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# Effects of Phosphodiesterase 5 Inhibition with Sildenafil on Atrial Contractile and Secretory Function

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**Selective inhibition of phosphodiesterase (PDE) 5 opened a new therapeutic approach for cardiovascular disorders. Therefore, the effect of PDE5 inhibition on the cardiac function should thoroughly be defined. The purpose of the present study was to define the effects of sildenafil, a selective inhibitor of PDE5, on the atrial cGMP efflux, atrial dynamics, and the release of atrial natriuretic peptide (ANP). By perfusing rabbit left atria to allow atrial pacing, changes in atrial stroke volume and pulse pressure, transmural extracellular fluid translocation, cGMP efflux, and ANP secretion were measured. SIN-1, an NO donor and soluble (s) guanylyl cyclase (GC) activator, and C-type natriuretic peptide (CNP), an activator of particulate (p) GC activator, were used. Sildenafil increased basal levels of cGMP efflux slightly but not significantly. Sildenafil in a therapeutic dose increased atrial dynamics (for atrial stroke volume, $2.84 \pm 1.71\%$, n=12, vs $-0.71 \pm 0.86\%$, n=21; $p < 0.05$) and decreased ANP release ($-9.02 \pm 3.36\%$, n=14, vs $1.35 \pm 3.25\%$, n=23; $p < 0.05$), however, it had no effect on the SIN-1- or CNP-induced increase of cGMP levels. Furthermore, sildenafil in a therapeutic dose accentuated SIN-1-induced, but not CNP-induced, decrease of atrial pulse pressure and ANP release. These data indicate that PDE5 inhibition with sildenafil has a minor effect on cGMP levels, but has a distinct effect on pGC-cGMP- and sGC-cGMP-induced contractile and secretory function.**

**Key Words: Atrial natriuretic peptide, Atrium, cGMP, Phosphodiesterase 5, Sildenafil**

## INTRODUCTION

The role of cGMP in the regulation of atrial contractile and secretory functions is shown to be compartmentalized (Wen et al, 2004): particulate (p) guanylyl cyclase (GC) activated with C-type natriuretic peptide (CNP) inhibits atrial dynamics and atrial natriuretic peptide (ANP) release via pGC-cGMP signaling, while soluble (s) GC activated with nitric oxide (NO) donor or direct sGC activator elicits only a minor effect on these parameters (Lee et al, 2000; Wen et al, 2004). cGMP has been shown to be a negative factor in the regulation of atrial ANP release (Lee et al, 2000; Wen et al, 2004), however, Ruskoaho et al (1986) found no effect of membrane permeable cGMP and sodium nitroprusside, an NO donor, on ANP secretion.

Because the level of intracellular cGMP is controlled by GC activation and degradation through phosphodiesterase (PDE) activity, it is possible that cGMP-specific PDE5 is involved in the control of atrial dynamics and secretory functions.

Sildenafil (Viagra) is a relatively selective and potent inhibitor of cGMP-specific PDE5, and has been shown to be an effective drug for the treatment of male erectile dysfunction (Boolell et al, 1996). The maximum plasma concentration of sildenafil with therapeutic doses (25~100 mg) is shown to be 127~560 ng/ml (up to ~ 1 $\mu$M) (Zusman et al, 1999).

PDE5 mRNA has been shown to be expressed in human heart (Loughney et al, 1998; Stacey et al, 1998; Yanaka et al, 1998), and PDE5 protein has also been shown to be expressed in mouse heart (Giordano et al, 2001), and cardiac myocytes (Takimoto et al, 2005), and canine ventricular cardiomyocytes (Das et al, 2005). Human ventricular cardiomyocytes are shown to express PDE5A (Senzaki et al, 2001). On the other hand, however, others found no PDE5 activity in human cardiac ventricle (Ito et al, 1996; Wallis et al, 1999). Furthermore, Cheitlin et al (1999) also found no PDE5 in cardiac myocytes. Therefore, the expression of PDE5 in the heart appears to be uncertain. Sildenafil with supratherapeutic doses has been shown to have no contractile effect on isolated canine isolated trabeculae carneae (Wallis et al, 1999) and human right atrial and left ventricular muscle strips (Cremers et al, 2003). The present study was designed to elucidate the effects of sildenafil on the atrial cGMP levels, and contractile and secretory functions in beating rabbit atria.

