*Korean J Physiol Pharmacol*
*Vol 11: 175-182, October, 2007*

# Prostaglandin $E_1$ Increases cGMP Levels in Beating Rabbit Atria: Lack of Effects of $PGE_1$-induced Cyclic Nucleotides on Secretory and Contractile Functions

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**Members of prostaglandin (PG) E-series elicit cellular effects mainly through adenylyl cyclase-cAMP signaling. The role of $PGE_2$-induced increase in cAMP has been shown to be compartmentalized in the cardiac myocytes: $PGE_2$-induced increase of cAMP is not involved in the control of cardiomyocytic contraction. The purpose of the present study was to define the effect of $PGE_1$ on the cGMP levels and the role of $PGE_1$ in the atrial secretory function. Experiments were performed in perfused beating rabbit atria and atrial contractile responses, cGMP and cAMP efflux, and atrial natriuretic peptide (ANP) secretion were measured. $PGE_1$ increased cGMP as well as cAMP efflux concentration in a concentration-dependent manner, however, no significant changes in atrial secretory responses were observed (with 1.0 $\mu$M $PGE_1$; for cGMP, 144.76±37.5%, n=11 versus −16.81±4.76%, n=6, control, $p<0.01$; for cAMP, 187.60±41.52%, n=11 versus 7.38±19.44%, n=6, control, $p<0.01$). $PGE_1$ decreased atrial dynamics slightly but transiently, whereas $PGE_2$ showed similar effects but with lower potency. Isoproterenol increased atrial cAMP efflux (with 2.0 nM; 145.71±41.89, n=5 versus 7.38±19.44%, n=6, control, $p<0.05$) and mechanical dynamics and decreased ANP secretion. The $PGE_1$-induced increase in cGMP efflux showed a bell-shaped concentration-response curve. $PGE_1$-induced increase of cGMP efflux was not observed in the presence of L-NAME, an inhibitor of nitric oxide (NO) synthase, or ODQ, an inhibitor of NO-sensitive guanylyl cyclase. L-NAME and ODQ showed no significant effect on the $PGE_1$-induced transient decrease of atrial dynamics. These data indicate that $PGE_1$ increases cGMP levels via NO-soluble GC signaling in the cardiac atrium and also show that $PGE_1$-induced increases in cGMP and cAMP levels are not involved in the regulation of atrial secretory and contractile functions.**

**Key Words: Atrial function, Atrial natriuretic peptide, cAMP, cGMP, Prostaglandin $E_1$**

## INTRODUCTION

Prostaglandins (PGs) exert biological effects via G protein-coupled receptors, receptors for prostanoid E type, EP receptors. $PGE_1$ and $PGE_2$ elicit variable cellular responses mainly through adenylyl cyclase-cAMP and $Ca^{2+}$ signaling via PG receptor subtypes, EP1, EP2, EP3, and EP4 (Narumiya et al, 1999). EP2 and EP4 subtypes couple to $G_s$ and activate adenylyl cyclase with increase in cAMP levels; EP3 subtype couples to $G_i$ and inhibits the increase in cAMP levels; and EP1 subtype couples to $G_q$ and increases intracellular $Ca^{2+}$. Furthermore, all EP receptor subtypes have been shown to be expressed in the heart (Honda et al, 1993; Breyer et al, 1994; Wolkowicz et al, 2002; Xiao et al, 2004).

It has earlier been reported that $PGE_1$ increases cGMP levels in the neuroblastoma cell line (Matsuzawa & Nirenberg, 1975), and $PGE_2$ and $PGE_1$ analogs have recently been shown to exert biological roles via an induction of eNOS expression in cerebral microvessels and umbilical vein endothelial cells (Gobeil et al, 2002; Haider et al, 2005). Also, $PGE_2$ was shown to stimulate NO production in spinal cord tissue (Sakai et al, 1998) and human umbilical vein endothelial cells (Namkoong et al, 2005). However, little is known about the effect of $PGE_1$ on cardiac cGMP levels which are implicated in the regulation of cardiovascular function.

Although PGs have been shown to protect cardiac dysfunction (Jugdutt et al, 1981; Hide et al, 1995; Thiemermann & Zacharowski, 2000), the precise mechanisms involved in the cardioprotective effects of PGs are to be clearly defined. It was shown that cardiac ischemia increases PGs, including PGE series (Alexander et al, 1975; Kraemer et al, 1976), and ischemia-induced increase of PGs has been suggested as one of factors protecting myocardiac

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ischemic injury (Fiedler, 1988). The cGMP system has been suggested as a protective factor in cardiovascular dysfunction (Booz, 2005).

Previously, it was shown that cAMP-elevating agents, $PGE_1$ and isoproterenol, have different roles in the control of mechanical responses in the heart: isoproterenol but not $PGE_1$ has a positive inotropic effect (Hayes et al, 1979; Brunton et al 1979). Compartmentalized roles of cAMP in the control of cellular responses are the subject of interests, and cAMP and cGMP are shown to be compartmentalized in the regulation of atrial secretory function (Cui et al, 2002a; Wen et al, 2004).

