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DIFFERENT EFFECTS OF VARIOUS BETA-ADRENOCEPTOR ANTAGONISTS ON ADENYLATE CYCLASE, GUANYLATE CYCLASE AND CALMODULIN-DEPENDENT PHOSPHODIESTERASE IN HEART

SHUZO KUDO and YOSHINORI NOZAWA
Department of Biochemistry, Gifu University School of Medicine, Tsukasamachi 40, Gifu, Japan

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Abstract—A series of six β-adrenergic blocking drugs including propranolol, bufetolol, bunitrolol, pindolol, labetalol and acebutolol were examined for effects on adenylate cyclase, guanylate cyclase and calmodulin-dependent phosphodiesterase from heart. The adrenergic blocking agents had no apparent effects on basal activities of adenylate cyclase, guanylate cyclase and phosphodiesterase. The drugs blocked the enhancement of adenylate cyclase activity by isoproterenol, but not by guanine nucleotide or fluoride. The inhibitory effects of β-antagonists were overcome by sufficiently large doses of isoproterenol. Sodium azide specifically required catalase whereas $NaNO_2$ required cysteine to activate myocardial guanylate cyclase. Among β-adrenergic blocking drugs tested, both pindolol and acebutolol inhibited the stimulation of guanylate cyclase by $NaNO_2$ or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). However, other β-blocking drugs did not significantly affect the activation by NaN_3, $NaNO_2$ and MNNG. Several β-antagonists, such as labetalol, bunitrolol, pindolol and acebutolol were also effective in blocking the activation of phosphodiesterase by calmodulin. The inhibitory effects of β-adrenergic blocking drugs, i.e. pindolol and acebutolol upon either nitroso compound-stimulated guanylate cyclase or calmodulin-activated phosphodiesterase display little correlation with their potency as β-adrenergic blocking agents. These data suggest that β-antagonists may have another site of action which is not directly related to the control of catecholamine metabolism.

Beta-adrenergic blocking agents have received major attention in the last decade because of their utility in the treatment of patients with hypertension, angina pectoris and cardiac arrythmia. Propranolol was the first β-adrenergic antagonist to come into wide clinical use and remains to be one of the most important agents. Other numerous β-adrenergic blockers have been developed but they differ from propranolol in their relative effects on cardiac and bronchial β-receptors. In the last several years, many new β-adrenergic receptor blocking agents have been developed and they may be divided into at least three subgroups on the basis of their specificity in blocking the receptors. The first group consists of agents that block β-receptors in all tissues with little or no specificity toward β_1 or β_2 receptors. Included in this group are propranolol, pindolol, bufetrolol, bunitrolol and labetalol. The second group consists of agents that block β_1 receptors more effectively than β_2 receptors, with a representative drug being acebutolol. The third group includes agents that block β_2 receptors more effectively than β_1 receptors, e.g. butoxamine. Some of these drugs also have direct actions on cell membranes, which are commonly described as local anesthetic and quinidine-like agents, but the molecular mechanism(s) of their pharmacological actions has not as yet been clarified [1].

Several lines of evidence suggest that cyclic nucleotides influence the function of heart, and indeed certain abnormalities of the heart functions can be modified by agents that alter the metabolism of cyclic nucleotides [2–4]. However, little information is available regarding the specific site(s) in and the mechanism(s) by which these nucleotides exert biochemical actions.

Accordingly, the present study was designed to examine the possible interaction between β-antagonist and cyclic nucleotide metabolism in the heart. The β-blocking activities of the drugs were monitored by the potency to inhibit the isoproterenol-stimulated adenylate cyclase. The effect of β-adrenergic drugs on cyclic GMP* metabolism were determined by changes in activities of the enzymes responsible for the biosynthesis (guanylate cyclase) and hydrolysis of cyclic GMP (phosphodiesterase).

