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Article

Gαq-RGS2 loop activator modulates the activity of vario us agonists on isolated heart and aorta of normal rats

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The Gαq-RGS2 loop activator, 1-(5-chloro-2-hydroxyphenyl)-3-(4-(trifluoromethyl)-phenyl)-1H-1,2,4-triazol-5(4H)-one has demonstrated Gαq signaling inhibitor activity. Therefore, we aimed to study the effect of Gαq-RGS2 loop activator on isolated heart and aorta of normal rats. Heart and aorta were isolated from the sacrificed rats (n=6) and mounted on the langendroff's and organ bath assembly, respectively. The effect of various receptor-dependent (acetylcholine, angiotensin II and adrenaline) and independent (calcium chloride and sodium nitroprusside) agonists in absence and presence of Gαq-RGS2 loop activator on left ventricular systolic pressure (LVSP) and the contractile responseswere evaluated in isolated heart and aorta, respectively. Gαq-RGS2 loop activator (100 µM) significantly attenuated the adrenaline ($p<0.001$,) and angiotensin II ($p<0.001$) induced increase in LVSP in isolated heart and contractile response of adrenaline ($p<0.01$) and angiotensin II ($p<0.01$) in the aorta. However, effect calcium chloride did not significantly alter by Gαq-RGS2 loop activator. The effect of acetylcholinewas significantly ($p<0.01$, $p<0.05$) increased by Gαq-RGS2 loop activator in isolated heart and aorta. The effect of sodium nitroprusside significantly ($p<0.01$) potentiated by Gαq-RGS2 loop activator (100 µM) in isolated heart while it did not significantly alters in the aorta. Ultimately, the Gαq-RGS2 loop activator modulated the action of receptor-dependent agonists in isolated heart and aorta.

Keywords: Gαq-RGS2 loop activator. Cardiovascular reactivity. Heart. Aorta.

INTRODUCTION

The pathogenesis of cardiovascular disease involved the abnormalities in activity of cardiovascular mediators. All the major cardiovascular mediators also produce their actions via G-protein coupled receptor (GPCR) signaling. GPCR is a transmembrane protein that regulates the number of cardiovascular functions such as heart rate and contractility in cardiac as well as vascular smooth muscle (Capote, Mendez Perez, 2015). GPCR transduces signals by three types of G-protein, stimulatory (Gαs), inhibitory (Gαi) and quiescent (Gαq)(Tuteja, 2009). An over activation of GPCR mediated Gαq signaling is predominantly attributed in development of cardiovascular diseases such as hypertension andcardiac hypertrophy. Previous study showed that vascular smooth muscle Gαq signaling was upregulated in renal artery stenosis induced hypertensive mice and genetic vascular smooth muscle-derived models of hypertension(Harris *et al*., 2007). It has reported that Gαq proteins are required to develop pressure overload cardiac hypertrophy(Wettschureck *et al*., 2001;Akhter *et al*., 1998). Thus, an over activity of Gαq signaling plays a significant role in development of cardiovascular abnormalities.Gαq signalingis negatively regulated by Regulator of G-protein signaling-2 (RGS2)(Heximer *et al*., 1997). RGS2 deficient mice developed phenotypes of hypertension owing to sympathetic hyperactivity and renovascular abnormalities(Gross *et al*., 2005;Osei-Owusu *et al*.,

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2012). The expression of RGS2 also decreases in saphenous artery of spontaneously hypertensive rats (Grayson *et al*., 2007). Gαq-RGS2 loop activator inhibits the Gαq signaling by stimulating RGS2 mediated Gαq bound GTP degradation(Fitzgerald *et al*., 2006). Therefore, we aimed to study Gαq-RGS2 loop activator induced modulation in action of various agonists on isolated heart and aorta. In present study, two types of agonists were selected those mediate their action via receptor dependent signaling pathway (adrenaline, angiotensin II and acetylcholine) and independent to receptor signaling pathways (calcium chloride and sodium nitroprusside).

