Defence reaction against nematobacterial infection in *Drosophila melanogaster*: A role for the adipokinetic hormone and adenosine

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INTRODUCTION:

Entomopathogenic nematodes (EPN) are multicellular insect parasites which are symbiotically associated with particular species of bacteria forming together highly pathogenic complex (1). During infection the EPN invade insect host and release the bacteria producing a variety of toxins into its body cavity. This is a classic example of general infection that upsets basic physiological processes in the body and is often terminated by insect dead. Nematobacterial infection of *Drosophila* larvae is characterized by the activation of antimicrobial and wound response with the primary focus on entrapment of the bacterial pathogens. It is indisputable that nematobacterial infection represents a severe stress for the infected insect that must activate its anti-stress defence system. It is supposed that adipokinetic hormone (AKH), important stress hormone responsible for keeping homeostasis in insect body (2,3), and adenosine, a purine nucleotide that serves as signalling factor in anti-stress reaction on both cellular and organismal levels (4), play a role in defence reaction against the infection.

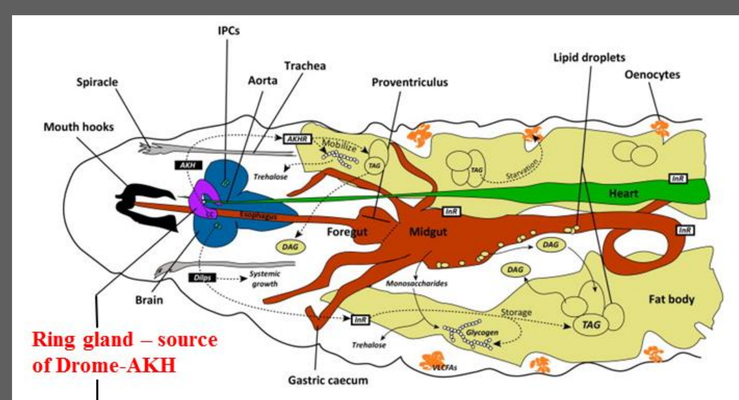
Material and Methods:

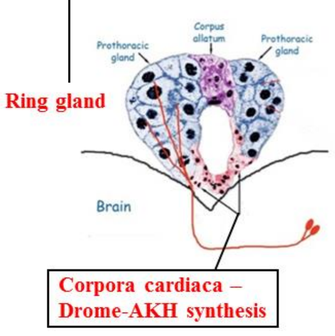
1. *Drosophila melanogaster* mutant larvae (w1118) producing (a) defect non-functional TALEN AKH (AKH-def, 5), (b) impaired adenosine receptor (AdoR-def, 6) and (c) mutant non-functional in both relevant genes (Double-def, 7), and two nematode species *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* with symbiotic bacteria *Xenorhabdus nematophila* and *Photorhabdus luminescens*, respectively, were used in the study.

2. Application of EPN – contact; application of Drome-AKH – dipping in solution 3 pmol Drome-AKH/µl 20% MeOH; determination of markers – 24 h after the treatments.

3. AKH level determination – using the specific antibody against Drome-AKH and competitive ELISA.

4. Spectrophotometric determination of nutrients (carbohydrates, lipids, proteins).





General AKH structure:

8 – 10 amino acids

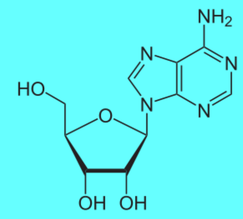
pGlu[1]-X[2]-X[3]-X[4]-X[5]-X[6]-X[7]-Trp[8]-Gly[9]-X[10]-NH_2

X[2] – Leu, Val, Ile
X[3] – Asn, Thr
X[4] – Phe, Tyr (arom. AA)
X[5] – Thr, Ser
X[6] – Pro, Ser, Thr, Ala
X[7] – Asn, Gly, Ser, Asp, Trp
X[10] – Thr, Asn, Ser, Tyr

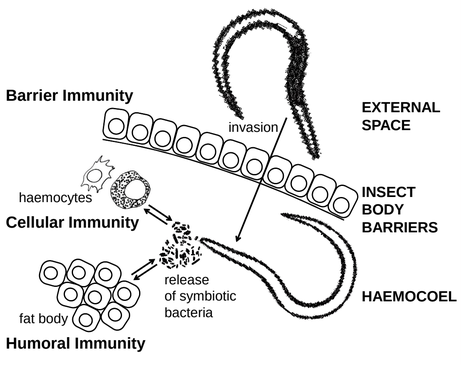
***D. melanogaster* AKH (Drome-AKH):**

pGlu-Leu-Thr-Phe-Ser-Pro-Asp-Trp-NH_2

DROSOPHILA ENDOCRINE SYSTEM (AKH)