MATERIALS AND METHODS

Materials. [2-^{3}H]ATP, [8-^{3}H]GTP and [8-^{3}H]-cyclic GMP were purchased from the Radiochemical Centre (Amersham, U.K.). Radioactive cyclic GMP was purified by column chromatography on Dowex 1-X2 resin before use. All unlabeled nucleotides, bovine liver catalase (−)isoproterenol and 5'-nucleosidase (*Crotalus atrox* venom) were obtained from Sigma Chemical Co. (St. Louis, MO). Creatine phosphate, creatine kinase and 5'-guanylyl-imidodiphosphate (GppNHp) were purchased from Boehringer Mannheim (F.R.G.). 3-Isobutyl-1-

* Abbreviations used: EGTA, ethylene glycol-bis (β-aminoethyl ether)-*N*,*N*'-tetraacetic acid; cyclic AMP, adenosine 3',5'-monophosphate; cyclic GMP, guanosine 3',5'-monophosphate.

methylxanthine was obtained from Aldrich Chemical Co. (Milwaukee, WI). Neutral alumium oxide was a product of Woelm Pharma (F.R.G.). Sodium azide, sodium nitrate and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) were from Nakarai Chemicals Co. (Kyoto, Japan).

Drugs were obtained from the following suppliers: propranolol hydrochloride from ICI Pharma. Ltd. (Osake, Japan); acebutolol hydrochloride from Chugai Pharmaceutical Co. Ltd. (Tokyo, Japan); pindolol from Sankyo Co. Ltd. (Tokyo, Japan); bunitrolol hydrochloride from Boehringer Ingelheim (Hyogo, Japan); labetalol hydrochloride from Takeda Chemical Industries Ltd. (Tokyo, Japan); bufetolol hydrochloride from Yoshitomi Pharmaceutical Co. (Osaka, Japan).

Propranolol and labetalol were solubilized in methanol, while pindolol was solubilized in acetic acid at a final concentration of 0.1% (v/v). This concentration of the solvent had no measurable effect on basal and stimulated activities of adenylate cyclase, guanylate cyclase and phosphodiesterase.

Other chemicals were obtained from commercial sources.

Preparation of adenylate cyclase. Adenylate cyclase was prepared from rat heart by the method of Tse *et al.* [5] with slight modification. Rats were killed by cervical dislocation and the heart was rapidly removed. The heart ventricles were dissected free of atria and washed with cold homogenizing buffer (0.25 M sucrose, 0.5 mM EDTA and 2 mM DTT in 10 mM Tris–HCl, pH 7.4). They were then minced with surgical scissors and homogenized with a polytron (Brickman) at a speed setting of 5 for 10 sec in 10 vol. of cold homogenizing buffer. The homogenate was filtered through two layers of cheese cloth and centrifuged at 8500 *g* for 20 min. The resulting pellet was washed by resuspension in homogenizing buffer and recentrifugation. The washed pellet was resuspended in the buffer and used as the enzyme preparation. The enzyme preparations were stored in small aliquots at −80°. Just prior to use, aliquots were thawed and diluted in the assay buffer to the appropriate concentration for linear assay conditions.

Preparation of guanylate cyclase. Guanylate cyclase was partially purified from pig myocardial soluble fraction by the method of Gruetter *et al.* [6]. Pig hearts were obtained from a local slaughterhouse. The ventricular muscle was cut into large pieces and homogenized with a prechilled Waring blender in 10 vol. of homogenizing buffer, and then rehomogenized with a polytron (Brickman) at speed setting of 5 for 10 sec in 10 vol. of cold homogenizing buffer. This homogenate was strained through two layers of cheese cloth and then centrifuged at 12,000 *g* for 20 min. The resultant supernatant fluid was filtered through a thin layer of glass wool and applied to a DEAE 52 cellulose column (2 × 20 cm) previously equilibrated with homogenizing buffer. The column was first eluted with five bed volume of homogenizing buffer and then with linear salt gradient 0–0.4 M NaCl in a flow rate 16 ml/hr (5 ml fraction). Fractions containing guanylate cyclase activity were pooled. To approximately 350 ml of the fraction, solid ammonium sulfate was added with constant stirring to give 40% saturation. The resulting precipitate was collected with centrifugation at 12,000 *g* for 20 min, suspended in 30 ml of a cold homogenizing buffer, and dialysed for 24 hr at 4° against 10 l. of homogenizing buffer. The dialysate was centrifuged at 12,000 *g* for 20 min and its clear supernatant fraction was stored at −80° or −20° for guanylate cyclase assay.

