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Direct actions of naringin on rat cardiac and vascular smooth muscle

[Acciones directas de la naringina sobre los músculos cardíaco y liso vascular de rata]

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Abstract: Naringin (NRG) is a flavanone glycoside present in grapefruit juice. Its biological activity has been only partially characterized and little is known about its potential effects in the cardiovascular system. We studied the effects of NRG on the electrical and contractile activities of isolated rat hearts and on the contraction of rat abdominal aortic rings. NRG exerted a negative inotropic action in hearts with an IC_{50} of 72.5 µmol/L but its effects on heart rate and surface electrogram were minimal. Surprisingly, NRG (10-100 µmol/L) was able to increase tension in aortic rings contracted by isotonic KCl or phenylephrine. This action of NRG was also evident in aortic rings in basal (resting) conditions but it was absent when resting aortic rings were previously perfused with ryanodine (30 µmol/L). Our results indicate that NRG has direct actions on cardiac and vascular smooth muscles that should be taken into account when considering this molecule either as a dietetic supplement or as a template to develop therapeutic agents for human diseases.

Keywords: flavonoids, naringin, naringenin, cardiac, vascular smooth muscle

Resumen: La naringina (NRG) es un glicósido de flavanona que se encuentra presente en el jugo de toronja. Su actividad biológica ha sido solo parcialmente caracterizada y poco se conoce acerca de sus efectos sobre el sistema cardiovascular. En la presente investigación estudiamos los efectos de la NRG sobre las actividades eléctrica y contráctil de corazones aislados de rata y sobre la contracción de anillos de aorta abdominal de rata. La NRG ejerció una acción inotropo-negativa en corazones con una IC_{50} de 72.5 µmol/L pero sus efectos sobre la frecuencia cardíaca y el electrograma de superficie fueron mínimos. Sorpresivamente, la NRG (10-100 µmol/L) incrementó la tensión en anillos de aorta contraídos por KCl isotónico o fenilefrina. Esta acción de la NRG ocurrió también en anillos de aorta en condiciones basales (en reposo) pero estuvo ausente cuando los anillos de aorta fueron previamente perfundidos con ryanodina (30 µmol/L). Nuestros resultados indican que la NRG tiene acciones directas sobre los músculos cardíaco y liso vascular que deben tenerse en cuenta al considerar esta molécula como suplemento dietético o como plantilla para el desarrollo de agentes terapéuticos para el tratamiento de enfermedades en humanos.

Palabras Clave: flavonoides, naringina, naringenina, cardíaco, músculo liso vascular

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Note
On July the 29th, 2013 Dr Francisco José Morón Rodríguez died suddenly. As a medical doctor and Second Degree Specialist in Pharmacology, Dr Morón had a fruitful career as a professor and researcher in the "Dr. Salvador Allende" Faculty of Medicine. He was member of the Cuban Society of Pharmacology and founder director of the Revista Cubana de Plantas Medicinales. He was member of the Editorial Advisory Board of the Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas (BLACPMA). Due to his competence, solidarity, modesty and dedication to work, he was an example of health professional. He stood out for its support to the research on therapeutic agents obtained from plants. At the time of his death, he was the Director of the Central Laboratory of Pharmacology of the "Dr. Salvador Allende" Faculty of Medical Sciences of the Havana University of Medical Sciences.

Nota
El 29 de julio de 2013 falleció súbitamente el Dr Francisco José Morón Rodríguez. Como médico y especialista de Segundo Grado en Farmacología, el Dr. Morón tuvo una fecunda trayectoria como profesor e investigador en la Facultad de Ciencias Médicas "Dr. Salvador Allende". Era miembro de la Sociedad Cubana de Farmacología y director fundador de la Revista Cubana de Plantas Medicinales. Era miembro del Consejo Editorial del Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas (BLACPMA). Por su capacidad, modestia, solidaridad y entrega al trabajo, fue ejemplo de profesional de la salud. Se destacó por el impulso a las investigaciones y aplicación de agentes terapéuticos obtenidos de plantas medicinales. Al momento de su fallecimiento ocupaba el cargo de Director del Laboratorio Central de Farmacología de la Facultad de Ciencias Médicas "Dr. Salvador Allende" de la Universidad de Ciencias Médicas de la Habana.

