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Pharmacological characteristics of the positive inotropic effect of angiotensin II in the rabbit ventricular myocardium

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1 In order to elucidate the mechanism underlying the positive inotropic effect (PIE) of angiotensin II (AII), we measured changes in phosphoinositide hydrolysis and contractile force induced by AII in the rabbit ventricular myocardium.

2 AII (1.0 nM–3 μM) produced a PIE in a concentration-dependent manner in the presence of bupranolol (0.3 μM) and prazosin (0.1 μM), the maximal response being about 40% of that to isoprenaline and the EC_{50} 30 nM.

3 The PIE of AII was associated with a concentration-dependent increase in the total duration of contraction; the time to peak force and the relaxation time were prolonged.

4 AII (10 nM–30 μM) elicited an accumulation of [^{3}H]-inositol monophosphate in a concentration-dependent manner in rabbit ventricular slices prelabelled with *myo*-[^{3}H]-inositol.

5 The PIE and the accumulation of [^{3}H]-inositol monophosphate induced by AII were inhibited by a non-selective AII receptor antagonist, saralasin (10 nM–1 μM) and by a selective AT_1 receptor antagonist, losartan (10 nM–1 μM), but not a selective AT_2 receptor antagonist, PD 123319 (1 μM).

6 A tumour-promoting phorbol ester, phorbol 12,13-dibutyrate (PDBu, 10–100 nM), inhibited the AII-induced PIE and [^{3}H]-inositol monophosphate accumulation in a concentration-dependent manner.

7 These results suggest that AII exerts a PIE through activation of AT_1 receptors and subsequent acceleration of phosphoinositide hydrolysis. Activation of protein kinase C by PDBu may inhibit the AII-induced stimulation of phosphoinositide hydrolysis and thereby the PIE of AII in the rabbit ventricular myocardium.

Keywords: Angiotensin II; saralasin; losartan; PD 123319; AT_1 receptor; positive inotropic effect; phosphoinositide hydrolysis; inositol phosphates; phorbol 12,13-dibutyrate; rabbit ventricular myocardium

Introduction

Angiotensin II (AII) plays an important role in the control of cardiovascular haemodynamic homeostasis through activation of the renin-angiotensin system. In addition to its well-established constrictor effect on the vascular smooth muscle, AII produces a positive inotropic effect (PIE) in mammalian ventricular myocardium including that of man (Schomisch *et al.*, 1990), dog (Kobayashi *et al.*, 1978) and rabbit (Dempsey *et al.*, 1971) and in the chick heart (Baker & Aceto, 1989). In whole animal experiments, AII modulates the myocardial contractility via a direct pathway and by an indirect pathway which is mediated by a cardiovascular reflex. Increased release of catecholamines may contribute to the indirect inotropic effect of AII (Ross & White, 1966). The mechanism of the direct PIE caused by AII has been postulated to be due to activation of specific AII receptors in myocardial cells (Dempsey *et al.*, 1971). Various pathways of intracellular signal transduction have been implicated as possible subcellular mechanisms of the action of AII: the activation of the slow Ca^{2+} channel (Freer *et al.*, 1976), acceleration of phosphoinositide hydrolysis (Baker & Aceto, 1989) and inhibition of adenylate cyclase (Anand-Srivastava, 1989). Furthermore, since AII stimulates protein synthesis in the myocardium, it has been suggested that AII may be involved in the pathogenesis of cardiac hypertrophy (Aceto & Baker, 1990). Recently, type 1 and type 2 AII receptor subtypes have been distinguished. Type 1 AII receptors (AT_1 receptors) have high affinity for the nonpeptide AT_1 receptor selective antagonist, losartan (also known as DuP 753 or MK 954) (Whitebread *et al.*, 1989). Type 2 angiotensin II receptors (AT_2 receptors) have a low affinity for losartan and a high affinity for the nonpeptide AT_2 receptor selective antagonists, PD 121981 (also known as WL-19) (Whitebread *et al.*, 1989) and PD 123319 (Dudley *et al.*, 1990). Although it has been shown that both subtypes of AII receptors exist in the rabbit ventricular myocardium (Chiu *et al.*, 1989), the role of receptor subtypes and subcellular mechanism of inotropic effects of AII have not yet been fully clarified.

In order to elucidate the mechanism of PIE of AII, experiments were designed to investigate the relationship between the PIE and acceleration of phosphoinositide hydrolysis induced by AII in the rabbit ventricular myocardium.

Methods

Isolation of rabbit papillary muscles and experimental procedures

Male albino rabbits weighing 1.5–2.2 kg were used for these experiments. The animals were anaesthetized with i.v. administration of sodium pentobarbitone (35 mg kg^{-1}; Tokyo Kasei, Tokyo, Japan) into a marginal ear vein. One thousand units per kg of heparin sodium was also administered simultaneously. After thoracotomy, the heart was removed immediately. Two or three papillary muscles were excised from the right ventricle of the rabbit and mounted in 20 ml organ baths containing Krebs-Henseleit solution with 0.057 mM ascorbic acid and 0.027 mM disodium EDTA. The solution was bubbled with 95% O_2 and 5% CO_2 at 37°C. The pH of the solution was 7.4 when bubbled with the gas mixture. The composition of the solution was as follows (mM): Na^+ 142.9, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, $H_2PO_4^-$ 1.2, HCO_3^- 24.9, SO_4^{2-} 1.2, Cl^- 127.8 and glucose 11.1. Papillary muscles were electrically stimulated by square wave pulses of 5 ms duration