ADENOSINE



STRESS (EPN INFECTION)

RESULTS:

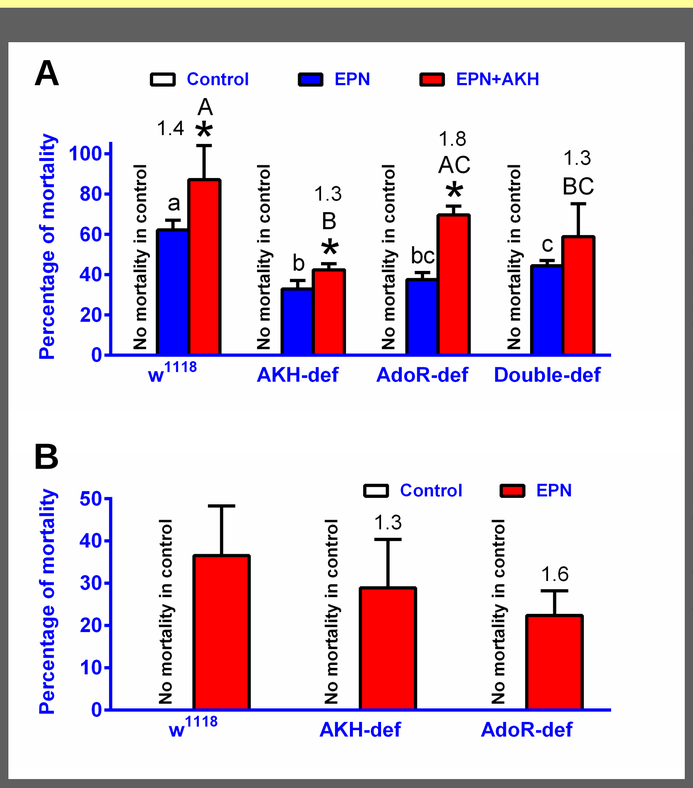


Fig. 1A. The effect of EPN *S. carpocapsae* and Drome-AKH treatment (AKH) on mortality of various *D. melanogaster* mutants 24 hours upon the infection. Statistically significant differences among the mutants at the 5% level evaluated by one-way ANOVA with Tukey's post-test are indicated by different letters (effect of mutation); differences between the experimental groups (EPN vs. EPN+AKH) within the mutant (effect of treatment) at the 5% level evaluated by Student's t-test are indicated by asterisks. The numbers above the columns represent fold-difference of mortality of Drome-AKH treated *Drosophila* larvae after infection by *S. carpocapsae* as compared with the nematode infection only (effect of AKH). Results further revealed significantly lower mortality in AKH-def, AdoR-def and Double-def mutants infected by EPN (1.9, 1.7 and 1.4 fold, respectively, blue columns) as compared to EPN infected w^{1118} control. The same results were observed in EPN+Drome-AKH treated mutants (2.0, 1.3 (NS) and 1.5-fold, respectively, red columns) as compared to EPN+AKH treated w^{1118} control. This suggests involvement of both Drome-AKH and adenosine into the metabolic pathways controlling vitality and mortality of *D. melanogaster* under the EPN infection.

Fig. 1B. The effect of EPN *H. bacteriophora* on mortality of various *D. melanogaster* mutants 24 hours upon the infection. No statistically significant differences among the mutants at the 5% level evaluated by one-way ANOVA with Tukey's post-test were found. The numbers above the columns represent fold-difference of mortality in AKH-def and AdoR-def mutants after infection by *H. bacteriophora* as compared with the treated w^{1118} control (effect of mutation). Results revealed slightly lower (nevertheless not-significant) mortality in the mutants than in the w^{1118} control. This supports the data obtained with *S. carpocapsae* (see Fig. 1B).

