MOLECULAR PHARMACOLOGY, 27:218–222

Age-Related Alterations in the Catecholamine-Sensitive Adenylate Cyclase System of the Prostate

SUMIO SHIMA, NOBU AKAMATSU, MASANAO HIRAI, AND HIROSHI KOUYAMA

Departments of Biochemistry and Pharmacology and Radioisotope Institute for Basic Medicine, St. Marianna University School of Medicine, 2095 Sugao, Miyamae-ku, Kawasaki, Kanagawa, 213, Japan

Received May 17, 1984; Accepted October 31, 1984

SUMMARY

Considerably reduced responses to stimulation by isoproterenol of adenylate cyclase activity of prostatic membranes were observed in 12- to 18-month-old rats, compared to 3-month-old animals. Plasma testosterone levels were significantly lower in 18-month-old rats, while 12-month-old animals showed levels similar to those present in young ones. A decrease in isoproterenol activation of adenylate cyclase was not associated with a fall in β-adrenergic receptor sites. Guanine triphosphate and 5′-guanylylimidodiphosphate (Gpp(NH)p) were effective in potentiation of isoproterenol activation of adenylate cyclase and altering the affinity of β-adrenergic receptors for the agonist in membranes from young rats but not from the aged. The age-induced refractoriness to isoproterenol or Gpp(NH)p was observed without a significant loss in NaF-stimulated activity. Prior incubation of aged membranes with isoproterenol and GMP restored subsequent stimulation by Gpp(NH)p, presumably due to the clearance of inhibitory GDP tightly bound to the guanine nucleotide regulatory components in aged membranes. These results indicate that the dysfunction in the adenylate cyclase system of old prostates may not be related to a modification in the β-adrenergic receptor *per se*, but to, in part, a defect in the interaction of activating guanine nucleotides with regulatory components of the adenylate cyclase system.

INTRODUCTION

Stimulation of the adenylate cyclase-cyclic AMP system in the rat prostate has been found to occur through a β-adrenoceptive mechanism (1, 2). Previous experiments (3) have demonstrated that castration chronically produces a significant decrease in the number of β-adrenergic receptors associated with a decreased response of adenylate cyclase to β-adrenergic agonists in prostatic membranes.

An age-related decrease in circulating androgen levels (4, 5) seems to produce effects similar to those of castration on the prostate, such as decreases in androgen receptors (6, 7), DNA, RNA, and protein synthesis (8).

The present experiments were designed to investigate effects of aging on the β-adrenergically regulated adenylate cyclase system in rat prostatic membranes.

MATERIALS AND METHODS

Prostatic tissues from young (3-month-old) and aged (12- to 18-month-old) rats of Donryu strains (Nippon Rats Co., Ltd., Tokyo, Japan) were minced and preincubated in Krebs-Ringer bicarbonate buffer solution (pH 7.4) containing 2 mg/ml glucose and 1 mg/ml bovine serum albumin in an atmosphere of 95% O_2/5% CO_2 at 37° for 30 min. For membrane preparation, the tissues were homogenized in 20 mM Tris-HCl buffer, pH 7.4, containing 0.24 M sucrose, 4 mM $MgCl_2$, 1 mM EDTA, and 3.25 mM 2-mercaptoethanol with a Potter-Elvehjem glass homogenizer (10–20 strokes), followed by filtration through two layers of gauze. The homogenates were centrifuged at 600 × *g* for 10 min. The supernatant was centrifuged at 10,000 × *g* for 30 min. The particulate fraction obtained at 10,000 × *g* is referred to as "membrane particles" (1–3).

Adenylate cyclase activity was assayed by incubation of the membranes in 40 mM Tris-HCl buffer (pH 7.4), containing 4 mM $MgCl_2$, 5 mM theophylline, 2 mM ATP, 100 μg/ml pyruvate kinase, and 5 mM phosphoenolpyruvate for 10 min (1, 2). Cyclic AMP formed during incubation was estimated by a competitive binding assay (9) using a protein purified from rabbit skeletal muscle.