The present study was designed to test the hypothesis that $PGE_1$ increases cGMP levels in the heart, and also to test the roles of cyclic nucleotides in the regulation of the cardiac secretory and mechanical contractile functions. Experiments were performed in beating rabbit atria.

## METHODS

### *Beating perfused atrial preparation*

New Zealand White rabbits were used. An isolated perfused atrial preparation was prepared by the method described previously (Cho et al, 1995; Cui et al, 2002b), allowing atrial pacing and measurements of changes in atrial volume during contraction (stroke volume), pulse pressure, transmural extracellular fluid (ECF) translocation, cAMP efflux, cGMP efflux and ANP secretion. Briefly, the hearts were rapidly removed and placed in oxygenated warm saline. The left atrium was then dissected. A calibrated transparent atrial cannula (8 cm long, 4 mm outer diameter), containing two small catheters within it, was inserted into the left atrium through the atrioventricular orifice. The cannula was secured by ligatures around the atrioventricular sulcus. The outer tip of the atrial cannula was open to allow for outflow from the atrium. One of the two catheters located in the atrium was for inflow, and the other catheter was used to record pressure changes in the atrium. The cannulated atrium was then transferred to an organ chamber containing buffer at 36.5°C. The pericardial space of the organ chamber was open to the air so as not to restrict atrial dynamics. The atrium was immediately perfused with Hepes buffer solution by means of a peristaltic pump (1 ml/min). The composition of the buffer was as follows (in mM): 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 25 $NaHCO_3$, 10.0 glucose, 10.0 HEPES (with NaOH pH 7.4) and 0.1% bovine serum albumin. Soon after setup of the perfused atrium, transmural electrical field stimulation with a luminal electrode was started at 1.3 Hz (duration, 0.3 ms; voltage, 2 times threshold intensity, 20~30 V; 6.1-cm$H_2O$ distension). The perfusate was prewarmed to 36.5°C by passage through a water bath and equilibrated with oxygen by passage through silicone tubing in a gas mixing chamber. The buffer in the organ chamber was oxygenated by passing oxygen through silicone tubing coils located inside the chamber. The changes in atrial stroke volume were monitored by reading the lowest level of the water column in the calibrated atrial cannula during end diastole. Atrial pulse pressure was measured via a pressure transducer connected to the intraatrial catheter and recorded on a physiograph.

Measurement of translocation of extracellular fluid (ECF): To estimate transendocardial ECF translocation, transmural atrial clearance of [$^3$H]inulin was measured as described previously (Cho et al, 1995). Radioactivity in the atrial perfusate and pericardial buffer solution was measured with a liquid scintillation system, and the amount of ECF translocated through the atrial wall was calculated as follows:

ECF translocated ($\mu l \cdot min^{-1} \cdot g$ atrial wet $wt^{-1}$) = (total radioactivity in the perfusate (cpm $\cdot min^{-1}$)× 1,000)/ (radioactivity in the pericardial reservoir (cpm $\cdot \mu l^{-1}$)× atrial wet wt (mg)).

### *Experimental protocols*

The beating atria were perfused for 60 min to stabilize atrial natriuretic peptide (ANP) secretion. [$^3$H]Inulin was introduced to the pericardial fluid 20 min before the start of the sample collection (Cho et al, 1995). The perfusate was collected for analyses at 2-min intervals at 4°C. Experiments were carried out using fourteen groups of atria. Control period (four 12-min period) was followed by an infusion of PGE1, (0.1 $\mu$M, group 1, n=2~3; 0.3 $\mu$M, group 2, n=3~5; 1.0 $\mu$M, group 3, n=11~12; 3.0 $\mu$M, group 4, n=5; 10.0 $\mu$M, group 5, n=4), $PGE_2$ (0.1 $\mu$M, group 6, n=7; 1.0 $\mu$M, group 7, n=6-8; 10.0 $\mu$M, group 8, n=9), isoproterenol (ISO), an activator for $\beta$-adrenergic receptor (2.0 nM, group 9, n=5) or vehicle (group 10, n=6~7) for 3 cycles (36 min). To analyze the effects of $PGE_1$, 36 min of $N^{\omega}$-nitro-L-arginine methyl ester hydrochloride (L-NAME), an inhibitor of NO synthase, or 1H-(1,2,4)oxadiazolo(4,3-a) quinoxalin-1-one (ODQ), a specific inhibitor of soluble guanylyl cyclase (sGC), was followed by an infusion of $PGE_1$ or vehicle [L-NAME (1 mM) plus $PGE_1$ (1.0 $\mu$M), group 11, n=11~12; L-NAME alone, group 12, n=5-8; ODQ (30.0 $\mu$M) plus $PGE_1$, group 13, n=11; ODQ alone, group 14, n=8-9]. $PGE_1$, $PGE_2$, and ODQ were dissolved in DMSO. The final concentration of DMSO was less than 0.1%. The effects were evaluated after one cycle (12 min) or three cycles (36 min) of administration of the agent. For the time-matched control, vehicle was introduced and values obtained in the control and experimental observations were compared.

