

Original Article

Positive inotropic effect of PHR0007 (2-(4-(4-(Benzyloxy)-3-methoxybenzyl)piperazin-1-)-N-(1-methyl-4,5-dihydro[1,2,4]triazolo[4,3-a]quinolin-7-yl)acetamide) on atrial dynamics in beating rabbit atria

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ABSTRACT: The aim of the present study was to examine the positive inotropic effects and mechanism of action of PHR0007 (2-(4-(4-(Benzyloxy)-3-methoxybenzyl)piperazin-1-)-N-(1-methyl-4,5-dihydro[1,2,4]triazolo[4,3-a]quinolin-7-yl)acetamide) on beating rabbit atria. Atria were obtained from New Zealand white rabbits, and experiments performed using a perfused beating atrial model. The effects of PHR0007 (1, 30, or 100 $\mu\text{mol/L}$), and of the protein kinase inhibitors, staurosporine (1.0 $\mu\text{mol/L}$) or H-89 (10 $\mu\text{mol/L}$), plus PHR0007 (30 $\mu\text{mol/L}$), on atrial pulse pressure and stroke volume were analyzed. PHR0007 significantly increased atrial pulse pressure and atrial stroke volume in beating rabbit atria compared with control baseline levels. These effects of PHR0007 were completely blocked by pretreatment with either staurosporine (a nonspecific protein kinase inhibitor) or H-89 (a cAMP-dependent protein kinase A inhibitor). In addition, 3-isobutyl-1-methylxanthine (IBMX), a non-specific inhibitor of phosphodiesterases (PDEs), completely blocked the positive inotropic effect of PHR0007 on atrial dynamics, but forskolin, an activator of adenylyl cyclases (AC), failed to modulate PHR0007-induced increases in atrial pulse pressure and stroke volume. In conclusion, these data suggest that PHR0007 produces a positive inotropic effect in rabbit atria via the PDE-cAMP-PKA signaling pathway.

Keywords: Inotropic effect, PHR0007, milrinone, protein kinase A (PKA), cAMP, PDE

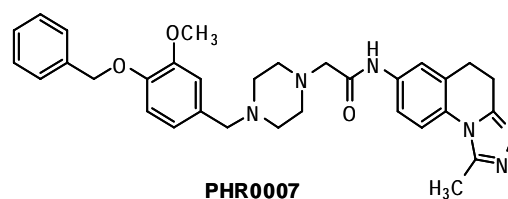
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1. Introduction

Medical treatment for severe heart failure commonly includes the use of digitalis glycosides, vasodilators, and catecholamines. However, these drugs exhibit only inotropic effects or only vasodilatory effects, while experimentally, a drug tolerance or arrhythmogenesis can also occur. Milrinone, a phosphodiesterase 3 (PDE3) inhibitor, is a potent cardiac bipyridine with inotropic and vasodilator properties (1,2), and is generally used for short-term management of heart failure (3). To develop more potent positive inotropic agents with less side effects, a series of positive inotropic agents were synthesized, and their biological activities were examined (4,5). Among the drugs tested, PHR0007 (2-(4-(4-(Benzyloxy)-3-methoxybenzyl)piperazin-1-)-N-(1-methyl-4,5-dihydro[1,2,4]triazolo[4,3-a]quinolin-7-yl)acetamide) exhibited a moderate positive inotropic activity.



However, to the best of our knowledge, a detailed study of the positive inotropic activity of PHR0007 within the atria and the mechanisms of action has not been reported. Herein, we examined the positive inotropic effect of PHR0007 on atrial dynamics and the mechanism of action in beating rabbit atria.

2. Materials and Methods

2.1. Preparation of rabbit perfused beating atria

Atria were obtained from New Zealand white rabbits. The mean atrial weight was 193.1 ± 7.4 mg.

