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Eicosanoids influence insect susceptibility to nucleopolyhedroviruses

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ABSTRACT

Nine pharmaceutical inhibitors of eicosanoid biosynthesis (e.g., bromophenacyl bromide, clotrimazole, diclofenamic acid, esculetin, flufenamic acid, indomethacin, nimesulide, sulindac, tolfenamic acid) that increased the susceptibility of the gypsy moth, *Lymantria dispar* (L.), to the nucleopolyhedrovirus LdMNPV were tested against the beet armyworm *Spodoptera exigua* (Hübner), the corn earworm *Helicoverpa zea* (Boddie) and the fall armyworm *Spodoptera frugiperda* (J.E. Smith) and their respective NPVs to determine whether these compounds also alter the susceptibility of these insects. The susceptibility of the beet armyworm was increased by six inhibitors (bromophenacyl bromide, clotrimazole, diclofenic acid, esculetin, flufenamic acid, nimesulide). The susceptibility of the fall armyworm was increased by seven inhibitors, (bromophenacyl bromide, diclofenamic acid, esculetin, indomethacin, nimesulide, sulindac, tolfenamic acid), whereas the susceptibility of the corn earworm was increased by only one inhibitor (sulindac). The influence of the cyclooxygenase inhibitor, indomethacin was expressed in a concentration-related manner in beet armyworms. We infer from these findings that eicosanoids, including prostaglandins and lipoxygenase products, act in insect anti-viral defenses.

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1. Introduction

Insect immune functions are usually resolved into two categories, humoral and cellular (or hemocytic). Humoral immunity involves induced expression of genes encoding a wide range of anti-microbial proteins and research in this area has unveiled important new signal transduction systems (Lemaitre and Hoffmann, 2007). Hemocytic immunity is characterized by direct interactions between circulating hemocytes and invaders (Lavine and Strand, 2002; Stanley, 2006). Hemocytic reactions begin immediately an infection is detected, while anti-microbial proteins do not appear in hemolymph of infected insects until hours after infection. While insect cellular defense functions are well known, there is far less information on the signal transduction mechanisms responsible for mediating these functions (Gillespie et al., 1997). Prostaglandins (PGs) and other eicosanoids mediate several aspects of inset cellular immunity, including phagocytosis, nodulation and specific cell actions that lead to nodulation (Stanley et al., 2009). Eicosanoids are oxygenated metabolites of arachidonic acid and two other C20 polyunsaturated fatty acids and they are crucial mediators of many immune reactions in mammals. Chemical structures and biosynthetic pathways for these compounds are detailed elsewhere (Stanley, 2000, 2006).

Insect immune responses to viral infections form a rapidly emerging frontier of insect science. Anti-viral mechanisms include hemocytic encapsulation of virus-infected cells (Washburn et al., 1996), apoptosis (Clem, 2005; Dougherty et al., 2006), RNAi mechanisms (Saumet and Lecellier, 2006) and the presence of a constitutive phenoloxidase in lepidopteran plasma (Hoover et al., 2000; Popham et al., 2004). Eicosanoids also act in anti-viral functions. In work with an established insect cell line, Goodman et al. (2006) reported that treating cells with inhibitors of eicosanoid biosynthesis increased viral replication in a normally non-permissive cell line. Also Stanley and Shapiro (2007) recently suggested that eicosanoids act in larval susceptibility to viral infection in gypsy moth, *Lymantria dispar*. Two reports indicate that eicosanoids also mediate melantoic nodule formation in response to challenge with a vertebrate virus, *Bovine herpes simplex virus-1*, in larvae of the waxmoth, *Galleria mellonella* (Büyükgüzel et al., 2007) and of the parasitic wasp, *Pimpla turionellae* (Durmuş et al., 2008).

Based on these considerations, we set the hypothesis that eicosanoids generally influence lepidopteran susceptibility to NPV infections. Here we report the outcome of our experiments with three larval pest species, the beet armyworm, *Spodoptera exigua*, the fall armyworm, *Spodoptera frugiperda* and the corn earworm, *Helicoverpa zea* designed to test our hypothesis.

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2. Materials and methods

2.1. Insects and virus inocula

The colonized strains of the beet armyworm, *S. exigua*, the corn earworm, *H. zea*, and the fall armyworm, *S. frugiperda*, established and maintained by USDA-ARS, Tifton, GA were used. Larvae were reared on a wheat germ diet developed for gypsy moth (Bell et al., 1981). Nucleopolyhedroviruses (NPVs) for both the beet armyworm (SeMNPV) and the corn earworm (HzSNPV) were obtained from Certis USA (Columbia, MD) and the NPV from the fall armyworm (SfMNPV) was obtained from Dr. John Hamm (USDA-ARS, Tifton, GA). For production of fresh NPV, each virus was diluted to a concentration of 10^6 occlusion bodies (OBs) and 100 *S. exigua*, *H. zea*, and *S .frugiperda* (6 d old) were infected with their respective viruses (0.1 ml of virus and 1 larva per 30 ml container) (=1338 mm^2 diet surface; Sweetheart Cup, Chicago, IL) . Larvae were reared at 29 °C, 50% RH, and a photoperiod of 12:12 (L:D) for 14 d. All virus-killed larvae were collected and frozen (−20 °C) before being assayed against *S. exigua*, *H. zea* and *S. frugiperda*.

