The biology of prostaglandins and related eicosanoids in invertebrates: cellular, organismal and ecological actions.

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Prostaglandins and related eicosanoids are oxygenated metabolites of C20 polyunsaturated fatty acids. These compounds have been detected in species representing all major animal phyla. The significance of eicosanoids lies in two broad areas of animal biology. In one, eicosanoids are involved in regulation of many cellular events. In the other, eicosanoids facilitate certain ecological interactions. Eicosanoids are known best in the narrow context of their clinical significance in human medicine. In this essay we suggest a new, broader paradigm for understanding the meaning of eicosanoids. Under this paradigm, called the biological paradigm, we note eicosanoids were recruited into roles as biological signal moieties long before the origins of the Metazoa. During the ensuing evolutionary diversification of animals, eicosanoids have been used in a vast diversity of biological roles, some of which occur only in invertebrates. We think this diversity endows eicosanoids with unusual explanatory power in apprehending biological phenomena. In this review, we recount the literature on eicosanoids in protozoans and procaryotes, then provide a detailed review of the roles of eicosanoids in invertebrate immunity. We draw upon recent work in parasitology to outline an ecological role of eicosanoids in host-parasite relationships. It appears to us that eicosanoids exert profound effects at the cellular, organismal and ecological levels of biological organization. We suggest that continued inquiry into the biological significance of eicosanoids will yield important new information on invertebrates.

Introduction

Prostaglandins (PGs) and related oxygenated fatty acids are collectively known as eicosanoids. These compounds are biosynthesized by enzymatic oxygenation of three C20 polyunsaturated fatty acids (PUFAs): homo-[gamma]-linolenate (20:3n-6), arachidonate (20:4n-6), or eicosapentaenoate (20:5n-3)(3). Eicosanoid structures and biosynthetic pathways are described in detail in recent reviews (Stanley-Samuelson, 1987, 1993, 1994a). The significance of eicosanoids is most appreciated in the contexts of human and veterinary medicine. Eicosanoids mediate a bewildering catalogue of physiological and pathophysiological actions. The intimate connection to human medicine drives a huge research enterprise, in private and public sectors, aimed at understanding and exploiting eicosanoid systems for human purposes. As might be expected, the intense focus on their medical significance has rather eclipsed a growing body of information on the broader biological significance of eicosanoids, particularly as they influence invertebrates. Perhaps more importantly, the information on eicosanoids in invertebrates seems to be interpreted within a framework of mammalian physiology and pathophysiology. While extremely useful in the large arena of palliating discomfort, the medical model does not accommodate all the biological phenomena, such as the ecological actions of eicosanoids, recorded from studies of invertebrates.

The goal of this essay is to suggest a broader paradigm for understanding the meaning of eicosanoids. Under this model, which we call the biological paradigm, we suggest eicosanoids were recruited into roles as biological signal moieties long before the origins of the Metazoa. In the course of the ensuing diversifying evolution, most, if not all, animals used eicosanoids to mediate events. As organisms and relationships among organisms became more complex, eicosanoids were drawn into a vast diversity of biological roles. The central point of our broader paradigm is the diversity of biological roles endows eicosanoids with unusual explanatory power. We suggest that work aimed at gaining a greater understanding of eicosanoids will yield valuable insights into animal biology. Part of the understanding will emerge from appreciation of the wide occurrence of eicosanoids in invertebrates.

The Occurrence of Eicosanoids in Multicellular Organisms

Stanley-Samuelson (1987) noted that the first discovery of eicosanoids in an invertebrate animal, the octocoral Plexaura homomella, had far more commercial than zoological significance. Weinheimer and Spraggins (1969) discovered this coral contains unusually high levels of certain PGs. The high levels may have to do with chemical defense against predatory coral fish events. As organisms and relationships among organisms became more complex, eicosanoids were drawn into a vast diversity of biological roles. The central point of our broader paradigm is the diversity of biological roles endows eicosanoids with unusual explanatory power. We suggest that work aimed

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at gaining a greater understanding of eicosanoids will yield valuable insights into animal biology. Part of the understanding will emerge from appreciation of the wide occurrence of eicosanoids in invertebrates.

The presence and significance of eicosanoids in invertebrates has been treated from various ponts of view in several reviews (Brady, 1983; Bundy, 1985; Stanley-Samuelson and Loher, 1986; Stanley-Samuelson, 1987, 1991, 1993, 1994a, b; Sauer et al., 1993; De Petrocellis and Di Marzo, 1994; Lamacka and Sajbidor, 1995; Stanley-Samuelson and Pedibhotla, 1996). Stanley-Samuelson considered the biological significance of eicosanoids in invertebrates three years ago in the American Zoologist (Stanley-Samuelson, 1994c). Reference to that review, cited as AZ, will help us highlight major areas of progress since early 1994. Some sections of the AZ paper, including ion transport, neurobiology and the ecological significance of eicosanoids, will not be repeated here because the essential learnings of these sections have not changed substantially.

The Occurrence of Eicosanoids in Unicellular Organisms

Stanley-Samuelson (1987) noted that the first discovery of eicosanoids in an invertebrate animal, the octocoral Plexaura homomella, had far more commercial than zoological significance. Weinheimer and Spraggins (1969) discovered this coral contains unusually high levels of certain PGs. While the high levels may have to do with chemical defense against predatory coral fish (Gerhart, 1991), P. homomella was harvested as a commercial source of PGs for research during the 1970s (Bundy, 1985). In those early days, economical chemical synthesis of eicosanoids lay in the future, and the discovery of a natural source of PGs launched a search for other natural sources. Work along these lines turned up the occurrence of PGs in hundreds of invertebrate species, representing all major phyla (Bundy, 1985; Stanley-Samuelson, 1987). In the early 1980s Corey and his colleagues discovered efficient strategies for chemical synthesis of eicosanoids (Corey et al., 1980), and research on natural sources of eicosanoids declined. Nonetheless, the major point to press is the general finding that eicosanoids occur in every invertebrate taxon taken under appropriate scrutiny. We infer that eicosanoids occur in most, if not all, animals.

