Myocardial $\alpha_1$-Adrenoceptors Mediate Positive Inotropic Effect and Changes in Phosphatidylinositol Metabolism

Species Differences in Receptor Distribution and the Intracellular Coupling Process in Mammalian Ventricular Myocardium

Masao Endoh, Tetsuya Hiramoto, Akira Ishihata, Masahiro Takanashi, and Jun Inui

Species-dependent variations of myocardial $\alpha_1$-adrenoceptor-mediated positive inotropic effects of epinephrine were assessed in relation to characteristics of $\alpha_1$-receptor bindings and acceleration of phosphatidylinositol metabolism in the isolated rat, rabbit, and dog ventricular myocardium. Epinephrine in the presence of the $\beta$-adrenoceptor antagonist bupranolol (10$^{-6}$ M) elicited a positive inotropic effect through activation of $\alpha_1$-adrenoceptors in rat and rabbit, whereas in dog ventricular myocardium, bupranolol abolished the positive inotropic effect of epinephrine. [3H]Prazosin bound to membrane fractions derived from rat, rabbit, and dog ventricular muscle with high affinities in a saturable and reversible manner. In dog, $B_{\text{max}}$ and $K_d$ values of $\alpha_1$-adrenoceptor binding sites were identical to those in rabbit ventricular muscle. The $B_{\text{max}}$ value of $\alpha_1$-adrenoceptors in rat ventricle was the highest, amounting to two to four times those in rabbit and dog. Epinephrine displacement curves for the specific binding of [3H]prazosin in the membrane fraction of these species showed high and low affinity sites with slope factors significantly less than unity, which were shifted to single low affinity sites with slope factors close to unity by addition of 5'-guanylylimidodiphosphate. Accumulation of [3H]inositol 1-phosphate ([3H]IP$_1$) in ventricular slices prelabeled with [3H]myo-inositol was increased by epinephrine in a time- and concentration-dependent manner in rat ventricular slices. [3H]IP$_1$ accumulation likewise was facilitated by $\alpha_1$-adrenoceptor stimulation in rabbit ventricular slices, whereas the extent of [3H]IP$_1$ accumulation was much less than that in rat. In dog ventricular slices, [3H]IP$_1$ was not accumulated by epinephrine. In rabbit papillary muscle, the time course of increase in contractile force induced by $\alpha_1$-adrenoceptors coincided with the prolongation of the action potential duration with a similar time course, which is in strong contrast to previous findings in rat that the contractile response was dissociated from the electrophysiological response to $\alpha_1$-adrenoceptor stimulation. The present results indicate that a wide range of variation of $\alpha_1$-adrenoceptor-mediated regulation of myocardial contractility may be ascribed to different contributions of facilitatory as well as inhibitory regulatory processes that lead to intracellular Ca$^{2+}$ mobilization subsequent to myocardial $\alpha_1$-adrenoceptor activation among mammalian species. (Circulation Research 1991;68:1179–1190)

Endogenous catecholamines play an important role in regulating myocardial pump function in situ. Epinephrine in circulating blood or norepinephrine released by excitation of sympathetic nerves binds specifically to adrenoceptors on the outer surface of myocardial cells and triggers a series of electrophysiological and biochemical processes leading to modulation of a wide range of cardiac functions such as inotropic, lusitropic, and chronotropic changes. Although adrenoceptors involved in these functional regulations are predominantly of the $\beta$-type, evidence has been accumulating that myocar-

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dial α₁-adrenoceptors likewise are involved in modulation of functions in most mammalian cardiac muscle (for reviews, see References 1–4). Subcellular mechanisms subsequent to activation of myocardial α₁-adrenoceptors have been studied extensively in the regulation of myocardial contractility. Stimulation of α-adrenoceptors by sympathomimetic amines results in a positive inotropic action. This action is associated with Ca²⁺ influx through myocardial cell membrane by direct activation of voltage-dependent Ca²⁺ channels⁵,⁶ or through prolongation of action potentials via modulation of K⁺ currents,⁷,⁸ and a resultant elevation of peak intracellular Ca²⁺ transients.⁹ Although it has been shown that neither cyclic AMP¹⁰ nor cyclic GMP¹¹ is involved in the intracellular signal transduction via myocardial α-adrenoceptors, the subcellular process that couples myocardial α-adrenoceptor stimulation to these electrophysiological and contractile responses has long been unclear.

More recently it has been shown clearly that α₁-adrenoceptors promote an acceleration of phosphatidylinositol (PI) metabolism in the cardiac muscle.¹²–¹⁴ Subsequently, these early findings were confirmed and extended to the potential intracellular biochemical relevance for the α-adrenoceptor-mediated increases in Ca²⁺ influx and release,¹⁵–¹⁸ PI metabolism and its functional role mostly have been studied in the rat heart, but myocardial α₁-adrenoceptors show a wide range of variation in eliciting a positive inotropic effect among mammalian species: rat heart responds to α₁-adrenoceptor stimulation with a characteristic biphasic inotropic response,¹⁵,¹⁶ whereas rabbit papillary muscle has a long-lasting monophasic response.¹⁰,¹¹ In guinea pig cardiac muscle, the α₁-adrenoceptor-mediated response is less pronounced¹⁰; in dog heart, β- but not α₁-adrenoceptors appear to mediate the positive inotropic effect of sympathomimetic amines.²⁰,²¹ In human atrial²²,²³ and ventricular myocardium, two main α₁-adrenoceptors are also coupled to the positive inotropic response and acceleration of PI metabolism.

