SCIENTIA PLENA
www.scientiaplena.org.br
VOL. 19, NUM. 02 2023
doi: 10.14808/sci.plena.2023.024901

Naringin promotes positive inotropism in atrial tissue through β-AR/PKA-dependent pathway

Naringina promove inotropismo positivo em tecido atrial por meio da via dependente de β-AR/PKA

L. R. Santos[1]; D. S. Souza[2]; T. R. R. Mesquita[3]; C. O. Dantas[1]; A. M. Araujo[1]; S. V. S. Cerqueira[1]; V. C. O. Santos[1]; K. O. Mota[1]; C. M. L. Vasconcelos[1*]

[1]*Departament of Physiology, Federal University of Sergipe, 49100-000, São Cristóvão-SE, Brazil*
[2]*Departament of Biophysics, Federal University of São Paulo, 04023-062, São Paulo-SP, Brazil*
[3]*Cedars-Sinai Medical Center, Smidt Heart Institute, 90048, Los Angeles-CA, United States*

**carlamlv@hotmail.com*
(Recebido em 20 de novembro de 2022, aceito em 06 de fevereiro de 2023)

Naringin is a flavonoid glycoside found in citrus fruits and grapes with a wide range of therapeutic actions. We aimed to study the inotropic effect of naringin on rat-isolated atria, dissecting intracellular mechanisms involved in this response. Concentration-response curves of naringin (0.003-6 mM) were obtained before and after pre-incubation with selective antagonists. Our results showed that naringin presented a biphasic inotropic response, with a positive inotropic effect at low and middle concentrations (0.003-2.0 mM), and a negative inotropic effect at high concentrations (above 3 mM). Pre-incubation with propranolol or atenolol (β-adrenergic receptors antagonists, β-AR), H89 (protein kinase-A inhibitor, PKA), nifedipine (L-type Ca^{2+} channel blocker), or ryanodine (ryanodine receptor inhibitor) fully abolished the positive inotropic effect induced by naringin. Pre-treatment of animals with reserpine, catecholamine-depleting drug, also prevented the increase of atrial contractility evoked by naringin. Altogether, we show that naringin causes a positive inotropic effect in isolated atria through β-AR/PKA-dependent pathway.
Keywords: flavonoids, atrium, β-adrenergic receptor.

A naringina é um glicosídeo flavonóide encontrado em frutas cítricas e uvas com uma ampla gama de ações terapêuticas. Nosso objetivo foi estudar o efeito inotrópico da naringina em átrios isolados de ratos, dissecando os mecanismos intracelulares envolvidos nessa resposta. As curvas de concentração-resposta de naringina (0,003-6 mM) foram obtidas antes e após a pré-incubação com antagonistas seletivos. Nossos resultados mostraram que a naringina apresentou uma resposta inotrópica bifásica, com efeito inotrópico positivo em baixas e médias concentrações (0,003-2,0 mM), e efeito inotrópico negativo em altas concentrações (acima de 3 mM). A pré-incubação com propranolol ou atenolol (antagonistas dos receptores β-adrenérgicos, β-AR), H89 (inibidor da proteína quinase-A, PKA), nifedipina (bloqueador do canal de Ca^{2+} tipo L) ou rianodina (inibidor do receptor de rianodina) aboliu totalmente o efeito inotrópico positivo induzido pela naringina. O pré-tratamento dos animais com reserpina, droga depletora de catecolaminas, também preveniu o aumento da contratilidade atrial evocada pela naringina. Ao todo, mostramos que a naringina causa um efeito inotrópico positivo em átrios isolados através da via β-AR/PKA-dependente
Palavras-chave: flavonóide, átrio, receptor β-adrenérgico.

1. INTRODUCTION

Flavonoids are a group of natural compounds with diverse biological and therapeutic activities attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties [1]. In the vascular system, this group can improve the function of failing cardiomyocytes and cardiac contractility through a positive inotropic action [2]. Positive inotropes are β1– and β2-adrenergic receptor agonists, restoring Ca^{2+} homeostasis by activation of L-type Ca^{2+} channels and Ca^{2+} release from the sarcoplasmic reticulum (SR) [3]. In this context, naringin, a flavonoid glycoside commonly found in grapes and citrus fruits, has been demonstrated to be efficacious for the treatment of metabolic and cardiac disorders [4, 5]. Accordingly, naringin has provided remarkable cardioprotection in isoproterenol-induced myocardial infarction [6], against cardiovascular dysfunction in high carbohydrate/fat-fed rats [7], hepatoprotective action [8], prevention of stroke-prone spontaneously hypertensive rats [9],

cardioprotective against from aging-dependent dysfunction [10], antioxidant, anti-inflammatory and antiapoptotic effects on cardiac damage induced by cisplatin [11], protective effect against diabetic cardiomyopathy [12] and protects H9C2 cardiomyocytes from chemical hypoxia-induced injury [13]. Furthermore, has been demonstrated that naringin can be used as an anti-inflammatory nutritional in many different diseases, such as SARS-CoV-1 [14].

