β-Adrenoceptors potentiate α_1-adrenoceptor-mediated inotropic response in rat left atria[1]

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KEY WORDS alpha-1 adrenergic receptors; beta adrenergic receptors; heart atrium; cardiac inotropism; inositol phosphates; adrenergic receptor antagonists; adrenergic agonists

ABSTRACT

AIM: To investigate whether stimulation of β-adrenoceptor (AR) and its subtypes augment α_1-AR-evoked positive inotropic response and inositol phosphate (InsP) accumulation in isolated rat left atria. **METHODS:** Inotropic response was determined by contractile function experiment in isolated electrically driven rat left atria. ^{3}H-InsP accumulations were measured by ^{3}H-inositol incorporation and column chromatography. **RESULTS:** (1) Stimulation of α_1-AR by phenylephrine (PE) or norepinephrine (NE) in the presence of propranolol (Prop) evoked positive inotropic response and ^{3}H-InsP accumulations, while stimulation of β-AR by isoprenaline (ISO) or NE in the presence of phentolamine (Phen) only evoked positive inotropic response, but not ^{3}H-InsP accumulations. (2) Simultaneous stimulation of α_1- and β-AR by NE or ISO plus PE significantly shifted the concentration-dependent inotropic response curves and ^{3}H-InsP accumulation curves to the left and upward compared with individual α_1-AR stimulation by PE or NE in the presence of Prop. (3) In the presence of ICI118551 (selective β_2-AR antagonist) or CGP12177 (selective β_1-AR antagonist), stimulation of either β_1- or β_2-AR did not change α_1-AR-evoked inotropic response and ^{3}H-InsP accumulations. **CONCLUSION:** Stimulation of β_1-AR and β_2-AR potentiates α_1-AR-mediated positive inotropic response and InsP accumulation in isolated rat left atria.

INTRODUCTION

The sympathetic nervous system plays a critical role in regulating the heart, including contraction, gene expression, hypertrophy, and apoptosis. Natural catecholamines bind to adrenoceptors (AR) localized on the myocardial cell membranes to trigger these effects. The positive inotropic response induced by stimulation of β_1-, β_2, or α_1-AR has been well documented. But the crosstalk between α_1- and β-AR stimulation in the cardiac inotropic response is at present only partly understood. Attenuation of β-AR-mediated inotropic response by α_1-AR appears to be better understood[1-4], but the influence of β-AR on α_1-AR-mediated inotropic response could not be fully elucidated[5-8]. It is as yet unknown whether, in left atria of adult rat under simultaneous stimulation of α_1-, β_1- or/and β_2-AR, β_1- or β_2-AR stimulation can modulate the inotropic response mediated by α_1-AR. To address this question, in the present study the positive inotropic response was determined by contractile function in isolated electrically

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driven rat left atria and ^{3}H-InsP accumulation was measured by ^{3}H-inositol incorporation and column chromatography in rat heart.

MATERIALS AND METHODS

Chemicals Norepinephrine (NE), isoprenaline (ISO), phenylephrine (PE), prazosin (Praz), phentolamine (Phen), propranolol (Prop), ICI 118551, and CGP 12177 were obtained from Sigma Co; ^{3}H-inositol was from Amersham Co.

Rats Male adult Wistar rats (160-200 g, Grade II, Certificate No 013056) were used. All procedures involving rat husbandry and manipulation were in accordance with the protocol of the committee on the Ethical Aspects of Research Involving Animals of Peking University Health Science Center.

Inotropic response Rats were sacrificed by cervical dislocation, the hearts were exposed, and the left atria were rapidly removed. Inotropic response of left atria was measured as described previously[9]. Briefly, tissues were placed in Krebs' solution (in mmol/L: NaCl, 120; KCl, 5.5; $CaCl_2$, 2.5; NaH_2PO_4, 1.2; $MgCl_2$, 1.2; $NaHCO_3$, 20; dextrose, 11; and Na_2 edetic acid, 0.029) equilibrated with 95 % O_2/5 % CO_2, and maintained at 37 ºC in an organ bath with a volume of 10 mL, containing desmethylimipramine 0.1 μmol/L and normetanephrine 1 μmol/L to block the uptake of NE by nerve endings and cardiac tissues. The tissues were attached to force-displacement transducer for measurement of isometric tension and stimulated by electrical pacing (1 Hz, 5 ms, 2 times threshold voltage). A resting tension of 0.5 g was applied to all of the preparations.

