

FORUM

Eicosanoids in Insect Biology

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Eicosanóides na Biologia de Insetos

RESUMO - Prostaglandinas e eicosanóides relacionados são metabolitos oxigenados de certos ácidos graxos poli-insaturados C20. Eicosanóides são melhor entendidos em relação à sua significância clínica em medicina humana. Sugerimos um novo e mais amplo significado para os eicosanóides, o qual chamamos 'paradigma biológico'. Sob esse aspecto, notamos que eicosanóides desempenhavam funções como sinalizadores celulares antes da origem dos Metazoa. Durante a diversificação evolucionária dos animais, os eicosanóides têm sido envolvidos em uma série de funções biológicas, algumas das quais ocorrendo somente em insetos e em outros invertebrados. Essas ações múltiplas dos eicosanóides permitem o melhor entendimento dos fenômenos biológicos. Para desenvolver essas idéias nós revisamos os papéis dos eicosanóides em duas áreas da biologia de invertebrados: mediação das reações da imunidade de insetos a bactérias e interações hospedeiro-parasita. Numa visão ampla, os eicosanóides desempenham importantes funções na organização biológica ao nível das células, dos organismos e das interações ecológicas. Sugerimos que os questionamentos contínuos sobre a significância dos eicosanóides levarão a novos entendimentos na biologia dos insetos.

PALAVRAS-CHAVE: Imunidade de insetos, parasitologia, prostaglandinas.

ABSTRACT - Prostaglandins and related eicosanoids are oxygenated metabolites of certain C20 polyunsaturated fatty acids. Eicosanoids are best understood in the context of their clinical significance in human medicine. We suggest a new and broader view of eicosanoids, which we have been calling a 'biological paradigm'. Under this view, we note that eicosanoids were taken into roles as cellular signal moieties long before the origins of the Metazoa. During the evolutionary diversification of animals, eicosanoids have been recruited into an array of biological roles, some of which occur only in insects and other invertebrates. These multiple actions endow eicosanoids with unusual explanatory power in understanding biological phenomena. We review the roles of eicosanoids in two areas of invertebrate biology: mediation of insect immune reactions to bacteria and host-parasite interactions. Seen broadly, eicosanoids play important roles at the cellular, organismal and ecological levels of biological organization. We suggest that continued inquiry into the significance of eicosanoids will yield important new insights into insect biology.

KEY WORDS: Insect immunity, parasitology, prostaglandins.

Most of us are familiar with prostaglandins (PGs) because they mediate discomforts associated with inflammation and minor injury, and because the over-the-counter analgesics we ingest to relieve discomforts, such as aspirin, act by inhibiting PG biosynthesis. PGs make up one group of a larger class of compounds collectively known as eicosanoids. The term *eicosanoid* was originally coined by Corey and his colleagues (Corey *et al.* 1980) to refer to all oxygenated metabolites of certain C20 polyunsaturated fatty acids. There are three major groups of eicosanoids, including the PGs, the epoxyeicosatrienoic acids, and the many lipoxigenase

products. Specific metabolic pathways are responsible for biosynthesis of each group of eicosanoids (Fig. 1).

Reference to pharmaceutical analgesics is meant to highlight the most widely appreciated significance of eicosanoids: these compounds exert potent actions in human physiology and pathophysiology. Indeed, the very term PG reflects the origins of research on eicosanoids, which arose in studies of human reproductive biology. Von Euler (1936) first published the term PG because the substances responsible for stimulating uterine muscle contraction were associated with the prostate gland. Similarly, another group of

