Anti-Inflammatory Effects of 4-Phenyl-3-butenoic Acid and 5-(Acetylamino)-4-oxo-6-phenyl-2-hexenoic Acid Methyl Ester, Potential Inhibitors of Neuropeptide Bioactivation

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ABSTRACT

Substance P (SP) and calcitonin gene-related peptide (CGRP) are well established mediators of inflammation. Therefore, inhibition of the biosynthesis of these neuropeptides is an attractive potential strategy for pharmacological intervention against a number of inflammatory diseases. The final step in the biosynthesis of SP and CGRP is the conversion of their glycine-extended precursors to the active amidated peptide, and this process is catalyzed by sequential action of the enzymes peptidylglycine α-monooxygenase (PAM) and peptidylamidoglycolate lyase. We have demonstrated previously that 4-phenyl-3-butenoic acid (PBA) is a PAM inhibitor, and we have also shown that in vivo inhibition of serum PAM by PBA correlates with this compound’s ability to inhibit carrageenan-induced edema in rats. Here we demonstrate the ability of PBA to inhibit all three phases of adjuvant-induced polyarthritis (AIP) in rats; this represents the first time that an amidation inhibitor has been shown to be active in a model of chronic inflammation. We recently introduced 5-(acetylamino)-4-oxo-6-phenyl-2-hexenoic acid (AOPHA) as one of a new series of mechanism-based amidation inhibitors. We now report for the first time that AOPHA and its methyl ester (AOPHA-Me) are active inhibitors of serum PAM in vivo, and we show that AOPHA-Me correspondingly inhibits carrageenan-induced edema in rats in a dose-dependent manner. Neither PBA nor AOPHA-Me exhibits significant cyclooxygenase (COX) inhibition in vitro; thus, the anti-inflammatory activities of PBA and AOPHA-Me are apparently not a consequence of COX inhibition. We discuss possible pharmacological mechanisms that may account for the activities of these new anti-inflammatory compounds.

A number of neuropeptides, such as calcitonin gene-related peptide (CGRP), neuropeptide Y, substance P (SP), and vasoactive intestinal polypeptide, are initially synthesized as glycine-extended precursors, which are then converted to the bioactive C-terminal amidated form. Amidation is a two-step process catalyzed by the sequential actions of peptidylglycine α-monooxygenase (PAM; EC 1.14.17.3) and peptidylamidoglycolate lyase (EC 4.3.2.5). The monooxygenase first catalyzes α-hydroxylation of the glycine-extended precursor, and the lyase then catalyzes conversion of this α-hydroxyglycine derivative to the C-terminally amidated peptide (Kataegis et al., 1990, 1991; Ping et al., 1992). In the case of SP and CGRP, their release from nerves, as well as inflammatory cells, has been shown to facilitate a number of inflammatory events, including increased vascular permeability, chemotaxis, and release of inflammatory mediators, such as cytokines, eicosanoids, and histamine (Pernow, 1983; Matucci-Cernic and Partsch, 1992; Haines et al., 1993; Holzer and Holzer-Petsche, 1997). Further evidence demonstrating the inflammatory capacity of SP was noted when SP receptor (neurokinin-1 receptor) knockout mice developed significantly less inflammation than controls after treatment with a phlogistic agent (Bozic et al., 1996).

Tissue levels of proinflammatory neuropeptides have been shown to change during the inflammatory process in animal models of acute and chronic inflammation as well as human...
1.2 of 5-acetylamino-4-oxo-6-phenyl-2-hexenoic acid (AOPHA) is amidation inhibitors known to date. Thus, the PAM-binding peptide moiety are the most potent irreversible that possess a C-terminal acrylate functionality linked to a human disease.