The present experiments were carried out to examine in more detail the relation between the acceleration of PI metabolism and the positive inotropic effect in response to myocardial α₁-adrenoceptors. For this purpose, we chose the ventricular myocardium of three species: the rat, in which the acceleration of PI metabolism by α₁-adrenoceptors has been shown most clearly; the rabbit, which exhibits a prominent positive inotropic effect mediated by α₁-adrenoceptor stimulation; and the dog, in which α₁-adrenoceptors do not seem to be involved in the positive inotropic effect of sympathomimetic amines. We attempted to determine 1) whether acceleration of PI metabolism occurs in parallel with the α₁-adrenoceptor-mediated positive inotropic effect in different mammalian species, and 2) whether the different characteristics of α₁-adrenoceptor binding sites or lack of intracellular coupling processes is responsible for the absence of α₁-adrenoceptor-mediated inotropic effect in dog heart.

Materials and Methods

Radioisogand Binding Assay

The experimental procedures essentially were the same as those described previously.²⁸ Mongrel dogs (7–10 kg), Japanese white rabbits (1.5–2.2 kg), and Wistar rats (180–250 g) were used in these experiments. The animals were anesthetized with pentobarbital (dogs) or diethyl ether (rabbits and rats), and the hearts were rapidly removed. Right and left ventricular free walls were excised separately and minced finely with scissors in ice-cold homogenization buffer (0.25 M sucrose containing 5 mM Tris-HCl and 1 mM MgCl₂, pH 7.4). Muscle preparations were homogenized in 10 volumes of homogenization buffer three times for 15 seconds at setting 7 of a Polytron (Kinematica, Lucerne, Switzerland). The homogenate then was centrifuged at 500g for 15 minutes at 4°C. The supernatant was filtered through a single layer of cheese cloth and centrifuged at 50,000g for 20 minutes at 4°C. The supernatant then was discarded, and the resulting pellets were washed twice with ice-cold incubation buffer (50 mM Tris-HCl, 10 mM MgCl₂, pH 7.5) by resuspension and centrifugation. The final pellet was resuspended in ice-cold incubation buffer, yielding the protein concentration of 1–2 mg/ml.

The binding assay was carried out as follows: [³H]Prazosin was used to characterize the α₁-adrenoceptors, and (+)-[³H]CGP-12177 was used for β-adrenoceptors. The incubation mixture contained 150 µl of membrane suspension, 50 µl of six increasing concentrations of [³H]Prazosin (0.0675–2.0 nM) or [³H]CGP-12177 (0.25–5.0 nM), and 50 µl of incubation buffer with and without adrenoceptor antagonists. Incubation was started by adding the membrane suspension to the assay mixture, was carried out for 20 minutes at 25°C, and was terminated by diluting the assay mixture with 2 ml ice-cold incubation buffer, followed by rapid vacuum filtration over a Whatman GF/C glass filter (Brandel Biochemical Research & Development Labs, Gaithersburg, Md.) by use of an M-24R type cell harvester (Brandel). The filter was washed rapidly twice with 2–3 ml ice-cold incubation buffer, dried for 1 hour at 95°C, and placed on scintillation vials. The radioactivity bound to the membrane was counted in ACS-II (Amersham, Arlington Heights, Ill.) at an efficiency of 50% by means of Tri-Carb 4530 (Packard Instrument Co., Inc., Downers Grove, Ill.).

Displacement experiments of the specific binding of [³H]Prazosin by epinephrine were carried out in the membrane fraction derived from rabbit, rat, and dog ventricular muscle, and the influence of the nonhydrolyzable GTP analogue 5'-guanylylimidodiphosphate (Gpp[NH]p) on the displacement curves was assessed. Adrenoceptor agonists in various concentrations were incubated with membranes and [³H]Prazosin (1.0 nM for rabbit and dog; 200 pM for rat heart membrane fraction) for 20 minutes at 25°C.
Nonspecific binding of ligands was defined as the radioactivity bound to membranes that was not displaced by 10^{-5} M phentolamine for α_{1}-adrenoceptors or by 10^{-6} M propranolol for β-adrenoceptors. Specific binding was defined as total radioactivity minus nonspecific binding. Incubations were prepared in triplicate. Displacement experiments were performed in duplicate. Protein concentrations were determined by the method of Lowry et al.29

**Determination of [%]H]inositol Phosphates**

The hearts were removed quickly from anesthetized dogs, rabbits, and rats and placed in Krebs-Henseleit solution with 0.057 mM ascorbic acid and 0.027 mM EDTA disodium. The solution was bubbled with 95% O_{2}-5% CO_{2} at 37°C; pH was 7.4. The composition of the solution was (mM) Na^{+} 142.9, K^{+} 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, H_{2}PO_{4}{}^{-} 1.2, HCO_{3}{}^{-} 24.9, SO_{4}{}^{2-} 1.2, Cl^{-} 127.8, and glucose 11.1. Right ventricular tissue slices (0.5 mm thick) were incubated with a tissue slicer (Arthur H. Thomas Co., Philadelphia) and equilibrated in Krebs-Henseleit solution for 30 minutes. After equilibration, the slices were preincubated with 24 μCi myo-[2-3H]inositol (specific activity, 18.7 Ci/mmol) (Amersham) in 4 ml Krebs-Henseleit solution for 60 minutes followed by 30 minutes of incubation in normal solution. The solution then was changed to fresh solution containing 5 mM myo-inositol and 10 mM LiCl, and all the experiments were performed in this Li^{+}-containing solution. Bupranolol (10^{-6} M) was added to the solutions 20 minutes before agonist administration to avoid any interference induced by β-adrenoceptor activation.

