Communications to the Editor

[Chem. Pharm. Bull. 34(9)3960—3963(1986)]

INHIBITION OF ARACHIDONATE 5-LIPOXYGENASE BY PHENOLIC COMPOUNDS

Satoshi Iwakami, Masaaki Shibuya, Chen-Fang Tseng, Fumio Hanaoka and Ushio Sankawa*

Faculty of Pharmaceutical Sciences, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113, Japan

Some cinnamoyl-β-phenethylamine derivatives, gingerols, diarylheptanoids and acyldopamines were tested for their inhibitory effects on the 5-lipoxygenase of RBL-1 cells. Compounds containing a catechol group together with a lipophilic group strongly inhibited 5-lipoxygenase. Significant inhibition was also observed in gingerols having guaiacol instead of catechol. Linoleoyl- and linolenoyl-dopamines were the strongest 5-lipoxygenase inhibitors.

KEYWORDS——5-lipoxygenase inhibitor; gingerol; diarylheptanoid; acyldopamine; cinnamoyl-β-phenethylamine derivative

Slow reacting substance of anaphylaxis (SRS-A) is a chemical mediator in asthma, which causes strong and long-lasting broncho-constraction. SRS-A has been shown to be identical to the leukotrienes (LTs) formed by leukocytes and mastocytoma cells.[1] The first-step enzyme of LT biosynthesis is arachidonate (AA) 5-lipoxygenase which catalyses the oxygenation of AA to yield 5-hydroperoxyeicosatetraenoic acid (5-HPETE). Since LTs have important roles in anaphylaxis and inflammatory response, the discovery of inhibitors for AA 5-lipoxygenase could lead to the development of new anti-allergic and anti-inflammatory drugs. Extensive studies have revealed that a variety of compounds inhibit lipoxygenases. Of the naturally occurring phenolics, dihydronorguaiaretic acid inhibits various kinds of lipoxygenases including 5-lipoxygenase.[2] Murota *et al.* reported that flavonoids and caffeic acid, isolated from a Chinese medicinal drug, inhibited the reaction of 5-lipoxygenase of mastocytoma cells (P-815).[3] Yoshimoto *et al.* reported the inhibition of 5-lipoxygenase of RBL-1 cells by flavonoids and discussed their structure-activity relationships.[4] In a previous paper,[5] we reported the inhibition and activation of prostaglandin (PG) biosynthesis by simple phenolic compounds and pointed out that the presence of phenol and lipophilic groups is essential for strong inhibition of PG biosynthesis. This structural feature seems to indicate that those phenolics are not strictly specific inhibitors of PG biosynthesis. Instead they inhibit various kinds of lipoxygenases, though the degree of inhibition varies with the enzymes. This communication deals with the inhibitory effect of some cinnamoyl-β-phenethylamines, diarylheptanoids, gingerols and acyldopamines against AA 5-lipoxygenase.

Materials and Methods

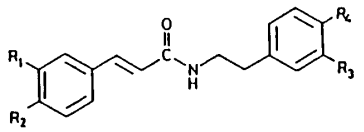
Assay of 5-Lipoxygenase——Rat basophilic leukemia cells (RBL-1) were purchased from Dainippon Pharmaceutical Co. and cultured in suspension. An enzyme solution was prepared as described by Jakschik et al.[6] A reported assay method[6] was modified as follows. The standard assay mixture (total volume 600 μl) consists of 29 mM phosphate buffer (pH 7.0), [1-^{14}C] arachidonic acid (1.3 μM, 10^5 dpm), indomethacin (10 μM), $CaCl_2$ (1.5 mM), EDTA (0.83 mM), gelatin (0.083%) and enzyme solution (from 2 x 10^6 cells). The mixture was incubated at 37° C for 15 min and the reaction was stopped by adding acetone (1.2 ml). The mixture was acidified with 2 N formic acid (0.1 ml) and extracted twice with $CHCl_3$ (1.8 ml). After removing the solvent, the reaction products were subjected to TLC with the organic phase of AcOEt/2,2,4-trimethylpentane/AcOH/H_2O=110/50/ 20/100 as a developing solvent. The zone corresponding to 5-HETE, which was derived from 5-HPETE by reduction with hydroperoxidase present in the enzyme solution, was scraped off and measured with a liquid scintillation counter. Substances for testing were added to the assay mixture as DMSO solutions.

Test Materials——Gingerols and diarylheptanoids were the kind gifts of Profs. T. Shioiri and H. Itokawa. Cinnamoyl-β-phenethylamines were synthetic compounds reported previously[5] or newly synthesized. Acyldopamines were synthesized by the condensation of dopamine and corresponding fatty acids.