Furthermore, an increase in SP concentration is observed in ankle joints of AIP rats during the later stages of this disease (Ahmed et al., 1995). In humans, the level of SP in both plasma and synovial fluid was shown to be elevated in patients with rheumatic diseases (Menkes et al., 1993). Moreover, neurokinin-1 receptor mRNA was only expressed by synoviocytes from patients with rheumatoid arthritis compared with synoviocytes from nonrheumatic patients (Krause et al., 1995).

Recognizing the potential pharmacological benefit of inhibiting the synthesis of proinflammatory neuropeptides, we have been developing new classes of mechanism-based inhibitors and transition-state analogs targeted at the post-translational amidation process (Katopodis and May, 1990; Mounier et al., 1997; Moore and May, 1999; Feng et al., 2000).

4-Phenyl-3-butenoic acid (PBA) is an olefinic substrate analog that has been shown to act as an irreversible inhibitor of PAM (Bradbury et al., 1990; Katopodis and May, 1990). In rats dosed chronically with PBA, there was a correlation among serum PAM inactivation, decreased tissue levels of SP, and the reduction of acute inflammation induced by carrageenan injection into rat hindpaws (Ogonowski et al., 1997). In the studies reported herein, we demonstrate for the first time the ability of PBA to significantly inhibit inflammation in AIP, an animal model that closely resembles the human disease.

We have reported that a novel series of compounds (Fig. 1) that possess a C-terminal acrylate functionality linked to a PAM-binding peptide moiety are the most potent irreversible amidation inhibitors known to date. Thus, the $k_{\text{max}}/K_m$ value of 5-acetylamino-4-oxo-6-phenyl-2-hexenoic acid (AOPHA) is $1.2 \times 10^5$ M/min, which is a potency two orders of magnitude greater than the analogs lacking the PAM-binding moiety (Moore and May, 1999; Feng et al., 2000). Results of studies conducted with AOPHA-Me, reported herein, clearly demonstrate its ability not only to inhibit PAM activity endogenously but also to produce an extremely potent inhibitory effect on carrageenan edema. We also demonstrate here that the anti-inflammatory activities of PBA and AOPHA-Me are not a consequence of COX inhibition, and we discuss alternate pharmacological mechanisms that may account for the activities of this new class of anti-inflammatory compounds.

### Materials and Methods

#### Experimental Animals.
Adult male, Sprague-Dawley rats (175–225 g) were purchased from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) and allowed to acclimate for at least 5 days in appropriate caging before experimentation. Animals were kept in the animal facility at Mercer University (Atlanta, GA) and received food and water ad libitum. All experiments were approved by the Mercer University Institutional Animal Care and Use Committee.

#### Drugs and Reagents.
PBA and isothiocyanate were obtained from Aldrich Chemical Co. (Milwaukee, WI). Before use, PBA was purified by dissolution in boiling hexane and precipitation overnight at 4°C. For in vivo experiments, PBA was titrated with sodium hydroxide in saline and adjusted to pH 7.4 using dilute hydrochloric acid. TNP-d-Tyr-Val-Gly, AOPHA, and AOPHA-Me were synthesized as described previously (Katopodis and May, 1990; Mounier et al., 1997; Moore and May, 1999; Feng et al., 2000). AOPHA-Me was dissolved in 10% DMSO in ethanol before use. *Mycobacterium butyricum* was purchased from Difco (Kansas City, MO). Indomethacin, EDTA, formamide, type IV lambda carrageenan, dithiothreitol, lipo polysaccharide (from *Escherichia coli* serotype 0111:B4), and Evans blue were all purchased from Sigma Chemical Co. (St. Louis, MO). Capsaicin was also purchased from Sigma and dissolved in a vehicle of 10% ethanol and 10% Tween 80 in saline before using. Thioetheramide-PC was purchased from Cayman Chemical (Ann Arbor, MI). Bovine serum albumin (BSA Fract V) was obtained from Fisher Scientific (Pittsburgh, PA). Osmotic pumps (2L1) were obtained from Alza Corporation (Palo Alto, CA). Heparinized Vacutainers were obtained from Becton, Dickinson and Company (Franklin Lakes, NJ). Ketamine and xylazine were obtained from Fort Dodge Laboratories (Fort Dodge, IA). All other chemicals, reagents, and solvents used in these experiments were of analytical grade.

