Prehispanolone, a novel platelet activating factor receptor antagonist from *Leonurus heterophyllus*

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1 Using an *in vitro* radioligand binding assay for the platelet activating factor (PAF) receptor, we have identified a novel, specific PAF antagonist, prehispanolone, from a Chinese medicinal herb *Leonurus heterophyllus*.

2 The presence of sodium ions inhibited specific [^{3}H]-PAF binding to rabbit platelet membrane with an IC_{50} of 5.2 mM, decreased the inhibitory potency of PAF but increased the inhibitory potency of prehispanolone.

3 Prehispanolone and several of its derivatives inhibited the binding of [^{3}H]-PAF to rabbit platelets with potencies closely resembling that of inhibition of PAF-induced aggregation.

4 The integrity of the tetrahydrofuran ring of prehispanolone is critical for its interaction with the PAF receptor.

5 By hydrogenating the dihydrofuran ring and replacing the keto group of prehispanolone with a hydroxyl group, we obtained a compound, LC5507, that is more stable and more active than prehispanolone as a PAF receptor antagonist.

Keywords: PAF receptor; rabbit platelet; BN52021; platelet aggregation; tetrahydrofuran; guanyl nucleotides; prehispanolone; *Leonurus heterophyllus*

Introduction

Platelet activating factor (PAF), also known as acetyl glyceryl ether phosphorylcholine (AGEPC), antihypertensive polar renal medullary lipid (APRL) or PAF-acether, was first identified as a potent mediator for aggregation and degranulation of platelets and neutrophils (Benveniste *et al.*, 1972). Its structure was determined in 1979 as 1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine (Benveniste *et al.*, 1979; Blank *et al.*, 1979; Demopoulos *et al.*, 1979) and is now known to produce a wide spectrum of biological responses in addition to platelet activation (Snyder, 1987; Winslow & Lee, 1987). There is considerable evidence suggesting its involvement in the pathogenesis of shock, ischaemia, glomerulonephritis, gastric ulcer formation, asthma and systemic anaphylaxis (Braquet *et al.*, 1987; Feuerstein & Hallenbeck, 1987; Grandel, 1987; Vargaftig & Braquet, 1987). Thus, specific antagonists of PAF may be useful therapeutic agents in a variety of inflammatory, respiratory, immunological and cardiovascular disorders (Saunders & Handley, 1987). Several PAF antagonists (including kadsurenone, BN52021 and WEB 2086) have indeed been reported to provide protection against the damage induced by ischaemia/reperfusion in the heart, kidney and brain in animal studies and to inhibit antigen-induced responses in asthmatic patients (Braquet *et al.*, 1987; 1989).

The presence of pharmacologically specific PAF receptors has been reported in cells that respond to PAF including human, dog and rabbit platelets (Valone *et al.*, 1982; Hwang *et al.*, 1983; Tahraoui *et al.*, 1988), human neutrophils (Valone & Goetzl, 1983), human lung tissues (Hwang *et al.*, 1985) and murine macrophages (Valone, 1988). The properties of PAF receptors and their interaction with drugs can be studied conveniently by radioligand binding assays using [^{3}H]-PAF with

high specific activity (Inarrea *et al.*, 1984; Hwang *et al.*, 1986a; Tahraoui *et al.*, 1988).

We have recently isolated a novel labdane diterpene, prehispanolone (9α, 13R; 15,16-diepoxy-labdane-14-en-7-one, LC5504), from a Chinese medicinal herb *Leonurus heterophyllus* (Figure 1, Hon *et al.*, 1990a). The aqueous extract of this herb can reduce blood viscosity and inhibit platelet aggregation (Zou *et al.*, 1989) and it has been used to treat myocardial ischaemia and oedema in chronic and acute nephritis (Wang *et al.*, 1985; Chang & But, 1987). In the present study, we provide radioligand binding and functional data to characterize prehispanolone as a specific PAF receptor antagonist. We have also performed a structure activity relationship study
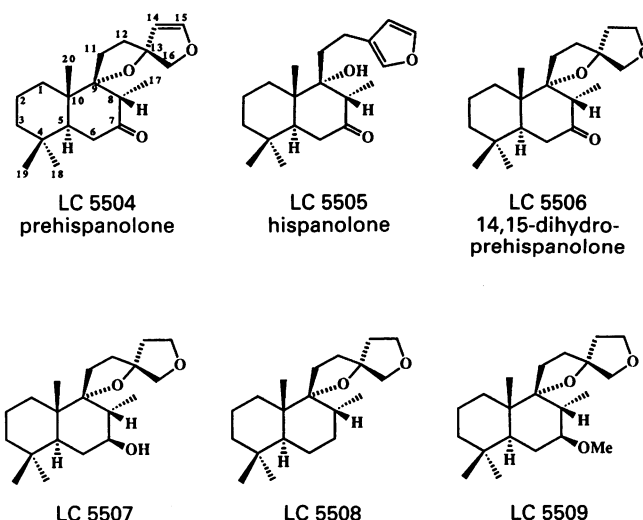
Figure 1 Chemical structure of prehispanolone and of its related analogues.

in the hope of improving its stability and identifying the key structural elements involved in its interaction with the PAF receptor.