Although the protective actions of naringin have been reported, the mechanism by which promotes its effects remains elusive. Moreover, all previous cardiac studies evaluated the actions of naringin in ventricular tissue or cells [5-7, 15, 16], while its effects in atrial tissue remained largely unexplored until now. Therefore, the present study aimed to study the inotropic effect of naringin on rat-isolated atria, dissecting intracellular mechanisms involved in this response.

2. MATERIALS AND METHODS

2.1 Animals

All experimental procedures were approved by the Ethical Committee for Animal Research of the Federal University of Sergipe (#19/15) and carried out in accordance with the Institutional Animal Care and Use. Male Wistar rats (250-300 g) were obtained from the Animal Care Facility of Federal University of Sergipe and housed under standard conditions.

2.2 Evaluation of the inotropic effect of naringin

The inotropic effect was evaluated in rat left atrium mounted in an organ chamber containing Krebs-Henseleit solution (in mM: NaCl 120, KCl 5.4, $MgCl_2$ 1.2, $NaHCO_3$ 27, $CaCl_2$ 1.25, Glucose 11, NaH_2PO_4 2.0) constantly oxygenated by carbogen mixture (95% O_2 and 5% CO_2) and maintained at 29° ± 0.1°C. The left atrium was stretched to 0.5 N and submitted to a field stimulation (1 Hz, 100 V, 0.5 ms) (STIMULATOR SD9 GRASS). Atrial force was recorded using an isometric force transducer (GRASS FT03). Concentration-response curves of naringin (0.003 to 6.0 mM) and 0.5% DMSO (used as diluent) were obtained to determine the contractile atrial response.

2.3 Effects of naringin on the β-adrenergic receptor (β-AR) and downstream targets of β-AR signaling pathway

To evaluate the involvement of β-AR on the inotropic response of naringin, concentration-response curves for naringin were obtained after pre-incubating the atria with propranolol (1 μM, non-selective β-AR antagonist) or atenolol (1 μM, selective β1-AR antagonist) during 20 min. To assess whether naringin affects atrial contractility via downstream targets of β-AR pathway, the involvement of protein kinase A (PKA), L-type calcium channel (LTCC), and ryanodine receptor (RyR) were evaluated pre-incubating atrial tissue, during 20 min, with H89 (PKA inhibitor, 1 μM), nifedipine (LTCC blocker, 1 μM), and ryanodine (RyR blocker, 1 μM), respectively. Then, concentration-response curves for naringin were obtained in the presence of these antagonists.

2.4 Reserpine treatment of animals

The effect of catecholamine-depleting pretreatments (reserpine 5 mg/kg, i.p, 24 h before the experiment) on the inotropic response to naringin was evaluated in the isolated atrium. Rats were divided into two groups: non-reserpinised and reserpinised. Each atrium was allowed to stabilize for 20 min. After the stabilization period, a cumulative concentration-response curve to naringin (0.003 to 6.0 mM) was obtained.

2.5 Data Analysis

All results are shown as means ± standard error of the mean (S.E.M). GraphPad Prism v.6.0 (GraphPad Software, CA, USA) was used for statistical analysis. Groups were compared using

the one-way or two-way analysis of variance (ANOVA) followed by Tukey's test. $P < 0.05$ was used as a significance level.

3. RESULTS

The inotropic effect of naringin (Figure 1A) was evaluated in left atrium submitted to electrical stimulation. Figure 1B shows representative traces of the concentration-response curve of naringin, in which it can be observed that this flavonoid presented a biphasic contractile effect. At concentrations ranging from 0.003 to 2.0 mM, naringin increased atrial contractility in a concentration-dependent manner, exhibiting a maximal effect at 2 mM (150%). At concentrations above 3 mM, a negative inotropic response was observed. From the concentration-response curve of naringin, it was possible to obtain the EC_{50} value of 0.32 ± 0.01 mM (Figure 1C). In addition, naringin also induced a decrease in the diastolic tension reaching its maximum effect at the highest concentration tested (6 mM), which as partially restored after washout (Figure 1D). As shown in the Figures 1E and 1F, the maximum and minimum dT/dt, which represent an index of contraction (+dT/dt) and relaxation (-dT/dt) velocities, were slowed down in the presence of naringin, 115% and 118%, respectively. However, after the washout, these effects were restored to control levels (Figures 1E-F).