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and a voltage approximately 20% above threshold at a frequency of 1 Hz. The developed tension was recorded on a thermal pen writing oscillograph (Recti-Horiz-8K, NEC-San-ei Instrument, Tokyo, Japan) by means of strain gauge transducers (Shinkoh UL 10 GR, Minebea, Tokyo, Japan). During the equilibration period (60 min) papillary muscles were stretched initially under a tension of 5 mN and the length was later adjusted to give 90% of the maximal contractile force. (±)-Bupranolol (0.3 μM) and prazosin (0.1 μM) were allowed to act for 20 min before the administration of AII and were present in the organ bath throughout the experiments to avoid any interference with α- and β-adrenoceptor stimulation by catecholamines (Dempsey *et al.*, 1971). In a series of experiments with AII antagonists, saralasin, losartan or PD 123319 were allowed to act for 20 min before the administration of AII. At the end of each experiment, the maximal contractile force was determined in each muscle by administration of isoprenaline (1–10 μM) after washout of other drugs for at least 30 min until the basal force of contraction returned to the control level. The concentration of isoprenaline was elevated stepwise until the maximal response was achieved. The maximal response to isoprenaline comparable to that achieved by an elevation of the extracellular Ca^{2+} to 9–12 mM was attained by isoprenaline 30 min after washout of the competitive β-adrenoceptor antagonist, (±)-bupranolol. Concentration-response curves for AII were determined by cumulative administration of AII dissolved in 0.1 ml of 0.9% NaCl. When the inotropic responses reached a steady level (usually 5–15 min), the next higher concentration was added. The response to AII was expressed as a percentage of the maximal response to isoprenaline. Concentration-response curves for AII were determined only once in each preparation to avoid possible occurrence of desensitization. This also applies to studies in which antagonists were administered. Therefore, for the calculation of the dose-ratio for AII in the absence and presence of various concentrations of losartan, the mean value for AII in the control experiment (in the absence of losartan) was used as the denominator.

Determination of [^{3}H]-inositol phosphates

The hearts were quickly removed from rabbits, and placed in Krebs-Henseleit solution that was bubbled with 95% O_2:5% CO_2 at 4°C. The experimental procedure was the same as that used in the previous study (Takanashi & Endoh, 1991). Briefly, ventricular slices (0.5 mm thick) were prepared with a Thomas tissue slicer (Thomas, Philadelphia, PA, U.S.A.) and were weighed and equilibrated in Krebs-Henseleit solution for 30 min. The experiment was carried out in duplicate. Each tube contained one ventricular slice cut and trimmed to appropriate size. The slice in each tube was preincubated with 24 μCi *myo*-[^{3}H]-inositol in 4 ml Krebs-Henseleit solution for 120 min. Then the solution was changed to fresh solution containing 5 mM *myo*-inositol and 10 mM LiCl, and all the experiments were performed in the Li^+-containing solution. (±)-Bupranolol (0.3 μM) and prazosin (0.1 μM) were added to the solution 20 min before agonist administration to avoid any interference induced by adrenoceptor activation. At the end of the incubation, the slices were quickly blotted and put into 1.0 ml of chloroform-methanol-12 N HCl (100:200:1 by volume) to terminate the reaction. After the addition of 0.2 ml of 5 mM EDTA, the tissue was homogenized (Polytron PT-10). The tip of the homogenizer was rinsed with 0.5 ml of chloroform-methanol-12 N HCl-5 mM EDTA (100:200:1:80 by volume), and the rinsing fluid was added to the original solution. Chloroform (0.4 ml) and 5 mM EDTA (0.5 ml) were added sequentially and the samples were centrifuged at 1,400 *g* for 20 min to separate the aqueous and organic phases. An aliquot of the aqueous layer was removed, neutralized with 1 M KOH and applied to columns containing a 50% slurry of AG 1-X8 (anion exchange resin, 100–200 mesh, formate form, Bio-Rad, Richmond, CA, U.S.A.). Each column was washed first with 20 ml of distilled water, and the resulting glycerophosphoryl esters were eluted with 8 ml of 5 mM sodium tetraborate-60 mM sodium formate as described previously (Berridge *et al.*, 1983; Takanashi & Endoh, 1991). Then [^{3}H]-inositol phosphates were eluted by addition of 0.2 M ammonium formate in 0.1 M formate as described by Berridge *et al.* (1983). Aliquots of the eluate were counted for radioactivity in a scintillation mixture (ACS-II, Amersham, Arlington Heights, IL, U.S.A.) by use of a Tri-Carb 1500 scintillation counter (Packard, Downers Grove, IL, U.S.A.) at an efficiency of 66%. Since it was found in the preliminary experiment with this method that the radioactivity of [^{3}H]-inositol trisphosphate (IP_3) or [^{3}H]-inositol bisphosphate was low, probably due to their rapid metabolism to [^{3}H]inositol monophosphate (IP_1), IP_1 was used as an indicator of phosphoinositide hydrolysis (Takanashi & Endoh, 1991).