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**ABBREVIATIONS:** PDE, phosphodiesterase; ANP, atrial natriuretic peptide; GC, guanylyl cyclase; p, particulate; s, soluble.

## METHODS

### *Preparation of beating perfused rabbit atrium*

New Zealand White rabbits were used. All experiments were carried out under the approval of the Ethics Committee at the Institute for Medical Sciences of Jeonbug National University. An isolated perfused atrium was prepared by the method described previously (Cho et al, 1995; 2002), allowing atrial pacing and measurements of changes in atrial volume during contraction (stroke volume), pulse pressure, transmural extracellular fluid (ECF) translocation, cGMP efflux and ANP secretion. The atrium was perfused with HEPES buffer solution by means of a peristaltic pump (at 36.5°C and 1 ml/min). The composition of the buffer was as follows (mM): 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 25 $NaHCO_3$, 10.0 glucose, and 10.0 HEPES (pH adjusted to 7.4 with NaOH).

### *Experimental protocols*

The atria were perfused for 60 min to stabilize cGMP efflux, ANP secretion and atrial dynamics. [$^3$H]inulin was introduced to the pericardial fluid 20 min before the start of sample collection (Cho et al, 1995; Wen et al, 2000). The perfusate was collected at 2-min intervals at 4°C for analyses. The atria were paced at 1.3 Hz. The control period (48 min) was followed by an infusion of SIN-1, a nitric oxide (NO) donor which is an activator of soluble (s) guanylyl cyclase (GC; 100 $\mu$M, n = 15), or C-type natriuretic peptide (CNP), an activator of particulate (p) GC (0.1 $\mu$M, n = 10). To analyze the effects of sildenafil on SIN-1- or CNP-induced changes in cGMP efflux, atrial dynamics, atrial stroke volume and pulse pressure, and ANP release, 36 min of sildenafil or vehicle treatment was followed by an infusion of SIN-1 or CNP [vehicle plus SIN-1 (100 $\mu$M), n= 13~15; vehicle plus CNP (0.1 $\mu$M), n=8~10; sildenafil (1 $\mu$M) plus SIN-1 (100 $\mu$M), n=8~9; Sildenafil (10 $\mu$M) plus SIN-1 (100 $\mu$M), n=8; sildenafil (1 $\mu$M) plus CNP (0.1 $\mu$M), n=3~5; sildenafil (10 $\mu$M) plus CNP (0.1 $\mu$M), n=9~10] or vehicle [sildenafil (10 $\mu$M) alone, n=4]. For the time-matched control experiments (n=11), vehicle was introduced and the values obtained in the control and experimental observations were compared.

### *Radioimmunoassay of ANP*

Immunoreactive ANP in the perfusate was measured by a specific radioimmunoassay, as described previously (Cho et al, 1995). The amount of immunoreactive ANP secreted was expressed in nanograms of ANP per minute per gram of atrial tissue. The molar concentration of immunoreactive ANP, calculated in terms of ECF translocation, which reflects the concentration of extracellular ANP in the atrium, therefore, indicates the rate of myocytic ANP released into the surrounding paracellular space (Cho et al, 1995), calculated as ANP released ($\mu$M)=immunoreactive ANP (in pg · min$^{-1}$ · g$^{-1}$)/ECF translocated (in $\mu$l · min$^{-1}$ · g$^{-1}$ 3063) [mol/wt., ANP- (1~28)]. Most of the ANP secreted are processed ANP (Cho et al, 1995).

### *Radioimmunoassay of cGMP*

cGMP was measured, calculated and expressed as described previously (Wen et al, 2004). Production of cGMP was measured by equilibrated radioimmunoassay. Briefly, standards or samples were incubated with antiserum for cGMP (Calbiochem, San Diego, CA, USA) and iodinated cGMP [2'-o-monosuccinyl guanosine-3',5'-cyclic monophosphate tyrosyl methyl ester (Sigma Chemical, St Louis, MO, USA) in 50 mM sodium acetate buffer (pH 4.85) containing 8 mM theophylline]. The bound form was separated from the free form by charcoal suspension. The amount of cGMP efflux was expressed as pmol cGMP per min per g of atrial tissue. The molar concentration of cGMP efflux in terms of ECF translocation, which may reflect the concentration of cGMP in the interstitial space fluid (Wen et al, 2004), was calculated as cGMP efflux concentration ($\mu$M) = cGMP (in pmol · min$^{-1}$ · g$^{-1}$)/ECF translocated (in $\mu$l · min$^{-1}$ · g$^{-1}$).

