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Inhibition of rat vas deferens contractions by flavonoids in-vitro

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Abstract

Flavonoids are a large heterogeneous group of benzo-γ-pyrone derivatives, which are abundantly present in our diet. In this study we investigated the effect of ten flavonoids (quercetin, kaempferol, morin, galangin, rutin, apigenin, flavone, naringenin, hesperitin and silybin) on the contractile response elicited by electrical field stimulation in the rat isolated vas deferens. All flavonoids tested inhibited vas deferens contractions. The relative order of potency of the tested flavonoids was naringenin > hesperitin > morin > kaempferol > apigenin > silybin > flavone > rutin > quercetin > galangin. Analysis of the chemical structures showed that the saturation of C-2-C-3 double bond and the presence of hydroxyl groups on the flavonoidic scaffold play an important role in the activity of flavonoids.

Introduction

Flavonoids occur naturally in fruit, vegetables, nuts, seeds, flowers and bark and are an integral part of the human diet. Over 5000 different flavonoids have been identified to date. The fundamental skeleton of flavonoids is formed as a 2-phenylbenzo-4*H*-pyran nucleus consisting of two benzene rings (A and B) linked through a heterocyclic pyrane ring (C) (Figure 1) (Taylor & Grotewold 2005). Flavonoids are classified into several subgroups, such as flavonols, flavones and flavanones. This classification is based on variations in the structure of the heterocyclic ring C. Individual subgroup flavonoids of the class differ in the number and distribution of the hydroxyl groups as well as in the nature and extent of glycosylation, alkylation or both. Positive health effects of these compounds have been described in several diseases, such as cardiovascular diseases and cancer (Neuhouser 2004; Stoclet et al 2004). Moreover, it has been demonstrated that flavonoids exert antioxidant, anti-inflammatory, antiviral, anti-allergic and anti-osteoporotic effects (Di Carlo et al 1999; Horvathova et al 2001; Moyers & Kumar 2004; Joseph et al 2005). However, due to differences in lipid solubility and to the presence of different substitutions on the carbon atoms of the basic skeleton (Itoigawa et al 1999), flavonoids may have different activity and potency.

We have recently shown that galangin, a member of the flavonol class of flavonoids, inhibited rat vas deferens contractility in vitro (Capasso & Mascolo 2003). In this paper, we report the effect of a series of flavonoids (quercetin, kaempferol, morin, galangin, rutin, apigenin, flavone, naringenin, hesperitin, silybin) as determined by measuring the inhibitory response on the contractions induced by electrical field stimulation (EFS) in the rat vas deferens.

Materials and Methods

Drugs

Quercetin, kaempferol, morin, galangin, rutin, apigenin, flavone, naringenin, hesperitin, silybin, prazosin hydrochloride, ω-conotoxin GVIA, α,β-methylene ATP and tetrodotoxin were purchased from Sigma-Aldrich (Milan, Italy). Kaempferol, morin, galangin, rutin,

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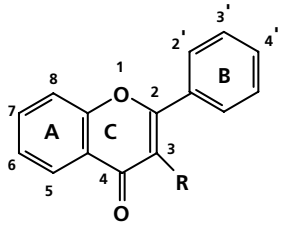

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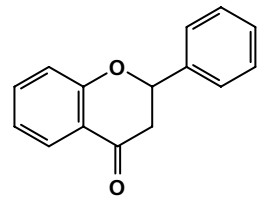
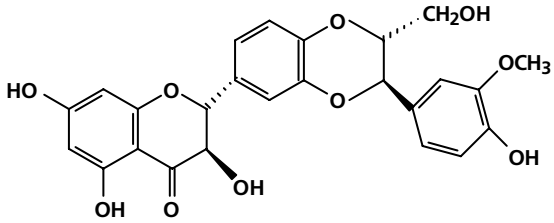
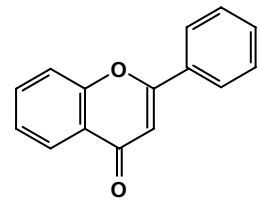


Flavanones



Silybin



Flavones

FLAVONOIDS	Substituent Position							
	3	5	6	7	8	2′	3′	4′
Flavonols								
Quercetin	OH	OH	H	OH	H	H	OH	OH
Kaempferol	OH	OH	H	OH	H	H	H	OH
Morin	OH	OH	H	OH	H	OH	H	OH
Galangin	OH	OH	H	OH	H	H	H	H
Rutin	O-Rh-Glc	OH	H	OH	H	H	OH	OH
Flavones								
Apigenin	H	OH	H	OH	H	H	H	OH
Flavone	H	H	H	H	H	H	H	H
Flavanones								
Hesperitin	H	OH	H	OH	H	H	OH	OCH_3
Naringenin	H	OH	H	OH	H	H	H	OH

Rh= rhamnose; Glc= glucose

Figure 1 Classification and structure of flavonoids.

apigenin, flavone, naringenin, hesperitin and silybin were dissolved in dimethyl sulfoxide (DMSO), while the other drugs were dissolved in distilled water. DMSO (less than 0.01%) did not modify EFS-induced contractions.