Aliquots of the membrane preparation were assayed for [^{3}H]DHA[1] binding as described previously (8, 9). Membranes were incubated with [^{3}H]DHA (1 to 20 nM, 55.6 Ci/nmol, New England Nuclear, Boston, MA) in a buffer (50 mM Tris-HCl, pH 7.4, and 2 mM $MgCl_2$) for 10 min at 37°. Incubations were terminated by dilution with 3 ml of ice-cold incubation buffer, followed by filtration through GF/C glass fiber filters. Specific binding to receptors was defined as the excess over blanks containing 50 μM propranolol.

Protein and DNA concentrations were measured by the method of Lowry *et al.* (10) and Burton (11), respectively.

Testosterone propionate (5 mg/kg) in sesame oil or the vehicle only was injected into old rats subcutaneously, three times per week for 6 months from 12 to 18 months of age. Plasma testosterone was measured by radioimmunoassay using an antibody to testosterone obtained from Sorin, Saluggia, Italy (5).

[1] The abbreviations used are: DHA, dihydroalprenolol; Gpp(NH)p, 5′-guanylylimidodiphosphate.

0026-895X/85/020218-05$02.00/0

The following drugs were generously donated: l-isoproterenol (Nicken Chemicals, Tokyo), l-propranolol (Japan ICI Pharma, Osaka), and cyclic AMP (Daiichi Sheiyaku, Tokyo). Gpp(NH)p and testosterone propionate were obtained from Japan Boehringer-Mannheim, Tokyo, and Sigma Chemicals, St. Louis, MO.

Student's *t* test was used for statistical analysis.

RESULTS

Levels of circulating testosterone were similar from 3 to 12 months of age and decreased significantly in 8-month-old rats (Table 1). Supplementation of testosterone propionate to rats from 12 to 18 months of age maintained increased plasma androgen levels and decreased the weight of their testes (Table 1). Stimulation of prostatic adenylate cyclase activity by isoproterenol was markedly reduced in aged animals (Fig. 1). Injections of testosterone propionate into old rats for 6 months failed to restore isoproterenol-stimulated adenylate cyclase activity (Fig. 1).

TABLE 1

Body, testicular, and prostatic weights, prostatic DNA contents, and plasma testosterone levels in 3-, 12-, and 18-month-old rats

Each result represents the mean ± SE of three to five rats. BW, body weight; TP, testosterone propionate treated for 6 months.

Age	Body weight	Testes weight	Prostate		Testosterone levels
			Weight	DNA	
	g	*mg/100 g BW*	*mg/100 g BW*	*μg/100 g BW*	*ng/ml*
3 months	350 ± 39	726 ± 80	67 ± 13	96 ± 13	3.78 ± 0.09
12 months	538 ± 52	571 ± 67	126 ± 16	181 ± 24	3.25 ± 0.12
18 months	525 ± 83	608 ± 118	166 ± 30	185 ± 33	1.21 ± 0.19
18 months + TP	562 ± 45	365 ± 53	172 ± 19	171 ± 22	14.2 ± 0.32

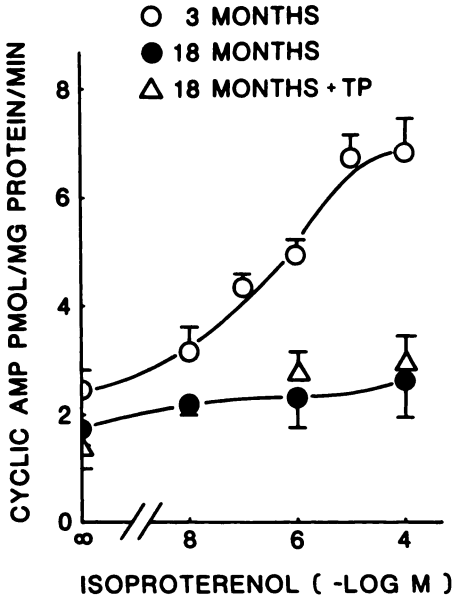

FIG. 1. *Effect of isoproterenol on adenylate cyclase activity in prostatic membranes*

Crude prostatic membranes from young (3-month-old), old (18-month-old), and testosterone-treated old rats were incubated with various concentrations of isoproterenol for 10 min. Results shown are the means ± SE (bars) of four incubations. *TP*, testosterone propionate-treated for 6 months.