Methods

Preparation of platelets

Adult New Zealand rabbits were anaesthetized with pentobarbitone (25 mg kg^{-1}, i.v.). Nine volumes (45 ml) of blood from the heart was drawn directly into 1 volume (5 ml) of ACD solution (0.8% citric acid, 2.2% sodium citrate and 2.45% glucose). The mixture was centrifuged at 270 g for 10 min at room temperature. The top platelet-rich plasma (PRP) was removed carefully and used in aggregation experiments.

For binding experiments, the PRP was centrifuged at 1100 g for 15 min at room temperature to obtain the platelet pellet and the supernatant was centrifuged again at 1800 g for 20 min to obtain the platelet-poor plasma (PPP). The platelet pellet was gently resuspended in buffer A (composition, mM: NaCl 100, MgCl$_2$ 15, EDTA 5 and Tris-HCl 50, pH 7.4) and centrifuged at 1100 g for 15 min. This washing procedure was repeated twice, first with buffer A followed by buffer B (Tris-HCl 50 mM, bovine serum albumin (BSA) 2.5 mg ml^{-1} and sucrose 0.3 M). The final pellet was resuspended in buffer B to give a concentration of 1–2 × 10^8 platelets ml^{-1} and used in binding experiments immediately. Platelets were counted with a haemocytometer (Neubauer improved) under a microscope.

Preparation of platelet membranes

PRP was prepared as described above except 3.8% sodium citrate was used in place of ACD solution since there was no need to provide glucose as an energy source. The PRP was centrifuged at 1100 g for 20 min to obtain the platelet pellet. The pellet was homogenized in 10 ml of ice cold buffer C (Tris-HCl 50 mM, BSA 2.5 mg ml^{-1}, pH 7.2) with a polytron (setting 6, 20–30 s). The homogenate was centrifuged at 39200 g for 10 min at 4°C. The supernatant was decanted and the pellet resuspended in 10 ml of ice cold buffer C by polytron (setting 6, 10 s) and centrifuged again. This washing procedure was repeated twice and the final pellet was stored at −70°C or resuspended in buffer C for receptor assay.

[^{3}H]-PAF and other radioligand binding experiments

Unless otherwise mentioned, the binding of [^{3}H]-PAF was routinely measured in duplicate in the absence of sodium. For intact platelet binding, about 2 × 10^7 platelets were incubated at 20°C for 60 min with 2 nM [^{3}H]-PAF in a final volume of 500 μl containing Tris-HCl 50 mM, pH 7.4, sucrose 0.3 M, BSA 2.5 mg ml^{-1} and various test drugs or the appropriate vehicle (0.36% dimethyl sulphoxide in buffer C). For platelet membrane binding, membrane aliquots (about 50 μg protein) were incubated at 0°C for 30 min with [^{3}H]-PAF 2 nM in a final volume of 200 μl containing Tris-HCl 50 mM, pH 7.2, BSA 0.31–5 mg ml^{-1} and various test drugs.

At the end of the incubation period, the reaction was stopped by rapid filtration under reduced pressure through Whatman GF/C glass fibre filters (presoaked in buffer C for 2 h before use) using a Brandel or a Skatron cell harvester. Filters were washed 3 times with 5 ml of ice cold buffer each. They were dried and radioactivity was measured by liquid scintillation spectrometry. Nonspecific binding was defined in the presence of 2.5 μM non-radioactive PAF. Specific binding was calculated by subtracting nonspecific binding from total binding.

Other radioligand binding assays were performed as described previously (Wong et al., 1983; Shi et al., 1984; Hon

et al., 1990b). The binding of [^{3}H]-nitrendipine to dihydropyridine-sensitive calcium channel was determined with rat cerebral cortex membranes. The binding of [^{3}H]-flunitrazepam and [^{3}H]-phenylisopropyl adenosine to central benzodiazepine and adenosine A$_1$ receptors respectively, was determined with bovine cerebral cortex membranes. The binding of [^{3}H]-Ro5-4864 and [^{3}H]-N-ethylcarboxamidoadenosine to peripheral benzodiazepine and adenosine A$_2$ receptors was determined with rat liver and striatum membranes respectively, whereas [^{3}H]-nitrobenzylthioinosine binding to nucleoside transporters was determined with guinea-pig lung membranes.

Rabbit platelet aggregation assay

Freshly prepared platelets were counted with a haemocytometer (Neubauer improved) under a microscope and adjusted with PPP to give a concentration of 1–2 × 10^8 platelet ml^{-1}. PRP was stored in a polypropylene tube with stopper at room temperature. Aggregation experiments were performed 30 min after PRP preparation and finished within 3 h.

Aggregation of platelets was monitored by measuring the changes in turbidity with a Chronolog platelet aggregometer, model 669 (Havertown, PA) at 37°C. Aliquots of PRP (200 μl) were incubated at 37°C for 5 min and then 25 μl of various test drugs or the appropriate vehicle (0.18% dimethyl sulphoxide in buffer C) were added and incubated for another min. Aggregation was induced by the addition of 25 μl of PAF (final concentration 2 nM in the aggregation cuvette) in BSA 2.5 mg ml^{-1}. The percentage of platelet aggregation was calculated from the change in light transmission by assigning the transmittance of unstimulated PRP to be 0% and that of PPP to be 100%. Typically, 2 nM PAF induced a 65% aggregation at the beginning of experiment. To control for time-dependent changes in platelet sensitivity to PAF, vehicle controls were included before and after every two drug tests and data were expressed as percentage of the mean of the controls.