MATERIAL AND METHODS

Ethical research approval

The experimental protocol (LMCP/COLOGY/16/12) was approved by the Institutional Animal Ethics Committee (IAEC), L. M. College of Pharmacy. An experiment on animals was conducted in accordance to guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Animals

Male wistar rats (200-250 g) were procured from Zydus research center (ZRC), Ahmedabad, India at 1 wk before the study. They were maintained at 22 ± 1°C, 55 ± 5% relative humidity and 12-hr light-dark cycle in the animal house facility of L. M. College of Pharmacy, Ahmedabad. Rats had free access to standard pellet diet and filtered tap water.

Chemicals

Phenylephrine, acetylcholine,adrenaline, angiotensin II, Sodium nitroprusside and CaCl2 were purchased from SigmaChemical Co. (St. Louis, MO, USA). NaCl, KCl, MgSO4, KH2PO4 and NaHCO3 were obtained from Merck (Mumbai, India). Gαq-RGS2 loop activator (1-(5-chloro-2-hydroxyphenyl)-3-(4-(trifluoromethyl) phenyl)-1H-1,2,4-triazol-5(4H)-one) was synthesized and purified in our laboratory according to reported data (Hewawasam *et al*., 2002).

Isolated perfused rat heart preparation

Rats were heparinized (500 IU heparin/rat) and sacrificed. Heart was rapidly isolated and placed in ice-cold Krebs–Henseleit (K-H) buffer. Heart was cannulated via aorta and perfused with non-recirculating K–H buffer (118 mMNaCl, 4.7 mMKCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4, 25 mM NaHCO3, 11 mM glucose, pH 7.4) at constant perfusion pressure 70 mmHg. The perfusate was equilibrated with 95% O2 and 5% CO2 and maintained at a temperature of 37 ºC. A fluid-fillled latex balloon was inserted in to the left ventricle to measure the left ventricular systolic pressure. Balloon was connected to a pressure transducer (Biopac-MP 100; Biopac, Santa Barbara, CA, USA) and inflated to achieve left vetricular end-diastolic pressure (LVEDP) of about 10 mm Hg. The biopac data acquisition software was used to record the left ventricular systolic pressure(Soni *et al*., 2010).

Isolated rat aorta preparation

Thoracic aorta was isolated and spirally cut strip (3-5mm width, 20-30mm length) was prepared. The strip was mounted in 35-ml organ tube containing Krebs-Henseleit buffer maintained at 37°C and oxygenated with 95% O2, CO2 mixture. The preparations were suspended under 1 g resting tension which was determined in the baseline studies and equilibrated for 60 min, with changes of bathing fluid every 15 min. Isometric tension studies were performed using Iworx data acquisition system (Iworx 304T, Iworx, Dover, NH, USA).

Experimental protocol

Effect of various receptor dependent agonists (adrenaline (100 μM), angiotensin II (100 μM) and acetycholine (100 μM)) and receptor independent agonist (Calcium chloride (1mM) and sodium nitroprusside (100 μM)) in absence and presence of Gαq-RGS2 loop activator (1, 10 and 100 μM)was evaluated on left ventricular systolic pressure isolated perfused heart.Contractile responses of same agonists (adrenaline, angiotensin II, acetycholine, Calcium chloride and sodium nitroprusside (10-9 to 10-4 M)) were taken in absence and presence of Gαq-RGS2 loop activator (100 μM)in isolated aortic preparation. In isolated aortic tissue preparation, aorta was pre-

constricted by phenylephrine to evaluate the effect vasodilators.

Statistical analysis

Data were expressed as mean ± SEM. Statistical evaluation was performed bystudent's two tailed paired t-test using Graph pad prism 5.0 software. $p < 0.05$ was considered statistically significant.

