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Short communication

α_{1D}-Adrenoceptors play little role in the positive inotropic action of phenylephrine

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Abstract

This study was designed to determine if the positive inotropic action of α_1-adrenergic stimulation in rat heart is mediated via α_{1D}-adrenoceptors. Isolated left atrial and papillary muscle were suspended in oxygenated Krebs-Henseleit buffer (37°C) containing 1 μM nadolol and paced at 3.0 Hz. Isometric tension was continuously monitored. Cumulative concentration-response curves for phenylephrine were obtained in the presence and absence of BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspirol[4.5]decane-7,9-dione), a selective competitive α_{1D}-adrenoceptor antagonist. BMY 7378 at concentrations up to 30 nM did not significantly affect the positive inotropic response to phenylephrine. In contrast, as reported by other investigators, α_{1D}-adrenoceptor-selective concentrations of this antagonist (3 and 10 nM) did elicit a concentration-dependent right-ward shift in the vasoconstrictor response to phenylephrine in rat abdominal aorta. These data suggest that α_{1D}-adrenoceptors do not play a major role in the positive inotropic action of α-adrenoceptor stimulation in rat cardiac muscle.

Keywords: α-Adrenoceptor, subtype; Atrial muscle; Papillary muscle; BMY 7378; Contractility; (Rat)

1. Introduction

Early pharmacological evidence suggested that there were two subtypes of α_1-adrenoceptors (classified as α_{1A} and α_{1B}), and both were shown to be present in the heart (Gascon et al., 1993; Lazou et al., 1994; Michel et al., 1994). However, continued studies identified three α_1-adrenoceptor subtypes (Cotecchia et al., 1988; Lefkowitz and Caron, 1990; Perez et al., 1991, 1994; Price et al., 1994; Blue et al., 1995), and according to standardized nomenclature recommended by the International Union of Pharmacology (Hieble et al., 1995) these three subtypes were designated as α_{1A}, α_{1B} and α_{1D}. α_{1A}-Adrenoceptors possess a high affinity for WB4101 (*N*-[2-(2,6-dimethoxyphenoxy)ethyl]-2,3-dihydro-1,4-benzodioxin-2-methanamine), 5-methylurapidil and (+)-niguldipine, whereas α_{1B}-adrenoceptors are selectively inactivated by chloroethylclonidine (CEC) (Bylund et al., 1994). BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl-8-azaspirol[4.5]-decane-7,9-dione) is a highly selective α_{1D}-adrenoceptor antagonist (Piascik et al., 1995; Goetz et al., 1995).

Previous studies in our laboratory and others have suggested that the positive inotropic effects of α_1-adrenoceptor stimulation in rat and rabbit myocardium are mediated by both α_{1A}- and α_{1B}-adrenoceptor subtypes (Endoh et al., 1992; Michel et al., 1994; Williamson et al., 1994a,b; Yu and Han, 1994; Deng et al., 1996). The mRNA for the α_{1D}-adrenoceptor can be detected in rat heart (see Graham et al., 1996); however, the relative contribution of the α_{1D}-adrenoceptor to the inotropic response has not been determined. Thus, current experiments examined effects of BMY 7378 on the concentration-dependent actions of phenylephrine in left atrial and papillary muscle isolated from rat hearts. Results were compared to data from rat abdominal aorta since it was recently reported that α_{1D}-adrenoceptors play a major role in regulating the contractile responses to α_1-adrenoceptor agonists in this tissue.

2. Materials and methods

All protocols in this study were approved by the Institutional Animal Care and Use Committee at the University

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of Arkansas for Medical Sciences and were in accordance with the *Guide for the Use of Laboratory Animals* put forth by the U.S. Department of Health and Human Services.

2.1. Isolated cardiac preparations

Male, Sprague-Dawley rats (300–400 g) were anesthetized. After thoracotomy, hearts were removed and immediately perfused through the aorta with a Krebs-Henseleit solution of the following composition (in mM): 118.0 NaCl, 27.1 $NaHCO_3$, 3.7 KCl, 1.4 $CaCl_2$, 1.2 $MgCl_2$, 1.0 KH_2PO_4, and 11.1 glucose. This solution was buffered to pH 7.4 by saturation with 95% O_2/5% CO_2 gas and maintained at 37°C.

