Alpha$_{1B}$-adrenoceptor-mediated positive inotropic and positive chronotropic actions in the mouse atrium

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Abstract

Modulation of cardiac contractility by α-adrenoceptor is well known in several mammals. Mice are useful experimental animals, but α-adrenoceptor-mediated responses have been examined only in the ventricles. To determine function of α-adrenoceptors in the atrium, effects of α-adrenoceptor agonists on spontaneous contraction and electrical-field stimulation (EFS)-induced contraction were examined. In addition, expression of $\alpha_{1A,}$ $\alpha_{1B,}$ α_{1D} and β_1-adrenoceptor mRNAs were examined. In the right atrium, noradrenaline and phenylephrine caused positive inotropic and positive chronotropic actions. However, methoxamine, clonidine and xylazine caused positive inotropic actions, but contractile frequency was decreased at high concentrations. Phenylephrine-induced positive inotropic and chronotropic actions were partially decreased by propranolol, and both actions remained in the presence of propranolol were inhibited by phentolamine or prazosin. A low concentration of silodosin (<100 nM) did not but a high concentration (1 μM) decreased the phenylephrine-induced chronotropic actions. Negative chronotropic actions of clonidine and xylazine were insensitive to propranolol and phentolamine. The EFS-induced contraction of the left atrium was potentiated by noradrenaline, phenylephrine and methoxamine but was not changed by clonidine or xylazine. Propranolol partially decreased the actions of phenylephrine, and prazosin caused additional inhibition. Expression of β_1-, α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor mRNAs was found in the atrium, and the expression level of β_1-adrenoceptor was the highest. Of α_1-adrenoceptors, the expression level of α_{1B} was higher than that of α_{1A} and α_{1D}. In conclusion, α_{1B}-adrenoceptors are expressed in the mouse atrium and mediate both positive chronotropic and inotropic actions. In contrast, the α_2-adrenoceptor is not functional in the isolated atrium.

Key words

Mouse atrium, inotropic action, chronotropic action, α_1-adrenoceptor, α_{1B}-adrenoceptor.

1. Introduction

Heart contraction is regulated by both parasympathetic (acetylcholine) and sympathetic nerves (noradrenaline). Acetylcholine acts on M_2-muscarinic receptors and decreases amplitude (negative inotropic) and heart rate (chronotropic actions). On the other hand, noradrenaline causes positive inotropic and chronotropic actions by activation of β_1-adrenoceptors (Broadley, 1982; Brodde and Michel M, 1999; Coote and Chauhan, 2016, Dyavanapalli et al., 2016). However, our previous study demonstrated that M_3-muscarinic receptors mediate positive inotropic and chronotropic actions braking M_2-receptor-induced actions in the mouse atrium (Kitazawa et al., 2009). Presence of the M_3-receptor and its function prompted us to investigate the functions of non-β_1 (α)-adrenoceptors in the atrial contraction.

Some functional studies using α-adrenoceptor selective agonists in the papillary muscles, left atrium, right atrium and ventricle muscles have already indicated that activation of α_1-adrenoceptors affects cardiac contractility in the isolated rabbit, rat, cat, guinea-pig, mouse and human hearts but the dog heart did not have functional α-adrenoceptors (Hattori and Kanno, 1982; Aass et al., 1983; Bruckner et al., 1984; Ask and Stene-Larsen, 1984; Williamson and Broadley, 1987; Chess-Williams et al., 1990; Endoh et al., 1991; Tanaka et al., 1995). In addition, binding sites of [^{3}H]-prazosin in the ventricles of various animals indicated presence of cardiac α_1-adrenoceptors (Steinfath et al., 1992).

Among the animals in which function of cardiac α-adrenoceptors **have been** examined, mice are interesting because activation of α_1-adrenoceptor caused positive inotropic actions in the ventricles of young mice but caused negative inotropic actions

92 in adult mice (Tanaka et al., 1995) and the inotropic actions were different in the right
93 ventricle (negative) and left ventricle (positive) (Wang et al., 2006). **Although** cardiac
94 region (atrium and ventricle)-**dependent different** actions of α-adrenoceptor agonists
95 have been **reported** in the rat and guinea-pig (Williamson and Broadley, 1987;
96 Chess-Williams et al., 1990), there has been little study concerning the inotropic and
97 chronotropic actions of α-adrenoceptor agonists in the mouse atrium.
98 Molecular biological studies have demonstrated the **expression** of distinct genes
99 coding for three α_1-adrenoceptors, α_{1A}, α_{1B} and α_{1D}, in various animal **tissues** (Bylund
100 et al., 1994; Hieble et al., 1995). In the human heart, α_{1A} and α_{1B} ($\alpha_{1B}>\alpha_{1A}$) are
101 abundant subtypes present in cardiomyocytes and are involved in the increase of
102 myocardial contractility, whereas α_{1D} is mainly expressed in coronary arterial cells to
103 mediate vasoconstriction (Jensen et al., 2011). Recently, the expression of α_{1A} and α_{1B}
104 mRNAs has been reported **in the mouse ventricle** (Myagmar et al., 2017), but the
105 expression of the α_1-adrenoceptor subtype regulating the mouse atrial contraction have
106 not been clarified.
107 In this study, **we hypothesized that the expression of** α_1-adrenoceptors **and the**
108 actions of α-adrenoceptor agonists in the mouse atrium might be different from those in
109 the ventricles. To characterize the α-adrenoceptor-mediated inotropic and chronotropic
110 actions in the mouse atrium, we examined effects of α-adrenoceptor-selective agonists
111 and antagonists on spontaneous contraction (right atrium) and electrical field
112 stimulation (EFS)-induced contraction (left atrium). In addition, the expression of β_1
113 and α_1 (α_{1A}, α_{1B} and α_{1D})-adrenoceptor mRNAs in the right and left atria and ventricles
114 was measured by quantitative RT-PCR.
115

2. Materials and Methods

All of the experiments were performed in accordance with the institutional guidelines approved by the Animal Ethics Committee of the School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan (VH17A9).

2.1. Animals and tissue preparations

Male DDY mice, aged more than 3 months and weighing 25-35 g, were **killed** by cervical dislocation. The beating heart was isolated from each animal and immersed in warmed bubbling Krebs solution. Both the right and left atria were dissected together from ventricles and their lumen was rinsed well to remove blood. The left and right atria were separated from each other under a microscope for use in functional and molecular biological studies. For functional study, one end of the right atrium was fastened with thread to a stationary glass rod and the other end was fixed to a force displacement transducer (SEN-6102, Nihon Kohden, Tokyo) to record spontaneous contraction. To induce myocardial contraction in the left atrium, the left atrium was placed between a pair of platinum rod electrodes and suspended vertically in an organ bath. The end of the preparation was tied and connected to a force–displacement transducer. EFS (1 Hz, 2 ms in duration, 1.5 threshold voltage; Kitazawa et al., 2009) was applied by an electrical stimulator. Both muscle preparations were suspended vertically in an organ bath filled with Krebs solution (NaCl, 118 mM; KCl, 4.75 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 1.2 mM; $CaCl_2$, 2.5 mM; $NaHCO_3$, 25 mM and glucose, 11.5 mM) warmed at 37℃ and gassed with 95% O_2 + 5% CO_2 .