#### AIP.
AIP was induced in rats by subplantar injection of 0.1 ml of Freund’s complete adjuvant (FCA) (1 mg/ml *M. butyricum* in mineral oil) into the right hindpaw. Contralateral hindpaws and control animals received subplantar injections of mineral oil only. Changes in hindpaw volume were determined plethysmographically by displacement of mercury at various time points after induction.

#### Carrageenan Edema.
Carrageenan edema was induced in male Sprague-Dawley rats (150–175 g) by a subplantar injection of 0.05 ml of carrageenan (20 mg/ml in saline). Contralateral paws received subplantar injections of saline only. Changes in hindpaw volume were measured plethysmographically by mercury displacement at various time points after injections.

#### Assay of Serum PAM Activity following in Vivo Dosing with PBA, AOPHA, and AOPHA-Me.
Serum was collected from anesthetized animals by clipping the tail, collecting 0.5 ml of blood from the tail vein in a microcentrifuge tube, allowing the blood to clot at room temperature for 20 min, and centrifuging at 16,000g in a refrigerated centrifuge for 5 min. Serum was collected from the tubes and stored at −80°C. Serum PAM activity was determined using a method previously described (Katopodis et al., 1991; Ping et al., 1995; Feng et al., 2000). In brief, 50 μl of serum sample was incubated with 200 μl of assay mixture containing 50 μM tripeptide TNP-d-Tyr-Val-Gly (substrate), 40 μM copper sulfate, 8 mM ascorbate, and 1 mg/ml catalase in 100 mM MES buffer, pH 6.5. After incubation for 45 min in a 37°C water bath, the reaction was halted by adding 100 μl of assay mixture to 10 μl of 3 M HClO₄. This

![Fig. 1. Chemical structures of PBA (1), AOPHA (2), and AOPHA-Me (3).](image-url)
solution was then centrifuged at 14,000g for 20 min. Twenty microliters was then removed and assayed for the α-hydroxyglycine and amidated products using reverse-phase high-performance liquid chromatography at 344 nm on a C8 column with a mobile phase of 56% water, 0.1% trifluoroacetic acid, and 44% acetonitrile at a flow rate of 1.5 ml/min. Both the amidated product and the glycine-extended intermediate were quantified simultaneously. PAM activity was expressed as milliunits per milliliter, which is the amount of enzyme required to produce a 1-nmol product/min.

Extraction and Measurement of SP from Rat Sciatic Nerves. Osmotic pumps slowly administered PBA (60 mg/kg/h) to animals for 7 days before the extraction of SP from sciatic nerves. AOPHA-Me (150 mg/kg) was administered to animals by s.c. injection 3 h before SP extraction from sciatic nerves. Capsaicin (50 mg/kg) was administered to animals by s.c. injection 24 h before SP extraction from sciatic nerves. SP was extracted from sciatic nerves using the method of Donnerer et al. (1996). In brief, animals were euthanized with CO2, and both sciatic nerves were excised and weighed. Each nerve was placed immediately in a microcentrifuge tube containing 500 μl of extraction solution (2 M acetic acid, 1 mM EDTA, 1 mM dithiothreitol, and 10 mM HCl). Nerves were boiled for 10 min, homogenized with a porcelain pestle at 400 rpm for 30 s, and centrifuged at 16,000 g for 5 min in a refrigerated centrifuge. The resulting supernatants were stored at −80°C until the concentration of SP was determined using a commercially available ELISA kit (Cayman Chemical; catalog no. 583751).