Of greater interest within the biological paradigm is discovery of eicosanoids in unicellular organisms. Here we discuss findings from representatives of two phyla of the organisms commonly known as protozoans. First we discuss amoebas (phylum Sarcomastigophora), then briefly consider ciliates (phylum Ciliophora). Das and Padma (1977) probably first reported on the presence of PGs in a protozoan, the amoeba Entamoeba histolytica. Hadas (1988a, b) reported on biosynthesis of PGs in a pathogenic and a non-pathogenic strain of the amoeba, Acanthamoeba castellanii. He noted different PG profiles in the two strains, and suggested continued work in this area would illuminate some aspects of pathogenicity of protozoans. Prusch et al. (1989) suggested a physiological role for PG in phagocytosis in the amoeba Amoeba proteus. They observed increased cytoplasmic streaming and pseudopod formation in the presence of micromolar concentrations of [PGE.sub.2]. They also recorded increased formation of food vacuoles in A. proteus exposed to [PGE.sub.2]. To test directly the idea that PGs mediate phagocytosis, they examined the influence of indomethacin on phagocytic uptake of the protozoan Tetrahymena pyriformis. Indomethacin reduced phagocytosis by about 40%. The authors suggest the physiological significance of PGs relates to coupling events at the cell surface to intracellular events in the initiation of phagocytosis.

The biosynthesis and presence of PGs in other protozoans, the ciliophorans (Tetrahymena), were also investigated (Szablewski and Hadas, 1991; Szablewski, 1993). These authors recorded PGs in T. pyriformis strain GL-C, a free-living form, and in T. rostrata, a parasitic ciliate. They recorded a single product, [PGE.sub.2], and found no difference between the two forms in quantities of [PGE.sub.2]. They also considered the influence of growth stage, and found no real difference in PG levels among stages.

From our perspective, the importance of these findings is the presence and physiological actions of PGs in representatives of two protozoan phyla. These findings support our view, expressed in the Introduction, that eicosanoids were recruited into regulatory processes very early in evolution. The point is made in stronger terms by reports of eicosanoids in procaryotic organisms. Kruger et al. (1990) recorded the presence of [PGF.sub.2[alpha]] in three cyanobacteria (Microcystis aeruginosa, Anacystis nidulans, Anabaena variabilis) and a bacterium (Pseudomonas spp.). This may be thought an isolated account from which little biological thought can be derived. However, more recently Lamacka and Sajbidor (1995) reviewed the evidence for the biosynthesis and occurrence of eicosanoids in many acellular forms, including bacteria, fungi and algae. As seen in similar papers on invertebrate animals over 20 years ago, the authors present little more than listings of eicosanoids and the organisms from which they were extracted. Virtually nothing is known about the physiological roles of eicosanoids in these organisms. Nonetheless, when considered within the biological paradigm, the presence of eicosanoids in procaryotes and

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acellular eucaryotes indicates eicosanoids were involved in cellular events in the very early stages of life.

The biological meaning of the occurrence of eicosanoids in animals is expressed in mediation of physiological events and in some ecological relationships, to which we now turn attention.

The Physiological Significance of Eicosanoids in Invertebrates

Eicosanoids in reproduction and development

The roles of eicosanoids in various aspects of reproduction were treated in the earlier reviews. It is worth repeating that the role of [PGE.sub.2] in releasing egg-laying behavior in newly-mated female crickets (Acheta domesticus) marks the first physiological role of an eicosanoid discovered in any invertebrate (Destephano and Brady, 1977). The review of PGs in insect reproduction by Stanley-Samuelson and Loher (1986) remains contemporary: releasing egg-laying behavior is the only known role of eicosanoids in insect reproduction. Releasing egg-laying behavior does not draw general interest, however, because PGs do not influence egg-laying in all, or even most, insect species. On the other hand, there are good reasons to suspect PGs influence other aspects of insect reproduction. For one, PGs have been recorded in reproductive tissues of several insect species, none of which responds to PG treatments with increased egg-laying. These include houseflies (Musca domestica), cabbage loopers (Trichoplusia ni), and locusts (Locusta migratoria) (Stanley-Samuelson and Loher, 1986). These and other such examples may represent many species, from which we infer PGs may be present in the generality of insect reproductive tissues.

While we cannot put forth biological roles of eicosanoids in reproduction, recent work by Ciapa et al. (1995) suggests eicosanoids may exert important, albeit subtle, actions in the reproductive physiology of insects and other invertebrates. Working with eggs of the sea urchins (Echinodermata) Abacia lixula and Paracentrotus lividus, Ciapa et al. (1995) found that arachidonic acid influenced intracellular events in newly fertilized, but not unfertilized, eggs. Specifically, arachidonic acid stimulated [Na.sup.+]/[H.sup.+] exchange, sharply depressed amino acid transport, and increased [Ca.sup.+2] influx. Connecting these new findings to older work on the roles of eicosanoids in preventing polyspermic fertilizations (Schuel et al., 1984, 1985), we suggest PGs and other eicosanoids are fundamental players in animal reproduction. From that point of view, we expect further research will show that eicosanoids exert quite important actions in invertebrate reproduction.

Eicosanoids in cellular defense mechanisms

Another fundamental aspect of animal biology lies in the ability to defend the body from parasites and pathogens. In most animals, a relatively impermeable integument forms the first, and often quite formidable, barrier to invasion by relatively small organisms. Once the outer barriers are breached, vertebrate and invertebrate animals mount effective cellular and humoral defense reactions. In mammals, eicosanoids play central roles, some stimulatory and other inhibitory, in inflammatory and immune reactions (Levine, 1988).

As just seen in reproductive biology, eicosanoids may fulfill fundamental roles. Evidence on this broad hypothesis would come in the form of similar biological actions in groups of animals of widely disparate phylogeny. Based on the background of eicosanoid actions in mammalian immunity, we posed the question of whether eicosanoids mediate cellular reactions in an invertebrate. We used fifth instar larvae of the tobacco hornworm (Manduca sexta) to test the question. The original work was based on probing eicosanoid biosynthetic pathways with eicosanoid biosynthesis inhibitors, then observing the influence of the inhibitors on the ability of the hornworms to clear bacterial infections from circulating hemolymph. We used a red pigmented strain of the bacterium Serratia marcescens, which could be counted easily on standard petri plates. Control insects were injected with the inhibitor vehicle (ethanol) and then with standard dosages of bacteria. We found the inhibitor treatments severely impaired the ability of the hornworms to clear bacterial infections from their hemolymph. On the basis of these experiments, we proposed that eicosanoids mediate one or more aspects of cellular immune reactions in insects and possibly other invertebrates (Stanley-Samuelson et al., 1991). This work, which was reviewed in AZ, now serves as a point of departure for the following summary of progress since then.