Preparation of calmodulin-dependent phosphodiesterase. Calmodulin-dependent phosphodiesterase was prepared from bovine heart by the method of Teo and Wang [7].

Preparation of calmodulin. Calmodulin was prepared from bovine brain as previously described [8].

Assays of adenylate and guanylate cyclase. Adenylate and guanylate cyclase activities were measured by the method of Nakazawa *et al.* [9]. Unless otherwise indicated, the standard assay mixture for adenylate cyclase contained 0.5 mM [^{3}H] ATP (4 Ci/mole), 0.8 mM cyclic AMP, 15 mM creatine phosphate, 20 μg cretine kinase, 1 mM dithiothreitol, 1.5 mM isobutylmethylxanthine, 30 mM $MgCl_2$, 0.2 mM EGTA, 25 mM Tris–HCl (pH 7.4) and 300 μg of enzyme protein in a total volume of 0.2 ml.

For the guanylate cyclase assay, the same assay conditions for adenylate cyclase were employed, except that 0.5 mM [^{3}H] GTP (5 Ci/mole), 1 mM cyclic GMP, 2 mM $MnCl_2$ and 100 μg of enzyme protein were substituted for ATP, cyclic AMP, $MgCl_2$ and 300 μg of enzyme protein, respectively.

After the assay mixture was incubated at 37° for 20 min, the reaction was terminated by heating for 2 min in a boiling bath, followed by the addition of 1 N HCl (40 μl). The radioactive cyclic AMP or cyclic GMP was isolated by the serial use of neutral aluminium oxide-Dowex 1-X2 column and the radioactivity was determined as described elsewhere [10].

Under the assay conditions employed, the rate of cyclic AMP (or cyclic GMP) production was directly proportional to time for 30 min at 37° and protein concentrations up to 400 μg per assay.

Assay of phosphodiesterase. The phosphodiesterase activity was assayed by the method of Thompson and Appleman [11] with slight modification [12]. Unless otherwise specified, the reaction mixture contained, in a total of 0.4 ml, 1 μM [^{3}H] cyclic GMP, 25 mM Tris–HCl (pH 7.4), 1 mM dithiothreitol, 5 mM $MgCl_2$, 50 μM $CaCl_2$ and 30 μg of enzyme protein. The reaction mixture was incubated at 30° for 10 min, and the reaction was stopped by being placed for 2 min in a boiling water bath. Subsequently, 0.1 ml of snake venom (1 mg/ml) from *Crotalus atrox*, was added to the reaction mixture, which was then incubated at 30° for the additional 10 min. Radioactive guanosine was isolated by the use of Dowex 50W column and the radioactivity was determined. The rate of cyclic GMP hydrolysis was found to be linear with respect to time of incubation (up to 30 min) and protein concentration (up to 200 μg).

Values reported are means of duplicate or triplicate determinations of representative experiments. The concentration at which 50% inhibition of the enzyme activity (IC_{50}) was determined by plotting percent inhibition vs the logarithm of inhibitor concentration (10^{-7}–10^{-3} M).

Protein determination. Protein was determined by the method of Lowry *et al.* [13], with bovine serum albumin as standard.

RESULTS

Effects of isoproterenol, guanine nucleotide and sodium fluoride on adenylate cyclase of myocardium

Dose–effect curves of adenylate cyclase activation in the presence of isoproterenol, 5'-guanylyl-imidodiphosphate (GppNHp) and sodium fluoride (NaF) are illustrated in Fig. 1. In rat heart, adenylate cyclase was markedly stimulated by GppNHp and NaF. Stimulation by isoproterenol was documented only when GppNHp was present in the incubation medium (Fig. 1). These data demonstrate that guanine nucleotide is required for isoproterenol stimulation of adenylate cyclase in heart.