### *Statistical analysis*

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

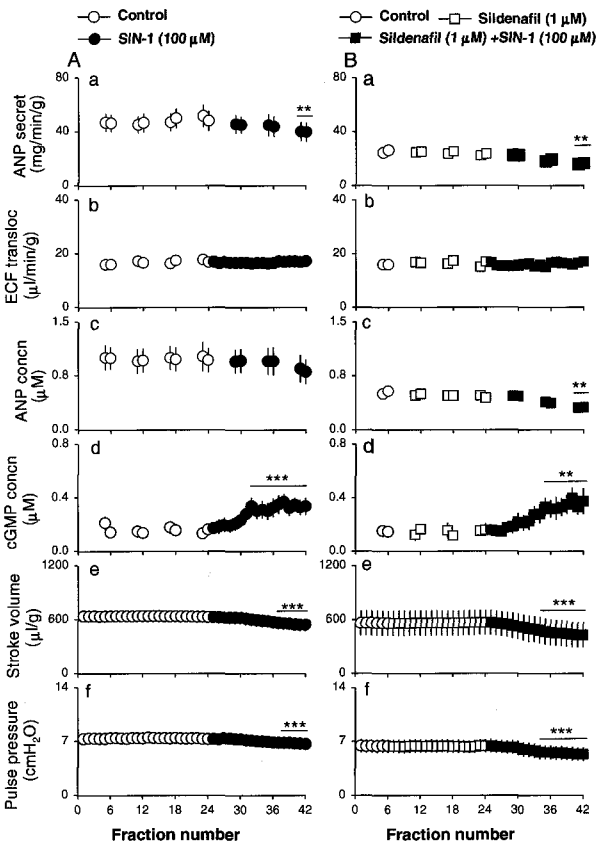


**Fig. 1.** Effects of SIN-1 and modification by sildenafil on atrial secretion and dynamics. (A) Effects of SIN-1 (100 $\mu$M) on ANP secretion (a), extracellular fluid (ECF) translocation (b), ANP concentration (c), cGMP efflux concentration (d), atrial stroke volume (e) and pulse pressure (f) in perfused beating rabbit atria (1.3 Hz; n=13~15). (B) Effects of sildenafil (1 $\mu$M) on SIN-1-induced changes in the same parameters (n=8~9). Values are means±SE. **$p<0.01$, ***$p<0.001$ vs. control or sildenafil.

## RESULTS

### *Effects of sildenafil on cGMP efflux concentration atrial dynamics and ANP release*

ANP secretion, ECF translocation, ANP concentration, which reflects the rate of atrial myocyte ANP released into the surrounding paracellular space of the atrium, and cGMP efflux concentration were stably maintained (Figs. 1A, 2A). Atrial dynamic changes, including stroke volume and pulse pressure, were also stable and repeatable. Sildenafil in a therapeutic concentration (1 $\mu$M, Zusman et al, 1999) slightly increased the basal level of cGMP efflux concentration, but not significantly (Figs. 1Bd, 3A). Sildenafil (1 $\mu$M) increased atrial stroke volume (Figs. 1Be, 3B), while decreased atrial ANP release (Figs. 1Bc, 3D).

Supratherapeutic dose (10 $\mu$M) of sildenafil further increased atrial stroke volume and pulse pressure (Figs. 2Be, Bf, 3B, C), while decreased ANP release, and the response was not different from that of 1 $\mu$M sildenafil (Figs. 2Bc, 3D). Sildenafil (10 $\mu$M) decreased cGMP efflux concentration slightly, but not significantly (Fig. 2Bd, 3A). These findings indicate that sildenafil in a therapeutic dose increases atrial dynamics and decreases atrial myocytic ANP release without significant change in cGMP efflux concentration.

