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DOI: 10.1590/S1519-69842003000300010

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EXPERIMENTALLY INDUCED HEAT- AND COLD-SHOCK TOLERANCE IN ADULT *Panstrongylus megistus* (BURMEISTER) (HEMIPTERA, REDUVIIDAE)

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Received February 22, 2002 – Accepted June 10, 2002 – Distributed August 31, 2003

(With 4 figures)

ABSTRACT

The survival rate of domestic male and female adult *Panstrongylus megistus* was studied after sequential heat and cold shocks in order to investigate shock tolerance compared to that previously reported for nymphs. Sequential shocks were such that a milder shock (0°C, 5°C, 35°C, or 40°C for 1 h) preceded a severe one (0°C or 40°C for 12 h), separated by intervals of 8, 18, 24, and 72 h at 28°C (control temperature). The preliminary thermal shock induced tolerance to the more severe one, although tolerance intensity depended on the initial shock temperature and the interval between treatments. Despite the observed tolerance, the survival rate for insects subjected to both shocks decreased when compared to that of individuals subjected to a single mild shock. When tolerance differed with sex, females showed greater values than males. In contrast to the response detected in nymphs, for which higher heat tolerance values were sustained for intervals of up to 24 h (preliminary shock, 35°C) or even longer (preliminary shock, 40°C) between sequential shocks, significant values were verified in adults only for shock intervals of up to 8 h (preliminary shock, 40°C). While findings for nymphs exhibited considerable cold-shock tolerance under conditions in which preliminary shocks were given at 5°C or 0°C and the periods between shocks were up to 72 h long, the adults were shown to be capable of acquiring a substantial tolerance response to a more severe cold shock only when the preliminary shock was given at 0°C and shock interval surpassed 18 h. It is assumed that the mechanisms involved in the cellular protection of *P. megistus* under sequential temperature shocks (heat shock protein action?) may lose effectiveness with insect development.

Key words: *Panstrongylus megistus*, adults, heat shock, cold shock, survival, tolerance.

RESUMO

Tolerância ao calor e ao frio experimentalmente induzida em alados de *Panstrongylus megistus* (Burmeister) (Hemiptera, Reduviidae)

A taxa de sobrevivência de alados domiciliares machos e fêmeas de *Panstrongylus megistus* foi analisada, após choques sequenciais quentes e frios, a fim de investigar aquisição de termotolerância em comparação a dados já reportados para ninfas. Os insetos foram submetidos a choques térmicos sequenciais, sendo que um choque mais suave (0°C, 5°C, 35°C ou 40°C por 1 h) precedeu o choque mais severo (0°C ou 40°C por 12 h). Estes foram separados por intervalos de 8, 18, 24 e 72 h a 28°C (temperatura-controle). Demonstrou-se que o choque térmico preliminar pode induzir tolerância ao mais severo, embora a intensidade de tolerância seja dependente da temperatura inicial do choque e do intervalo entre os tratamentos. Apesar da tolerância observada, a taxa de sobrevivência diminuiu

em insetos submetidos a ambos os choques quando comparados àqueles espécimes submetidos a um único choque suave. Quando a tolerância diferiu com o sexo, as fêmeas apresentaram tolerância maior. Diferentemente da resposta observada em ninfas, para as quais a tolerância a choques quentes se estende até quando o período entre os choques é de 24 h (choque inicial a 35°C) ou mais (choque inicial a 40°C), taxa expressiva de tolerância nos adultos foi verificada apenas em condições de até 8 h de intervalo entre os choques, sendo o choque inicial dado a 40°C. Também diferindo do que acontece às ninfas, cuja tolerância ao choque frio se expressa em todas as condições semelhantemente estudadas, nos adultos observou-se expressiva tolerância ao choque frio apenas quando o choque inicial foi dado a 0°C e o tempo entre choques seguidos ultrapassou 18 h. Presume-se que em *P. megistus* os mecanismos que desempenham papel na proteção celular a choques seqüenciais de temperatura (proteínas de choque?) possam perder a eficácia com o desenvolvimento do inseto.

Palavras-chave: *Panstrongylus megistus*, adultos, choque quente, choque frio, sobrevivência, tolerância.