2.2. Experimental protocols

Right atrium: After establishment of steady spontaneous contraction, α-adrenoceptor agonists were cumulatively added to an organ bath at 5-min intervals with 10-folds increasing concentrations. The spontaneous contractions were analyzed by both amplitude of contraction from the baseline before application of the agonists (Some α-adrenoceptor agonists increased the baseline tonus.) and frequency of spontaneous contraction (heart rate, beats/min). Amplitude of the contraction was used to determine the inotropic actions and frequency of spontaneous contraction (heart rate) was used to determine the chronotropic actions. Changes in amplitude and heart rate were normalized using the control values before application of the agonists and expressed as % change in amplitude or heart rate.

Left atrium: After establishment of steady EFS-induced contraction (generally 60–70 min of equilibration time), noradrenaline and α-adrenoceptor agonists were applied cumulatively to the organ bath at 5-min intervals and their effects on the amplitude of EFS-induced contraction were observed and characterized. For determination of the effects of the receptor antagonists, the antagonist was applied about 20 min before application of the agonists.

2.3. Real-time PCR for quantitation of adrenoceptor mRNAs

We measured the mRNA expression levels of α_{1A}, α_{1B}, α_{1D}, and β_1-adrenoceptors in different regions of the heart such as the left atrium, right atrium and whole ventricle in

six mice. Total RNA was extracted from respective tissues (less than 0.1 g) with Trizol (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized from total RNA (500 ng) with a Rever Tra Ace qPCR RT Kit (Toyobo, Osaka, Japan). Real-time RT-PCR analysis was performed using a real-time PCR detector (LightCycler480: NIPPON Genetics, Tokyo, Japan) with Thunderbird qPCR mix containing SYBR Green (Toyobo). The primer sets used for detection of the four adrenoceptors were as follows: α_{1A}-adrenoceptor (Forward: CAGAGGCATGGTGCGTATCC, Reverse: ATAAAAGCCCTAGTGTCATCCCT [335 bp], in Exon2-Exon3), α_{1B}-adrenoceptor (Forward: CGGTCATCCTGGTCATGTACT, Reverse: TACAATGCCCAAGGTTTTGGC [248 bp], in Exon1-Exon2), α_{1D}-adrenoceptor (Forward: CAGGGACACAGAGTAGCAAGG, Reverse: TAGATGAGCGGGTTCACACAG [250 bp], in Exon1-Exon2), β_1-adrenoceptor (Forward: CAAGGACCCGAGTGGAAACT, Reverse: CAGAGTGAGGTAGAGGACCCA [357 bp], including the 5'-UTR). Amplification conditions were initial incubation at 95℃ for 1 min followed by 40 cycles of 95℃ for 15 s and 60℃ for 1 min. To estimate mRNA copy numbers, the normal PCR product in the agarose gel was quantified with Image J (Schneider et al., 2012) and copy numbers were determined in each sample that was serially diluted using as standards. Copy numbers are shown as 1000 copies per 100 ng total mRNA.

2.4.*Chemicals*

The following drugs were used in the experiments: noradrenaline bitartrate (Wako), phenylephrine hydrochloride (Sigma), methoxamine hydrochloride (Sigma), clonidine

hydrochloride (Sigma), xylazine hydrochloride (Wako), DL-propranolol hydrochloride (Wako), phentolamine mesylate (Novartis Pharma), prazosin hydrochloride (Sigma) and silodosin (Chemscene LLC). All of the drugs except for silodosin were dissolved in water and applied directly to an organ bath. Silodosin was dissolved in dimethyl sulfoxide (DMSO) and diluted with distilled water. The volume of application was set to less than 10% of the bath volume (200 μl). The highest concentration of DMSO was set to less than 0.01%.

2.5.Statistical analysis

The results of experiments are expressed as means± S.E.M of at least three experiments using muscle preparations from different mice. Statistical significance was assessed by Student's t-test for comparison between two groups or by analysis of variance (ANOVA) followed by Dunnet's or Tukey's test for comparison of more than three groups using Origin software (Version 7.0, Origin Lab. USA). **P** <0.05 was considered to be statistically significant.

3. Results

3.1. Effects of α-adrenoceptor agonists in the spontaneous beating right atrium

Contractile frequency (heart rate) of the right atrium was 347±13.5 beats/min (n=23). Noradrenaline (1nM-10 μM) increased the frequency. The EC_{50} value was 35±17 nM (n=4) and the maximum response was 167±7.1% (n=4) (Fig. 1A). Phenylephrine (100

212 nM – 100 μM) also increased the frequency, and the maximum response (100 μM, 161
213 ±8.4%, n=8) was comparable to that of noradrenaline, but the EC_{50} value (4.1±0.8
214 μM, n=8) was higher than that of noradrenaline. On the other hand, methoxamine did
215 not change the frequency until 10 μM but significantly decreased heart rate at 100 μM
216 (82.6±5.3%, n=6). The α_2-adrenoceptor agonists, xylazine and clonidine did not affect
217 the frequency up to10 μM but tended to decrease the frequency at 100 μM. Inhibition
218 by xylazine (32.7±5.9%, n=6) was marked compared with that by clonidine (10.5±
219 10.7 %, n=5) (Fig. 1A).
220 Noradrenaline increased the amplitude of spontaneous contraction. The inotropic
221 response reached a peak at 1 μM (139.3±16.5%, n=4) and the EC_{50} value was 222±70
222 nM (n=4). Phenylephrine also significantly increased the amplitude of spontaneous
223 contraction. The maximum response was 141.8±10.2% (n=6) and the EC_{50} value was
224 2.2±0.5 μM (n=6). Methoxamine increased the contractile amplitude, but the response
225 (129.7±8.3% at 100 μM, n=5) was weak compared with that of phenylephrine.
226 Xylazine and clonidine tended to increase the amplitude. The relative amplitudes at 10
227 and 100 μM were 141±7.5% and 121±13% for xylazine (n=6) and 122±20.6% and
228 126.5±28.2% for clonidine (n=5) (Fig. 1B).
229
230 ***3.2. Pharmacological characterization of phenylephrine-, clonidine- and***
231 ***xylazine-induced responses***
232
233 First, pharmacological properties of the chronotropic actions by the α-adrenoceptor
234 agonists were examined. The phenylephrine-induced positive chronotropic actions were
235 partially but significantly decreased by propranolol (1μM). In the presence of

propranolol, phentolamine (3 μM) or prazosin (1 μM) significantly decreased the positive chronotropic actions of phenylephrine (Fig. 2). As described above, methoxamine caused negative chronotropic actions. Propranolol did not affect the negative chronotropic actions of methoxamine (100 μM: control=82.6±5.3%, propranolol=82.5±9.6%, n=6). The combination of propranolol and phentolamine did not affect the responses to methoxamine (70±19%, n=4). The negative chronotropic actions of xylazine at 100 μM were not affected by propranolol or propranolol plus phentolamine (Fig. 3A). The responses to clonidine were also not affected by propranolol or propranolol plus phentolamine (Fig. 3B). In addition, atropine (1 μM) did not decrease the negative chronotropic actions of xylazine and clonidine (data not shown).