An isolated perfused atrial preparation was used as previously described (7). Briefly, hearts were removed from rabbits and the left atria were dissected free. A calibrated transparent atrial cannula containing two small catheters was inserted into the left atrium. The cannulated atrium was transferred to an organ chamber and perfused immediately with HEPES buffer solution (1.25 mL/min) at 34°C. The perfusate contained 0.1% bovine serum albumin, 118 mmol/L NaCl, 4.7 mmol/L KCl, 2.5 mmol/L CaCl₂, 1.2 mmol/L MgCl₂, 25 mmol/L NaHCO₃, 10.0 mmol/L glucose, and 10.0 mmol/L HEPES (pH adjusted to 7.4 with NaOH). Within 2-3 sec after the perfused atrium was set up, transmural electrical field stimulation with a luminal electrode was started at 1.5 Hz (duration 0.3 msec; voltage 30-40 V). The changes in atrial pulse pressure were measured by an electrophysiograph, and changes in atrial stroke volume were monitored by reading the lowest levels of the water column in the calibrated atrial cannula during end diastole (6,7).

2.2. Drugs

PHR0007 was provided by Yanbian University College of Pharmacy. Milrinone, staurosporine, H-89, IBMX (3-isobutyl-1-methylxanthine) and forskolin were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Experimental protocol

Atria were perfused for 60 min to stabilize atrial pulse pressure and atrial stroke volume, and the atria were paced at 1.5 Hz. The control period was followed by infusions of 1, 30, and 100 $\mu\text{mol/L}$ PHR0007 in each atria, each for 12 min as one experimental cycle, and changes of atrial pulse pressure and stroke volume were recorded at each dose, and fractions were collected at 2 min intervals. The effects of 30 $\mu\text{mol/L}$ PHR0007 on atrial pulse pressure and stroke volume were also directly compared with those of 30 $\mu\text{mol/L}$ milrinone.

In another series of experiments to determine the mechanism of PHR0007 on atrial dynamics, 30 $\mu\text{mol/L}$ forskolin [an activator of adenylyl cyclases (AC)] or 30 $\mu\text{mol/L}$ IBMX [a non-specific inhibitor of phosphodiesterases (PDEs)] were infused for 12 min prior to infusion of 30 $\mu\text{mol/L}$ PHR0007, and 1.0 $\mu\text{mol/L}$ staurosporine (a non-specific protein kinase inhibitor) or 10 $\mu\text{mol/L}$ H-89 [a specific inhibitor of cAMP-dependent protein kinase A (PKA)] were infused for 24 min prior to infusion of 30 $\mu\text{mol/L}$ PHR0007. Atrial pulse pressure and stroke volume during the control period were compared with those during PHR0007 infusion.

2.4. Statistical analysis

Data are presented as means \pm SEM. Differences

between groups were determined by one-way ANOVA and student's unpaired *t*-test. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Effect of PHR0007 on atrial pulse pressure and stroke volume

There were time- and dose-dependent effects of PHR0007 on both atrial pulse pressure and stroke volume, with a significant increase in both parameters compared with the control period at 30 $\mu\text{mol/L}$ and 100 $\mu\text{mol/L}$ PHR0007 (Figure 1, $n = 6/\text{group}$, $p < 0.001$), which peaked at 48 min of PHR0007 infusion.

3.2. Role of phosphodiesterase (PDE)-cAMP-PKA pathway in the PHR0007-induced increase in atrial pulse pressure and stroke volume

