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Molecular Design toward Biologically Significant Compounds Based on Platelet Activating Factor: A Highly Selective Agonist as a Potential Antihypertensive Agent

Sir:

Since the identification of platelet activating factor (PAF)[1] and an antihypertensive lipid[2] as alkylacetylglycerophosphocholine, a number of constitutional analogues have been prepared by many laboratories, and we also recently reported that acetyl glyceryl ether phosphorylcholines, their enantiomers, and their analogues were efficiently synthesized in a stereochemically unambiguous manner starting from D- and L-tartaric acids as chiral synthons.[3] The accumulated study on the biological activities of various synthetic phospholipids with acylic structures showed that the irreversible platelet aggregation always parallels the antihypertensive activity,[3b,4] although several selective antagonists have been found recently and are now investigated intensively.[5] It can be said that PAF analogues are structurally simple compounds but are conformationally complex. Therefore, a molecular design

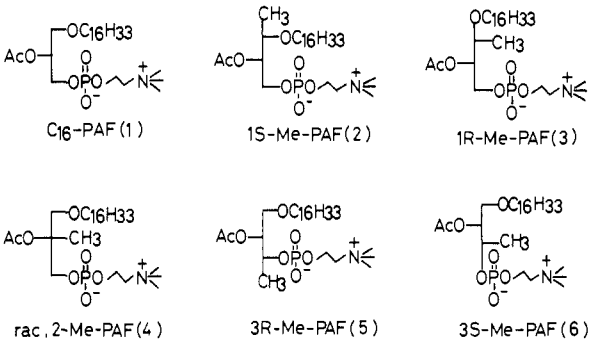

Figure 1. Platelet activating factor and the analogues.

of a PAF analogue has been carried out in such a way as to localize the conformational isomers of PAF by introducing a methyl group[6] in the glycerine moiety, since we assumed that the multiple biological activities of PAF may be mediated by the different stereochemical environment of a receptor or by the multiple receptors[7] with different stereochemical requirements. From this point of view, we attempted to design an analogue with a high selective hypotensive activity but with a limited ability to cause platelet activation. In this paper, we describe a highly selective synthetic agonist with orally potential antihypertensive activity. A methyl group was introduced at C_1 and C_3 diastereoselectively[8] and enantioselectively by our tartaric acid strategy[9] (Figures 1 and 2). For comparison, a methyl group was also introduced at C_2 to afford 2-Me-PAF in racemic form.[9] The simple conformational analysis by using Newman projection formulas shows that the more stable conformers of C_{16}-PAF, 1(*S*)-Me-PAF, 1(*R*)-Me-PAF, 3(*S*)-Me-PAF, and 3(*R*)-Me-PAF are different from one another in the spacial orientation of the methyl, alkyl ether, and phosphocholine groups.[10] Biological activities (platelet activation and antihypertensive) of the synthetic methyl derivatives are described in this paper. The derivatives were assessed for activity to induce platelet activation by measuring the release of [^{14}C]serotonin from rabbit platelets,[11] and the effect of PAF and the methyl derivatives on blood pressure was examined by injecting male Wistar rats with the test compounds intravenously. The results as shown in Table I clearly demonstrate that 1(*S*)-Me-PAF is more important bio-

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(9) Details about the synthetic study of other compounds described here will be published elsewhere. 1(*S*)-Me-C_{16}-PAF (**2**): mp 222–230 °C; $[\alpha]^{21}_D$ −1.11° (*c* 1.80, $CHCl_3$–MeOH); FAB-MS 538 ($M^+ + 1$).

(10) A study on the relative stability of the conformers is now in progress by computer-assisted calculation.

(11) The details of the biological test of platelet aggregation and serotonin release activities are essentially followed by the method described in the following: Hayashi, H.; Kudo, I.; Inoue, K.; Onozaki, K.; Tsushima, S.; Nomura, H.; Nojima, S. *J. Biochem. (Tokyo)* **1985**, *97*, 1737–1745.