Next, pharmacological properties of α-adrenoceptor agonist-induced inotropic actions were examined. Propranolol decreased the positive inotropic actions of phenylephrine in ten atrial preparations. In five of ten preparations, propranolol completely abolished the positive inotropic actions (Fig. 4A, Type A). In the other five preparations, the inhibition by propranolol was partial and phenylephrine caused a significant increase even in the presence of propranolol (Fig. 4A, Type B). The relative amplitudes were 104±1.7% for 1 nM, 102±1.6% for 10 nM, 104±1.6% for 100 nM, 105±1.7% for 1 μM, 117±1.8% for 10 μM and 125±4.8% for 100 μM. Additional treatment with phentolamine or prazosin completely decreased the phenylephrine-induced inotropic actions (Fig. 4B). On the other hand, the methoxamine-induced inotropic actions were not inhibited by propranolol. The relative amplitudes of contraction in the control were 106±2.6 % for 10 μM and 129.7±8.4% for 100 μM (n=5), and those in the presence of propranolol were 108±6.3% for 10 μM

and 117.5±11.3% for 100 μM (n=4). Prazosin decreased the methoxamine-induced actions, but the inhibition did not reach statistical significance (Relative amplitudes: 97 ±3.8% for 10 μM and 89±12.5% for 100 μM, n=3).

3.3. Effects of α-adrenoceptor agonists on EFS-induced contraction of the left atrium.

EFS-induced contractions (1 Hz, 2 ms duration) were potentiated by noradrenaline (EC_{50}=597±98 nM, n=9). Phenylephrine and methoxamine also increased the amplitude of EFS-induced contraction, but the effect of phenylephrine was stronger than that of methoxamine. The maximum responses at 100 μM were 115±2.3% for methoxamine (n=5) and 143±7.9% for phenylephrine (n=11). The EC_{50} value of phenylephrine was 5.6±1.3 μM (n=9). Clonidine and xylazine sometimes caused small increases in EFS-induced contraction, but the increases were not significant (Fig. 5A).

Phenylephrine-induced positive inotropic actions were significantly attenuated by propranolol (1 μM). Prazosin (1 μM) decreased the responses to phenylephrine in propranolol-treated preparations (Fig. 5B).

3.4. Expression of β_1- and α_{1A}, α_{1B} and α_{1D} adrenoceptor mRNAs

The expression levels of β_1-adrenoceptor mRNA were significantly higher than those of the three α_1-adrenoceptor mRNAs in the left atrium, right atrium and ventricle (Fig. 6). Among the α_1-adrenoceptor subtype mRNAs, the expression levels of α_{1B} were higher than those of α_{1A} and α_{1D} in all regions of the heart (Fig. 6).

3.5. Effects of silodosin on the positive chronotropic actions of phenylephrine

Silodosin (10 nM and 100 nM) did not decrease the phenylephrine-induced positive chronotropic actions in the propranolol-treated right atrium. However, a higher concentration of silodosin (1 μM) significantly decreased the responses to phenylephrine (Fig. 7).

4. Discussion

Acetylcholine causes negative inotropic and chronotropic actions by stimulation of the M_2-muscarinic receptor, whereas, noradrenaline causes positive inotropic and chronotropic actions through activation of β_1-adrenoceptors. However, non-M_2-muscarinic receptors and non-β_1-adrenoceptors have been reported to express in the heart (Broadley, 1982; Pe´rez et al., 2006; Jensen et al., 2011; Myagmar et al., 2017). The M_3-muscarinic receptor on the myocardial endothelium causes positive inotropic and chronotropic actions to antagonize the M_2-receptor-mediated actions (Kitazawa et al., 2009). In the sympathetic nervous system, α-adrenoceptor-mediated actions have been demonstrated using isolated rat, human, rabbit, guinea-pig, mouse and dog cardiac preparations, such as the left and right atria, ventricles and papillary muscles. α-Adrenoceptors are distributed heterogeneously among species and cardiac regions and induce the species- and region-dependent inotropic and chronotropic actions (Broadley, 1982; Bruckner et al., 1984; Ask and Stene-Larsen, 1984; Williamson and Broadley, 1987; Chess-Williams et al., 1990; Endoh et al., 1991). Among animals, the

mouse is an interesting species because of the age-, experimental condition-, and region-dependent different α-adrenoceptor-mediated inotropic actions in the ventricles (Tanaka et al., 1995; Nishimura et al., 1999; Wang et al., 2006), but there has been little study concerning α-adrenoceptor-mediated inotropic and chronotropic actions in the atrium.

In the present study of the mouse atrium, noradrenaline and phenylephrine caused only positive inotropic actions (right and left atria) and positive chronotropic actions (right atrium). A part of the phenylephrine-induced actions in both atria was decreased by propranolol, indicating that phenylephrine can act on β-adrenoceptors as previously reported in the rabbit and guinea-pig papillary muscles (Sanchez-Chapula, 1981; Chess-Williams et al., 1990). The β-adrenoceptor-mediated action by phenylephrine was different from that of another α_1-adrenoceptor agonist, methoxamine. Methoxamine caused positive inotropic actions (right and left atria) but decreased frequency of the spontaneous contractions in the right atrium as previously reported (Gorelik et al., 1988). Different pharmacological actions of methoxamine and phenylephrine have already been demonstrated in the guinea-pig papillary muscles (Chess-Williams et al., 1990). Positive inotropic actions of methoxamine were not decreased by propranolol but tended to be decreased by prazosin in this study. Therefore, it is thought that methoxamine is a pure selective α_1-adrenoceptor agonist, being different from phenylephrine. Although both inotropic and chronotropic actions by phenylephrine were decreased by propranolol, the inhibition by propranolol was marked in the inotropic responses compared with the chronotropic responses, suggesting heterogeneous expression of β_1-adrenoceptors in the pacemaker and other atrial regions.

In the presence of propranolol, phentolamine and prazosin significantly decreased

both positive inotropic and chronotropic actions by phenylephrine in left and right atria, and the results indicated that the α_1-adrenoceptor mediates positive chronotropic and inotropic responses in the mouse atrium, being different from the results of previous studies demonstrating negative inotropic actions in the ventricles (Tanaka et al., 1995; Nishimura et al., 1999;Verma et al., 2003). Different α_1-adrenoceptor-mediated actions observed in the atrium and ventricle might be explained by the different expression pattern of α_{1A}, α_{1B} and α_{1D} receptor subtypes. However, the levels and pattern of expression of α_1-adrenoceptors ($\alpha_{1B} > \alpha_{1A} = \alpha_{1D}$) were almost the same in the atria and ventricles in the present study. Therefore, the opposite actions by α_1-adrenoceptor activation were not due to different expression pattern of α_1-adrenoceptor subtypes between the atrium and ventricle.