Drugs used

The drugs used were obtained from the following sources: prazosin HCl (Pfizer Taito, Tokyo); (±)-bupranolol HCl (Kaken Kagaku, Tokyo); AII (Peptide Institute, Osaka, Japan); saralasin ([Sar^1, Val^5, Ala^8]angiotensin II, Sigma Chemical, St. Louis, MO, U.S.A.); losartan (2-n-butyl-4-chloro-5-hydroxymethyl-1-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl) methyl]imidazol, potassium salt, Du Pont, Wilmington, DE, U.S.A.); phorbol 12,13-dibutyrate (PDBu), (−)-isoprenaline hydrochloride (Sigmal Chemical, St. Louis, MO, U.S.A.); *myo*-[^{3}H]-inositol (specific activity 115.5 Ci mmol^{-1}) (Amersham, Buckinghamshire, U.K.); PD 123319-121b (Warner-Lambert Co., Ann Arbor, MI, U.S.A.). PDBu was dissolved in 100% DMSO, and the stock solution (20 mM) was diluted with 0.9% NaCl to the desired concentration. The stock solution of isoprenaline was prepared in 1% ascorbic acid, further diluted with 0.9% NaCl solution and kept ice-cold.

Statistics

Experimental values are presented as means ± s.e.mean. Significant differences between mean values were estimated by means of Student's *t* test. A *P* value of smaller than 0.05 was considered to indicate significance.

Results

Positive inotropic effect of angiotensin II on the rabbit ventricular myocardium

AII (1 nM–3 μM), in the presence of (±)-bupranolol (0.3 μM) and prazosin (0.1 μM), produced a PIE in a concentration-dependent manner on rabbit isolated papillary muscles (Figure 1). The pD_2 value [−log 50% effective concentration (EC_{50})] was 7.50 ± 0.24, and the maximum response to AII was achieved at 1–3 μM of AII and was 40.85 ± 4.57% of the maximal response to isprenaline ($n = 7$). The slope of Hill plot for the concentration-response curve for AII was 0.88 ± 0.04, which was not significantly different from 1.0 ($P > 0.05$).

The PIE of AII was associated with characteristic changes in the time course of isometric contractions. Typical tracings of isometric contraction are shown in Figure 2a. The total duration of contraction was prolonged in a concentration-dependent manner to 281 ± 8.0 ms from 236 ± 7.5 ms by AII (0.3 μM) (Figure 2b). The time to peak force was prolonged by AII (0.3 μM) to 125 ± 4.6 ms from 105 ± 5.8 ms and the half relaxation time to 63.6 ± 2.4 ms from 54.3 ± 2.4 ms, respectively (Figure 2b).

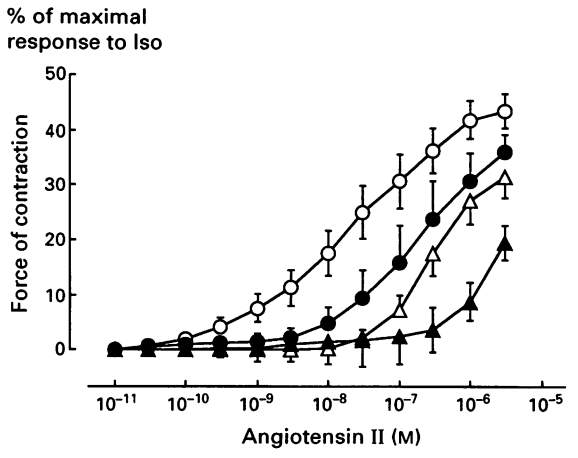

Figure 1 The influence of saralasin on the positive inotropic effect of angiotensin II (AII) in the rabbit isolated papillary muscle in the presence of (±)-bupranolol (0.3 μM) and prazosin (0.1 μM). Saralasin was allowed to act for 20 min before the determination of concentration-response curves for AII and experiments were carried out in its presence. (○) Control ($n = 14$); (●) saralasin 10 nM ($n = 6$); (△) saralasin 100 nM ($n = 6$); (▲) saralasin 1 μM ($n = 6$). Saralasin (10 nM–1 μM) did not significantly affect the basal force of contraction. All values represent means ± s.e.mean. The basal force and maximal response to isoprenaline (Iso) in this series of experiments were 2.85 ± 0.29 and 12.4 ± 0.9 mN ($n = 32$), respectively.

Influence of saralasin, losartan and PD 123319 on the positive inotropic effect of angiotensin II

Figure 1 shows the influence of a nonselective AII receptor antagonist, saralasin on the PIE of AII in the presence of (±)-bupranolol and prazosin. Saralasin up to a concentration of 1 μM did not affect the basal force of contraction. Saralasin, at a concentration of 10 nM, partially inhibited the PIE induced by AII, and at a higher concentration of 1 μM, shifted further the concentration-response curve for AII. Figure 3a shows the influence of the nonpeptide AT_1 receptor selective antagonist, losartan on the PIE of AII in the presence of (±)-bupranolol and prazosin. Losartan (10 nM–1 μM) did not affect the basal force of contraction, but inhibited the PIE of AII concentration-dependently (Figure 3a). Schild analysis using the dose ratio at the AII concentration eliciting 20% of the maximal response to isoprenaline indicated that the slope was not different from unity (0.98 ± 0.11) and the pA_2 value was 9.00 ± 0.84 (inset of Figure 3a). It was not shown whether the maximal response to AII in the presence of losartan (0.1–1 μM) could reach that of control, because of the availability of AII. On the other hand, the nonpeptide AT_2 receptor selective antagonist, PD 123319 (1 μM) did not influence the PIE of AII (Figure 3b).

