Inhibition of eicosanoid signaling leads to increased lipid peroxidation in a host/parasitoid system

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Running title: Eicosanoids and oxidative response

Abstract

We posed the hypothesis that inhibition of eicosanoid biosynthesis leads to increased lipid peroxidation in insects. Here we report that rearing the greater wax moth, *Galleria mellonella*, on media supplemented with selected inhibitors of eicosanoid biosynthesis throughout the larval, pupal and adult life led to major alterations in selected oxidative and antioxidative parameters of wax moth and its ectoparasitoid, *Bracon hebetor*. The highest dietary dexamethasone (Dex), esculetin (Esc) and phenidone (Phe) led to increased malondialdehyde (MDA) levels and to elevated catalase (CAT) and glutathione-S-transferase (GST) activities in all developmental stages of host larvae. Dietary Phe resulted in increased MDA levels, and CAT activity in *G. mellonella* adults by about 4-fold and about 2-fold, respectively. The Phe effect on GST activity in all stages of the wax moth was expressed in a dose-dependent manner, increased to 140 nmol/mg protein/min in larvae. MDA levels were increased by over 30-fold in adult wasps reared on Dex- and Esc-treated hosts. CAT and GST activities were increased in adult parasitoids reared on Esc- and Phe-treated hosts. GST activity of Dex-treated parasitoid larvae increased from about 4 to over 30 nmol/mg protein/min. Dietary Phe led to increased GST activity, by about 25-fold, in adult wasps. These data indicate that chronic inhibition of eicosanoid biosynthesis leads to increased oxidative stress, strongly supporting our hypothesis. The significance of this work lies in understanding the roles of eicosanoids in insect biology. Aside from other well-known eicosanoids actions, we propose that eicosanoids mediate reductions in oxidative stress.

Keywords:

G. mellonella

B. hebetor

GST

CAT

oxidative stress

eicosanoids

1. Introduction

Eicosanoids are biologically active oxygenation products of arachidonic acid and two other C20 polyunsaturated fatty acids (Tunaz et al., 2003; Stanley and Kim, 2014). The major groups of eicosanoids include prostaglandins (PGs), thromboxanes, epoxyeicosatrienoic acids and the many lipoxygenase (LOX) products. Eicosanoid structures and biosynthetic pathways are treated in detail elsewhere (Stanley and Kim, 2014). All animals biosynthesize a wide range of eicosanoids, which serve in molecular, physiological and ecological actions (Büyükgüzel et al., 2002; Stanley, 2000, 2006). Eicosanoids mediate insect cellular immune reactions including hemocyte-spreading and migration, microaggregation, nodulation, encapsulation and phagocytosis, to microbial and metazoan infections (Tunaz, 2006; Büyükgüzel et al., 2007; Durmuş et al., 2008; Stanley and Kim, 2014). Eicosanoids also mediate biosynthesis of antimicrobial peptides that contribute to humoral immunity by directly killing invading microbes (Morishima et al., 1997; García Gil de Muñoz et al., 2008) as well as the activation of prophenoloxidase (PPO) in *Galleria mellonella* (L.) larvae (Kanost and Gorman, 2008). PGs act in insect renal physiology in Malpighian tubules and rectum (Stanley and Kim, 2014). Tootle et al. (2011) reported that PGs act in fundamental aspects of egg development in *Drosophila*. At the ecological level, some corals produce high tissue concentrations of PGs, which protect them from fish predation (Gerhart, 1984). We anticipate additional eicosanoid actions in insects will come to light.

Eurykarotic cells produce reactive oxygen species (ROS) via ordinary mitochondrial operation of the electron transport chain. Plant pro-oxidant secondary metabolites are another major source of ROS and these metabolites act in the chemical ecology of insect herbivore/host plant interactions. Many studies indicate these metabolites make up a powerful defense arsenal responsible for exerting serious, lethal damage to insect alimentary canals, particularly in the midgut cells (Felton and Summers, 1995; Krishnan and Sehnal, 2006). The

86 damage results from their strong pro-oxidant activities. Pro-oxidant damage mechanisms
87 include lipid peroxidation, protein carbonylation, DNA oxidation, and glutathione depletion.
88 These metabolites may also act in tritrophic food webs, where they influence predators that
89 consume the plant prooxidants (Johnson, 2011).