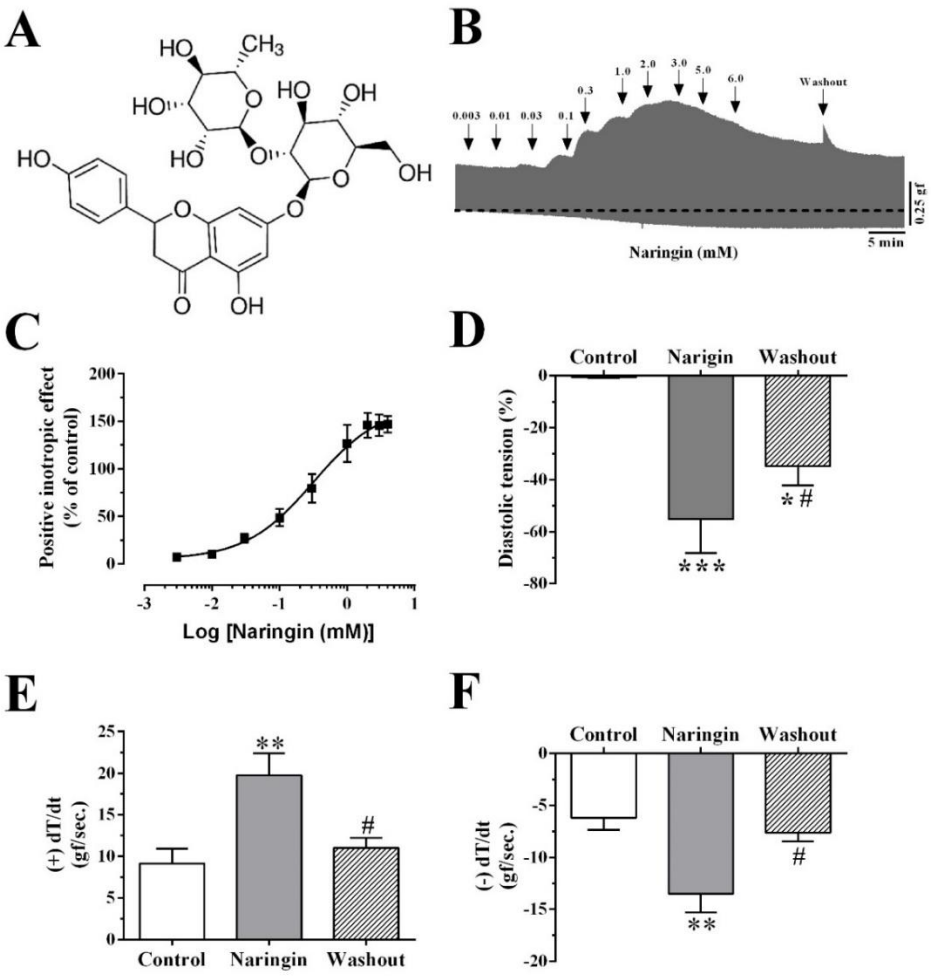

*Figure 1. Effect of naringin on the contractility of rat left atria. A, Chemical structure of naringin. B, Representative trace of contractile force in response to naringin. C, Concentration-response curve to naringin. Effects of naringin at the highest concentration tested (6 mM) on the diastolic relaxation (D), +dT/dt (E), and -dT/dt (F). Data are represented as means ± SEM (n = 6). One-way ANOVA followed by Tukey post-test. **p < 0.01 and ***p < 0.01 vs. control. #p < 0.05 vs. naringin.*

Taking into account that the increase of atrial contractility induced by naringin could be related to activation of β-ARs, concentration-response curves of naringin were performed in the presence of propranolol or atenolol. Figure 2 shows representative traces of atrial force in all experimental conditions (A) showing that pre-incubation with both β-ARs antagonists was able to abolish the positive inotropic response evoked by naringin (B). Therefore, as our results strongly indicate that naringin activates β-AR, we further investigated the involvement of PKA, a key effector of the β-AR signaling pathway. As shown in Figure 3, pre-incubation with H89 (PKA inhibitor) fully abolished the positive inotropic effect of naringin.