INTRODUCTION

According to the World Health Organization, Chagas' disease affects 20 million people, mostly in Central and South America. In Brazil, Chagas' disease is the third greatest cause of death by infectious parasitic diseases (Silveira & Rezende, 1994; Dos Reis, 1997), with vectorial transmission being the principal route of infection. Studies of the responses of blood-sucking insects to various stress factors represent an important approach to understanding how to control these species (Rodrigues *et al.*, 1991; Silva & Silva, 1993; Garcia *et al.*, 1999; Schmuñis, 2000).

Panstrongylus megistus (Burmeister), a blood-sucking hemipteran, is an important vector of Chagas' disease in Brazil because of its wide geographical distribution, high rates of infection, and its ability to adapt to artificial ecotopes (Forattini, 1980). *P. megistus* is native to Brazil and increasingly associated with human habitations because of the extensive destruction of its natural habitats (Silveira, 2000).

Following exposure to stress agents, changes in the survival rate, molting incidence, and nuclear phenotypes of *P. megistus* have been observed (Mello, 1978; Mello & Raymundo, 1980; Garcia *et al.*, 1999, 2000a, b). Similarly, heat and cold shock tolerance has been reported for *P. megistus* nymphs when they are subjected to sequential thermal shocks (Garcia *et al.*, 2001a, b). However, cold-shock tolerance differed from heat-shock tolerance in nymphs of *P. megistus*. Heat-shock tolerance varied with the time between sequential shocks, thus being transiently controlled, whereas cold-shock tolerance did not change when intervals of 8-72 h between

shocks were considered. In addition, sequential cold shocks produced a higher molting incidence when compared to sequential heat shocks (Garcia *et al.*, 2001a, b).

Considering that differences in survival rates after single heat or cold shocks have been reported when adults are compared to nymphs and that thermal shock response in adults varies with sex (Garcia *et al.*, 1999), the survival rate of *P. megistus* adults after sequential heat and cold shocks may also differ. In the present study, therefore, survival rates after sequential heat and cold shocks, in which a mild shock precedes a severe one, were investigated in male and female adults of *P. megistus*.

MATERIAL AND METHODS

Domestic adult male and female *P. megistus* specimens and reared in the laboratory at Sucen (Mogi-Guaçu, SP) of domestic habit were used. The insects were separated into groups of 50 specimens, each of which was put into glass cylinders (total: 21) which were then covered with cheesecloth and fastened with elastic bands. The insects were fasted for 15 days before being subjected to heat shock (35°C or 40°C for 1 h) or cold shock (5°C or 0°C for 1 h) followed by a return to their optimal rearing temperature (28°C). After various intervals (8 h, 18 h, 24 h, 72 h) following the mild shock, the insects were subjected to a more severe treatment (12 h at 40°C or 0°C).

The temperatures of 40°C and 0°C were chosen because of the need to use relatively extreme temperatures in comparison to those of the control (28°C), the operational facilities, and for comparison

with previous data on *P. megistus* (Garcia *et al.*, 1999; 2001a, b). Based on previous results, temperatures of 35°C and 5°C were chosen as milder shock temperatures (Garcia *et al.*, 2001a, b). A single shock at 40°C for 1 h has been reported to induce a less severe fall in the survival of adult specimens compared to that brought on by a single shock at 0°C for the same period (Garcia *et al.*, 1999). Single shocks at 40°C and 0°C for 12 h are lethal to both male and female *P. megistus* adults (Garcia *et al.*, 1999).

The choice of a moderate fasting condition prior to the shock assays was based on previous data which demonstrated a slightly better opportunity for survival of males, thus nourished and of domestic habit, under cold-shock conditions (Garcia *et al.*, 1999). Immediately after the shock assays the insects were returned to the 28°C temperature, fed once a week on hen's blood, and monitored daily for 30 days to investigate survival rates changes.

Five control groups were used: 1. insects maintained at 28°C but not subjected to heat or cold shock; 2. insects subjected to a single cold shock at 0°C (1 h); 3. insects subjected to a single cold shock at 5°C (1 h); 4. insects subjected to a single heat shock at 35°C (1 h); and 5. insects subjected to a single heat shock at 40°C (1 h).