Drug sources

[^{3}H]-PAF (1-O-[^{3}H]-hexadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine) was purchased from New England Nuclear, Boston, MA, U.S.A. with a specific activity of 56.7 Ci mmol^{-1}. [^{3}H]-nitrendipine and [^{3}H]-Ro 5-4864 were also from New England Nuclear. [^{3}H]-N-ethylcarboxamidoadenosine, [^{3}H]-phenylisopropyladenosine and [^{3}H]-flunitrazepam were from Amersham, Buckinghamshire, England. [^{3}H]-nitrobenzylthioinosine was purchased from Moravek Biochemicals, Brea, CA, U.S.A. Unlabelled PAF was obtained from Calbiochem Corporation, San Diego, CA, U.S.A. and RIA grade BSA, thrombin, collagen, Gpp NHp, ADP and ATP were from Sigma Chemical Co., St. Louis, MO, U.S.A. BN52021 (Ginkgolide B) was a generous gift from Dr P. Braquet (Institut Henri Beaufour, Le Plessis, France). Prehispanolone and its derivatives were prepared in our laboratory (Hon et al., 1990a). Details of the chemistry involved in their preparation will be published elsewhere. Prehispanolone and its derivatives were dissolved in dimethyl sulphoxide to give 15 mM stock solutions. They were diluted with buffer C to give the appropriate working solutions for binding and aggregation experiments. Stock solutions of [^{3}H]-PAF and PAF in ethanol were also diluted with buffer C to give the appropriate working solutions of 10 nM [^{3}H]-PAF (0.41% ethanol) and 10 μM PAF (0.10% ethanol).

Statistical analysis

The results, unless otherwise stated, are expressed as the mean ± s.d.. Data, where appropriate, were compared by

Student's paired t test and were considered to be significantly different when $P < 0.05$. Statistical analysis of correlation was done with SPSS/PS+ statistical package.

Results

General properties of [³H]-PAF binding

Initial experiments showed little difference between total and nonspecific binding of [³H]-PAF to rabbit platelet membranes in the absence of BSA. However, reproducible specific binding could be detected in the presence of BSA. As illustrated in Figure 2, there was a marked reduction of nonspecific binding accompanied by a graduate increase in specific binding when the BSA concentration was increased from 0.31 to $5 \, mg \, ml^{-1}$. This was not a result of [³H]-PAF binding to BSA since no specific binding was observed when platelet membranes were omitted.

Initial experiments also indicated that [³H]-PAF binding to rabbit platelet membranes and intact platelets reached equilibrium after 20 min at 0°C and after 45 min at 20°C respectively. Thus, incubation periods of 30 min at 0°C and 60 min at 20°C were adopted for all subsequent experiments.

Typically, with washed rabbit platelet membranes incubated with 2 nM [³H]-PAF at 0°C in the presence of $2.5 \, mg \, ml^{-1}$ BSA, total binding was about 8450 c.p.m. whereas nonspecific binding assayed in the presence of 2.5 μM PAF was about 2850 c.p.m. Thus under standard binding conditions, two-thirds of the total binding of [³H]-PAF was specific.

The presence of sodium chloride was found to inhibit the specific binding of [³H]-PAF to rabbit platelet membranes in a concentration-dependent manner with an IC_{50} (concentration which inhibited 50% of specific [³H]-PAF binding) of 5.2 mM (Figure 3). This effect seemed to be specific for sodium because potassium chloride tested between 1.5 and 150 mM did not significantly affect [³H]-PAF binding (Figure 3). Both NaCl and KCl had no significant effect on nonspe-
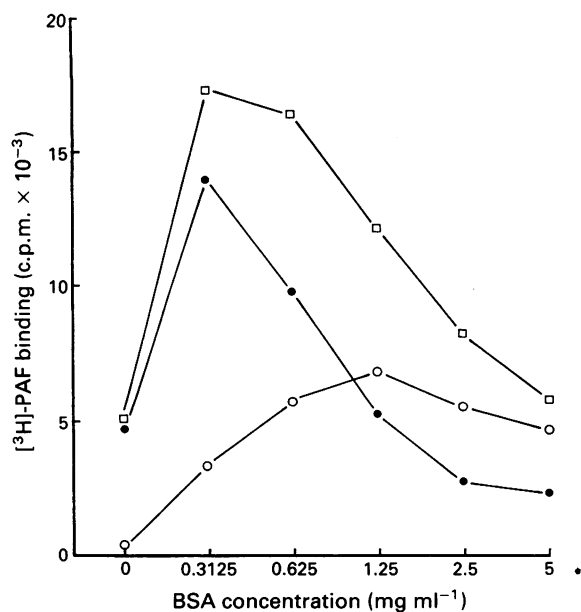
Figure 3 Effects of different concentrations of NaCl (○) and KCl (●) on the specific binding of [³H]-PAF to rabbit platelet membranes. Results are the mean (with s.d. indicated by vertical bars) of 3 separate experiments performed in duplicate. * $P < 0.05$.

cific binding of [³H]-PAF at the concentration range examined.

Similar to results obtained with other G-protein (guanine nucleotide dependent protein)-coupled receptors (Snyder, 1979), the specific binding of [³H]-PAF to platelet membranes at 0°C was inhibited by GppNHp (guanylyl-5'-imidophosphate), a metabolically stable analogue of GTP (guanosine-5'-triphosphate). The inhibition was concentration-dependent and up to 58% inhibition was obtained at 1000 μM (Figure 4). Other nucleotides such as ATP (adenosine triphosphate) and ADP (adenosine diphosphate) were inactive at similar concentrations (Figure 4).