INTRODUCTION

Flavonoids constitute a complex chemical group of natural compounds comprising the flavanols, flavanones, flavones, isoflavones, flavonols, anthocyanidin and proanthocyanidin subgroups (McNaught and Wilkinson, 1997). Early in the nineties epidemiological studies showed that an association seemed to exist between dietary intake of flavonoids and the reduction of myocardial infarction and stroke in some populations (Hertog *et al*., 1993; Keli *et al*., 1996). Since then, evidence arose suggesting that flavonoids could have a potential therapeutic value in the prevention and treatment of cardiovascular diseases (Benavente-García and Castillo, 2008; Habauzit and Morand, 2012). Several clinical trials have been conducted to explore the possible beneficial effects of flavonoids intake. Most results are suggestive of a reduced cardiovascular risk in individuals having flavonoid-rich diets. The beneficial effects of flavonoids have been usually associated to their anti-oxidant, anti-inflammatory, anti-proliferative and anti-thrombotic actions (see for review Wright *et al*., 2013). However, intracellular modulator actions of flavonoids could be quite diverse and complex (Wright *et al*., 2013). In addition, as pointed out by Wrigth and co-workers physiological and pharmacological actions of flavonoids can be affected by sex, lifestyle, disease states and interactions with drugs thus limiting their impact on human health (see also Chanet *et al*., 2012). Nevertheless, these compounds are extremely interesting as molecular templates to design drugs with better pharmacological profiles for the treatment of human cardiovascular diseases.

The flavanone naringin is the major flavonoid in grapefruit juice (Peterson *et al*., 2006; Rouseff 1988), an important dietary source of flavonoids, and for this reason studies have been conducted to study the pharmacological actions of naringin and its aglycone naringenin. Naringin was reported as being effective in protecting against bone loss in ovariectomised mice, an effect that is probably mediated through ligand-independent activation of œstrogen receptor in osteoblastic cells (Pang *et al*., 2010). Moreover, naringin can improve bone quality in senescent rats (Habauzit *et al*., 2011). Its antioxidant action could be responsible, at least in part, for its neuroprotective effect in human dopaminergic cells SH-SY5Y (Choi *et al*., 2010) and PC12 cells (Lu *et al*., 2010), for its blockade of the proinflammatory effect of interferon-γ and histamin in human keratinocytes (Cardile *et al*., 2010; Graziano *et al*., 2012) and for its anti-inflamatory action in the rat air pouch model of inflammation (Jain and Parmar, 2011). Interestingly, naringin but not its aglycone naringenin had a CNS depressing action in mice suggesting that the sugar moiety could be important for this action following systemic administration of the flavonoid (Fernández *et al*., 2006). It has also been reported that naringin (and other flavonoids) possess hypouricemic action in mice, an effect that seems to be related to the inhibition of liver xanthyne oxidase activity (Mo *et al*., 2007), and reduces radiation-induced damage to DNA (Jagetia *et al*., 2003). In addition, naringin decreases the release of the angiogenic vascular endothelial growth factor (Schindler and Mentlein, 2006) and protects against ethanol injury in rats (Martin *et al*., 1994). It could also decrease total cholesterol levels (da Silva *et al*., 2001; Lee *et al*., 2001; Ueng *et al*., 1999) and possesses anti-apoptotic properties (Gordon *et al*., 1995).

Naringin also seems to have a cardioprotective action in isoproterenol-induced myocardial infarction in rats (Rajadurai and Prince, 2007a; Rajadurai and Prince, 2007b). Although the mechanism of cardioprotection was no elucidated, it seems to involve complex pathways. In stroke-prone spontaneously hypertensive rats, orally-administered naringin (as well as hesperidin and glucosyl-hesperidin) was reported to suppress the age related increase in blood pressure, to significantly decrease thrombotic tendency and to increase nitric oxide (NO) production thus improving vasodilation induced by an acetylcholine-mediated NO production in the endothelium (Ikemura *et al*., 2012). These results could explain in part its beneficial effect on the mechanisms of hypertension and thrombosis. However, most probably the effects were due to the actions of naringenin, the aglycone formed during the cleavage of the sugar moiety of naringin after its ingestion (Fuhr and Kummert, 1995). It should be noted here that the aglycone naringenin was among the less efficient flavonoids in releasing NO in porcine coronary arteries (Taubert *et al*., 2002). In a recent report it was suggested that naringin could inhibit high glucose-induced apoptosis by attenuating mitochondrial dysfunction and modulating the activation of the p38 signaling pathway (Huang *et al*., 2013). However, little is known about the possible direct cardiovascular physiological actions of naringin. Saponara *et al.* (2006) reported that naringin and its aglycone naringenin could increase the conductance of vascular smooth muscle calcium-activated potassium ion channels ($BK_{(Ca)}$); naringin showed, however, a poor vasorelaxing action in rat aortic rings compared to naringenin a fact that could be linked to its