90 In biochemical terms, oxidized lipid moieties can decompose to malondialdehyde
91 (MDA) or 4-hydroxynonenal (4-HNE), both strong pro-oxidants (Cheeseman, 1993). Insects
92 express a suite of antioxidant enzymes, including superoxide dismutase (SOD), catalase
93 (CAT), glutathione peroxidase (GPx), glutathione S transferases (GSTs) and ascorbate
94 peroxidase (APOX) (Ahmad, 1995), thought to metabolize ROS into relatively harmless by-
95 products (Hyršl et al., 2007; Liu et al., 2015). Endogenous mitochondrial ROS production
96 occurs as a function of metabolic rates, which in lepidoptran larvae are relatively low
97 compared to the powerful flight muscles of some insect groups such as the Hymenoptera and
98 Diptera. We infer that plant secondary compounds are probably the major sources of ROS in
99 larvae.

100 Most of our understanding of eicosanoid actions in antioxidant systems comes from a
101 very large literature on the biomedical significance of these compounds in humans and other
102 mammals. For example, some PGs mediate expression of GST isozymes in vertebrates
103 (Kawamoto et al., 2000). Although studies with eicosanoid biosynthesis inhibitors (EBIs)
104 showed that eicosanoids are among the mediators responsible for ROS metabolism (Hatamoto
105 et al., 2006), the point has not been extensively studied in insects. Parasitoid insects can be
106 exposed to dietary prooxidants within their hosts (Ortel et al., 1993; Büyükgüzel, 2006), an
107 idea we tested directly in our model host/parasitoid, *G. mellonella*/ *Bracon hebetor*, system by
108 rearing wax moth larvae on standard culture media amended with EBIs. We maintained its
109 ectoparasitoid on the experimental larvae which led to substantially reduced fecundity,
110 recorded as numbers of eggs laid and egg hatch rates, in the parasitoids (Büyükgüzel et al.,

2010, 2011). We drew two inferences from our results, one, that the dietary prooxidants negatively influence the fitness of the experimental parasitoids and two, that eicosanoids are one of the mediators of the mechanisms responsible for reducing oxidative stress. We posed this as the hypothesis that inhibition of eicosanoid biosynthesis leads to increased lipid peroxidation in our model host-parasitoid system. Here we report on the outcomes of experiments designed to test our hypothesis.

2. Materials and methods

2.1. Insect Stock Cultures

Greater wax moth, *G. mellonella* (L.) larvae were reared on an artificial diet (Bronskill, 1961) in 1,000-ml glass jars at $30 \pm 1^\circ\text{C}$ in constant darkness. The standard diet was composed of 420 g of bran, 150 ml of filtered honey, 150 ml of glycerol, 20 g of ground old dark honey comb, and 30 ml of distilled water. Fifteen newly emerged females were placed in the jars and they were provided a piece of old honeycomb on the diet for egg deposition and feeding of newly hatched larvae. Experimental neonate larvae were reared on the artificial diet amended with concentrations of either Dexamethasone (Dex), Esculetin (Esc) or Phenadone (Phe) at concentrations indicated in results. The larvae developed through their 7th-larval instars in similar glass jars at $30 \pm 1^\circ\text{C}$ and constant dark in an incubator (Nuve ES500 Nuve Co. Ankara, Turkey). Seventh-instar larvae were used for experiments (Büyükgüzel et al., 2007, 2011).

Couples of the ectoparasitoid, *B. hebetor* Say, were individually placed in 30-ml cylindrical plastic cups (35 by 55 mm, OrLab Ltd., Ankara, Turkey, used in all experiments) covered with screen lids, and maintained on their natural host, healthy late 7th-instar wax moth larvae, maintained on a 50 % honey solution (wt:v) soaked in cotton pads. After 24h, parasitized host larvae were transferred to another clean plastic cup and maintained at 28 ± 2

°C, 70 ± 10% RH, and 16h photophase until the adult parasitoids emerged. Newly emerged wasps were used for the biochemical analyses.

2.2. Chemicals

All reagents were analytical grade and all abbreviations are defined in Table 1. Phenylmethylsulphonyl fluoride (PMSF), dithiotreitol (DTT), β-nicotinamide adenine dinucleotide phosphate (NADPH), butylated hydroxytoluene (BHT), bovine serum albumin (BSA), Folin-Ciocalteu reagent, ethylenediaminetetraacetic acid (EDTA), thiobarbituric acid (TBA), dipotassium hydrogen phosphate (K₂HPO₄), hydrogen peroxide (H₂O₂), glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), ethanol (EtOH), potassium chloride (KCl), and esculetin (6,7-dihydroxycoumarin), phenidone (4-Methyl-1-phenyl-3-pyrazolidinon), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dexamethasone [(11^β,16^α)-9-fluoro-11-17,21-trihydroxy-16-methylpregna-1,4-diene-3, 20-dione] was a kind gift from Deva Holding (Levent, İstanbul).