### *Effects of sildenafil on SIN-1-induced changes in cGMP efflux concentration, atrial dynamics and ANP release*

To further clarify the effects of sildenafil on sGC-activation-induced changes in cGMP levels, atrial dynamics and ANP release, sildenafil was followed by SIN-1, an NO donor which is an activator for sGC. As seen in Figs. 1A and 4, SIN-1 increased cGMP level and decreased atrial stroke volume, pulse pressure and ANP release. One $\mu$M Sildenafil had no significant effect on SIN-1-induced increase of cGMP levels (Figs. 1Bd, 4A). With higher concentration (10 $\mu$M), sildenafil attenuated the SIN-1-induced changes of cGMP levels, however, not significantly (Fig. 4A). Sildenafil

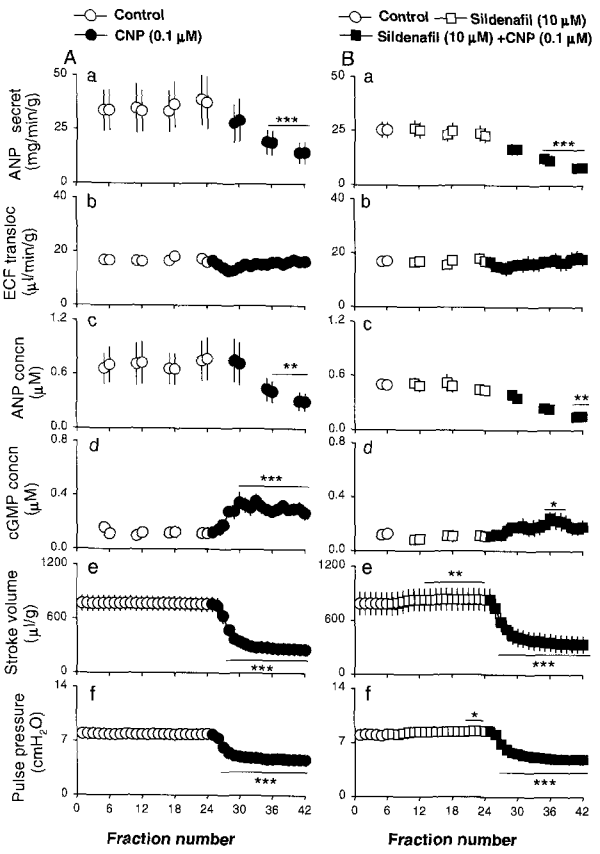


**Fig. 2.** Effects of CNP and modification by sildenafil on atrial secretion and dynamics. (A) Effects of CNP (0.1 $\mu$M) on ANP secretion (a), extracellular fluid (ECF) translocation (b), ANP concentration (c), cGMP efflux concentration (d), atrial stroke volume (e) and pulse pressure (f) in perfused beating rabbit atria (1.3 Hz; n=8~10). (B) Effects of sildenafil (10 $\mu$M) on CNP-induced changes in the same parameters (n=9~10). Values are means±SE. **p<0.01, ***p<0.001 vs. control or sildenafil.

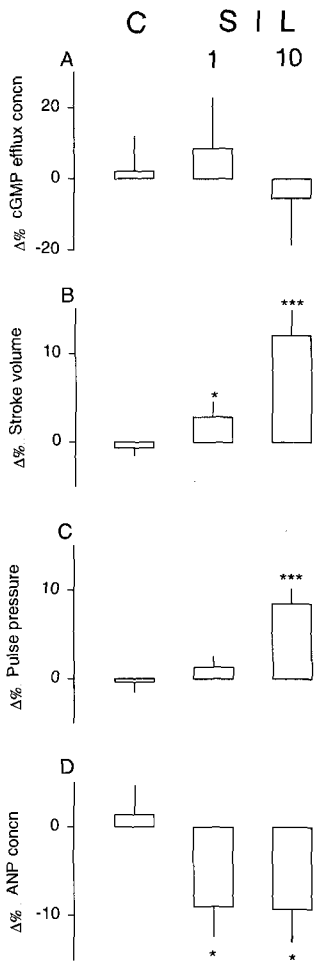


**Fig. 3.** Summarized data for the effects of sildenafil (SIL) on atrial cGMP efflux concentration (A), atrial stroke volume (B), pulse pressure (C), and ANP concentration (D). The responses were compared with differences of mean values of 2 fractions before (fraction number 5 and 6) and after 3 cycles (fraction number 23 and 24) of sildenafil (1, 10 $\mu$M) or vehicle (C). Number of experiments: control, n=21~25; sildenafil (1 $\mu$M), n=11~14; sildenafil (10 $\mu$M), n=17~18. Values are means±SE. *p<0.05, ***p<0.001 vs. control.