Effects of angiotensin II on phosphoinositide hydrolysis

After administration of 10 μM AII, the accumulation of [^{3}H]-IP_1 in rabbit ventricular slices in the presence of 10 mM LiCl, (±)-bupranolol (0.3 μM) and prazosin (0.1 μM) reached a significant level at 4 min and then increased time-dependently to reach about 200% of the control level at 30 min (Figure 4a).

The concentration-response curve for the effect of AII on the [^{3}H]-IP_1 accumulation was determined in ventricular slices incubated with various concentrations of AII for 30 min. The accumulation of [^{3}H]-IP_1 increased with AII in a concentration-dependent manner (10 nM–30 μM) and the maximal response reached about 200% of the basal level at 30 μM (Figure 4b).

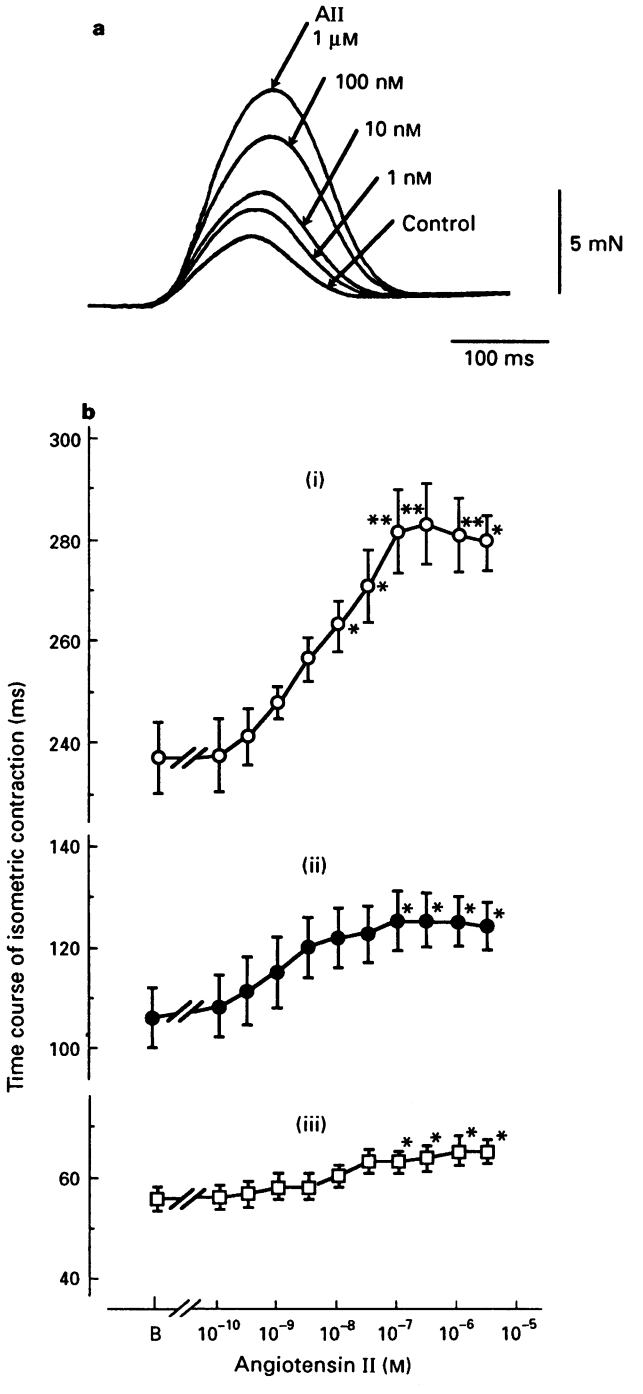

Figure 2 (a) Representative tracings showing the change in time course of isometric contractions induced by angiotensin II (AII). (b) Concentration-response curve for AII in the rabbit isolated papillary muscle in the presence of (±)-bupranolol (0.3 μM) and prazosin (0.1 μM). Total duration of contraction (i), time to peak force (ii) and half relaxation time (iii) are plotted against the concentration of AII. (B) Basal values. All values represent means ± s.e.mean ($n = 7$). $^*P < 0.05$, $^{**}P < 0.01$ vs. the corresponding control values.

Influence of saralasin, losartan, PD 123319 and PDBu on the accumulation of [^{3}H]-IP_1 induced by angiotensin II

Figure 5a shows the influence of saralasin on the [^{3}H]-IP_1 accumulation induced by AII (10 μM) in rabbit ventricular slices. AII at a concentration of 10 μM caused an increase in the [^{3}H]-IP_1 level to 183.2 ± 15.1% ($n = 8$) of the control. Saralasin at a concentration of 10 nM attenuated the AII-induced accumulation of [^{3}H]-IP_1, but the reduction was not