Inhibitory effects of β-adrenergic antagonists on isoproterenol-activated adenylate cyclase

A β-adrenergic blocking agent, propranolol, completely abolished the stimulatory action of isoproterenol in combination with GppNHp, whereas both the basal and stimulated activities by NaF or GppNHp were hardly affected by the drug (Fig. 2). These findings confirm that isoproterenol-stimulated adenylate cyclase activity in rat heart preparation is affected by β-agonist action. Similar results were obtained in studies using other adrenergic blocking agents such as bufetolol, labetalol, bunitrolol, pindolol and acebutolol. The effects of increasing concentrations of the antagonists on adenylate cyclase activity were tested in the presence of a fixed concentration of GppNHp (10 μM) and isoproterenol (10 μM). The results, given in Table 1, imply that there is a significant decrease in adenylate cyclase activation by isoproterenol with GppNHp. On the other hand, NaF-stimulated or GppNHp-stimulated activity was not altered by the addition of these drugs. The concentration at which 50% inhibition of the isoproterenol-stimulated enzyme activity (IC_{50} values) was determined as described in Methods section and shown in Table 1.

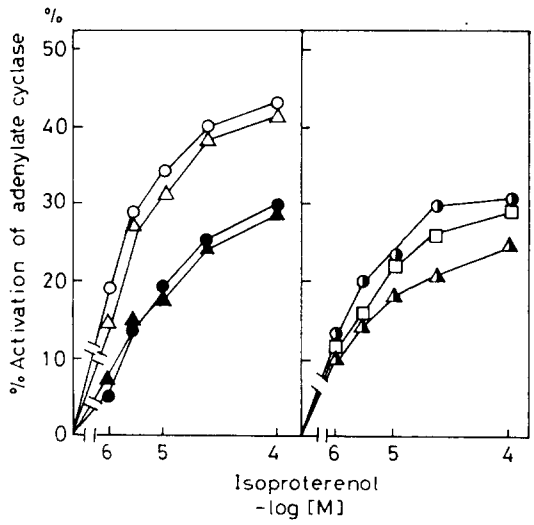

Fig. 1. Dose–effect curves of adenylate cyclase activity by GppNHp, isoproterenol and NaF. Adenylate cyclase activity was assayed in the absence (○) and presence (●) of 10 μM isoproterenol with various concentrations of 5'-guanyl-imidodiphosphate (GppNHp) (left panel); in the absence (○) and presence of 10 μM GppNHp (●) with various concentrations of isoproterenol (middle panel); in the presence of various concentrations of NaF (○) (right panel). Isoproterenol was used at 10 μM and GppNHp at 10 μM. Data are from a representative experiment of three separate experiments.

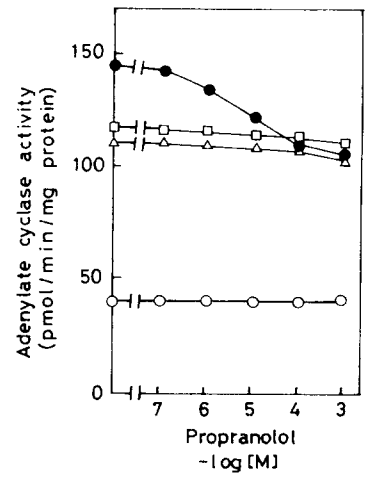

Fig. 2. Effect of propranol on adenylate cyclase. Adenylate cyclase activity was determined at various concentrations of propranolol in the absence (○) and presence of 10 μM isoproterenol plus 10 μM GppNHp (●), 10 μM GppNHp alone (□) or 4 mM NaF (△). Data are from a representative experiment of three separate experiments.

Dose–effect curves of isoproterenol were examined in the absence and presence of 10 μM of each antagonist. In each experiment, the inhibitory effects of β-antagonists were overcome by sufficiently large doses of isoproterenol. Epinephrine, an α- and β-adrenergic agonist, was also observed to reverse adenylate cyclase inhibition by β-antagonist. The potency of isoproterenol to stimulate adenylate cyclase was about 10-fold higher than that of epinephrine in the presence of 10 μM propranolol.