In the experiments examining α_1-AR- or β-AR-mediated positive inotropic response, cumulative concentration-response curves for PE (1 nmol/L-300 μmol/L), ISO (0.1 nmol/L-1 μmol/L) or NE (1 nmol/L-300 μmol/L) in the presence of Prop or Phen were generated. The potency of the drugs was expressed pD_2, which was the negative logarithm to base 10 of the EC_{50}.

In the experiments examining the effect of β-AR stimulation on α_1-AR-mediated positive inotropic response, cumulative concentration-response curves for PE were generated first. Preparations were washed and equilibrated for 30 min, and the contractile tension for ISO (1 nmol/L) was recorded. After regulating the tension to baseline, cumulative concentration-response curves for PE were repeated.

In the experiments examining the effect of ICI 118551 or/and CGP12177, the cumulative concentration-response curves for PE were generated first. After washing and 40-min equilibration, preparations were incubated with or without ICI 118551 30 nmol/L or/and CGP 12177 30 nmol/L for 40 min. Preparations were then washed and equilibrated for 40 more min, and contractile tension for ISO (1 nmol/L) was recorded. After regulating this tension to baseline, cumulative concentration-response curves for PE were repeated.

^{3}H-InsP accumulation ^{3}H-inositol phosphate (^{3}H-InsP) accumulation in whole left atrium was measured by ^{3}H-inositol incorporation and column chromatography, as described previously[9]. Atria were incubated in Krebs' solution equilibrated with 95 % O_2/5 % CO_2 at 37 ºC for 20 min. The atria were then incubated in Krebs solution containing 37 MBq/L [^{3}H]myoinositol under 95 % O_2/5 % CO_2 for 120 min at 37 ºC. After being washed 5 times, the atria were incubated in Krebs solution containing LiCl 10 mmol/L (NaCl changed to 110 mmol/L) with or without adrenergic receptor agonists or/and antagonists under the same conditions for 120 min. Reaction was stopped by 3 washes with Krebs solution containing LiCl and addition of 0.62 mL chloroform, 0.62 mL methanol, and 0.31 mL water. The sample were homogenized and then centrifuged at 2000×*g* to separate the aqueous and organic phases. The aqueous layer was added to a column containing packed DOWEX anion exchange resin in formate form (100-200 dry mesh). Each column was washed 4 times with 10 mL myoinositol 5 mmol/L and subsequently eluted with 1 mL of ammonium formate 1 mmol/L formic acid 0.1 mmol/L into a scintillation vial; Bq was detected.

Statistical analysis Values were expressed as mean±SD. Significance was tested by the paired *t*-test or analysis of variance.

RESULTS

Roles of β- and α_1-AR in inotropic response in isolated rat left atria PE (activating α_1-AR) caused a dose-dependent positive inotropic response with a pD_2 value of (5.3±0.4) and R_{max} of (242±86) mg; NE in the presence of Prop 1 μmol/L (activating α_1-AR) showed a similar dose-response curve with a pD_2 value of (5.1±0.3) and R_{max} of (270±66) mg in isolated electrically driven left atria. However, ISO (activating β-AR) produced a curve with pD_2 value of (8.0±0.4) and R_{max} of (412±57) mg. Similarly, dose-response curve induced by NE in the presence of Phen 1 μmol/L (activating β-AR) showed a pD_2 value of (6.9±0.4)

[P<0.05 *vs* (5.1±0.3)] and R_{max} of (450±75) mg [P<0.05 *vs* (270±66) mg] (Fig 1).

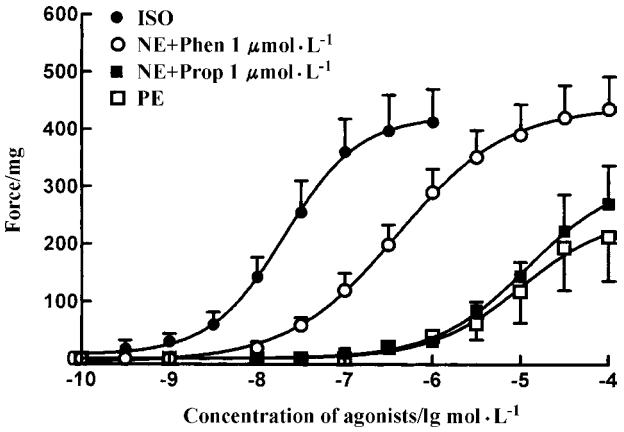

Fig 1. β- and α_1-AR mediated positive inotropic response in rat left atria. n=9. Mean±SD.