We begin with a brief description of insect immunity, which is generally dissected into two major forms (Dunn, 1986; Gupta, 1991; Chernysh et al., 1996), humoral and cellular. Humoral immunity involves induced synthesis of anti-bacterial proteins and peptides. Lysozymes act by enzymatically hydrolyzing peptidoglycan components of bacterial cell walls. Lysozymes are present in hemolymph in low levels. Lysozyme levels increase in the event of bacterial infections. These enzymes also appear in other tissues (Russell and Dunn, 1996). Anti-bacterial peptides include cecropins, attacins, and defensins (Chernysh et al., 1996). These induced peptides exhibit detergent

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properties, thought to facilitate disruption of bacterial cell membranes by the peptides. Cellular immunity is characterized by direct interactions between circulating hemocytes and bacterial cells; bacterial cells are sequestered from circulation through these interactions (Russell and Dunn, 1996). The interactions are phagocytosis of bacterial cells and nodulation (Fig. 1). Nodulation results from formation of microaggregates of hemocytes and entrapped bacterial cells. The microaggregates grow into nodules by addition of hemocytes and bacterial cells. The mature nodules usually adhere to tissues.

Nodulation is responsible for clearing large numbers of bacterial cells from hemolymph circulation (Horohov and Dunn, 1983), and we hypothesized that nodulation is an identifiable cellular immune reaction mediated, at least in part, by eicosanoid biosynthesis. We refer to this as the eicosanoid hypothesis. The idea was first tested in tobacco hornworms following protocols analogous to the original work on bacterial clearance (Stanley-Samuelson et al., 1991). Briefly, hornworms were treated with an eicosanoid-biosynthesis inhibitor, then injected with [10.sup.5] cells of a non-pigmented strain of S. marcescens. After selected incubation periods, the larvae were anesthetized by chilling them on ice, then assayed for either microaggregation or for nodule formation. For microaggregation (Fig. 1), hemolymph was withdrawn. Total hemocytes and numbers of microaggregates were counted on a hemacytometer under phase contrast optics. For nodulation, the hemocoels were exposed, and melanized, dark nodules were counted under a stereomicroscope. After initial counts were made, the alimentary canal was removed and nodules in the previously unexposed areas were counted.

[FIGURE 1 ILLUSTRATION OMITTED]

We observed the time course of nodulation in control and inhibitor-treated insects. By 6 hours post-infection (PI), control larvae formed about 122 nodules/larva, compared to 30 nodules/larva in inhibitor-treated larvae. Nodulation did not increase significantly in the following 18 hours. In subsequent experiments nodulation was assessed at 6 hours PI. We found the effects of, dexamethasone, an inhibitor of eicosanoid biosynthesis, were expressed in a dose-dependent manner, indicating that inhibition of eicosanoid biosynthesis acted in a physiological, rather than pharmacological, mode. We tested the influence of eight eicosanoid-biosynthesis inhibitors, that express differing modes of action. All the inhibitors uniformly impaired the ability of hornworms to form nodules. We infer the influence of the inhibitors was expressed through their effects on eicosanoid biosynthesis rather than other mechanisms not connected to eicosanoids. Together, these data support our hypothesis that nodulation is mediated by eicosanoids (Miller et al., 1994).

We obtained additional support from the outcomes of reversal experiments. Dexamethasone is thought to inhibit eicosanoid biosynthesis by indirectly inhibiting the first step in eicosanoid biosynthesis, namely release of arachidonate from cellular phospholipids by action of an intracellular phospholipase [A.sub.2] ([PLA.sub.2]). If dexamethasone acts as expected, we reasoned the dexamethasone effect could be reversed by treating dexamethasone-injected larvae with an eicosanoid-precursor PUFA immediately after injecting a standard dose of bacteria. In these experiments, larvae were first treated with dexamethasone, then with the standard dose of bacteria. The larvae were then injected with arachidonate. We also conducted several control experiments. To control for the possibility that ethanol impaired the immune response, one group of larvae was injected with the dexamethasone vehicle, then with bacteria. One experimental group was injected with dexamethasone, then bacteria. One group was injected with dexamethasone, then with palmitate to control for the influence of a lipid on nodulation. One group was injected with dexamethasone, then with bacteria, then with ethanol to control for the effects of handling and injections on nodulation. The control larvae produced about 120 nodules/larva compared to about 40 nodules/larva in dexamethasone-treated experimentals. The arachidonate-treated larvae produced about 140 nodules/larva, not different from controls. The palmitate-treated and ethanol-treated control larvae produced significantly fewer nodules. These findings show that eicosanoid-precursor fatty acids specifically returned the ability to form nodules in response to bacterial infections. Again, these findings strongly support the eicosanoid hypothesis.

We also considered the influence of dexamethasone on formation of microaggregates. Experimental larvae were injected with dexamethasone, then with bacteria, and controls were injected with ethanol, then bacteria. At 1 hour PI, hemolymph was withdrawn, and numbers of circulating microaggregates and total hemocytes were assessed. Microaggregation was reduced by more than 4-fold in experimental larvae. We infer from these findings that the influence of eicosanoid biosynthesis inhibitors on nodulation is expressed early in the cellular defense reactions, during the microaggregation phase. This idea is also supported by total hemocyte counts. Many circulating hemocytes are invested in nodule formation, and circulating hemocyte populations are typically depleted following bacterial infections. In our experiments, the

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experimental larvae, in which microaggregation was reduced, had significantly larger populations of circulating hemocytes than control larvae, in which microaggregation was not reduced (Miller et al., 1994).