Saturation curves of specific binding of [^{3}H]DHA to the membranes were similar in young and old rats, indicating that the density of β-adrenergic receptors in prostatic membranes remains unchanged through the adult life-span of rats (Fig. 2). Fig. 3 shows effects of GTP on isoproterenol competition with radioactive DHA in prostatic membranes from young and aged rats. The affinity of the β-adrenergic receptor of young prostates for isoproterenol was decreased by GTP about 1 order of magnitude. On the other hand, GTP was without effect on binding to membranes from either old or androgen-treated old rats (Fig. 3).

GTP and Gpp(NH)p greatly enhanced activation of adenylate cyclase by isoproterenol in membranes from

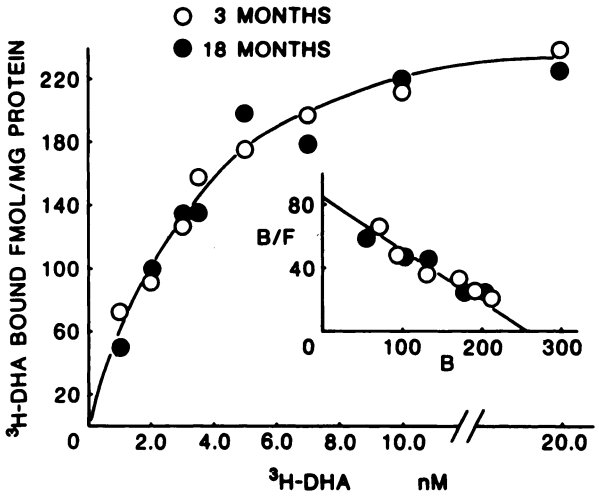

FIG. 2. *Specific [^{3}H]DHA binding and Scatchard analysis in prostatic membranes as a function of the radioligand concentration*

Crude prostatic membranes from young (3-month-old) and old (18-month-old) rats were incubated with 1 to 20 nM [^{3}H]DHA for 10 min. Inset, Scatchard plot of the data. Values shown represent the means of triplicate determinations. The bound/free ratio is calculated as B = femtomoles/mg of protein and F = nanomoles.

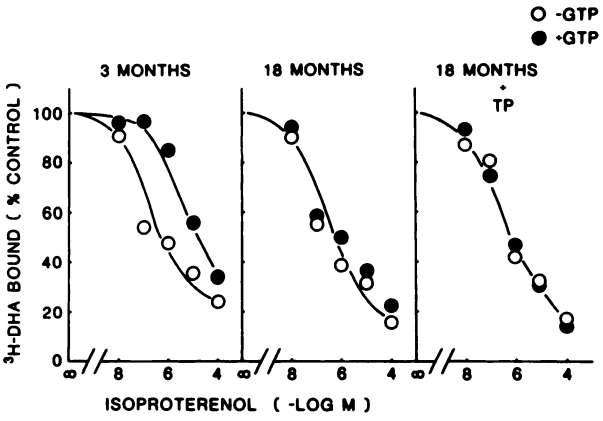

FIG. 3. *Isoproterenol competition for [^{3}H]DHA-binding sites*

Crude prostatic membranes from young (3-month-old), old (18-month-old), and testosterone-treated old rats were incubated with 5 nM [^{3}H]DHA in the presence of the indicated concentrations of isoproterenol with or without 50 μM GTP. Maximum specific binding to membranes from young, old, and testosterone-treated rats in the absence of isoproterenol was taken as 100%. Values shown represent the means of triplicate determinations. *TP*, testosterone propionate-treated for 6 months.

young prostates, but not in prostatic membranes from untreated and androgen-treated old animals (Figs. 4 and 5). The response of prostatic membranes from aged animals to Gpp(NH)p was negligible, contrasting with the marked increment in adenylate cyclase activity by sodium fluoride (Fig. 5 and Table 2).