After the heart was free of residual blood, left atrial and papillary muscle were dissected and hung vertically in muscle baths containing the oxygenated solution described above. Nadolol (1 μM; a β-adrenoceptor antagonist) was included in the buffer to prevent potential effects of endogenous catecholamines and to insure that phenylephrine was not acting via β-adrenoceptor stimulation. Preparations were paced via platinum contact electrodes at a frequency of 3.0 Hz by 1.0 ms square wave pulses set at 125% threshold voltage. Force of resting tension and isometric contraction were monitored by force-displacement transducers and recorded continuously on a polygraph. A length-tension relationship was determined for each preparation, and resting tension was subsequently maintained at that which elicited 90% of maximum observed contractile force. Tissues were equilibrated for 60 min, during which time the bathing solution was changed every 15 min.

These preparations were used to examine the effects of BMY 7378 on the inotropic responses to phenylephrine, an α_1-adrenoceptor agonist. BMY 7378 (10, 30 or 100 nM) or control solvent (distilled water) was added to the bathing solution immediately after the equilibration period and remained in the solution for the duration of the experiment. Concentration-dependent inotropic actions of phenylephrine (10^{-7}–10^{-4} M) were monitored via cumulative addition; each subsequent concentration of agonist was added to the bathing solution only after the preparations achieved a steady state response to the previous dose.

2.2. Isolated vascular preparations

Abdominal aortae were isolated from the same animals described above. After being cleaned of fat and connective tissue, the vessels were cut into rings (3–4 mm in length) and mounted between stainless steel hooks (22 gauge) in tissue baths (37°C) containing the oxygenated Krebs-Henseleit solution. The upper hooks were connected to force-displacement transducers, and isometric tension was recorded continuously on a polygraph. The rings were maintained at 1.5 g and allowed to equilibrate for 120 min during which time the contractile response to 70 mM extracellular KCl was examined every 15 min; the maximum tension elicited by this KCl-induced depolarization became constant after 90–105 min.

After stabilization, the ring preparations were used to monitor vasoconstrictor responsiveness to phenylephrine in the presence and absence of 3 and 10 nM BMY 7378. The antagonist or control solvent (distilled water) was added to the bathing solution immediately after the equilibration period and remained in the solution for the duration of the experiment. Concentration-dependent actions of phenylephrine (10^{-9}–10^{-4} M) were monitored via cumulative addition.

2.3. Materials

BMY 7378 dihydrochloride was purchased from Research Biochemicals International (Natick, MA, USA). L-Phenylephrine hydrochloride and nadolol were purchased from Sigma (St. Louis, MO, USA). All other chemicals were reagent grade.

2.4. Statistical analysis

Data were analyzed by analysis of variance (ANOVA) with Duncan's multiple range test or by Student's *t*-test where appropriate. EC_{50} (the concentration of agonist eliciting a half-maximal response) values were obtained by graphical evaluation of individual concentration-response curves. All data are presented as mean ± S.E.M. Criterion for significance was a P value less than 0.05.

3. Results

3.1. Effects of BMY 7378 on phenylephrine responses in atrial and papillary muscle

Experiments examined effects of 10, 30 and 100 nM BMY 7378 on the positive inotropic responses to phenylephrine in left atrial and papillary muscle. The basal developed tension recorded immediately before addition of phenylephrine was not altered by BMY 7378 (left atria: 0.28 ± 0.04, 0.20 ± 0.05, 0.26 ± 0.05 and 0.21 ± 0.04 g in the absence and presence of 10, 30 and 100 nM BMY 7378, respectively; papillary muscle: 0.55 ± 0.06, 0.48 ± 0.06, 0.63 ± 0.06 and 0.48 ± 0.06 g in the absence and presence of 10, 30 and 100 nM BMY 7378). Similarly, the maximum increase in contractile force elicited by phenylephrine was not significantly affected by BMY 7378 (left atria: 0.74 ± 0.06, 0.60 ± 0.14, 0.54 ± 0.10 and 0.72 ± 0.12 g in the absence and presence of 10, 30 and 100 nM BMY 7378, respectively; papillary muscle: 0.36 ± 0.05, 0.31 ± 0.09, 0.36 ± 0.05 and 0.29 ± 0.04 g in the absence and presence of 10, 30 and 100 nM BMY 7378). The two lower concentrations of the antagonist (10 and 30 nM) did not significantly shift the phenylephrine concentration-re-