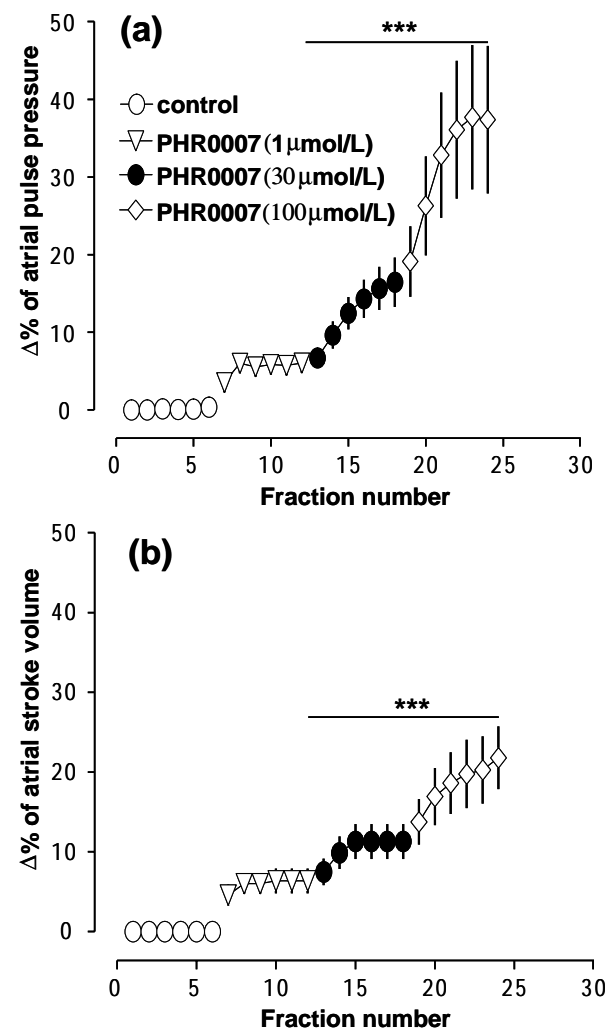


Figure 1. Effect of PHR0007 on atrial pulse pressure and stroke volume. Effects of 1, 30, and 100 $\mu\text{mol/L}$ PHR0007 on atrial pulse pressure (a) and atrial stroke volume (b). Fractions were collected at 2 min intervals. Data are mean \pm SEM ($n = 6/\text{group}$). *** $p < 0.001$ compared with the control period.

Staurosporine (1.0 $\mu\text{mol/L}$) or H-89 (1.0 $\mu\text{mol/L}$) significantly decreased atrial pulse pressure and stroke volume compared with the control period respectively (Figure 2, $n = 6$, both $p < 0.001$; Figure 3, $n = 6$, both $p < 0.05$). In the presence of staurosporine or H-89 plus PHR0007 (30 $\mu\text{mol/L}$) significantly decreased atrial pulse pressure and stroke volume compared with staurosporine or H-89 alone (Figure 2, $n = 6$, both $p < 0.001$; Figure 3, $n = 6$, both $p < 0.05$). Forskolin (30 $\mu\text{mol/L}$), an activator of adenylyl cyclases (AC), significantly increased atrial pulse pressure and stroke volume, but had no effect on PHR0007-induced increases in atrial pulse pressure and atrial stroke volume ($n = 6$; Figure 4). However, IBMX (30 $\mu\text{mol/L}$) completely blocked the positive inotropic effect of PHR0007 on atrial dynamics ($n = 6$; Figure 5).