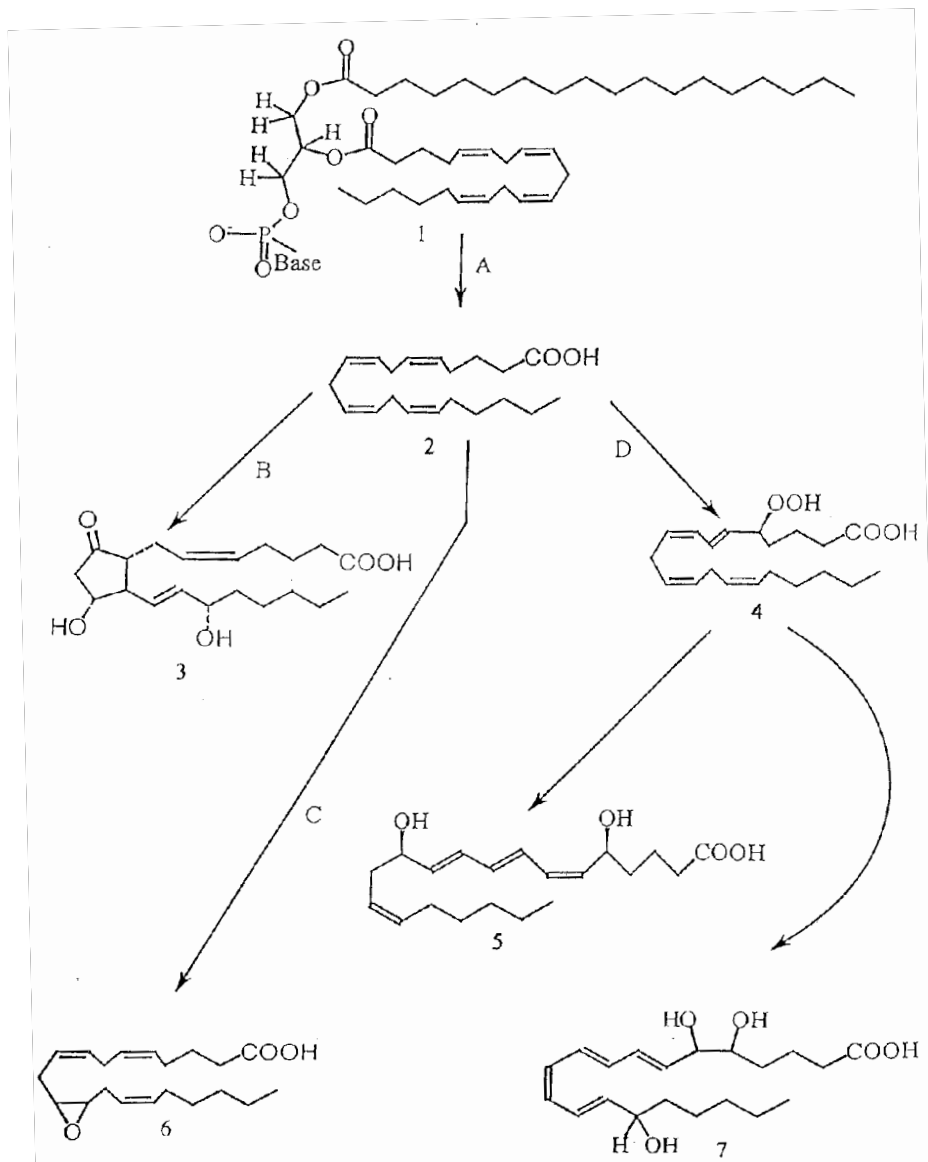


Figure 1. An overview of 20: 4n-6 metabolism as understood from the mammalian background. Three polyunsaturated fatty acids, 20: 3n-6, 20: 4n-6 and 20: 5n-3 are potential substrates for eicosanoid biosynthesis. Of these, metabolism of 20: 4n-6 is most well studied. Chemical structures are denoted by numerals. 1 = a cellular phospholipid. 2 = hydrolyzed 20: 4n-6. 3 = prostaglandin E₂. 4 = 5-hydroperoxyeicosatetraenoic acid. 5 = leukotriene B₄. 6 = 11,12-epoxyeicosatrienoic acid. 7 = lipoxin A. Capital letters indicate major enzyme systems responsible for eicosanoid biosynthesis. A = phospholipase A₂; B = cyclooxygenase and associated enzyme steps; C = cytochrome P₄₅₀ epoxygenase; D = lipoxygenase.

eicosanoids, the leukotrienes, were so named because they are trienoic acids produced in human leukocytes (Samuelsson 1983). The chemical structures of some PGs were first determined in the 1960's (Bergstrom *et al.* 1962), from which it was inferred that PGs must be biosynthesized by oxygenation of C20 polyunsaturated fatty acids, particularly arachidonic acid (20: 4n-6). In contemporary idiom, studies of eicosanoid biosynthesis are often called arachidonate metabolism. Recognition of the pathophysiological significance of eicosanoids emerged from a now-classic series of experiments by Robert Vane. Vane showed that aspirin inhibits PG biosynthesis in a dose-dependent way. Bergstrom, Samuelsson and Vane later shared a Nobel Prize in Medicine

or Physiology for their work on eicosanoids (Oates 1982). Again, these comments emphasize the human- or perhaps mammal-centered appreciation of the significance of eicosanoids.

The goal of this essay is to substantially broaden appreciation and interest in the biological significance of eicosanoids. In our view which we have been calling a 'biological paradigm', eicosanoids were biosynthesized and exerted regulatory actions in the earliest forms of animal life, those representing the unicellular phyla. By the time metazoan animals evolved, cells already had considerable experience in the synthesis and information content of eicosanoids. Recognizing that multicellular animals are far more complex

than their unicellular progenitors, animal evolution provided abundant opportunity for recruiting eicosanoids into many biological roles. Virtually all known eicosanoid actions involve some form of signaling. The key point is that eicosanoids can signal events within cells, between cells, and between organisms. Eicosanoids act at several levels of biological organization, and they exert varying degrees of influence from subtle so-called "housekeeping" actions in homeostatic physiology to crucial events in life cycles. While the traditional mammal-centered view would suggest a similar appreciation for mammals, the biological paradigm guides us in thinking that eicosanoids act in all animals, and in some cases between populations of animals.