At the end of the incubation, slices were blotted quickly and put into 0.5 ml of 5% trichloroacetic acid to stop the reaction. The tissue was homogenized in 5% trichloroacetic acid, and homogenizer was washed by the same volume of 5% trichloroacetic acid. Then the homogenate was centrifuged at 400g for 5 minutes, the supernatant was transferred to a tube, and trichloroacetic acid was removed by several ether washings. Samples of the aqueous phase obtained as described above that contained water-soluble inositol phosphates were diluted to 5 ml and applied to a column containing anion-exchange resin (Bio-Rad AG1-X8, 100–200 mesh, formate form, Richmond, Calif.). Then [%]H]inositol phosphates were eluted by stepwise addition of increasing concentrations of formate as described by Berridge et al.30,31 Aliquots of the eluate were counted for radioactivity by Tri-Carb 4530. Although [%]H]inositol 1-phosphate (IP_{1}), inositol 1,4-bisphosphate (IP_{2}), and inositol 1,4,5-trisphosphate (IP_{3}) were collected separately and counted, the low radioactivity of the latter two products prevented detection of reliable and reproducible α_{1}-adrenoceptor-mediated changes. Therefore, we used [%]H]IP as an indicator of PI turnover acceleration. The pellet of homogenates was dissolved in 0.1N NaOH and neutralized by addition of 0.1N HCl, and its protein concentration was determined by the method of Lowry et al.29

**Measurement of Inotropic Responses**

Papillary muscles from rats and rabbits and ventricular trabeculae from dogs were excised from the right ventricles and mounted in 20-ml organ baths containing the same Krebs-Henseleit solution, except that in rat papillary muscles, the extracellular Ca^{2+} concentration was reduced to 1.25 mM.

Isolated muscle preparations were stimulated electrically by square wave pulses of 5-msec duration and voltage approximately 20% above threshold at a rate of 1 Hz in rats and rabbits and 0.5 Hz in dogs. Muscles were stretched to a length to give the developed tension of 90% of the maximum during the equilibration period of 60 minutes. Muscle tension was recorded on a thermal pen writing oscillograph (Recti-Horiz-8K, San-ei Instrument, Tokyo) or an ink writing oscillograph (WTR-331, Graphtec, Tokyo) by means of strain-gauge transducers (Shinkoh UL 10 GR, Minebea, Tokyo). Bupranolol (10^{-5} M) was present for more than 20 minutes before the administration of epinephrine and throughout the experiments. The concentration–response curve for epinephrine was determined by cumulative administration of the drug dissolved in 0.1 ml of 0.9% NaCl. When the inotropic responses reached a steady level (usually after 5–15 minutes), the next higher concentration was added.

**Measurement of Electrophysiological Responses**

Rabbits of either sex (1.7–2.5 kg) were used in these experiments. Papillary muscles were excised from the right ventricle and were mounted in a recording chamber that was perfused at a constant rate of 10 ml/min with Tyrode’s solution of the following composition (mM): Na^{+} 149.4, K^{+} 2.2, Mg^{2+} 1.1, Ca^{2+} 1.8, H_{2}PO_{4}{}^{-} 0.4, HCO_{3}{}^{-} 12.0, Cl^{-} 145.0, and glucose 5.5. The solution was bubbled with 95% O_{2}-5% CO_{2} and maintained at 37.0±0.1°C. The preparation was driven electrically by rectangular pulses (frequency, 0.5 Hz; duration, 5 msec; current, twice threshold) under the resting tension of 5 mN. Membrane potentials were measured with 3 M KCl-filled microelectrodes (resistance, 20–30 MΩ). Potentials were led to an amplifier with high input resistance and negative capacitance adjustment (model 101102, Philbrick/Nexus Research, Dedham, Mass.). Developed tension was measured by means of strain-gauge transducers. Action potential and contraction were monitored on an oscilloscope (model 5115, Tektronix, Beaverton, Ore.) and photographed. The maximum rate of rise of the action potential (V_{max}) was measured with an electric differentiator. Action potential duration was measured at 50% and 90% repolarization in the case of off-line monitoring or at −40 and −70 mV in the case of continuous monitoring with a comparator, digital counter (model 5300A-5302A, Suginami, Tokyo) and digital-to-analog converter (model DAC 80-CCD, Burr-Brown Research Corp., Tucson, Ariz.). Effects of phenylephrine (10^{-7} to 10^{-5} M) were observed 15 minutes after perfusion of the drug.
solution when the response reached a steady level. All experiments in this series were carried out in the presence of pindolol (10^{-7} \text{ M}).

**Assessment of Membrane Marker Enzymes**

Because the size of the hearts from the three species used in the present experiments differed considerably from each other, we tried to confirm that application of the same purification procedure would be equally effective in each species. To check this, we determined Na\(^+\),K\(^+\)-ATPase\(^{32}\) and 5'-nucleotidase activities\(^{33}\) of the homogenates and purified membrane fractions. Homogenates or membrane fractions were dissolved in 0.25 M sucrose with 10 mM DL-histidine (6–8 mg protein/ml). The samples were incubated with alamethicin, 1.2 mg/mg protein, for 20 minutes at 37°C before the assay. The difference of activities in the absence or presence of 10 mM ouabain was taken as the Na\(^+\),K\(^+\)-ATPase activity.

**Data Analysis and Statistics**

In the radioligand binding assay, B\(_{\text{max}}\) and the equilibrium dissociation constant (K\(_{\text{d}}\)) were determined from a Scatchard plot. Analysis of the curve for the epinephrine-induced displacement of \([\text{\textsuperscript{3}H}]\text{prazosin}\) by nonlinear curve-fitting was performed by use of the Ligand program. Experimental values were presented as mean±SEM. For multiple comparisons, two- or three-way analysis of variance including Scheffé’s test for variables was applied. The slope of the regression line was calculated by least-squares methods. Differences between two groups were considered to be significant when the F value from the analysis of variance indicated a significant difference among groups at the level of p<0.05.