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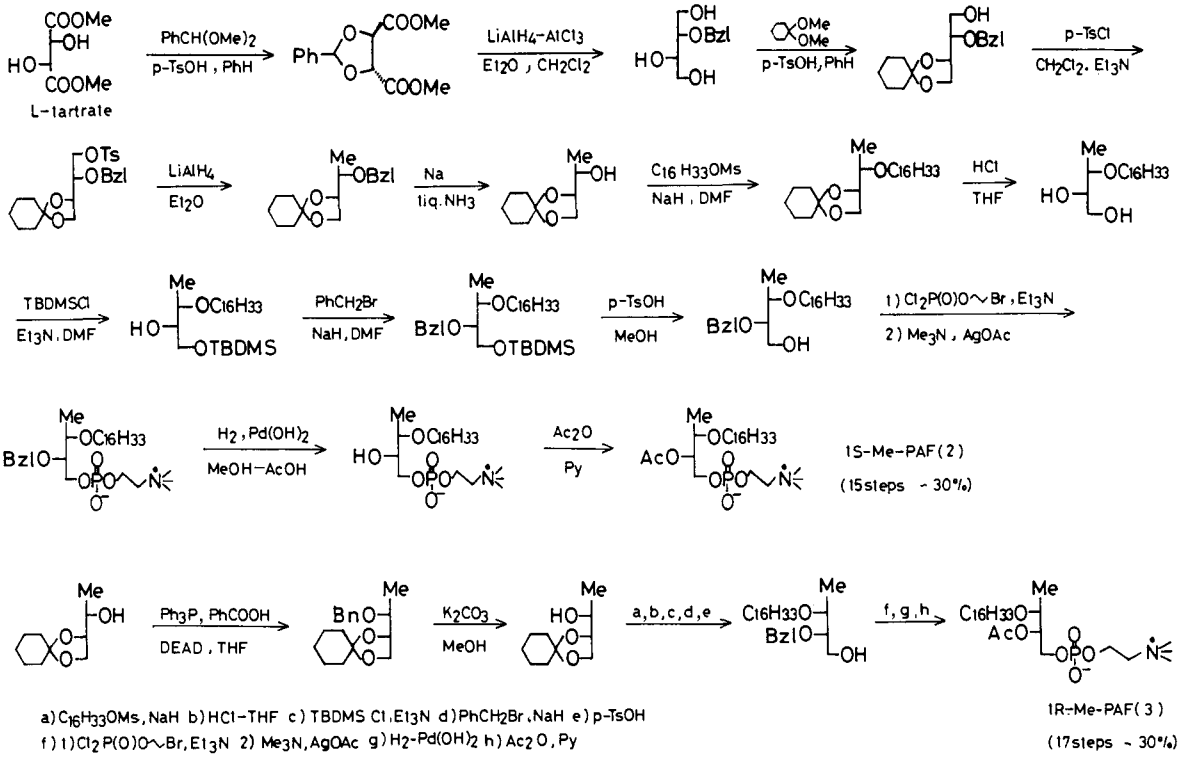

Figure 2. Enantioselective synthesis of 1(*S*)-Me-PAF (**2**) and 1(*R*)-Me-PAF (**3**).

logically than any other methyl derivatives. All other methyl derivatives (1(*R*)-Me-PAF, 3(*S*)-Me-PAF, 3(*R*)-Me-PAF, 2-Me-PAF) and their enantiomers showed no significant platelet activation or antihypertensive properties. However, it was remarkable to find that the 1-(*S*)-Me-PAF showed only weak platelet activation (about $^1/_{115}$ of C_{16}-PAF) but still held a reasonable ability to reduce blood pressure intravenously (about $^1/_{20}$ of C_{16}-PAF).[12] Encouraged by this discovery, we used spontaneously hypertensive rats for evaluation of the activity of the orally administered 1(*S*)-Me-PAF analogue on blood pressure.[13] The results are summarized in Table II. Surprisingly, 1(*S*)-Me-PAF caused a significant reduction in blood pressure in 3 h after drug administration even at a very small dose (0.1 and 1.0 mg/kg po) without any change in heart rate. On the contrary, such a significant reduction by PAF was obtained only at a rather high dose (25 mg/kg po), and the blood pressure was not significantly affected at lower doses of PAF (0.3 and 3.0 mg/kg po). At higher doses of PAF (25 mg/kg po) and 1(*S*)-Me-PAF (10 mg/kg po), one of four animals and all four animals in the experiment were dead, respectively, before measurement of blood pressure 3 h after drug administration. The biological activities obtained here have clearly demonstrated that 1(*S*)-Me-PAF is a biologically significant compound,

Table I. Biological Activities of Methyl-Substituted PAF Analogues

compound	relative activity: platelet activation (A)[11]	relative activity: antihypertension (B)[12]	B/A
C_{16}-PAF (1)	1.0[a]	1.0[b]	1.0
1(*S*)-Me-PAF (**2**)	8.7×10^{-3}	5.9×10^{-2}	6.78
1(*R*)-Me-PAF (**3**)		3.3×10^{-4}	
ent of **2**		2.0×10^{-4}	
ent of **3**	5.6×10^{-4}	1.0×10^{-4}	0.18
rac 2-Me-PAF (**4**)		1.0×10^{-4}	
3(*R*)-Me-PAF (**5**)	5.5×10^{-4}	2.0×10^{-3}	3.64
3(*S*)-Me-PAF (**6**)	8.9×10^{-4}	1.4×10^{-3}	1.57
ent of **5**		2.0×10^{-4}	
ent of **6**	5.5×10^{-4}	1.0×10^{-3}	1.82