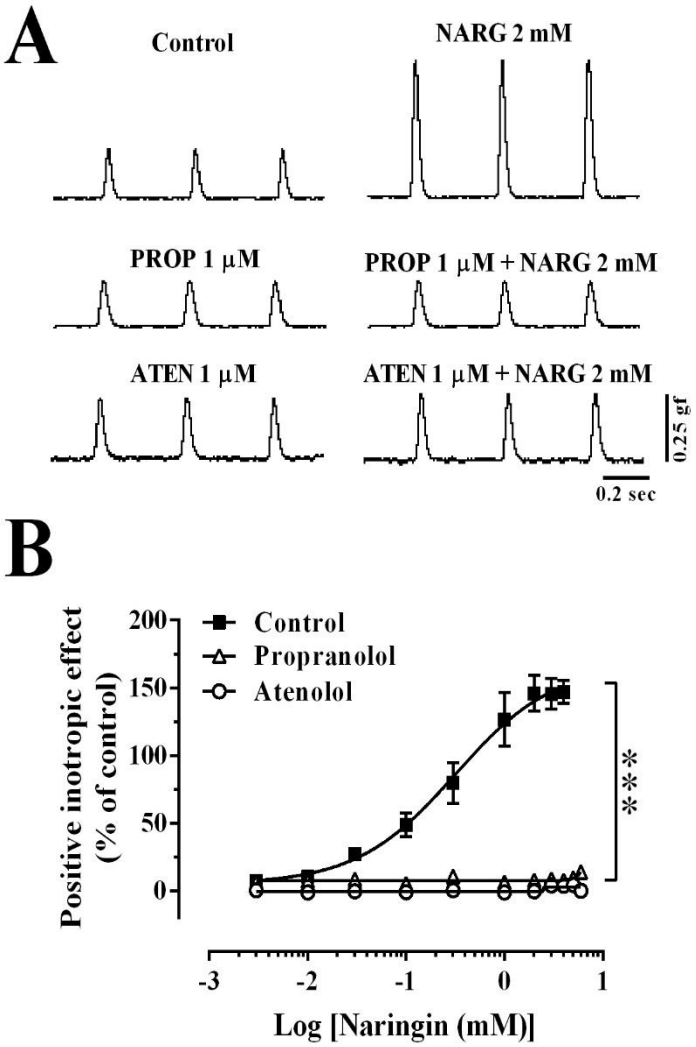

*Figure 2. Positive inotropic effect of naringin was abolished in the presence of β-AR antagonists. A, Representative traces of inotropic effect of naringin (NARG) in the absence and presence of propranolol (PROP, non-selective β-AR antagonist) or atenolol (ATEN, a selective β1-AR antagonist). B, Concentration-response curve to naringin in the absence and presence of presence β-AR antagonists. Data are represented as means ± SEM (n = 4). Two-way ANOVA followed by Tukey post-test. ***p < 0.01 vs. control.*

Once active, PKA phosphorylates a wide range of critical proteins related to the excitation-contraction (EC) coupling, which include LTCC and RyR. Therefore, to assess whether the inotropic effect of naringin involves the modulation of these downstream targets, we evaluated the response of naringin in the presence of nifedipine (LTCC blocker) and ryanodine (RyR

inhibitor). Accordingly, we demonstrated that nifedipine and ryanodine also prevented the positive inotropic effect evoked by naringin (Fig. 3).

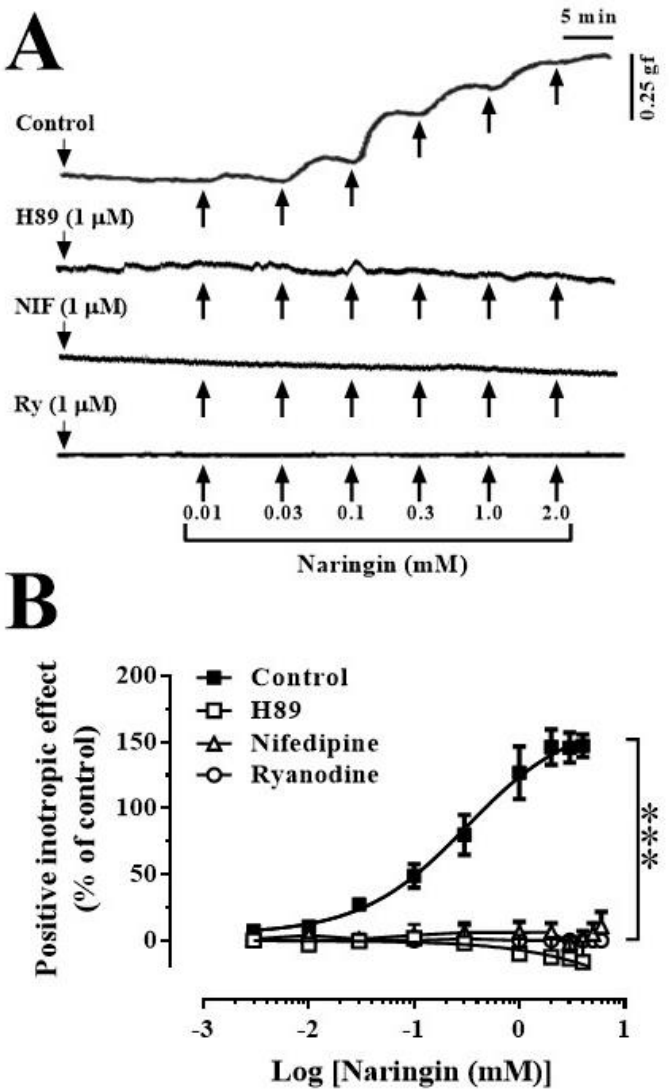

*Figure 3. Positive inotropic effect of naringin was abolished in the presence of inhibitors of downstream effectors and targets of β-AR signaling pathway. A, Representative traces of inotropic effect of naringin in the absence and presence of H89 (PKA inhibitor), nifedipine (LTCC blocker), or ryanodine (ryanodine receptor inhibitor). B, Concentration-response curve to naringin in the absence and presence of drugs mentioned above. Data are represented as means ± SEM (n = 3). Two-way ANOVA followed by Tukey post-test. ***p < 0.01 vs. control.*