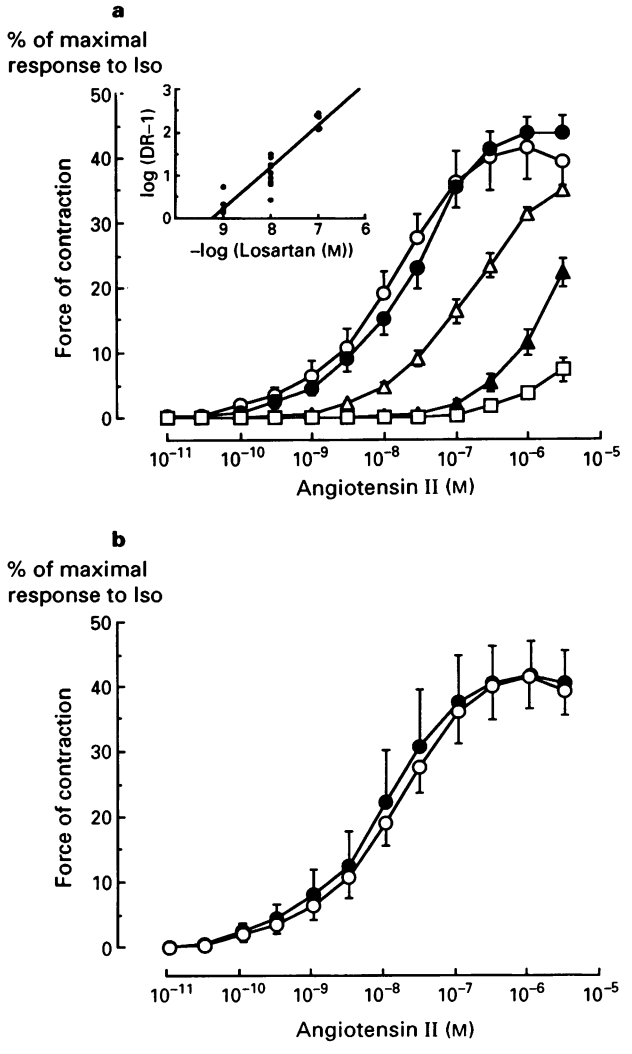

Figure 3 Effects of losartan and PD 123319 on the positive inotropic effect of angiotensin II (AII) in the rabbit isolated ventricular papillary muscle in the presence of (±)-bupranolol (0.3 μM) and prazosin (0.1 μM). Losartan (1 nM–1 μM) or PD 123319 (1 μM) was allowed to act for 20 min before the determination of concentration-response curves for AII and experiments were carried out in its presence. (a) (○) Control ($n = 6$); (●) losartan 1 nM ($n = 8$); (△) losartan 10 nM ($n = 9$); (▲) losartan 100 nM ($n = 5$); (□) losartan 1 μM ($n = 5$). Losartan (1 μM) did not significantly affect the basal force of contraction. All values represent means ± s.e.mean. The basal force and maximal response to isoprenaline (Iso) in this series were 2.9 ± 0.3 and 12.0 ± 1.0 mN ($n = 33$), respectively. Inset: Schild plot calculated with the dose-ratio at the level of 20% in (a). DR; AII dose-ratio. (b) (○) Control ($n = 6$); (●), PD 123319 1 μM ($n = 6$). PD123319 1 μM did not significantly affect the basal force of contraction. The basal force and maximal response to isoprenaline in this series were 4.3 ± 0.3 and 13.9 ± 1.3 mN ($n = 12$), respectively.