Effects of β-adrenergic blocking drugs on guanylate cyclase activity

Guanylate cyclase was partially purified from pig myocardial soluble fraction which is free of hemoglobulin and myoglobulin, since these hemoproteins are known to inhibit the basal guanylate cyclase activity and to repress the cyclase activation by sodium nitrate and nitric oxide [14].

Sodium azide or $NaNO_2$ had no effect on purified guanylate cyclase from pig heart unless catalase or cysteine was added (Table 2). In the presence of catalase, 1 mM azide increased myocardial guanylate cyclase about 1.3-fold. Catalase alone did not increase cardiac guanylate cyclase activity. In the presence of 5 mM cysteine, guanylate cyclase activity was stimulated about six-fold by 20 mM $NaNO_2$ whereas cysteine alone did not affect the enzyme activity. Guanylate cyclase activity was also stimulated to approximately five-fold by a carcinogenic nitrosamine.

Table 1. Effects of β-adrenergic blocking drugs on adenylate cyclase activity

Additions	(mM)	Adenylate cyclase activity* (pmoles/min/mg protein) None	NaF	GppNHp†	GppNHp + isoproterenol	IC_{50} (μM)
None		37 ± 2	101 ± 14	121 ± 10	147 ± 6	—
Propranolol	0.01	37 ± 2	100 ± 11	121 ± 9	125 ± 5‡	8
	1.0	35 ± 2	99 ± 13	109 ± 8	105 ± 4‡	
Bufetorol	0.01	36 ± 1	105 ± 14	121 ± 10	126 ± 5‡	20
	1.0	38 ± 3	104 ± 14	116 ± 10	119 ± 5‡	
Labetalol	0.01	37 ± 2	103 ± 14	116 ± 10	128 ± 5‡	90
	0.1	35 ± 2	107 ± 13	105 ± 8	100 ± 2‡	
Bunitrolol	0.01	36 ± 2	99 ± 13	109 ± 9	125 ± 5‡	0.8
	0.1	38 ± 2	92 ± 13	111 ± 9	119 ± 5‡	
Pindolol	0.01	37 ± 2	101 ± 14	123 ± 10	126 ± 5‡	60
Acebutolol	0.01	36 ± 2	105 ± 14	116 ± 9	140 ± 5	400
	0.1	36 ± 2	97 ± 13	116 ± 9	125 ± 5‡	

* Adenylate cyclase activity was measured in the absence and presence of NaF (10 μM), GppNHp (10 μM) or isoproterenol (10 μM) plus GppNHp (10 μM). Data are expressed as the mean ± standard deviation in three to four separate experiments.

† GppNHp; 5′-guanyl-imidodiphosphate.

‡ $P < 0.01$.

Table 2. Effects of catalase and cysteine on activation of guanylate cyclase by NaN_3, $NaNO_2$ and MNNG

Additions	(mM)	Guanylate cyclase activity* (pmoles/min/mg protein) None	Catalase	Cysteine
None		97 ± 18	91 ± 13	109 ± 15
NaN_3	3	116 ± 17	160 ± 12‡	116 ± 17
$NaNO_2$	20	108 ± 15	674 ± 32‡	720 ± 32‡
MNNG†	1	582 ± 26‡	88 ± 12	109 ± 14

* Guanylate cyclase activity was measured in the absence and presence of 10 μg of catalase or 5 mM cysteine. Data are expressed as the mean ± standard deviation in three separate experiments.

† MNNG; *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine.

‡ $P < 0.01$.

N-*methyl*-N′-*nitro*-N-*nitroguanidine (MNNG)*

Catalase and cysteine were found to be required for enhancement of guanylate cyclase by NaN_3 and $NaNO_2$, respectively, but they inhibited MNNG-induced activation of this enzyme system. At concentrations where the isoproterenol-induced activation of adenylate cyclase was blocked (10^{-5} or 10^{-3} M), two β-adrenergic blocking drugs, pindolol and acebutolol, inhibited the stimulation of guanylate cyclase by $NaNO_2$ or MNNG. However, other β-blocking drugs did not significantly affect the enzyme activity enhanced by NaN_3, $NaNO_2$ or MNNG (Table 3).