The survival rates were compared using the Mantel-Hantzel non-parametric test, the Cox proportional hazard semi-parametric test, and a Weibull parametric test (Kalbfleisch & Prentice, 1980).

RESULTS

For consistency with previous papers (Garcia *et al.*, 2001 a, b), only results obtained with Mantel-Hantzel non-parametric test are presented, although all three statistical tests gave similar results.

Total sample (1050 specimens)

There was strong evidence of a difference among treatments when all the subgroups ($W = 69.1$, $p = 0.000$) and both sexes ($W = 9.6$, $p = 0.002$) were considered.

Control samples of each subgroup (250 specimens)

When control groups 1 to 5 were compared among themselves, there was strong evidence of a difference in survival ($W = 14.4$, $p = 0.006$). However, there was no difference for sex ($W = 1.2$, $p = 0.280$).

Insect survival in each control group was 70% for 1 h at 0°C; 68% for 1 h at 5°C; 96% for 1 h at

35°C; 76% for 1 h at 40°C; and 80% for unshocked insects. Each shock (heat or cold, short or long) decreased insect survival (Figs. 1.2, 2.2, 3.2, and 4.2). The groups in which most insects survived were those which received no shock or which were subjected to a shock at 35°C, followed by those which received shocks at 40°C, 0°C, and 5°C.

Insects subjected to a single heat (35°C and 40°C) or cold (0°C and 5°C) shock

Insects subjected to a single shock at 35°C (group 4) showed results that differed significantly from the ones of those subjected to a single shock at 40°C (group 5) ($W = 394.8$, $p = 0.000$). A similar result was obtained with a cold shock at 0°C (group 2) compared with that at 5°C (group 3) ($W = 307.5$, $p = 0.000$). There was also a difference when the survival of insects subjected to single heat shocks (groups 4 and 5) was compared with that of insects subjected to single cold shocks (groups 2 and 3).

The survival for males plus females subjected to sequential heat shocks was 37%, whereas the survival for those subjected to cold shocks was 67%. However, when comparing control groups that received single heat shocks (4 + 5) with control groups that received single cold shocks (2 + 3), the survival rate was greater for heat-shocked insects (86%; cold-shocked insects, 69%).

Insects subjected to an initial shock at 0°C for 1 h

Since the survival curves obtained after all the treatments differed significantly at $p \leq 0.05$, multiple comparisons were necessary to identify the different curves. To guarantee an overall significance level of 0.05, a p value of 0.01 was used for subsequent tests which compared the curves.

There was significant difference when a single shock at 0°C (group 2) was compared with sequential shocks at this temperature ($W = 248.2$, $p = 0.000$). The insects subjected to sequential cold shocks at 0°C differed significantly from each other ($W = 15.9$, $p = 0.001$). When the shocks were separated by intervals of 18, 24, or 72 h at 28°C, these insects survived longer than those subjected to shocks separated by an 8-h interval. The survival rates of insects subjected to sequential cold shocks with intervals of 18 h and 24 h at 28°C between shocks were similar to each other ($W = 0.8$, $p = 0.359$). The survival curve of insects whose interval between shocks was 72 h and that of insects subjected to a single shock at 0°C were similar (Fig.

1.2; $W = 0.7$, $p = 0.397$). Survival was not affected by sex ($W = 2.8$, $p = 0.092$; Fig. 1.1). The relative frequency of survival confirmed the difference between control group 2 and that of the insects subjected to two shocks.

Insects subjected to an initial shock at 5°C for 1 h

Although the survival rate of insects subjected to cold shock 18 h after the first shock was apparently higher than of the remaining groups, including control group 3, the difference was not statistically consistent ($W = 7.8$, $p = 0.100$; Fig. 2.2).