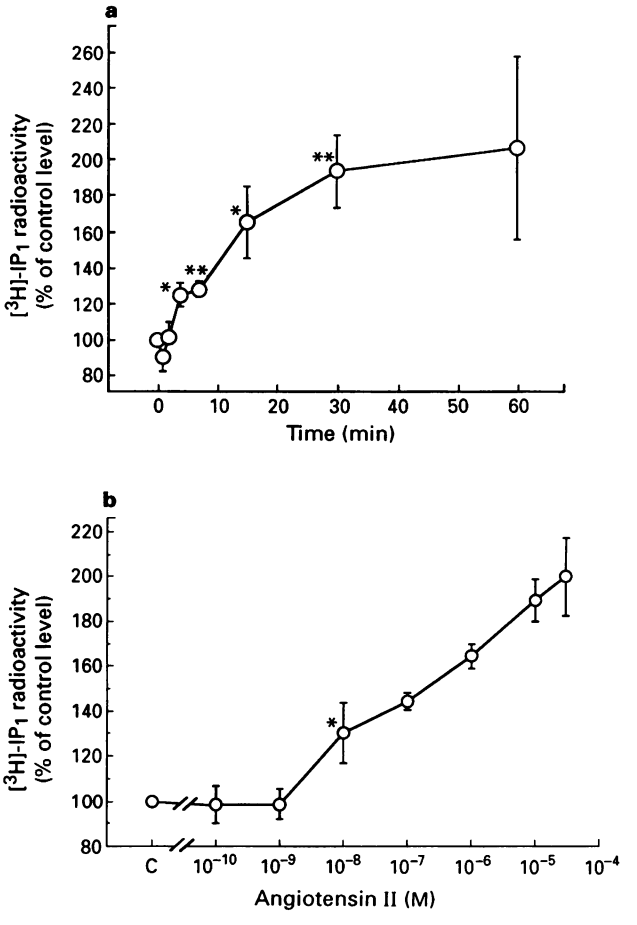

Figure 4 The angiotensin II (AII)-induced accumulation of [^{3}H]-inositol monophosphate ([^{3}H]-IP$_1$) in rabbit ventricular slices prelabelled with *myo*-[^{3}H]-inositol. (a) Time-response curve for [^{3}H]-IP$_1$ accumulation. AII (10 μM) was allowed to act for periods of time from 0 to 60 min and then [^{3}H]-IP$_1$ levels were determined. Actual radioactivity of 100% was 40.6 ± 6.8 d.p.m. mg^{-1} of wet weight of the tissue ($n = 6$). Values represent means ± s.e.mean ($n = 6$ for each determination). $^*P < 0.05$, $^{**}P < 0.01$ vs. the corresponding control values. Average wet weight of slices was 84.69 ± 4.03 mg ($n = 48$). (b) Concentration-response curve for the accumulation of [^{3}H]-IP$_1$. Accumulation of [^{3}H]-IP$_1$ was assessed 30 min after the addition of AII in the presence of 10 mM LiCl. Actual radioactivity of 100% was 28.3 ± 3.8 d.p.m. mg^{-1} of wet weight of the tissue ($n = 4$). Values represent means ± s.e.mean ($n = 4$ in duplicate for each concentration). Asterisk represents the threshold concentration ($P < 0.05$). Average wet weight of slices was 53.0 ± 2.2 mg ($n = 32$).

statistically significant. Saralasin at 100 nM significantly ($P < 0.01$, $n = 8$) decreased the accumulation of [^{3}H]-IP$_1$ induced by AII (Figure 5a). The concentration-dependent inhibition by saralasin of the AII-induced [^{3}H]-IP$_1$ accumulation indicates that the AII-induced acceleration of phosphoinositide hydrolysis is caused through activation of AII receptors in the rabbit ventricular myocardium.

The selective AT$_1$ receptor antagonist, losartan, inhibited significantly the AII-induced accumulation of [^{3}H]-IP$_1$ at concentrations of 100 nM and higher ($n = 8$ each, Figure 5b). On the other hand, a selective AT$_2$ receptor antagonist, PD 123319 (0.1–1.0 μM) did not affect the AII-induced accumulation of [^{3}H]-IP$_1$ ($n = 6$ each, Figure 5c).

Influence of PDBu on the positive inotropic effect and phosphoinositide hydrolysis induced by angiotensin II

Previously we have found that the phorbol esters, phorbol 12-myristate 13-acetate and PDBu, antagonize selectively the PIE mediated by myocardial α_1-adrenoceptors or endothelin receptors that was elicited in association with acceleration of phosphoinositide hydrolysis in the rabbit papillary muscle (Kushida *et al.*, 1988; Endoh *et al.*, 1991; Takanashi & Endoh, 1991). Therefore, we examined the influence of PDBu on the PIE of AII. The PIE of AII was antagonized by PDBu in a concentration-dependent manner (Figure 6a) over a similar concentration-range that inhibited the PIE of phenylephrine and endothelin-1 (Takanashi & Endoh, 1991). PDBu (10 nM) inhibited the PIE of AII by approximately 65% without affecting the basal force of contraction. At a concentration of 100 nM, PDBu decreased the basal force of contraction ca. 20% and abolished the PIE of AII.

The effects of PDBu on the accumulation of [^{3}H]-IP$_1$

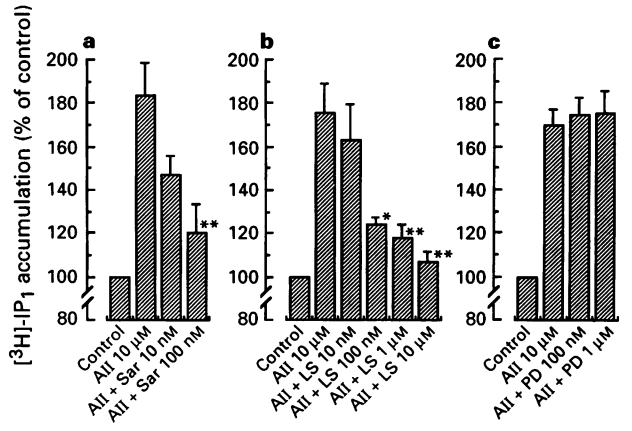

Figure 5 The influence of saralasin (Sar), losartan (LS) and PD 123319 (PD) on the angiotensin II (AII)-induced accumulation of [^{3}H]-inositol monophosphate ([^{3}H]-IP$_1$) in rabbit ventricular slices prelabelled with *myo*-[^{3}H]-inositol. Sar, LS, PD or 0.9% NaCl was added 20 min before the administration of AII and experiments were carried out in their presence. AII (10 μM) was allowed to act for 30 min in the absence (control) or presence of Sar (a), LS (b) or PD (c) and then the [^{3}H]-IP$_1$ level was determined. Actual radioactivities of 100% were 54.3 ± 4.3 (a, $n = 8$), 45.1 ± 5.3 (b, $n = 8$) and 61.4 ± 4.8 (c, $n = 6$) d.p.m. mg^{-1} of wet weight of the tissue, respectively. All experiments were determined in duplicate. Values represent means ± s.e.mean. The effect of Sar, LS or PD on the response to AII was compared to the corresponding value with AII alone. $^*P<0.05$, $^{**}P<0.01$ vs. the value with AII alone. Average wet weight of slices was 70.9 ± 5.2 mg (a, $n = 32$), 82.2 ± 3.4 mg (b, $n = 40$), 39.8 ± 1.9 mg (c, $n = 24$).

induced by AII are shown in Figure 6b. Pretreatment with 10 nM PDBu for 20 min, the concentration at which it inhibited significantly the PIE of AII, significantly reduced the accumulation of [^{3}H]-IP$_1$ caused by AII ($P<0.001$, $n = 8$). Pretreatment with a higher concentration of PDBu (100 nM), which abolished the PIE of AII, further attenuated the accumulation of [^{3}H]-IP$_1$. The level of [^{3}H]-IP$_1$ in the presence of 100 nM PDBu was not significantly different from the control.