The inhibitory effect was observed when the nitroso compounds, catalase and cysteine were tested at concentrations producing maximal or submaximal

Table 3. Effects of β-antagonists on activation of guanylate cyclase by nitroso compounds

Additions	None	Guanylate cyclase activity* (pmoles/min/mg protein) NaN_3	$NaNO_2$ (IC_{50}) (μM)	MNNG† (IC_{50}) (μM)
None	110 ± 8	167 ± 9	707 ± 50	585 ± 25
Propranolol	118 ± 6	161 ± 6	554 ± 95	589 ± 76
Bufetolol	107 ± 7	157 ± 3	653 ± 23	589 ± 32
Labetalol	111 ± 5	152 ± 10	679 ± 51	556 ± 21
Bunitrolol	109 ± 8	169 ± 20	674 ± 45	548 ± 43
Pindolol	111 ± 12	171 ± 17	232 ± 37‡ (0.3)	448 ± 26‡ (20)
Acebutolol	105 ± 9	141 ± 19	372 ± 3‡ (6.0)	459 ± 25‡ (40)

* Guanylate cyclase activity was assayed with various β-antagonists in the presence and absence of NaN_3 (3 mM), $NaNO_2$ (20 mM) and MNNG (1 mM). All antagonists were at a final concentration of 10 μM. All values represent the mean ± standard deviation for three determinations in three separate experiments.

† MNNG; *N*-methyl-*N*′-nitro-*N*-nitroguanidine.

‡ $P < 0.01$.

Table 4. Effects of β-adrenergic blockers and isoproterenol on calmodulin-dependent phosphodiesterase

Additions	Phosphodiesterase activity* (pmoles/min/mg protein)				
	None	Calmodulin (2 μg)	Calmodulin (4 μg)	Isoproterenol (10 μM)	Isoproterenol + calmodulin (4 μg)
None	242 ± 30	1104 ± 36	1059 ± 34	193 ± 18	1029 ± 20
Propranolol	195 ± 10	1001 ± 52	1043 ± 43	205 ± 11	1030 ± 10
Bufetolol	253 ± 21	995 ± 32	1049 ± 24	213 ± 31	1028 ± 14
Labetalol	210 ± 10	639 ± 31†	1049 ± 42	213 ± 11	906 ± 24†
Bunitrolol	223 ± 32	867 ± 14†	1102 ± 2	212 ± 21	919 ± 20†
Pindolol	231 ± 30	802 ± 63†	910 ± 48[b]	238 ± 31	896 ± 27†
Acebutolol	204 ± 27	786 ± 88†	929 ± 45[b]	224 ± 22	919 ± 28†

* Phosphodiesterase activity was measured in the presence and absence of calmodulin. Concentration of each antagonist was 10 μM. Values are expressed as the mean ± standard deviation in three separate experiments.

† $P < 0.01$.

response. The potency of agents to block the β-receptor did not parallel the ability to inhibit guanylate cyclase activation. In addition, the inhibition of guanylate cyclase activity seemed to be independent of β-adrenergic receptor blockade, since the inhibition was not diminished by isoproterenol and epinephrine. The addition of the two agonists themselves tended to decrease the basal and activated guanylate cyclase activity.

Effects of β-adrenergic agents on phosphodiesterase activity in the presence and absence of calmodulin

Calmodulin-dependent phosphodiesterase was prepared from the soluble fraction of bovine heart. The phosphodiesterase activity can be maximally stimulated by 2 μg of calmodulin to an approximately 4.5-fold increase above its basal activity in the presence of 50 μM $CaCl_2$, when assayed with 1 μM cyclic GMP. All β-adrenergic blockers tested had no demonstrable effect on phosphodiesterase in the absence of calmodulin (Table 4). Propranolol and bufetolol, in concentration up to 10^{-5} M, exerted no detectable effect on the phosphodiesterase activity in the presence of calmodulin, whereas labetalol, bunitrolol, pindolol and acebutolol produced a dose-dependent inhibition of the calmodulin-mediated stimulation of phosphodiesterase. The potency order was labetanolol (K_i; 10^{-5} M) > acebutanolol (2×10^{-4} M) > pindolol (4×10^{-4} M) > bunitrolol (>10^{-3} M) which is not consistent with IC_{50} values for adenylate cyclase.