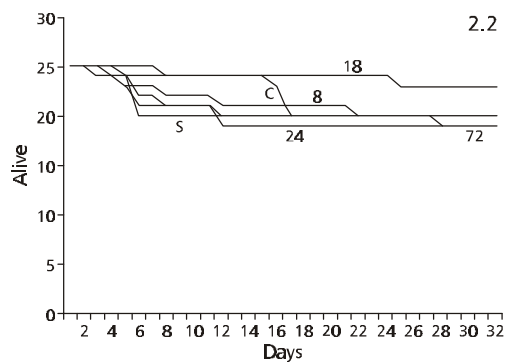
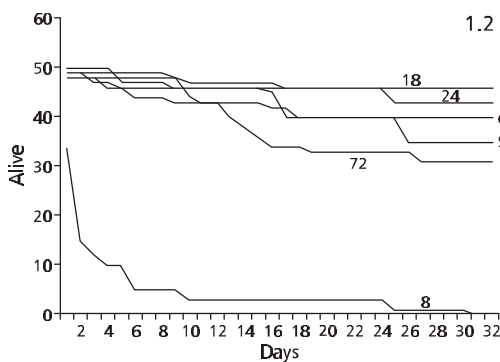
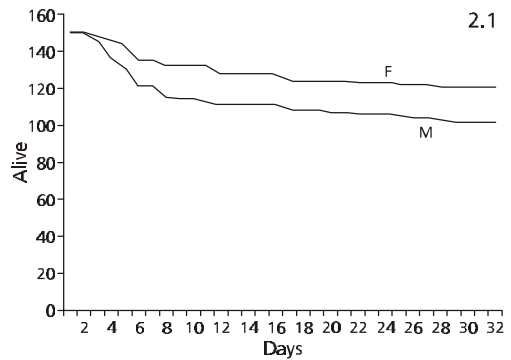
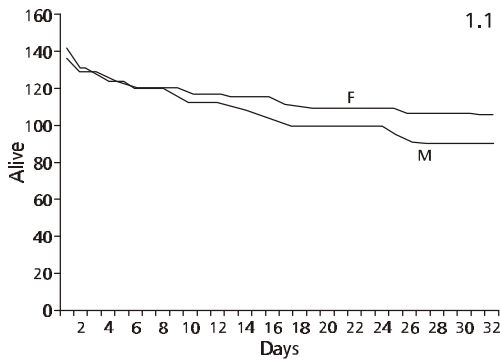
Comparison of control group 3 with insects subjected to sequential shocks showed that the female survival rate was greater than that of males ($W = 7.8$, $p = 0.005$; Fig. 2.1).

Insects subjected to an initial shock at 35°C for 1 h

There was a significant difference between survival of control group 4 and that of insects subjected to sequential shocks separated by various periods at 28°C ($W = 55.2$, $p = 0.000$), with control group 4 surviving longer than insects subjected to sequential shocks. There was no difference among insects subjected to sequential shocks separated by 8, 18, 24, and 72 h at 28°C ($W = 1.0$, $p = 0.801$; Fig. 3.2), although females always survived longer than males (Fig. 3.1).

Insects subjected to an initial shock at 40°C for 1 h

There was strong evidence of a difference in the survival rate of control group 5 compared to that of insects subjected to sequential heat shocks ($W = 164.8$, $p = 0.000$).