glycoside moeity (Saponara *et al*., 2006). Naringenin also blocks the cardiac hERG channels (Scholz *et al*., 2005; Zitron *et al*., 2005). In one report, the effects naringin were studied on recombinant human $K_{IR}3.1–3.4$ and $K_{IR}3.1–3.2$ expressed in *Xenopus* oocytes using the two-electrode voltage clamp method (Yow *et al*., 2011; see also Oki *et al*., 2012 in HAC15 cells). The results indicated that naringin (but not its aglycone naringenin) is a direct activator of inward rectifying K currents, an effect that could have an impact on heart rate at least in isolated heart or spontaneous activity in single cell preparations.

The aim of the present investigation was to characterize the possible direct actions of naringin on electrical and contractile activities of rat isolated hearts and on the contraction of endothelium-deprived rat aortic rings.

MATERIALS AND METHODS

Naringin extraction and purification

Naringin was obtained according to the method of Ikan (Ikan, 1991). In brief, naringin was extracted in hot water from the grapefruit (*Citrus maxima* (Burm.) Merrill) peel along with a small quantity of pectin. The extract was concentrated to ~1/9 of the original volume yielding naringin as an octahydrate. Recrystallization from isopropanol gave the pure dihydrate. Both purified and commercial naringin (Sigma, USA; kindly provided by Prof K. Talavera; Leuven) were dissolved in ethanol:water (1:1) at 0.2 mmol/L and subjected to reversed-phase C18 HPLC in an Ultropac Spherisorb ODS2 column (3 µm particle size, 100 × 4.6 mm i.d.). Separations were performed at 1 mL/min employing an increasing linear gradient of acetonitrile from 85/5/10 to 45/45/10 (%A/%B/%C) in 40 min, being A: water, B: acetonitrile and C: 0.1% ortho-phosphoric acid in water. The eluting compounds were online detected by UV absorption at 254 nm. The separation was carried out on a Knauer HPLC system (Knauer, Germany).

Isolated hearts

Experiments were performed on male adult Wistar rats according to the guidelines of the National Center for Laboratory Animal Reproduction (CENPALAB). As previously reported (Galán *et al*., 1998), under pentobarbital anaesthesia hearts were removed and placed in cold Tyrode (see below). They were carefully dissected, mounted on a Langendorff column and perfused at constant flow (10 mL/min) with a Tyrode solution of the following composition (mmol/L): NaCl 140; KCl 2.5; $MgCl_2$ 0.5; $CaCl_2$ 2; Tris-hydroxymethylaminomethane 10; Glucose 5 (pH = 7.4, gassed with O_2; T = 35° C). A bipolar platinum recording electrode was placed on the right ventricular epicardium and the surface electrocardiogram (ECG) was recorded with a biophysical amplifier (AVB-10; Nihon Kohden, Japan). Another bipolar platinum electrode was placed near the atrioventricular ring and was connected to an electronic stimulator (SEN-7103; Nihon Kohden, Japan) through a stimulus-isolating unit (SS-102J; Nihon Kohden, Japan). Cardiac apex was fixed to a force-displacement transducer (SB-1T; Nihon Kohden, Japan) with a surgical 5-0 or 6-0 silk thread. The force of contraction (FC) was recorded using a low-level DC preamplifier (ACH-8; Nihon Kohden, Japan). Analogue signals (ECG and FC) were digitized (100 µs sampling interval) using an AD/DA converter (Labmaster TL-1-125, Mentor, Ohio, USA), stored on a computer and analyzed off-line with the ACQUIS1 (version 2.0, CNRS License, France) and SciDAVis (Debian GNU/Linux; version 0.2.4) softwares. ECG and FC values were recorded at the spontaneous heart rate and at a fixed stimulus rate (200 bpm). Hearts were let to stabilize for at least 30 min before beginning the experiments.