In view of the fact that turkey erythrocyte membranes, in contrast to other tissue, require preliminary incubation with isoproterenol alone or with GMP before full activation by Gpp(NH)p can occur (12–14), effects of preincubation of membranes from old animals with isoproterenol and GMP on subsequent basal, Gpp(NH)p, and fluoride-dependent adenylate cyclase activity were studied (Table 3). These prior incubation conditions induced a remarkable stimulation by Gpp(NH)p and a decline in stimulation by fluoride (Table 3).

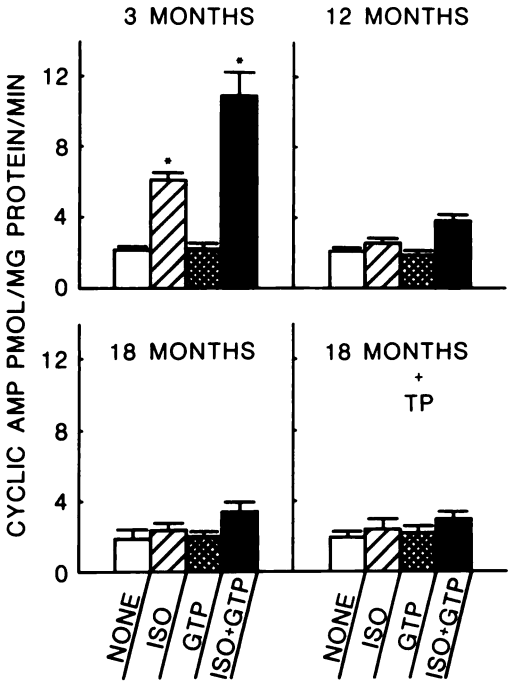

FIG. 4. *Effects of isoproterenol and GTP on adenylate cyclase activity in prostatic membranes*

Crude prostatic membranes from young (3-month-old), middle age (12-month-old), old (18-month-old), and testosterone-treated old rats were incubated with or without 10 μM isoproterenol (*ISO*) and/or 50 μM GTP for 10 min. Values shown represent the means ± SE (bars) of three separate incubations. * Significantly different from no addition ($p < 0.01$). *TP*, testosterone-treated for 6 months.

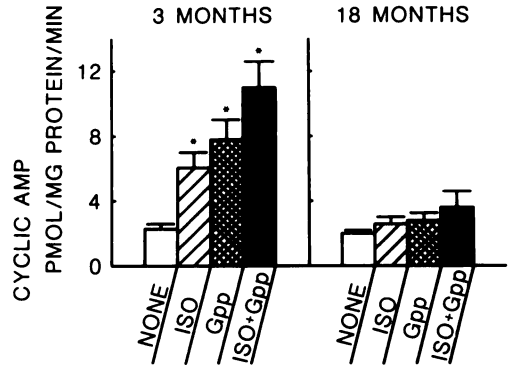

FIG. 5. *Effects of isoproterenol and Gpp(NH)p on adenylate cyclase activity in prostatic membranes*

Crude prostatic membranes from young (3-month-old) and old (18-month-old) rats were incubated with or without 10 μM isoproterenol (*ISO*) and/or 10 μM Gpp(NH)p for 10 min. Values shown represent the mean ± SE (bars) of three separate incubations. *TP*, testosterone propionate-treated for 6 months. *Gpp*, Gpp(NH)p. * Significantly different from no addition ($p < 0.01$).

TABLE 2

Effects of sodium fluoride and Gpp(NH)p on adenylate cyclase activity in prostatic membranes

Crude prostatic membranes were incubated with 10 mM sodium fluoride or 10 μM Gpp(NH)p for 10 min. Values shown represent the means ± SE of four separate incubations. TP, testosterone propionate treated for 6 months.