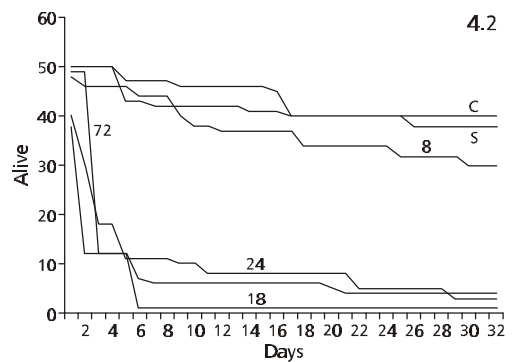
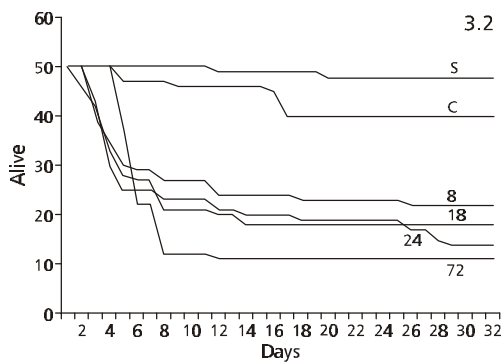
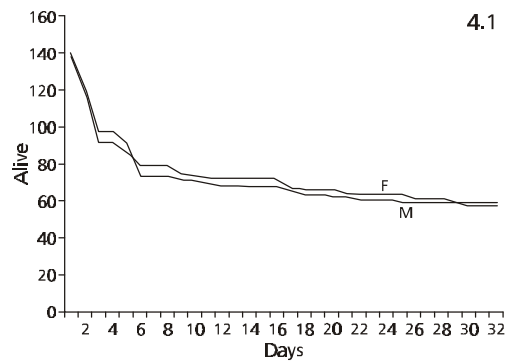
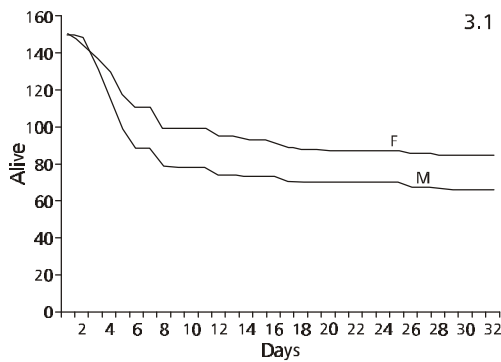
Figs. 1.1-2.2 — Survival curves for cold-shocked adult *P. megistus*. **Fig. 1.1** — A single shock at 0°C (1 h) in males (M) and females (F). **Fig. 1.2** — Sequential shocks at 0°C (first one for 1 h; second one for 12 h) separated by different periods at 28°C. Controls: 28°C (C) and a single shock at 0°C for 1 h (S). **Fig. 2.1** — A single shock at 5°C (1 h) in males (M) and females (F). **Fig. 2.2** — Shock at 5°C (1 h) followed by a shock at 0°C (12 h), separated by different periods at 28°C. Controls: 28°C (C) and a single shock at 5°C for 1 h (S).

Only the results for insects subjected to a second shock 8 h after the first were similar to those of the control ($W = 2.6$, $p = 0.105$). These two conditions produced a survival rate greater than the remaining treatments (control, 76%; 8 h, 60%; 18 h, 2%; 24 h, 6%; 72 h, 8%) (Fig. 4.2). The survival rate of insects subjected to a second shock after an interval of 24 and 72 h at 28°C was similar in both groups ($W = 1.3$, $p = 0.247$). Although there was a significant difference among all groups subjected to sequential heat shocks, the results were not affected by sex ($W = 0.0$, $p = 0.993$; Fig. 4.1).

Control group at 28°C

There was no difference in the survival rates of males and females in control group 1 ($W = 0.0$, $p =$

0.954). Comparison of the groups that received one or two shocks with group 1 showed that the insects in group 2 survived for lesser periods than those in group 1 whereas insects of almost all groups subjected to sequential cold shocks had survival curves similar to that of control group 1 (Fig. 1.2). The insects in group 3 and all samples subjected to sequential cold shocks had survival curves similar to that of control group 1. However, control 3 insects survived less than insects belonging to control 1 (Fig. 2.2). Control group 4 insects survived more than control group 1 insects, although the survival curves of both were similar (Fig. 3.2). Control group 5 and insects subjected to heat sequential shocks in which the shocks were separated by 18, 24, and 72 h at 28°C, survived less than control group 1 insects (Fig. 4.2).



Figs. 3.1-4.2 — Survival curves for heat-shocked adult *P. megistus*. **Fig. 3.1** — A single shock at 35°C (1 h) in males (M) and females (F). **Fig. 3.2** — Preliminary shock at 35°C (1 h) followed by a shock at 40°C (12 h), separated by different periods at 28°C. Controls: 28°C (C) and a single shock at 35°C for 1 h (S). **Fig. 4.1** — A single shock at 40°C (1 h) in males (M) and females (F). **Fig. 4.2** — Sequential shocks at 40°C (first one for 1 h; second one for 12 h) separated by different periods at 28°C. Controls: 28°C (C) and a single shock at 40°C for 1 h (S).