2.2. Chemicals

The following chemicals (pharmaceutical inhibitors of eicosanoid biosynthesis in mammals) were obtained from Sigma–Aldrich, St. Louis, MO: bromophenacyl bromide (0.036 M; CAS 93-73-0), clotrimazole (0.033 M; CAS 23593-75-1), diclofenac (0.031 M; CAS 15307-79-6), esculetin (0.056 M; CAS 305-01-1), flufenamic acid (0.036 M; CAS 530-78-9), indomethacin (0.028 M; CAS 53-86-1), nimesulide (0.032 M; CAS 51803-7802), sulindac (0.028 M; CAS 38194-50-2), tolfenamic acid (0.038 M; CAS 13710-19-5). All chemicals were diluted in distilled water.

2.3. Virus and test protocol

Occlusion bodies (OBs) from each virus were extracted from virus-killed larvae using standardized methodology (Shapiro et al., 1981). The insects were homogenized (e.g., each gram of tissue was blended in 9 gm distilled water and filtered through cheesecloth). The filtrate was collected (=stock virus suspension) and 1 ml of the stock suspension was diluted in 9 ml distilled water. A sample was removed by Pasteur pipette and the concentration of the suspension (1:10) was determined using a double-line hemacytometer with improved Neubauer ruling and phase microscopy (430× magnification).

Dilutions were made from the stock suspension either in distilled water (standard treatment) or in a chemical (test treatment) to produce concentrations ranging from 10^1 to 10^6 OBs/ml and 0.1 ml of a virus suspension was pipetted onto the diet surface (30-ml container; 1338 mm^2 = surface area). In addition, controls (e.g., distilled water, test chemical) were used. Second instars (4 d old) were placed individually in each container and were reared for 14 d at 29 °C, 50% RH, and a photoperiod of 12:12 (L:D). Tests were repeated eight times with 10 larvae per virus dilution per treatment per replicate (=$\sum$ 400 larvae per treatment) and 10 control (=$\sum$ 80 larvae) and 10 test control larvae per treatment per replicate (=$\sum$ 80 larvae per test chemical only).

2.4. Concentration–response study for Indomethacin

In a second series of tests, indomethacin was tested at different concentrations (e.g., 0.0035–0.056 M) against beet armyworm larvae, following procedures previously described. Tests were repeated six times with 10 larvae per virus dilution per treatment per replicate (=300 larvae per treatment; = 1500 total number of larvae) and 10 untreated control (=60 larvae) and 10 indomethacin-only larvae (=60 larvae per dilution; = 300 total larvae).

2.5. Statistical methods

Concentration-mortality regressions were calculated to determine the effects of eicosanoid inhibitors on virus activities. Slopes and LC_{50} s were obtained with the probit option of POLO (LeOra Software, 1987). Means were separated for significance according to Fisher's protected least significant difference (LSD) at $P \leqslant 0.05$ (Steel and Torrie, 1960).

3. Results

3.1. Toxicity studies

Direct observations of larval growth, turgidity and development indicate that none of the test compounds used in this study was toxic to the beet armyworms, fall armyworms or the corn earworms.

3.2. Single-concentration studies with inhibitors of eicosanoid biosynthesis

The influence of selected pharmaceutical inhibitors of eicosanoid biosynthesis on SeMNPV virulence toward beet armyworms is shown in Table 1. We report susceptibility to viral challenges

Table 1
The influence of pharmaceutical inhibitors of eicosanoid biosynthesis on the virulence of BAWNPV in beet army worm. LC_{50} values marked with * are significantly different from control values obtained by treating larvae with BAWNPV alone. LC_{50} values were calculated as OB/ml ÷ container surface area = OB/mm^2.

Treatment	Concentration (M)	LC_{50} (OB/mm^2)	95% CL	Activity ratio
BAWNPV alone		0.48	0.21–1.23	1.00
BAWNPV plus				
NSAID COX inhibitors				
Clotrimazole	0.033	0.11*	0.07–0.16	4.36*
Flufenamic acid	0.036	0.04*	0.03–0.06	10.75*
Indomethacin	0.028	0.36	0.26–0.50	1.33
Tolfenamic acid	0.038	0.21	0.16–0.27	2.28
Diclofenac	0.031	0.09*	0.07–0.12	5.01*
NSAID COX-2 inhibitors				
Nimesulide	0.032	0.11*	0.08–0.15	4.54*
Sulindac	0.028	0.18	0.13–0.25	2.67
LOX inhibitor				
Esculetin	0.056	0.12*	0.09–0.16	3.94*
PLA_2 inhibitors				
Bromophenacyl Bromide	0.036	0.09*	0.07–0.16	5.37*