Flavonoid compounds are potent inhibitors of cyclic AMP phosphodiesterase[1]

A. Beretz, R. Anton and J.C. Stoclet

Faculté de Pharmacie, Université Louis Pasteur, Laboratoire de Pharmacognosie, and Laboratoire de Pharmacodynamie (CNRS, ERA 787) F-67083 Strasbourg (France), 29 December 1977

Summary. The inhibitory activity of 19 flavonoid molecules on cyclic AMP breakdown by a commercial beef heart phosphodiesterase preparation is reported. 7 compounds are active in the micromolar range, 2 of which have a potency equivalent to that of papaverine. Some structure activity relationships are drawn.

Flavonoids are a large group of naturally occurring substances which are used as therapeutic agents. They have a wide spectrum of pharmacological properties[2], the mechanisms of which remain to a large extent unknown. Several of their effects, including smooth muscle relaxation and inotropic, anti-inflammatory, or diuretic effects, are similar to those of many inhibitors of cyclic nucleotide phosphodiesterase (PDE)[3,4]. Recently quercetin has been found to increase the cyclic AMP level in Ehrlich ascites tumor cells[5]. These observations are consistent with the hypothesis that cyclic AMP may play a part in the mechanism of some of the actions of flavonoids. It was suggested that the elevation of cyclic AMP level induced by quercetin may result indirectly from the inhibition of the $(Na^+ + K^+)$ ATPase[5]. We report here that flavonoid compounds are also potent inhibitors of cyclic AMP PDE. The structure-activity relationships were demonstrated by studying the inhibition of a commercial PDE preparation by several flavonols, flavones, anthocyanidins, flavanones, flavanonols, and catechins (figure).

Material and methods. The PDE preparation used (Sigma Chemical Co.) was the soluble PDE from beef heart, partially purified according to Butcher and Sutherland[6]. This preparation displays non-linear kinetics leading to different apparent K_m values, depending on the substrate and enzyme concentrations present in the assay medium[7]. In the experimental conditions used here, the apparent K_m value was 1.2 µM (determined using cyclic AMP concentrations ranging from 0.5 to 10.0 µM and 3.5 µg of enzyme protein in 400 µl of assay medium).

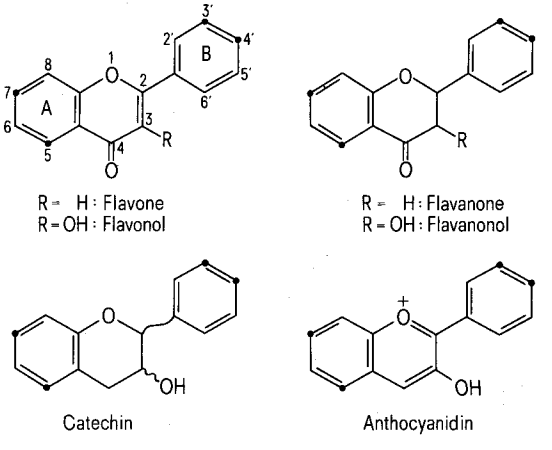
PDE activity was assayed using a modification of the method initially described by Thompson and Appleman[8]. Briefly, this method consists in measuring [^{3}H] products resulting from the incubation of [^{3}H]-cyclic AMP with the PDE preparation and with an excess of nucleotidase. An anion exchange resin was used in a batch process to remove the residual substrate after the end of the enzyme reactions. In the experiments reported here, [^{14}C]-adenosine was added with the resin in each tube in order to measure the recovery of the nucleoside, and to correct each result for the binding of the reaction products to the resin. In preliminary experiments it was verified that identical results are obtained on crude PDE preparations using this method and measuring all the reaction products separated by chromatography[9]. Unless otherwise mentioned, the experimental conditions used for the assay of PDE activity were those described by Thompson and Appleman[8]. The anion exchange resin was QAE Sephadex A25 (Pharmacia, 1 ml of a 50% suspension in deionized water). [^{14}C]-adenosine (10,000 cpm in 100 µl) was added with the resin in each tube. Enough beef heart PDE was added to achieve 5–20% hydrolysis after 10 min of incubation.

The inhibition of PDE by flavonoids was studied in experiments in which the concentration of the drug was varied while the initial concentration of cyclic AMP was held constant at 1 µM. The I_{50} (concentration of flavonoid resulting in a 50% inhibition of cyclic AMP breakdown) was calculated by interpolating 2 values of inhibition ranging from 35 to 75% against the logarithm of the dose of flavonoid added.

All flavonoids were solubilized in ethanol (Merck, reagent grade) and brought to the adequate concentration using the incubation medium. The amount of ethanol in the assay tubes (always $\leq 1\%$) did not significantly influence the enzyme activity. Luteolin, pentaacetylquercetin, apigenin, quercetin and naringenin were extracted and prepared in the laboratory. Rhamnetin and pelargonidin chloride were obtained from Fluka, Buchs, all other flavonoids from Carl Roth, Karlsruhe.

Results and discussion. The figure diagrams the chemical groups studied. The data contained in the table show that most of these molecules inhibited PDE. Comparison of the I_{50} values obtained under identical conditions allows us to determine the relative efficacy of the inhibitors[10]. 7 compounds were active in the micromolar range. The order of their inhibitory potency was flavonols > flavones or anthocyanidins > flavanones or flavanonols > catechins. The main features of the chemical structure influencing activity are the following.

