Cold shock and heat shock: a comparison of the protection generated by brief pretreatment at less severe temperatures

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Abstract. Brief exposure to low (0°C) or high (40°C) temperature elicits a protective response that prevents injury when the flesh fly, Sarcophaga crassipalpis Macquart, is subjected to more severe cold (−10°C) or heat (45°C). Both the low and high temperature responses were found in all developmental stages of the fly, but were most pronounced in the pupal and pharate adult stages. The protective responses generated by brief exposure to 0 or 40°C appear similar in that both result in a rapid acquisition of cold or heat tolerance and a loss of protection after the flies are returned to 25°C. The protection generated by chilling is obvious within 10 min of exposure to 0°C while a 30 min exposure to 40°C is required to induce the high temperature protection. High temperature protects against cold shock injury within a narrow range (around 36°C) but we have no evidence that low temperature can protect against heat injury. We previously demonstrated that the rapid increase in cold tolerance correlates with concomitant increases in glycerol concentration, but in this study we found no significant elevation in glycerol in heat-shocked flies. Thus the physiological and biochemical bases for the rapid responses to cold and heat appear to be different.

Key words. Cold shock, heat shock, glycerol, flesh flies, Sarcophaga crassipalpis.

Introduction

Two types of cold injury are generally recognized in cryobiology: freezing injury which results from ice formation and chilling injury which occurs without ice nucleation. Cold shock is a form of chilling injury observed after rapid cooling but in the absence of ice formation in extracellular fluids. The severity of cold shock injury increases with higher rates of cooling, increased time of exposure, and the final low temperatures to which the organism is exposed. This form of injury has been described in a variety of organisms ranging from bacteria to mammalian embryos (Morris et al., 1983; Watson & Morris, 1987). A number of insects have a rapid cold-hardening response that protects against cold shock injury (Chen et al., 1987; Lee et al., 1987a). For example, pharate adult flesh flies cannot tolerate exposure to −10°C for 2 h even though their supercooling point is −23°C, but they can readily survive
-10°C for 2 h if they are first exposed briefly to 0°C. A rapid accumulation of glycerol appears to provide at least a partial basis for this cryoprotective response against cold shock injury.

In concordance with cold shock, heat shock is the form of stress caused by rapid exposure to high temperatures. A brief pretreatment at high temperatures results in protection against lethal high temperature injury in Drosophila and other organisms (Petersen & Mitchell, 1987). Expression of the heat shock genes and synthesis of heat shock proteins appears to be a universal response of organisms to high temperature exposure (Schlesinger et al., 1982; Sheldon & Berger, 1988), and many researchers have suggested a protective role for heat shock proteins against thermal injury.

Though exposure to certain high or low temperatures can cause a rapid biological and biochemical response for protection against heat or cold injury, the nature of these responses and the relationship between the mechanisms for these protections remains largely undefined. In our work with flesh flies we demonstrate that although the rapid cold and heat responses have many similar attributes, they may have different physiological and biochemical bases.

**Materials and Methods**

Experimental samples were from our laboratory colony of the flesh fly, Sarcophaga crassipalpis Macquart. Adults and their progeny were reared in a LD 15:9 h photocycle, and at 25°C. Under these long-day conditions, no pupae entered diapause. Test tubes (10 x 1.5 cm) containing fifteen samples from different stages were either pretreated (at 0 or 40°C) or remained at the control temperature (25°C) before exposure to extreme low (-10°C) or high (45°C) temperatures (see details about timings of the pretreatments and subsequent treatments in the results section). Each treatment consisted of three replicates of fifteen flies. Pretreatments at 0°C were conducted in a insulated container filled with ice, and -10°C was obtained using a Lauda RMT-20 (Brinkmann) low-temperature bath filled with water and ethylene glycol (1:1). High temperature treatments (40°C and 45°C) were carried out in a regulated water bath. All samples were returned to room temperature (25°C), and the emergence percentage was recorded.

Low molecular weight polyols were analysed by high performance liquid chromatography (Waters Associates) as described by Lee et al. (1983) and Chen et al. (1987). Glycerol concentrations were expressed in mu units based on water-content data reported by Adedokun & Denlinger (1985) for corresponding developmental stages of the same species.

**Results**

**Optimal temperatures for eliciting protection**

To determine the temperatures that provide protection against cold or heat shock injury, pharate adults of S. crassipalpis were exposed to various temperatures (0–45°C) for 2 h before subjecting the flies to a 2 h exposure to -10 or 45°C (Fig. 1). Optimal temperatures for generating protection against cold shock injury occur between 0 and 5°C. Temperatures that generate protection against heat shock injury range between 36 and 40°C. A 2 h exposure to 36°C also protects against cold shock injury (40% survival). However, no temperatures below 25°C protected against heat shock injury.