The idea that eicosanoids occur in invertebrates surfaced in 1969 with the discovery of high levels of certain PGs in a gorgonian octacoral, *Plexaura homomalla*, (Weinheimer & Spraggins 1969). Methods for the chemical synthesis of PGs for research had not yet been invented, and the octacoral paper launched an intense search for other natural, and commercially useful, sources of PGs. Although none were found, the many ensuing reports inform us that PGs and other eicosanoids can be detected in virtually any animal taken under appropriate scrutiny.

The occurrence of PGs and other eicosanoids in invertebrates eventually begged the question of the biological significance of these compounds. What do these compounds do in invertebrates? Insects yielded the first insight into a biological role of eicosanoids in invertebrates. The late U.E. Brady suggested that certain PGs are partially responsible for inducing egg-laying behavior in newly-mated house crickets, *Acheta domesticus* (L.) (Destephano & Brady 1977). We now know that eicosanoids act in many areas of invertebrate biology, including reproduction, ion transport, and peptide hormone signal transduction (Stanley 2000 and reviews cited therein). In this essay we discuss recent advances in two other important areas of insect biology, namely immunity and in host-parasite relationships.

Eicosanoids in Insect Immunity

Whereas some of the earliest experiments in immunity were carried out with invertebrates (Rowley 1996), their lack of specific acquired, or adaptive, immunity shifted interest from invertebrate to vertebrate immune systems. Nonetheless, it remains that both invertebrates and vertebrates express generalized innate, and very effective, immune reactions to infection. In insects and many other invertebrates these reactions include immediate cellular reactions such as phagocytosis, nodule formation, and encapsulation (Gillespie *et al.* 1997, Strand & Pech 1995). Phagocytosis involves internalization of small particles, such as bacterial cells, by circulating hemocytes. Nodule formation clears large numbers of bacteria from hemolymph by entrapping the bacterial cells in microaggregates of hemocytes and bacterial cells. The microaggregates and their entrapped bacteria grow by attracting additional hemocytes and bacterial cells. They finally mature into nodules with a final layer of hemocytes, which are responsible for melanization of the nodules by activation of prophenoloxidase. Millions of circulating

hemocytes are typically invested in these clearing reactions. Larger foreign bodies, such as the eggs of parasitoid insects, are encapsulated by succeeding layers of hemocytes. These, too, are melanized by a final layer of hemocytes.

The melanization step is driven via activation of prophenoloxidase, which entrains a complex series of biochemical pathways. In larvae of *Drosophila melanogaster* (Meigen), the innate cellular immune response that results in the formation of melanotic capsules around intrahemocoelic wasps (parasitoids) is accompanied by elevated levels of nitric oxide, superoxide anion and hydrogen peroxide, molecules implicated along with certain reactive quinonoid intermediates of melanin in the killing process (Carton & Nappi 2001). Collectively, these host responses not only destroy the parasitoids, but also confine the cytotoxic activity within pigmented capsules, thereby protecting endogenous tissues from the biochemically hostile reactions directed against nonself entities. Invertebrates also express humoral reactions to infections which mostly involves induced biosynthesis of anti-bacterial proteins (Dunn 1986, Chernysh *et al.* 1996).

These observable immune reactions are well known, however, the signal transduction mechanisms responsible for initiating and coordinating these reactions are more obscure. We made the first inquiry into the biochemical signals that operate in invertebrate cellular immunity by investigating the roles of eicosanoids in clearing bacterial infections from hemolymph circulation in fifth stage tobacco hornworm, *Manduca sexta* L. We inferred that eicosanoids mediate cellular defense reactions to bacterial infections in insects and other invertebrates (Stanley-Samuelson *et al.* 1991). Nodulation is responsible for clearing the majority of invading bacterial cells from insect hemolymph circulation during the first 2h post-infection (Horohov & Dunn 1983), and we next hypothesized that nodulation is one of the cellular defense reactions mediated by eicosanoids (Miller *et al.* 1994). We tested this idea by treating experimental hornworms with an eicosanoid biosynthesis inhibitor, then infecting them with bacteria. Controls were treated with ethanol. At selected times post-infection, nodulation was assessed by counting the numbers of nodules. We recorded about 120 nodules/hornworm in ethanol-treated controls, and about 40 nodules/hornworm in experimental insects. The influences of the inhibitors were expressed in dose-dependent manners and could be reversed by treating experimental insects with arachidonic acid. Again, we inferred that eicosanoids mediate nodulation reactions to bacterial infections in insects (Miller *et al.* 1994).