Effect of sodium on the inhibition of [³H]-PAF binding to platelet membranes by prehispanolone and PAF

Prehispanolone (LC5504) inhibited specific [³H]-PAF binding to rabbit platelet membranes with an IC_{50} of 9 μM in the absence of NaCl (Figure 5). The presence of 5 and 10 mM NaCl in the incubation mixture increased the inhibitory potency of prehispanolone to 3.6 and 3.3 μM respectively. On

Figure 2 Effects of different concentrations of bovine serum albumin (BSA) on the total (□), nonspecific (●) and specific (○) binding of [³H]-PAF (2 nM, 30 min, 0°C) to rabbit platelet membranes. Data shown are those of a typical experiment. Similar results were obtained in 3 separate experiments performed in duplicate.
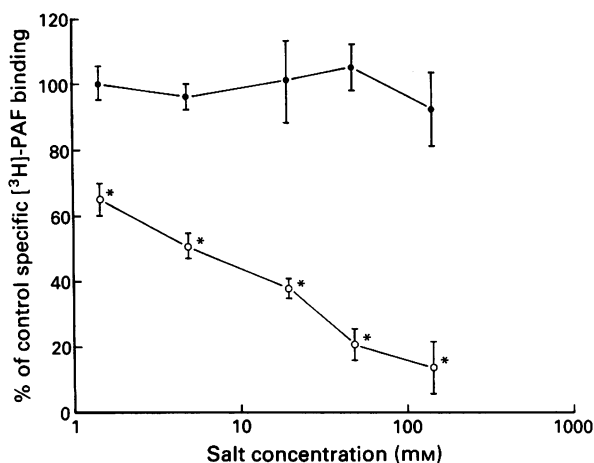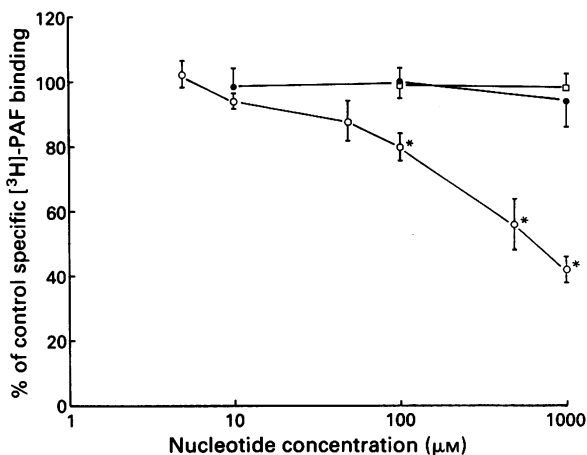
Figure 4 Effects of Gpp NHp (○), ADP (□) and ATP (●) on specific [³H]-PAF (2 nM) binding to rabbit platelet membranes at 0°C. Data represent the mean (s.d. indicated by vertical bars) of 3 separate experiments performed in duplicate. * $P < 0.05$.
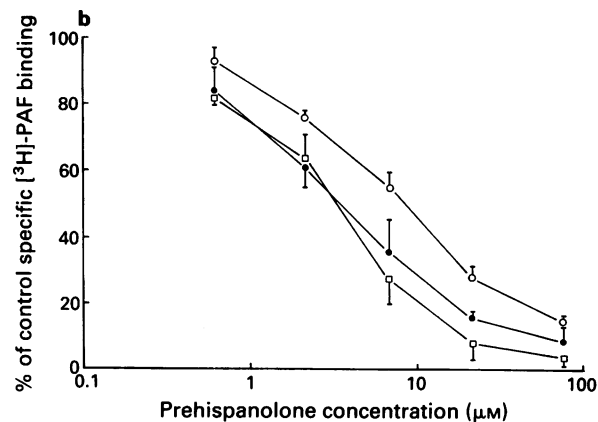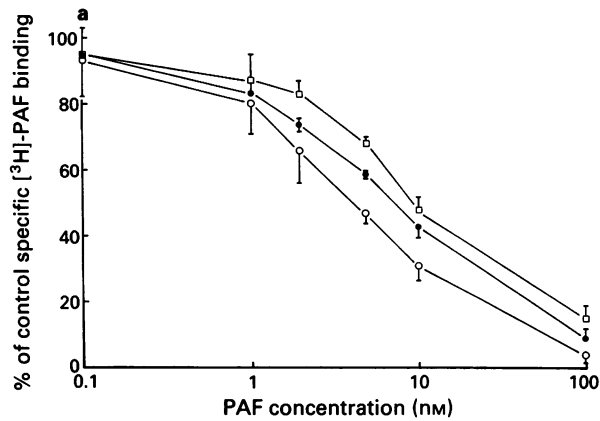
Figure 5 Effects of 5mM (●) and 10mM (□) NaCl on the inhibition of specific [³H]-PAF binding to rabbit platelet membranes by PAF (a) and prehispanolone (b); (○), control. Results are the mean (s.d. indicated by vertical bars) of (n) separate experiments performed in duplicate. In (a), control, n = 8; 5mM NaCl, n = 4; 10mM NaCl, n = 3. In (b), control, n = 4; 5mM NaCl, n = 3; 10mM NaCl, n = 3.

the other hand, the presence of 5 and 10mM NaCl decreased the inhibitory potency of PAF from 4.1nM (no NaCl) to 7.3 and 9.2nM respectively (Figure 5).