To determine subtypes of the α_1-adrenoceptor, silodosin was used in the present study. Prazosin is a non-selective antagonist for three α_1-adrenoceptor types (pK_d=9.82 for α_{1A}, 10.6 for α_{1B} and 10.1 for α_{1D}), but silodosin is a potent α_{1A}-adrenoceptor antagonist (pK_d=10.4 for α_{1A}, 8.12 for α_{1B} and 8.64 for α_{1D}, Murata et al., 1999). In the propranolol-treated mouse right atrium, the positive chronotropic actions by phenylephrine were not affected by low concentrations of silodosin (10 - 100 nM), concentrations of which are sufficient to block the α_{1A} receptor subtype, indicating that the α_{1A} is not involved in the positive chronotropic actions. However, a high concentration of silodosin (1 μM), which can act on both α_{1B} and α_{1D}-adrenoceptors, significantly decreased the responses to phenylephrine. The α_{1D}-adrenoceptor is mainly expressed in the coronary artery and α_{1B} is expressed in cardiomyocytes (Jensen et al., 2011; Myagma et al., 2017), and α_{1D}-adrenoceptors have been reported to be not involved in the positive inotropic action of phenylephrine in the rat heart (Wang et al.,

1997). Therefore, α_{1B}-receptor, not α_{1D}-receptor, is thought to be a functional α_1-adrenoceptor in the mouse atrium to induce positive chronotropic and inotropic actions. The molecular biological results showing that α_{1B} is the dominant receptor subtype in the mouse atrium support the physiological significance of α_{1B} receptor. Since an α-adrenoceptor agonist has been reported to induce negative inotropic responses by activation of the α_{1A} subtype, not the α_{1B} subtype, in the mouse ventricles (Varma et al., 2003), the different inotropic responses to an α_1-adrenoceptor agonist in the atrium and ventricle might be explained by the difference in the α_1-adrenoceptor subtype (α_{1A} and α_{1B}) mediating the actions. The opposite inotropic actions of α_{1A} and α_{1B} subtypes might be caused by different intracellular signaling pathways coupling with the respective α_{1A}- and α_{1B}-adrenoceptor subtypes (Jensen et al., 2011). However, inconsistent with the results of the present study, α_{1B}-receptor was shown not to have a significant role in the inotropic actions but indirectly to decrease the inotropic actions of α_{1A}-adrenoceptors through down-regulation of α_{1A}-adrenoceptors in a mouse Langendorff heart study (Ross et al., 2003). In the Langendorff study, pressure of the left ventricle, reflecting contraction of the left ventricle, was measured to evaluate the inotropic actions, but contraction of the isolated atrium was evaluated in this study. Therefore, different contributions of α_{1A} and α_{1B} receptors in the α_1-adrenoceptor-mediated inotropic actions are suggested to be difference in the cardiac regions examining the inotropic actions.

In the present study, positive inotropic and chronotropic actions by activation of α_{1B}-adrenoceptors in the mouse atrium were demonstrated. The α_1-adrenoceptor-mediated inotropic mechanisms might be clinically important in a case of chronic heart failure when endogenous catecholamine concentrations are elevated

and β_1-adrenoceptors are down-regulated and their inotropic actions are dysfunctional (Jensen et al., 2014). In heart failure, α_1-adrenoceptor-mediated inotropic responses in the right ventricle have been reported to shift from negative to positive actions (Wang et al., 2010), suggesting heart failure-dependent changes in α_1-adrenoceptor-mediated inotropic functions. In addition to their inotropic actions, α_1-adrenoceptors have been demonstrated to have numerous adaptive functions such as physiological hypertrophy, survival signaling, ischemic preconditioning and protection against multiple injuries (Jensen et al., 2011; 2014). Therefore, to extend the findings of the present basic pharmacological study, changes in the expression and inotropic/chronotropic functions of α_{1A}- and α_{1B}-adrenoceptors in the atrium should be compared between normal and heart failure conditions in a future study.

In the present experimental conditions, clonidine and xylazine did not cause any inotropic and chronotropic actions up to a concentration of 1 μM. Since the action of clonidine mediated by the α_2-adrenoceptor has been reported to appear at concentrations of 30 nM -100 nM (Musgrave et al., 1987), the results of the present study indicated that there were no changes in contractility of the mouse atrium caused by α_2-adrenoceptor stimulation. At high concentrations of clonidine and xylazine (10-100 μM), both agonists caused negative chronotropic actions and positive inotropic actions. Neither propranolol nor phentolamine affected the negative chronotropic actions in the right atrium. Therefore, the negative chronotropic actions were thought not to be induced by activation of α- and β-adrenoceptors. Gorelik et al. (1988) reported that the negative chronotropic action of clonidine in the mouse atrium was decreased by atropine. However, atropine did not affect the negative chronotropic action of either clonidine or xylazine in the present study. The EFS-induced contractions of the left atrium were not

potentiated by clonidine and xylazine in the present study, suggesting that clonidine and xylazine do not cause positive inotropic actions by themselves. Therefore, it is thought that the positive inotropic actions were indirect actions due to the decrease in heart rate caused by high concentrations of clonidine and xylazine. However, the mechanisms of decrease in the heart rate were not investigated in the present study.

In conclusion, α_1 adrenoceptors but not α_2 adrenoceptors, in the mouse atrium cause positive chronotropic and inotropic actions. Among the α_1 adrenoceptor subtypes, α_{1B} is a dominant subtype regulating mouse heart contractility in the normal conditions.

We have no conflict of interest.

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544 **Figure Legends**

545 Fig. 1

546 Effects of α-adrenoceptor agonists on the frequency and amplitude of the spontaneous
547 contraction in the mouse right atrium. A: Chronotropic actions of noradrenaline (Nor,
548 ◆), phenylephrine (Pheny, ●), methoxamine (Meth, ○), clonidine (Clon, ▲) and
549 xylazine (Xyl, △). B: Inotropic actions of noradrenaline (Nor,◆), phenylephrine
550 (Pheny, ●), methoxamine (Meth, ○), clonidine (Clon, ▲) and xylazine (Xyl, △).
551 The ordinate axis shows relative changes in heart rate (A) and in amplitude (B) of
552 spontaneous contraction. The heart rate and amplitude of spontaneous contraction in the
553 absence of agonists were considered as 100%. The abscissa axis shows the
554 concentration of each agonist (LogM). Each symbol indicates the mean±**S.E.M** of
555 more than four experiments.