Of course, it is difficult to extrapolate from findings based on a single moth species to the remainder of the class Insecta and from there to other invertebrate taxa. The protocols for assessing the roles of eicosanoids in nodulation reactions to bacterial infections are fairly straightforward (Miller & Stanley 1998a), and we applied these protocols to insect species representing several major orders. Results from these exercises support the view that eicosanoids mediate nodulation reactions to bacterial infections in other lepidopterans, including silkworms, *Bombyx mori* (L.) (Stanley-Samuelson *et al.* 1997), true armyworms, *Pseudaletia unipuncta* (Haworth) and black cutworms *Agrotis ipsilon* (Hufnagel) (Jurenka *et al.* 1997),

and larvae of the butterfly, *Colias eurytheme* L. (Stanley *et al.* 1999), as well as larvae of the tenebrionid beetle, *Zophobas atratus* (Fabricius) (Miller *et al.* 1996). These species are all members of the Endopterygota (the term preferred by Daly *et al.* [1998] over Holometabola). We also investigated the Exopterygota, including adults of the cricket, *Gryllus assimilis* (Fabricius) (Miller *et al.* 1999), adults of two species of 17-year periodical cicadas, *Magicicada septendecim* L. and *M. cassini* L. (Tunaz *et al.* 1999), and adult cockroaches *Periplaneta americana* (L.) (Tunaz & Stanley, 2000). The results of these experiments similarly support the idea that eicosanoids mediate microaggregation and nodulation reactions to bacterial infections.

Microaggregation and nodulation reactions are complex cellular activities, which entail a series of discrete steps, the exact number of which remains unknown. Work with waxmoths, *Galleria mellonella* L. showed that eicosanoids mediate two steps in the overall process (Mandato *et al.* 1997). One is cell spreading, which involves cytoskeleton rearrangements and the other is the final melanization step, which depends on activation of prophenyloxidase. In the same report, they noted that phagocytosis also depends on eicosanoids. Morishima *et al.* (1997) added a new level of information by investigating the possibility that expression of genes encoding anti-bacterial proteins also requires eicosanoid biosynthesis. Experimental larvae of the silkworm, *B. mori*, were injected with a pharmaceutical inhibitor of eicosanoid biosynthesis after which immune genes were stimulated by injecting soluble peptidoglycan prepared from the bacterium *Bacillus licheniformis*. After 1h incubation, total RNA was extracted from fat body and blotted onto membranes. The membranes were probed for the presence of mRNA encoding the antibacterial protein cecropin B and the antibacterial enzyme lysozyme. They found that expression of the genes for these two proteins was reduced by approximately 50% in experimental larvae. The authors also recorded similar results using *in vitro* preparations of isolated fat body. They interpreted their findings to suggest that eicosanoids are among the signaling moieties involved in expression of two genes encoding immune proteins.

While all results have so far bolstered the idea that eicosanoids mediate immune reactions in insects, and likely other invertebrates, the experimental protocols are attended by theoretical problems. For one, we assessed the possibility that eicosanoids are involved in immune reactions by treating the experimental insects with more or less selective pharmaceutical inhibitors of eicosanoid biosynthesis (Miller & Stanley 1998a). So far, the pharmacology of only one of these inhibitors has been studied in an invertebrate. Miller & Stanley (1998b) described the uptake, metabolism and elimination of indomethacin, a PG biosynthesis inhibitor, by tobacco hornworms. Whereas indomethacin is rapidly metabolized in mammals, most of the injected indomethacin recovered from hornworm tissues was intact. Indomethacin was metabolized into polar products after coming into contact with microbes in frass, from which we inferred that this pharmaceutical product is rapidly distributed among hornworm tissues, where it undergoes very little metabolism until it is cleared from the tissues and moved into the alimentary canal

for excretion. In biochemical experiments we also found that indomethacin inhibits PG biosynthesis by *in vitro* enzyme preparations (Stanley-Samuels & Ogg 1994). Hence, we proposed that indomethacin is an appropriate probe for PG biosynthesis in insects (Miller & Stanley 1998b).

Another theoretical issue is even more basic to assessing the roles of eicosanoids in insect biology. A base assumption is that the experimental organisms are competent to biosynthesize eicosanoids. We addressed this issue by documenting the presence of major elements of eicosanoid biosynthesis systems in insect immune tissues (Fig. 1). The first step in eicosanoid biosynthesis is thought to be the release of 20: 4n-6 from cellular phospholipids by action of a cytosolic phospholipase A₂ (cPLA₂). We characterized a cPLA₂ in tobacco hornworm fat body (Uscian & Stanley-Samuels 1993) and hemocytes (Schleusener & Stanley-Samuels 1996). These exercises revealed the intracellular cPLA₂s associated with these tissues are calcium-independent enzymes, which in mammals are thought to regulate eicosanoid biosynthesis (Dennis 1997, Balsinde *et al.* 1999).