### *Radioimmunoassay of cAMP*

cAMP was measured by equilibrated radioimmunoassay (Cui et al, 2002b; Wen et al, 2004). The amount of cAMP efflux was expressed as pmol of cAMP per min per g of atrial tissue. The molar concentration of cAMP efflux in terms of ECF translocation, which may reflect the concentration of cAMP in the interstitial space fluid (Cui et al, 2002b), was calculated as cAMP efflux concentration ($\mu$M); cAMP (in pmol $\cdot min^{-1} \cdot g^{-1}$)/ECF translocated (in $\mu l \cdot min^{-1} \cdot g^{-1}$). Nonspecific binding was <2.0%. The intra- and interassay coefficients of variation were 5.0 (n=10) and 9.6% (n=10), respectively.

### *Radioimmunoassay of cGMP*

cGMP was measured, calculated and expressed as described previously (Lee et al, 2000; Wen et al, 2004). Production of cGMP was measured by equilibrated radioimmunoassay (Lee et al, 2000; Wen et al, 2004). Briefly, standards or samples were incubated with antiserum for cGMP (Calbiochem-Novabiochem, San Diego, CA) and iodinated cGMP (guanosine 3',5'-cyclic phosphoric acid, 2'-o-succinyl [$^{125}$I]iodotyrosine methylester) in a sodium ace-

tate buffer (50 mM, pH 4.85) containing theophylline (8 mM). The bound form was separated from the free form by charcoal suspension. Nonspecific binding was <2.4%. The intra- and interassay coefficients of variation were 4.2 (n=15) and 7.1% (n=8), respectively.

### *Radioimmunoassay of ANP*

ANP in the perfusate was measured by a specific radioimmunoassay, as described previously (Cho et al, 1995). The amount of ANP secreted was expressed as nanogram of ANP · $min^{-1}$ · $g^{-1}$ of atrial tissue. The molar concentration of ANP in terms of the ECF translocation reflects the concentration of ANP in the interstitial space of the atrium and, therefore, indicates the rate of myocytic release of ANP into the surrounding paracellular space (Cho et al, 1995). It was calculated by the following formula: ANP released ($\mu$M) = ANP (in pg · $min^{-1}$ · $g^{-1}$)/ECF translocated (in $\mu$l · $min^{-1}$ · $g^{-1}$) · 3063 [mol wt of ANP-(1-28)].

### *Statistical analysis*

Significant difference was compared using repeated measures ANOVA followed by Bonferroni's multiple-comparison test (Fig. 1, 3, 4 and 5). Student's *t*-test for unpaired data (Fig. 2, 6 and 7) was also applied. Statistical significance was defined as $p<0.05$. The results are given as means±SE.

## RESULTS

### *$PGE_1$ increases cGMP as well as cAMP levels*

Atrial efflux of cGMP and cAMP, expressed as efflux concentration ($\mu$M) of cGMP and cAMP in perfusate in terms of the ECF translocation which reflects the concentration of cGMP and cAMP in the interstitial space fluid (Cui et al, 2002a; 2002b), were steady and stable during the control periods (Fig. 1, Aa, Ab, Ba and Bb). Atrial dynamics (stroke volume and pulse pressure), ANP secretion, and the concentration of ANP in perfusate in terms of the ECF translocation, which reflects the rate of atrial myocytic ANP release, were also steady and stable (Fig. 1, Ac-Ae and Bc-Be). $PGE_1$ (1.0 $\mu$M) increased cGMP and cAMP efflux concentrations (Fig. 1, Aa and Ab, respectively). $PGE_1$ caused a transient decrease of atrial dynamics (Fig. 1, Ac and Ad), however, $PGE_1$ had no significant effect on myocytic ANP release (Fig. 1Ae). $PGE_1$ (1.0

