Regulation of Anti-oxidative Stress Response in *Drosophila melanogaster*: Involvement of the Adipokinetic Hormone and Adenosine

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Introduction

Animals including insects have developed effective defence system to damages caused by reactive oxidative species. The main antioxidative response includes the activity of the enzymes (superoxide dismutase, catalase, glutathione-S-transferase) and utility of low molecular weight antioxidants (glutathione, ascorbate...).
In the present study, the involvement of the insect adipokinetic hormone (AKH) and nucleoside adenosine (Ado) into the protection was investigated.

The AKH is a typical stress hormone; recently it has been proven the AKH stimulates the actions of the antioxidative defence and reparation. Both AKH and Ado pathways are mediated by G-protein coupled receptors, and are known to activate cAMP and calcium signalling. To study possible functional relationship between both factors the fruit flies *Drosophila melanogaster* with malfunctions of AKH production and with dysfunctional Ado receptor were prepared and studied under the oxidative stress caused by herbicide paraquat (PQ).

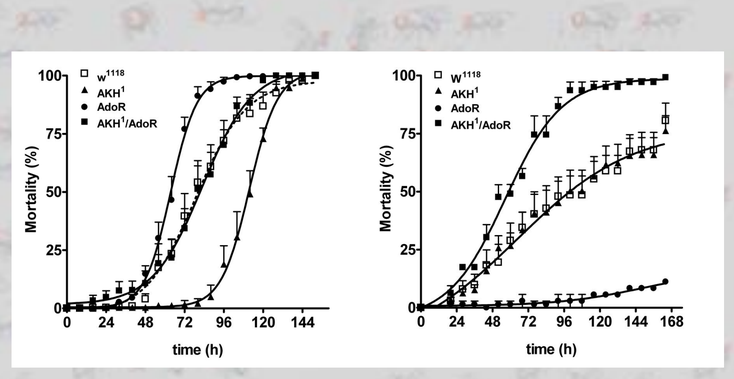
Methods

Flies w^{1118} **- white control flies (Bloomington)**
AKH^1 **- AKH deficient (Sajwan et al. 2015)**
AKH-RNAi - AKH deficient
AdoR - dysfunctional Ado receptor (Žuberová et al. 2010)
AKH^1**/AdoR - double mutant (Stašková, unpubl.)**

The vitality tests were perfomed in adult males and pupariation rate was observed in 3rd-instar larvae.

To examine the antioxidative response the adult flies were homogenised in potassium phosphate buffer, or haemolyph of the 3rd-instar larvae was collected. For estimating the level of reduced glutathione (GSH) the sample were mixed with NADPH and glutathione reductase and 5,5′-dithiobis(2-nitrobenzoic acid), the change of absorbance was measured (Griffith 1980, Krishnan et al. 2007). Then the Glutathione-S-Transferase Assay Kit (Sigma-Aldrich) was used.
To estimate the level of AKH the brains (CNS) of 3rd-instar larvae were dissected and the competitive ELISA (Enzyme-Linked ImmunoSorbent Assay) using specific anti-AKH antibody (kindly provided by Prof. J. Veenstra, University of Bordeaux) was employed.

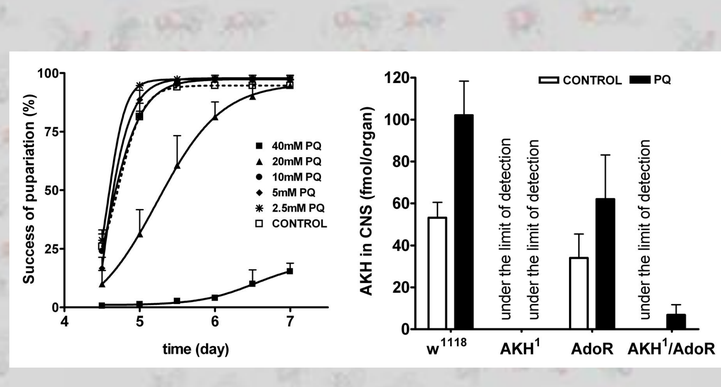
Results



Left. The starving test of 5-day old adult males kept on 5% agarose as a source of water. Thirty flies were treated in one trial of 7 repetitions.