Animals

Male Wistar rats, 200–220 g, purchased from Harlan Italy (S. Pietro al Natisone, UD, Italy) were maintained under controlled conditions of temperature (21 ± 1°C) and humidity (60–65%) until used. The rats had free access to water and food. All animal experiments complied with the Italian D.L. no. 116 of 27 January 1992 and associated guidelines in the European Communities Council Directive of 24 November 1986 (86/609/ECC).

Vas deferens preparations

Rats were killed by asphyxiation with CO_2. Both vas deferens were removed and placed in Krebs solution (composition in mM: NaCl 119; KCl 4.75; KH_2PO_4 1.2; $NaHCO_3$ 25; $MgSO_4$ 1.5; $CaCl_2$ 2.5; and glucose 11). The vas deferens was prepared as previously reported (Capasso et al 2005). Briefly, the isolated organ was set up in an organ

bath containing 20 mL Krebs solution equilibrated with 95% O_2 and 5% CO_2 at 37°C. The tissues were connected to an isometric transducer (load 0.5 g) connected to Gemini recording apparatus (Ugo Basile, Comerio, Italy). All experiments commenced after a minimal 60-min equilibration period and the strips were subjected to EFS (2.5 Hz for 1 s, 500 mA, 0.25-ms pulse duration), delivered via electrodes placed around the tissue.

Stable and reproducible contractions for a time-period of 4 h were obtained with stimulation every 2 s. After stable control contractions evoked by EFS had been recorded, the responses were observed in the presence of increasing cumulative concentrations of flavonoids (10^{-9} to 3×10^{-4} M). The contact time for each concentration of flavonoids varied between 30 and 45 min (until the inhibitory effect reached a plateau). In preliminary experiments the effect of tetrodotoxin (3×10^{-7} M), prazosin (10^{-6} M), α,β-methylene ATP (10^{-6} M) and ω-conotoxin GVIA (3×10^{-8} M) on EFS-induced contractions was evaluated after a contact time of 30 min.

Statistics

Results are expressed as means ± s.e.m. Comparisons between two sets of data were made by Student's *t*-test for paired data. When multiple comparisons against a single control were made, analysis of variance was used followed by the Tukey–Kramer multiple comparisons test. A value of $P<0.05$ was considered significant. The concentration of flavonoids that produced 50% inhibition of EFS-induced contractions (IC50) was used to characterise their potency. IC50 values (geometric means ± 95% confidence limits (c.l.)) were calculated using the computer program of Tallarida & Murray (1996).

Results and Discussion

Flavonoids are a large heterogenic group of benzo-γ-pyrone derivatives that possess numerous biological actions. However, structure–activity relationships have been proposed only for the antioxidant and the antispasmodic effects (Di Carlo et al 1993; Heijnen et al 2002; Heim et al 2002). In this paper, we have evaluated the effect of 10 flavonoids on EFS-induced vas deferens contraction.

EFS of the rat vas deferens evoked twitch contractions that were abolished by tetrodotoxin (a toxin that blocks Na^+ channels on neurons and consequently blocks neural activity) or ω-conotoxin (a toxin that blocks N-type Ca^{2+} channels on neurons and consequently blocks neurotransmitter release) and reduced by prazosin (an α_1-adrenergic receptor antagonist) (n = 5 for each drug investigated) (Table 1). The prazosin-insensitive response was abolished by desensitization with α,β-methylene ATP. These results indicate that the contractions are predominantly due to the release of noradrenaline and purines from noradrenergic and purinergic neurones, respectively, and that neurotransmitter release occurs through N-type Ca^{2+} channels.

Table 1 Effect of prazosin, prazosin + desensitization with α,β-metilene ATP (ATPdesens), tetrodotoxin and ω-conotoxin on EFS-induced contractions in rat isolated vas deferens

	Amplitude (g)	% Inhibition
Control	1.20 ± 3	—
Prazosin (10^{-6} M)	0.45 ± 7	62
Prazosin (10^{-6} M) + ATPdesens (10^{-6} M)	0	100
Tetrodotoxin (3×10^{-7} M)	0	100
ω-Conotoxin (3×10^{-8} M)	0	100

Results are means ± s.e. m. of 5 rats for each experimental group.

