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PRELIMINARY COMMUNICATIONS

FLAVONOIDS ARE SELECTIVE CYCLIC GMP PHOSPHODIESTERASE INHIBITORS

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The inhibitory activity of flavonoid molecules on cyclic AMP breakdown by a commercial beef-heart phosphodiesterase preparation has been recently reported (1). One of these molecules has been found to increase the cyclic AMP level in Ehrlich ascites tumor cells (2). These observations are consistent with the hypothesis that cyclic AMP may play a part in the mechanism of some of the actions of flavonoids, as several of the effects of flavonoids are similar to those of many inhibitors of cyclic nucleotide phosphodiesterase currently used in therapeutics. However cyclic nucleotide phosphodiesterase exists in several molecular forms which are unequally distributed in tissues (3), suggesting that selective inhibitors of the different isozymes might be used to selectively raise the level of cyclic AMP or GMP, the biological effets of which appear to be in some instances mutually opposed (4). The present work shows that most flavonoid compounds more effectively inhibit the breakdown of cyclic GMP than of cyclic AMP. Moreover one of the flavonoids tested, amentoflavone, exhibited a potency 100 times higher than flavonoids tested previously (1), and 5 to 10 times higher than papaverine.

To test the activity of flavonoids on the breakdown of cyclic AMP and cyclic GMP, we used a 105 000 x g supernatant from bovine lung, which, like guinea pig lung (5, 6), contains two distinct main cyclic nucleotide phosphodiesterases hydrolysing specifically cyclic AMP and cyclic GMP (7). Fresh bovine lung was minced , thoroughly washed with a medium containing 2 mM $MgCl_2$- $6H_20$, 3 mM B mercaptoethanol, and 20 mM Tris-HCl pH 7.5;and homogenized with one volume of this medium. The homogenate was centrifuged at 3000 x g for 20 min and at 105 000 x g for 60 min. The last supernatant was frozen at -20°C in small volumes and used to test the phosphodiesterase inhibitors.

Phosphodiesterase activities were assayed using a modified (8) Thompson and Appleman technic (9), with 1 μM ^{3}H -cyclic AMP or GMP as substrates and ^{14}C-adenosine or guanosine added with resin to measure the recovery of the nucleoside and to correct each result for the binding of the reaction products to the resin (QAE Sephadex A 25). The reaction, performed at 37°C for 3 min, was initiated by adding enzyme to the reaction medium (after preincubation for 2 min at 37°C) and stopped by immersing the test tubes in boiling water for 1 min. Under these conditions and with respect to appropriate amounts of 105 000 x g supernatant (40 μg protein for cGMP-PDE and 80 μg protein for cAMP-PDE), linear kinetics were observed and none of the compounds used induced analytical interference phenomena. All assays were performed in duplicate and simultaneously with both substrates.

Table I shows the I_{50}s for both cAMP and cGMP phosphodiesterase activities of flavonoids compared to reference substances. The great selectivity of 2'-deoxy-substrates for each corresponding enzyme had been observed before with purified enzymes and crude extract from guinea pig lung, whereas the selectivities tested with crude extracts from others tissues were smaller (10), indicating the interest of mammal lung in studies of the selectivity of cyclic nucleotide phosphodiesterase inhibitors.

Among the flavonoids tested, (+)- catechin exhibited a selectivity similar to that previously shown for methylisobutylxanthine (11,12) and disodium cromoglycate (13) ; this result differs from the result obtained with papaverine, which, in a study made with rat heart (14), more selectively inhibited cAMP breakdown (14). The other flavonoids tested also inhibited the cGMP phosphodiesterase more than they did the cAMP phosphodiesterase, but with less selectivity. As shown before with a cAMP phosphodiesterase preparation from bovine heart (1), the potency of most flavonoids, for both cAMP and cGMP phosphodiesterases, is of the same order of magnitude as that of the widely used papaverine and theophylline. The flavonoids could be ranked as follows : flavonol > flavone and anthocyanidin > flavanonol and catechin . Considering the present results, we can add, concerning apigenin and amentoflavone (a di-apigenin), that doubling the flavonoid molecule increases the inhibition potency for both enzymes by two orders of magnitude and partially preserves the selectivity. Other studies with biflavonoids and structurally related compounds are in progress.

