Journal of Invertebrate Pathology 138 (2016) 18–23

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Effects of abnormal temperature and starvation on the internal defense system of the schistosome-transmitting snail *Biomphalaria glabrata*

Molly K. Nelson, Brandon C. Cruz, Kevin L. Buena, Hai Nguyen, John T. Sullivan *

Department of Biology, University of San Francisco, 2130 Fulton Street, San Francisco, CA 94117, United States

ARTICLE INFO

Article history:
Received 15 March 2016
Revised 20 May 2016
Accepted 30 May 2016
Available online 31 May 2016

Keywords:
Climate change
Internal defense system
Hemocyte
Hemopoiesis
Mollusca

ABSTRACT

Climate change may affect the internal defense system (IDS) of freshwater snails, and as a result their capacity to transmit disease. We examined effects of short-term exposure to supra- and sub-optimal temperatures or starvation on 3 parameters of the IDS of the schistosome-resistant Salvador strain of *Biomphalaria glabrata* — hemocyte concentrations, cell division in the amebocyte-producing organ (APO), and resistance to infection with *Schistosoma mansoni*. Adult snails were exposed to 1 of 3 temperatures, 20 °C, 27 °C (controls), or 33 °C, for 1 or 2 weeks, with food. A fourth group was maintained at 27 °C, but without food. Compared to the controls, starved snails had significantly higher hemocyte counts at both 1 and 2 weeks, although mitotic activity in the APO was significantly lower at both time periods. Exposure to 20 °C or 33 °C for 1 or 2 weeks did not affect hemocyte numbers. However, APO mitotic activity in snails exposed to 20 °C was significantly higher at both 1 and 2 weeks, whereas mitotic activity in snails exposed to 33 °C was significantly lower at 1 week but normal at 2 weeks. None of the treatments altered the resistance phenotype of Salvador snails. In a follow-up experiment, exposure to 33 °C for 4–5 h, a treatment previously reported to both induce expression of heat shock proteins (Hsps) and abrogate resistance to infection, caused immediate upregulation of Hsp 70 and Hsp 90 expression, but did not alter resistance, and Hsp expression levels returned to baseline after 2 weeks at 33 °C. Results of this study indicate that abnormal environmental conditions can have both stimulatory and inhibitory effects on the IDS in adult *B. glabrata*, and that some degree of acclimation to abnormal temperatures may occur.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Changes in global climate may affect the biology of disease-transmitting snails (Mas-Coma et al., 2009), presumably through effects on water temperature, water chemistry, food availability, and aquatic community structure. Several studies have attempted to predict the impact of climate change on schistosomiasis transmission (reviewed by McCreesh and Booth, 2013), and using an agent-based model incorporating effects of increasing temperature on both the snail host and larval parasite, McCreesh et al. (2015) have hypothesized that an increase in prevalence and intensity of schistosomiasis may occur in some parts of eastern Africa over the next several decades.

In *Biomphalaria glabrata*, an intermediate host of *Schistosoma mansoni* in the New World, a potential effect of climate change that could impact disease transmission is alteration of the immune or internal defense system (IDS), which largely determines the outcome of invasion by a larval trematode. The IDS of *B. glabrata* consists of both phagocytic hemocytes and plasma immune factors, and in incompatible *B. glabrata*–*S. mansoni* relationships, snail hemocytes recognize, then rapidly encapsulate and kill schistosome sporocysts (reviewed by Coustau et al., 2015). Hemocyte production occurs in a region of the anterior pericardial wall called the amebocyte-producing organ (APO) (Lie et al., 1975; Pila et al., 2016b), and is stimulated by an endogenous growth factor, *B. glabrata* granulin (Pila et al., 2016a). Hypothetically, environmentally-induced physiological stress could affect hemocytes, plasma factors, or hemopoiesis in the APO, and as a result host vector competence, making *B. glabrata* either less or more susceptible to infection with *S. mansoni* as well as microbial snail pathogens. For example, Ittiprasert and Knight (2012) have reported that exposure of 4–6 mm juvenile BS-90 (schistosome-resistant) *B. glabrata* to high temperature (32 °C) for 4 or more h causes upregulated expression of heat shock proteins (Hsps) 70 and 90. Moreover, this heat treatment abrogates resistance to infection with *S. mansoni*, apparently due to increased expression of Hsp

* Corresponding author.
E-mail address: sullivan@usfca.edu (J.T. Sullivan).

http://dx.doi.org/10.1016/j.jip.2016.05.009
0022-2011/© 2016 Elsevier Inc. All rights reserved.

90, as shown by reversal of this effect by geldanamycin, an inhibitor of Hsp 90.

The concentration of circulating phagocytic leukocytes is a commonly measured indicator of immune status in animals. For example, in humans prolonged and very low blood concentrations of neutrophils (chronic severe neutropenia) is associated with increased risk of microbial infection, and among several extrinsic causes of this condition are certain types of nutritional deficiencies that impair hemopoiesis (Newburger and Dale, 2013). In marine bivalves, a large number of studies have investigated effects of abnormal environmental conditions, e.g., toxicants, temperature, salinity, and starvation, on hemocyte concentrations, as well as on other parameters of the IDS, including susceptibility to infection with pathogens (partially reviewed by Raftos et al., 2014). Among gastropods, Al-Rawadeh (2010) reported a significant decrease in the number of hemocytes in *Helix aspersa* after 3 weeks of starvation. Stumpf and Gilbertson (1978) exposed *B. glabrata* to a range of temperatures, from 12 °C to 36 °C, for 3 days and observed a bell-shaped effect on hemocyte concentrations, with hemocyte numbers increasing from 12 °C to 27 °C and then decreasing at higher temperatures. Suresh et al. (1994) exposed 30 °C-acclimated *Lymnaea acuminata* and *Indoplanorbis exustus* to 20 °C, 25 °C, 35 °C, and 40 °C for up to 24 h, and found elevated cell counts at 20 °C and 25 °C after 2 and 12 h in *I. exustus* and at all time periods in *L. acuminata*. The highest temperature did not affect hemocyte numbers in *I. exustus* but in *L. acuminata* caused an increase after 2 h of exposure and a sharp decrease at 24 h. Hypothetically, the observed changes in hemocyte numbers could result from direct effects on hemocyte survival and/or distribution between hemolymph and tissues, as well as effects on hemocyte production.