The inhibition of the calmodulin-activated phosphodiesterase by labetanolol and bunitrolol was reversed by addition of calmodulin. In the presence of 4 μg of calmodulin, a small reduction in maximum was noted in the presence of pindolol and acebutanolol, indicating a noncompetitive component of the β-blockers antagonism (Table 4). Isoproterenol and epinephrine also inhibited the phosphodiesterase activity and did not reverse the inhibited enzyme activity by β-blockades. These results imply that inhibition of calmodulin-stimulated phosphodiesterase by β-blockers is unlikely to result from adrenergic receptor blocking activity.

DISCUSSION

Isoproterenol, guanine nucleotide (GppNHp) and fluoride (NaF) increase adenylate cyclase activity in particulate fractions from rat heart. Isoproterenol-stimulated adenylate cyclase activation was repressed by addition of β-antagonists, whereas there was no change in basal, fluoride- or GppNHp-stimulated activities (Table 1). The effects of β-antagonists were typical for β-receptor-mediated response, because the inhibitory action of β-antagonist on isoproterenol-stimulated adenylate cyclase was overcome by the addition of isoproterenol and epinephrine.

In the experiments using pig cardiac muscle supernatant fraction, we observed that the guanylate cyclase activity was stimulated by NaN_3, $NaNO_2$ and MNNG. Sodium azide and $NaNO_2$ required catalase and cysteine, respectively, for activation of guanylate cyclase partially purified from pig heart. Enzyme activation by MNNG required no further additions (Table 2). These findings are consistent with earlier observations indicating that several nitroso compounds are potent stimulants for guanylate cyclase in heart [18], and that the oxidation of SH groups leading to SH to S—S transformation is involved in enzyme activation [19]. Two β-adrenergic blocking drugs, pindolol and acebutolol inhibited the stimulation of guanylate cyclase by $NaNO_2$ or MNNG. However, other β-blocking drugs failed to repress the cyclase activation by NaN_3, $NaNO_2$ and MNNG. It is possible that pindolol and acebutanolol could exert some protection against cyclic GMP formation induced by nitroso compounds (Table 3). A variety of vasodilators such as nitroglycerin, nitroprusside and $NaNO_2$ elevate the level of cyclic GMP [20]. In addition, the finding that 8-bromo cyclic GMP markedly relaxes smooth muscle suggested that smooth muscle relaxation could involve cyclic GMP [21]. At present stage, however, very little information is available on the mechanism of regulation of cyclic GMP in heart at physiological and pathological conditions.

The present study indicates that β-adrenergic blocking drugs, such as labetolol, bunitrolol, pin-

dolol and acebutolol, are capable of inhibiting calmodulin-induced activation of phosphodiesterase. However, other β-adrenergic blocking drugs tested do not undergo any blockade of phosphodiesterase activation by calmodulin. The inhibition of calmodulin-stimulated phosphodiesterase by β-antagonists was reversed by addition of calmodulin but not isoproterenol and epinephrine. These results raise the possibility that the blockade of the calmodulin-induced stimulation of phosphodiesterase may not be related to the α- and β-blocking activity but rather to the interaction of β-antagonists with calmodulin.

Although β-adrenergic blocking drugs are widely used, relatively little is known of the precise mechanism of their actions. The results obtained from this work indicated the possibility that cyclic GMP and calmodulin would be site(s) of action of the drugs. It is, however, unclear at present whether the inhibition by some β-adrenergic blockers of enzyme activities involving cyclic GMP formation is related to the pharmacological effects of the drugs. The answer must await establishment of roles for cyclic GMP and calmodulin in heart functions.

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