Assay for Inhibition of COX-1 Enzyme Activity in Human Whole Blood. COX-1 activity was determined by measuring the production of thromboxane B2, which occurs during the spontaneous clotting of human whole blood (Brideau et al., 1996). In brief, human venous blood was collected in Vacutainers without anticoagulant from nonfasted healthy male volunteers who had not taken any aspirin or nonsteroidal anti-inflammatory drugs for 14 days. Aliquots of blood (500 μl) were immediately transferred to glass tubes containing 2 μl of inhibitor or vehicle (DMSO). Tubes were then vortexed and incubated for 1 h at 37°C to allow clot formation. After incubation, tubes were centrifuged, and 100 μl of serum was mixed with 400 μl of methanol for protein precipitation. The supernatants were used to assay for thromboxane B2 using a commercially available ELISA kit (catalog number EA 25; Oxford Biomedical, Oxford, MI).

Assay for Inhibition of COX-2 Enzyme Activity in Human Whole Blood. COX-2 activity was determined by measuring the formation of PGE2, which occurs after incubation of blood samples for 24 h with lipopolysaccharide (L-2630 from E. coli serotype 0111:B4; Sigma Chemical) according to a method described previously (Brideau et al., 1996). In brief, fresh human venous blood from nonfasted healthy male volunteers who had taken aspirin for 2 days before (81 mg daily) was centrifuged at 16,000 g for 10 min, homogenized with a porcelain pestle at 400 rpm for 30 s, and centrifuged at 16,000 g for 5 min in a refrigerated centrifuge. The resulting supernatants were stored at −80°C until the concentration of SP was determined using a commercially available ELISA kit (Cayman Chemical; catalog no. 583751).

Assay for the Inhibition of Human Secretory Phospholipase A2 (sPLA2) Enzyme Activity. Inhibition of human secretory phospholipase A2 (sPLA2) (type V) enzyme activity was measured using a colorimetric sPLA2 (type V) inhibitor screening assay (catalog no. 10004883; Cayman Chemical).

Statistical Analysis. Data are presented as the mean ± S.E.M. Significance was determined using the Student’s t test to compare the means between two groups. One-way analysis of variance was performed to test for significance among repeated measures. Comparison of means was performed using Tukey’s post hoc test. A probability of p < 0.05 was considered statistically significant.

Results

Anti-Inflammatory Effect of PBA in Adjuvant-Induced Polyarthritis. We have shown previously that PBA possesses anti-inflammatory activity in carrageenan edema, a model of acute inflammation. Here, we evaluated this compound for anti-inflammatory activity in AIP, a model of chronic inflammation. AIP is a three-phase, cell-mediated immune response that develops over several weeks in response to a hindpaw injection of FCA (Szekanez et al., 2000; Waksman, 2002). In these experiments, PBA (60 mg/kg/h) was administered continuously from surgically implanted osmotic pumps for 7 days during each of the three phases of the disease through day 2 (recognition phase), days 2 through 9 (proliferative phase), and days 10 through 16 (effector phase). Control animals and untreated arthritic animals were sham-treated.

The administration of PBA during the recognition phase produced a significant reduction in the acute inflammation of the injected hindpaw on day 2 compared with untreated arthritic controls (Fig. 2a). Furthermore, the injected hindpaw of PBA-treated animals remained significantly less swollen than arthritic control animals through day 9, 7 days after discontinuation of drug administration. However, by day 14, the inhibitory effect produced by PBA during the early phase of the disease was no longer present. There was no significant difference between the uninjected contralateral hindpaws of the PBA-treated and arthritic control animals at any time point.

After the recognition phase, the acute inflammatory response subsided for 7 to 10 days, characteristic of the proliferative phase of AIP. During this period, the FCA-injected hindpaws remained significantly larger compared with nonarthritic controls (Fig. 2b). The administration of PBA during the proliferative phase produced a significant decrease in the acute inflammation that had developed in the FCA-injected hindpaw during the recognition phase. In addition, the anti-inflammatory effect of PBA produced during this phase persisted through the effector phase (day 16) of this disease because the swelling of the FCA-injected hindpaw during this late phase was significantly less in PBA-treated animals compared with untreated arthritic controls. Interestingly, a significant decrease in swelling of the contralateral hindpaws was observed in PBA-treated animals on day 16.