556

557 Fig. 2

558 Effects of propranolol, phentolamine and prazosin on the positive chronotropic actions
559 of phenylephrine in the spontaneous beating right atrium.
560 Symbols indicate positive chronotropic actions of phenylephrine in the absence (control,
561 ●) and presence of propranolol (Prop, 1 μM, ○), propranolol + phentolamine (Phent,
562 3 μM, △) and propranolol + prazosin (Praz, 1 μM, □). The ordinate axis shows
563 relative changes in heart rate (%). The abscissa axis shows concentration of
564 phenylephrine (LogM). Each symbol indicates the mean±**S.E.M** of four to eight
565 experiments. #; P<0.05 compared with control preparations.*; **P**<0.05, ****; P<0.01**
566 compared with propranolol-treated preparations.

567

Fig. 3

Chronotropic actions of xylazine and clonidine in the spontaneously beating right atrium. Each symbol indicates chronotropic actions of xylazine (A) and clonidine (B) in the absence (control, ■) and presence of propranolol (1 μM, ●) and propranolol (1 μM) + phentolamine (1 μM) (▲). The ordinate axis shows relative changes in heart rate (%). The abscissa axis shows concentrations of clonidine and xylazine (LogM). Each symbol indicates the mean±**S.E.M** of at least three experiments.

Fig. 4

Effects of propranolol, phentolamine and prazosin on the positive inotropic actions of phenylephrine in the spontaneous beating right atrium.

A: Positive inotropic actions of phenylephrine in the absence (control, ●) and presence of propranolol (1 μM, ○, n=10). In 5 of 10 preparations, propranolol completely abolished the responses of phenylephrine (Type B, △). However, propranolol partially decreased the phenylephrine-induced positive inotropic actions in the other 5 preparations (Type A, ▲). B: Positive inotropic actions of phenylephrine in the presence of propranolol (Type A, ●) were decreased by phentolamine (Phent. 3 μM, ○) or prazosin (Praz, 1 μM, △). The ordinate axis shows relative changes in contraction amplitude (%). The abscissa axis shows concentrations of phenylephrine (LogM). Each symbol indicates the mean±**S.E.M** of more than four experiments. **##; P<0.01, ###; P<0.001** compared with control preparations.*; **P<0.05, **; P<0.01, ***; P<0.001** compared with propranolol-treated preparations.

Fig. 5

592 Positive inotropic effects of α-adrenoceptor agonists on EFS-induced contraction of the
593 left atrium. A: The symbols indicate concentration-response curves for noradrenaline
594 (Nor,◆), phenylephrine (Pheny, ●), methoxamine (Meth, ○), clonidine (Clon, ▲)
595 and xylazine (Xyl, △) in the electrically stimulated left atrium. B: Effects of
596 propranolol (Pro, 1 μM ○) and prazosin (Praz, 1 μM △) on the
597 phenylephrine-induced positive inotropic actions in the left atrium (●). Each symbol
598 indicates the mean±**S.E.M** of more than four experiments. #; **P**<0.05 compared with
599 control preparations.*; **P**<0.05, ****; P<0.01, ***; P<0.001** compared with
600 propranolol-treated preparations.
601
602 Fig.6
603 Comparison of the expression levels of four adrenoceptor mRNAs in the mouse heart.
604 Each figure shows the expression of α_{1A}, α_{1B}, α_{1D} and β_1-adrenoceptor mRNAs in the
605 left atrium (A), right atrium (B) and ventricle of the mouse (C). The abscissa axis shows
606 the expression levels of adrenoceptor mRNAs (1,000 copies/100 ng total RNA). Each
607 column indicates the mean±**S.E.M** of six experiments. *****; P<0.001 compared with**
608 **the expression level of β_1-adrenoceptor mRNAs.**
609
610 Fig. 7
611 Effects of silodosin on the positive chronotropic action of phenylephrine in
612 propranolol-treated right atrium. The symbols show the concentration-response curves
613 for phenylephrine in the absence (●) and presence of an increasing concentration of
614 silodosin (10nM: ○, 100 nM: △, 1000 nM: □). The ordinate axis shows relative
615 changes in heart rate (%). The abscissa axis shows concentrations of phenylephrine

616 (LogM). Each symbol indicates the mean±**S.E.M** of more than four experiments. ***;**

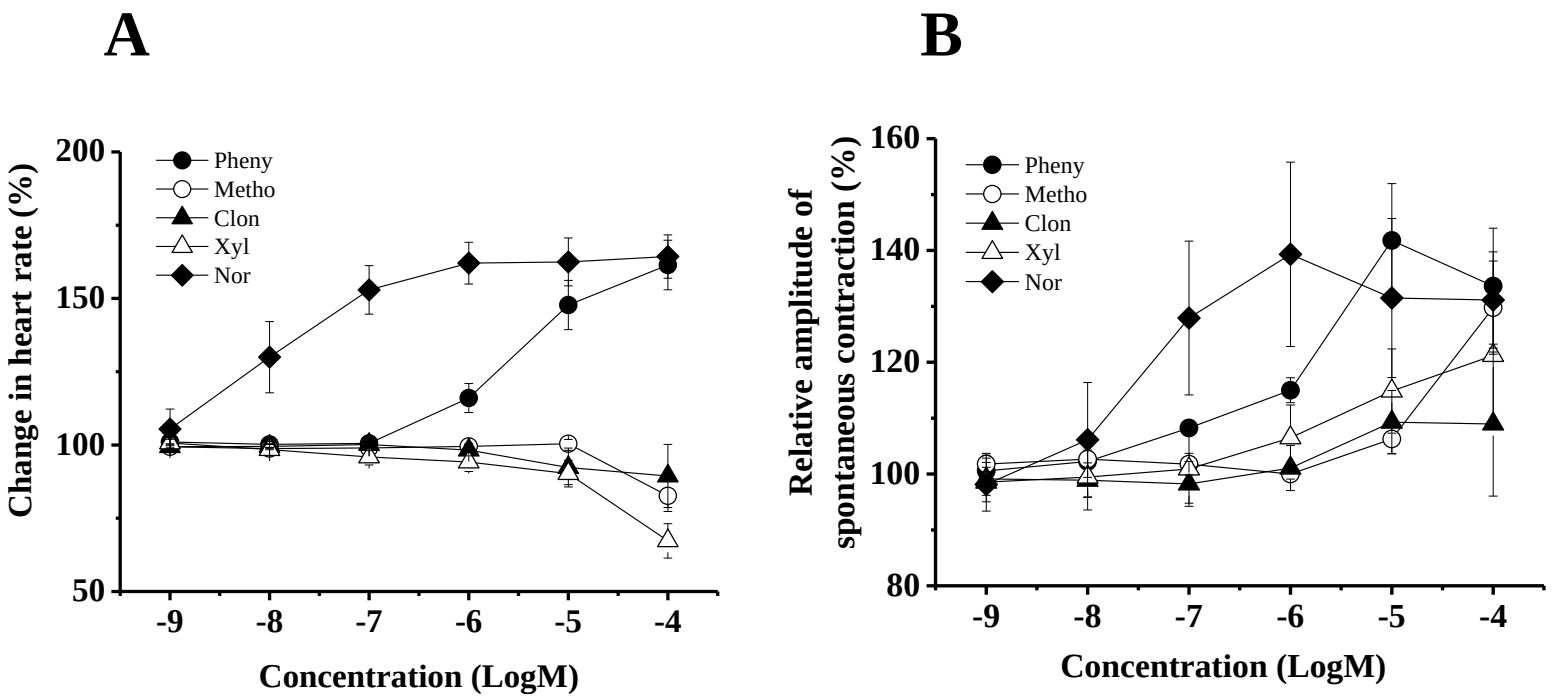
617 **P<0.05, **; P<0.01** compared with the response before silodosin treatment.