Results and Discussion

In our previous paper,[5] we reported the inhibition of PG biosynthesis by cinnamoyl-β-phenethylamine derivatives. Since some of these derivatives contained caffeic acid residue, 12 amides (**1-12**) prepared by synthesis were tested for their inhibitory effects against 5-lipoxygenase. Of the amides tested, compounds having the catechol group, at least on one of the aromatic rings, inhibited 5-lipoxygenase with IC_{50} values of 0.12 - 0.48 μM. The amides are a hundred times more potent inhibitors of 5-lipoxygenase than caffeic acid, whose IC_{50} value was 20 M

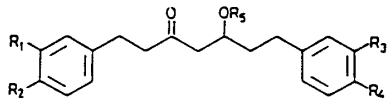
Table I. IC_{50} Values of Aromatic Amides against 5-Lipoxygenase and Prostaglandin Synthetase



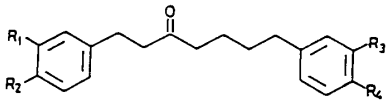
R_1	R_2	R_3	R_4	Compounds		IC_{50} (μM) 5-LIP*1	PG-ase*2
H	H	OH	OH	Cinnamoyldopamine	(1)	0.20	90
H	H	H	OH	Cinnamoyltyramine	(2)	--	120
H	H	H	H	Cinnamoylphenethylamine	(3)	--	180
H	OH	OH	OH	p-Coumaroyldopamine	(4)	0.36	230
H	OH	H	OH	p-Coumaroyltyramine	(5)	--	280
H	OH	H	H	p-Coumaroylphenethylamine	(6)	--	100
OH	OH	OH	OH	Caffeoyldopamine	(7)	0.23	270
OH	OH	H	OH	Caffeoyltyramine	(8)	0.16	210
OH	OH	H	H	Caffeoylphenethylamine	(9)	0.12	80
OMe	OH	OH	OH	Feruloyldopamine	(10)	0.48	440
OMe	OH	H	OH	Feruloyltyramine	(11)	--	270
OMe	OH	H	H	Feruloylphenethylamine	(12)	21%(1μM)	90

*1: 5-lipoxygenase, *2: prostaglandin synthetase, --: less than 7% inhibition at 1μM.

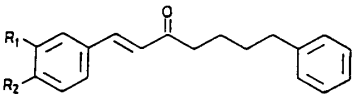
Table II. IC_{50} Values of Diarylheptanoids against RBL-1 Lipoxygenase



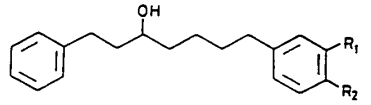
Compounds	R_1	R_2	R_3	R_4	R_5	IC_{50} (μM)
13	H	H	H	OH	H	--
14	H	H	OMe	OH	H	1.6
15	H	H	OMe	H	H	--
16	H	H	OMe	OMe	H	--
17	H	H	OMe	OH	Me	--
18	H	OH	H	H	H	--
19	OH	OH	H	OH	H	0.26
20	OMe	OH	OMe	OH	H	3.2



	R_1	R_2	R_3	R_4	IC_{50} (μM)
21	H	H	H	H	--
22	H	H	OMe	OH	0.24
23	OMe	OAc	H	H	0.94
24	OMe	OH	H	H	0.41



	R_1	R_2	IC_{50} (μM)
25	H	OH	--
26	OMe	OH	0.26
27	OH	OMe	--
28	OH	OH	0.018

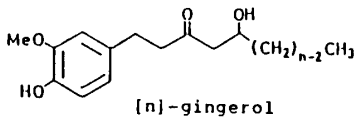


	R_1	R_2	IC_{50} (μM)
29	OMe	OH	0.21

--: less than 10% inhibition at 1μM.

under the same assay conditions. (Table I) Next, we tested the inhibitory effect of the diarylheptanoids (**13-29**), because they are inhibitors of PG biosynthesis[7] and their structures are somewhat similar to those of cinnamoyl-β-phenethylamines. Of the diarylheptanoids tested, 7 compounds inhibited 5-lipoxygenase with IC_{50} values less than 1 μM, (Table II). The IC_{50} value of the most potent diarylheptanoid (**28**) was 18 nM. Significant inhibitory effects were observed in the compounds having guaiacol (3-methoxy-4-hydroxyphenol) instead of catechol: compounds **22**, **24**, **26** and **29**. Then we tested the inhibitory effects of gingerols, pungent principles of ginger, since they were regarded as alkyl analogues of the diarylheptanoids. The results are summarized in Table III. The gingerols with longer side chains, [10-16]-gingerols, strongly inhibited 5-lipoxygenase. As can be seen from Table III, the inhibitory effect of the gingerols was sharply less in the compounds whose side chain lengths were less than C_{12}, [2-8]-gingerols. In order to compare the inhibitory effect with that of the gingerols, we synthesized several acyldopamines, which can be regarded as gingerol analogues. The IC_{50}