**Drugs**

Drugs were obtained from the following sources: (–)-epinephrine bitartrate, (–)-phénylèphrine HCl, (±)-propranolol HCl, phenotamine HCl, myo-inositol, lithium chloride, and Gpp(NH)p were from Sigma Chemical Co., St. Louis; diethyl ether, trichloroacetic acid, and ammonium formate were from Wako, Tokyo; prazosin HCl was from Pfizer Taito, Tokyo; (±)-buprano

**Results**

**Binding Characteristics of \(\alpha\) and \(\beta\)-Adrenoceptors in the Ventricular Myocardium**

Before the receptor binding assay, the extent of the enrichment of membrane fractions obtained with the purification procedure was assessed by Na\(^+\),K\(^+\)-ATPase and 5'-nucleotidase activities (Table 1). 5'-Nucleotidase activity gave a more consistent purification ratio than Na\(^+\),K\(^+\)-ATPase (3.4–3.6 and 2–4, respectively) in the three species. There was no difference in the extent of purification among the membrane fractions prepared from the ventricular muscles from the three species.

Figure 1 shows a typical experiment for binding of \([\text{\textsuperscript{3}H}]\text{prazosin}\) to the membrane fraction derived from the dog right ventricular muscle. The binding of \([\text{\textsuperscript{3}H}]\text{prazosin}\) was saturable, with an apparent single class of binding sites by Scatchard analysis. Similar binding characteristics were obtained in membrane fractions from rabbit and rat ventricles. The B\(_{\text{max}}\) and K\(_{\text{d}}\) values for \(\alpha\)- and \(\beta\)-adrenoceptor binding in individual membrane preparations from the three species are summarized in Table 2. The B\(_{\text{max}}\) values of \(\alpha\)-adrenoceptors in dog and rabbit were identical, whereas the value in rat was higher than those in rabbit and dog (p<0.05) (Table 2). In contrast, the B\(_{\text{max}}\) value of \(\beta\)-adrenoceptors in rat was lower than the values in rabbit and dog (p<0.05) (Table 2). Thus, the ratio of \(\alpha\)-receptors to \(\beta\)-receptors in rat was more than five times higher than the ratio in rabbit and dog.

Characteristics of \(\alpha\)-adrenoceptors in the membrane fractions of these species were analyzed further by the displacement experiments, and the results are presented in Table 3. In the membrane fraction derived from rat heart, epinephrine displaced the \([\text{\textsuperscript{3}H}]\text{prazosin}\) specific binding (200 pM) in a concentration-dependent manner with two different K\(_{\text{i}}\) values: the percentage of the high affinity binding site was 10.42\% (K\(_{\text{hi}}\)=63.0 nM), and that of the low affinity binding site was 89.58\% (K\(_{\text{lo}}\)=3.5 \text{ \mu M}). In the presence of 1 mM Gpp(NH)p, the curve showed the binding with a single low affinity (K\(_{\text{lo}}\)=3.8 \text{ \mu M}). The slope factor for the epinephrine-induced displacement curve was increased to 1.54 by addition of Gpp(NH)p from the control value of less than unity (0.692). In the membrane fraction derived from rabbit heart, epinephrine similarly displaced the \([\text{\textsuperscript{3}H}]\text{prazosin}\) specific binding (1.0 nM) with two different K\(_{\text{i}}\) values: the percentage of the high affinity binding site was 28.46\% (K\(_{\text{hi}}\)=256.7 nM), and that of the low affinity binding site was 71.54\% (K\(_{\text{lo}}\)=5.5 \text{ \mu M}). In the presence of 1 mM Gpp(NH)p, the curve showed the binding with a single low affinity (K\(_{\text{lo}}\)=8.8 \text{ \mu M}). The slope factor for the epinephrine-induced displacement curve in rabbit was increased to 0.921 by addition of Gpp(NH)p from the control value of 0.614. In the membrane fraction derived from dog heart, epinephrine again displaced the \([\text{\textsuperscript{3}H}]\text{prazosin}\) specific binding (1.0 nM) with two different K\(_{\text{i}}\) values. The characteristics of displacement and the shift of the curve to the low affinity site and of the slope factor induced by addition of Gpp(NH)p were similar to those in the rabbit membrane fraction (Table 3).
Concentration–Response Relation for the 
$\alpha_1$-Adrenoceptor–Mediated Accumulation of IP$_1$ and 
Positive Inotropic Effect

Accumulation of IP$_1$ in the presence of Li$^+$ reflects the $\alpha_1$-adrenoceptor–mediated phosphoinositide breakdown. The concentration–response curve for the effect of epinephrine by $\alpha_1$-adrenoceptor stimulation on the formation of [H]IP$_1$ was determined in these three species (Figure 2). Ventricular slices were incubated with epinephrine in the presence of $10^{-6}$ M bupranolol and 10 mM Li$^+$ for 30 minutes. A concentration-dependent increase of [H]IP$_1$ was observed in rat ventricular slices but not in dog. In rabbit ventricular slices, [H]IP$_1$ accumulated with epinephrine at concentrations of 10$^{-6}$ M and higher, whereas the extent of [H]IP$_1$ accumulation was much less than that in rat ventricular slices. These concentration–response curves for epinephrine in the three species were significantly different from each other ($p<0.05$). Prazosin (10$^{-5}$ M) abolished the accumulation of [H]IP$_1$ produced by epinephrine at concentrations lower than 10$^{-4}$ M (Figure 3). The influence of prazosin was statistically significant ($p<0.05$).