The tobacco hornworm is often regarded as a model for studies of insect and even arthropod biochemistry and physiology, and it is not unreasonable to extrapolate ideas supported by work on hornworms to other insects. On the basis of our findings with hornworms, we set forth the hypothesis that eicosanoids will prove to be crucial mediators of nodulation in insects, and perhaps in arthropods generally. We began testing this idea by conducting similar lines of experimentation with other insect species. In our first exploration, we worked with larvae of the tenebrionid beetle Zophobas atratus (Miller et al., 1996). Larvae of Z. atratus are large insects, and we proceeded as just described in our work with hornworms. The findings were virtually identical. Treating Z. atratus larvae with inhibitors of eicosanoid biosynthesis prior to injecting them with heat-killed bacteria resulted in significantly reduced nodule formation, and as in M. sexta, both cyclooxygenase and lipoxygenase products are involved in nodulation. We also found that the influence of dexamethasone could be reversed by treating dexamethsone-treated larvae with arachidonate. We inferred from these results that eicosanoids mediate nodule formation in representatives of two large groups of insects, which considerably strengthened our assertion.

We have continued testing our hypothesis. A similar line of experimentation supported our view that eicosanoids mediate nodulation in silkworms (Bombyx mori) (Stanley-Samuelson et al., 1997). From there, we moved to two other lepidopterans, black cutworms (Agrotis ipsilon) and true army-worms (Pseudaletia unipuncta). Again, the results of similar experiments support the eicosanoid hypothesis (Jurenka et al., 1997). We have recently gained similar findings with a hemimetabolous insect, the cricket Gryllus assimilus (unpublished data, DWS). When taken together, similar results from all these insect species amount to solid support of the eicosanoid hypothesis.

Mandato et al. (1997) provided additional support by investigating the role of eicosanoids during three discrete immunocyte processes in the wax moth (Galleria mellonella). These authors found that prophenoloxidase activation, cell spreading, and phagocytosis depend on eicosanoid biosynthesis. This is the first work to provide insight into eicosanoid actions in identified phases of the overall process of nodulation, namely prophenoloxidase activation and cell spreading.

Nonetheless, in the absence of additional information, it can still be argued that the summed evidence is circumstantial (Stanley-Samuelson, 1994b). First, while eicosanoids have been detected in tissue extracts from many invertebrate species (Bundy, 1985; Stanley-Samuelson, 1987, 1991), the biochemistry of eicosanoid formation has been described in very few of them. We require detailed characterization of eicosanoid systems in insects. Second, it has not been shown that eicosanoid biosynethesis is changed in any way relative to bacterial infections. We are aware of these important lacunae in the eicosanoid hypothesis. In the following paragraphs we summarize our work to characterize the basic elements of eicosanoid systems in insect immune tissues.

A fundamental element of eicosanoid systems is the presence of arachidonic acid, or other eicosanoid precursor PUFAs, in cellular phospholipids. These components occur in high abundance in phospholipids from mammals, most invertebrates and aquatic insects. However, the situation is quite different for many terrestrial insects. C20 PUFAs are often found in very low levels, often less than 0.1% of phospholipid fatty acids (Stanley-Samuelson et al., 1988). Because they occur in very low levels, C20 PUFAs were often overlooked in early gas chromatographic analyses of insect lipids. There finally emerged a mind set predisposed to regard insects as generally lacking in eicosanoid precursor PUFAs (Bade, 1964). Based on analytical and literature surveys, Stanley-Samuelson and Dadd (1983) suggested C20 PUFAs generally occur in insect lipids, albeit in low proportionalities.

The eicosanoid hypothesis: The biochemistry of eicosanoid systems in immune tissues

To address the basic point of C20 PUFAs in insect eicosanoid systems, we have conducted detailed gas chromatographic and gas chromatographic/mass spectrometric analysis of the fatty acids associated with selected insect tissues. We detected arachidonate at [is less than] 0.1% of total fatty acids in phospholipids prepared from thoracic muscles, abdominal dorsal glands, and Malpighian tubules of the cicada Tibicen deal-batus (Stanley-Samuelson et al., 1990). Similarly, we found arachidonate at [is less than] 0.1% of PL fatty acids of Malpighian tubules from the yellow mealworm beetle Tenebrio molitor (Howard et al., 1992). We concluded that eicosanoid precursor PUFAs were probably present in most, if not all, insect tissues, albeit at quite low levels. The main issue, then, would be merely a technical one: detecting trace levels of these fatty acids.

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We analyzed the phospholipid fatty acids in hemocytes from tobacco hornworms, which yielded no more than trace levels of eicosanoid precursor PUFAs (Ogg et al., 1991). More detailed analyses of all developmental stages and nine separate tissues similarly revealed no more than trace levels, many of which could be detected only with combined gas chromatography/mass spectrometry (Ogg and Stanley-Samuelson, 1992). These very low levels could be an artifact of environmental factors, such as availability in diet, or they could be the outcome of selective phospholipid modelling processes. Analyses of the artificial diet in our routine hornworm culture showed the diet provided more of the C20 PUFAs than we found in the insect tissues. This suggested the low proportions of C20 PUFAs result from selective phospholipid remodelling.

We investigated this possibility by tracing the incorporation of radiolabeled fatty acids into hemocyte phospholipids in vitro (Gadelhak and Stanley-Samuelson, 1994). Hemocytes were collected without immunological activation, then the hemolymph was diluted 1:1 with Manduca saline buffer. The cells were diluted to a constant number (ca. 3.2 x [10.sup.6] hemocytes/tube), then incubations were initiated by adding a radiolabeled fatty acid to the tube. After selected incubation periods, the hemocytes were homogenized by sonication, and total lipids were extracted. Lipid classes were isolated by thin-layer chromatography and the amount of radioactivity in each fraction was estimated by liquid scintillation counting. Two major points emerged from these experiments. First, unlike C18:1n-9 and C18: 2n-6, the C20 PUFAs 20:4n-6 and 20:5n-8 were rapidly incorporated into cellular phospholipids, then selectively moved from phospholipids to triacylglycerols over the following 2 hours. This indicates hemocytes are able to remodel phospholipids by selectively moving C20 PUFAs into other lipid pools. Second, the incorporated radiolabeled fatty acids were also redistributed among phospholipid fractions. For example, arachidonate appeared to be redistributed from phosphatidylcholine to phosphatidylethanolamine. These data indicate the very low levels of C20 PUFAs in hemocytes result from selective remodelling events and not from environmental constraints.