Free hydroxyl substituents are necessary (see the very low activity of flavone and pentaacetylquercetin, and compare acacetin and apigenin, rhamnetin and quercetin). The 2 hydroxyl groups at C-5 and C-7 are not sufficient to confer a significant activity to chrysin. However, the omission of the hydroxyl at C-5 or the methoxylation at C-7 reduces the activity (quercetin is 10 times as potent as fisetin and 3 times as potent as rhamnetin). The addition of 1 hydroxyl at C-3 or C-4' on chrysin greatly increases the activity (galangin, apigenin); the presence of a 2nd hydroxyl in these positions still increases the potency, although less markedly (kaempferol). An additional hydroxyl at C-3'on the B-ring does not affect the activity (compare apigenin with luteolin and kaempferol with quercetin). The



Types of flavonoids studied.
● marks the various positions for hydroxyl substitution.

Effect of flavonoids on the breakdown of cyclic AMP by beef heart phosphodiesterase

Type*	Compound	Substituents*					I_{50} (μM)
		Ring A			Ring B		
		3	5	7	3′	4′	
Flavonol	Kaempferol	OH	OH	OH	-	OH	2.7
	Quercetin	OH	OH	OH	OH	OH	3.6
	Rhamnetin	OH	OH	OCH_3	OH	OH	8.3
	Galangin	OH	OH	OH	-	-	8.6
	Fisetin	OH	-	OH	OH	OH	36
	Pentaacetylquercetin	OAc	OAc	OAc	OAc	OAc	> 100**
Flavone	Apigenin	-	OH	OH	-	OH	9.2
	Luteolin	-	OH	OH	OH	OH	8.7
	Chrysin	-	OH	OH	-	-	> 100**
	Acacetin	-	OH	OH	-	OCH_3	> 100**
	Flavone	-	-	-	-	-	> 100**
Anthocyanidin	Pelargonidin chloride	OH	OH	OH	-	OH	7.8
Flavanone	Hesperetin	-	OH	OH	OH	OCH_3	26
	Naringenin	-	OH	OH	-	OH	45
Flavanonol	(+)-Dihydroquercetin	OH	OH	OH	OH	OH	94
	Dihydrofisetin	OH	-	OH	OH	OH	320
Catechin	(−)-Catechin	OH	OH	OH	OH	OH	300
	(+)-Catechin	OH	OH	OH	OH	OH	500
	(−)-Epicatechin	OH	OH	OH	OH	OH	500

* Molecule types and substituents numbering are given in the figure. ** The lower solubility of some compounds interfered with the determination of their I_{50}; the value was more than 100 μM (> 100). These molecules show an inhibitory potency of approximately 20% at 100 μM.

carbonyl at C-4 increases the potency (compare (+)-dihydroquercetin and (+)-catechin).

Reduced molecules lacking the double bond between C-2 and C-3 (i.e. flavanones and flavanonols) are less potent than their unsaturated homologues (naringenin is a 5th as potent as apigenin, dihydrofisetin a 10th as potent as fisetin, (+)-dihydroquercetin a 30th as potent as quercetin). Catechins, which lack this double bond, have a low activity. The double bond gives the molecule a plane structure and also creates an extended conjugated resonating system; one or both of these features may thus play a role in the interaction with the enzyme.

The stereochemistry of catechins does not influence their activity (compare the isomers (+)-catechin, (−)-catechin, (−)-epicatechin). Because pelargonidin, with its distinctive skeleton (flavylium ion), is active in the micromolar range, we infer that the complete flavone nucleus is not necessary for the inhibition of PDE.

The main characteristics, then, that give the flavone nucleus its inhibitory potency against beef heart PDE are the hydroxyl groups at C-3, C-5, C-7 and C-4', and the presence of the double bond between C-2 and C-3. These structural requirements are found in kaempferol and quercetin, whose inhibitory activities are comparable to those of papaverine[11] and of the best inhibitors previously described[4].

These results show that in addition to their previously reported indirect effect on the cyclic nucleotide level[5], flavonoid compounds may also directly alter the breakdown of cyclic AMP by PDE. Hare and Skidmore (unpublished observations quoted by Fewtrell and Gomperts[12]) found that quercetin only slightly inhibited PDE from the human lung. Some authors have recently reported selective effects of inhibitors on the PDEs from different tissues[3,13]. Thus the inhibition of PDE may or may not play a part in the quercetin-induced elevation of cyclic AMP level, depending on the sensitivity of the tissue PDE to the drug.

An inhibitory effect of flavonoids on various enzyme activities has been reported[5,12,14–20]. In most cases the link between the enzyme activity and the physiological properties is unknown. It is noteworthy that the concentration of flavonoid compounds that inhibited PDE in the present study were no more than $^1/_{10}$ as high as those used in most earlier studies to inhibit other enzyme systems (except in the case of COMT[15], lactic dehydrogenase[16], and lens aldose reductase[20]). Therefore how PDE inhibition enters into the pharmacological properties of flavonoids deserves further investigation.

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