To evaluate whether naringin induces the release of pre-synaptic catecholamines and, therefore, increases the contractile force, we carried out experiments in animals pretreated with reserpine, an alkaloid that depletes catecholamines at the sympathetic nerve terminal. As shown in Figure 4, a typical recording demonstrates the positive inotropic effects produced by naringin in control animals (A), while the increase of contractile force was not observed in the atria of animals previously treated with reserpine (B).

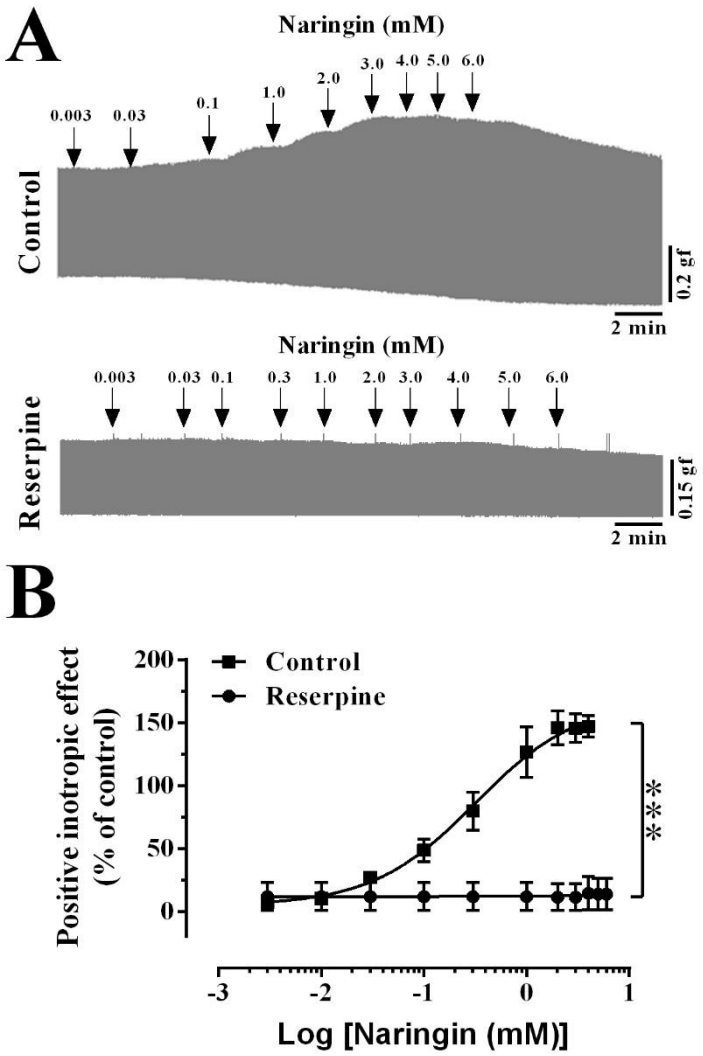

*Figure 4. Positive inotropic effect of naringin was abolished in reserpine-treated rats. A, Representative traces of inotropic effect of naringin in atrial tissue of non-reserpinised (control) and reserpinised rats. B, Concentration-response curves to naringin in atrial tissue of non-reserpinised and reserpinised rats. Data are represented as means ± SEM (n = 4). Two-way ANOVA followed by Tukey post-test. ***p < 0.01 vs. control.*

4. DISCUSSION

Naringin presents some biological properties such as antioxidant and anti-inflammatory activities [6]. However, many studies have shown that the biological actions of this flavonoid mainly depend on the modulation of ion channels and G-coupled protein receptors [17, 18]. From this point of view, the literature is very scarce regarding the effects of naringin on the cardiovascular system. Our results showed that naringin exerted a two-phase contractile effect, increasing the atrial force at low concentrations (0.003 to 2 mM) and reducing the contractile force at concentrations above 3 mM. The calculated EC_{50} of naringin was 0.32 ± 0.01 mM, which is 10-fold less potent compared to quercetin (0.032 mM), another flavonoid with similar properties, as we recently reported [18]. Moreover, our results demonstrated that the increased atrial force induced by naringin involves, at least in part, by a significant improvement of diastolic relaxation, possibly mediated by enhanced Ca^{2+} reuptake to the sarcoplasmic reticulum (SR).