Aortic rings

Abdominal aortic rings were dissected from rats under pentobarbital anaesthesia. Care was taken to mechanically remove the endothelium. They were fixed to a force transducer and placed in bath chamber continuously perfused (10 mL/min) with the same Tyrode solution used for isolated hearts (pH = 7.4, gassed with O_2; T = 35° C) and stabilized, under a load of 0.5 g, for 30 min before the beginning of the experiment. Contraction was induced by replacing all NaCl by KCI (140 mmol/L) or by phenylephrine (PE) 10 µmol/L (Galán *et al*., 1998).

Nifedipine (Sigma, USA; kindly provided by Prof G. Vassort; Montpellier) was prepared as a stock solution (10 mmol/L) in ethanol and was used as reference compound. Results were analyzed using a Student's t-test for paired or unpaired samples as required using the Gnumeric Spreadsheet (Gnome Project, version 1.10.17). Differences were considered statistically significant for $p < 0.05$. Results are expressed as Means ± Standard Errors of Means.

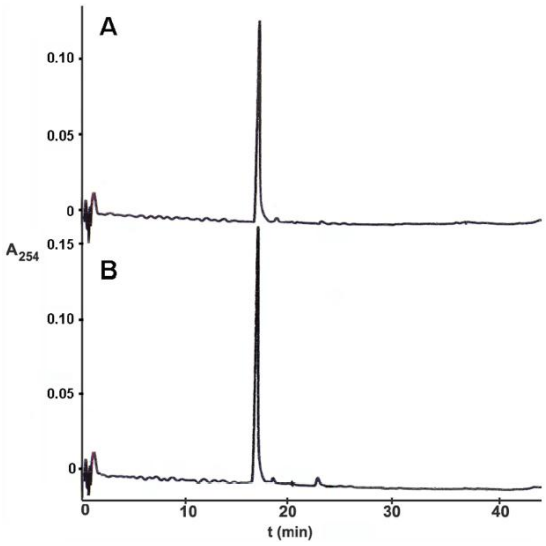
RESULTS

Extracted and purified naringin

The obtained naringin was analyzed by RP18-HPLC. The resulting chromatographic profile (Figure 1A) yielded a very dominant, narrow and symmetric peak

that eluted at 17.50 min, indicating the high purity of the compound. Moreover, its retention time exactly matched the behaviour of commercial naringin (Figure 1B), confirming the presence of naringin in the purified sample. Only extracted and purified naringin (NRG) was used for the pharmacological experiments.

Figure 1

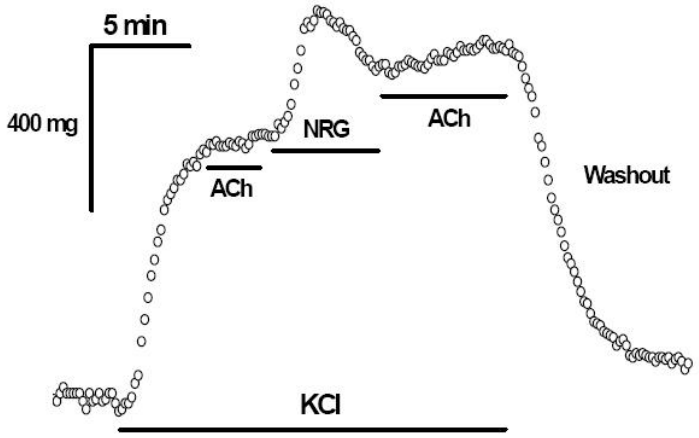


Extracted naringin is of high purity. Reversed-phase C18 chromatographic profiles of naringin obtained as reported in Methods (A) and commercial naringin (B). Both chromatographic separations were performed in identical conditions. Column: Ultropac Spherisorb ODS2 (3 µm particle size, 100 × 4.6 mm i.d.), flow rate: 1 mL/min, gradient program: 85/5/10 to 45/45/10 (%A/%B/%C) in 40 min, being A: water, B: acetonitrile and C: 0.1% ortho-phosphoric acid in water. Sample volume: 100 µL.

Effects on cardiac electrical and mechanical activities.