Table III. IC_{50} Values of Gingerols against RBL-1 5-Lipoxygenase and Rabbit Kidney Prostaglandin Synthetase

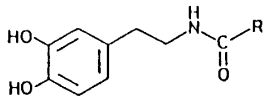


[n]-gingerol

Compounds	IC_{50} (μM) 5-LIP*1	PG-ase*2
[2]-Gingerol (30)	34%(10μM)	7%(100μM)
[4]-Gingerol (31)	39%(10μM)	35%(100μM)
[6]-Gingerol (32)	3	4.6
[8]-Gingerol (33)	0.36	5.0
[10]-Gingerol (34)	0.053	2.5
[12]-Gingerol (35)	0.046	4.1
[14]-Gingerol (36)	0.042	5.7
[16]-Gingerol (37)	0.055	8.6

*1: 5-lipoxygenase, *2: prostaglandin synthetase.

Table IV. IC_{50} Values of Acyldopamines against RBL-1 5-Lipoxygenase



R	Compounds		IC_{50} (nM)
$n\text{-}C_8H_{17}$	Pelargonoyldopamine	(38)	45
$n\text{-}C_8F_{17}$	Perfluoropelargonoyldopamine	(39)	66
$n\text{-}C_{10}H_{21}$	Undecyloyldopamine	(40)	17
$n\text{-}C_{17}H_{35}$	Stearoyldopamine	(41)	16
$n\text{-}C_{17}H_{33}$	Oleanoyldopamine	(42)	7.5
$n\text{-}C_{17}H_{31}$	Linoleoyldopamine	(43)	2.7
$n\text{-}C_{17}H_{29}$	Linolenoyldopamine	(44)	3.2

values of undecyloyl- and stearoyl-dopamines were basically the same. Undecyloyldopamine corresponds to [10]-gingerol in its side chain length, so the effect of side chain length would be the same in the both series of compounds. The side chain length corresponding to C_{14} alkyl seems to be the minimum length for strong activity and elongation of the side chain did not affect the inhibitory activity. Introduction of a double bond contributed to the potentiation of the inhibitory effect. Linoleoyl- and linolenoyl-dopamines are the strongest inhibitors so far found in this study. Their inhibitory activities are comparable to those of the synthetic inhibitors of 5-lipoxygenase such as AA-861[8] and 5,6-methano-LTA_4.[9] Since the IC_{50} values[10] of acyldopamines for PG biosynthesis are two to three orders of magnitude higher than those for 5-lipoxygenase, the acyldopamines can be regarded as specific inhibitors of 5-lipoxygenase. The results obtained in this study clearly demonstrate that the presence of the catechol or guaiacol group together with a lipophilic group, aryl or alkyl, is essential for strong inhibitory activity, and compounds having the catechol group have more specific and potent effect against arachidonate 5-lipoxygenase.

ACKNOWLEDGEMENT We are grateful to Profs. T. Shioiri of Nagoya City University and H. Itokawa of Tokyo College of Pharmacy for the generous gifts of gingerols and diarylheptanoids. The authors thank the Ministry of Education, Science and Culture for providing a Grant-in-Aid.

REFERENCES AND NOTES

1) B.Samuelsson, Angew.Chem.Int.Ed.Engl., **21**, 902 (1982).
2) L.Levine, Biochem.Pharmacol., **32**, 293 (1983).
3) Y.Koshihara, T.Neichi, S.Murota, A.Lao, Y.Fujimoto and T.Tatsuno, FEBS Lett., **158**, 41 (1983).
4) T.Yoshimoto, M.Furukawa, S.Yamamoto, T.Horie and S.Watanabe-Kohno, Biochem. Biophys.Res.Commun., **116**, 612 (1983).
5) C.-F.Tseng, A.Mikajiri, M.Shibuya, Y.Goda, Y.Ebizuka, K.Padmawinata and U.Sankawa, Chem.Pharm.Bull., **34**, 1380 (1986).
6) B.A.Jakschik, T.Harper and R.C.Murphy, "Methods in Enzymology", Vol. 86, ed. by W.E.Lands and W.L.Smith, Academic Press, New York, pp 30-37 (1982).
7) F.Kiuchi, M.Shibuya, and U.Sankawa, Chem.Pharm.Bull., **30**, 2279 (1982).
8) Y.Koshihara, S.Murota, N.A.Petasis and K.C.Nicolaou, FEBS Letters, **143**, 13 (1982).
9) T.Yoshimoto, C.Yokoyama, K.Ochi, S.Yamamoto, Y.Maki, Y.Ashida, S.Terao and M.Shiraishi, Biochem.Biophys.Acta, **713**, 470 (1982).
10) The IC_{50} values of acyldopamines against PG biosynthesis are 4.8 to 21 μM.

(Received June 30, 1986)