PhD Thesis of RNDr.Glenda Alquicer

Contribution to the study of insect stress hormones

This is a generally well-written and well-presented account. The candidate is to be congratulated on producing a thesis that contains only a few typographical and/or grammatical errors, and is easy and interesting to read. I draw attention only to the inconsistent inclusion/exclusion of reference indices in/from sentences (whether they are placed before or after the full stop). I was grateful eventually to discover the list of abbreviations (not "abbreviatures") or glossary, but I might have found it earlier if it had been placed at the beginning of the thesis.

The thesis comprises three rather distinct series of experiments that have each generated publishable data, but these different experimental studies are only weakly interconnected. The research as a whole does not appear to be tightly focussed around a single theme, and there seems little progressive development of the research, in the way that (as results are obtained) one study leads to another in greater depth.

The general commentaries that accompany each of the published studies are informative, and the relevant literature is discussed/evaluated in an adequate manner.

General questions

1. Contrary to what is stated in the introduction (3rd sentence) that "It is left to the target tissues to schedule their responses to these chemical stimuli…", is it not more the case that cells are pre-programmed (in their genome) to undertake various tasks, but need to be told **when** it is appropriate to perform these tasks?

2. The role of the **neuroendocrine** system (2nd paragraph on page 1) is mentioned briefly but the text seems to miss the point. Many endocrine organs are not innervated. Is it not a major function of the neuroendocrine system to relay interoceptive and exteroceptive information to endocrine cells?

3. What are the roles of ecdysone and JH in energy metabolism alluded to at the beginning of Chapter 2 (Third sentence, page 10)?

4. Is there a reason why in the analogy drawn between the vertebrate hypothalamic-pituitary system and insect retrocerebral endocrine system, the embryological evidence is not discussed?

5. Why in these studies is the dose-response relationships for the effects of AKH not studied? It seems to me that without this information the experimental data may only be of pharmacological rather than physiological relevance.

Specific comments and questions

CHAPTER 1

6. PQ treatment causes a fivefold increase in AKH titre in the haemolymph of *Pyrrhocoris*. However, this increase is from 0.3 – 1.5 **femtomoles** per µl of haemolymph. If the blood volume of this insect is not greater than 50 µl, this means that a maximum total of 75 fmol of AKH is present in the circulation. How does the candidate justify injection of 40 **picomoles** of AKH (more than 500 times that present even in these stressed bugs)?

7. If AKH is released into the haemolymph during oxidative stress, why is extraneous AKH effective in reducing the effect of PQ?

CHAPTER 3

8. The candidate shows that glucagon-like immunoreactive material is present in various tissues of *Pyrrhocoris*, but it is below the level of reliable detection in the haemolymph. Why did the candidate not test to see whether these levels change under conditions of oxidative stress? Without this information, we are left uncertain as to whether the experiments using porcine glucagon are of any physiological significance.

CHAPTER 4

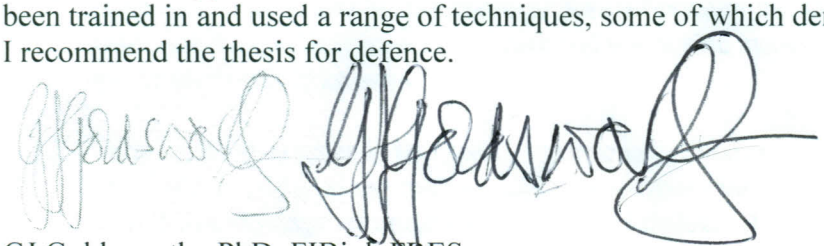
In this section of the thesis, the candidate shows data suggesting that there is a short-lived reduction in the concentration of lipid in the haemolymph of *Pyrrhocoris* after topical application of methoprene.

9. Was this experiment undertaken just once, or was it repeated several times with the same result?

The candidate writes in several places that methoprene treatment increases the amount of AKH in the CNS. However (see Fig 2A), the amount of AKH in the CNS at 24 h after methoprene treatment is actually **less** than at 6h.

10. Is it not more accurate to state that the methoprene limits the **reduction** in AKH content apparent in the control insects?

These studies appear to have been undertaken in a careful manner, and the student has been trained in and used a range of techniques, some of which demand considerable care. I recommend the thesis for defence.



GJ Goldsworthy PhD, FIBiol, FRES
November 2009

Review of the Dissertation

Glenda Paola Alquicer Barrera: Contribution to the study of insect stress hormones. PhD. Thesis. The University of South Bohemia, Faculty of Sciences, České Budějovice.