3.3. Effect of PHR0007 and milrinone on atrial pulse pressure and stroke volume

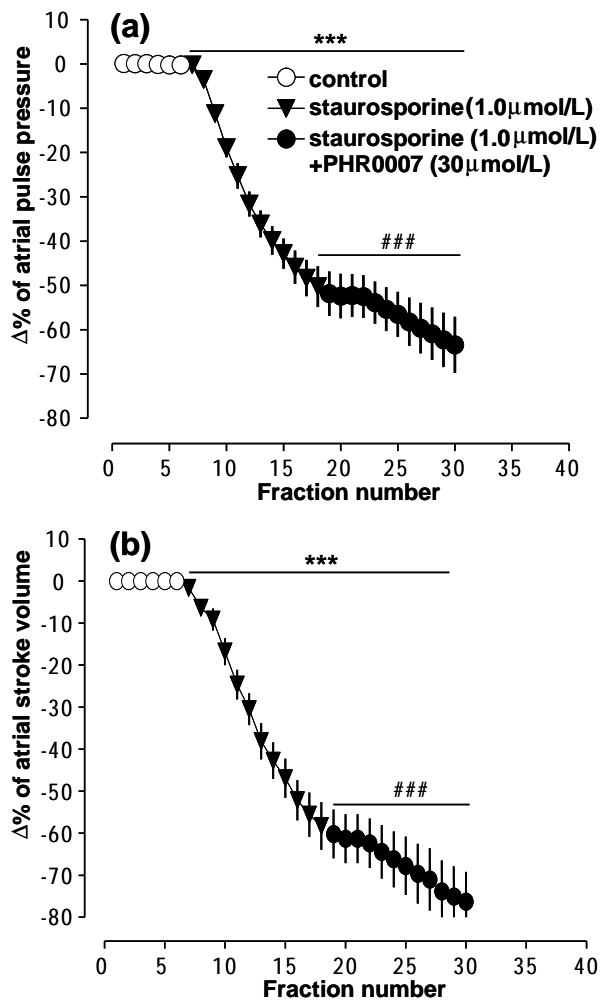


Figure 2. Role of phosphodiesterase (PDE)-cAMP-PKA pathway in the PHR0007-induced increase in atrial pulse pressure and stroke volume. Effects of the non-specific protein kinase inhibitor staurosporine (1.0 $\mu\text{mol/L}$) on PHR0007-induced atrial pulse pressure (a) and atrial stroke volume (b). Data are mean \pm SEM ($n = 6$). *** $p < 0.001$ compared with the control period. ### $p < 0.001$ compared with staurosporine alone.

PHR0007 (30 $\mu\text{mol/L}$) significantly increased atrial pulse pressure and atrial stroke volume compared with milrinone (30 $\mu\text{mol/L}$) in perfused beating atria (Figure 6; $n = 6/\text{group}$). The PHR0007-induced increase in atrial pulse pressure was time-dependent, and peaked at 12 min of PHR0007 infusion (Figure 6a; $n = 6$), then remained significantly higher than in the control group until the fourth experimental cycle. In contrast, the milrinone-induced increase in atrial pulse pressure peaked at 8 min of milrinone infusion, and then recovered throughout the remainder of the milrinone infusion (Figure 6a; $n = 6$). As such, both atrial pulse pressure and stroke volume were significantly higher in the PHR0007 group compared with the milrinone group in the fourth cycle ($p < 0.001$; Figure 6b).

4. Discussion

The present study showed that PHR0007 significantly increased atrial pulse pressure and atrial stroke