After day 10, the effector phase of AIP begins with swelling in both the FCA-injected and noninjected hindpaws resulting in pain, inflammation, and, eventually, joint destruction (Walz et al., 1971). When administered during this time, PBA produced a significant decrease in the swelling of both hindpaws as early as 3 days after initiating dosing (Fig. 2c).

Anti-Inflammatory Effect of AOPHA-Me in Carrageenan-Induced Edema in the Rat. We evaluated the ability of AOPHA-Me to inhibit carrageenan-induced edema in the rat. Remarkably, a single s.c. dose of AOPHA-Me (150 mg/kg) afforded practically 100% inhibition of hindpaw edema for the entire 6 h postcarrageenan injection (Fig. 3). Furthermore, this response by AOPHA-Me was dose-dependent (10–150 mg/kg) with all doses significantly inhibiting carrageenan edema 1 h postcarrageenan injection. Of the evaluated doses, 50 mg/kg was the lowest dose capable of significantly inhibiting hindpaw edema for the entire 6 h postcarrageenan injection.
Inhibitory Effects of PBA on Serum PAM Activity in Vivo. To demonstrate whether the anti-inflammatory effects of PBA observed in AIP were related to inhibition of PAM activity, three different doses of PBA (25, 50, and 75 mg/kg/h) were administered continuously to normal animals by surgically implanted osmotic pumps for 7 days, whereas control animals were sham-treated. Adjuvant arthritis was induced on day 0 as described under Materials and Methods. Volumes of injected and contralateral paws were measured at various time points through day 16. Change in paw volume was calculated as the difference between the volumes of arthritic control or PBA-treated hindpaws and volumes of nonarthritic control hindpaws. Bar, period of administration during each phase; ▫, arthritic injected hindpaw; ○, PBA-treated injected hindpaw; ■, arthritic contralateral hindpaw; □, PBA-treated contralateral hindpaw. Data are presented as the mean ± S.E.M. for each group (n = 6). *, statistically significant (p < 0.05) compared with arthritic controls (Student's t test).

Inhibitory Effects of AOPHA and AOPHA-Me on Serum PAM Activity in Vivo. The inhibitory effects of AOPHA and AOPHA-Me were also evaluated for their effects on serum PAM activity. After a single s.c. dose, AOPHA-Me was able to sustain the inhibition of serum PAM activity to a greater extent than AOPHA in vivo (Fig. 5). In this experiment, AOPHA-Me (100 mg/kg s.c.) maintained approximately 38% inhibition of PAM activity for 3 h postinjection with significant inhibition (~22%) still present 6 h after drug administration.

Extraction and Measurement of SP Levels from Rat Sciatic Nerves. Experiments were conducted to determine whether both the doses of PBA and AOPHA-Me, and the dosing regimens used to inhibit chronic inflammation in AIP and carrageenan edema in rats, respectively, were capable of inhibiting the endogenous synthesis of SP. Capsaicin was used to demonstrate that our method of SP extraction was capable of detecting changes in SP levels within the rat sciatic nerve. Levels of SP detected in vehicle and capsaicin treated animals were 143 ± 18 pg/mg tissue, respectively. Capsaicin significantly (p < 0.05) reduced levels of SP in rat sciatic nerves by 45%. However, neither PBA nor AOPHA-Me were found capable of significantly reducing endogenous levels of SP in the sciatic nerve compared with their respective controls at doses and dosing regimens shown previously to be effective in inhibiting inflammation (Figs. 2 and 3).