2.3. Experimental designs

EBIs, Esc (98 %), Dex (98 %) and Phe (97 %) were diluted in 1 ml of EtOH (70 %) and completed with distilled water to prepare solutions at 0.001, 0.01, 0.1 or 1.0 g per 100 g of diets, selected based on previous study (Büyükgüzel et al., 2007). Individual EBIs were first desolved in EtOH, then final solutions made up in water. The solutions were added into the larval diets as water component during preparation. Control larvae were reared on the normal diet without EBIs. Preliminary experiments showed that diluted EtOH did not affect life table parameters (Büyükgüzel et al., 2011). Larvae were reared from first through last instars and, as in our standard protocols, late last instars were used as hosts for experimental parasitoids.

Some of the wax moth seventh-instars, pupae and adults and some parasitoids, 3rd instars, pupae and adults were weighed and kept at -80°C until biochemical analysis. Ten experimental insects in each development stage were used for determination of MDA quantities and CAT and GST activities.

2.4. Sample preparation and determination of MDA content and antioxidant enzymes activities

Wax moth and parasitoid samples were collected into chilled eppendorf tubes charged with cold homogenisation buffer (w/v 1.15% KCL, 25 mM K_2HPO_4 , 5 mM ethylenediaminetetraacetic acid EDTA, 2 mM phenylmethylsulphonyl fluoride PMSF, 2mM dithiotreitol DTT, pH: 7.4) and stored -80°C . Whole insects were prepared at 4°C by an ultrasonic homogenizer (Bandelin Sonoplus, HD2070, Berlin, Germany) at 30 W, 10 s in homogenisation buffer, then centrifuged (Hettich Zentrifugen Mikro200R, Germany) at $10,000 \times g$ for 15 min at 4°C . The resulting cell-free extracts were collected for biochemical analysis. Supernatants were centrifuged at either $1000 \times g$ for 10 min at 4°C (CAT assays), $16,000 \times g$ for 20 min (GST) or $2000 \times g$ for 15 min (MDA content). MDA contents and antioxidant enzymes activities were determined by measuring the absorbance of the samples in a dual beam spectrophotometer (Shimadzu 1700, UV/VIS Spectrophotometer, Kyoto, Japan). Assays were replicated four times each with ten insects. Protein concentrations were determined according to Lowry et al. (1951) using BSA as a quantitative standard.

MDA contents were assayed according to Jain and Levine (1995), determined after incubation at 95°C , 45 min with TBA (1% wt/v). Absorbances were measured at 532 nm and data expressed as nmol MDA/mg protein using $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$ as extinction coefficient.

GST (EC 2.5.1.18) activity was assayed by measuring the formation of the GSH and 1-chloro-2,4-dinitrobenzene (CDNB) conjugate (Habig et al., 1974). The increase in absorbance was recorded at 340 nm for 3 min. The specific activity of GST was expressed as 1 mol GSH-CDNB conjugate formed/min/mg protein using an extinction coefficient of $9.6 \text{ mM}^{-1}\text{cm}^{-1}$. All assays were corrected for non-enzymatic conjugation using 25 mM CDNB and 20 mM GSH in 50 mM phosphate buffer, pH 7.0.

Before the determination of CAT (EC 1.11.1.6) activity, samples were diluted 1:9 with 1% v/v Triton X-100. Enzyme activity was measured according to Aebi (1984), assaying the hydrolysis of H_2O_2 and decreasing absorbance at 240 nm over a 3 min period at 25 °C. CAT activity was expressed as mmol H_2O_2 reduced/min/mg protein, extinction coefficient = $0.0394 \text{ mM}^{-1}\text{cm}^{-1}$. A blank without homogenate was used as a control for non-enzymatic hydrolysis of peroxide in phosphate buffer (50 mM, pH 7.0).

2.5. Statistical analysis

Data were evaluated by one-way analysis of the variance (ANOVA). The least significant difference (LSD) (PROC GLM, SAS Institute, 1989) test was used for significant differences at $P \leq 0.05$.