Influence of β-AR stimulation on α_1-AR mediated positive inotropic response The effect of ISO 1 nmol/L on PE-induced positive inotropic response in the left atria was studied. PE (activating α_1-AR) caused a concentration-dependent positive inotropic response with a pD_2 value of (5.3±0.4) and R_{max} of (242±86) mg. Pretreatment of preparations with ISO 1 nmol/L shifted the concentration-response curve for PE (simultaneous activation of both α_1- and β-AR) to the left and upward compared with that of PE alone. The pD_2 value was increased to (5.8±0.5) (P<0.05 *vs* 5.3±0.4), while R_{max} did not change [(252±79) mg *vs* (242±86) mg] (Fig 2).

ISO (1 nmol/L) induced 30-70 mg contractile tension in the left atria. To rule out influence of ISO 1 nmol/L increased contractile tension, 50 mg and 100 mg basal tension were added respectively before generating the concentration-response curve for PE, and then the pD_2 values (5.28±0.24 and 5.26±0.21) of the concentration-contraction response curves for PE were not changed, compared with that without adding basal tension (5.30±0.26, P>0.05, n=9). This suggested that there be no influence of increasing contractile tension caused by ISO 1 nmol/L on PE-mediated contractile response.

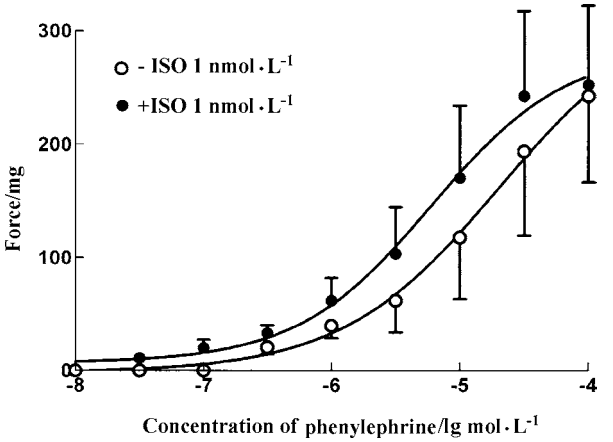

Fig 2. Effect of β-AR stimulation on α_1-AR mediated positive inotropic response in rat left atria. n=15. Mean±SD.

Effects of β-AR subtype stimulation on α_1-AR-mediated positive inotropic response To study which β-AR subtype might be involved in potentiating PE-induced positive inotropic response, the selective β_1-AR antagonist CGP12177 (30 nmol/L) and the selective β_2-AR antagonist ICI 118551 (30 nmol/L) were used as mentioned in methods. In the presence of ICI 118551 and CGP12177, respectively, ISO 1 nmol/L did not significantly affect the pD_2 values and R_{max} of the concentration-response curve for PE compared with the control (Tab 1).

Tab 1. Effects of β-AR subtype stimulation on α_1-AR mediated positive inotropic response in rat left atria. n=7. Mean±SD.

Groups	pD_2 values	Dr-1	R_{max} /mg
PE (α_1)	5.42±0.17		242±70
PE+ISO+ICI (α_1+β_1)	5.72±0.15	1.0	233±60
PE+ISO+CGP (α_1+β_2)	5.77±0.14	1.2	241±48
PE+ISO+ICI+CGP (α_1)	5.52±0.23	0.2	221±56

Intracellular signaling involved in the crosstalk between β-AR and α_1-AR Basal value of ^{3}H-InsP accumulations in rat left atria in the absence of any agonists and antagonists was 27 %±4 % (Fig 3). Stimulation of β-AR by ISO 100 nmol/L or NE 1 μmol/L in the presence of Praz 100 nmol/L did not alter basal accumulation of ^{3}H-InsP [(26 %±5 %) and (31 %±5 %) respectively, P>0.05]. While stimulation of α_1-AR by NE 1 μmol/L in the presence of Prop 10 μmol/L increased ^{3}H-InsP accumulations to 58 %±4 %, which was significantly higher than basal value (P<0.05). Moreover, NE stimulated concentration-dependent ^{3}H-InsP accumulation with a pD_2 value of 6.59±0.26 and R_{max} of (35 %±3 %) (subtracted basal value).