RESULTS

In present study, adrenaline induced increase in left ventricle systolic pressure significantly ($p < 0.001$) attenuated in the presence of Gαq-RGS2 loop activator (10, 100 μM)in the pefused heart. Contractile response of adrenaline significantly ($p < 0.01$) attenuated in the presence Gαq-RGS2 loop activator in isolated aorta (Figure1). Similarly, angiotensin II induced increase in left ventricle systolic pressure significantly ($p < 0.001$) attenuated in the presence of Gαq-RGS2 loop activator (100 μM) in the pefused heart and contractile response of angiotensin II significantly ($p < 0.01$) attenuated in the presence Gαq-RGS2 loop activator in isolated aorta (Figure2).The calcium chloride induced increase in left ventricular systolic pressure in isolated heart and contractile response in aortic tissue did not alter in the presence of Gαq-RGS2 loop activator (Figure3). The acetylcholine induced decrease in left ventricular systolic pressure significantly ($p < 0.05$, $p < 0.001$) increased in the presence of Gαq-RGS2 loop activator (10, 100 μM, respectively) in the isolated heart and vasorelaxant effect of acetylcholine was significantly ($p < 0.05$) increased in the presence of Gαq-RGS2 loop activator in the aortic tissue (Figure4). The sodium nitroprusside induced decrease in the left ventricular systolic pressure in the isolated heart significantly increased by Gαq-RGS2 loop activator while it did not modulate by Gαq-RGS2 loop activator in the isolate aortic tissue (Figure5).