In contrast to our results, López-Medina et al. (2014) [19] have shown that naringin induced, in isolated rat heart, negative inotropism in the concentration range of 1 to 100 μM. Moreover,

the same group of researchers demonstrated a similar effect in isolated mouse hearts, justifying this effect due to the reduction of sodium and LTCC currents [16]. A reasonable explanation for this discrepancy is the difference in tissues studied, since our results were obtained in atrial tissue, while previous studies were carried out using ventricular tissue or cells. Therefore, albeit these cells comprise cardiomyocytes, they differ from each other in terms of morphology, EC coupling, and gene expression [20].

Although several mechanisms have been described to enhance cardiac contractility, activation of β-AR remains the main signaling pathway in the heart [21]. Indeed, 3 types of β-ARs (β_1-, β_2-, and β_3-) have been identified in human atrial and ventricular tissue at mRNA and protein level [22, 23]. However, the positive inotropic effect in the human heart is generally attributed to β_1- and β_2-AR stimulation, while activation of β_3-ARs has provided inconsistent data in atrial tissue leading to positive [24] and negative [25] inotropic effects. Accordingly, the inhibition of the concentration-effect curve to naringin in the presence of propranolol (a non-specific antagonist of β-receptors) or atenolol (a specific β1-receptor antagonist) support that its positive inotropic effect occurs through a β-AR-dependent mechanism.

Downstream, activation of β-AR signaling pathway results in immediate PKA-dependent phosphorylation of effectors, such as RyR and LTCC, increasing cardiac contractile function [26]. In atrial myocytes, which transverse tubules are not present or poorly developed compared to ventricular myocytes [27], the EC coupling is initiated through Ca^{2+} release units (CRUs) at the surface sarcolemma, each including approximately 6 LTCCs opposite a cluster of approximately 50 RyRs Ca^{2+} release channels [28]. Therefore, Ca^{2+} transients and contractions are initiated through subsarcolemmal CRUs, leading initially to a peripheral elevation of Ca^{2+} via LTCCs, which travels toward the myocyte center through propagated Ca^{2+}-induced Ca^{2+} release via RyRs [28, 29]. On the other hand, phospholamban, which is an endogenous SERCA inhibitor, once phosphorylated, increases Ca^{+2} reuptake to SR increasing the contraction force, and improving diastolic relaxation [30]. In line with our results, inhibition of PKA, as well as its downstream targets, RyR and LTCC, entirely abolished the positive inotropism evoked by naringin. Therefore, our results validate that naringin enhances contractile function through a β-AR-dependent mechanism, which stimulates a PKA-mediated activation of proteins involved in the control of Ca^{2+} handling and EC coupling of atrial tissue.

Thereafter, experiments were performed to evaluate the influence of reserpine pre-treatment naringin inotropic effect. It is well known that reserpine inhibits the vesicular monoamine transporter, preventing norepinephrine and dopamine uptake into storage vesicles and, consequently, enhancing catecholamines degradation by monoamine oxidase [31]. Using this approach, our data clearly showed that naringin was unable to increase atrial contractility. Thus, this result suggests that this flavonoid is able to release catecholamines present in the isolated atrium, which in turn, bind to β-AR promoting the increase of cardiac contractility. Interestingly, catecholamine release-dependent activity has also been observed in other natural compounds. For instance, silymarin, a flavonolignan extracted from the seeds of *Silybum marianum*, exerted positive inotropic response in isolated rat heart through endogenous catecholamines release [32]. Although it is well known that endogenous catecholamines release increases both heart muscle contraction and heart rate, positive inotropic agents may be beneficial for patients that need adrenergic stimulation to improve cardiac function, such as acute heart failure, especially with severe hypoperfusion and cardiogenic shock [33].

5. CONCLUSION

In summary, our data show that naringin exerts positive inotropism in atrial tissue by stimulation of adrenergic pathway through β-AR/PKA-dependent mechanism. However, it seems that part of this effect occurs by endogenous catecholamines release from sympathetic nerve terminals.

6. ACKNOWLEDGMENT

This study was supported by FAPITEC (Edital Universal 07/2008) ELETROBRAS (Process #23113.009351/03-67), CNPq (Process #478581/2008-4). DSS and COD are recipients of CAPES, SVSC and VCOS are recipients of CNPq and KOM is recipient of FAPITEC.