618

619

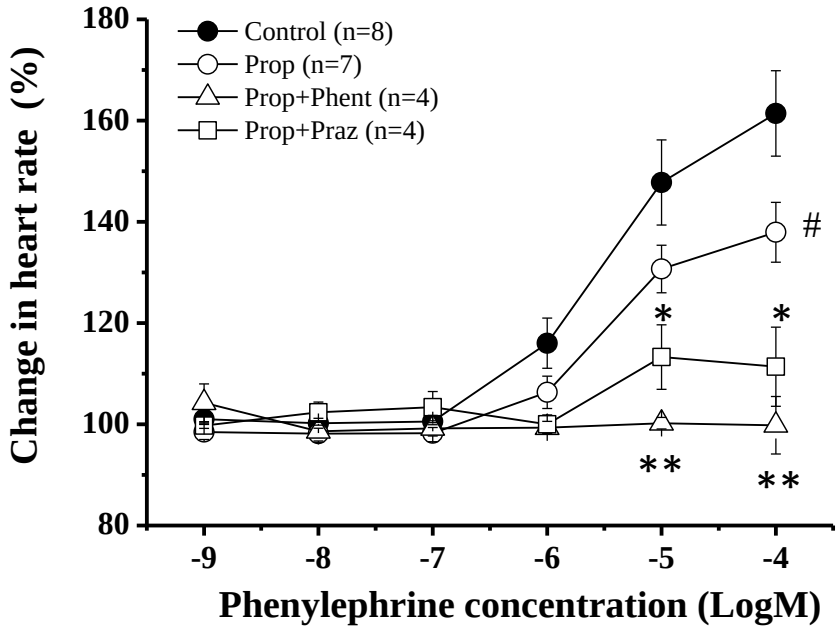
Figure

Fig.1


A
B
Change in heart rate (%)
Relative amplitude of spontaneous contraction (%)
Concentration (LogM)
Concentration (LogM)
Pheny
Metho
Clon
Xyl
Nor
200
150
100
50
160
140
120
100
80
-9
-8
-7
-6
-5
-4

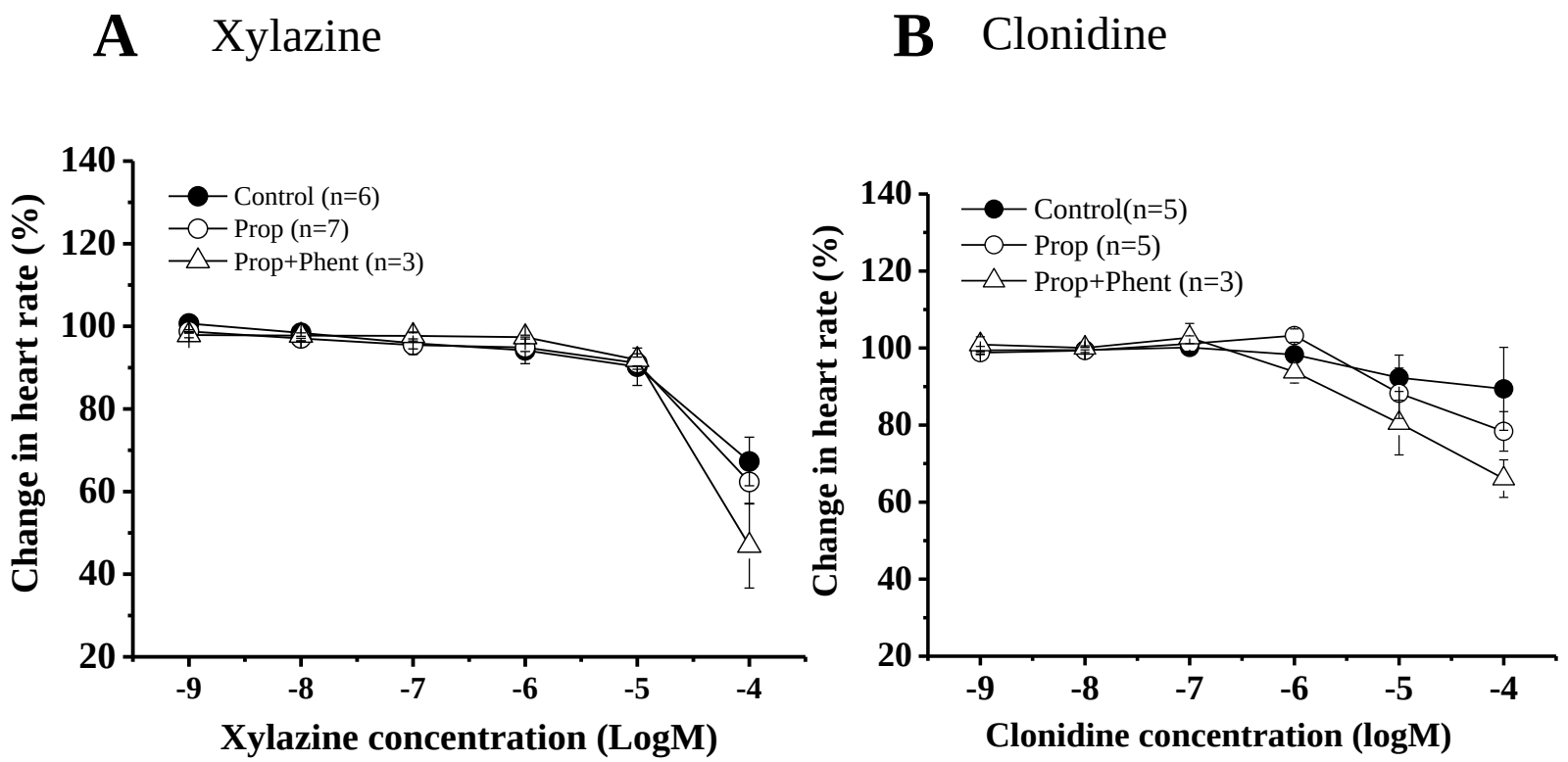
Figure

Fig.2


Control (n=8)
Prop (n=7)
Prop+Phent (n=4)
Prop+Praz (n=4)
Change in heart rate (%)
180
160
140
120
100
80
-9
-8
-7
-6
-5
-4
Phenylephrine concentration (LogM)
#
*
*
**
**