Age and additions	Cyclic AMP formed		
	None	NaF	Gpp(NH)p
	pmol/mg protein/min		
3 months	2.28 ± 0.24	12.2 ± 1.56[a]	7.33 ± 1.21[a]
18 months	2.01 ± 0.16	11.8 ± 1.44[a]	3.61 ± 0.38
18 months + TP	1.98 ± 0.32	11.0 ± 1.68[a]	2.95 ± 0.57

[a] Significantly different from no addition ($p < 0.01$).

TABLE 3

Effects of pretreatment with isoproterenol and GMP on adenylate cyclase activity of prostatic membranes from old rats

Crude prostatic membranes from 18-month-old rats were preincubated with isoproterenol (50 μM) and GMP (1 mM) in a medium containing 4 mM $MgCl_2$, 3.25 mM 2-mercaptoethanol in 20 mM Tris-HCl buffer (pH 7.4) for 20 min at 37°. Following three washes in the same cold buffer, the membranes were incubated with 10 μM Gpp(NH)p or 10 mM NaF as described under Materials and Methods. Values are means ± SE.

Pretreatment	Additions in assay	Cyclic AMP formed
		pmol/mg protein/min
None	None	3.09 ± 0.22
	Gpp(NH)p (10 μM)	3.91 ± 0.19
	NaF (10 mM)	12.9 ± 0.70[a]
Isoproterenol + GMP	None	2.33 ± 0.15
	Gpp(NH)p (10 μM)	8.99 ± 0.28[a]
	NaF (10 mM)	5.86 ± 0.41[a]

[a] Significantly different from no addition ($p < 0.01$).

DISCUSSION

Activation of prostatic adenylate cyclase by isoproterenol was found to be decreased in aging rats. The decreased activity was observed not only in 18-month-old rats with reduced plasma androgen levels, but also in 12-month-old animals with similar hormone levels to young ones. It is of interest that, in spite of the decreased response of the enzyme to isoproterenol, the density of β-adrenergic receptors in the prostatic membrane from old rats was similar to that in membranes from young rats. This indicates that, unlike complete deprivation of androgens by castration (3), low circulating levels of androgens in old rats are not a crucial factor for refractoriness of prostatic adenylate cyclase to β-adrenergic agonists. The major defects of the old prostate response to catecholamines probably are beyond the receptor site.

The absence of a change in receptor affinity for isoproterenol by addition of GTP and ineffectiveness of GTP to enhance isoproterenol-stimulated adenylate cyclase activity suggests some defects in coupling efficiency of the guanine nucleotide regulatory sites between the receptor and the catalytic moiety in the old membrane. Inability of the cyclase to respond to the agonist with or without GTP was observed in 12-month-old rats, an age when plasma androgens are maintained at levels similar to those in young animals. Furthermore, supplementation of testosterone propionate for 6 months, started from 12 months of age, failed to restore the decreased β-adrenergic response of adenylate cyclase. Addition of GTP was also ineffective in altering the receptor affinity for isoproterenol and in enhancing the agonist-stimulated enzyme activity in prostatic membranes from androgen-treated old rats. These results suggest that a functional lesion on the guanine nucleotide regulatory component is not due to androgen deficiency during senescence.

Reduced responsiveness to adenylate cyclase to catecholamines associated with aging has been reported in the brain (15), liver (16), and lymphocyte (17, 18). A decrease in β-adrenergic receptors as a function of aging also has been demonstrated in several tissues (19–21). The present experiments on the rat prostate showed no change in the number of β-adrenergic receptors and alterations in the concentration or state of the guanine nucleotide-coupling factors associated with senescence. Also, no changes in the β-adrenergic receptor system in regulating exocrine protein secretion has been reported in the rat parotid gland during aging (22). The decreased effectiveness of GTP may reflect a decreased number of guanine nucleotide regulatory sites (24).