We have also documented eicosanoid biosynthesis by hornworm hemocytes and fat body, the major immunity-conferring tissues in insects (Stanley-Samuelson and Ogg, 1994; Gadelhak et al., 1995). Using standardized radiometric techniques, fat body yielded mainly PGs, of which [PGA.sub.2] was the predominant product. PG biosynthesis was sensitive to two cyclooxygenase inhibitors, indomethacin and naproxin. Interestingly, inhibition of cyclooxygenase activity revealed the presence of lipoxygenase activity, which yielded a major product tentatively identified as 15-hydroxyeicosatetraenoic acid. Relative to fat body, the hemocyte preparations expressed a strong lipoxygenase activity and less cycloxygenase activity. Again, [PGA.sub.2] was the major cyclooxygenase product and the putative 15-hydroxyeicosatetraenoic acid was the major lipoxygenase product. These findings are presented in greater detail in the primary reports (Stanley-Samuelson and Ogg, 1994; Gadelhak et al., 1995), all of which serve to document the presence of eicosanoid biosynthetic enzyme in insect immune tissues.

[PLA.sub.2]s are responsible for hydrolyzing the fatty acid associated with the sn-2 position of phospholipids. Research on the biology and biochemistry of [PLA.sub.2]s is a very active arena. [PLA.sub.2]s are most generally categorized as intracellular and extracellular, or secretory, enzymes. The detailed picture is more complicated, and there are now at least five categories of [PLA.sub.2]s (Dennis, 1994). Most extracellular [PLA.sub.2]s are low molecular weight, globular proteins linked with six or more cysteine bridges. All known extracellular [PLA.sub.2]s require millimolar calcium concentrations for activity. Various extracellular [PLA.sub.2]s are involved in phospholipid digestion, secreted in pathophysiological conditions (such as some forms of arthritis), and occur in insect and snake venoms. Intracellular [PLA.sub.2]s are critical elements of phospholipid metabolism and remodelling within cells. Most of them are high molecular weight proteins requiring micromolar calcium concentrations for activity. A few high molecular weight intracellular [PLA.sub.2]s are calcium independent. In mammalian systems, some [PLA.sub.2]s are thought to be the first, and rate limiting, step in eicosanoid biosynthesis (Dennis, 1994). Three points help to understand this idea. One is the regulation of eicosanoid biosynthetic enzymes, which are thought to be limited by the availability of free C20 PUFA substate. Another is the general asymmetry of cellular phospholipids. PUFAs, such as arachidonate, are preferentially associated with the sn-2, but not sn-1, position of phospholipids. Finally, some [PLA.sub.2]s display a preference for arachidonyllinked PLs.

We have investigated the presence of an intracellular [PLA.sub.2] in fat body and hemocytes from tobacco hornworms. The fat body expresses a [PLA.sub.2] that can hydrolyze arachidonate from the sn-2 position of phosphatidylcholine, a major cellular phospholipid (Uscian and Stanley-Samuelson, 1993). This enzyme appears to be calcium-independent, another feature of some [PLA.sub.2]s thought to regulate eicosanoid biosynthesis. We conducted preliminary work aimed at partial purification of the fat body [PLA.sub.2] (unpublished data,

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D.W.S.). Size exclusion chromatography indicates the enzyme is approximately 42 kDa, with properties similar to a mammalian myocardial [PLA.sub.2] (Hazen et al., 1990). The hemocyte [PLA.sub.2] is similarly calcium-independent. The hemocyte enzyme showed a marked preference for sn-2-arachidonyl-phosphatidylcholine, compared to sn-2-palmitoyl-phosphatidylcholine (Schleusener and Stanley-Samuelson, 1996). Again, this is a property of intracellular [PLA.sub.2]s thought to regulate eicosanoid biosynthesis.

The determination of C20 PUFAS, of intracellular [PLA.sub.2]s that can hydrolyze arachidonate from phospholipids, and of eicosanoid-biosynthetic enzymes indicates several major elements of eicosanoids systems are present in M. sexta fat body and hemocytes, the immunity-conferring tissues in insects. We have also determined, albeit in less detail, the presence of these elements in fat body from the other insect species for which we postulated the eicosanoid hypothesis (Miller et al., 1996; Jurenka et al., 1997; Stanley-Samuelson et al., 1997). When coupled with biological studies, these findings amount to quite substantial support for the eicosanoid hypothesis. However, the data supporting the hypothesis remain rather circumstantial (Stanley-Samuelson, 1994b). We are currently working to determine quantities of PGs and other eicosanoids in immunity-conferring tissues, and to determine how the quantities are influenced by bacterial infections. We anticipate these and other new initiatives will bolster the eicosanoid hypothesis.

The Ecological Significance of Eicosanoids

Studies in chemical ecology are rooted in recognition that many ecological interactions between and among populations are mediated by chemicals. The idea that eicosanoids are among the major players in chemical ecology broadens our appreciation of the biological significance of eicosanoids (AZ). The roles of eicosanoids as sex pheromones, in predator-prey relationships, and in host-parasite interactions were briefly outlined in AZ. We note a very detailed review of the presence and significance of PGs in tick saliva by Bowman and colleagues (1996), which supercedes the condensed treatment of this aspect of host-parasite interactions in AZ Here, we briefly outline the emergent picture of eicosanoid actions in host-parasites relationships.

The idea that eicosanoids may be involved in host-parasite interactions dates back to the early 1980s. Leid and McConnell (1983a, b) reported on the biosynthesis and release of eicosanoids by larvae of the cat tapeworm, Taenia taeniaeformis (Cestoda). They first recorded the release of thromboxane [A.sub.2], the stable breakdown product of thromboxane [B.sub.2], into medium in which larval worms were incubated with arachidonate. In additional work they found the larval worms released [PGE.sub.2] into the medium, while [PGI.sub.2] was biosynthesized but not released. In both papers, while giving due recognition to the speculative nature of their insights, the authors suggested endoparasites may form and utilize eicosanoids to evade host immune reactions to parastization (Leid and McConnel, 1983a, b). There soon followed a large body of work by Salafsky and his colleagues on the biochemistry and biology of eicosanoids in blood flukes, Schistosoma mansoni (reviewed in AZ and elsewhere). Lipoxygenase products are thought to mediate skin penetration by cercarial stages of S. mansoni, and cyclooxygenase products were associated with transformation of cercaria into schistosomules.