Figure 4 shows the concentration–response curve for the positive inotropic effect of epinephrine mediated by $\alpha_1$-adrenoceptors in the presence of bupranolol (10$^{-5}$ M) in rat, rabbit, and dog right ventricular myocardium determined in experimental conditions identical to those in which the $\alpha_1$-mediated PI metabolism was assessed. The concentration-dependent positive inotropic effect was elicited in rat and rabbit right ventricular papillary muscles. In rat papillary muscle, a characteristic biphasic positive inotropic effect was elicited as reported previously. The steady-state response (late phase) is plotted in Figure 4. The percentage increase in force in rabbit papillary muscle was more pronounced than that in rat. The concentration–response curves for rat were significantly different from those for rabbit ($p<0.05$). The $pD_2$ value ($−\log EC_50$) in rabbit (6.54) was approximately log 1 unit (0.88) higher than that in rat (5.66). Epinephrine up to 3×10$^{-6}$ M did not affect contractile force in the presence of 10$^{-6}$ M bupranolol in dog right ventricular trabeculae; at 10$^{-5}$ M and higher, it induced a positive inotropic effect that was abolished by further addition of bupranolol (Figure 4).

Time Course of $\alpha$-Mediated [H]IP$_1$ Accumulation 
and Positive Inotropic Effect

Figure 5 shows the time course of changes in contractile force in papillary muscles and ventricular trabeculae, and of [H]IP$_1$ accumulation in ventricular slices induced by 10$^{-5}$ M epinephrine through $\alpha_1$-adrenoceptors. In rat ventricle, accumulation of [H]IP$_1$ and increase in contractile force developed substantially in a similar time course. The contractile force was, however, decreased transiently at 1 minute after the administration of epinephrine. The force reached a steady level approximately 10–20 minutes after the administration, whereas [H]IP$_1$ accumulated gradually up to 30 minutes. In rabbit ventricular myocardium, contractile force increased rapidly within 3 minutes to reach a maximal steady level at 5 minutes after epinephrine administration; [H]IP$_1$ accumulated slightly at 10 minutes. In dog ventricular myocardium, epinephrine affected neither force nor [H]IP$_1$ accumulation.

We also examined the effects of phenylephrine on [H]IP$_1$ accumulation and contractile force in the presence of 10$^{-6}$ M bupranolol in rat, rabbit, and dog ventricular muscle. Phenylephrine (10$^{-5}$ M) at 30 minutes after administration facilitated both [H]IP$_1$ accumulation and contractile force in rat heart. In rabbit, it markedly increased contractile force and caused a slight increase in [H]IP$_1$ accumulation. In dog ventricle, phenylephrine did not affect contractile force but did reduce the extent of [H]IP$_1$ accumulation.

Time Course of $\alpha$-Mediated Prolongation 
of Action Potential Duration

Figure 6A shows the time course of changes in action potential duration and the positive inotropic effect of phenylephrine (3×10$^{-6}$ M) mediated by $\alpha$-adrenoceptors in the presence of pindolol (10$^{-7}$ M). Figure 6B presents the actual oscilloscope tracings in the absence and presence of 10$^{-5}$ M phenylephrine. Action potential duration was prolonged markedly in association with the $\alpha$-mediated increase in contractile force (Figure 6B). Also, the time course of the positive inotropic effect of phenylephrine and the recovery to control after washout of the drug coincided with the time course of prolongation and recovery of the action potential duration, respectively, measured at −40 and −70 mV of repolarization (Figure 6A), indicating a strong relation between the electrophysiological and contractile responses in rabbit papillary muscle. The effect of

<table>
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<th>Species</th>
<th>Na$^+$,K$^+$-ATPase activity (μmol P$_1$/mg protein/hr)</th>
<th>5′-Nucleotidase activity (nmol P$_1$/mg protein/hr)</th>
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Values are mean±SEM. Crude, activities immediately after homogenization; Purified, activities after the purification procedure; Ratio, Purified/Crude. Figures in parentheses are number of preparations.
FIGURE 1. Panel A: Saturation binding curves for [3H]prazosin to the membrane fraction derived from dog right ventricular myocardium. Specific binding (○) was defined as total binding (△) minus nonspecific binding (■). Nonspecific binding was determined as the radioactivity bound to the membrane preparation that was not displaced by phentolamine (10⁻⁵ M). B/F, bound/free fraction. Panel B: Scatchard plot of data calculated from the values in panel A. The slope of the regression line was determined by least-squares methods. Values presented are means of triplicate determinations in a single experiment.

Discussion

Subcellular Signal Transduction Process of Myocardial α₁-Adrenoceptors

It is increasingly accepted that α₁-adrenoceptor stimulation results in a positive inotropic effect in the heart of most mammalian species.¹⁻⁴ β-Adrenoceptors have been shown to be uniquely coupled to adenylate cyclase and L-type Ca²⁺ channels⁵⁻⁶ via stimulatory GTP binding protein. On the other hand, it has been revealed that activation of myocardial α₁-adrenoceptors is coupled to a number of diverse subcellular processes. The amplitude of intracellular Ca²⁺ transients was increased during induction of the positive inotropic effect by α₁-adrenoceptor stimulation in rabbit⁹ and rat³⁷ myocardium. It is postulated that the activation of myocardial α₁-adrenoceptors elicits the positive inotropic effect through at least two subcellular mechanisms in respect to Ca²⁺-mediated processes: an increase in the intracellular Ca²⁺ transient and an increase in the Ca²⁺ sensitivity of myofibrils in rabbit papillary muscle.⁹ Recently, a pronounced elevation of the diastolic Ca²⁺ level and an increase of Ca²⁺ transients have been reported after administration of dobutamine, which the authors postulated to be mediated by α-adrenoceptors in the rat perfused heart loaded with indo 1.³⁷ The pharmacological characteristics of the α-adrenoceptor-mediated changes in Ca²⁺ transients, however, have not been analyzed yet in the rat.