In contrast with stimulation by sodium fluoride, an inability of Gpp(NH)p to activate adenylate cyclase in aged membranes is surprising. The guanine nucleotide regulatory protein is thought to be necessary for activation of adenylate cyclase by both fluoride and the guanine nucleotide (13).

Turkey erythrocyte membrane adenylate cyclase is relatively less sensitive to Gpp(NH)p (25, 26). However, in membranes preincubated with isoproterenol and GMP, Gpp(NH)p stimulates the enzyme activity (13, 14). This is apparently due to the replacement of tightly bound GDP by GMP at the guanine nucleotide regulatory site (27, 28). Once bound at the regulatory site, GMP can be readily replaced by Gpp(NH)p to give the persistently active enzyme in the absence of hormones. These mechanisms in turkey erythrocyte membranes seem to be analogous to those in prostatic membranes from aged rats. An inability of Gpp(NH)p to activate adenylate cyclase in aged membranes would not be attributed to a defect in the guanine nucleotide regulatory protein per se, but to an inhibitory GDP tightly bound to the protein during senescence.

Krall *et al.* (18) have found elevated plasma catecholamine levels in old men, which in turn might result in an age-dependent deterioration of lymphocyte responsiveness to catecholamines. Refractoriness of prostatic adenylate cyclase system with aging would not reflect on androgen deficiency but might reflect on alteration in sympathetic nervous activity in old rats.