NRG, in the concentration range from 1 to 100 µmol/L, barely affected spontaneous electrical activity of isolated rat hearts (n = 9 hearts). NRG changed neither QRS duration nor its amplitude. RR interval was also not significantly changed by NRG within this concentration range. Corrected QT (QTc = QT/√RR) was not significantly affected by NRG at concentrations from 1 to 10 µmol/L. However, at 30 and 100 µmol/L concentrations, NRG showed a tendency to decrease QTc (from 9.4 ± 1 ms in control to 7.1 ± 1 ms and 7.0 ± 2 ms, respectively) but without statistical significance. On the other hand, NRG significantly decreased the force of contraction (FC) in isolated hearts. Although RR interval was not significantly changed by NRG, hearts were paced at 200-ms stimulus interval (slightly over the spontaneous RR interval under control condition; 210 ± 2 ms) in order to avoid any frequency-dependent change in FC. At 1 µmol/L concentration, NRG had not significant effect on FC (4.9 ± 3 % decrease); however at higher concentrations, it significantly decreased FC ($p < 0.05$; 97 ± 3 % maximal decrease at 300 µmol/L). Experimental data were fitted to a Hill function and the estimated IC_{50} for inhibition of contraction was 72.5 µmol/L (Hill number = 1.1). Compared to the reference compound nifedipine (IC_{50} = 0.26 µmol/L; Hill number = 1.5), NRG was less potent as antagonist of cardiac contraction. The action of NRG on FC was reversible upon washout with normal Tyrode solution.

Figure 2



Naringin potentiates KCl-induced contraction in rat aortic rings
Contraction was induced by replacing all NaCl by KCl (140 mmol/L). ACh (10 µmol/L) was perfused together with KCl in order to confirm the lack of endothelium activity. Upon washout of ACh, NRG (30 µmol/L) was perfused. As can be seen, naringin (NRG) rapidly potentiated KCl-induced contraction but only transiently. Application of ACh after NRG washout, corroborated that no functional endothelium was present.

Effects on aortic contraction.
To our surprise, NRG increased the force of contraction of rat aortic rings pre-contracted with isotonic KCl. The effect was transient in some preparations at the highest concentrations (e. g. Figure 2). Table 1 shows the action of different concentrations of NRG on KCl-induced contraction; the values represent the maximal (peak) effect of NRG as percent increase over the effect of KCl. As can be seen in Table 1, the effect of NRG was statistically significant ($p < 0.05$) at 10 µmol/L concentration but physiological relevant increases (~20%) were only obtained at 30 and 100 µmol/L concentrations. On the other hand nifedipine, a classic vasodilator, exerted, as expected, a concentration-dependent depression of KCl-induced contraction of aortic rings with an IC_{50} of 10.1 nmol/L (Hill number = 0.6). NRG also increased contraction in aortic rings pre-contracted by phenylephrine (PE; 10 µmol/L), however, the effect was statistically significant only at 30 and 100 µmol/L (10.5 ± 2% and 11.3 ± 2%, respectively, of maximal PE-induced contraction; n = 5). The action of NRG on the PE-induced contraction was less marked than on KCl-induced contraction. This could be related to the fact that KCl-induced contraction was less potent than PE-induced contraction in our conditions (99.7 ± 17 % vs 360.0 ± 196.0 %). More strikingly, NRG was able to contract aortic rings in basal conditions (not depolarized by isotonic KCl), an effect that was statistically significant only at 30 and 100 µmol/L concentrations (Table 1) and also in a transient manner. Figure 3 shows an example of this effect of NRG. It is to note that for the same concentrations, the effects of NRG on contraction were more ample in aortic rings in basal conditions than in KCl- (Table 1) or PE-contracted ones.

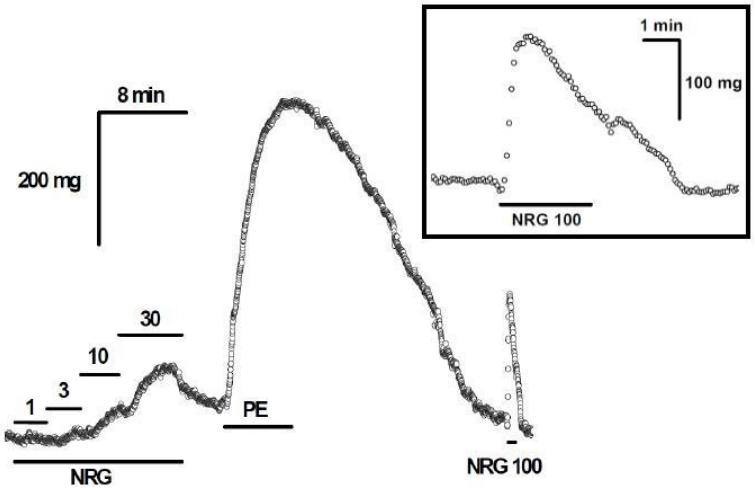
In some experiments Ca^{2+} release through ryanodine receptors (RyR) in the sarcoplasmic reticulum, was blocked using ryanodine (RYA; 30 µmol/L). Aortic rings were let to stabilize for at least 5 min in normal Tyrode solution with 30 µmol/L RYA and then, they were perfused with the same Tyrode (+ RYA) but adding 30 µmol/L NRG. In contrast to what we described above, in three experiments NRG (30 µmol/L) did not induce contraction of the aortic rings in basal conditions. In each case PE (10 µmol/L) triggered a strong contraction when it was applied after washout of RYA. Figure 4 shows a typical experiment.