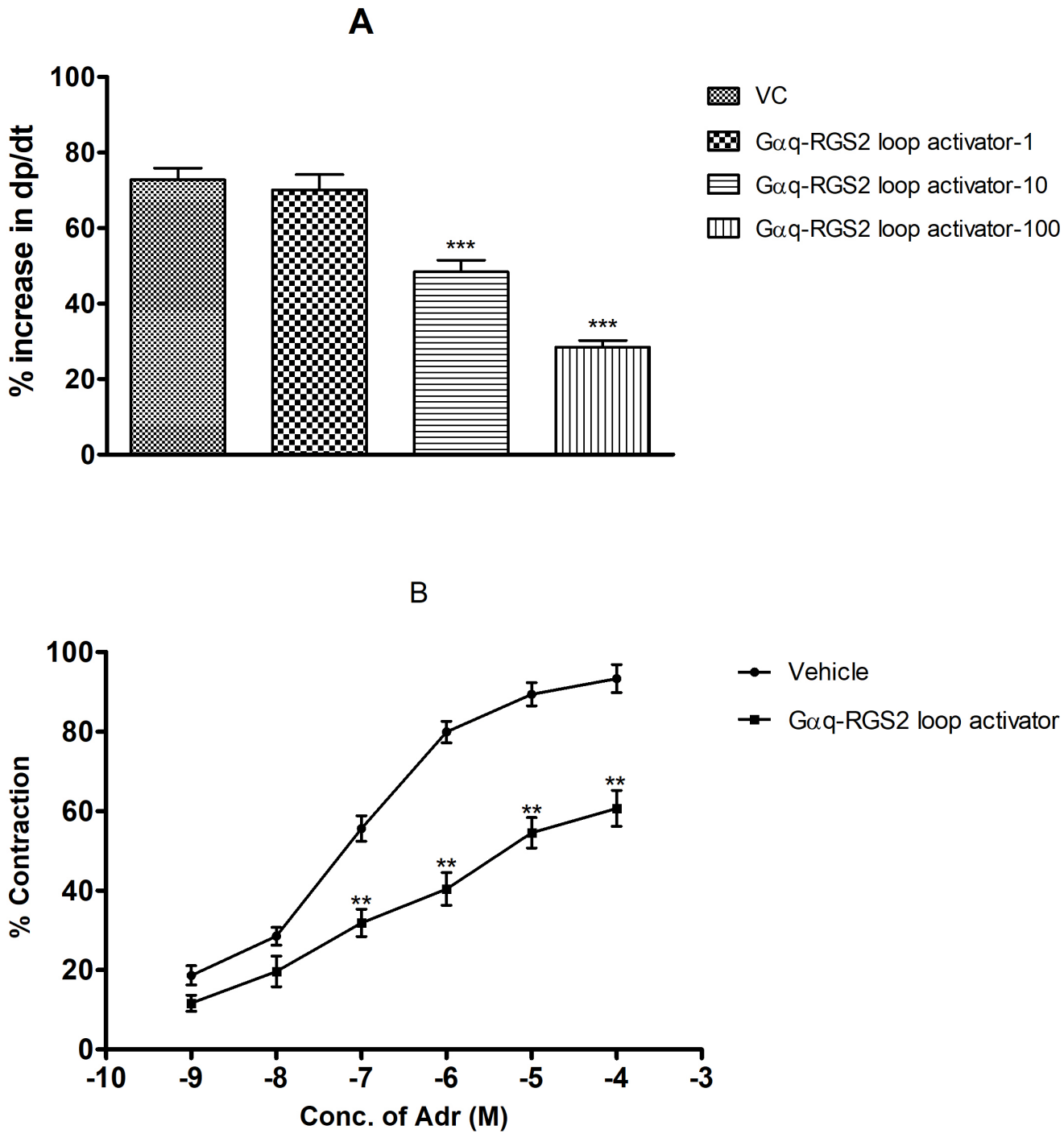

FIGURE 1 - Effect of adrenaline in absence and presence of Gαq-RGS2 loop activator on (A) Heart and (B) Aorta. Vehicle = in absence of Gαq-RGS2 loop activator, Gαq-RGS2 loop activator = in presence of Gαq-RGS2 loop activator. (1, 10 and 100 represents concentration μM). **p<0.01, ***p<0.001 vs. Vehicle.