Table 2. Distribution of α₁- and β-Adrenoceptors as Determined by [3H]Prazosin and (±)-[3H]CGP-12177 in Membrane Fractions Derived From Rat, Rabbit, and Dog Ventricular Myocardium

<table>
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<th>Kd (nM)</th>
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<td>β</td>
<td>5</td>
<td>85.5±7.40</td>
<td>1.345±0.220</td>
</tr>
<tr>
<td>LV</td>
<td>α</td>
<td>5</td>
<td>19.3±1.35</td>
<td>0.246±0.012</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>5</td>
<td>133.0±19.0</td>
<td>1.120±0.206</td>
</tr>
<tr>
<td>Dog</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RV</td>
<td>α</td>
<td>5</td>
<td>24.0±3.90</td>
<td>0.266±0.028</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>5</td>
<td>104.2±16.0</td>
<td>2.220±0.018</td>
</tr>
<tr>
<td>LV</td>
<td>α</td>
<td>5</td>
<td>25.7±3.20</td>
<td>0.264±0.022</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>5</td>
<td>124.7±17.0</td>
<td>2.470±0.211</td>
</tr>
</tbody>
</table>

Values are mean±SEM. RV, right ventricle; LV, left ventricle.
Epinephrine elicited a more pronounced and rapidly developing positive inotropic effect in rabbit than in rat ventricular myocardium, whereas binding data disclosed a higher density of \( \alpha_1 \)-adrenoceptors in rat than in rabbit heart. The pD\textsubscript{2} value for the positive inotropic effect of epinephrine mediated by \( \alpha_1 \)-adrenoceptors in the presence of \( \beta \)-adrenoceptor antagonist was higher in rabbit papillary muscle by 0.88 log units than in rat. This implies a dissociation of functional response from binding characteristics in rabbit from that of rat ventricular myocardium. Acceleration of PI metabolism appears to be related more closely to the density of \( \alpha_1 \)-adrenoceptors among species. However, the relation between PI metabolism and positive inotropic effect induced by \( \alpha_1 \)-adrenoceptor stimulation is not straightforward. There was a significant concentration-dependent increase of \( [\text{H}] \)P\textsubscript{i}, by \( \alpha_1 \)-adrenoceptor in rat ventricular slices, but this increase was much less in rabbit (Figure 3). On the other hand, the positive inotropic effect by the \( \alpha_1 \)-adrenoceptor agonist epinephrine was more pronounced in rabbit than in rat (Figure 5). Therefore, the link between the degree of PI metabolism and positive inotropic effect seems quite weak for these two species. This phenomenon also was observed in Figure 6, which indicated that the time course for IP\textsubscript{1} accumulation and positive inotropic effect matched well only in rat and not in rabbit and dog. Modulation of \( \alpha \)-adrenoceptor–mediated positive inotropic effect by activation of protein kinase C with tumor-promoting phorbol esters likewise is different between rat and rabbit heart. In rat heart, production of 1,2-diacylglycerol and subsequent activation of protein kinase C has been reported to be responsible for maintaining the sustained component of the positive inotropic effect of \( \alpha_1 \)-adrenoceptor stimulation: phorbol 12,13-dibutyrate, a protein ki-

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Concentration-response curves for the \( \alpha_1 \)-adrenoceptor–mediated phosphatidylinositol metabolism in right ventricular slices of rat, rabbit, and dog. Accumulation of \( [\text{H}] \)inositol 1-phosphate was assessed 30 minutes after the addition of \((-)\)-epinephrine in the presence of 10 mM LiCl. Values are expressed as mean±SEM. Figures in parentheses are numbers of experiments. C, control.

![Figure 3](http://circres.ahajournals.org/)

**Figure 3.** Effects of \( 10^{-7} \) M prazosin on \( [\text{H}] \)inositol 1-phosphate accumulation induced by epinephrine in rat right ventricular slices. Each value was obtained after 30 minutes of incubation with epinephrine in the presence of 10 mM LiCl without (●) and with (○) \( 10^{-7} \) M prazosin. C, control.

### Table 3. Displacement Characteristics of \( [\text{H}] \)Prazosin Binding by Epinephrine From Membrane Fractions Derived From Rat, Rabbit, and Dog Ventricular Myocardium

<table>
<thead>
<tr>
<th>Species</th>
<th>( n )</th>
<th>Gpp(NH)p</th>
<th>Slope factor</th>
<th>( K_{\text{high}} ) (nM)</th>
<th>( K_{\text{low}} ) (( \mu )M)</th>
<th>( R_H ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>8</td>
<td>–</td>
<td>0.692±0.015</td>
<td>163.0±67.1</td>
<td>3.5±0.4</td>
<td>10.42±2.02</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>+</td>
<td>1.154±0.056</td>
<td>...</td>
<td>3.8±0.2</td>
<td>0</td>
</tr>
<tr>
<td>Rabbit</td>
<td>5</td>
<td>–</td>
<td>0.614±0.045</td>
<td>256.7±116.2</td>
<td>5.5±0.4</td>
<td>28.46±7.58</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+</td>
<td>0.921±0.036</td>
<td>...</td>
<td>8.19±3.3</td>
<td>0</td>
</tr>
<tr>
<td>Dog</td>
<td>5</td>
<td>–</td>
<td>0.622±0.062</td>
<td>253.0±129.2</td>
<td>11.0±4.8</td>
<td>24.04±6.78</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>+</td>
<td>0.981±0.052</td>
<td>...</td>
<td>7.5±1.1</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Each experiment was carried out in duplicate using myocardial membrane fractions derived from four rats, one rabbit, and one dog. Data presented were obtained from the concentration-dependent displacement curve for epinephrine to inhibit specific \( [\text{H}] \)prazosin binding (200 nM for rat and 1.0 nM for rabbit and dog). Analysis by nonlinear curve-fitting was performed by use of the LIGAND program. \( R_H \) (%) represents the production of binding sites with high affinity as the percentage of total binding sites. Gpp(NH)p, 5'-guanylimidodiphosphate; \( K_{\text{high}} \), \( K_{\text{low}} \) values for the high affinity binding sites; \( K_{\text{low}} \), \( K_{\text{low}} \) values for the low affinity binding sites.
nase C activator, enhanced the inotropic effect of α₁-adrenoceptor stimulation. In contrast, in rabbit papillary muscle, 12-O-tetradecanoylphorbol 13-acetate and phorbol 12,13-dibutyrate at concentrations that influenced neither the basal force of contraction nor the β-adrenoceptor-mediated positive inotropic effect, suppressed selectively the α₁-adrenoceptor-mediated positive inotropic effect. Tumor-promoting phorbol esters likewise have been shown to inhibit myocardial contractility by decreasing the intracellular calcium transients in cultured chick embryo ventricular myocytes. Although the mechanism responsible for these variable actions of phorbol esters is not clear, activation of protein kinase C may have different sites of action, including processes such as downregulation of protein kinase C or modulation at the level of α₁-receptors.