DISCUSSION

Overall results indicated induced tolerance to heat and cold shocks in adults of *P. megistus* in terms of their survival of sequential shocks in which a mild shock preceded a more severe one. Shocks at 40°C or 0°C for 12 h are both lethal to these insects when given isolatedly (Garcia *et al.*, 1999). The tolerance rate was found to be predominantly a function of the temperature of the preliminary shock and time elapsed between sequential shocks. The different responses to sequential thermal shocks probably elicit different cellular protection mechanisms against stress.

Significant values of heat-shock tolerance were elicited in adults only when they were subjected to sequential treatments in which the preliminary shock was given at 40°C and the second one 8 h after the first shock, results which differ from findings reported for nymphs (Garcia *et al.*, 2001b). In the case of nymphs, higher values of heat-shock tolerance were sustained for intervals between shocks extending up to 24 h (preliminary shock, 35°C) or even longer (preliminary shock, 40°C) (Garcia *et al.*, 2001b).

The mechanism(s) involved in heat-shock tolerance in adults probably requires(e) that the shock being given must drastically shift from 28°C to 40°C in order to elicit efficient protective action. This is possibly necessary to activate significant changes in genomic expression, with specialized metabolic responses thus being triggered (Gasch *et al.*, 2000). Even so, this result is very transient, unlike that reported for nymphs (Garcia *et al.*, 2001b).

The cold-shock tolerance response in adults of *P. megistus* also differed from the response described for nymphs of the same species under identical experimental conditions (Garcia *et al.*, 2001a). *P. megistus* nymphs exhibit expressive cold-shock tolerance under various sequential shock conditions (preliminary shock at 5°C or 0°C; periods at 28°C extending from 8 to 72 h long between shocks) (Garcia *et al.*, 2001a). In the present study, adults were found to acquire substantial tolerance to a severe cold shock at 0°C for 12 h but only when this shock was preceded by a mild shock at 0°C for 1 h and the time between shocks surpassed 18 h. Maybe in this case, because of protective mechanisms being elicited effectively, adults require a longer period than do nymphs. Even so, tolerance

in adults was much lower than that elicited in nymphs. The 5°C temperature was not found to be a good elicitor of protective mechanisms in adults, a finding completely different from that verified for nymphs subjected to the same experimental conditions (Garcia *et al.*, 2001a).

It was concluded that the mechanisms involved in cellular protection against sequential temperature shocks in *P. megistus* may lose effectiveness with insect development. A certain disadvantage was also found for males in the adult phase.

Acquisition of heat-shock tolerance is attributed to involvement in a wide sense with heat-shock proteins (HSP) (Welch, 1993). These may also be involved with cold-shock tolerance in some insects (Denlinger *et al.*, 1991). A preliminary immunocytochemical investigation of HSP70 in Malpighian tubules of *P. megistus* subjected to heat shock at 40°C for 1 h failed to identify an increase in this protein type (Garcia, 2002), nor did another study on Malpighian tubules of *Drosophila* (Singh & Lakhotia, 1995). In the latter case, it was assumed that a rapid fall in concentration of HSP70 as mediated by proteolytic action might affect the immunocytochemical results and so does not mean absence of this protein (Singh & Lakhotia, 1995). Additional investigation on HSP is thus required on *P. megistus* subjected to thermal shocks.

In any case, although not discarding a differential participation of HSP types in the heat- and cold-shock tolerance responses of *P. megistus*, other mechanisms should also be considered, among which are modulation of DNA topoisomerase activity, and even expression of stress genes unrelated to the heat-shock response as well as genetic variation in metabolic traits (Clark & Fucito, 1998; Lopes-Garcia & Forterre, 2000).

The different patterns of heat- and cold-shock tolerance of *P. megistus* as a function of its different developmental phases may have favored this species' adaptation to different environments, especially when considering its nymphal phases, and could explain its increase and wide distribution throughout Brazil. Considering the marked susceptibility of *P. megistus* to *Trypanosoma cruzi*, present results may be relevant to our understanding of the biological responses to stress in these insects in either their natural environment or the laboratory.

Acknowledgments — This work was partially supported by the State of São Paulo Research Foundation (Fapesp, grant nos. 98/0826-4 and 99/2547-8) and the Brazilian National Research & Development Council (CNPq). The authors are indebted to Mr. Pedro Ribeiro da Silva for his technical support.

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