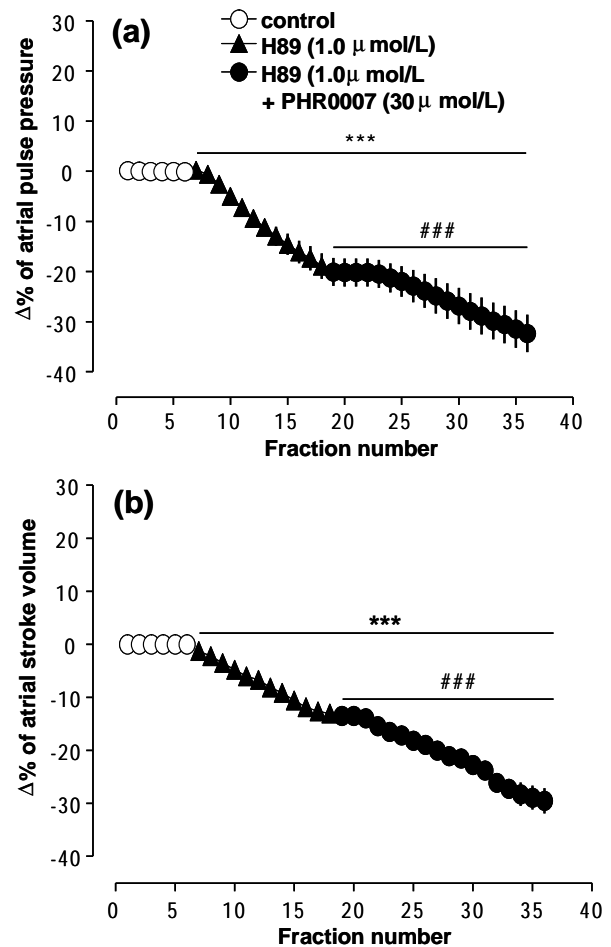


Figure 3. Role of phosphodiesterase (PDE)-cAMP-PKA pathway in the PHR0007-induced increase in atrial pulse pressure and stroke volume. Effects of the cAMP-dependent protein kinase inhibitor H-89 (1.0 $\mu\text{mol/L}$) on PHR0007-induced atrial pulse pressure (a) and atrial stroke volume (b). Data are mean \pm SEM ($n = 6$). *** $p < 0.001$ compared with the control period. ### $p < 0.001$ compared with H-89 alone.

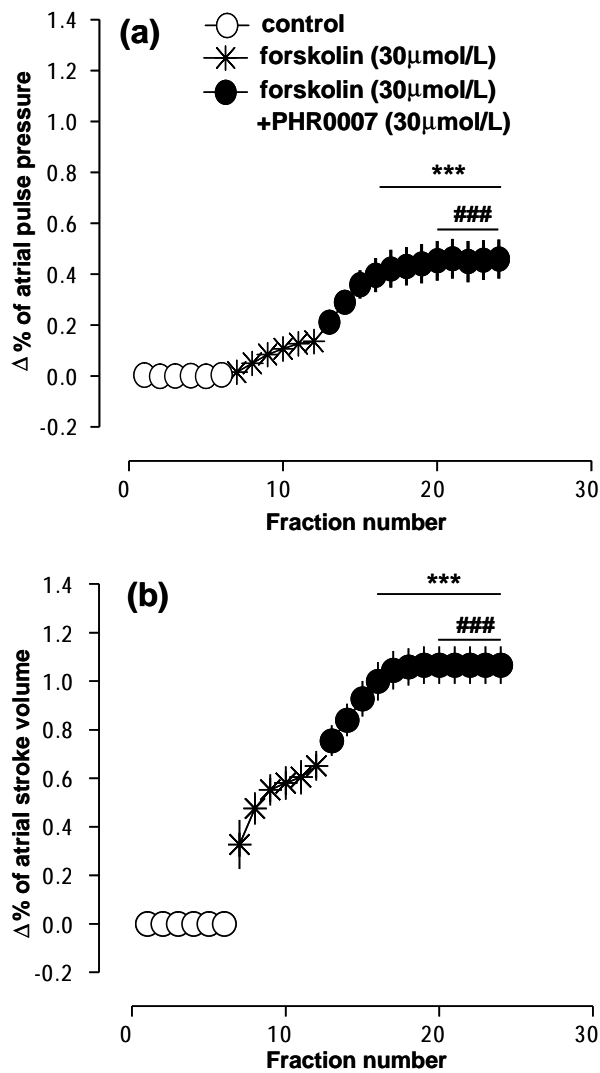


Figure 4. Role of phosphodiesterase (PDE)-cAMP-PKA pathway in the PHR0007-induced increase in atrial pulse pressure and stroke volume. Effects of the adenylyl cyclases (AC) potent activator forskolin (30 $\mu\text{mol/L}$) on PHR0007-induced atrial pulse pressure (a) and atrial stroke volume (b). Data are mean \pm SEM ($n = 6$). *** $p < 0.001$ compared with the control period. ## $p < 0.001$ compared with forskolin alone.

volume, and general inhibition of protein kinases by staurosporine caused a reduction in both atrial pulse pressure and stroke volume that were further reduced using staurosporine plus PHR0007. Furthermore, selective inhibition of PKA by H-89 completely inhibited the PHR0007-induced increase in atrial pulse pressure and stroke volume. Because the cAMP signaling pathway is closely related to the protein kinases A (8,9) which increase cardiac contractility in beating atria, we hypothesized that cAMP would be related, *via* protein kinases, to the PHR0007-induced increase in atrial dynamics. Inhibition of protein kinases with staurosporine, or of PKA with H-89 (10), both blocked the PHR0007-induced increase in atrial pulse pressure and stroke volume, suggesting that the PHR0007-induced positive inotropic effect is cAMP-protein kinase A (PKA) pathway-dependent.

