{-5}$ M isoproterenol + $10^{-5}$ M epinephrine | 7.56 ± 2.12 |
| $10^{-6}$ M propranolol | 2.16 ± 0.22 |
| $10^{-4}$ M haloperidol | 2.21 ± 0.16 |

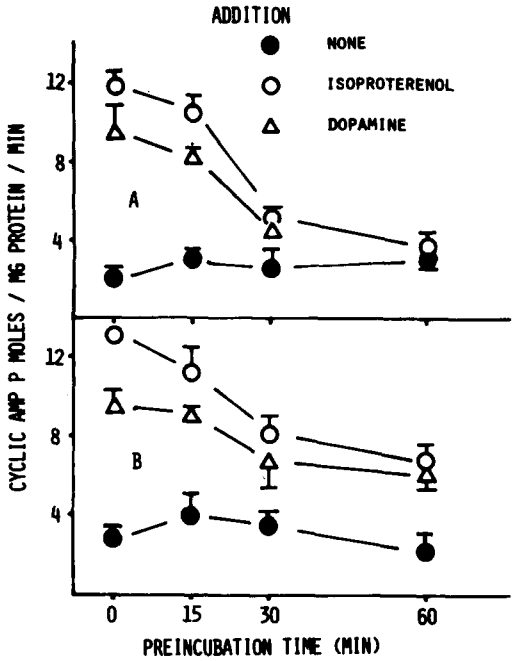


Fig. 3. Effects of preincubation of catecholamines on adenylate cyclase activity. Maximum catecholamine-sensitive adenylate cyclase activity in the presence of $10^{-3}$ M isoproterenol or dopamine, as a function of time of preincubation of the prostate gland with $10^{-4}$ M isoproterenol (A) or $10^{-4}$ M dopamine (B). Each result represents the mean ± S.E. (vertical lines) of four to five separate incubations.

not by phentolamine. The adrenergic stimulation of adenylate cyclase activity was confirmed in the particular fraction sedimented by centrifugation of 10 000 × *g* (Fig. 1). With respect to their ability to stimulate adenylate cyclase activity, the beta-hydroxylated catecholamines demonstrated the greatest potency, with isoproterenol > epinephrine ≃ salbutamol > norepinephrine. Graphical estimation of the concentration resulting in half maximal stimulation gave $K_a$ values for isoproterenol, epinephrine, salbutamol and norepinephrine of $8.5 \cdot 10^{-8}$, $1.2 \cdot 10^{-7}$, $2.1 \cdot 10^{-7}$ and $8.9 \cdot 10^{-7}$ M respectively. The non-beta-hydroxylated catecholamine, dopamine was much less potent with $K_a$ of $3.5 \cdot 10^{-6}$ M. Dose response curve of adenylate cyclase activity stimulated by isoproterenol was shifted to right by addition of propranolol in the manner of competitive inhibition (Fig. 2). A beta-1-adrenergic antagonist, atenolol was without effect on isoproterenol-stimulated activity. Isoproterenol-stimulated activity was inhibited by propranolol but not haloperidol, while dopamine-stimulation was exclusively prevented by haloperidol (Table II and Fig. 3). No 'additive stimulatory effect' or significant enhancement of the cyclase activation was demonstrated when dopamine was combined with saturable concentration of isoproterenol or epinephrine. Particulate preparations from slices previously incubated with $10^{-4}$ M isoproterenol for 30 min displayed a great reduction of the maximal catecholamine (isoproterenol or dopamine)-stimulated adenylate cyclase activity (Fig. 4). Pretreatment with $10^{-4}$ M dopamine also reduced the maximum of catecholamine-response to a lesser extent