Inhibitory Effects of PBA and AOPHA-Me on Human Whole-Blood COX Activity in Vitro. Due to recent controversy regarding adverse cardiovascular events associated with the chronic inhibition of COX activity, we evaluated the ability of both PBA and AOPHA-Me to inhibit COX-mediated...
prostaglandin formation in vitro. Neither PBA nor AOPHA-Me (≤1 mM) produced any inhibitory effect on COX-1 activity in vitro. Furthermore, PBA (1 mM) and AOPHA-Me (0.5 mM) significantly (p < 0.05) inhibited COX-2 activity by 82 and 63%, respectively. The high concentrations required to significantly inhibit COX-2 enzyme activity suggest that PBA and AOPHA-Me are only weak inhibitors.

**Inhibitory Effects of AOPHA-Me on Human sPLA₂ Activity in Vitro.** sPLA₂ (type V) is another important cellular enzyme involved in the formation of prostaglandins. This enzyme has been shown to be involved in eicosanoid formation in inflammatory cells, such as macrophages and mast cells (Reddy et al., 1997; Han et al., 1998). Experiments were conducted in vitro to determine whether AOPHA-Me was capable of inhibiting the activity of this enzyme. Thioetheramide-PC was used as a positive control, because it is a structurally modified phospholipid that functions as a competitive, reversible inhibitor of sPLA₂ (Yu et al., 1990). AOPHA-Me produced a significant dose-dependent inhibition of human type V sPLA₂ activity (Fig. 6).

**Discussion**

Interfering with endogenous levels of SP and CGRP holds promising medicinal value, especially when considering the number of inflammatory cascades mediated by these proinflammatory neuropeptides (Pernow, 1983; Matucci-Cernic and Partsch, 1992; Haines et al., 1993; Holzer and Holzer-Petsche, 1997). There are continuing efforts to explore the therapeutic potential of novel compounds that interfere with the endogenous effects of these neuropeptides by either blocking the endogenous receptors for SP and CGRP or by causing a depletion of SP from sensory nerve endings, thus indirectly reducing its inflammatory activity (Appendino et al., 2005; Seto et al., 2005; Ambalavanar et al., 2006). In contrast, our approach for reducing inflammation caused by SP and CGRP focuses on interfering with the synthesis of these neuropeptides by inhibiting the activity of PAM, the rate-limiting enzyme required for their endogenous bioactivation.

We have demonstrated previously in vivo that continuous administration of the PAM inhibitor PBA inhibits serum PAM activity. Furthermore, we showed that PBA inhibits carrageenan edema, an acute model of inflammation associated with increased levels of SP, and that this anti-inflammatory effect correlated with decreased levels of endogenous SP in rat hindpaw tissue over the entire course of the inflammatory response (Ogonowski et al., 1997). Because SP has been shown to be involved during different stages in the pathogenesis of AIP (Donaldson et al., 1995; Walker, 2003), we reasoned that PBA should be effective in the treatment of this inflammatory disease.

Our results in Fig. 2 clearly demonstrate the anti-inflammatory effect of a PAM inhibitor when dosed during both the acute and chronic phases of AIP. To our knowledge, this is the first time that an inhibitor of PAM has been shown to exhibit anti-inflammatory activity in this model of inflammation. Binder et al. (1999) proposed that SP is involved in all three phases of AIP, taking a full 21 days for maximum levels to develop. This is consistent with our data in which we have demonstrated the anti-inflammatory effects of PBA when administered during all three phases of AIP (Fig. 2). Moreover, this activity correlated with decreased levels of endogenous SP, such as the release of interleukin-1, interleukin-6, tumor necrosis factor α, and other proinflammatory mediators from macrophages and CD4⁺ helper T cells, as well as the direct killing of antigen-containing target cells by CD8⁺ cytotoxic T cells (Szekanecz et al., 2000; Waksman, 2002). At present, it is not known whether PBA interferes with these other mechanisms.