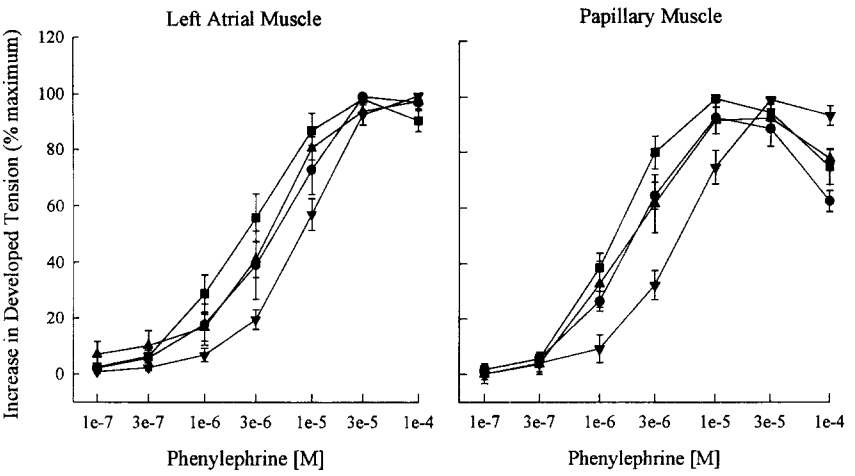

Fig. 1. Effects of phenylephrine on isometric developed tension in isolated rat left atrial (left panel) and papillary muscle (right panel) in the absence (squares) and presence of 10 nM (●), 30 nM (▲) and 100 nM (▼) BMY 7378. Preparations were bathed in an oxygenated Krebs-Henseleit buffer (37°C) containing 1 μM nadolol and paced at 3.0 Hz. Concentration-response curves were obtained by cumulative addition. Values on the ordinate are presented as a percentage of the maximum response to phenylephrine in each preparation. Vertical bars represent S.E.M. Each group represents an '*n*' of 5–6.

sponse curve; however, 100 nM BMY 7378 shifted the curve to the right (Fig. 1) and increased the EC_{50} values for the α-adrenoceptor agonist (log EC_{50} values – left atria: -5.62 ± 0.12, -5.40 ± 0.16, -5.43 ± 0.09 and -5.08 ± 0.07 in the absence and presence of 10, 30 and 100 nM BMY 7378, respectively; papillary muscle: -5.86 ± 0.05, -5.70 ± 0.06, -5.77 ± 0.09 and -5.31 ± 0.06 in the absence and presence of 10, 30 and 100 nM BMY 7378).

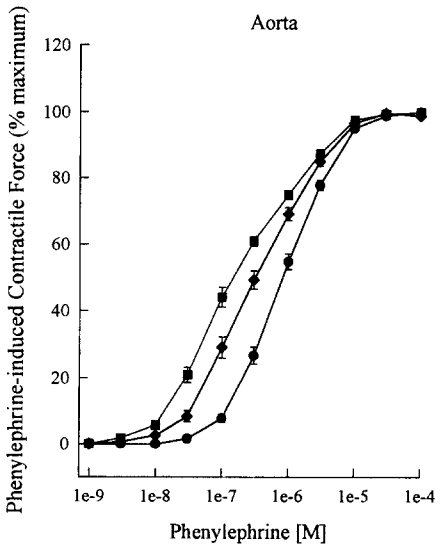

Fig. 2. Effects of phenylephrine on isometric contractile force in rat abdominal aorta in the absence (■) and presence of 3 nM (◆) and 10 nM (●) BMY 7378. Preparations were bathed in an oxygenated Krebs-Henseleit buffer (37°C) containing 1 μM nadolol. Concentration-response curves were obtained by cumulative addition. Values on the ordinate are presented as a percentage of the maximum response to phenylephrine in each preparation. Vertical bars represent S.E.M. Each group represents an '*n*' of 5–6.