Inhibition of [³H]-PAF binding and PAF-induced aggregation of intact platelets by prehispanolone and its analogues

Prehispanolone (LC5504) inhibited specific [³H]-PAF (2nM) binding to intact rabbit platelets with an IC_{50} of 14.1 ± 7.9μM (mean ± s.d., n = 4, Table 1). It also inhibited aggregation induced by 2nM PAF in a concentration-dependent manner, with an IC_{50} of 28.4 ± 7.3μM (mean ± s.d., n = 3, Table 1, Figure 6). As shown in Figure 7, prehispanolone concentration-dependently inhibited both the shape change and platelet aggregation induced by 2nM PAF. These effects were specific since prehispanolone and 14,15-dihydroprehispanolone (50μM) did not inhibit the aggregation induced by thrombin (0.1iuml⁻¹), ADP (1μM) and collagen (12.5μgml⁻¹) (Table 2). Prehispanolone (50μM) also had little or no effect on [³H]-nitrendipine binding to rat cerebral cortex calcium channels, [³H]-flunitrazepam binding to bovine cerebral cortex central benzodiazepine receptors, [³H]-NECA binding to rat striatum A_2 adenosine receptors, [³H]-Ro5-4864 binding to rat liver peripheral benzodiazepine receptors, [³H]-phenylisopropyladenosine binding to bovine

Table 1 Inhibition of specific [³H]-PAF binding to intact rabbit platelets and PAF-induced aggregation by prehispanolone (LC5504) and its derivatives

	IC_{50} (μM)	
Inhibitors	[³H]-PAF binding	Aggregation
BN52021	4.8 ± 1.6 (n = 4)	3.3 ± 1.2 (n = 3)
LC5504	14.1 ± 7.9 (n = 4)	28.4 ± 7.3 (n = 3)
LC5505	Inactive*	Inactive*
LC5506	13.4 ± 7.4 (n = 4)	19.0 ± 7.9 (n = 3)
LC5507	1.2 ± 0.7 (n = 4)	4.6 ± 1.9 (n = 3)
LC5508	14.2 ± 7.7 (n = 4)	59.7 ± 1.2 (n = 3)
LC5509	5.7 ± 2.7 (n = 4)	11.4 ± 2.1 (n = 3)

IC_{50} is the concentration of drug required to give 50% inhibition of specific [³H]-PAF binding or platelet aggregation induced by 2nM PAF. Results are the mean ± s.d. of (n) separate determinations performed in duplicate. *Inactive when tested at 20–100μM.

cerebral cortex A_1 adenosine receptors and [³H]-nitrobenzylthioinosine binding to guinea-pig lung nucleoside transporter (Table 3).

In order to understand the key structural elements involved in the interaction of prehispanolone with the PAF receptor,
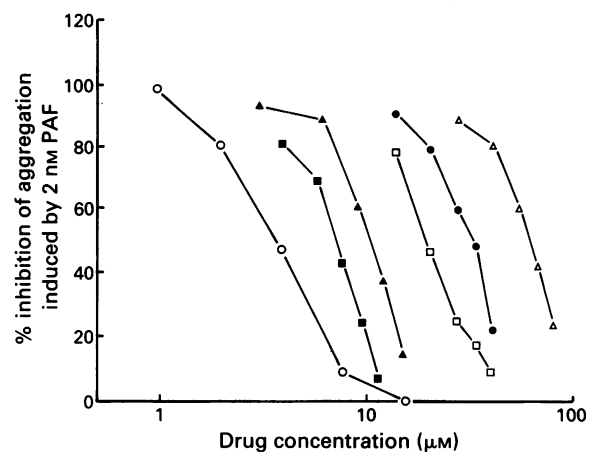

Figure 6 Inhibition of rabbit platelet aggregation induced by 2nM PAF by BN52021 (○), prehispanolone (●), LC5506 (□), LC5507 (■), LC5508 (△) and LC5509 (▲). Data are those of a typical experiment. Similar results were obtained in 3 separate experiments.
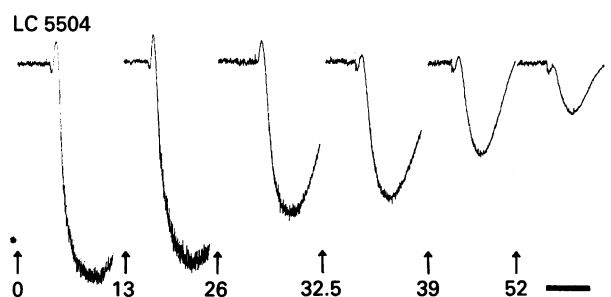

Figure 7 A typical tracing to illustrate the inhibitory effect of prehispanolone (LC5504) on rabbit platelet aggregation induced by 2nM PAF. LC5504 was added at the arrows. Numerals indicate the concentrations of prehispanolone in μM. Bar indicates 1 min.