3. Results

3.1. The effects of dietary EBIs on MDA content, CAT activity, GST activity of *G. mellonella* developmental stages

The influence of dietary EBIs on host MDA contents is summarized in Fig. 1A. For *Galleria*, control larvae had about 0.5 nmol MDA/mg protein. Dietary Dex and Esc did not influence MDA levels, with exception of larvae reared on the highest Dex and Esc dosages, at about 4.0 and 4.5 nmol MDA/mg protein. High dietary doses of Phe, a dual

cyclooxygenase/lipoxygenase (COX/LOX) inhibitor, (0.01 and 0.1 g/100 g diet) led to increased MDA contents in larvae and pupae, above 5 nmol MDA/mg protein in pupae. Dietary Phe led to higher MDA contents in larvae only at the highest dose, with about 11 nmol MDA/mg protein. In comparison with the controls, increased MDA content were identified in adult of host fed on diets containing high concentrations of Dex, Esc, Phe.

Larval CAT activity increased from about 2.5 nmol/mg protein/min in controls to about 5.5 nmol or more for larvae reared in the presence of 0.1 % or higher concentrations of Dex. Dietary Esc at the highest dose significantly increased CAT activities by about 2.5-fold in all developmental stages of wax moth, relative to controls (Fig. 2A). Dietary Phe led to about 2-fold increases in CAT activity in larvae reared on the highest, but not lower doses. The CAT activities increase with Dex, Esc, Phe were dose-dependent with activities in pupa and adult stage of *Galleria*.

Dex at the highest dose led to significantly increased larval and pupal wax moth GST activity, which increased from 115 nmol/mg protein/min for controls to about 155 nmol/mg protein/min for larvae and from 55 to 75 nmol/mg protein/min for pupae (Fig. 3A). High dietary Esc doses (0.1 and 1%) led to significantly increased GST activities in pupae and adults. The highest Esc dose Esc resulted in increased larval GST activity from about 90 to 150 nmol/mg protein/min relative to control. The Phe effect on GST activity in larval, pupal and adult stages was expressed in a dose-dependent manner, which reached 140 nmol/mg protein/min at the highest concentration and for larvae.

3. 2 The effects of dietary EBIs on MDA content, CAT activity, GST activity of *B. hebetor* developmental stages

The highest dietary Dex and Esc led to increased MDA concentrations, by over 30-fold in pupal and adult wasps reared on Dex- and Esc-treated hosts. MDA levels were not strongly influenced by dietary Phe except for hosts reared in the presence of the highest concentration (Fig. 1B). The highest Phe dose led to increased MDA concentrations, up by 2.5-fold to about 4.2 nmol MDA/mg protein in adult wasps and by about 6-fold in larvae and pupae.

Dietary Dex led to increased parasitoid larval and adult CAT activities at low concentrations, but not higher dietary concentrations. The highest Esc dose led to significantly increased CAT activity (Fig. 2B). All dietary Esc led to increased CAT activity in adult, but larval stage of parasitoids were significantly increased at highest concentration of Esc. Dietary Phe was associated with significantly increased CAT activity in parasitoid larvae, pupae and adults. The highest dose of Phe increased CAT activity in larval parasitoid by 7-fold, to over 7 nmol/mg protein/min.

GST activity in parasitoid larvae increased from about 4 nmol/mg protein/min to above 30 nmol/mg protein/min (Fig. 3B) for all dietary Dex concentrations. Pupal and adult wasp GST activity increased following development on experimental hosts reared in the presence of Dex at 0.1 and 1%. Wasp larvae expressed increased GST activity, about 4 nmol/mg protein/min for controls and about 8 nmol/mg protein/min for the highest Esc concentration. The Esc effect on pupal and adult GST activity was expressed in a dose-dependent manner, reaching a significant increase at high dose. The highest Phe concentration led to increased GST activity in adults, up by about 25-fold relative to controls. GST activity in pupal and adult wasps exposed to the highest Phe concentration was significantly increased.