The hypothesis for this study is that short-term abnormal environmental conditions will cause physiological stress, broadly defined as a condition that challenges homeostasis (Kagias et al., 2012), and that such stress may affect the IDS of *B. glabrata*. Relative to "normal" and "abnormal" environmental conditions, *B. glabrata*, which is endemic to South America, has been raised in the laboratory at temperatures ranging from 20 to 29 °C (Bruce et al., 1971), with an optimal temperature in the range of 24–28 °C (Eveland and Haseeb, 2011). Survival is limited to several days at 7 °C and only 4 h at 42 °C (Maldonado, 1967). Freshwater pulmonate snails normally feed on algae (Palmieri et al., 1978) and decaying plant matter (Rollinson, 2011), but in the laboratory *B. glabrata* grows and reproduces well on a diet of Romaine lettuce leaves. Young adult snails (10.1–13 mm, shell diameter) are able to survive without food for at least 22 days, although their O_2 consumption declines significantly (van Aardt et al., 2003).

In this study, we examined effects of 1- and 2-week exposures to supra- and sub-optimal temperatures or starvation on 3 parameters of the IDS of the schistosome-resistant Salvador (BS-90) strain of *B. glabrata*, i.e., hemocyte concentrations, mitotic activity in the APO as a measure of hemocyte production, and resistance to infection with *S. mansoni.*

2. Materials and methods

2.1. Snails

Schistosome-resistant Salvador (Paraense and Correa, 1963) and schistosome-susceptible M-line (Newton, 1955) *B. glabrata* were reared in aerated aquaria at room temperature, which varied from 22 to 25 °C during this study, and were fed a diet of Romaine lettuce. Because acquiring sufficient hemolymph from small snails may require pooling samples (Jeong et al., 1980), adult snails, measuring 10.5–13 mm, were used for all experiments in order to obtain individual hemocyte counts.

2.2. Miracidia

Livers were removed from mice experimentally infected with the NMRI strain of *S. mansoni* at the Biomedical Research Institute (Rockville, MD), chilled with refrigerant gel cold packs, and shipped overnight to the University of San Francisco (USF). Upon receipt of the livers, miracidia were harvested by a previously described method (Sullivan and Richards, 1981).

2.3. Exposure to different environmental conditions

Salvador snails were individually exposed in 500-ml jars in incubators maintained at 20 °C, 27 °C, or 33 °C for 1 or 2 weeks, with food. Incubators were not equipped with illumination, and consequently snails were exposed in the dark. Water was brought to the appropriate temperature before snails were added. A fourth group was maintained at 27 °C, but without food. A total of 30–31 snails were used for each treatment. For this study, 27 °C with food was considered the normal or control environmental condition.

2.4. Hemocyte and mitotic figure counts

After 1 or 2 weeks of exposure to each treatment, a total hemocyte count was obtained for each snail by counting all adherent cells in a 2-µl sample of hemolymph, as described previously (Sullivan et al., 2016). The bleeding technique involved puncturing the body wall through a hole made in the shell overlying the ventral side of the digestive gland and collecting hemolymph that welled up in the shell depression (Jeong et al., 1980). After snails were bled for hemocyte counts, the pericardial sac was dissected, fixed in 1/3-strength Bouin's fluid for at least 24 h, before being dehydrated in an isopropanol-xylene series and embedded in paraffin. Tissues were then serially sectioned at 7 µm. Sections were mounted on microscope slides, and stained with Delafield's hematoxylin and eosin. Rows of stained sections were scanned at 100× to locate the section that appeared to possess the most hemopoietic tissue (identifiable by its location and staining), and then mitotic activity was estimated by counting total numbers of mitotic figures in that section and the 2 sections on either side at 1000× (5 sections/APO). Counts were obtained for 25–30 APOs from each treatment.

2.5. Exposure to miracidia

Groups of 11–15 adult *B. glabrata,* pooled in 2 L of water in covered plastic rectangular containers, were subjected to the above 4 treatments for 1 or 2 weeks, and were then individually challenged with 25 miracidia of *S. mansoni.* Incubations were set up such that 1-and 2-week treatment groups were exposed simultaneously to the same batch of miracidia. This experiment was conducted on 3 separate occasions, using 3 different batches of miracidia. Infection controls consisted of 3 groups of 15 schistosome-susceptible M-line strain adult snails that had been reared in aquaria prior to miracidial challenge. Two of the 3 control groups were exposed simultaneously with the experimental groups; the third control group was exposed to a separate batch of miracidia. Snails were exposed to miracidia overnight at room temperature in 4 ml of water in 35-mm plastic Petri dishes immediately upon removal from the treatment temperature. Unlike the procedure of Ittiprasert and Knight (2012), we did not incubate the snails overnight in distilled water containing ampicillin beforehand. Following exposure to miracidia, snails were maintained at 27 °C with food, again in the dark, until they were dissected and assessed

Download English Version:

https://daneshyari.com/en/article/4557494

Download Persian Version:

https://daneshyari.com/article/4557494

Daneshyari.com