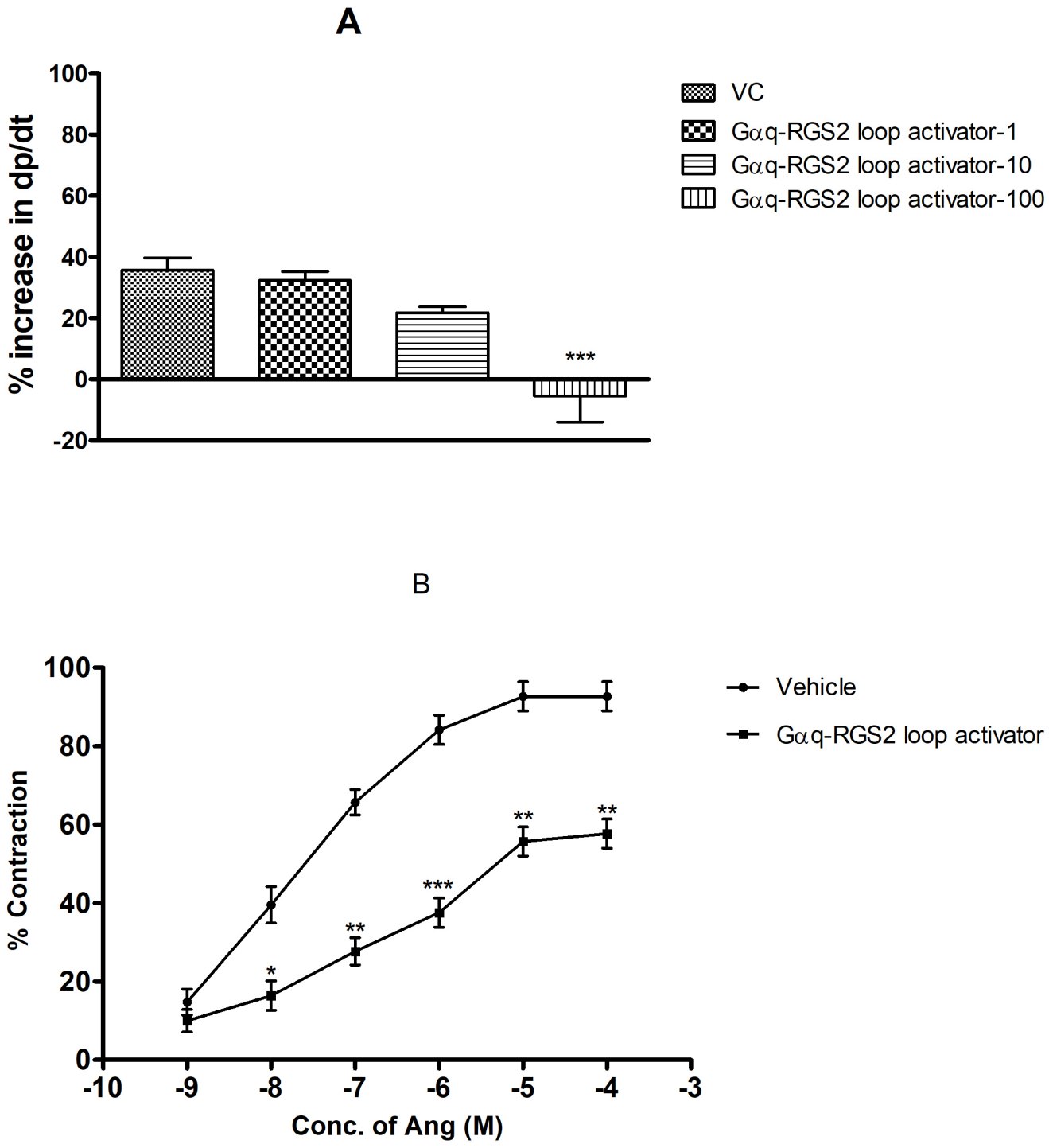

FIGURE 2 - Effect of angiotensin II in absence and presence of Gαq-RGS2 loop activator on (A) Heart and (B) Aorta. Vehicle = in absence of Gαq-RGS2 loop activator, Gαq-RGS2 loop activator = in presence of Gαq-RGS2 loop activator. (1, 10 and 100 represents concentration μM). ***p<0.001 vs. Vehicle.