[a] [^{14}C]Serotonin-preloaded rabbit platelets were included with various amounts of PAF and PAF analogues at 20 °C for 2 min, and the release of [^{14}C]serotonin from the platelets was measured and compared with the ED_{50} of C_{16}-PAF (2.8×10^{-9} M). [b] C_{16}-PAF produced a dose-dependent hypotension of Wistar rats by intravenous doses ranging from 1 to 10 nmol/kg, and the blood pressures lowered by PAF analogues were compared with those lowered by C_{16}-PAF, and the relative activities were obtained.

and, to our knowledge, it is the first agonist with a potential antihypertensive activity (about 200 times stronger than PAF, orally). Furthermore, we believe that the design of this unique conformational drug has opened new avenues in the design of even more potentially useful PAF analogues.[14]

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(12) Three to six male Wistar rats weighing 350–430 g were used throughout the experiments. Animals were anesthetized with sodium pentobarbital (60 mg/kg intrapectorally). Blood pressure was measured via a catheter from the femoral artery by using a pressure transducer, and heart rate was counted from the blood pressure pulse waves. Drug injection was performed through a catheter inserted in the abdominal vena cava via the femoral vein. After stable values of blood pressure and heart rate were obtained, test drugs were injected intravenously, and changes in both parameters were measured at 5-min intervals for 40 min after dosing.

(13) Experiments were performed on male SHR rats (18–20 weeks of age). Food was withdrawn 24 h before the animals were used, but they had free access to water. Systolic blood pressure in the tail artery was detected indirectly by use of a tail-cuff method, and heart rate was also measured simultaneously.

(14) The other biological tests on **2** (hydrolysis with acetylhydrolase and phospholipase A_2) are now extensively studied, and the results and other synthetic homologues of **2** will be reported in the near future.

Table II. Hypotensive Activity of PAF and 1(*S*)-Me-PAF in Spontaneously Hypertensive Rats (SHR)[13]

drug	dose, mg/kg (po)	n^a	decrease in systolic blood pressure,[b] mmHg
C_{16}-PAF	0.3	5	4 ± 1.9
	3	5	5 ± 7.7
	25[c]	4	53 ± 3.9
1(*S*)-Me-PAF	0.1	5	35 ± 11.4
	1	4	69 ± 11.9
	10	5	*d*

[a] Number of SHRs in the experiments. [b] Values were given in mmHg 3 h after oral administration (po). [c] One of four animals was dead before measurements of blood pressure. [d] All SHRs used in the experiment were dead within 3 h after oral administration.

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Articles

Synthesis and Inhibitory Activity on Carbonic Anhydrase of Some New Sulpiride Analogues Studied by Means of a New Method

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The pharmacological activity of several new sulpiride analogues was studied by means of a new approach, based on a potentiometric technique with a pCO_2 sensor, capable of detecting carbonic anhydrase inhibition at equilibrium conditions. This procedure gives results stated as percent of inhibition of enzymatic activity (IP, inhibitory power). To prove the reliability of the proposed approach and to study structure–activity relationships, several new molecules were synthesized and tested in comparison with the two sulpiride enantiomers. A possible inhibition mechanism is discussed in terms of experimental evidence obtained from the interactions between the molecular structures of the new synthesized compounds and carbonic anhydrase.

Carbonic anhydrase (CA) is a well-known metalloenzyme[1,2] prevalent in plant and animal tissues as well as in bacteria; it is stable in solution under a wide range of conditions, for example from pH 4 to pH 11. Carbonic anhydrase in man is mainly located in the blood in a concentration of 1–2 g/L, but it is also present in many organs and tissues like the pancreas, kidneys, lungs, eyes, arterial walls, gastric mucosa, and the central nervous system. In mammalian red cells, the enzyme is constituted by a polypeptide chain with a molecular weight of about 30 000, and it contains one zinc atom per molecule.[2] Its role is to reversibly catalyze the so-called "hydration reaction" of carbon dioxide to carbonic acid (eq 1) at very

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^- \quad (1)$$

high rates. In particular, an "acid form" of the enzyme is required for the "dehydration", and a "basic form", for the "hydration" reaction. This implies a proton binding of the enzyme at some step of the catalytic pathway (eq 2), where

$$CO_2 + H_2O + E \rightleftharpoons HCO_3^- + EH^+ \rightleftharpoons HCO_3^- + E + H^+ \quad (2)$$

EH^+ and E represent respectively the acid and the basic forms of the enzyme.[2] Another possible aspect of these equilibrium reactions has recently been proposed[3] where the net classic reaction (1) is now the result of the addition of reactions 3 and 4.

$$EH_2O \rightleftharpoons EOH^- + H^+ \quad (3)$$

$$EOH^- + CO_2 + H_2O \rightleftharpoons HCO_3^- + EH_2O \quad (4)$$

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^- \quad (1)$$

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