**Fig. 1.** Success of adult emergence when pharate adults (red-eye stage) were pretreated for 2 h at the various temperatures shown on the x axis and then exposed for 2 h to either -10°C (solid bars) or 45°C (open bars). x ±SE, per cent adult survival to emergence (three replicates of fifteen flies each).
Fig. 2. Ontogenetic patterns of the cold and heat shock responses. Nondiapause flies (LD 15:9 h) were tested for their tolerance to various durations of exposure to −10°C or 45°C (as indicated on the x-axis). Flies were either transferred directly from 25°C to −10 or 45°C (open circles) or first chilled (0°C) or preheated (40°C) for 2 h before being transferred to −10 or 45°C (solid circles). A, B = feeding phase of the third larval instar; C, D = wandering phase of the third larval instar; E, F = 4 days after pupariation; G, H = 10 days after pupariation; I, J = 1-day-old adults. x ± SE, per cent survival to adult emergence (three replicates of fifteen flies each).
Ontogenetic patterns of the cold shock and heat shock responses

Different developmental stages of the flesh flies were exposed to -10°C (cold shock) or 45°C (heat shock) for various durations (open circles in Fig. 2). Pupae (Fig. 2, E and F) and stages thereafter have a higher cold and heat tolerance than larvae (Fig. 2, A–D). All stages increased cold or heat tolerance when they were first chilled (0°C) or preheated (40°C) for 2 h (solid circles in Fig. 2), but the largest increases in tolerance were observed in pupal and pharate adult stages. The protection elicited by pre-exposure to low or high temperatures tended to diminish as exposure time to the extreme temperatures (−10 or 45°C) was increased.

Duration of protection generated by brief periods of chilling and heating

Though pharate adults (red-eye stage) died when they were transferred from 25 to −10°C for 2 h (LD50 = 83 min, Fig. 2G, open circles), they successfully tolerated −10°C beyond 4 h if they were chilled 2 h at 0°C immediately before exposure to −10°C (Fig. 3). Similarly, pharate adults reared at 25°C could not tolerate a 2 h exposure to 45°C (LD50 = 85 min, Fig. 2H, open circles), but a 2 h pre-exposure to 40°C protected against injury at 45°C. However, the protection generated by a 2 h exposure to 40°C dropped rapidly when flies were exposed to 45°C for longer than 3 h (Fig. 3). Thus, under these experimental conditions the high temperature protection generated at 40°C is eroded more rapidly than the low temperature protection generated at 0°C.

Initiation of the protective responses

Cold tolerance increased markedly within 30 min (90% survival) of exposure to 0°C (Fig. 4). In contrast, a 30 min exposure to 40°C was not sufficient to provide protection against heat injury at 45°C. A 60 min exposure to 40°C achieved only about 50% survival at 45°C, and maximum protection was achieved only after a 2 h exposure to 40°C. These results indicate that, for the experimental conditions we describe, the protective response of chilling is achieved much more rapidly than the protective response elicited by heating.

Decay of protection

Pharate adults were chilled 2 h at 0°C or heated 2 h at 40°C and then, following various times of exposure to 25°C, were exposed for 2 h to −10 or 45°C. The results from this experiment

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**Fig. 3.** Duration of protection generated by a 2 h exposure to 0 or 40°C when flies were then exposed to −10 or 45°C for the various durations shown on the x axis. Flies were tested as pharate adults (red-eye stage). x ± SE, per cent survival to adult emergence (three replicates of fifteen flies each). ○, Cold shock; ●, heat shock.

**Fig. 4.** Initiation of the protective responses generated by chilling (0°C) and heating (40°C). After various periods at 0 or 40°C (as indicated on the x axis) flies were transferred for 2 h to −10 or 45°C. Flies were tested as pharate adults (red-eye stage). x ± SE, per cent survival to adult emergence (three replicates of fifteen flies each). ○, Cold shock; ●, heat shock.
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decay rates for the protective responses. Protection against both cold and heat injury decreased rapidly within 2 h at 25°C (Fig. 5).

**Restoration of protection**

Protection generated by brief chilling or heating can be repeatedly lost and regenerated (Fig. 6). Exposure of flies to 0°C for 2 h immediately before transfer to -10°C increased cold tolerance markedly, regardless of the fly's previous temperature history. In contrast, no survival was observed at -10°C if the previous 2 h were spent at 25°C. The results for high temperature were similar, i.e. all the treatments with a 2 h exposure to 40°C immediately before exposure to 45°C increased heat tolerance, and the protection could be repeatedly lost and regained.