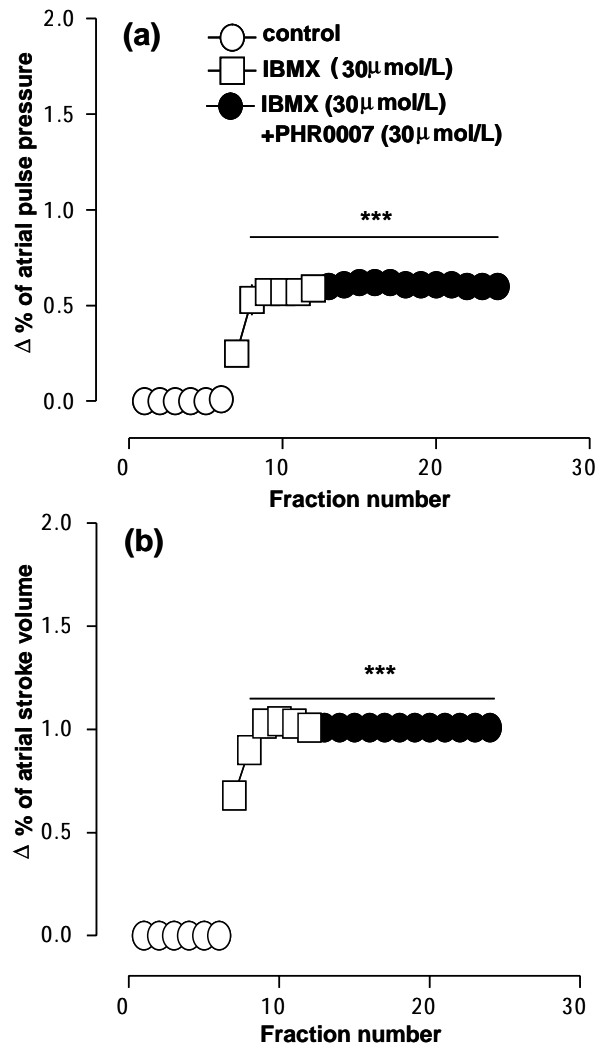


Figure 5. Role of phosphodiesterase (PDE)-cAMP-PKA pathway in the PHR0007-induced increase in atrial pulse pressure and stroke volume. Effects of the potent phosphodiesterase (PDE) inhibitor IBMX (30 $\mu\text{mol/L}$) on PHR0007-induced atrial pulse pressure (a) and atrial stroke volume (b). Data are mean \pm SEM ($n = 6$). *** $p < 0.001$ compared with the control period.

Growing evidence suggests that multiple spatially, temporally, and functionally distinct pools of cyclic nucleotides exist and regulate cardiac performance, from acute myocardial contractility to chronic gene expression and cardiac structural remodeling. The adenylyl cyclase (AC)-cAMP-PKA pathway has been shown to play an important role in determining the cellular response to outside stimuli (11,12). AC activity is inhibited or activated when a cell accepts an external stimulus, resulting in the regulation of intracellular cAMP levels (13,14) and the corresponding functional changes in cellular external metabolic signaling (15, 16). cAMP is a catalytic agent of PKA acting as a second messenger, which causes the regulation of cell function (17-19). Cyclic nucleotide phosphodiesterases (PDEs), by hydrolyzing cAMP and cyclic GMP, regulate the amplitude, duration, and compartmentation