We also characterized eicosanoid biosynthesis in the hornworm fat body (Stanley-Samuels & Ogg 1994) and hemocytes (Gadelhak *et al.* 1995), the main immunity-conferring tissues. PGA₂, the major fat body product, is thought to influence gene expression in mammalian systems (Negishi *et al.* 1995). Taken with the work of Morishima *et al.* (1997), the biosynthesis of PGA₂ in fat body suggests the intriguing possibility that PGA₂ regulates expression of genes encoding the antibacterial proteins cecropin D and lysozyme. Eicosanoid biosynthesis in hemocytes differs from the findings with fat body. The major hemocyte product was tentatively identified as the lipoxygenase product 15-hydroxyeicosatetraenoic acid (Gadelhak *et al.* 1995). These studies document the capability of tobacco hornworm immune tissues to biosynthesize eicosanoids.

While this work supports the eicosanoid hypothesis, the supporting arguments are circumstantial. First, treating insects with pharmaceutical inhibitors of eicosanoid biosynthesis impaired various immune reactions to bacterial infections. Second, the biochemical studies document the presence of eicosanoid biosynthetic systems. This reasoning does not make the crucial connection between a bacterial infection and a subsequent alteration in eicosanoid biosynthesis. We developed a new protocol to assess the influence of bacterial infections on eicosanoid biosynthesis (Jurenka *et al.* 1999). True armyworms, *P. unipuncta*, were injected with heat-killed bacteria, *Serratia marcescens*. After 30-minute incubations, hemolymph was collected and eicosanoids extracted. The extracts were cleaned up, then fluorescent derivatives were prepared by reacting the samples with 9-anthryldiazomethane. Individual components were separated on a high-pressure liquid chromatograph, and detected on a flow-through fluorescent detector. PGF_{2a} was the major hemolymph PG. Samples taken from saline-injected controls yielded 0.9 pg/ml hemolymph of PGF_{2a} compared to 4.0 ng/ml hemolymph for bacteria-injected experimental larvae. The fluorescent chromatograms also indicated increases in other eicosanoids, which remain to be identified. We concluded that increased amounts of PGs and other eicosanoids are biosynthesized

following bacterial infections. This conclusion strongly supports the eicosanoid hypothesis.

Another issue in the work with PGs is careful identification of the compounds. In the work just mentioned, we confirmed the identification of PGF_{2a} by analysis on gas chromatography-mass spectrometry (GC-MS). We similarly determined the structure of PGA_2 in our studies with true armyworm (Tunaz *et al.* 2001). PGs are present in very low amounts in insect tissues, which calls for careful attention to experimental details. The PGs must be derivatized before GC-MS analysis, and due to the charged nature of the compounds, the analytes are easily lost during the preparation procedures. We found, for example, that sample recovery after clean up on silica columns is enhanced by using acidified silica in the columns. A representative mass spectrum of PGE_2 from midgut tissue of the tobacco hornworm, *M. sexta*, is shown in Fig. 2.

One of our current efforts is directed toward identifying specific bacterial components, which elicit eicosanoid-mediated cellular defense reactions. Toward this end, we recently reported that the lipopolysaccharide (LPS) component of the bacterium *S. marcescens* stimulates microaggregation and nodulation reactions within minutes following injection into the hemocoels of larval beetles, *Z. atratus* (Bedick *et al.* 2000). More important to our work, eicosanoids mediate these reactions in response to a purified chemical. In vertebrates

and invertebrates LPS is recognized by interaction with specific binding proteins (Charalambidis *et al.* 1996).

We are now poised to investigate the immediate biochemical reactions that follow the interaction between cellular binding proteins and LPS. Our preliminary work reveals increased hemocyte cPLA_2 activity immediately following infection. As seen in Fig. 1, the cPLA_2 step is thought to be the first step in eicosanoid biosynthesis, and we recorded increased cPLA_2 activity within 15 seconds following infection. While it is clearly important, the mechanism of activating this enzyme has not yet been investigated.

Eicosanoids in Host-Parasite Relationships

Possibly one of the most profound - and least explored - roles of eicosanoids in animal biology relates to their actions in mediating certain host-parasite relationships. Relationships of this sort are very intimate and complex. Eicosanoids, as is true for many other chemicals, could potentially act in many ways. In this section we mention a few eicosanoid actions in parasitology, then give an overview of current work in our laboratory on an insect host-parasite relationship.