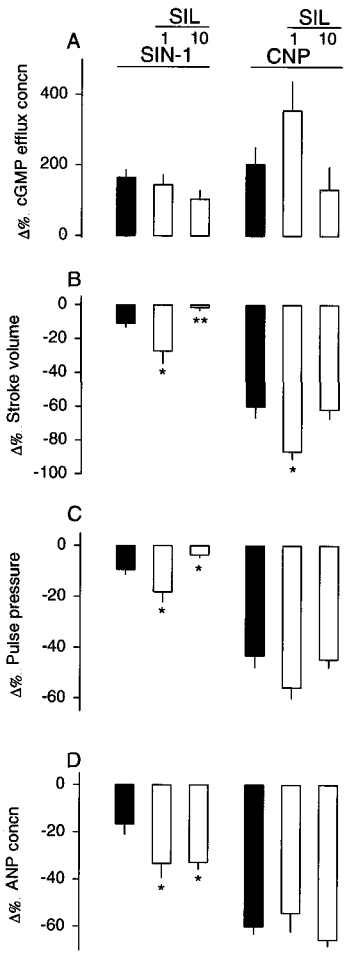


**Fig. 4.** Summarized data for the effects of CNP or SIN-1 and modification by sildenafil (SIL) on atrial cGMP efflux concentration (A), atrial stroke volume (B), pulse pressure (C), and ANP concentration (D). Thirty-six minutes of sildenafil (1, 10 $\mu$M) or vehicle was followed by CNP or SIN-1. The responses were compared with differences of mean values of 2 fractions before (fraction number 23 and 24) and after 3 cycles (fraction number 41 and 42) of CNP (0.1 $\mu$M) or SIN-1 (100 $\mu$M). Number of experiments: CNP, n=8~10; sildenafil (1 $\mu$M) plus CNP, n=3~5; sildenafil (10 $\mu$M) plus CNP, n=9~10; SIN-1, n=13~15; sildenafil (1 $\mu$M) plus SIN-1, n=8~9; sildenafil (10 $\mu$M) plus SIN-1, n=8. Values are means±SE. *$p<0.05$, **$p<0.01$ vs. corresponding control.

in a therapeutic concentration accentuated the SIN-1-induced decrease of atrial dynamics (Figs. 1Be, Bf, 4B, C), and higher concentration (10 $\mu$M) of sildenafil rather attenuated the SIN-1-induced decrease of atrial dynamics (Fig. 4B, C). Sildenafil accentuated the SIN-1-induced decrease of ANP release (Fig. 1Ba, Bc, 4D). These findings indicate that sildenafil in a therapeutic dose accentuates SIN-1-induced decrease of atrial dynamics and ANP release.

### *Effects of sildenafil on CNP-induced changes in cGMP efflux concentration, atrial dynamics and ANP release*

Next, to define the effects of sildenafil on pGC-activation-induced changes in cGMP levels, atrial dynamics, and ANP release, sildenafil was followed by CNP. It was earlier shown that CNP decreased atrial dynamics and ANP release via pGC-coupled natriuretic peptide receptor (NPR)-B-cGMP signaling (Lee et al, 2000). Fig. 4A shows that 1 $\mu$M sildenafil accentuated CNP-induced increase of cGMP levels slightly, but not significantly. Higher concentration (10 $\mu$M) of sildenafil rather attenuated CNP-induced increase of cGMP levels, but not significantly (Figs. 2Bd, 4A). Sildenafil (1 $\mu$M) slightly accentuated the CNP-induced decrease of atrial stroke volume (Fig. 4B). The response to CNP of pulse pressure was not modified by sildenafil (Fig. 2Bf, 4C). Sildenafil had no effect on the CNP-induced decrease of ANP release (Figs. 2Ba, Bc, 4D). These findings indicate that sildenafil has a minor effect on CNP-induced changes of atrial contractile and secretory functions.