4. Discussion

Büyükgüzel et al. (2010) reported that rearing wax moth larvae on diets amended with selected EBIs led to increased MDA and protein carbonyl content, and increased activities of the antioxidant enzymes, SOD, GST, GPx in larval hemolymph. The results reported here extend that idea and support our hypothesis that inhibition of eicosanoid biosynthesis leads to increased lipid peroxidation in our model host/parasitoid system because chronic dietary exposure to EBIs in host larvae led to increased MDA concentrations, and to increased activities of two antioxidant enzymes, CAT and GST, in hosts and their parasitoids. More to the point, the influence of dietary EBIs on hosts translated into reduced biological fitness parameters in wax moths and their parasitoids (Büyükgüzel et al., 2011). Survival of experimental wax moths declined with exposure to Dex, Esc and Phe. Dex treatments reduced survival of parasitoid adults and Esc exposure influenced survival of parasitoids. All three EBIs reduced fecundity and egg hatch in experimental parasitoids (Büyükgüzel et al., 2011). The outcomes of control experiments in this work and many other reports on eicosanoid actions in insects (Stanley and Kim, 2014) indicate the pharmaceuticals themselves do not influence insect fitness, from which it can be taken that eicosanoids mediate defense mechanisms against internal (aerobic metabolism) or exogenous (dietary) prooxidants, although the mediating mechanisms remain unclear.

It is not unexpected to find that eicosanoid signaling acts in protecting insect from oxidative damage. Eicosanoids mediate fundamental actions in insect biology (Stanley, 2006; Stanley and Kim, 2014). In insect reproduction, certain PGs act in follicle development (Tootle and Spradling, 2008), correct temporal expression of genes encoding egg shell proteins (Tootle et al., 2011) and in intracellular localization of Fascin, an actin-bundling protein (Groen et al., 2015). PGs may mediate such actions in all insects. PGs also release egg-laying behavioral programs in some species, particularly crickets (Stanley and Kim,

2014). Eicosanoids mediate a wide range of immunological actions, such as nodulation reactions to bacterial infection and responses to viral infections (Stanley and Shapiro, 2007; Büyükgüzel et al., 2007; Büyükgüzel, 2012; Durmuş et al., 2008). PGs act in insect renal function, modulating the rates of primary urine formation in Malpighian tubules from mosquitoes and ants (Petzel and Stanley-Samuelson, 1992; Van Kerkhove et al., 1995). Eicosanoids may also act in detoxification systems because cytochrome P450 activities were strongly increased in response to *Bt* endotoxins in larvae of *Manduca sexta* (L.), *Choristoneura fumiferana* (Clemens) and *Plodia interpunctella* (Hübner) exposed to LOX and COX inhibitors (Johnson and Howard, 1996). We expect the list of known eicosanoid actions in insects will increase over time.

Our data indicate that the influences of Dex, Esc and Phe occurred in dose-related manners in some, but not all experiments. The influence of dietary Dex on whole body MDA concentrations occurred in dose-response manners in adult and pupal hosts and in parasitoid pupae. There were virtually no dose-response connections for CAT activities, and only a few for GST activities. Several points help understand these results. First, the three EBIs used in this work operate at different points in eicosanoid biosynthesis. Dex is a glucocorticoid that inhibits PLA₂ activities in an indirect mechanism, Esc is a direct competitive inhibitor of certain LOXs and Phe a dual COX/LOX inhibitor. Second, the experiments were designed in a tritrophic format, the compounds moving from larval media to larvae and on to the parasitoids. There is very little information on insect metabolism and clearance of EBIs in insects. Miller and Stanley-Samuelson (1996) investigated the pharmacology of the COX inhibitor, indomethacin (Indo), in tobacco hornworms. They injected radioactive Indo into 5th-instar hornworms and found most radioactivity, still in the form of intact Indo, was cleared from circulation via Malpighian tubules by about 12 h after injection. The Indo was metabolized into more polar products by microbes within the frass. We do not have

information on the fates of dietary Dex, Esc and Phe in wax moth larvae, nor in the wasps, and it is not unreasonable to see experiments with these compounds do not yield uniformly linear results.

In our experiments, dietary EBIs led to increased CAT and GST activities, albeit in different ways among the developmental stages in hosts and parasitoids. In some stages the enzyme activities increased substantially. For example, GST activity increased by 8-fold in wasp larvae reared on Dex-treated hosts and by 25-fold in adults exposed to Phe. However, such large increases were not uniformly expressed in hosts nor parasitoids. On the understanding that the EBIs used in this work are not themselves pro-oxidant compounds, these irregular changes in anti-oxidant enzyme activities indicate to us that insects lacking eicosanoid biosynthesis do not modulate CAT and GST activities in the expected tightly regulated manner. This opens questions on the mechanisms of eicosanoid actions in modulating activities of the enzymes. PG treatments led to large changes in expression of protein-encoding genes in a lepidopteran cell line, HzAM1 cells (Stanley et al., 2008; 2012). Subsequent work with the same cell line shows that PG treatments also led to changes in relative phosphorylation of existing proteins (Stanley et al., in preparation). It follows that PGs can influence enzyme activities at transcriptional and post-transcriptional levels. As a working hypothesis, we suggest PGs can influence CAT and GST activities at more than one level.