Leid & McConnell (1983a,b) investigated the possibility that an endoparasitic worm, the tapeworm *Taenia taeniaeformis* (Platyhelminthes), was competent to

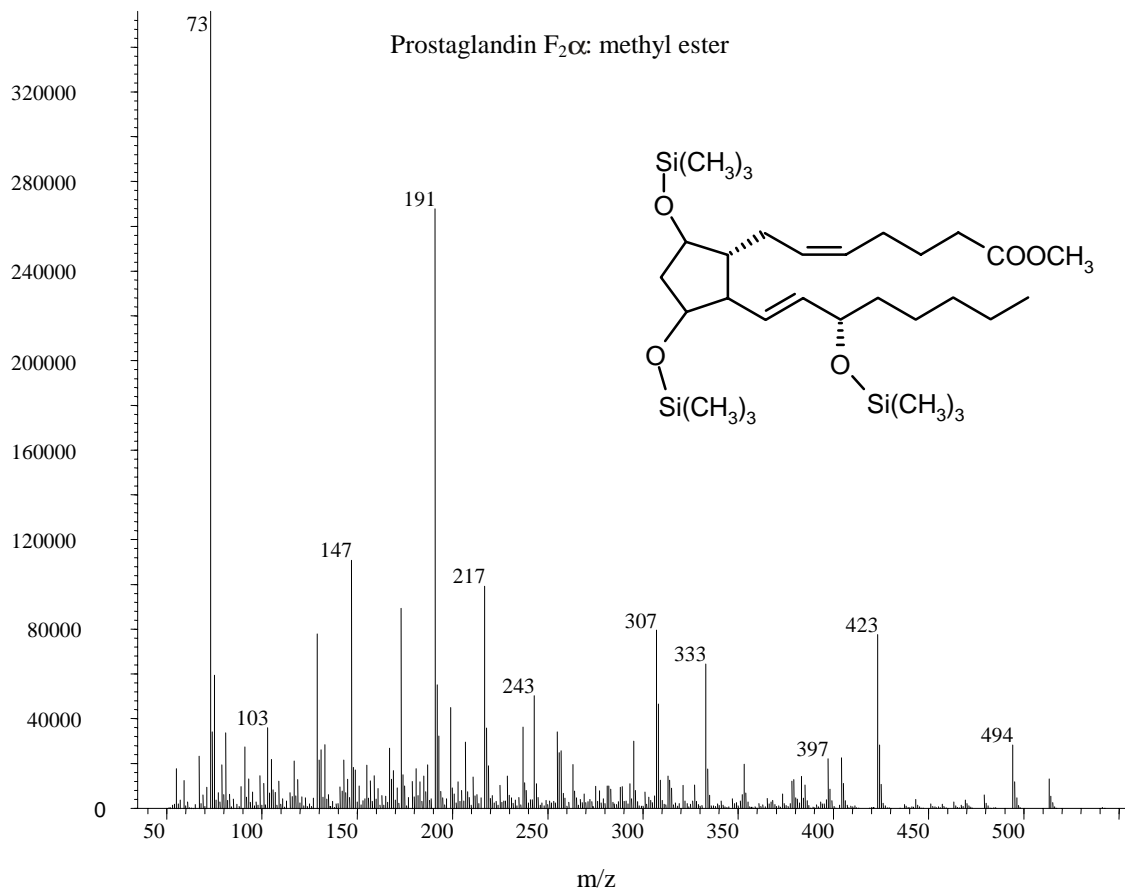


Figure 2. A total ion scan of the methyl ester trimethylsilyl derivative of PGF_{2a} prepared from midguts of the tobacco hornworm, *M. sexta*.

biosynthesize eicosanoids. They showed that the tapeworms produced and secreted three eicosanoids, thromboxane B₂ (a stable product of thromboxane A₂), PGE₂ and PGL₂. The importance of this work, however, lies in their hypothesis relative to the biological significance of eicosanoid biosynthesis in the worms. They suggested that the eicosanoids were released by the worms into host tissues, where they act to attenuate host cellular defense reactions to the presence of the worm (Leid & McConnell 1983a). The idea that parasites might manipulate host immunity by secreting eicosanoids into host tissues now echoes, albeit faintly, throughout the field of parasitology.

Subsequent workers came to similar conclusions with several species of endoparasites, including the cestodes (Platyhelminthes) *Spirometra erinacei* Fukushima *et al.* (1993) and *T. multiceps* (Rakha *et al.* 1996), the blood fluke *Schistosoma mansoni* (Trematoda; Fusco *et al.* 1993), three species of microfilariae (the nematodes responsible for lymphatic filariasis), *Wuchereria bancrofti*, *Brugia malayi* and *B. timori* (Liu & Weller 1992), and another nematode, *Oesophagostomum dentatum* (Dauguschies 1996). The idea takes on considerable urgency with the report from Kubata *et al.* (1998), showing that *Plasmodium falciparum*, the organism responsible for the most serious form of human malaria, secretes immunosuppressive PGs. Again, the authors speculated that the PGs secreted by the parasite act to manipulate host defense mechanisms.