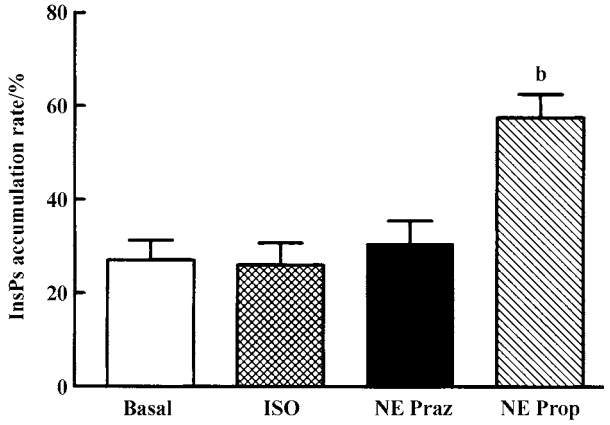

Fig 3. β- and α_1-AR mediated ^{3}H-InsP accumulation response in rat left atria. *n*=6. Mean±SD. ^{b}*P*<0.05 *vs* basal value.

Pretreatment with Prop 10 μmol/L did not change basal ^{3}H-InsP accumulations, but shifted the concentration-response curve for NE to the right. The pD_2 value was reduced to (5.71±0.16) (*P*<0.05), but R_{max} (34 %± 3 %) did not change compared with NE in the absence of Prop (Fig 4).

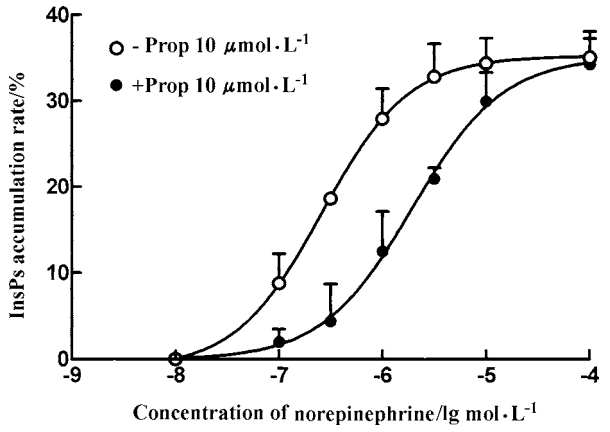

Fig 4. Effect of β-AR on α_1-AR-mediated ^{3}H-InsP accumulations in rat left atria. *n*=5. Mean±SD.

Influences of β-AR subtype stimulation on α_1-AR-mediated ^{3}H-InsP accumulations It was further investigated which β-AR subtype might be involved in this potentiating α_1-AR-mediated ^{3}H-InsP accumulations. ^{3}H-InsP accumulations for NE (simulta-neously activating α_1-AR and β-AR) were (36 %±3 %). In the presence of ICI 118551 30 nmol/L, CGP12177 30 nmol/L, and ICI 118551 30 nmol/L plus CGP12177 30 nmol/L, respectively, NE-induced ^{3}H-InsP accumulations were significantly suppressed [(26 %±3 %), (27 %± 5 %), and (24 %±2 %), respectively, *P*<0.05] compared with that induced by NE, which were not different among the three groups (Fig 5).

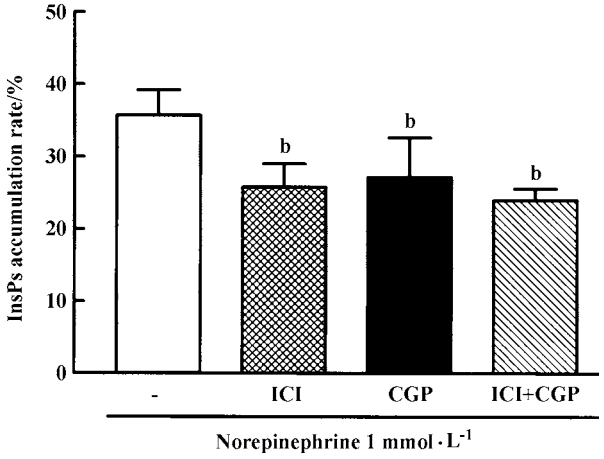

Fig 5. Effects of β-AR subtypes on α_1-AR mediated ^{3}H-InsP-accumulation response in rat left atria. *n*=6. Mean±SD. ^{b}*P*<0.05 *vs* NE group.