shown in other types of cells and tissues. Thus, although the present observations in the mammalian ventricular myocardium of these three species together with previous findings imply a close association of PI metabolism and its product with the α₁-adrenoceptor-mediated inotropic effect, the cause–effect relation between these changes requires further examination.

The variation observed in rat and rabbit heart may be partly explained by the difference in α₁-adrenoceptor-mediated changes in electrophysiological properties and ion transport mechanisms in these species. Namely, involvement of different types of membrane ion channels and ion transport systems has been advocated for α₁-adrenoceptor-mediated functional changes among various species. It has been believed that activation of α₁-adrenoceptors facilitates Ca influx directly through L-type Ca channels based on findings in voltage-clamp experiments and slow response action potentials in K⁺-depolarized multicellular cardiac muscle preparations. However, it has been revealed more recently that activation of α₁-adrenoceptors scarcely or only slightly facilitates L-type Ca channels on patch-clamp experiments in single cardiomyocytes. Instead, α₁-adrenoceptor agonists have been shown to result in an inhibition of the transient outward K⁺ current, which may lead to an elevation of [Ca²⁺], by prolongation of the action potential duration. This mechanism appears to be important and responsible for a marked increase in contraction in rabbit heart under α₁-activation as long as the time course of changes in contractile force showed an excellent coincidence with those of action potential duration (Figure 6). In rat heart, however, it has been shown that the change in contractile force apparently is dissociated from the electrophysiological change. On the other hand, it recently has been demonstrated that in quiescent rat myocytes loaded with fura-2 and the pH-sensitive fluorescent dye 2',7'-bis(2-carboxylethyl)-5(6)-carboxyfluorescein, the Na⁺-H⁺ exchange system is facilitated by α₁-adrenoceptor stimulation to result in an elevation of [Ca²⁺], probably through an activation of Na⁺-Ca²⁺ exchange.

**FIGURE 4.** Concentration–response curves for α-adrenoceptor–mediated positive inotropic effect in rat and rabbit right ventricular papillary muscles and in dog right ventricular trabeculae. Epinephrine was administered cumulatively, and each value was expressed as mean±SEM. Figures in parentheses are numbers of experiments. C, control.
TABLE 4. Effects of Phenylephrine on Action Potential Parameters and Developed Tension Mediated by α-Adrenoceptors in the Presence of Pindolol in Isolated Rabbit Papillary Muscle

<table>
<thead>
<tr>
<th>Phenylephrine (M)</th>
<th>Resting potential (mV)</th>
<th>Overshoot potential (mV)</th>
<th>Amplitude (mV)</th>
<th>Repolarization (msec) 50%</th>
<th>Repolarization (msec) 90%</th>
<th>Vmax (V/sec)</th>
<th>Developed tension (mN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>81.3±0.9</td>
<td>26.9±0.8</td>
<td>108.3±1.3</td>
<td>141.9±9.1</td>
<td>183.5±9.0</td>
<td>102.8±18.0</td>
<td>0.8±0.2</td>
</tr>
<tr>
<td>10^-7</td>
<td>81.2±0.7</td>
<td>27.1±0.6</td>
<td>108.3±1.1</td>
<td>143.6±9.1</td>
<td>185.6±9.3</td>
<td>98.6±16.2</td>
<td>1.0±0.3*</td>
</tr>
<tr>
<td>10^-6</td>
<td>82.1±0.5</td>
<td>26.8±0.8</td>
<td>108.9±1.0</td>
<td>147.5±9.2*</td>
<td>191.2±9.4*</td>
<td>100.7±17.4</td>
<td>2.0±0.6*</td>
</tr>
<tr>
<td>10^-5</td>
<td>83.2±0.8</td>
<td>27.1±1.0</td>
<td>109.5±1.3</td>
<td>156.7±9.5*</td>
<td>203.2±9.9*</td>
<td>100.0±9.9</td>
<td>4.0±0.9*</td>
</tr>
</tbody>
</table>

Values are mean±SEM of seven isolated rabbit papillary muscles (n=7 each).