The idea is not limited to endoparasitic invertebrates. Ticks are obligate ectoparasites which feed on vertebrate blood. Tick saliva is rich in PGs, and ticks secrete copious amounts of saliva into the wounds of their hosts. The idea that the PGs serve the ticks by attenuating host immune reactions to the presence of the ticks has been put forth very clearly by Ribeiro (1987) and Sauer *et al.* (1993).

Of course invertebrate parasites are also physiological entities, and eicosanoids serve several physiological roles in invertebrates. Fusco *et al.* (1986) reported that rates of penetrating host skin by cercarial larvae of *S. mansoni* is influenced by lipoxygenase products. Similarly, eicosanoids influence migration behavior in *O. dentatum* (Dauguschies & Ruttkowski 1998). Hence, eicosanoids influence parasite behaviors that somehow facilitate gaining entry to potential hosts. Dauguschies (1996) also suggested that eicosanoids mediate unspecified physiological actions within parasites, which can be registered in terms of growth and development.

The larval forms of some dipteran insects live as parasites in mammalian tissues, and this form of parasitic life history produces a disease condition known as myiasis. The primary screwworm, *Cochliomyia hominivorax* (Coquerel) is a serious myiasis-causing pest of cattle and humans in the New World tropics. Adults of this species lay eggs near openings in host skin, usually wounds. The insects develop through three larval stages in their hosts, then drop to the ground, where they go through the pupal stage and emerge as free-living adults. The screwworms seem to go through their developmental excursion in very little contact with host immune cells. On the basis of this observation, we erected the hypothesis that

screwworms produce and secrete immuno-suppressive PGs into their vertebrate hosts.

The primary screwworm is a quarantine species, now eradicated from North America. Screwworms are maintained in a biocontainment facility by the USDA-ARS Midwest Livestock Insect Laboratory, and our work on this species is done in collaboration with the USDA laboratory.

We began our investigation by determining the presence of arachidonic acid in screwworms. Analyses on GC revealed relatively high proportions of arachidonic acid in phospholipids prepared from larvae and adults (Nor Aliza *et al.* 1999). On the understanding that the screwworms were reared on an artificial diet based on bovine blood and whole egg, we considered the idea that screwworms must elaborate a digestive phospholipase A₂ that is responsible for hydrolyzing arachidonic acid from dietary phospholipids for absorption across the midgut epithelium of the screwworms. Characterization studies showed that this enzyme is sensitive to pH and temperature; it requires calcium for full catalytic potential and is sensitive to a site-specific inhibitor, oleyloxyethylphosphorylcholine. By these criteria, the digestive phospholipase A₂ from screwworm midguts is similar to mammalian digestive phospholipases A₂ (Nor Aliza *et al.* 1999).

We are now assessing PG biosynthesis in third-stage screwworms. For these studies, the alimentary canals are removed from the screwworms, and homogenates of the remaining tissues are prepared for enzyme sources. These studies are producing evidence of PG biosynthesis by screwworm tissues. The major products are PGE₂, PGD₂, PGF_{2a} and PGA₂; so far, there is no evidence of lipoxygenase activities. We also have preliminary data on the secretion of PGs into the media surrounding screwworms. The outcomes of these exercises add fuel to a smoldering fire: eicosanoids are very important mediators of relationships between mammalian hosts and representatives of most major invertebrate parasites, including insect parasites of mammals.

The Significance of Research into Eicosanoids in Insect Biology

In this essay we discussed past and current work on the roles of eicosanoids in two important areas of insect biology, immunity and host-parasite relationships. Eicosanoids act as signal moieties, and thereby serve in the regulation, integration and coordination of cellular and organismal activities. Research on chemicals which act as signals has three main lines of significance. For one, the research will yield insights into the mechanisms which regulate and coordinate events. For another, specific pharmaceutical compounds can be used as tools with which to identify and isolate in time specific cellular actions, such as discrete actions in the nodulation process. Finally, the idea that eicosanoids mediate both insect immune reactions and host-parasite relationships opens widely a new window onto the potentials to recognize new biochemical targets that may be exploited in future research on insect pest management.