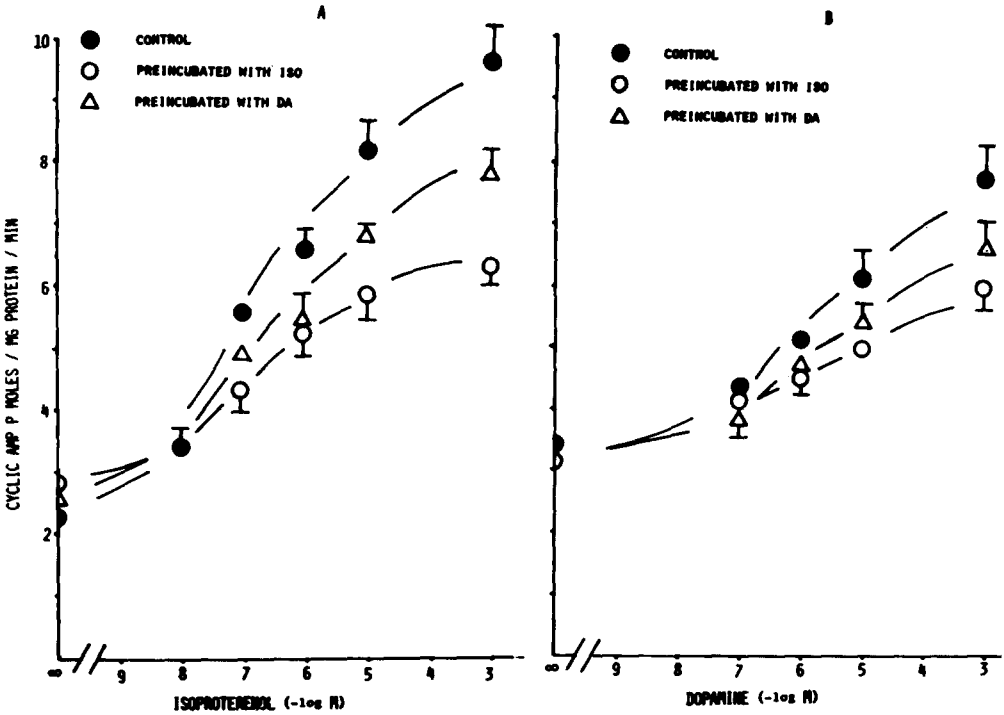


Fig. 4. Effect of preincubation of catecholamines on adenylate cyclase activity. Isoproterenol (A) and dopamine (B) stimulation of adenylate cyclase in prostatic membranes from tissue preincubated with (open circles, $10^{-4}$ M isoproterenol; triangles, $10^{-4}$ M dopamine) or without (closed circles) catecholamines.

than seen in the pretreatment with isoproterenol. Decrements in adenylate cyclase activity induced by prior exposure to both agonists were minimal before 15 min and nearly complete at 30 min without any change in fluoride-stimulated activity.

## Discussion

Stimulatory effects of catecholamines on cyclic AMP accumulation and adenylate cyclase system in rat prostate confirmed the adrenergic innervation of male sex accessory organs [20]. The observed order of potency for beta-hydroxylated catecholamines in stimulating cyclic AMP accumulation and adenylate cyclase activity in prostate tissue was isoproterenol > epinephrine ≃ salbutamol > norepinephrine, indicating properties characteristic of a typical beta-2-adrenergic receptor-sensitive adenylate cyclase. In comparison, non-beta-hydroxylated catecholamine, dopamine, was much less potent. Dopamine-stimulation, however, was inhibited by a dopaminergic antagonist, haloperidol but not a beta-adrenergic antagonist, propranolol, which competitively blocked isoproterenol-stimulated cyclase activity. These results suggest that prostates exhibit properties characteristic of dopaminergic receptor-coupled adenylate cyclase. Additive effects on activation by combination of isoproterenol, epi-

nephrine and dopamine at their saturable concentrations failed to be observed, suggesting that two distinct receptors for beta-adrenergic and dopaminergic agonists share a common enzyme unit. Prior exposure of the prostate gland to isoproterenol caused a marked inactivation of beta-adrenergic receptor-adenylate cyclase system (Fig. 4). Functional desensitization or 'tolerance' of the beta-adrenergic receptor to catecholamines has been intensively studied [12—15, 22,23]. The properties of desensitization of the prostatic membranes by the beta-adrenergic agonist are similar to those of the frog erythrocyte [13,15], characterized by a decrease in $V$ with no change in $K_a$. The time course was earlier than that required for the frog erythrocyte membranes [12,13,15]. Higher hormonal affinity in the adenylate cyclase activity of the prostate than that of the frog erythrocytes [13,15], might be one of the explanations for difference in time course of desensitization.

It is of interest that desensitization induced by isoproterenol was observed in dopamine-sensitive adenylate cyclase system (Fig. 4). Conversely, refractoriness induced by dopamine occurred on isoproterenol- or dopamine-sensitive system (Fig. 4). Functional inactivation of the beta-adrenergic receptors by their agonist may be involved in some conformational change in receptors occupied by the agonists [13,15,24]. In the prostatic adenylate cyclase system which is coupled to two distinct receptors, the beta-adrenergic and dopaminergic receptors, conformational change in an agonist specific receptors appears to interact with the receptors or receptor-enzyme complex for another agonist, resulting in inactivation of the enzyme. The kinetics of isoproterenol-refractoriness in human astrocytoma cells indicated the existence of two processes: an early process of an agonist-specific desensitization and a late process of a hormone-nonspecific desensitization [25]. The present studies also could not rule out a possibility of an agonist-nonspecific desensitization in the adenylate cyclase system distal to the hormone-receptor interaction. Desensitization of the beta-adrenegic receptors has been characterized by the reduction of the concentration of functional receptors in membranes [12—15,22—24]. Although direct identification of the receptors by radioligand-binding techniques was not made in the present experiments, isoproterenol-desensitization of the prostatic adenylate cyclase system might be involved in decreased number of the beta-adrenergic receptors on the prostatic membranes. It would be of interest to examine the influence of the beta-adrenergic and/or dopaminergic agonist on the population of their own receptors. Studies on effects of both agonists, isoproterenol and dopamine on the receptor binding properties in prostatic membranes are in progress.

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