7. REFERENCES

1. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci [Internet]. 2016 Dec 29 [cited 2018 Feb 22];5:1-15. doi: 10.1017/jns.2016.41
2. Usmanov PB, Jumayev I, Rustamov SY, Zaripov A, Esimbetov AT, Zhurakulov S. The combined inotropic and vasorelaxant effect of DHQ-11, A conjugate of flavonoid dihydroquercetin with isoquinoline alkaloid 1-Aryl-6,7-Dimethoxy-1,2.3,4-Tetrahydroisoquinoline. Biomed Pharmacol J. 2021 Jun 30;14(2):651-61. doi: 10.13005/bpj/2167
3. Petersen JW, Felker GM. Inotropes in the management of acute heart failure. Crit Care Med. 2008 Jan;36(1 Suppl):S106-111. doi: 10.1097/01.CCM.0000296273.72952.39
4. de Lourdes Mata Bilbao M, Andrés-Lacueva C, Jáuregui O, Lamuela-Raventós RM. Determination of flavonoids in a *Citrus* fruit extract by LC–DAD and LC–MS. Food Chem. 2007 Jan 1;101(4):1742-7. doi: 10.1016/j.foodchem.2006.01.032
5. Alam MA, Subhan N, Rahman MM, Uddin SJ, Reza HM, Sarker SD. Effect of citrus flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action. Adv Nutr Bethesda Md. 2014 Jul;5(4):404-17. doi: 10.3945/an.113.005603
6. Rajadurai M, Prince PSM. Preventive effect of naringin on isoproterenol-induced cardiotoxicity in Wistar rats: an in vivo and in vitro study. Toxicology. 2007 Apr 11;232(3):216-25. doi: 10.1016/j.tox.2007.01.006
7. Alam MdA, Kauter K, Brown L. Naringin improves diet-induced cardiovascular dysfunction and obesity in high carbohydrate, high fat diet-fed rats. Nutrients. 2013 Feb 27;5(3):637-50. doi: 10.3390/nu5030637
8. Yadav M, Sehrawat N, Singh M, Upadhyay SK, Aggarwal D, Sharma AK. Cardioprotective and hepatoprotective potential of citrus flavonoid naringin: Current status and future perspectives for health benefits. Asian J Biol Life Sci [Internet]. 2020 [cited 2022 Oct 7];9(1):1-5. doi: 10.5530/ajbls.2020.9.1
9. Ikemura M, Sasaki Y, Giddings JC, Yamamoto J. Preventive effects of hesperidin, glucosyl hesperidin and naringin on hypertension and cerebral thrombosis in stroke-prone spontaneously hypertensive rats. Phytother Res PTR. 2012 Sep;26(9):1272-7. doi: 10.1002/ptr.3724
10. Testai L, Piragine E, Piano I, Flori L, Da Pozzo E, Miragliotta V, et al. The citrus flavonoid naringenin protects the myocardium from ageing-dependent dysfunction: Potential role of SIRT1. Oxid Med Cell Longev. 2020 Jan 25;2020:1-15. doi: 10.1155/2020/4650207
11. Gelen V, Şengül E. Antioxidant, anti-inflammatory and antiapoptotic effects of Naringin on cardiac damage induced by cisplatin. Indian J Tradit Knowl IJTK. 2020 May 15;19(2):459-65. doi: 10.56042/ijtk.v19i2.35371
12. Uryash A, Mijares A, Flores V, Adams JA, Lopez JR. Effects of naringin on cardiomyocytes from a rodent model of type 2 diabetes. Front Pharmacol [Internet]. 2021 [cited 2022 Oct 7];12:719268. doi: 10.3389/fphar.2021.719268
13. Li S, Jiang J, Fang J, Li X, Huang C, Liang W, et al. Naringin protects H9C2 cardiomyocytes from chemical hypoxia-induced injury by promoting the autophagic flux via the activation of the HIF-1α/BNIP3 signaling pathway. Int J Mol Med. 2021 Jun 1;47(6):1-11. doi: 10.3892/ijmm.2021.4935
14. Alberca RW, Teixeira FME, Beserra DR, de Oliveira EA, Andrade MMS, Pietrobon AJ, et al. Perspective: The potential effects of naringenin in COVID-19. Front Immunol. 2020 Sep;11:570919 doi: 10.3389/fimmu.2020.570919
15. Saponara S, Carosati E, Mugnai P, Sgaragli G, Fusi F. The flavonoid scaffold as a template for the design of modulators of the vascular Cav1.2 channels. Br J Pharmacol. 2011 Nov;164(6):1684-97. doi: 10.1111/j.1476-5381.2011.01476.x
16. Alvarez-Collazo J, López-Medina AI, Rodríguez AA, Alvarez JL. Mechanism of the negative inotropic effect of naringin in mouse heart. J Pharm Pharmacogn Res. 2014;2(5):148-57.
17. Li W, Du L, Li M. Alkaloids and flavonoids as α(1)-adrenergic receptor antagonists. Curr Med Chem. 2011;18(32):4923-32. doi: 10.2174/092986711797535209
18. Santos MS, Oliveira ED, Santos-Miranda A, Cruz JS, Gondim ANS, Menezes-Filho JER, et al. Dissection of the effects of quercetin on mouse myocardium. Basic Clin Pharmacol Toxicol. 2017 Jun;120(6):550-9. doi: 10.1111/bcpt.12743