Table 2 Comparison of the effects of prehispanolone (LC5504) and 14,15-dihydroprehispanolone (LC5506) on rabbit platelet aggregation induced by thrombin, collagen, ADP and PAF

| | Platelet aggregation (%) | | |
Aggregating agents	Control	LC5504	LC5506
Thrombin $(0.1 \text{ i u ml}^{-1})$	57.7 ± 1.2	58.0 ± 2.6	57.7 ± 0.58
Collagen $(12.5 \mu\text{g ml}^{-1})$	86.8 ± 0.23	87.0 ± 2.6	87.0 ± 3.6
ADP $(1 \mu\text{M})$	62.7 ± 1.2	63.0 ± 1.0	62.7 ± 0.58
PAF (2 nM)	64.3 ± 2.7	$30.3 \pm 12.5^*$	$21.0 \pm 11.5^*$

Results are the mean $\pm$ s.d. of 3 separate experiments. LC5504 and LC5506 were tested at $50 \mu\text{M}$ for thrombin, collagen and ADP but at $30 \mu\text{M}$ for PAF. $* P < 0.05$ when compared with control by Student's paired t test.

we have prepared several derivatives from it and studied their structure activity relationship. The chemical structures of these compounds are shown in Figure 1. The ability of these compounds and BN 52021, a classical PAF receptor antagonist, to inhibit [^{3}H]-PAF binding and PAF-induced aggregation in intact rabbit platelets are shown in Table 1. Opening up the tetrahydrofuran ring in LC5504 by acid treatment resulted in LC5505 which is inactive in these assays even when tested at $100 \mu\text{M}$. Hydrogenation of the dihydrofuran ring of LC5504, on the other hand, did not alter its bioactivity (compare LC5504 and LC5506). The replacement of the keto group in LC5504 by a hydrogen was similarly without significant effect (compare LC5504 and LC5508). Activity of LC5504 was actually improved when its keto group was replaced with a methoxy (LC5509) or a hydroxyl (LC5507) group. The hydroxy analogue (LC5507) was the most potent compound in this series and its activity was comparable to that of BN52021 (Table 1). The rank order of potencies of BN52021, prehispanolone and its analogues in inhibiting specific [^{3}H]-PAF binding to rabbit platelets was LC5507 > BN52021 > LC5509 > LC5506 $\geqslant$ LC5504 $\geqslant$ LC5508 $\gg$ LC5505. This correlated significantly with their rank order of potencies in inhibiting PAF-induced platelet aggregation, BN52021 $\geqslant$ LC5507 > LC5509 > LC5506 > LC5504 > LC5508 $\gg$ LC5505 ($r = 0.77$, $P < 0.01$). The positive correlation of the ability of these drugs in inhibiting [^{3}H]-PAF binding and PAF-induced aggregation supports the notion that they may block PAF-induced aggregation by inhibiting PAF binding to its receptors.

Discussion

Sodium ions and guanyl nucleotides have been shown to regulate differentially the receptor binding of agonists and antagonists at G-protein coupled receptors including the opiate receptor, α-adrenoceptor, bradykinin, histamine H_1 and adenosine A_1 receptors (Snyder, 1979; 1983). Typically, the presence of sodium ions or guanyl nucleotides decreases the

affinity of agonists while having little or no effect on the affinity of antagonists. Similar observations have been made for the PAF receptor where the presence of sodium ions lowered agonist affinity but increased or had no effect on antagonist affinity (Hwang et al., 1986a,b). In the present study, we have confirmed the inhibitory effect of sodium ions and guanyl nucleotide on specific binding of [^{3}H]-PAF to rabbit platelet membranes. Moreover, the inhibitory potency of PAF was lower in the presence of sodium ions indicating a decrease in its affinity for the PAF receptor. On the other hand, the inhibitory potency of prehispanolone was actually higher in the presence of sodium ions. This behaviour of prehispanolone resembles that of several PAF antagonists including CV3988, Ono-6240, BN52021, L-651,142, alprazolam, kadsurenone and dihydrokadsurenone but differs from RP48740 and RP52770 which are insensitive to the presence of sodium ions (Hwang et al., 1986a,b). Indeed, prehispanolone as predicted by its positive sodium shift in the radioligand binding assay, behaved as a PAF receptor antagonist and inhibited platelet aggregation induced by PAF. This effect was specific as it did not inhibit the aggregation of platelets induced by thrombin, ADP and collagen. Prehispanolone, up to $50 \mu\text{M}$, displayed no interaction at the dihydropyridine-sensitive calcium channel and central benzodiazepine receptor. This distinguished it from several other PAF receptor antagonists which are calcium channel blockers (e.g. gallopamil and diltiazem) and benzodiazepine receptor ligands (e.g. alprazolam and triazolam) (Braquet et al., 1987).

All of the natural PAF receptor antagonists which have been identified from plants to date are furanoid compounds. For instance, the ginkgolides (e.g. BN52021) from Ginkgo biloba contain a tetrahydrofuran cycle; kadsurenone, kadsurin A and B and piperenone from Piper futokadsurae as well as mirandine, burchellein and chrysophyllin from Brazilian Lauraceas are benzofuranoid compounds; veraguensin and calopiptin from Magnolia acuminata, galbelgin and galgravin from Himantandra belgravena, grandisin from Litsea grandis and nectandrin A and B from Nectandra rigida are 2,3,4,5-tetra-substituted furanoid lignans; burseran from Bursera microphylla as well as presteganes A and B and other butanolides

Table 3 Effects of $50 \mu\text{M}$ prehispanolone in various radioligand binding assays