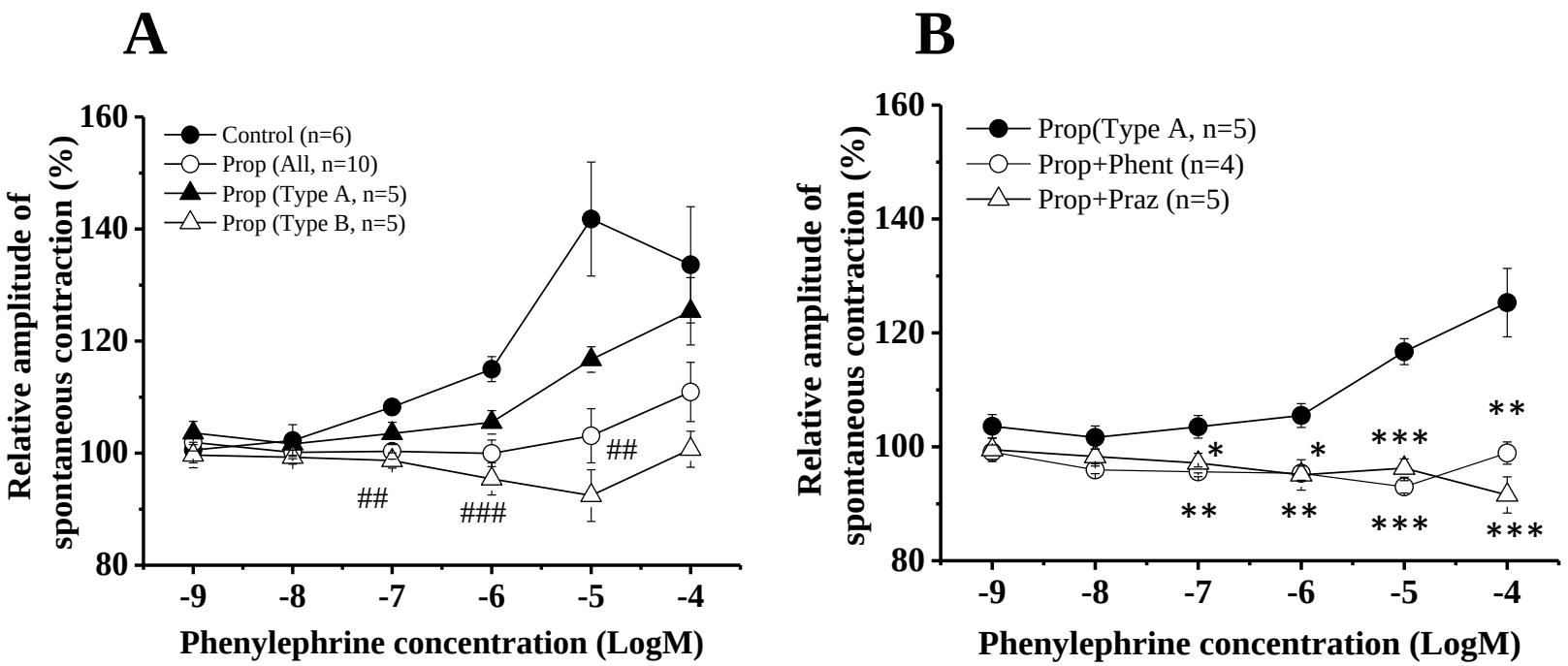
Figure

Fig.3


A
Xylazine
Control (n=6)
Prop (n=7)
Prop+Phent (n=3)
Change in heart rate (%)
140
120
100
80
60
40
20
-9
-8
-7
-6
-5
-4
Xylazine concentration (LogM)
B
Clonidine
Control(n=5)
Prop (n=5)
Prop+Phent (n=3)
Change in heart rate (%)
Clonidine concentration (logM)