19. López-Medina AI, Alvarez-Collazo J, Rodríguez AA, Morón-Rodríguez F, Cabrera-Suárez H, Alvarez JL. Direct actions of naringin on rat cardiac and vascular smooth muscle. Bol Latinoam Caribe Plantas Med Aromát. 2014 May;13(3):238-48.
20. Walden AP, Dibb KM, Trafford AW. Differences in intracellular calcium homeostasis between atrial and ventricular myocytes. J Mol Cell Cardiol. 2009 Apr;46(4):463-73. doi: 10.1016/j.yjmcc.2008.11.003
21. Grimm M, Brown JH. β-Adrenergic receptor signaling in the heart: Role of CaMKII. J Mol Cell Cardiol. 2010 Feb;48(2):322-30. doi: 10.1016/j.yjmcc.2009.10.016
22. Ho D, Yan L, Iwatsubo K, Vatner DE, Vatner SF. Modulation of β-adrenergic receptor signaling in heart failure and longevity: Targeting adenylyl cyclase type 5. Heart Fail Rev. 2010 Sep;15(5):495-512. doi: 10.1007/s10741-010-9183-5
23. Cannavo A, Koch WJ. Targeting β3-adrenergic receptors in the heart: selective agonism and β-blockade. J Cardiovasc Pharmacol. 2017 Feb;69(2):71-8. doi: 10.1097/FJC.0000000000000444
24. Skeberdis VA, Gendvilienė V, Zablockaitė D, Treinys R, Mačianskienė R, Bogdelis A, et al. β3-adrenergic receptor activation increases human atrial tissue contractility and stimulates the L-type Ca2+ current. J Clin Invest. 2008 Sep 2;118(9):3219-27. doi: 10.1172/JCI32519
25. Kitamura T, Onishi K, Dohi K, Okinaka T, Isaka N, Nakano T. The negative inotropic effect of beta3-adrenoceptor stimulation in the beating guinea pig heart. J Cardiovasc Pharmacol. 2000 May;35(5):786-90. doi: 10.1097/00005344-200005000-00016
26. Galaz-Montoya M, Wright SJ, Rodriguez GJ, Lichtarge O, Wensel TG. Beta-2 adrenergic receptor activation mobilizes intracellular calcium via a non-canonical cAMP-independent signaling pathway. J Biol Chem. 2017 Apr 25;292(24):9967-74. doi: 10.1074/jbc.M117.787119
27. Sheehan KA, Blatter LA. Regulation of junctional and non-junctional sarcoplasmic reticulum calcium release in excitation-contraction coupling in cat atrial myocytes. J Physiol. 2003 Jan 1;546(Pt 1):119-35. doi: 10.1113/jphysiol.2002.026963
28. Bootman MD, Higazi DR, Coombes S, Roderick HL. Calcium signalling during excitation-contraction coupling in mammalian atrial myocytes. J Cell Sci. 2006 Oct 1;119(19):3915-25. doi: 10.1242/jcs.03223
29. Blatter LA. The intricacies of atrial calcium cycling during excitation-contraction coupling. J Gen Physiol. 2017 Sep 4;149(9):857-65. doi: 10.1085/jgp.201711809
30. Bers DM. Cardiac excitation-contraction coupling. Nature. 2002 Jan 10;415(6868):198-205.
31. Kreusser MM, Lehmann LH, Haass M, Buss SJ, Katus HA, Lossnitzer D. Depletion of cardiac catecholamine stores impairs cardiac norepinephrine re-uptake by downregulation of the norepinephrine transporter. PLoS ONE [Internet]. 2017 Mar 10 [cited 2018 Feb 22];12(3):e0172070. doi: 10.1371/journal.pone.0172070
32. Gabrielová E, Zholobenko AV, Bartošíková L, Nečas J, Modriansky M. Silymarin constituent 2,3-dehydrosilybin triggers reserpine-sensitive positive inotropic effect in perfused rat heart. PLOS ONE. 2015 Sep 29;10(9):e0139208. doi: 10.1371/journal.pone.0139208
33. Triposkiadis F, Karayannis G, Giamouzis G, Skoularigis J, Louridas G, Butler J. The sympathetic nervous system in heart failure physiology, pathophysiology, and clinical implications. J Am Coll Cardiol. 2009 Nov 3;54(19):1747-62. doi: 10.1016/j.jacc.2009.05.015