A Research Prospectus - Future Eicosanoid Research

A more complete understanding of the biology of eicosanoids draws attention to the cellular mechanisms of eicosanoid actions (Fig. 3). Here, we consider the mechanism of PG signal transduction which is drawn from the literature on mammals. Most PGs interact with specific G-protein coupled receptors located on cell surfaces (Negishi *et al.* 1995). When present at the cell surface, a PG can act as the natural agonist for its receptor, which in turn influences a related G-protein. The G-protein then influences an effector protein which may alter the intracellular concentration of a second messenger. PGE₂, for example, interacts with at least four surface receptors. The EP₁ receptor increases phosphoinositol turnover, with

elevation in intracellular free calcium ions. The EP₂ and EP₄ receptors increase adenylate cyclase activity, which increases intracellular cAMP concentrations, while most EP₃ receptors have the opposite effect. Inquiry into eicosanoid signal transduction mechanisms is one of the key frontiers, which until now remains virtually unexplored (Fig. 3).

Similarly, while the last decade has seen tremendous progress in the molecular biology of mammalian eicosanoid systems, this is another dark frontier awaiting the illuminating work of entomologists. We have begun some work in this area, with a collaborative effort to clone the gene for cyclooxygenase in the tobacco hornworm fat body. So far, work with primers based on the mammalian genes has not revealed the insect gene. Nonetheless, we anticipate considerable progress in this vein.

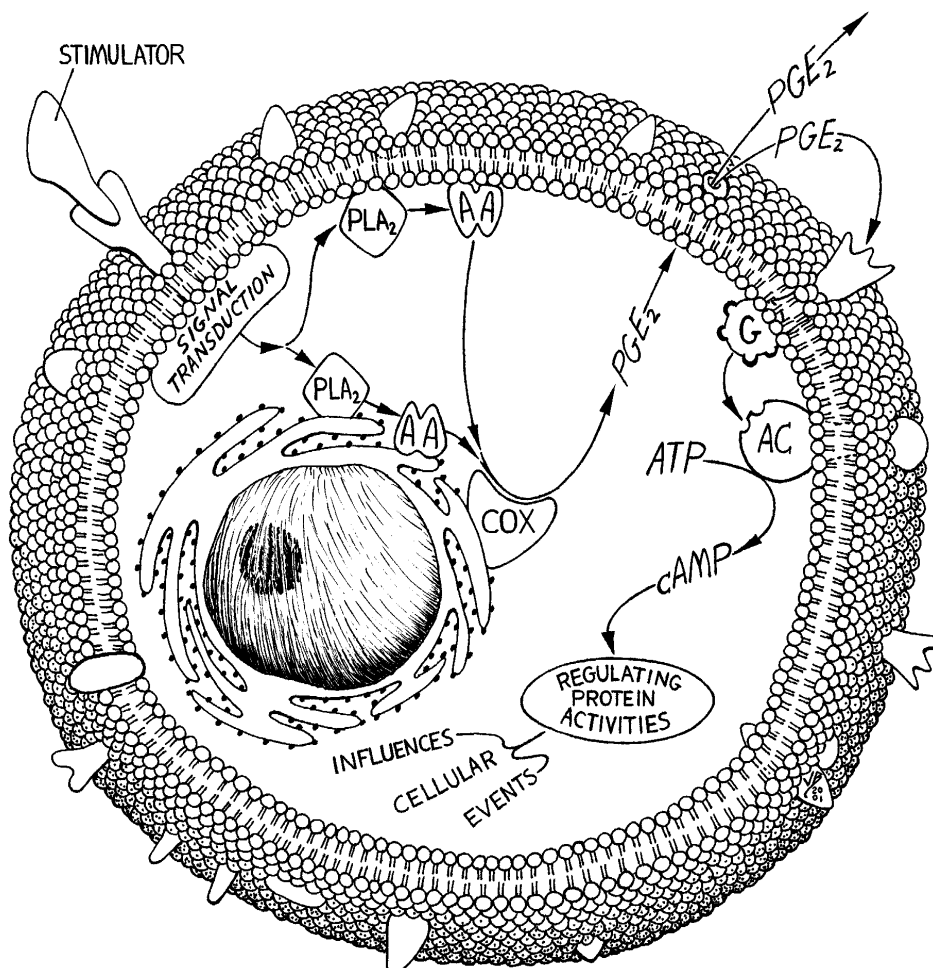


Figure 3. An overview of the cellular actions of PGs, one group of eicosanoids. In this view, a stimulator (which could be a hormone or other physiological mediator or a chemical associated with an infectious agent) interacts with specific cell surface receptors. This interaction stimulates cPLA₂ activity, which releases arachidonic acid (AA) from phospholipid components of cellular membranes. The AA is oxygenated into a PG, in this illustration PGE₂, by cyclooxygenase (COX), the central enzyme in PG biosynthesis. Via action of specific PG transporter proteins, the PGs can be exported from the cell, where they might function in an autocrine mode by interacting with PG receptors on the external surface of the cell, or work in a paracrine mode by interacting with receptors on other cells. The PG signals are transduced to the intracellular space through receptor coupling to G proteins, which in this case shown to influence intracellular concentrations of cAMP.

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