Discussion

In the rabbit isolated papillary muscle, AII elicited a concentration-dependent PIE in the presence of α- and β-adrenoceptor blockade. The inotropic effect of AII was antagonized by saralasin, a nonselective angiotensin II receptor antagonist (Figure 1). It has been demonstrated that angiotensin receptors exist in myocardium of various experimental animals (Wright *et al.*, 1983; Rogers, 1984; Aceto & Baker, 1990). AII has been shown to produce a PIE in some species including man, cat, and rabbit, but not in guinea-pig and ferret (Dempsey *et al.*, 1971; Baker & Aceto, 1989; Schomisch *et al.*, 1990). This implies that AII shows a wide range of species-dependent difference in its inotropic effect. In this respect, rabbit is characteristic in the mammalian species in that its cardiac muscle responds to AII with a pronounced positive inotropy. The selective AT$_1$ receptor antagonist, losartan, inhibited concentration-dependently the PIE of AII, whereas the selective AT$_2$ receptor antagonist, PD 123319, did not influence the PIE (Figure 3), indicating that the AT$_1$ receptor subtype may be involved in the PIE of AII in the rabbit ventricular myocardium.

A possible subcellular mechanism for the intropic action of AII is an increase in phosphoinositide hydrolysis catalyzed via AT$_1$ receptor activation (Figures 4 and 5). This view is supported by previous findings showing that AII stimulates phosphoinositide hydrolysis in various tissues including vascular smooth muscle (Smith *et al.*, 1984), liver (Burgess *et al.*, 1991), kidney (Kurtz & Penner, 1989) and chick cardiac muscle (Baker & Aceto, 1989).

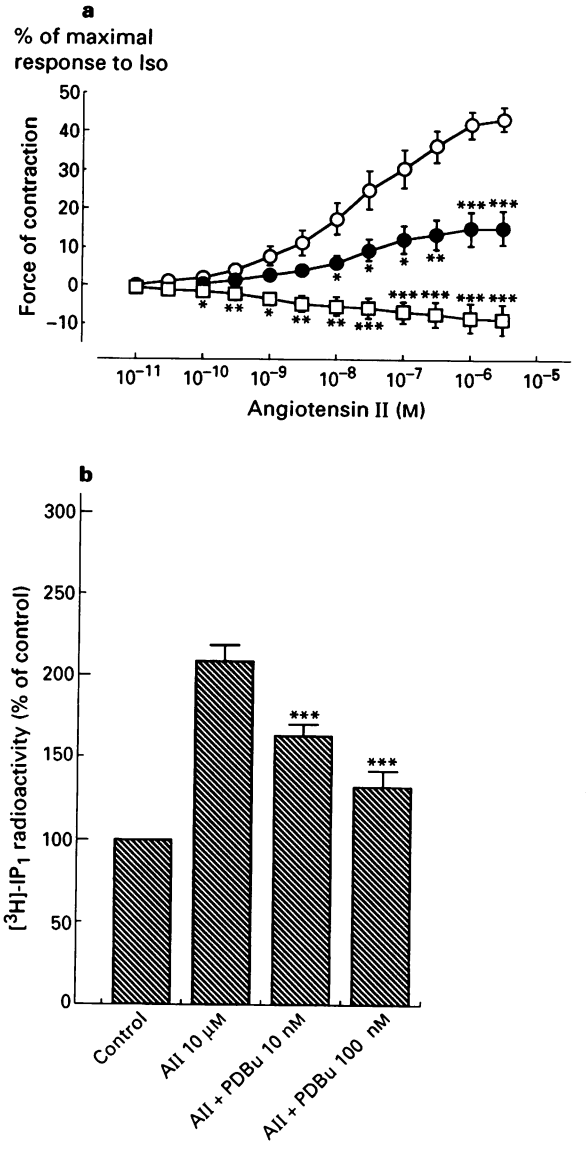

Figure 6 (a) The influence of phorbol 12, 13-dibutyrate (PDBu) on the positive inotropic effect of angiotensin II (AII) in the rabbit isolated ventricular papillary muscle in the presence of (±)-bupranolol (0.3 μM) and prazosin (0.1 μM). PDBu was applied 20 min before the determination of concentration-response curves for addition of AII and experiments carried out in its presence. (○) Control ($n = 14$); (●) PDBu 10 nM ($n = 6$); (□) PDBu 100 nM ($n = 6$). All values represent means ± s.e.mean. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. the corresponding control values. At 20 min after the administration of 10 or 100 nM PDBu, the contractile force was 90.9 ± 3.3% ($n = 6$) or 78.6 ± 6.3% ($n = 6$; $P<0.05$) of the control basal force of contraction. The basal force and maximal response to isoprenaline (Iso) in this series were 3.35 ± 0.62 and 12.4 ± 0.9 mN ($n = 26$), respectively. (b) The influence of PDBu on the AII (10 μM)-induced accumulation of [^{3}H]-inositol monophosphate ([^{3}H]-IP$_1$) in rabbit ventricular slices prelabelled with *myo*-[^{3}H]-inositol. PDBu or 0.9% NaCl was added 20 min before the administration of AII and experiments were carried out in their presence. AII (10 μM) was allowed to act for 30 min in the absence (control) or presence of 10 or 100 nM PDBu, and then the [^{3}H]-IP$_1$ level was determined. Actual radioactivity of 100% was 38.1 ± 4.5 d.p.m. mg^{-1} of wet weight of the tissue ($n = 8$). Values represent means ± s.e.mean ($n = 8$ in duplicate for each column). The effect of PDBu on the response to AII was compared to the value with AII alone. $^{***}P<0.001$ vs. the value with AII alone. Average wet weight of slices was 46.5 ± 2.6 mg ($n = 32$).