TABLE I INHIBITION OF cAMP and cGMP PHOSPHODIESTERASES FROM BOVINE LUNG

Compounds	I_{50} (uM)		$\frac{I_{50}\ cAMP}{I_{50}\ cGMP}$
	cAMP	cGMP	
Reference substances			
2'-Deoxy cAMP	12	380	0.03
2'-Deoxy cGMP	1100	7	157
Papaverine	5	11	0.45
Theophylline	300	310	1
1-Methyl-3-isobutylxanthine	35	7	5
Disodium cromoglycate	>2000	250	>8
Flavonoids			
(+)-Catechin	640	170	3.8
Pelargonidin chloride	70	23	3
Quercetin	23	15	1.5
(+)-Dihydroquercetin	320	170	1.9
Apigenin	53	35	1.5
Amentoflavone	0.66	0.54	1.2

I_{50} is the concentration of compound required to give 50 % inhibition of phosphodiesterase activity, determined using 1 uM substrate and various concentrations of inhibitors. It was calculated by interpolating of at least four values of inhibition, ranging from 35 to 75 %, against the logarithm of inhibitor concentrations. All compounds were solubilized in ethanol (Merck, reagent grade). The amount of ethanol in final concentration did not exceed 0.5 % and did not influence the enzyme activity. 2'-Deoxy cAMP and cGMP were purchased from Boehringer, Mannheim (FRG) ; papaverine, from Synthelabo, Paris (F) ; theophylline from Merck, Darmstadt (FRG). 1-Methyl-3-isobutyl-xanthine was obtained from Aldrich Chem. Corp (USA) ; catechin, quercetin, dihydroquercetol, and a apigenin from Carl Roth, Karlsruhe (FRG) ; pelargonidin chloride from Fluka, Buchs (CH). Disodium cromoglycate was a gift from Fisons (F), and amentoflavone, the gift from Dr. H. Wagner, Munich (FRG)

These results suggest that cyclic GMP may play a part in the mechanism of action of flavonoids currently used in therapeutics. The selectivity of the tested flavonoids is smaller than recently reported for others compounds (13). However, as shown with amentoflavone the potency of flavonoids for the inhibition of cyclic nucleotide phosphodiesterase can be highly increased by doubling the molecule, reaching potencies 5 to 10 times higher than observed with papaverine. These first data show that flavonoid analogs might be valuable candidates in the search of potent cyclic GMP phosphodiesterase inhibitors.

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REFERENCES

1. A. BERETZ, R. ANTON and J.C. STOCLET, Experientia 34, 1054 (1978).

2. Y. GRAZIANI and R. CHAVOTH, Biochem. Pharmac . 26, 1259 (1977).

3. J.N. WELLS and J.G. HARDMAN, Adv. Cyclic. Nucl. Res. 8, 119 (1977).

4. B. WEISS and W.N. HAIT, Ann. Rev. Pharmacol. Toxicol. 17, 441 (1977).

5. C.W. DAVIS and J.F. KUO, Biochem. Biophys. Acta 444, 554 (1976).

6. C.W. DAVIS and J.F. KUO, J. Biol. Chem. 252, 4078 (1977).

7. B. ILIEN, A. STIERLE, C. LUGNIER, J.C. STOCLET and Y. LANDRY, Biochem. Biophys. Res. Comm. 83, 486 (1978).

8. C. LUGNIER, T. MICHON and J.C. STOCLET, Br. J. Pharmac . 58, 434 P (1976)

9. W.J. THOMPSON and M.M. APPLEMAN, Biochemistry, 10, 311 (1971).

10. C.W. DAVIS and J.F. KUO, Biochem. Pharmac . 27, 89 (1978).

11. J.N. WELLS, Y.J. WU, C.E. BAIRD and J.G. HARDMAN, Molec. Pharmac . 11, 775 (1975).

12. G.L. KRAMER, J.E. GARST, S.S. MITCHEL and J.N. WELLS, Biochemistry 16, 3316 (1977).

13. H. BERGSTRAND, J. KRISTOFFERSSON, B. LUNDQUIST and A. SCHURMANN, Molec. Pharmac . 13, 38 (1977).

14. C. LUGNIER and J.C. STOCLET, Biochem. Pharmac . 23, 3071 (1974).