3.2. Effects of BMY 7378 on phenylephrine responses in abdominal aorta

Concentration-response curves for phenylephrine in rat abdominal aorta were compared in the presence and absence of 3 and 10 nM BMY 7378. The maximum contractile force elicited by phenylephrine was not significantly altered in the presence of BMY 7378 (2.29 ± 0.10, 2.02 ± 0.11 and 2.47 ± 0.24 g in the absence and presence of 3 and 10 nM BMY 7378, respectively); however, the antagonist did shift the curve to the right (Fig. 2). Log EC_{50} values for phenylephrine were increased in a concentration-dependent manner from -6.86 ± 0.07 in the absence of BMY 7378 to -6.51 ± 0.07 and -6.09 ± 0.04 in the presence of 3 and 10 nM BMY 7378, respectively.

4. Discussion

Results of the present study suggest that α_{1D}-adrenoceptors play little role in the positive inotropic action of phenylephrine in rat heart. As reported by other investigators (Piascik et al., 1995; Testa et al., 1995; Villalobos-Molina and Ibarra, 1996), α_{1D}-adrenoceptor-selective concentrations of BMY 7378 (3 and 10 nM) elicited a concentration-dependent rightward shift in the dose-response curve for phenylephrine-induced vasoconstrictive actions in rat abdominal aorta. However, the same concentrations of the antagonist did not significantly affect the dose-response curve in atrial and papillary muscle. The first statistically significant shift in the inotropic response to phenylephrine in cardiac muscle was observed at 100 nM BMY 7378 with no effect seen at 30 nM.

The observed antagonist action of BMY 7378 in aorta corresponds closely with reported K_i values ($\approx$ 2–6 nM)

that were determined by others in binding studies using the cloned rat α_{1D}-adrenoceptor expressed in COS-1, COS-7 and rat-1 fibroblast cell membranes (Piascik et al., 1995; Testa et al., 1995; Goetz et al., 1995). In contrast, reported K_i values for BMY 7378 binding to cloned rat and bovine α_{1A}-adrenoceptors range from approximately 500 to 800 nM, while values for the cloned hamster α_{1B}-adrenoceptor range from 400 to 650 nM. These values for the α_{1A} and α_{1B} subtypes are somewhat higher than that estimated from the BMY 7378-induced antagonism of the response to phenylephrine in rat cardiac muscle where more than a 2-fold increase in the EC_{50} value for agonist was produced by 100 nM BMY 7378. This suggests that α_{1D}-adrenoceptors may play a small role in the positive inotropic action. If one assumes (1) that the K_i values of BMY 7378 for the α_{1D}- and α_{1A}/α_{1B}-adrenoceptors are 2 and 600 nM, respectively, in rat cardiac muscle, and (2) that the data can be fit by the sum of two Michaelis-Menten equations, current results suggest that the α_{1D}-adrenoceptor subtype may contribute 5–10% of the positive inotropic response. The presence of α_{1D}-adrenoceptors in rat heart is supported by studies showing that the corresponding mRNA can be identified in this tissue (Graham et al., 1996).

Alternatively, it is possible that α_{1D}-adrenoceptors have no role in the inotropic response to phenylephrine. The difference between present data and reported K_i values (Piascik et al., 1995; Testa et al., 1995; Goetz et al., 1995) may result from the possibility that binding to cloned α_1-adrenoceptors in isolated membranes is not totally representative of binding in whole tissue. Furthermore, species differences may be involved. Studies by Goetz et al. (1995) showed that BMY 7378 binds to cloned hamster and human α_{1B}-adrenoceptors with K_i values of approximately 600 and 60 nM, respectively. Villalobos-Molina and Ibarra (1996) recently reported that the K_i value for the BMY 7378-induced antagonism of the response to methoxamine in rat caudal artery is approximately 100–130 nM and concluded that the vasoconstrictor response in this vessel is mediated primarily via the α_{1A}-adrenoceptor subtype.

In summary, when considered in light of previous work done by our laboratory (Williamson et al., 1994a,b) and others (Endoh et al., 1992; Michel et al., 1994; Yu and Han, 1994; Deng et al., 1996), present data suggest that the effects of α_1-adrenoceptor stimulation on contractility in rat heart are mediated primarily by the α_{1A}- and α_{1B}-adrenoceptors with a minor, if any, contribution of the α_{1D}-adrenoceptor subtype.

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