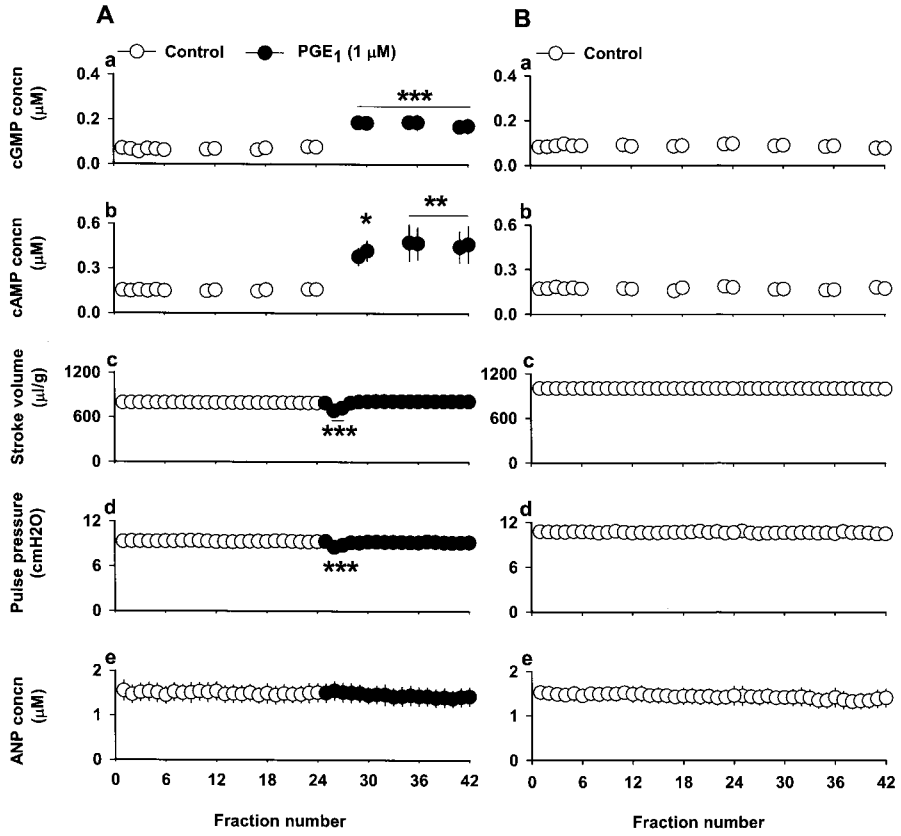


**Fig. 1.** Effects of PGE1 (1.0 $\mu$M) on the levels of cGMP and cAMP efflux concentration, and contractile and secretory functions in beating rabbit atria. (A) $PGE_1$-induced changes in cGMP efflux concentration (a), cAMP efflux concentration (b), stroke volume (c), pulse pressure (d) and ANP concentration (e) (n=11~12). (B) Time-matched controls (n=6~7). *$p<0.05$, **$p<0.01$, ***$p<0.001$ versus control values before the addition of $PGE_1$. Values are means±SE. Some of error bars are not seen because the amplitudes are less than the size of dots.

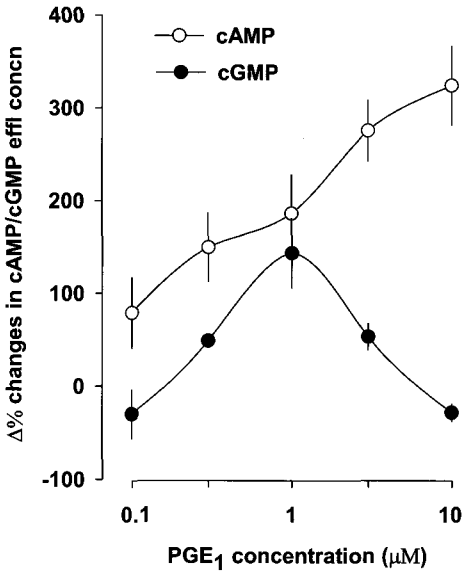


**Fig. 2.** Concentration-response curves for cGMP efflux concentration and cAMP efflux concentration by $PGE_1$. The values are the results obtained at third cycle (36 min) after PGE1 administration. Number of experiments: 0.1 $\mu$M, n=2~3; 0.3 $\mu$M, n=3~5; 1.0 $\mu$M, n=11; 3.0 $\mu$M, n=5; 10.0 $\mu$M, n=4. Values are means±SE.

$\mu$M) increased cGMP efflux levels by 176.23±45.36% from 0.077±0.013 $\mu$M (mean of two periods, fraction number 23 and 24) to 0.189±0.028 $\mu$M (fraction number 29 and 30) at first 12 min cycle of an infusion of agent and then maintained the levels thereafter up to third cycle (at 36 min, 144.76±37.50%) without significant decrease (Fig. 1Aa, n=11). $PGE_1$-induced increase of cGMP levels was significant as early as ~8 min of an exposure of the agent ($p<0.001$ versus control; Fig. 1Aa). With $PGE_1$, cAMP efflux concentration increased by 175.88±35.23% from 0.155±0.024 $\mu$M to 0.405±0.069 $\mu$M at first 12 min cycle and then maintained the levels thereafter up to 36 min (at 36 min, 187.60±41.52%; n=11). For the time-matched control, changes in the parameters were constant and stable (Fig. 1B). The responses were reproducible (differences between the periods were not significant)

Three $\mu$M $PGE_1$ increased cGMP levels by 54. 64± 14.19%, but 10 $\mu$M $PGE_1$ slightly decreased them by −26 96±9.45%. A lower concentration of $PGE_1$ (0.1 $\mu$M) showed not significant changes in cGMP levels (−28.69±26.16%) and 0.3 $\mu$M $PGE_1$ increased cGMP levels by 50.29±7.16%. $PGE_1$ showed a bell-shaped concentration-response curve for cGMP levels (Fig. 2). $PGE_1$ increased cAMP efflux in a concentration-dependent manner (Fig. 2). Neither higher nor lower concentration of $PGE_1$ resulted in any significant changes in ANP secretion (data not shown).