Although PBA can be considered a first generation PAM inhibitor, we have recently developed a more potent series of amidation inhibitors that possess a C-terminal acrylate functionality linked to a PAM-binding peptide moiety (Moore and May, 1999; Feng et al., 2000). As shown in Fig. 5, we now report that two compounds of this series, AOPHA and AOPHA-Me, significantly inhibit serum PAM activity. Moreover, when AOPHA-Me was evaluated against carrageenan edema (Fig. 3), a well established model of acute inflammation, it produced a dose-dependent inhibition of hindpaw edema, with virtually complete inhibition at a dose of 150 mg/kg for up to 6 h after its administration. The duration of inhibition (6 h) of PAM enzyme activity in vivo by AOPHA-Me correlates well with its demonstrated anti-inflammatory capacity in carrageenan edema. Therefore, the acute anti-inflammatory response produced by AOPHA-Me is consistent with a mechanism that entails the inhibition of...
PAM, as we have previously suggested with the less potent inhibitor, PBA (Ogonowski et al., 1997). Although we attempted to evaluate AOPHA-Me in the adjuvant model of arthritis, we could not successfully administer this compound chronically using standard dosing procedures due to its lack of solubility in conventional vehicles. It is worth noting that we were unable to detect any significant changes in levels of SP excised from normal animals dosed with either PBA or AOPHA-Me. It is presently unknown whether similar results would be obtained in animals subjected to various inflammatory conditions.

The rapid onset of action of AOPHA-Me in carrageenan edema suggests that this compound may possess other pharmacological mechanisms, which could contribute to its anti-inflammatory effect independent of its ability to inhibit neuropetide bioactivation. Other inflammatory mediators, such as prostaglandins, have been shown to be increased in the inflamed hindpaws of rats with carrageenan edema (Pinheiro and Calixto, 2002). Recently, there has been controversy regarding adverse cardiovascular events associated with the therapeutic use of nonsteroidal anti-inflammatory agents, which inhibit prostaglandin production via COX. Therefore, it was of interest to determine whether PBA and AOPHA-Me would inhibit COX activity. Results from these studies showed that both PBA and AOPHA-Me did not significantly inhibit COX isozyme activity (IC_{50} > 10^{-4} M), suggesting that these compounds may provide a safer alternative over traditional nonsteroidal anti-inflammatory drugs in the management of inflammatory diseases. Another important enzyme, phospholipase A_{2}, is thought to initiate proinflammatory cascades by releasing arachidonic acid from membrane phospholipids, which can then be utilized by COX for the biosynthesis of eicosanoids such as prostaglandins (Murakami and Kudo, 2002). Although AOPHA-Me was found to not inhibit COX, it was shown to produce a significant inhibitory effect on sPLA_{2} activity in vitro (Fig. 6). This ability to inhibit sPLA_{2} activity may contribute, in part, to the acute anti-inflammatory effect produced by AOPHA-Me in carrageenan edema.

In summary, we have demonstrated the anti-inflammatory capacity of PBA, an irreversible PAM inhibitor, in AIP during both the acute and chronic stages of this disease. Furthermore, AOPHA-Me, a much more potent PAM inhibitor, inhibited carrageenan edema in a dose-dependent manner and completely inhibited this acute inflammatory response at the highest dose. However, we were unable to detect changes in levels of sciatic nerve SP in normal animals dosed with either PBA or AOPHA-Me, despite their abilities to inhibit serum PAM in vivo. Neither PBA nor AOPHA-Me exhibited significant inhibition of COX activity; AOPHA-Me did exhibit some inhibition of sPLA_{2} activity, which may contribute to its rapid anti-inflammatory effect in carrageenan edema. In view of recent controversies regarding adverse effects associated with anti-inflammatory agents that inhibit COX, further development of the anti-inflammatory agents described here and their derivatives are of obvious interest from a therapeutic perspective.

References


and CGRP, but not somatostatin content of innervating dorsal root ganglia in adjuvant monoaarthritis in the rat. **Neurosci Lett** **137**:257–260.


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