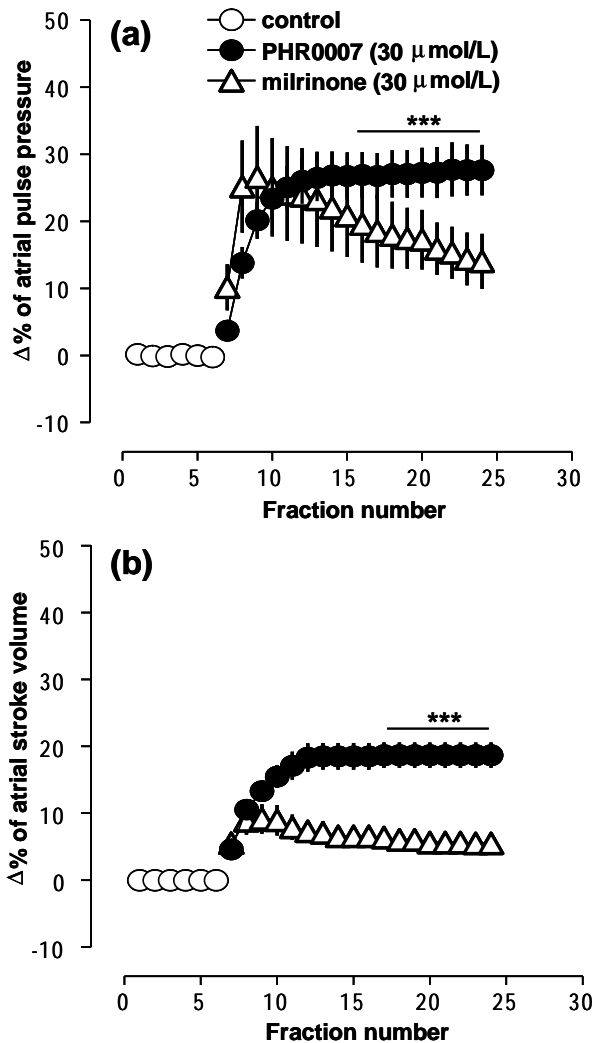


Figure 6. Effect of PHR0007 and milrinone on atrial pulse pressure and stroke volume. Effects of 30 $\mu\text{mol/L}$ PHR0007 on atrial pulse pressure (a) and atrial stroke volume (b) compared with 30 $\mu\text{mol/L}$ milrinone. Fractions were collected at 2 min intervals. Data are mean \pm SEM ($n = 6/\text{group}$). *** $p < 0.001$ compared with milrinone.

of cyclic nucleotide-mediated signaling. In cardiac myocytes, multiple PDE isozymes from at least 5 different families have been described. These include PDE1, PDE2, PDE3, PDE4, and PDE5. In particular, PDE3 enzymes play a major role in regulating cAMP metabolism in the cardiovascular system. PDE3 inhibitors, by raising cAMP content, have acute inotropic and vasodilatory effects in treating congestive heart failure (20). Our data indicated that IBMX, a potent phosphodiesterase (PDE) inhibitor, completely blocked the positive inotropic effect of PHR0007 on atrial dynamics, but Forskolin, a potent activator of adenylyl cyclases (AC), had no effect on PHR0007-induced increases in atrial pulse pressure and atrial stroke volume. Therefore, the inotropic effect of PHR0007 is closely related to PDE but not AC.

In the present study, we compared the positive inotropic effect of PHR0007 *versus* milrinone on atrial

dynamics in rabbit perfused beating atria. Milrinone is a PDE3 inhibitor known to enhance ventricular (LV) contractility by inhibiting the breakdown of cAMP *via* inhibition of PDE3 (21,22), and is used for short-term management of acutely decompensated heart failure, chronic hyperemia heart failure, and refractory heart failure. PHR0007 markedly increased both the atrial pulse pressure and stroke volume in beating rabbit atria in a dose-dependent manner. Furthermore, compared with 30 $\mu\text{mol/L}$ milrinone, which itself increased atrial pulse pressure and stroke volume, these effects were significantly prolonged with 30 $\mu\text{mol/L}$ PHR0007. These data suggest that the positive inotropic effect of PHR0007 is dependent on the PDE-cAMP-PKA signaling pathway. However, why did the general inhibition of protein kinases by staurosporine or H-89 cause a reduction in both atrial pulse pressure and stroke volume, that were further reduced using staurosporine or H-89 plus PHR0007. Those facts should be further investigated.

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