Effects of $PGE_2$ were similar to those of $PGE_1$ but with lower potency (Fig. 3). $PGE_2$ (10.0 $\mu$M) increased cGMP and cAMP efflux concentrations (Fig. 3, a and b). The responses by $PGE_2$ were less than those by $PGE_1$ (1.0 $\mu$M) (Fig. 7). $PGE_2$ caused a transient negative inotropic response (Fig. 3, c and d), and had no effect on ANP release (Fig. 3e). $PGE_2$ (1.0 $\mu$M) increased cAMP but not cGMP efflux (Fig. 7). $PGE_2$ increased cGMP and cAMP in a concentration-dependent manner (data not shown). These results indicate that $PGE_2$ has a similar pattern of effects to $PGE_1$ in

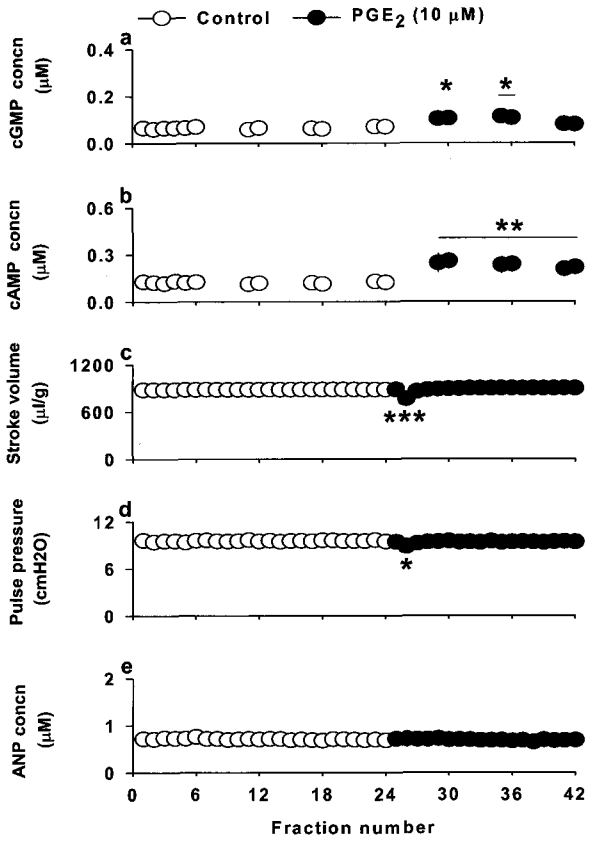


**Fig. 3.** Effects of $PGE_2$ (10.0 $\mu$M) on the levels of cGMP and cAMP efflux concentration, and contractile and secretory functions in beating rabbit atria. $PGE_2$-induced changes in cGMP efflux concentration (a), cAMP efflux concentration (b), stroke volume (c), pulse pressure (d) and ANP concentration (e) (n=9). *$p<0.05$, **$p<0.01$, ***$p<0.001$ versus control values before the addition of $PGE_2$. Values are means±SE.

beating rabbit atria.

### *$PGE_1$ increases cGMP via NO-sGC signaling*

To define the role of NO-sGC pathway in the $PGE_1$-induced increase in cGMP production, effects of L-NAME, an inhibitor of NO synthase, and ODQ, an inhibitor of sGC, were examined.

L-NAME showed no significant changes in basal levels of cGMP and cAMP, and contactile and secretory functions (Fig. 4). An inhibition of NO synthase with L-NAME completely blocked PGE1-induced increase in cGMP, but not cAMP levels (Fig. 4, Aa and Ab and 6). L-NAME slightly attenuated $PGE_1$-induced increase in cAMP levels but not significantly. L-NAME had no effect on the $PGE_1$-induced transient decrease in pulse pressure (Fig. 4Ac), and showed no significant changes in the parameters during the period corresponding to experimental observations (Fig. 4B). These results indicate that NO is involved in the regulation of $PGE_1$-induced increase in cGMP levels.