Table 1
Effects of NRG on aortic ring contractions

Condition	NRG concentration (µmol/L)				
	1	3	10	30	100
KCl-induced contraction	1.3 ± 0.9	3.6 ± 1.8	10.0 ± 3.2 *	22.4 ± 2.6 *	20.6 ± 1.0 *
Basal	9.9 ± 8	10.4 ± 4.5	25.7 ± 5.7 *	49.8 ± 6.5 *	53.5 ± 13.5 *

Values represent the maximal (peak) effect of NRG as percent increase over the effect of KCl or basal (resting tension). * $p < 0.05$ with respect to control; $n \geq 5$ for each condition.

Figure 3



Naringin is able to contract aortic rings in basal conditions
The preparation was stabilized with normal Tyrode solution (not shown here; see Methods) and naringin (NRG) was applied at increasing concentrations. Low concentrations (1 and 3 µmol/L) were without any significant effect on resting passive force but a clear increase in force was evident at 10 and 30 µmol/L concentrations. NRG was washed out by perfusing the preparation with Tyrode solution. Phenylephrine (PE; 10 µmol/L) was then applied to confirm that the ring could elicit a strong contraction. After washout of PE, NRG was again applied but at a concentration of 100 µmol/L. The inset shows, in expanded scales, the effect of 100 µmol/L NRG. Note the huge increase in force by NRG in this preparation.

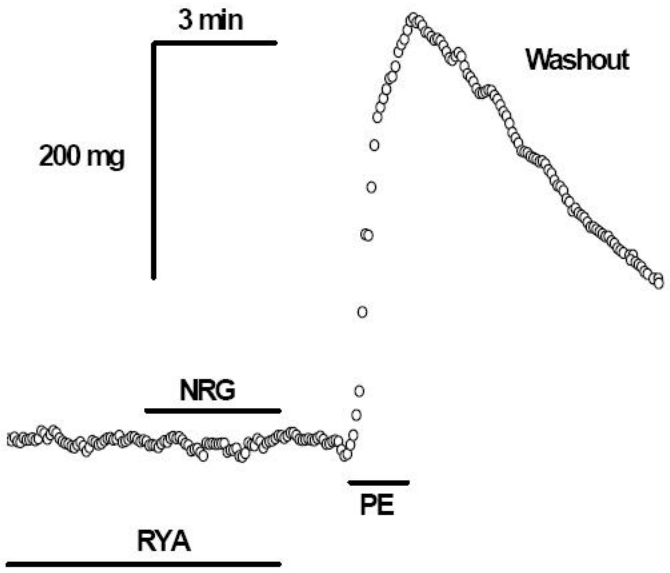
DISCUSSION

The major conclusion of this paper is that naringin (NRG) has direct effects on cardiac and vascular smooth muscles. A clear negative inotropic effect could be demonstrated in isolated hearts. The negative effect on cardiac contraction was, however, less important than that of the classic "Ca-antagonist" nifedipine and not accompanied by changes in the surface electrogram or heart rate. Strikingly NRG, at the same concentrations that it exerted a negative inotropic action in heart, increased the vasomotor tone of aortic rings precontracted with either isotonic KCl or phenylephrine (PE). This vasoconstrictor action of NRG was also manifested in resting aortic rings.