*p<0.05, †p<0.01 vs. corresponding control values in the absence of phenylephrine.

subsequent to accumulation of [Na⁺]; the resultant intracellular alkalinization may explain the increase in the Ca²⁺ sensitivity of contractile proteins.⁵⁹ Thus, it appears to be possible that different subcellular mechanisms activated by α-adrenoceptor stimulation may contribute to the functional changes in rabbit and rat. In this context, as in other mechanisms regulated by myocardial α₁-adrenoceptors, activation of the delayed rectifier K⁺ current,⁵⁴ muscarinic K⁺ channel,⁵⁵ and Na⁺,K⁺-ATPase⁵⁶,⁵⁷ have been demonstrated, which may counteract the positive inotropic effect mediated by α₁-adrenoceptors. In addition, protein kinase C has been shown to cause phosphorylation of troponin I and T⁵⁸,⁵⁹ in vitro, although it was recently shown that this mechanism may be less important in the regulation of in vivo guinea pig heart.⁶⁰ Thus, the intracellular coupling process subsequent to the agonist binding to myocardial α₁-adrenoceptors may be divergent not only in quantitative but also in qualitative respects. The modulation of myocardial contractility by α₁-adrenoceptors may be achieved by the balance of these divergent regulatory interventions.

The epinephrine-induced displacement curve of the specific [³H]prazosin binding displayed high and low affinity sites, and Gpp(NH)p was capable of shifting the displacement curve for epinephrine to the right and increased the slope factor of the curve to unity in the three species (Table 3). These results are essentially consistent with previous findings in rat heart.⁶¹-⁶⁴ Although in the early experiments,⁶¹ clear effects of Gpp(NH)p on the agonist displacement curve were not seen, it has been confirmed that Gpp(NH)p shifts the α-adrenoceptor agonist displacement curve to the low affinity site.⁶²,⁶³ These findings suggest that the GTP binding protein is involved in coupling the receptor activation to subsequent subcellular processes under myocardial α₁-adrenoceptor activation. The treatment of animals with pertussis toxin, which inhibited muscarinic and adenosine receptor-mediated negative inotropic effects through ADP-ribosylation of the inhibitory GTP binding protein, did not affect either the positive inotropic effect of α-adrenoceptor agonist in rabbit papillary muscle⁶⁵ and rat left auricle⁶⁶ or acceleration of PI turnover mediated by α₁-adrenoceptors in the latter tissue.⁶⁷ Therefore, the GTP binding protein involved is not considered to be a substrate for ADP-ribosylation by pertussis toxin.

Absence of α-Adrenoceptor-Mediated Positive Inotropic Effect in Dog Heart

In dog heart, the positive inotropic effect of sympathomimetic amines is not mediated by α₁-adrenoceptors, as shown in the previous studies with phenylephrine, norepinephrine, and dopamine⁵⁵,⁵⁶ and with epinephrine in the present study. In spite of the lack of the α₁-adrenoceptor-mediated positive inotropic effect, the results in this study are consistent with previous studies in rat heart.⁶¹-⁶⁴

Figure 6. Panel A: Time course of changes in action potential duration (APD) and developed tension (Ten.) induced by phenylephrine (PE) (3x10⁻⁶ M) in the presence of pindolol (10⁻⁷ M) in isolated rabbit papillary muscle. APD was monitored continuously at –40 and –70 mV with a comparator, digital counter, and digital-to-analog converter. AP, action potential. Panel B: Typical oscilloscope tracings of the effect of phenylephrine (10⁻⁵ M) in the presence of pindolol (10⁻⁷ M), showing that phenylephrine prolonged APD and increased developed tension at 15 minutes after administration.
tropic effect, there were characteristic α₁-adrenoceptor binding sites in the dog heart, with B\text{max} values and affinity no different from those in rabbit heart determined under the same experimental conditions. The experimental conditions chosen in the present study might have affected the detection of the α₁-adrenoceptor-mediated positive inotropic effect in dog heart; the optimal condition for the assay of dog tissue may be different from that used. We varied the temperature and frequencies of stimulation and also used blood-perfused dog right ventricular papillary muscles. Phenylephrine, norepinephrine, and dopamine all caused a concentration-dependent positive inotropic effect, which was antagonized by a β-adrenoceptor antagonist, pinodolol, but not by phentolamine; methoxamine did not elicit the positive inotropic effect in dog heart either.\textsuperscript{20,68} It therefore is unlikely that incorrect experimental conditions were chosen.

Although in dog Purkinje fibers\textsuperscript{69} and adult dog ventricular myocytes\textsuperscript{70} α-adrenoceptor stimulation is able to prolong action potential duration, the prolongation is not reflected to an increase in contractile force in this species. The finding that the epinephrine displacement curve of the [\(^{3}H\)]prazosin specific binding was shifted by Gpp(NH)p in the dog implies that α₁-receptors in this species are coupled to subcellular processes via GTP binding protein. It has been shown in the dog heart that myocardial α₁-adrenoceptors are coupled to the cellular signal transduction pathways such as activation of Na⁺, K⁺-ATPase\textsuperscript{56,57}; therefore, this would cause a fall in [Na⁺]\textsuperscript{57} and thus a fall in [Ca\textsuperscript{2⁺}] via Na⁺-Ca\textsuperscript{2⁺} exchange, which may account for the lack of positive inotropic effect by α₁-adrenoceptor activation in the dog.

In summary, both an acceleration of PI metabolism and positive inotropic effect in response to activation of myocardial α₁-adrenoceptors occurred in rat and rabbit but were absent in dog. The difference in the number of α₁-adrenoceptors among the mammalian species examined does not totally account for species difference in the positive inotropic effect mediated by myocardial α₁-adrenoceptors. Differences in the coupling process subsequent to agonist binding to the myocardial α₁-adrenoceptor, which results in a wide range of modulation of electrophysiological properties and ion transport mechanisms in various species, may be crucial in explaining the species differences in α₁-adrenoceptor-mediated functional changes.

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Keywords: alpha1-adrenoceptors • positive inotropic effect • phosphatidylinositol metabolism • mammalian ventricular myocardium
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