REFERENCES

1. Shima, S., Y. Kawashima, M. Hirai, M. Asakura, and H. Kouyama. Effect of adrenergic stimulation on adenylate cyclase activity in rat prostate. *Biochim. Biophys. Acta* **628:** 255–262 (1980).
2. Shima, S., M. Hirai, J. Asakura, and H. Kouyama. Regulation of the adenylate cyclase system by catecholamines in the rat prostate. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **312:**19–22 (1980).
3. Shima, S., Y. Kawashima, M. Hirai, M. Asakura, and H. Kouyama. Effects of androgens on isoproterenol-sensitive adenylate cyclase system of the prostate. *Mol. Pharmacol.* **18:**45–48 (1980).
4. Harman, S. J., R. L. Danner, and G. S. Roth. Testosterone secretion in the rat in response to human chorionic gonadotropins: alterations with age. *Endocrinology* **102:**540–544 (1978).
5. Lin, T., E. Murono, J. Osterman, D. O. Allen, and H. R. Nankin. The aging Leydig cell. 1. Testosterone and adenosine 3′,5′-monophosphate responses to gonadotropin stimulation in rat. *Steroids* **35:**653–663 (1980).
6. Shain, S. A., and R. W. Boesel. Aging-associated diminished rat prostate androgen receptor content concurrent with decreased androgen dependence. *Mech. Aging Dev.* **6:**219–232 (1977).
7. Shain, S. A., and W. M. Nitchuk. Testosterone metabolism by the prostate of the aging AXC rat. *Mech. Aging Dev.* **11:**9–22 (1979).
8. Boesel, R. W., R. W. Klipper, and S. A. Shain. Androgen regulation of androgen receptor content and distribution in the ventral and dorsalateral prostate of aging AXC rats. *Steroids* **35:**157–177 (1980).
9. Gilman, A. G. A protein binding assay for cyclic AMP. *Proc. Natl. Acad. Sci. U. S. A.* **67:**305–312 (1970).
10. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:**265–275 (1951).
11. Burton, K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62:**315–323 (1956).
12. Lad, P. M., T. B. Neilsen, M. S. Preston, and M. Rodbell. The role of the guanine nucleotides exchange reaction in the regulation of the β-adrenergic receptor in the actions of catecholamine and cholera toxin on adenylate cyclase in turkey erythrocyte membranes. *J. Biol. Chem.* **255:**988–995 (1980).
13. Downs, R. W., A. M. Spiegel, M. Singer, S. Reen, and G. D. Aurbach. Fluoride stimulation of adenylate cyclase is dependent on the guanine nucleotide regulatory protein. *J. Biol. Chem.* **255:**949–954 (1980).
14. Morris, S. A., and J. P. Bilizikian. Evidence that forskoline activates turkey erythrocyte adenylate cyclase through a noncatalytic site. *Arch. Biochem. Biophys.* **220:**628–636 (1983).
15. Schmidt, M. J., and J. F. Thornberry. Cyclic AMP and cyclic GMP accumulation in vitro in brain regions of young, old and aged rats. *Brain Res.* **139:**169–177 (1978).
16. Bitensky, M. W., V. Russell, and M. Blanco. Independent variation of glucagon and epinephrine responsive components of hepatic adenyl cyclase as a function of age, sex and steroid hormones. *Endocrinology* **86:**154–159 (1970).
17. Krall, J. F., M. Connelly, and M. L. Tuck. Evidence for reversability of age-related decrease in human lymphocyte adenylate cyclase activity. *Biochem. Biophys. Res. Commun.* **99:**1028–1034 (1981).
18. Krall, J. F., M. Connelly, R. Weisbart, and M. L. Tuck. Age-related elevation of plasma catecholamine concentrations and reduced responsiveness of lymphocyte adenylate cyclase activity. *J. Clin. Endocrinol. Metab.* **52:**863–867 (1981).
19. Lakatta, E. G., G. Gerstenblith, C. S. Angell, N. W. Shock, and M. L. Weisfeldt. Diminished inotropic response of aged myocardium to catecholamines. *Circ. Res.* **36:**262–269 (1975).
20. Shocken, D. D., and G. S. Roth. Reduced β-adrenergic receptor concentrations in aging man. *Nature* **267:**856–858 (1977).
21. Greenberg, L. H., and B. Weiss. β-Adrenergic receptors in aged rat brain: reduced number and capacity of pineal gland to develop supersensitivity. *Science* **201:**61–63 (1978).
22. Ito, H., B. J. Baum, and G. S. Roth. β-Regulation of rat parotid gland exocrine protein secretion during aging. *Mech. Aging Dev.* **15:**177–188 (1981).
23. Limbird, L. E., D. M. Gill, and A. R. Lefkowitz. Loss of β-adrenergic receptor-guanine nucleotide regulatory protein interactions accompanies decline in catecholamine responsiveness of adenylate cyclase in maturing rat erythrocytes. *J. Biol. Chem.* **255:**1854–1861 (1980).
24. Limbird, L. E., D. M. Gill, and R. J. Lefkowitz. Agonist-promoted coupling of the beta-adrenergic receptor with the guanine nucleotide regulatory protein of the adenylate cyclase system. *Proc. Natl. Acad. Sci. U. S. A.* **77:**775–779 (1980).
25. Spiegel, A. M., and G. D. Aurbach. Binding of 5′-guanylylimidodiphosphate

to turkey erythrocyte membranes and effects on β-adrenergic-activated adenylate cyclase. *J. Biol. Chem.* **249**:7630–7636 (1974).
26. Sevilla, N., M. L. Steer, and A. Levitzki. Synergistic activation of adenylate cyclase by Gpp(NH)p and epinephrine. *Biochemistry* **15**:3493–3499 (1976).
27. Cassel, D., Z. Selinger. Mechanism of adenylate cyclase activation through the β-adrenergic receptor: catecholamine-induced displacement of bound GDP by GTP. *Proc. Natl. Acad. Sci. U. S. A.* **75**:4155–4159 (1978).
28. Pike, L. J., and R. J. Lefkowitz. Correlation of β-adrenergic receptor-stimulated [^{3}H]GDP release and adenylate cyclase activation. *J. Biol. Chem.* **256**:2207–2212 (1981).

Send reprint requests to: Sumio Shima, Department of Biochemistry, St. Marianna University Schol of Medicine, Miyamae-ku, Kawasaki, Kanagawa 213, Japan.