Previous investigators have documented the effect of some flavonoids (e.g. flavones from *Thymus vulgaris*, naringenin, naringin, flavonoids fraction from *Amburana cearensis*) on vas deferens activity (Van Den Broucke & Lemli 1983; Herrera & Marhuenda 1993; Leal et al 2003). In this study we have shown that all flavonoids tested (quercetin, kaempferol, morin, galangin, rutin, apigenin, flavone, naringenin, hesperitin, silybin) inhibited EFS-induced contractions (Table 2). Among these flavonoids, naringenin and hesperitin showed a greater potency (Table 3).

As reported for other effects of flavonoids (Di Carlo et al 1993; Hammad & Abdalla 1997), there is a tight correlation between the considered structures and the observed pharmacological effects. In fact, the saturation of the C-2-C-3 double bond without the simultaneous presence of a hydroxyl group in position 3 (flavonones) provides the most active compounds (naringenin and hesperitin); on the other hand, flavonols, characterized by unsaturation of the C-2-C-3 double bond and the contemporary presence of the 3-hydroxyl group, provide less active compounds, with the exception of morin and kaempferol, which still present an interesting inhibition. Glycosylation on the 3-hydroxyl group (rutin), the presence of the 3′-hydroxyl group (quercetin) or the elimination of the 4′-hydroxyl group furnish, in all cases, less active compounds (flavonols). Similarly, silybin, a flavonol where the 3′ and 4′-OH are cyclizated in a 2,3-dihydro-benzo[1,4]dioxine ring (Figure 1), showed a lower activity. As consequence of these data, it is suggested that the hydroxyl groups on the flavonoidic scaffold play a crucial role in the inhibition of the contractile response in the rat isolated vas deferens; this is confirmed by flavone, where the complete absence of substituents gave one of the less active derivatives.

Conclusions

While in previous studies on intestinal motility and on papillary muscle activity (Di Carlo et al 1993; Itoigawa et al 1999), the presence in the flavonoidic scaffold of the double bond between C-2 and C-3 increased the biological activity of the compounds, in the case of contractile response on rat isolated vas deferens the activity is increased by the reduction of this double bond. Then, differences in the flexibility of the molecules induced by saturation or unsaturation between C-2 and C-3 could suggest some differences in the interaction with the molecular target of these compounds.

Table 2 Effect of flavonoids on EFS-induced contractions in isolated rat vas deferens

	Concentration of flavonoids (M)							
	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	3×10^{-4}
Flavonols								
Quercetin	1 ± 2	2 ± 2	3 ± 3	5 ± 4	18 ± 5	42 ± 5 *	72 ± 3**	89 ± 4***
Kaempferol	1 ± 2	2 ± 2	9 ± 3	15 ± 5	33 ± 8	65 ± 7 **	80 ± 8**	89 ± 5***
Morin	5 ± 2	14 ± 3	28 ± 5	42 ± 7*	50 ± 9*	70 ± 8 **	95 ± 4***	97 ± 3***
Galangin	2 ± 2	2 ± 3	3 ± 2	4 ± 3	19 ± 3	35 ± 8	50 ± 9*	99 ± 4***
Rutin	2 ± 2	3 ± 2	3 ± 3	9 ± 3	15 ± 3	21 ± 5	32 ± 8	47 ± 7*
Flavones								
Apigenin	1 ± 3	2 ± 3	6 ± 3	16 ± 5	29 ± 5	61 ± 6**	77 ± 8**	89 ± 5***
Flavone	1 ± 2	3 ± 2	15 ± 4	22 ± 5	29 ± 5	43 ± 6*	52 ± 8*	83 ± 6***
Flavanones								
Hesperitin	1 ± 2	2 ± 4	6 ± 3	12 ± 4	24 ± 6	29 ± 7	40 ± 8*	49 ± 9*
Naringenin	6 ± 4	19 ± 3	28 ± 6	42 ± 5*	47 ± 7*	66 ± 7**	70 ± 8**	76 ± 9**
Flavanolols								
Silybin	1 ± 2	2 ± 3	5 ± 3	10 ± 2	21 ± 5	59 ± 7**	85 ± 9***	96 ± 8***

Results (means ± s.e. m. of 8 rats for each compound) are expressed as % of inhibition of the corresponding control. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs corresponding control.