In the rabbit ventricular myocardium, striking similarities have been noted between the inotropic effect of AII and myocardial α_1-adrenoceptor and endothelin receptor stimulation, all of which stimulate phosphoinositide hydrolysis. Firstly, characteristics of contractile regulation through these classes of receptors are very similar. Figure 2 shows the changes in time course of isometric contractions induced by AII, which are akin to those produced by α_1- or endothelin receptor activation in the rabbit papillary muscle (Takanashi & Endoh, 1991). Stimulation of myocardial α_1-, endothelin and AII receptors consistently results in prolongation of action potential duration (Dempsey *et al.*, 1971; Argentieri *et al.*, 1990; Dirksen & Sheu, 1990). These changes were in strong contrast to those produced by β-adrenoceptor stimulation (Endoh & Blinks, 1988). The maxial response to α_1- or endothelin receptor agonists was about 50–60% of that to isoprenaline (Takanashi & Endoh, 1991), which was slightly higher than that of AII.

Secondly, a tumour-promoting phorbol ester, PDBu (10–100 nM) inhibited the PIE and phosphoinositide hydrolysis induced by AII in rabbit ventricular muscle (Figure 6). The effects of α_1-adrenoceptor agonists and endothelin-1 were also inhibited by PDBu or PMA (Kushida *et al.*, 1988; Endoh *et al.*, 1991; Takanashi & Endoh, 1991). It is noteworthy that the inhibitory action of PDBu was selective for the inotropic effect associated with phosphoinositide hydrolysis: the basal force of contraction, and the inotropic effects of isoprenaline and Bay K 8644 were scarcely affected by PDBu.

Inhibition by PDBu of the agonist-induced phosphoinositide hydrolysis and functional changes have been demonstrated in a variety of cells, including smooth muscle cells (Brock *et al.*, 1985), platelets (Kagaya *et al.*, 1990) and myocardial cells (Yuan *et al.*, 1987). In addition, in chick embryo myocytes, PDBu decreased Ca^{2+} transients increased by AII (Baker *et al.*, 1989) and PMA inhibited the AII-mediated increase in Ca^{2+} transients in rat myocytes (Kem *et al.*, 1991). These observations indicate that the externally administered tumour-promoting phorbol esters may inhibit the receptor-mediated stimulation of phosphoinositide hydrolysis and thereby antagonize the inotropic effect. Phosphorylation of receptors (Resink *et al.*, 1990), and/or of GTP binding proteins (Katada *et al.*, 1985) due to protein kinase C activation have been postulated to be responsible for the subcellular mechanism for uncoupling of the GTP binding protein to phospholipase C activation in noncardiac cells.

A wide range of species-dependent variation of the positive inotropic effects of α_1-adrenoceptor agonists (Endoh, 1982), endothelin-1 (Takanashi & Endoh, 1991) and AII is seen among mammalian species. The species-dependent variation may be largely due to difference in coupling processes, because the presence of receptors has been demonstrated in the species in which the inotropic effect was not induced by these receptor agonists (Baker & Singer, 1988; Endoh *et al.*, 1991; Takanashi & Endoh, 1991).

Finally, activation of AII receptors as well as α_1- and endothelin receptors may be involved in the development of myocardial hypertrophy (Simpson, 1985; Aceto & Baker, 1990; Ito *et al.*, 1991). Thus, receptors that stimulate phosphoinositide hydrolysis may be coupled to divergent physiological and pathophysiological signal transduction in cardiac muscle.

In summary, while the subcellular mechanism of the PIE of AII is not yet fully clarified, we have noted marked similarities among the AII (AT_1), α_1-adrenoceptor and endothelin receptor-mediated modulation of myocardial contractility and phosphoinositide hydrolysis. The present findings together with earlier experimental evidence strongly suggest that acceleration of phosphoinositide hydrolysis induced by AT_1 receptor activation may contribute to the receptor-mediated regulation of myocardial contractility.

Note added in proof

After our manuscript had been submitted, a pertinent paper by Scott *et al.*, appeared, in which it was shown that in the rabbit papillary muscle the PIE of angiotensin II was antagonized by losartan in a competitive manner (Scott, A.L., Chang, R.L.S., Lotti, V.J. & Siegl, P.K.S. (1992). Cardiac angiotensin receptors: effects of selective angiotensin II receptor antagonists, DUP 753 and PD 121981, in rabbit heart. *J. Pharmacol. Exp. Ther.*, **261**, 931–935).

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