In the presented PhD Thesis the author is dealing with three main topics. The first one was devoted to the research of oxidative stress through the action of paraquat, a herbicide which is a potent agent causing oxidative stress. The second topic involves the investigation of a functional homology between adipokinetic hormone and glucagon based on the effects on haemolymph lipids and on the level of AKH in the central nervous system and haemolymph. The aim of the third topic was to characterize the action of juvenile hormone analogue methoprene on the functioning of AKH.

The dissertation contains 69 pages of text and figures, and the list of used references. The title hits the contents of the work well, with AKH being the most thoroughly studied hormone. The text is well understandable, and all parts of the text are related to the subject matter. The text is divided into four chapters, and the first of them, the "Introduction to the study of stress," is related to methods of oxidative stress research. The consequent chapters deal with adipokinetic hormones, glucagon and the interaction of AKH and methoprene. Each of these chapters contains literature review of the topic and paper or manuscript published by Glenda and co-workers. The final parts of the thesis are Conclusion remarks, Future perspectives and References.

Experimental procedures and methods used in the work are adequate to the aim of the work and reflect the department orientation on the study of insect hormones. The conclusions emerging from the results of experiments presented in the section "Conclusion Remarks" are competent and provide interesting data. The first one is about the action of AKH applied together with paraquat which resulted in the increase of total antioxidant capacity in *Pyrrhocoris apterus*. It indicated the involvement of AKH in the activation of antioxidant protection mechanisms. The second interesting result concerns the effect of glucagon on antioxidant stress reaction when the antioxidant response was manifested by increasing the level of glutathione in haemolymph and decreasing protein carbonyl concentrations. Last result refers to juvenile hormone analogue action on AKH in central nervous system and in haemolymph. In this case methoprene did not influence the lipid mobilizing function of AKH but caused distinctive changes of AKH levels in these tissues by suppressing its release from nervous system to the haemolymph.

I have no critical remarks to the thesis; after all these results were published or were accepted for publication in good scientific journals. I would like only to ask

a) about the possible influence of trehalose, sugar occurring in insect haemolymph in large quantities, on antioxidative mechanisms in insects. It is known that under certain conditions trehalose enhanced the resistance of cells to the oxygen stress;

b) when determining the possible effects of methoprene on AKH content in central nervous system it was stated that time intervals used (6 and 72 hours) were too short and too long, respectively. Have you tried some time interval in between sometimes later?

Finally, it can be stated that the presented dissertation work according to the provision of the act No. 111/1998 contains original scientific results published in three scientific papers, so the prerequisites for awarding the academic title Ph.D. have been fulfilled.

Date: December 2, 2009

J. Šula

Opponent review on doctoral dissertation thesis: Contribution to the study of insect hormones from RNDr. Glenda Paola Alquicer Barrera

In her doctoral thesis, Ms. Barrera deals with the problem of stress which is the complex factor that is unfortunately well known to all living organisms. However, as the author mentions, the stress is the engine of evolution and understanding of how organisms cope with the stress may bring new insights into general mechanisms of adaptation as well as valuable practical applications. Pest control or human medicine are two of enormously important fields where knowledge of impacts of stress may open the way for diverse practical outputs. Ms. Barrera presents the problems of stress in four chapters where the first is devoted to general introduction into the topic while three following deal with particular experimental investigations each well documented by three already published papers.

Since hormones provide the biochemical basis of stress reactions, the roles of adipokinetic hormone and glukagon in insect metabolism under the stress were addressed. Moreover, the interconnections between juvenoid methoprene and AKH were investigated. As a model of stressing agent the oxidative stress was chosen.

The results of a team the author was a member show convincingly that both AKH and glucagon may be involved in antioxidant protection mechanisms. The role of methoprene in stress response seems to be less clear. However its interferences with AKH were well documented.

The text introducing and accompanying the enclosed papers is concise and very well written which show both research and narrative skills of the author. Being an opponent, I appreciate Ms. Barrera's authorship of other scientific papers showing her as an expert also on neurobiology of rat's brain.

While reading the thesis, I became curious about some minor points which I mention bellow to be responded.

1) There are diverse kinds of biological stress such as light or food or sleep deprivation or extreme temperatures, xenobiotics etc. Can oxidative stress be supposed as a common factor of all kinds of the other stresses or as a specific kind? Consequently, can conclusions drawn from ROS stress responses be applicable to other stressors?

2) On page 5 bellow, the resistance to xenobiotics and to oxidative stress are mentioned. Could the author discuss if possible relationships or evolution links between these systems exist?

3) The third paper indicates impacts of methoprene on AKH content. Could the author discuss this finding in the light of antioxidant protection or general anti-stress response?

Conclusions: I consider the thesis entirely fulfilling demands of high quality doctoral work and I recommend it to be accepted.

Brno, December 3,

Martin Vácha, Ph.D