Right. The mortality test of 5-day old adult males kept on PQ food (20mM). Thirty flies were treated in one trial of 5 repetitions.

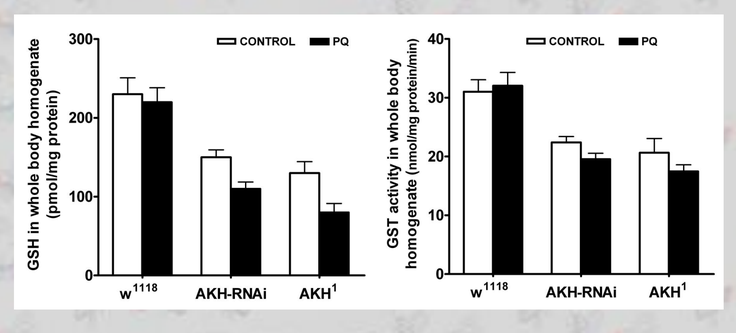
Data represent mean SEM



Left. The effect of PQ treatment on pupariation of w^{1118} **larvae kept on food with various PQ content. Fifty larvae were treated in one trial of 3 repetitions.**

Right. The effect of PQ treatment on the AKH content in larval brain (CNS). Two CNS were examined in one trial of 6 repetitions.

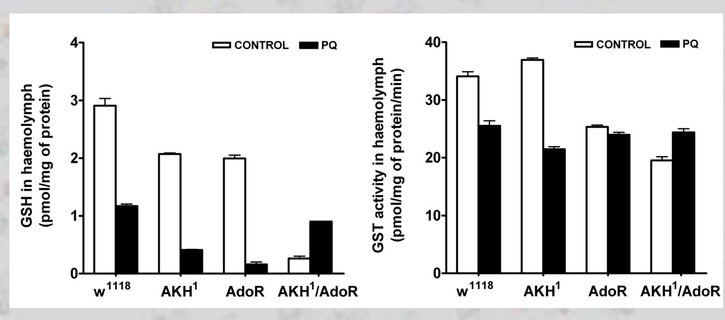
Data represent mean SEM



Left. The effect of PQ treatment on glutathione (GSH) content in whole body homogenate. About 50 adults were treated in one trial of 12 repetitions.

Right. The effect of PQ treatment on activtity of glutathione-S-transferase enzyme (GST) in whole body homogenate. About 50 adults were treated in one trial of 20 repetitions.

Data represent mean SEM



Left. The effect of PQ treatment on glutathione (GSH) content in larval haemolymph. About 500 larvae were treated.

Right. The effect of PQ treatment on activtity of glutathione-S-transferase enzyme (GST) in larval haemolymph. About 500 larvae were treated.

Data represent mean SEM

References

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Conclusions

PQ treatment elicited oxidative stress in both adult flies and larvae, increased adult mortality and delayed larval pupariation

Absence of AKH (AKH^1 **flies) enhanced the fly resistance to starving, while dysfunctional adenosine receptor (AdoR flies) increased resistance to PQ toxicity**

PQ treatment increased AKH titre in CNS of control (w^{1118}**) and AdoR larvae**

Differences in reduced glutathione (GSH) content and in glutathione-S-transferase (GST) activity between control (w^{1118}**) and the other flies (**AKH^1**, AdoR,** AKH^1**/AdoR) suggest involvement of both the AKH and adenosine signalling into the anti-oxidative defence system**

June 2015

Supported by the GACR project. No. 14-07172S, and by the Postdok BIOGLOBE CZ.1.07/2.3.00/30.0032 project co-financed by the European Social Fund and the state budget of the CR.