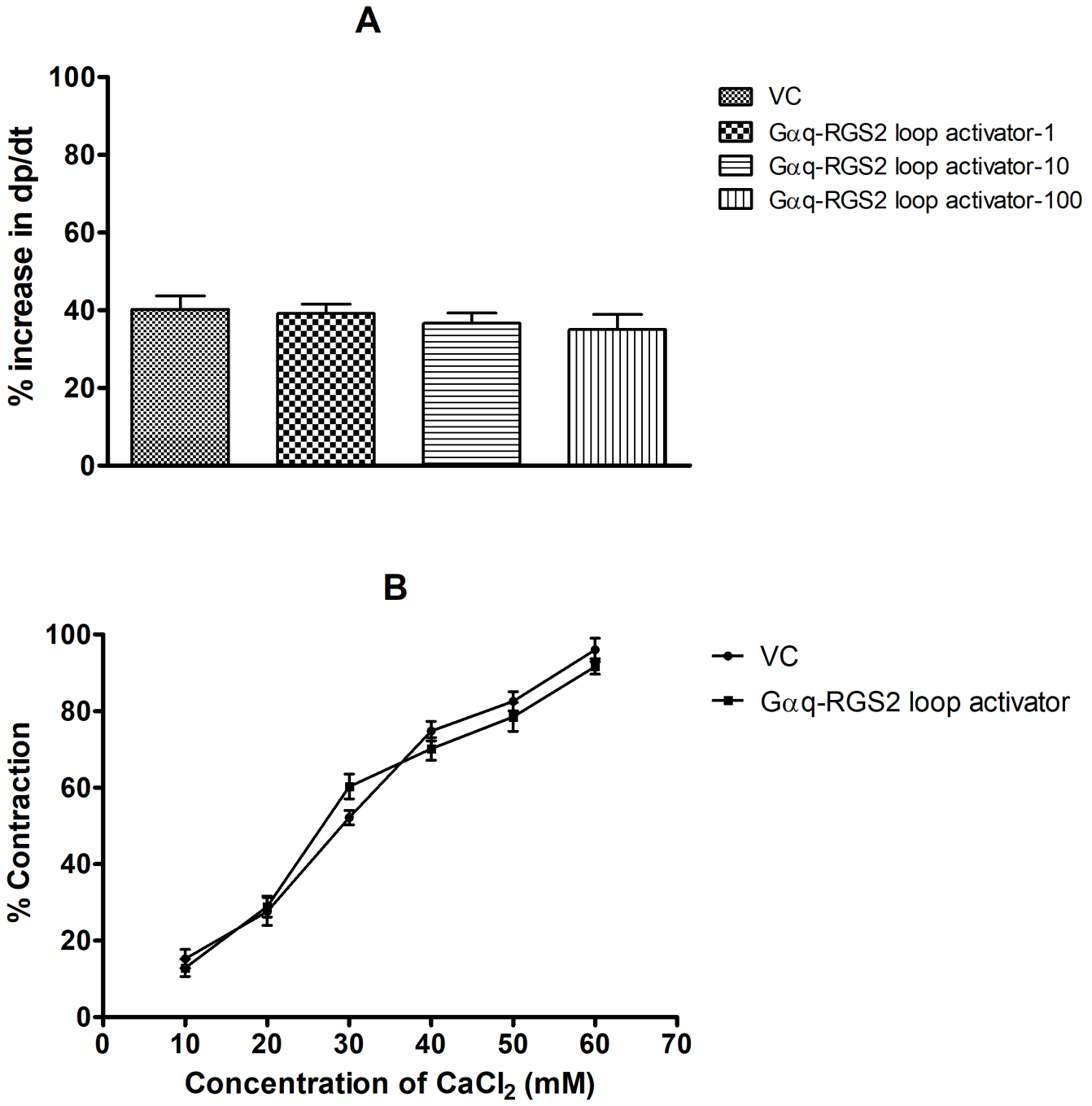

FIGURE 3 - Effect of calcium chloride in absence and presence of Gαq-RGS2 loop activator on (A) Heart and (B) Aorta. Vehicle = in absence of Gαq-RGS2 loop activator, Gαq-RGS2 loop activator = in presence of Gαq-RGS2 loop activator. (1, 10 and 100 represents concentration μM). ***p<0.001 vs. Vehicle.