Eicosanoids modulate antioxidant defence reactions in various mammalian species (Upmacis et al., 2006; Sanchez-Gomez et al., 2007). Studies with COX and LOX inhibitors including aspirin, NS-398, Esc, Indo and Phe, and the glucocorticoid, Dex, indicate that eicosanoids act in defense against oxidative stress-induced cytotoxicity, leading to neurodegenerative disorders (Kim et al., 2000), reduced cell viability (Schwenger et al., 1997; Hatamoto et al., 2006) and decreased neuronal survival (Wie et al., 1999) in some vertebrates.

With respect to invertebrates, PGE₂, at physiological concentrations (10⁻⁶ and 10⁻⁷M) decreased apoptosis, while a lower dose (10⁻⁸ M) increased apoptosis in a marine holothurian *Eupentacta fraudatrix* (Dolmatova and Zaika, 2007) as evidenced by decreased SOD, CAT and glutathione reductase activities. We infer eicosanoids generally operate in modulating the antioxidant defences in vertebrates and invertebrates.

MDA is a biological marker of oxidative stress, which we investigated in our system (Büyükgüzel et al., 2013). The data reported here indicate that pharmaceutical inhibition of eicosanoid biosynthesis in host larvae leads to increased tissue MDA concentrations in their parasitoids. We infer from this finding that eicosanoids mediate mechanisms operating in the clearance of MDA from cells. MDA can be formed as side products of certain enzymes involved in biosynthesis of some eicosanoids or via non-enzymatic processes. It is metabolized to CO₂ and H₂O via aldehyde dehydrogenase, decarboxylase, acetyl Co synthase and, finally, enzymes in the Krebs's cycle (Ayala et al., 2014). Based on earlier findings that some PGs influence gene and protein expression in an established insect cell line (Stanley et al., 2008; 2012), we suggest that EBIs lead to increased MDA accumulation by down-regulating synthesis of eicosanoids required to modulate expression of genes encoding one or more of the enzymes responsible for MDA metabolism. Our data also show that dietary EBIs lead to increased activities of two enzymes, CAT and GST, responsible for metabolizing ROS. As just outlined, we propose that PGs or other eicosanoids modulate activities of antioxidant enzymes via their influence on gene expression or protein phosphorylation.

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FIGURE LEGENDS

Fig. 1. The influence of three anti-inflammatory pharmaceuticals (Dex = Dexamethasone; Esc = Esculetin; Phe= Phenidone) on MDA content (nmol/mg protein) in the greater wax moth, *Galleria mellonella* (Panel A) and the parasitoid wasp *Bracon hebetor* (Panel B). Histogram bars indicate mean amount of MDA (± 1 SE; n = four biological replicates, 10 insects per replicate) at the indicated developmental stages. Values labeled with the same letter are not significantly different from each other within each developmental stages, $P \leq 0.05$, LSD test. Control diet (without eicosanoid inhibitor)

Fig. 2. The influence of three anti-inflammatory pharmaceuticals (defined in Fig. 1 legend) on CAT activity (nmol/mg protein/min) in the greater wax moth, *Galleria mellonella* (Panel A) and the parasitoid wasp *Bracon hebetor* (Panel B). Histogram bars indicate mean CAT activity (± 1 SE; n = four biological replicates, 10 insects per replicate) in the indicated developmental stages. Values labeled with the same letter are not significantly different from each other within each developmental stages, $P \leq 0.05$, LSD test. Control diet (without eicosanoid inhibitor).

Fig. 3. The influence of three anti-inflammatory pharmaceuticals (defined in Fig. 1 legend) on GST activity (nmol/mg protein/min) in the greater wax moth, *Galleria mellonella* (Panel A) and the parasitoid wasp *Bracon hebetor* (Panel B). Histogram bars depict mean GST activity (± 1 SE; n = four biological replicates, 10 insects per replicate) in the indicated developmental stages. Values labeled with the same letter are not significantly different from each other within each developmental stages, $P \leq 0.05$, LSD test. Control diet (without eicosanoid inhibitor).

Fig. 1. MDA

(A) *G. mellonella*

(B) *B. hebetor*

Fig. 2. CAT

(A) *G. mellonella*

(B) *B. hebetor*

Fig. 3. GST

(A) *G. mellonella*

(B) *B. hebetor*