Table 3 The IC50 (i.e., the concentration of flavonoids that produced 50% inhibition of electrically induced contractions) values of flavonoids on the rat vas deferens

Flavonoids	n	IC50 (M) (95% Confidence Intervals)
Flavonols		
Quercetin	8	1.02×10^{-5} (6.30×10^{-6} to 1.65×10^{-5})
Kaempferol	8	2.05×10^{-6} (1.33×10^{-6} to 3.15×10^{-6})
Morin	8	1.67×10^{-6} (9.04×10^{-7} to 3.10×10^{-6})
Galangin	8	1.47×10^{-4} (6.53×10^{-5} to 3.31×10^{-4})
Rutin	8	1.13×10^{-5} (3.98×10^{-6} to 3.20×10^{-5})
Flavones		
Apigenin	8	2.95×10^{-6} (1.68×10^{-6} to 5.16×10^{-6})
Flavone	8	7.49×10^{-6} (2.68×10^{-6} to 2.09×10^{-5})
Flavanones		
Hesperitin	8	1.06×10^{-6} (2.99×10^{-7} to 3.76×10^{-6})
Naringenin	8	2.72×10^{-7} (8.83×10^{-8} to 8.37×10^{-6})
Flavanolols		
Silybin	8	6.03×10^{-6} (4.05×10^{-6} to 8.98×10^{-6})

n, No. of experiments.

References

Capasso, R., Mascolo, N. (2003) Inhibitory effect of the plant flavonoid galangin on rat vas deferens in vitro. *Life Sci*. **72**: 2993–3001

Capasso, R., Borrelli, F., Montanaro, V., Altieri, V., Capasso, F., Izzo, A. A. (2005) Effects of the antidepressant St. John's wort (*Hypericum perforatum*) on rat and human vas deferens contractility. *J. Urol*. **173**: 2194–2197

Di Carlo, G., Autore, G., Izzo, A. A., Maiolino, P., Mascolo, N., Viola, P., Diurno, M. V., Capasso, F. (1993) Inhibition of intestinal motility and secretion by flavonoids in mice and rats: structure–activity relationships. *J. Pharm. Pharmacol*. **45**: 1054–1059

Di Carlo, G., Mascolo, N., Izzo, A. A., Capasso, F. (1999) Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life Sci*. **65**: 337–353

Hammad, H. M., Abdalla, S. S. (1997) Pharmacological effects of selected flavonoids on rat isolated ileum: structure-activity relationship. *Gen. Pharmacol*. **28**: 767–771

Heim, K. E., Tagliaferro, A. R., Bobilya, D. J. (2002) Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J. Nutr. Biochem*. **13**: 572–584

Heijnen, C. G., Haenen, G. R., Oostveen, R. M., Stalpers, E. M., Bast, A. (2002) Protection of flavonoids against lipid peroxidation: the structure activity relationship revisited. *Free Radic. Res*. **36**: 575–581

Herrera, M. D., Marhuenda, E. (1993) Effect of naringin and naringenin on contractions induced by noradrenaline in rat vas deferens-I. Evidence for postsynaptic alpha-2 adrenergic receptor. *Gen. Pharmacol*. **24**: 739–742

Horvathova, K., Vachalkova, A., Novotny, L. (2001) Flavonoids as the chemoprotective agents for civilization diseases. *Neoplasma* **48**: 435–441

Itoigawa, M., Takeya, K., Ito, C., Furukawa, H. (1999) Structure–activity relationship of cardiotonic flavonoids in guinea-pig papillary muscle. *J. Ethnopharmacol*. **65**: 267–272

Joseph, J. A., Shukitt-Hale, B., Casadesus, G. (2005) Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds. *Am. J. Clin. Nutr*. **81** (Suppl. 1): 313S–316S

Leal, L. K., Nechio, M., Silveira, E. R., Canuto, K. M., Fontenele, J. B., Ribeiro, R. A., Viana, G. S. (2003) Anti-inflammatory and smooth muscle relaxant activities of the hydroalcoholic extract and chemical constituents from *Amburana cearensis* A C Smith. *Phytother. Res*. **17**: 335–340

Moyers, S. B., Kumar. N. B. (2004) Green tea polyphenols and cancer chemoprevention: multiple mechanisms and endpoints for phase II trials. *Nutr. Rev*. **62**: 204–211

Neuhouser M. L. (2004) Dietary flavonoids and cancer risk: evidence from human population studies. *Nutr. Cancer* **50**: 1–7

Stoclet, J. C., Chataigneau, T., Ndiaye, M., Oak, M. H., El Bedoui, J., Chataigneau, M., Schini-Kerth, V. B. (2004) Vascular protection by dietary polyphenols. *Eur. J. Pharmacol*. **500**: 299–313

Tallarita, R. J., Murray, R. B. (1996) *Manual of pharmacological calculations with computer programs*. Springer, Berlin

Taylor, L. P., Grotewold, E. (2005) Flavonoids as developmental regulators. *Curr. Opin. Plant Biol*. **8**: 317–323

Van Den Broucke, C. O., Lemli, J. A. (1983) Spasmolytic activity of the flavonoids from *Thymus vulgaris*. *Pharm. Weekbl. Sci*. **25**: 9–14