Investigations into the occurrence and roles of eicosanoids in parasites is now an active area. Liu et al. (1990) showed that microfilariae of the nematode Brugia malavi biosynthesize and release two eicosanoids, [PGI.sub.2], and [PGE.sub.2]. Again, they speculated these products may influence host immune reactions. Baskova et al. (1995) reported on the influence of eicosanoids from the leech Hirudo medicinalis on another defense reaction, thrombus formation. Taken as a fraction extracted from the leeches, the eicosanoid fraction inhibited thrombus formation in rat mesentery arterioles. Most recently, Daugschies (1996) reported on leukotriene biosynthesis by the parasitic nematode Oesophagostomum dentatum. Leukotriene [B.sub.4] was detected in homogenates and in supernatants of the larvae. Experiments with diethylcarbamazine, a leukotriene biosynthesis inhibitor, yielded results showing the endogenous leukotrienes are regulatory elements in growth and development of the larve. He suggested the precise function of the role of parasite-producted leukotrienes in the host-parasite interaction remains to be investigated.

On the basis of these reports, we speculate eicosanoids will be subjects of intense study in efforts to gain greater understanding of host-parasite interactions. Seen from our bias, such work illustrates the explanatory power of eicosanoids.

Another Prospectus

Stanley-Samuelson (1994a; AZ) noted a major lacuna in our understanding of eicosanoids in invertebrates: in early 1994 there was virtually no information on eicosanoid receptors and the biochemical mechanisms of eicosanoid actions in invertebrates. The only hint of eicosanoid

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receptors came from work on Malpighian tubules. Based on this work, [PGE.sub.2] is thought to modulate fluid secretion rates in Malpighian tubules of two insect species, the mosquito Aedes aegypti (Petzel and Stanley-Samuelson, 1992) and the ant, Formica polyctena (Van Kerkhove et al., 1995). PGs may act through G-protein coupled receptors because exogenous [PGE.sub.2] stimulated increased intracellular cAMP concentrations in Aedes aegypti Malpighian tubules (Parrish et al., 1992). Sauer and his colleagues have now reported on a specific [PGE.sub.2] receptor in the plasma membrane fraction of salivary glands from female ticks, Amblyomma americanum (Qian et al., 1997). The receptor was saturable, reversible, and specific for [PGE.sub.2]. As to biochemical mechanism of action, the receptor is coupled to a cholera toxin-sensitive G-protein. In this case, the [PGE.sub.2] appears to serve an autocoid role, modulating salivary secretion rates. This report marks a first solid step forward in understanding modes of eicosanoid action in invertebrates. We anticipate considerable advances on this frontier in future.

With the progress just noted in biochemical mechanisms of eicosanoid action, we close this essay with a brief comment on another untouched frontier of our understanding of eicosanoids in invertebrates. The advanced work on mammalian systems has made considerable headway in the molecular biology of eicosanoids. Genes for eicosanoid biosynthetic enzymes and for eicosanoid receptors have been cloned from many mammalian sources. This work facilitates understanding regulation of gene expression. More important to understanding the biology of invertebrates, the mammalian literature sets a stage for comparisons to similar genes from invertebrate sources.

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References

Bade, M. L. 1964. Biosynthesis of fatty acids in the roach Eurycotis floridana. J. Insect Physiol. 10: 333-341.

Baskova, I. P., L. L. Zavalova, A. V. Basanova, O. M. Aquejouf, F. Azougagh-Oualane, and C. Doutremepuich. 1995. Antithrombotic effect of piyavit (a preparation from the medicinal leech Hirudo medicinalis), of its prostanoid fraction, and of the enzyme destabliase. Biochem. (Moscow) 60:1355-1360.

Bowman, A. S., J. W. Dillwith, and J. R. Sauer. 1996. Tick salivary prostaglandins: Presence, origin and significance. Parasitol. Today 12:388-396.

Brady, E. U. 1983. Prostaglandins in insects. Insect Biochem. 13:443-451.

Bundy, G. L. 1985. Nonmammalian sources of eicosanoids. In J. E. Pike and D. R. Morton, Jr. (eds.), Advances in prostaglandin, thromboxane, and leukotriene research, Vol. 14, pp. 229-262. Raven Press, New York.

Chernysh, S., S. Cociancich, J.-P. Briand, C. Hetru, and P. Bulet. 1996. The inducible antibacterial peptides of the hemipteran insect Palomena prasina: Identification of a unique family of proline-rich peptides and of a novel insect defensin. J. Insect Physiol. 42:81-89.

Ciapa, B., D. Allemand, and G. De Renzis. 1995. Effect of arachidonic acid on Na+/H+ exchange and neutral amino acid transport in sea urchin eggs. Exp. Cell Res. 218:248-254.

Corey, E. J., J. O. Albright, A. E. Barton, and S. Hashimoto. 1980. Chemical and enzymic syntheses of 5-HPETE, a key biological precursor of slow-reacting substance of anaphylaxix (SRS) and 5-HETE. J. Am. Chem. Soc. 102:1435-1436.

Das, U. N. and M. C. Padma. 1977. Presence of prostaglandins A and E in Entamoeba histolytica. Indian J. Exp. Biol. 15:1227-1228.

Daugschies, A. 1996. Investigations into the production and function of leukotrienes during histotropic development of Oesophagostomum dentatum. Parasitol. Res. 82:416-422.

Dennis, E. A. 1994. Diversity of group types, regulation and function of phospholipase [A.sub.2] J. Biol. Chem. 269:13057-13060.