Next, to define the role of sGC in the PGE1-induced increase in cGMP efflux levels, an inhibitor of sGC was applied. ODQ decreased basal levels of cGMP in the first 12 min cycle (Fig. 5, Aa and Ba). The response returned

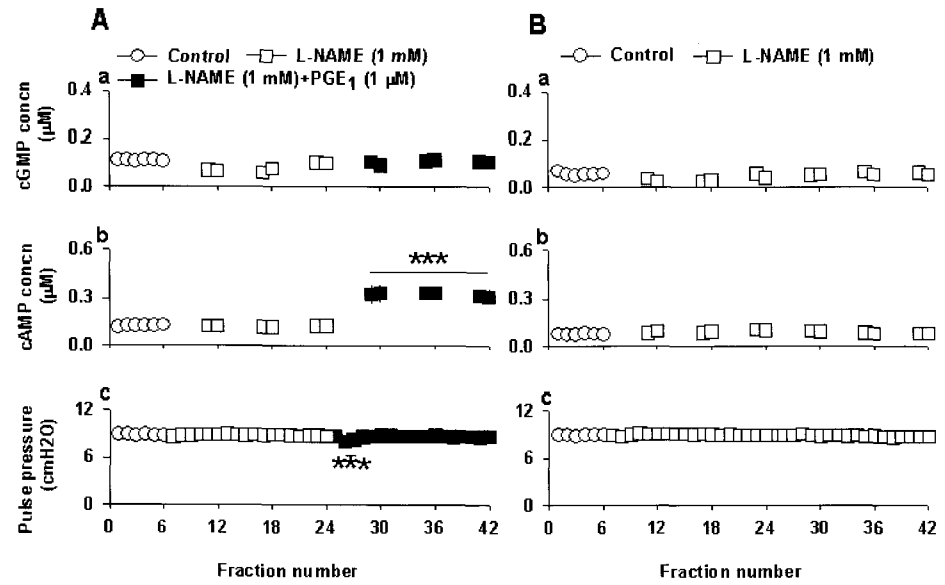


**Fig. 4.** Effects of NO blocker on the $PGE_1$-induced changes. (A) Effects of L-NAME (1.0 mM) on $PGE_1$-induced changes in cGMP efflux concentration (a), cAMP efflux concentration (b) and pulse pressure (c) (n=11~12). (B) L-NAME-induced changes in the same parameters (n=5~8). ***P<0.01 versus L-NAME. Values are means±SE.

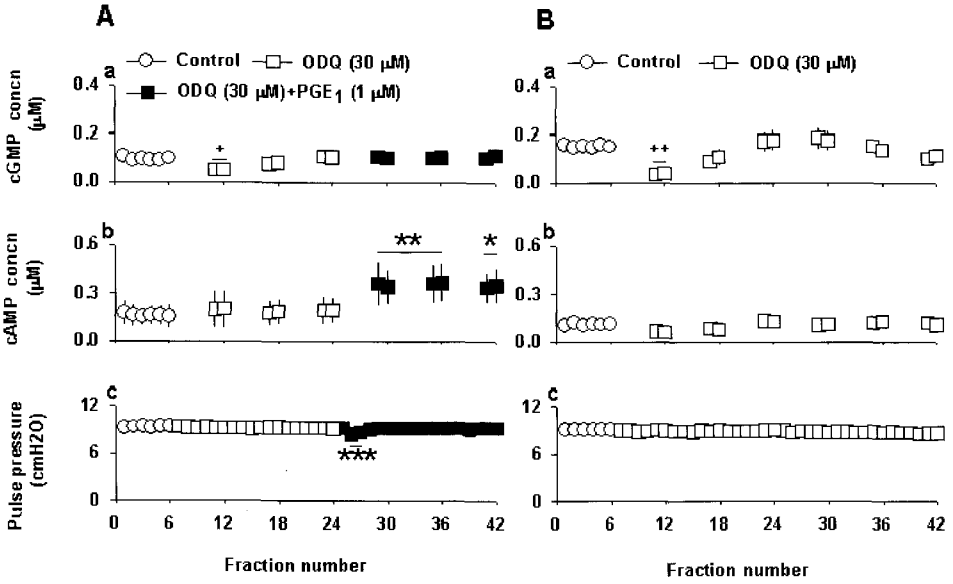


**Fig. 5.** Effects of sGC blocker on the $PGE_1$-induced changes. (A) Effects of ODQ (30.0 $\mu$M) on $PGE_1$-induced changes in cGMP efflux concentration (a), cAMP efflux concentration (b) and ANP concentration (c) (n=11). (B) ODQ-induced changes in the same parameters (n=8~9). +p<0.05, ++p<0.01 versus control; *p<0.05, **p<0.01, ***p<0.001 versus ODQ. Values are means±SE.

to control levels during the third cycle and was then maintained thereafter without significant changes (ODQ alone; Fig. 5B). The inhibition of sGC with ODQ completely blocked $PGE_1$-induced increase in cGMP levels, but not cAMP levels (Fig. 5, Aa and Ab, and 6). These results indicate that sGC is involved in the regulation of basal levels of cGMP and $PGE_1$-induced increase in cGMP levels.