Receptor type	^{3}H-ligand	Radioligand concentration (nM)	Binding (% of control)
Dihydropyridine-sensitive calcium channel	[^{3}H]-nitrendipine	0.3	89 ± 8 (3)
Central benzodiazepine	[^{3}H]-flunitrazepam	1	102 ± 5 (4)
Peripheral benzodiazepine	[^{3}H]-Ro 5-4864	1	98 ± 4 (4)
Adenosine A_1	[^{3}H]-phenylisopropyladenosine	1	92 ± 7 (3)
Adenosine A_2	[^{3}H]-N-ethylcarboxamidoadenosine	20	100 ± 4 (3)
Nucleoside transporter	[^{3}H]-nitrobenzylthioinosine	0.5	90 ± 7 (3)

Values presented are the mean $\pm$ s.d. of (n) separate experiments performed in duplicate. Nonspecific binding was defined as follows: [^{3}H]-nitrendipine, $1 \mu\text{M}$ nifedipine; [^{3}H]-flunitrazepam, $2 \mu\text{M}$ diazepam; [^{3}H]-Ro 5-4864, $1 \mu\text{M}$ Ro 5-4864; [^{3}H]-phenylisopropyladenosine, $10 \mu\text{M}$ cyclohexyladenosine; [^{3}H]-ethylcarboxamido-adenosine, $100 \mu\text{M}$ N-ethylcarboxamidoadenosine; [^{3}H]-nitrobenzyl-thioinosine, $20 \mu\text{M}$ nitrobenzylthioguanosine.

from *Steganotaenia araliacea* are 3,4-disubstituted furanoid lignans; liroresinol-B dimethyl ether, magnolin, pinoresinol dimethyl ether, fargesin, demethoxyaschantin and aschantin from *Magnolia biondi* are substituted furofurans (Braquet *et al.*, 1987; Cordeiro *et al.*, 1989). Prehispanolone from *Leonurus heterophyllus* is no exception as it also contains a tetra-hydrofuran ring. In the present study, we have also demonstrated the importance of the structural integrity of the natural tetrahydrofuran framework in its interaction with the PAF receptor. Opening up the tetrahydrofuran ring of prehispanolone by mild acid treatment resulted in a loss of PAF receptor antagonist activity as determined by radioligand binding and functional assays.

Prehispanolone (LC5504) was not stable in acidic conditions and can be converted into the inactive hispanolone (LC5505) readily by dilute hydrochloric acid treatment (Hon *et al.*, 1990a). In an attempt to stabilize prehispanolone, we converted it into 14,15-dihydroprehispanolone (LC5506) by hydrogenation. Indeed, 14,15-dihydroprehispanolone was stable and did not undergo the same rearrangement reaction

as prehispanolone in acidic conditions. Moreover it displayed more or less the same antagonistic activity at the PAF receptor as prehispanolone.

The keto group appears to be non-essential since its replacement by a hydrogen (LC5508) or a methoxy group (LC5509) had a minimal effect on the activity of 14,15-dihydroprehispanolone. On the other hand, the replacement of the keto group by a hydroxyl group (LC5507) actually increased its potency as a PAF receptor antagonist.

In conclusion, we have provided radioligand binding and biological data to support the role of prehispanolone as a specific PAF receptor antagonist. Moreover, by hydrogenating the dihydrofuran ring and replacing the keto group of prehispanolone with a hydroxyl group we obtained a compound LC5507 which is more stable and more active than prehispanolone as a PAF receptor antagonist.

References

BENVENISTE, J., HENSON, P.M. & COCHRANE, C.G. (1972). Leukocyte-dependent histamine release from rabbit platelets: the role of IgE, basophils and a platelet activating factor. *J. Exp. Med.*, **136**, 1356–1377.

BENVENISTE, J., TENCE, M., VARENNE, P., BIDAULT, J., BOULLET, C. & POLONSKY, J. (1979). Semi-synthesis and proposed structure of platelet-activating factor (PAF): PAF-acether, an alkyl ether analog of lysophosphatidylcholine. *C.R. Acad. Sci.*, **289D**, 1037–1040.

BLANK, M.L., SNYDER, F., BYERS, L.W., BROOKS, B. & MUIRHEAD, E.E. (1979). Antihypertensive activity of an alkyl ether analog of phosphatidylcholine. *Biochem. Biophys. Res. Commun.*, **90**, 1194–1200.

BRAQUET, P., PAUBERT-BRAQUET, M., KOLTAI, M., BOURGAIN, R., BUSSOLINO, F. & HOSFORD, D. (1989). Is there a case for PAF antagonists in the treatment of ischemic states? *Trends Pharmacol. Sci.*, **10**, 23–30.

BRAQUET, P., TOUQUI, L., SHEN, T.Y. & VARGAFTIG, B.B. (1987). Perspectives in platelet-activating factor research. *Pharmacol. Rev.*, **39**, 97–145.

CHANG, H.M. & BUT, P.P.H. (1987). *Pharmacology and Applications of Chinese Materia Medica*, **2**, 989–993.

CORDEIRO, R.S.B., AMORIM, C.Z., MARTINS, M.A., RODRIGUES E SILVA, P.M. & HENRIQUES, M.G.M.O. (1989). PAF antagonists from plants. *Drug News & Perspect.*, **2**, 287–291.

DEMOPOULOS, C.A., PINCKARD, R.N. & HANAHAN, D.J. (1979). Platelet activating factor: evidence for 1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine as the active compound (a new class of lipid chemical mediators). *J. Biol. Chem.*, **254**, 9355–9358.