De Petrocellis, L. and V. Di Marzo. 1994. Aquatic invertebrates open up new perspectives in eicosanoid research: Biosynthesis and bioactivity. Prostaglandins

The biology of prostaglandins and related eicosanoids in invertebrates: cellular, organismal and ecological actions.

Leukotrienes and essential fatty acids 51:215-229.

Destephano, D. B. and U. E. Brady. 1977. Prostaglandin and prostaglandin synthetase in the cricket, Acheta domesticus. J. Insect Physiol. 23:905-911.

Dunn, P. E. 1986. Biochemical aspects of insect immunity. Annu. Rev. Entomol. 31:321-339.

Gadelhak, G. G. and D. W. Stanley-Samuelson. 1994. Incorporation of polyunsaturated fatty acids into phospholipids of hemocytes from the tobacco hornworm, Manduca sexta. Insect Biochem. Mol. Biol. 24:775-785.

Gadelhak, G. G., V. K. Pedibhotla, and D. W. Stanley-Samuelson. Eicosanoid biosynthesis by hemocytes from the tobacco hornworm, Manduca sexta. Insect Biochem. Mol. Biol. 25:743-749.

Gerhart, D. J. 1991, Emesis, learned aversion, and chemical defense in octocorals: A central role for prostaglandins? Am. J. Physiol. 260:R839-R843.

Gupta A. P. (ed.) 1991. Immunology of insects and other arthropods. CRC Press, Boca Raton, Florida.

Hadas, E. 1988a. Biosynthesis of prostaglandins in pathogenic and non-pathogenic strains of Acanthamoeba castellanii. Acta Protozool. 27:61-66.

Hadas, E. 1988b. Factors conditioning biosynthesis of prostaglandins in pathogenic and non-pathogenic strains of Acanthamoeba castellanii. Acta Protozool. 27:239-247.

Hazen, S. L., R. J. Stupy, and R. W. Gross. 1990. Purification and characterization of canine myocardial cytosolic phospholipase [A.sub.2]. J. Biol. Chem. 265:10622-10630.

Horohov, D. W. and P. E. Dunn. 1983. Phagocytosis and nodule formation by hemocytes of Manduca sexta larvae following injection of Pseudomonas aeruginosa. J. Invert. Path. 41:203-213.

Howard, R. W., N. A. Witters, and D. W. Stanley-Samuelson. 1992. Phospholipid fatty acid composition and distribution patterns of prostaglandins in Malpighian tubules of the yellow mealworm (Coleoptera; Tenebrionidae). Ann. Entomol. Soc. Am. 85:489-498.

Jurenka, R. A., J. S. Miller, V. K. Pedibhotla, R. L. Rana, and D. W. Stanley-Samuelson. 1997. Eicosanoids mediate microaggregation and nodulation responses to bacterial infections in black cutworms, Agrotis ipsilon, and true armyworms, Pseudaletia unipuncta. J. Insect Physiol. 43:125-133.

Kruger, G. H. J., E. G. Broenewald, H. de Wet, and P. J. Botes. 1990. The occurrence of prostaglandin [F.sub.2[Alpha] in procaryotic organisms. S.-Afr. Tydskr. Plantk. 56:150-153.

Lamacka, M. and J. Sajbidor. 1995. The occurrence of prostaglandins and related compounds in lower organisms. Prostaglandins, Leukotrienes Essential Fatty Acids 52:357-364.

Leid, R. W. and L. A. McConnell. 1983a. Thromboxane [A.sub.2] generation by the larval cestode, Taenia taeniaeformis. Clinical Immunol. Immunopathol. 28:67-76.

Leid, R. W. and L. A. McConnell. 1983b. [PGE.sub.2] generation and release by the larval stage of the cestode, Taenia taeniaeformis. Prostaglandins, Leukotrienes Med. 11:317-323.

Levine, L. (ed.) 1988. Arachidonate metabolism in immunologic systems. Karger, Basel.

Liu, L. X., C. N. Serhan, and P. F. Weller. 1990. Intravascular filarial parasites elaborate cyclooxygenase-derived eicosanoids. J. Exp. Medic. 172:993-996.

Mandato, C. A., W. L. Diehl-Jones, S. J. Moore, and R. G. H. Downer. 1997. The effects of eicosanoid biosynthesis inhibitors on prophenoloxidase activation, phagocytosis and cell spreading in Galleria mellonella. J. Insect Physiol. 43:1-8.

Miller J. S., T. Nguyen, and D. W. Stanley-Samuelson. 1994. Eicosanoids mediate insect nodulation responses to bacterial infections. Proc. Natl. Acad. Sci U.S.A. 91:12418-12422.

Miller, J. S., R. W. Howard, T. Nguyen, A. Nguyen, R. M. T. Rosario, and D. W. Stanley-Samuelson. 1996. Eicosanoids mediate nodulatoin responses to bacterial infection in larvae of the tenebrionid beetle, Zophobas atratus. J. Insect Physiol. 42:3-12.

Ogg, C. L. and D. W. Stanley-Samuelson. 1992. Phospholipid and triacylglycerol fatty acid compositions of the major life stages and selected tissues of the tobacco hornworm Manduca sexta. Comp. Biochem. Physiol. 101B:345-351.

Ogg, C. L., R. W. Howard, and D. W. Stanley-Samuelson.

The biology of prostaglandins and related eicosanoids in invertebrates: cellular, organismal and ecological actions.

1991. Fatty acid composition and incorporation of arachidonic acid into phospholipids of hemocytes from the tobacco hornworm, Manduca sexta. Insect Biochem. 21:809-814.

Parrish, A. E., D. Petzel, and D. W. Stanley-Samuelson. 1992. Prostaglandin [E.sub.2] stimulates increased intracellular cAMP concentrations in Malpighian tubules of the yellow fever mosquito (Aedes aegypti). FASEB J. 6:A1747.

Petzel, D. H. and D. W. Stanley-Samuelson. 1992. Inhibition of eicosanoid biosynthesis modulates basal fluid secretion in the Malpighian tubules of the yellow fever mosquito (Aedes aegypti). J. Insect Physiol. 38:1-8.

Prusch, R. D., S.-M. Goete, and P. Haberman. 1989. Prostaglandins may play a signal-coupling role during phagocytosis in Amoeba proteus. Cell Tissue Res. 255:553-557.