DISCUSSION

The critical aim of this study is to determine whether stimulation of β-AR and its subtypes could modulate the positive inotropic response mediated by α_1-AR on rat left atria. The results showed that either stimulation of α_1-AR or β-AR alone could produce the positive inotropic response as previously reported[1]. And we demonstrated that stimulation of β-AR potentiated α_1-AR-mediated positive inotropic response in isolated electrically driven rat left atria. This conclusion is based on that the low concentration of ISO (1 nmol/L, activating β-AR) did not induce the positive inotropic response but significantly shifted the positive inotropic response curve induced by PE (activating α_1-AR) to the left and upward. In order to further confirm the results of contractile function, the effects of β-AR activation on α_1-AR-induced InsP accumulation in rat left atria were studied. It is well known that the positive inotropic response to NE is mediated by β-AR and α_1-AR in adult rat left atria. β-AR-induced inotropic effect is mediated by the cyclic-AMP (cyclic adenosine monophosphate)-dependent protein kinase (PKA) pathway rather than by inositol phosphates pathway. In this study we further confirmed that stimulation of β-AR alone did not induce ^{3}H-inositol phosphates (^{3}H-InsP)

accumulation. But simultaneous stimulation of α_1- and β-AR by NE markedly shifted concentration-dependent ^{3}H-InsP accumulation curve to the left and upward compared to individual α_1-AR stimulation by NE in the presence of Prop. Thus in rat left atria, stimulation of β-AR potentiates α_1-AR-induced inositol phosphates formation, which is known to be an essential step in α_1-AR-mediated positive inotropic response[10]. This indicates that β-AR stimulation potentiates, rather than attenuates α_1-AR-induced positive inotropic response in rat left atria and the crosstalk between α_1- and β-AR signaling pathways occurs at or above the level of inositol phosphates formation, and further confirms that stimulation of β-AR potentiates the positive inotropic response mediated by activating α_1-AR at the level of intracellular signals.

Our results are apparently contrary to the other studies on isolated rat ventricular myocardium, in which β-AR attenuated the α_1-adrenergic inotropic response[1,5]. Their conclusion was drawn when comparing the results from concomitant stimulation of both α_1- and β-AR with the β-AR agonist and timolol 10 μmol/L or/and prazosin 100 nmol/L. But Curiel R *et al*[6] reported that the positive inotropic effects of α_1-adrenergic stimulation do not appear to be significantly inhibited by β-adrenergic tone by measuring the slope of the end-systolic pressure/end-diastolic dimension relationship in intact human subjects after injecting the α_1-AR agonist methoxamine. This effect was attenuated by α_1-AR blockade, but not modified by pretreatment with propranolol. The different tissue and different agonist and antagonist used in the experiments may interpret the inconsistent conclusions. Moreover, it was not supported by intracellular signals involved in β-AR mediated inhibitory effect in which simultaneous β-AR stimulation did not suppressed α_1-AR-stimulated increase of inositol-1,4,5-trisphosphate (IP_3)[11]. Furthermore, high concentration of β-AR agonist may cause β-AR desensitization and then affect the calculated magnitude of AR-induced inotropic response. This problem was considered in our study. In the pre-experiment, desensitization of β-AR was tested upon incubation of isolated rat left atria by different concentration of ISO. ISO 1 nmol/L could not induce desensitization of β-AR on mediation of positive inotropic response (data not shown).

To rule out the possibility that the tension induced by ISO 1 nmol/L maybe enhance α_1-AR sensitivity to PE, basal tension was added by mechanical method in the left atria to mimic the tension increased by ISO, and then PE-induced concentration-response curve was compared with that without additional basal tension. We found that the increase of the contractile tension caused by ISO 1 nmol/L *per se* or by additional mechanical basal tension did not affect the positive inotropic response to PE. Moreover, we removed the tension increase induced by ISO 1 nmol/L through adjusting basal tension in the experiment.

Subsequently the further question arises that which β-AR subtype is responsible for the effect of β-AR on α_1-AR-mediated positive inotropic response. To answer this question, additional experiments were performed. The results showed that the positive inotropic response to PE was not enhanced by β_1-AR stimulation or β_2-AR stimulation. Stimulation of β_1- or β_2-AR respectively also could not change the ^{3}H-InsP accumulation induced by α_1-AR. This suggests that β-AR stimulation, rather than β_1-AR stimulation alone or β_2-AR stimulation alone, potentiates α_1-AR-induced inositol phosphates formation. This suggests that there is some synergism between β_1-AR and β_2-AR. However, this hypothesis needs to be further confirmed in intracellular signaling.

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