**Changes in glycerol concentration**

Glycerol appears to be the major low molecular weight cryoprotectant used by *Sarcophaga* (Lee et al., 1987b), and glycerol concentrations of non-diapause pharate adults of *S. crassipalpis* increase 2–3-fold in response to a 2 h exposure to 0°C (Chen et al., 1987). However, in this experiment we failed to detect a significant difference (Student’s t-test, P>0.05) of glycerol concentrations between controls at 25°C (10.7±1.8 μM, mean ±SE, n = 6) and pharate adults exposed to 40°C for 2 h (7.8±1.4 μM, n = 6).

**Discussion**

Brief exposure to a mildly low temperature stimulates insects to undergo a rapid physiological adjustment enabling them to survive short-term exposure to even lower temperatures that would otherwise be lethal (Chen et al., 1987; Lee et al., 1987a; Czajka & Lee, 1990). At high temperatures a similar response is well documented, especially for *Drosophila melanogaster* (Mitchell et al., 1987); mildly high temperature provides protection against injury at higher temperature. Our results demonstrate the protective properties of both mild low and high temperature responses in the flesh fly. The patterns of protection generated by short-term cold and heat exposure appear quite similar in *S. crassipalpis*: both are rapid responses that provide protection from injury at more extreme temperatures. The capacity to generate protection is found in all developmental stages but is expressed to a greater extent in certain stages. Under our experimental conditions the greatest protection was attained by pharate adults. Over 90% of the pharate adults pretreated at 0 or 40°C survived while flies transferred directly from 25°C could not survive a 2 h exposure to low (-10°C) or high (45°C) temperatures.
Thus, both mild cold and heat exposure elicit physiological adjustments that provide protection against injury at more severe temperatures. Though mild cold and heat exposure markedly increase cold and heat tolerance, the protection generated only persists for about 2 h. Protection, however, could be repeatedly induced. This type of physiological response thus differs from the long-term physiological adaptations of many arthropods that have evolved some form of diapause or dormancy to tolerate long periods of extreme cold or heat.

In several aspects, the responses to high and low temperature differ. While we demonstrated that the protective responses can be invoked rapidly, a 10 min exposure to 0°C allows nearly a 3-fold increase of survival in flesh flies exposed to -10°C, but heat tolerance is less rapid: a 30 min exposure to 40°C is required to provide protection against injury at 45°C. The optimal temperature range for generating protection against cold injury is 0–5°C in S.crasipalpis. The best temperatures to protect flies against high lethal temperature is 36–40°C. Though heat exposure can provide protection against cold shock injury, low temperature exposure did not prevent heat injury in our experimental conditions. In D.melanogaster, high temperature can also protect against cold injury (Burton et al., 1988): exposure to 34°C dramatically increased survival of larvae at 0°C.

We previously demonstrated that glycerol
concentration is rapidly elevated at low temperature and is thus likely to be at least partially responsible for the protective action of chilling in *S. crassipalpis* (Chen et al., 1987). A 2 h exposure of flies to 0°C was adequate to elevate glycerol levels 2–3-fold, but we find no significant elevation of glycerol in response to high temperature. This result, of course, does not rule out the possibility that other low molecular weight substances may play a role in heat shock protection.

The heat shock proteins, which have been well studied in many organisms (Petersen & Mitchell, 1985; Sheldon & Berger, 1988), have also been characterized in *Sarcophaga* (Bultmann, 1986; Joplin & Denlinger, 1990). These proteins appear to play a protective role against injury at high temperatures, but exactly how the protection is achieved remains unresolved. Protection is perhaps accomplished by the prevention of protein denaturation or by maintaining polypeptides and proteins in conformations suitable for translocation across membranes (Chirico et al., 1988; Deshaies et al., 1988). Heat shock proteins may also play a role in lysosomal degradation of intracellular proteins (Chiang et al., 1989). Cold stress can also elicit the synthesis of heat shock proteins in *D. melanogaster* (Burton et al., 1985) and unique proteins in higher plants (Guy et al., 1985). In *Dicyostelium discoideum*, both heat (30°C) and cold (4°C) exposure can induce a developmentally regulated membrane protein gene (Maniak & Nellen, 1988). Thus, the possibility that high and low temperatures both elicit expression of the same stress-related proteins remains a viable option in *Sarcophaga*, and this possibility is currently being tested. Our failure to detect elevation of glycerol in heat-shocked flies, and the fact that chilling does not offer protection from heat injury implies, however, that the physiological bases for these two responses to thermal stress are likely to be different.

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References


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