FEUERSTEIN, G. & HALLENBECK, J.M. (1987). Prostaglandins, leukotrienes and platelet activating factor in shock. *Annu. Rev. Pharmacol. Toxicol.*, **27**, 301–313.

GRANDEL, K.E. (1987). Platelet activating factor. *Drugs Today*, **23**, 257–268.

HON, P.M., LEE, C.M., SHANG, H.S., CUI, Y.X., WONG, H.N.C. & CHANG, H.M. (1990a). Prehispanolone, a new labdane diterpene from *Leonurus heterophyllus*. *Phytochemistry*, (in press).

HON, P.M., LEE, C.M., CHOANG, T.F., CHUI, K.Y. & WONG, H.N.C. (1990b). Structure of a new ligustilide dimer from *Angelica sinensis*. *Phytochemistry*, **29**, 1189–1191.

HWANG, S.B., LEE, C.S.C., CHEAH. M.J. & SHEN, T.Y. (1983). Specific receptor sites for 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine (platelet activating factor) on rabbit platelet and guinea pig smooth muscle membranes. *Biochemistry*, **22**, 4756–4763.

HWANG, S.B., LAM, M.H. & SHEN, T.Y. (1985). Specific binding sites for platelet activating factor in human lung tissues. *Biochem. Biophys. Res. Commun.*, **128**, 972–979.

HWANG, S.B., LAM, M.Y. & PONG, S.S. (1986a). Ionic and GTP regulation of binding of platelet activating factor to receptors and platelet activating factor induced activation of GTPase in rabbit platelet membranes. *J. Biol. Chem.*, **261**, 532–537.

HWANG, S.B., LAM, M.H. & CHANG, M.N. (1986b). Specific binding of

[³H]Dihydrokadsurenone to rabbit platelet membranes and its inhibition by the receptor agonists and antagonists of platelet activating factor. *J. Biol. Chem.*, **261**, 13720–13726.

INARREA, P., GOMEZ-CAMBRONERO, J., NIETO, M. & SANCHEZ CRESPO, M. (1984). Characteristics of the binding of platelet activating factor to platelets of different animal species. *Eur. J. Pharmacol.*, **105**, 309–315.

SAUNDERS, R.N. & HANDLEY, D.A. (1987). Platelet activating factor antagonists. *Annu. Rev. Pharmacol. Toxicol.*, **27**, 237–255.

SHI, M.M., WU, J.S., LEE, C.M. & YOUNG, J.D. (1984). Nucleoside transport. Photoaffinity labelling of high affinity nitrobenzylthioinosine binding sites in rat and guinea pig lung. *Biochem. Biophys. Res. Commun.*, **119**, 594–600.

SNYDER, F. (1987). *Platelet Activating Factor and Related Lipid Mediators*. New York: Plenum Press.

SNYDER, S.H. (1979). Peptide and neurotransmitter receptors in the brain: regulation by ions and guanyl nucleotides. In *Central Regulation of the Endocrine System*. ed. Fuxe, K., Hokfelt, T. & Luft, R., pp. 109–117. New York: Plenum Press.

SNYDER, S.H. (1983). Molecular aspects of neurotransmitter receptors: an overview. In *Handbook of Psychopharmacology*, Vol. 17, ed. Iversen, L., Iversen, S.D. & Snyder, S.H. pp. 1–12. New York: Plenum Press.

TAHRAOUI, L., FLOCH, A., MONDOT, S. & CAVERO, I. (1988). High affinity specific binding sites for tritiated platelet factor in canine platelet membranes: counterparts of platelet activating factor receptors mediating platelet aggregation. *Mol. Pharmacol.*, **34**, 145–151.

VALONE, F.H. (1988). Identification of platelet-activating factor receptors in P388D1 murine macrophages. *J. Immunol.*, **140**, 2389–2394.

VALONE, F.H., COLES, E., REINHOLD, V.R. & GOETZL, E.J. (1982). Specific binding of phospholipid platelet activating factor by human platelets. *J. Immunol.*, **129**, 1637–1641.

VALONE, F.H. & GOETZL, E.J. (1983). Specific binding by human polymorphonuclear leukocytes of the immunological mediator 1-O-hexadexyl/octadecyl-2-acetyl-sn-glycero-3-phosphorylcholine (AGEPC). *Immunology*, **48**, 141–149.

VARGAFTIG, B.B. & BRAQUET, P.G. (1987). PAF-acether today – relevence for acute experimental anaphylaxis. *Br. Med. Bull.*, **43**, 312–335.

WANG, Z.S., LI, D.W., XIA, W.J., YIN, H.Q., ZHU, L.Y., YE, J.H. & YU, Y. (1985). Observations of 100 cases of myocardial ischaemia treated with *Leonurus heterophyllus*. *J. Tradit. Chin. Med.*, **3**, 29–30.

WINSLOW, C.M. & LEE, M.L. (1987). *New Horizons in Platelet Activating Factor Research*. London: John Wiley & Sons Ltd.

WONG, D.T., BYMASTER, F.P., REID, L.R. & THRELKELD, P.G. (1983). Fluoxetine and two other serotonin uptake inhibitors without affinity for normal receptors. *Biochem. Pharmacol.*, **32**, 1287–1293.

ZOU, Q.Z., BI, R.G., LI, J.M., FENG, J.B., YU, A.M., CHAN, H.P. & ZHEN, M.X. (1989). Effect of Motherwort on blood hyperviscosity. *Am. J. Chin. Med.*, **17**, 65–70.