### *Isoproterenol, another cAMP-elevating agent, increases atrial dynamics and decreases ANP release*

To compare the effects of $PGE_1$ on contractile and secretory function, $\beta$-adrenergic receptor activator, ISO, was introduced. ISO (2 nM) increased cAMP efflux levels from 0.064±0.012 $\mu$M to 0.160±0.021 $\mu$M at first 12 min cycle (by 170.59±36.68%) and then maintained up to third cycle (at

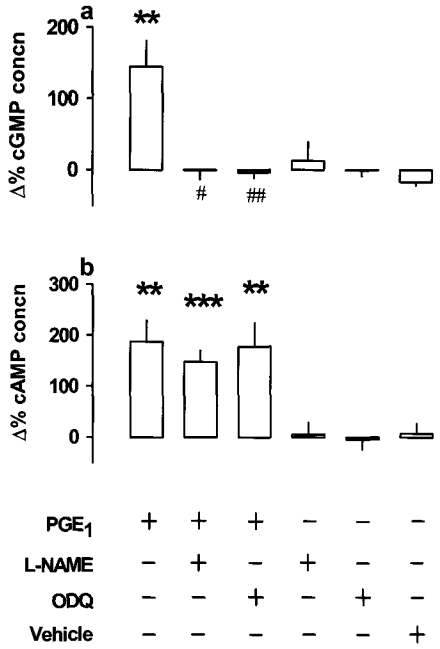


**Fig. 6.** Summarized data showing the effects of L-NAME and ODQ on the $PGE_1$-induced changes. $PGE_1$-induced changes in cGMP efflux concentration (a) and cAMP efflux concentration (b). The data were derived from Figs. 1, 4 and 5. **p<0.01, ***p<0.001 versus corresponding controls; #p<0.01, ##p<0.001 versus PGE1. Values are means±SE.

36 min, 145.71±41.89%). Increases in cAMP levels by $PGE_1$, $PGE_2$ and ISO were not significantly different from each other (Fig. 7). ISO increased atrial pulse pressure and decreased atrial ANP release. Atrial responses by ISO of contratile and secretory function were significantly different from those by $PGE_1$ and $PGE_2$ (Fig. 7, c and d).

## DISCUSSION

The present study clearly shows for the first time that $PGE_1$ increases cGMP via NO-sGC signaling pathway in the cardiac atrium, and also that $PGE_1$- and $PGE_2$-induced increases in cGMP and cAMP levels are not involved in the regulation of atrial secretory and contractile functions in beating rabbit atria.

### *$PGE_1$ increases cGMP levels via NO-sGC signaling*

The present findings that $PGE_1$-induced increase of cGMP was completely blocked by both L-NAME and ODQ indicate that increase of cGMP levels by $PGE_1$ is mediated by NO-sGC signaling. In the present protocol, it seems likely that sGC but not NO is activated to increase basal levels of cGMP, because ODQ but not L-NAME decreases basal levels of cGMP (Fig. 4 and 5).

$PGE_1$-induced increase of cGMP levels showed a bell-shaped concentration-response curve, while $PGE_1$-induced increase of cAMP levels showed a monotonous concentration-dependent response. Higher concentrations of $PGE_1$ generated lower cGMP levels. This finding indicates that the mechanism involved in the $PGE_1$-induced increase of cGMP

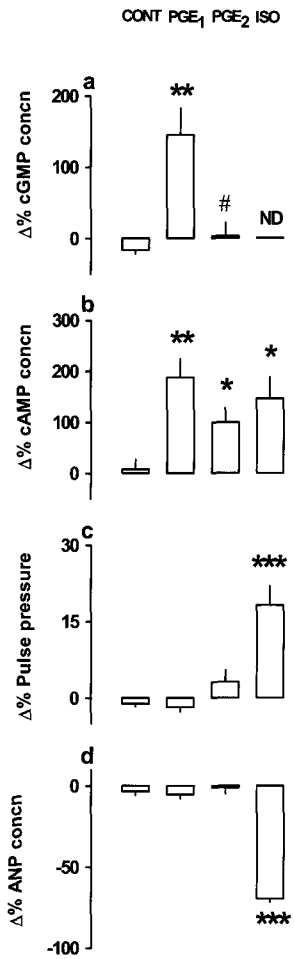


**Fig. 7.** Summarized data showing the effects of cAMP-elevating agents on atrial secretory and contractile functions. Effects on cGMP efflux concentration (a), cAMP efflux concentration (b), pulse pressure (c) and ANP concentration (d). The values are the results obtained at third cycle after the administration of agents. The data for $PGE_1$ were from Fig. 1. Number of experiments: $PGE_1$ (1.0 μM), n=11~12; $PGE_2$ (1.0 μM), n=6-8; isoproterenol (ISO, 2.0 nM), n=5; control, n=6~7. *p<0.05, **p<0.01, ***p<0.001 versus control; #p<0.01 versus PGE1. ND, not determined. Values are means±SE.