Qian, Y., R. C. Essenberg, J. W. Dillwith, A. S. Bowman, and J. R. Sauer. 1997. A specific prostaglandin [E.sub.2] receptor and its role in modulating salivary secretion in the female tick, Amblyomma americanum (L.). Insect Biochem. Molec. Biol. (In press)

Russell, V. and P. E. Dunn. 1996. Antibacterial proteins in the midgut of Manduca sexta during metamorphosis. J. Insect Physiol. 42:65-71.

Sauer, J. R., A. S. Bowman, M. M. Shipley, C. L. Gengler, M. R. Surdick, J. L. McSwain, C. Luo, R. C. Essenberg and J. W. Dillwith. 1993. Arachidonate metabolism in tick salivary glands. In: D. W. Stanley-Samuelson and D. R. Nelson (eds), Insect lipids: Chemistry, biochemistry and biology, pp. 99-138. University of Nebraska Press, Lincoln, Nebraska.

Schleusener, D. R. and D. W. Stanley-Samuelson. 1996. Phospholipase [A.sub.2] in hemocytes of the tobacco hornworm, Manduca sexta. Arch. Insect Biochem. Physiol. 33:63-74.

Schuel, H., E. Traeger, R. Schuel, and J. Boldt. 1984. Anti-inflammatory drugs promote polyspermic fertilization in sea urchins. Gamete Res. 10:9-19.

Schuel, H., R. Moss, and R. Schuel. 1985. Induction of polyspermic fertilization in sea urchins by the leukotriene antagonist FPL-55712 and the 5-lipoxygenase inhibitor BW755C. Gamete Res. 11:41-50.

Stanley-Samuelson, D. W. 1987. Physiological roles of prostaglandins and other eicosanoids in invertebrates. Biol. Bull. 173:92-109.

Stanley-Samuelson. D. W. 1991. Comparative eicosanoid physiology in invertebrate animals. Am. J. Physiol. 260:R849-R853.

Stanley-Samuelson, D. W. 1993. The biological significance of prostaglandins and related eicosanoids in insects. In D. W. Stanley-Samuelson and D. R. Nelson (eds.), Insect lipids: Chemistry, biochemist and biology, pp. 45-97. University of Nebraska Press, Lincoln, Nebraska.

Stanley-Samuelson, D. W. 1994a. Prostaglandins and related eicosanoids in insects. Adv. Insect Physiol. 24:115-212.

Stanley-Samuelson, D. W. 1994b. Assessing the significance of prostaglandins and other eicosanoids in insect physiology. J. Insect Phyiol. 40:3-11.

Stanley-Samuelson, D. W. 1994c. The biological significance of prostaglandins and related eicosanoids in invertebrates. Amer. Zool. 34:589-598.

Stanley-Samuelson D. W. and R. H. Dadd. 1983. Long-chain polyunsaturated fatty acids: Patterns of occurrence in insects. Insect Biochem. 14:321-327.

Stanley-Samuelson, D. W. and W. Loher 1986. Prostaglandins in insect reproduction. Ann. Entomol. Soc. Am. 79:841-853.

Stanley-Samuelson, D. W. and C. L. Ogg. 1994. Prostaglandin biosynthesis by fat body from the tobacco hornworm, Manduca sexta. Insect Biochem. Molec. Biol. 24:481-491.

Stanley-Samuelson, D. W. and V. K. Pedibhotla. 1996. What can we learn from prostaglandins and related eicosanoids in insects? Insect Biochem. Molec. Biol. 26:223-234.

Stanley-Samuelson, D. W., R. A. Jurenka, C. Cripps, G. J. Blomquist, and M. de Renobales. 1988. Fatty acids in insects: composition, metabolism, and biological significance. Arch. Insect Biochem. Physiol. 9:1-33.

Stanley-Samuelson. D. W., E. Jensen, K. W. Nickerson, K. Tiebel, C. L. Ogg. and R. W. Howard. 1991. Insect immune response to bacterial infection is mediated by eicosanoids. Proc. Natl. Acad. Sci. U.S.A. 88:1064-1068.

Stanley-Samuelson, D. W., R. W. Howard, and E. C.

The biology of prostaglandins and related eicosanoids in invertebrates: cellular, organismal and ecological actions.

Toolson. 1990. Phospholipid fatty acid composition and arachidonic acid uptake and metabolism by the cicada Tibicen dealbatus (Homoptera: Cicadidae). Comp. Biochem. Physiol. 97B:285-289.

Stanley-Samuelson, D. W., V. K. Pedibhotla, R. L. Rana, A. R. Nor Aliza, W. W. Hoback, and J. S. Miller. 1997. Eicosanoids mediate nodulation responses to bacterial infections in larvae of the silkmoth, Bombyx mori. Comp. Biochem. Physiol. 118A:93-100.

Szablewski, L. 1993. Activity of prostaglandin synthetase in Tetrahymena rostrata and Tetrahymena pyriformis GL-C. Acta Protozool. 32:33-36.

Szablewski, L. and E. Hadas. 1991. Prostaglandins of Tetrahymena pyriformis GL-C and T. rostrata. Acta Protozool. 30:165-168.

Uscian, J. M. and D. W. Stanley-Samuelson. 1993. Phospholipase [A.sub.2] activity in the fat body of the tobacco hornworm Manduca sexta. Arch. Insect Biochem. Physiol. 24:187-201.

Van Kerkhove, E., P. Pirotte, D. H. Petzel, and D. W. Stanley-Samuelson. 1995. Eicosanoid biosynthesis inhibitors modulate basal fluid secretion rates in the Malpighian tubules of the ant, Formica polyctena. J. Insect Phyiol. 41:435-441.

Weinheimer, A. J. and R. L. Spraggins. 1969. The occurrence of two new prostaglandin derivatives (15-epi-[PGA.sub.2] and its acetate methyl ester) in the gorgonian Plexaura homomalla. Chemistry of coelentrates XV. Tetrahedron Lett. 59:5185-5188.

(4) sn-1 and sn-2 refer, respectively, to the first and second carbons of the glycerol backbone of phospholipids.