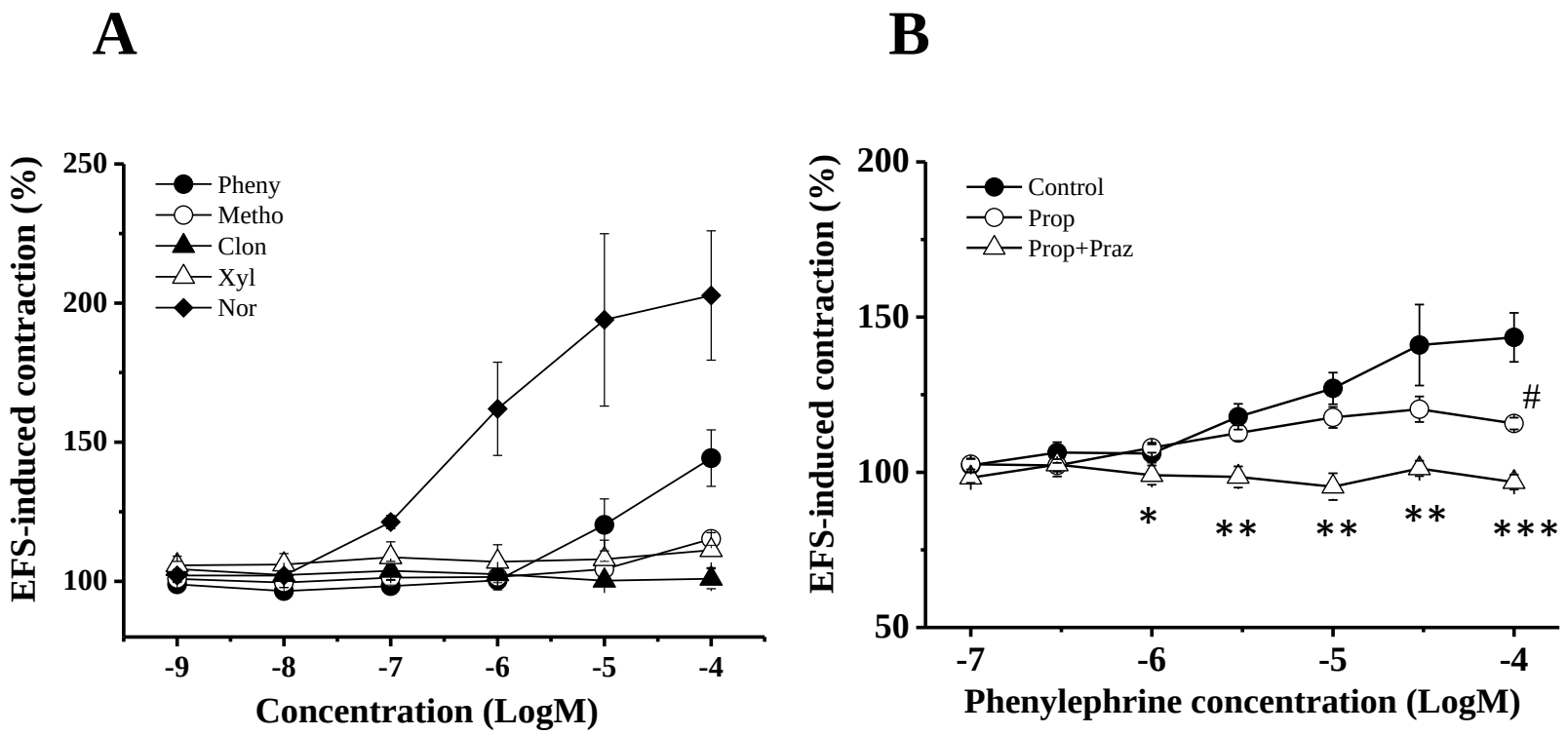
Figure

Fig.4


A
Relative amplitude of spontaneous contraction (%)
160
140
120
100
80
Control (n=6)
Prop (All, n=10)
Prop (Type A, n=5)
Prop (Type B, n=5)
##
###
##
-9
-8
-7
-6
-5
-4
Phenylephrine concentration (LogM)
B
Relative amplitude of spontaneous contraction (%)
160
140
120
100
80
Prop(Type A, n=5)
Prop+Phent (n=4)
Prop+Praz (n=5)
*
**
*
**
***
***
**
***
-9
-8
-7
-6
-5
-4
Phenylephrine concentration (LogM)

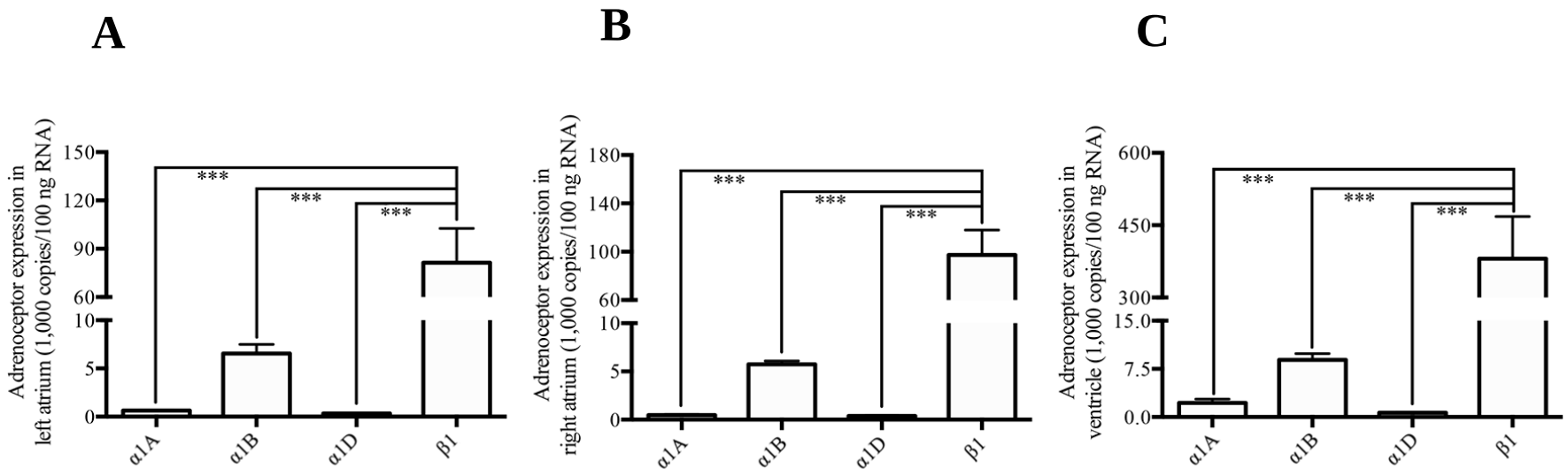
Figure

Fig.5


A
B
EFS-induced contraction (%)
Concentration (LogM)
Pheny
Metho
Clon
Xyl
Nor
250
200
150
100
-9
-8
-7
-6
-5
-4
EFS-induced contraction (%)
Phenylephrine concentration (LogM)
Control
Prop
Prop+Praz
200
150
100
50
-7
-6
-5
-4
*
**
**
**
***
#

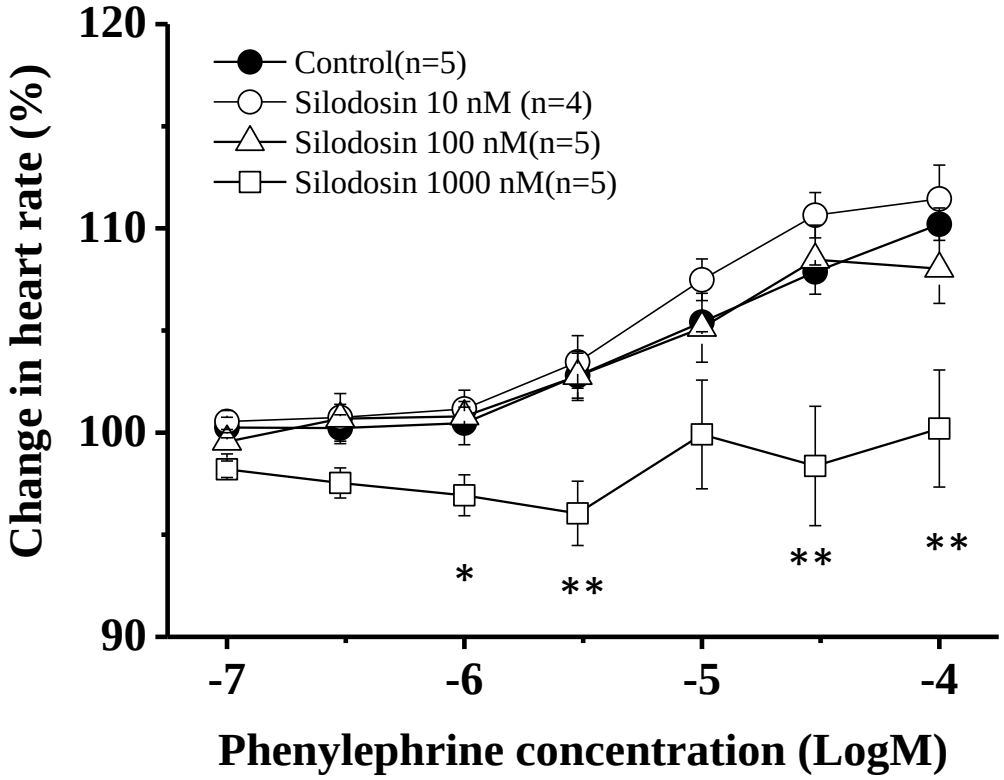
Figure

Fig.6


A
B
C
Adrenoceptor expression in left atrium (1,000 copies/100 ng RNA)
Adrenoceptor expression in right atrium (1,000 copies/100 ng RNA)
Adrenoceptor expression in ventricle (1,000 copies/100 ng RNA)
***
α1A
α1B
α1D
β1

Figure

Fig.7


Control(n=5)
Silodosin 10 nM (n=4)
Silodosin 100 nM(n=5)
Silodosin 1000 nM(n=5)
Change in heart rate (%)
120
110
100
90
-7
-6
-5
-4
Phenylephrine concentration (LogM)
*
**
**
**