levels is not the same as that for the increase of cAMP levels. However, whether specific cell types are responsible for the $PGE_1$-induced increase of cGMP levels are not clear at present, and exact signaling pathway to increase cGMP levels should be defined. EP2 and EP4 receptors are shown to be expressed in the heart (Castleberry et al, 2001; Guan et al, 2002; Wolkowicz et al, 2002; Xiao et al, 2004). The bell-shaped concentration-response curve for cGMP by $PGE_1$ may be related to rapid desensitization of sGC. Gibb et al (2003) showed a bell-shaped cGMP generation curve for different concentrations of NO; higher NO concentration generated lower cGMP levels in GC isozyme expressing COS-7 cells. Rapid desensitization of NO receptor sGC has been suggested to be a possible cause of the bell-shaped concentration-response curve for NO in intact cells (Bellamy et al, 2000; Gibb et al, 2003).

In increasing levels of cyclic nucleotides in the beating rabbit cardiac atrium, $PGE_1$ was more potent than $PGE_2$.

This finding contrasts with the report of Wilson et al (2004) that $PGE_1$ and $PGE_2$ are equally potent in the stimulation of cAMP formation in the transfected cell lines with EP2 or EP4 receptor subtypes.

***$PGE_1$-induced increases of cGMP and cAMP levels are not involved in the regulation of atrial secretory and contractile responses***

In contrast to ISO, $PGE_1$ and $PGE_2$ which similarly increased cAMP levels had no effect on atrial dynamics. This is consistent with the previous reports showing that $PGE_1$-induced increase of cAMP is not involved in the positive inotropic effect on the heart (Hayes et al, 1979; Brunton et al, 1979). Similarly, as shown in the present study, $PGE_1$- and $PGE_2$-induced increase of cGMP levels was not involved in the regulation of atrial dynamics. Previously, we have shown that an elevation of cGMP levels by sGC activation is not sensitive to regulation of atrial dynamics (Wen et al, 2004), and the present finding is consistent with the report.

It has been shown that an increase of cAMP (Cui et al, 2002a; 2002b; Li et al, 2003) and cGMP (Lee et al, 2000; Wen et al, 2004) inhibits atrial ANP release, and that cAMP and cGMP are compartmentalized in the regulation of atrial ANP release. An elevation of cAMP by inhibition of PDE3 phosphodiesterase, but not PDE4 phosphodiesterase, inhibits ANP release (Cui et al, 2002a). And an elevation of cGMP by activation of particulate GC coupled NPR-B, but not of sGC, is sensitive to inhibition of ANP release (Wen et al, 2004). The present finding that increase of cGMP via NO-sGC signaling had no effect on the regulation of ANP release is consistent with the previous report (Wen et al, 2004). However, the present finding is in contrast with previous reports that PGE2 increased ANP release in cultured atrial cardiocytes (Gardner & Schultz, 1990) and sliced rabbit atria (Azizi et al, 1995). These data suggest that roles of cAMP and cGMP increased by $PGE_1$ or $PGE_2$ and ISO are compartmentalized in the regulation of atrial secretory and contactile functions.

The mechanism by which $PGE_1$ or $PGE_2$ elicits a transient negative inotropic effect is not clear at present. $PGE_1$-induced increase of cGMP may not be related to the effect, because NO or sGC inhibitor did not modify the $PGE_1$-induced response.

The present study provides a rationale for a novel signaling pathway in the physiological and pathological roles of PGE1 in the heart. Although $PGE_1$-induced increases of cGMP and cAMP levels are not involved in the regulation of atrial secretory and contractile functions, it is, however, possible that $PGE_1$-induced activation of NO-cGMP signaling may be involved in cardioprotection against ischemic cardiomyopathy. Recently, it has been shown that NO-cGMP signaling is involved in protection of cardiomyocytes against apoptosis and necrosis caused by ischemia and reperfusion injury (Das et al, 2005), and Xiao et al (2004) showed that endogenous $PGE_2$ protects the heart from ischemia-reperfusion injury. Furthermore, $PGE_1$ has been shown to have cardioprotective effects (Jugdutt et al, 1981; Hide et al, 1995; Thiemermann & Zacharowski, 2000).

In summary, our results demonstrate that $PGE_1$ increases cGMP levels via NO-sGC signaling in the cardiac atrium, and also that $PGE_1$- and $PGE_2$-induced increases of cGMP and cAMP are compartmentalized in the regulation of contractile and secretory functions.

## ACKNOWLEDGMENT

This work was supported by research grants from the Korea Science and Engineering Foundation (R01-2003-000-10507-0), and the Ministry of Health and Welfare (03-PJ9-PG6-SO02-0001 to H.S.L.). The authors thank Kyong Sook Kim for secretarial work.

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