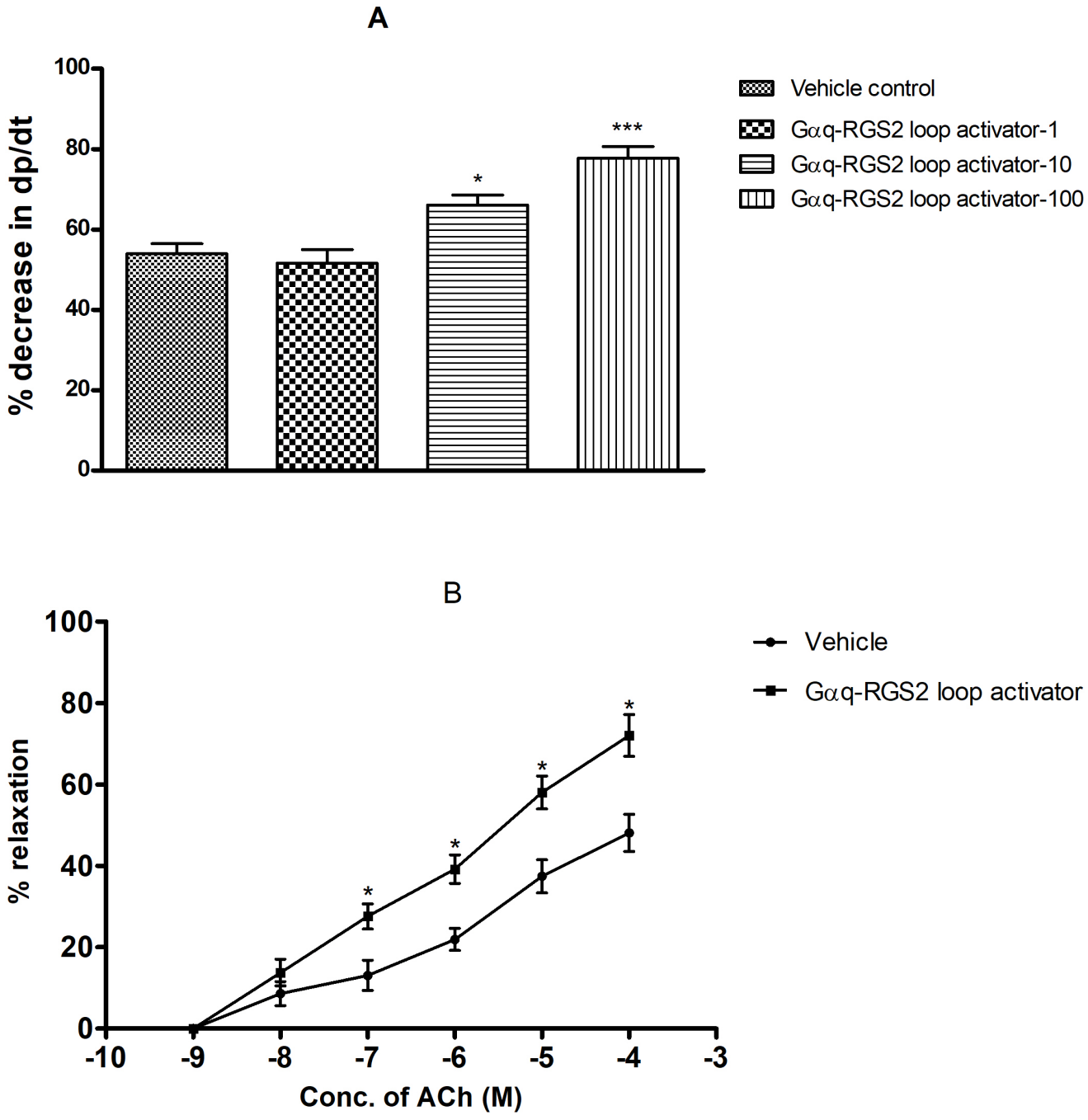

FIGURE 4 - Effect of acetylcholine in absence and presence of Gαq-RGS2 loop activator on (A) Heart and (B) Aorta. Vehicle = in absence of Gαq-RGS2 loop activator, Gαq-RGS2 loop activator = in presence of Gαq-RGS2 loop activator. (1, 10 and 100 represents concentration μM). ***p<0.001 vs. Vehicle.