## DISCUSSION

The present study shows that therapeutic and supratherapeutic doses of sildenafil increased atrial dynamics and decreased ANP release without any significant change of atrial cGMP efflux concentration. Sildenafil modified the effects of SIN-1 and CNP, and accentuated SIN-1-induced decrease of atrial dynamics and ANP release. The effect of sildenafil on CNP-induced changes was not prominent as much as that on SIN-1-induced changes. SIN-1- and CNP-induced increase of cGMP efflux levels was not significantly modified by sildenafil. These results suggest that sildenafil has distinct effects on sGC-cGMP and pGC-cGMP signalings, in agreement with the result that sGC-cGMP and pGC-cGMP signalings have distinct roles in the regulation of cGMP levels, atrial dynamics and secretory function (Wen et al, 2004). It has been shown that pGC-cGMP and sGC-cGMP signalings function as high- and low-gain switches, respectively, in the regulation of cyclic nucleotides levels, contractile and secretory functions.

PDE5, a target molecule of sildenafil, has been shown to be expressed in heart (Stacey et al, 1998; Yanaka et al, 1998; Giordano et al, 2001), whereas it may not exist in heart (Cheitlin et al, 1999; Wallis et al, 1999). Therefore, the presence of PDE5 in the heart is the matter of controversy. Therefore, the presence of PDE5 in the cardiac atrium should clearly be defined. Therapeutic and supratherapeutic doses of sildenafil, had no effect on atrial cGMP efflux levels. High concentration of sildenafil may inhibit atrial cGMP efflux, because it is shown that multidrug resistance protein 5 (MRP5), a cellular export pump for cyclic nucleotides, is expressed in human cardiac auricular tissue (Dazert et al, 2003) and sildenafil inhibits MRP5-mediated transport of cGMP in the V79 cell membrane vesicles (Jedlitschky et al, 2000). In the present study, high concentration of sildenafil decreased cGMP efflux concentration slightly, but not significantly. Therapeutic dose of sildenafil did not modify GC activation-induced cGMP efflux levels. Because GC-cGMP signaling is compartmentalized in the cardiac atrium (Wen et al, 2004) and PDE5A is confined to the certain site of cardiomyocytes (Takimoto et al, 2005), the effect of sildenafil on cGMP metabolism may possibly be restricted to a microdomain.

Sildenafil has been shown to increase cAMP levels (Stief et al, 2000; Sugiyama et al, 2002; Du Toit et al, 2005). If this is the case, sildenafil-induced increase of atrial

dynamics and decrease of ANP release in the present study is conceivable, since cAMP is a stimulatory (Fischmeister and Hartzell, 1991) and inhibitory (Iida & Page, 1989; Cui et al, 2002) factor for contractile and secretory function of the heart, respectively. Alternatively, because supratherapeutic dose of sildenafil blocks delayed rectifier $K^+$ channel and prolongs cardiac repolarization (Geelen et al, 2000; Chiang et al, 2002; Sarazan et al, 2004), it is possible that sildenafil has a positive inotropic effect as shown in the present study. In contrast, Chiang et al (2002) observed no effect of a therapeutic dose of sildenafil on cardiac repolarization and the delayed rectifier $K^+$ currents. Because an increase of $Ca^{2+}$ influx is a negative regulator of atrial ANP release (Ito et al, 1988; De Bold & De Bold, 1989; Ruskoaho et al, 1990; Deng & Lang, 1992; Kim et al, 1997; Wen et al, 2000; Li et al, 2005), the present finding which showed decrease of ANP release by sildenafil is consistent with the above reports. In the presence of sildenafil, SIN-1-induced changes in atrial dynamics were different in terms of the concentrations of sildenafil; a low concentration of sildenafil accentuated SIN-1-induced decrease of atrial dynamics whereas a high concentration attenuated it. Possible reason for the difference in the effects of sildenafil due to it's concentration may be related to the notion mentioned above: sildenafil may have two opposing effects in terms of the concentration: effects on PDE5 and cAMP metabolism or delayed rectifier $K^+$ channels.

In summary, the present study shows that sildenafil has a positive inotropic effect and modifies sGC-activation induced changes of atrial dynamics and secretory responses in rabbit cardiac atrium.

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