As stated above, minor effects on electrical activity accompanied the concentration-dependent negative inotropic action of NRG in cardiac muscle. A small, not statistically significant, shortening of QTc

was seen only at the highest concentrations. This could suggest that NRG had no (or a minor) effect on ionic channels controlling cardiac automaticity, conduction and repolarization. However, it should be also considered that it could exert multiple actions on different ionic channels that counterbalance to each other, resulting in an "apparent" absence of effects on cardiac surface electrogram. This is an essential issue that deserves further investigation since information about the actions of NRG on ionic channels is extremely limited. There are two reports showing that NRG is an agonist of inward rectifiers (including cardiac), expressed in *Xenopus* oocytes (Yow *et al.*, 2011) and in HAC15 cells (Oki *et al.*, 2012). In addition, Saponara *et al.* (2006) showed that NRG (and naringenin) is a $BK_{(Ca)}$ agonist but with a poor vasorelaxing action. On the other hand, naringenin, the aglycone of NRG, inhibits the cardiac hERG channels (Scholz *et al.*, 2005; Zitron *et al.*, 2005). In the absence of information on this issue, we can only speculate that the negative inotropic effect of NRG could be due to a Ca^{2+} channel blocking action of this molecule. Nevertheless, effects of NRG on Na^{+} channels and/or on the Na-Ca exchanger cannot be excluded. We might discard *a priori* a blocking effect of NRG on the sarcoplasmic reticulum Ca^{2+} channel ("ryanodine receptor", RyR) as a cause of the negative inotropic action. We obtained evidence suggesting that NRG could act as an agonist of RyR channels (see below).

Figure 4



Naringin is not able to contract aortic rings perfused with ryanodine
The preparation was stabilized (not shown) and then it was perfused with normal Tyrode solution plus ryanodine (RYA; 30 µmol/L) for at least 5 min. After this period, NRG (10 µmol/L) was applied (always in the presence of RYA) for ~ 3 min. No contraction was elicited by NRG in this condition. RYA and NRG were washed out and phenylephrine (PE; 10 µmol/L) was applied evoking a strong contraction.

One striking property of NRG is that in our experimental conditions, it could increase the vasomotor tone in both resting and precontracted aortic rings. According to the published evidences, we might discard any hypothesis involving K^{+} channels (e.g. $BK_{(Ca)}$) in the smooth muscle cell sarcolemma, since NRG, in any case, is a $BK_{(Ca)}$ opener (Saponara *et al.*, 2006). It is interesting to stress here that Saponara et al., showed that NRG exhibited the same potency as naringenin over $BK_{(Ca)}$ channels, but it had a poor vasorelaxing action in aortic rings contracted by 20 mmol/L KCl. NRG was devoid of a vasorelaxing

effect when KCl was 60 mmol/L. Our experiments with 140 mmol/L KCl uncovered an unusual vasomotor action for NRG.

When aortic ring contraction was induced by PE, the effects of NRG were less ample than with isotonic KCl, a result that could be related to the amount of Ca^{2+} release in each case. This suggests that the effect of NRG is limited by the amount of Ca^{2+} previously released. According to the strength developed in each case (see Results), it could be possible that PE-induced Ca^{2+} release was greater than with KCl and then, less Ca^{2+} could be additionally released by NRG. Two lines of evidence suggest that this might be the case. First, the greatest NRG-induced contractions were observed in basal (resting) aortic rings (see Table 1) where Ca^{2+} release mechanisms are not operating. Second, when aortic rings were perfused with ryanodine (RYA), a well-known RyR blocker, NRG was not able to induce contraction. In our experimental conditions (long exposure to 30 µmol/L RYA), RYA is known to fully block the RyR (Pessah and Zimanyi, 1991; Ostrovskaya *et al.*, 2007). Summarizing, although further research is obviously needed, the present results suggest that NRG could act as a sarcoplasmic Ca^{2+} release channel (RyR) agonist and have as targets several voltage-dependent ionic channels.

Naringin is an active ingredient identified in citrus fruits, a major source of dietetic flavonoids. As stated in Introduction, there is a longstanding interest on these compounds not only as dietetic supplements but also as possible therapeutic agents for the treatment of several human diseases. It is thus of interest to study the pharmacological properties of these compounds and to identify not only their beneficial profiles but also their potential undesirable side effects. In this sense the present results show that NRG could have a negative inotropic action in heart and a potential vasoconstrictor action (aorta). Whether these effects are relevant or not to human health requires further investigation since NRG is in part metabolized to naringenin by enteral bacteria that play an important role in this metabolic pathway. Nonetheless, the fate of NRG not metabolized intraluminally to naringenin is not clear. Naringin could be also transformed to naringenin by gut wall or hepatic enzymes but this remains to be examined (Fuhr and Kummert, 1995).

CONCLUSION

We may conclude that NRG has direct actions on cardiac and smooth muscles that should be taken into account when considering this molecule either as a dietetic supplement or as a template to develop therapeutic agents for human diseases.

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