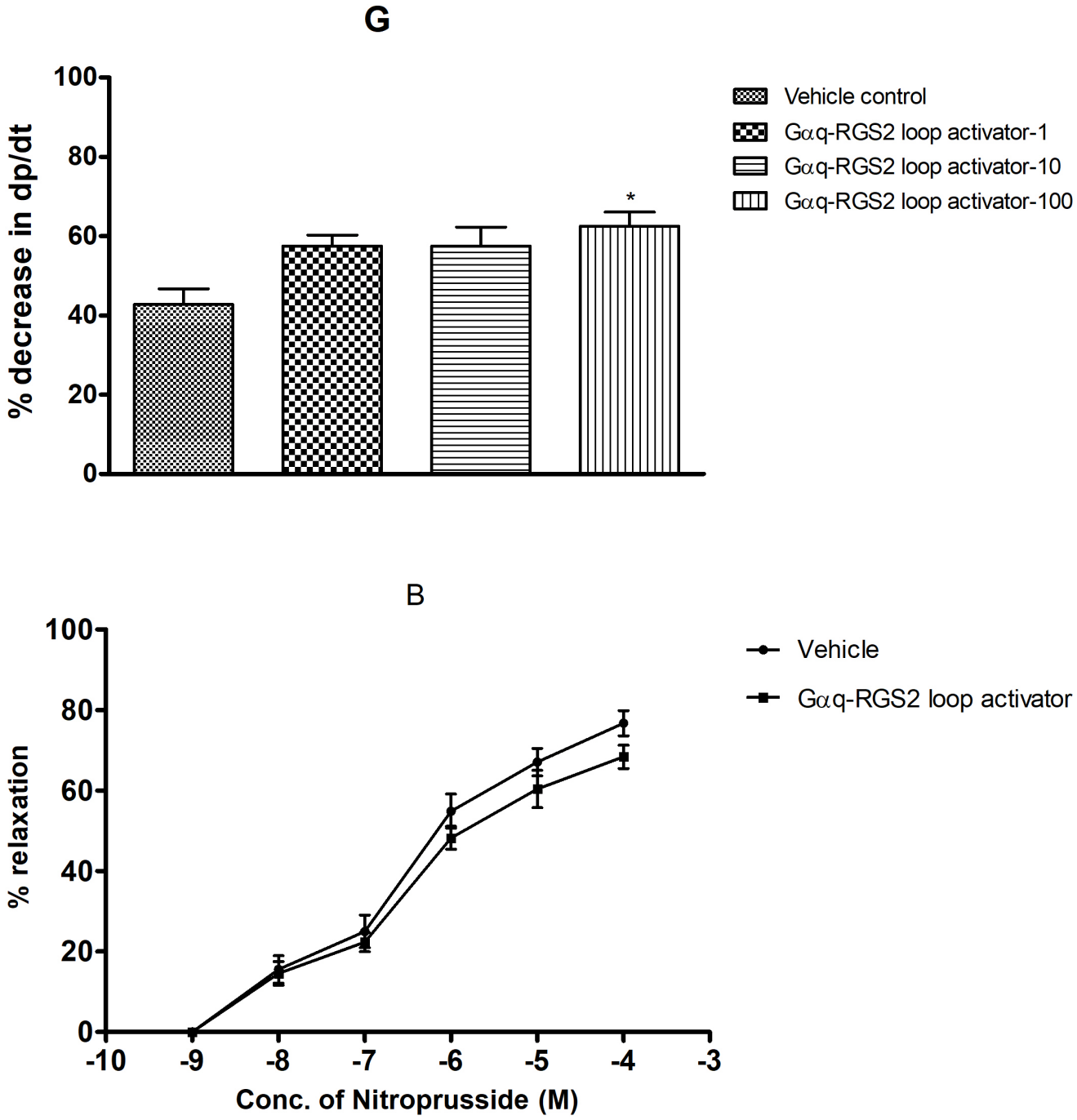

FIGURE 5 - Effect of sodium nitroprusside in absence and presence of Gαq-RGS2 loop activator on (A) Heart and (B) Aorta. Vehicle = in absence of Gαq-RGS2 loop activator, Gαq-RGS2 loop activator = in presence of Gαq-RGS2 loop activator. (1, 10 and 100 represents concentration μM). **p<0.01 vs. Vehicle.

DISCUSSION

In current study, Gαq-RGS2 loop activator attenuated the effect of adrenaline and angiotensin II on isolated heart and aorta.Adrenaline produces their action via principally 1 and α1 receptor in myocardial and vascular smooth muscle cells, respectively(Rockman, Koch, Leftkowitz, 2002;Brodde, Michel, 1999). An angiotensin II produces their action through AT1 receptor in myocardial and vascular smooth muscle cells(Griendling *et al*., 1997;Exton, 1985). Gαq-RGS2 loop activator mimicked the action of acetylcholine in isolated heart and aortic. Acetlycholine produces their action via M2 and M3 receptor in myocardial and vascular smooth muscle cells, respectively(Brodde, Michel, 1999). Gαq-RGS2 loop activator did not modulate the action of calcium chloride and sodium nitroprusside in isolated heart and aorta. Both calcium chloride and sodium nitroprusside produce their action independent to receptor. Ultimately, Gαq-RGS2 loop activator modulated the action of receptor dependent agonists (adrenaline, angiotensin II and acetylcholine) in isolated heat and aorta.

The action of these receptor dependent agonists is regulated by GPCR and their intracellular signaling pathway. Adrenaline, angiotensin II and acetylcholine produce their action through GPCR mediated Gαs, Gαq and Gαi signaling pathway, respectively in myocardium(Salazar, Chen, Rockman, 2007). In vascular smooth muscle cells, adrenaline, angiotensin II and acetylcholine mediate their action through Gαq signaling of GPCR(Osei-Owusu, Blumer, 2015). The compound, 1-(5-chloro-2-hydroxyphenyl)-3-(4-(trifluoromethyl)-phenyl)-1H-1,2,4-triazol-5(4H)-one (Gαq-RGS2 loop activator) has demonstrated Gαq inhibitor activity by stimulating RGS2 mediated Gαq bound GTP degradation(Fitzgerald *et al*., 2006). However, Gαq-RGS2 loop activator decreased the action of adrenaline in isolated heart and increased the activity of acetylcholine in isolated heart and aorta in present study which showed contraindication with hypothesis. Therefore, there is need of further study to explore the exact mechanism of action of Gαq-RGS2 loop activator. Based on these data, the effect of Gαq-RGS2 loop activator in various cardiovascular disease and possible mechanism for the action is a great interest of study in future.

CONCLUSION

In conclusion, Gαq-RGS2 loop activator modulated the action of receptor dependent agonists in the isolated heart and aorta. However, the effect of receptor independent agonists did not modulate by the Gαq-RGS2 loop activator. The mechanism of the Gαq-RGS2 loop activator for modulation in the action of various receptor dependent agonists is a great interest of study in future.

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