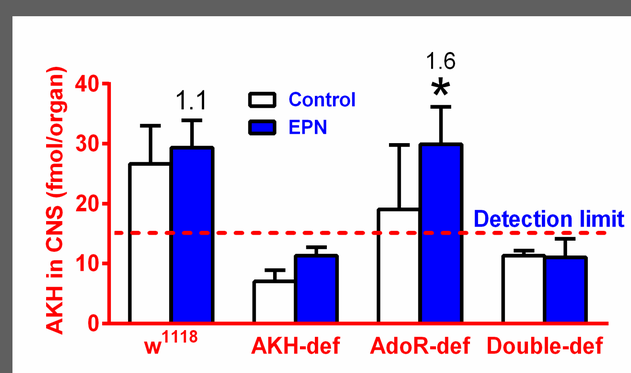


Fig. 2. The effect of EPN *S. carpocapsae* on Drome-AKH level in various *D. melanogaster* mutants 24 hours upon the infection. The levels of AKH in the mutants producing deficient AKH - not recognised by used antibody against normal AKH - was under the ELISA detection limit (15 fmol) therefore they were not included into the statistics. Statistically significant difference between the EPN treatment and corresponding untreated control at the 5% level evaluated by Student's t-test is indicated by asterisk (effect of treatment); no significant differences were recorded between the AdoR-def mutant and w^{1118} control (both for infected and non-infected pairs; again using Student's t-test). The numbers above the columns represent fold-difference of AKH level after infection by *S. carpocapsae* as compared with untreated controls; certain increase of the AKH level after the infection was recorded in both mutants with normal Drome-AKH production, however, only in AdoR-def was significant.

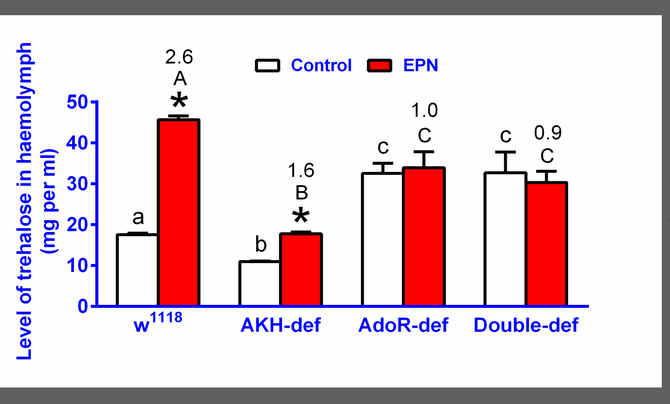


Fig. 3. The effect of EPN *S. carpocapsae* on trehalose level in haemolymph of various *D. melanogaster* mutants 24 hours upon the infection. Statistically significant differences among the mutants at the 5% level evaluated by one-way ANOVA with Tukey's post-test are indicated by different letters (effect of mutation); differences between the experimental groups within the mutant (effect of treatment) at the 5% level evaluated by Student's t-test are indicated by asterisks. The numbers above the columns represent fold-difference of trehalose level after the infection by *S. carpocapsae* as compared with the controls: however those differences were significant just in mutants with AdoR normal function (w^{1118} and AKH-def) and they were more distinct in control w^{1118} than those in AKH-def.
Further, level of trehalose was significantly lower (1.6 fold) in untreated AKH-def, and higher in untreated AdoR-def and Double-def (both 1.8 and 1.9 fold, respectively) *Drosophila* mutants than that in control w^{1118}. In EPN infected mutants the trehalose level was significantly lower in all tree '-def' mutants: 2.6, 1.3 and 1.5 fold in AKH-def, AdoR-def and Double-def ones, respectively. Those indicate clear stimulatory role of AKH, and minor modulatory role of adenosine in regulation of trehalose level in *Drosophila* haemolymph.

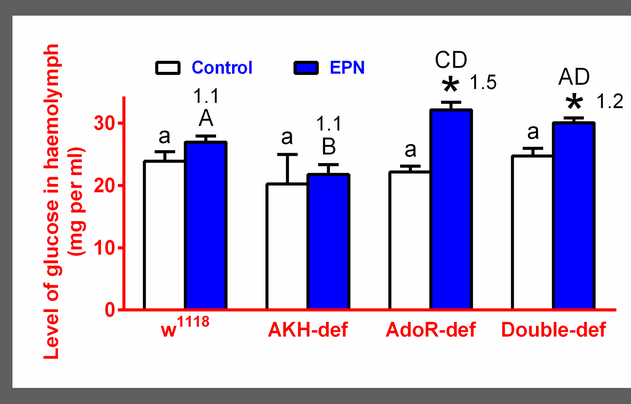


Fig. 4. The effect of EPN *S. carpocapsae* on glucose level in haemolymph of various *D. melanogaster* mutants 24 hours upon the infection. Statistically significant differences among the mutants at the 5% level evaluated by one-way ANOVA with Tukey's post-test are indicated by different letters (effect of mutation); differences between the experimental groups within the mutant (effect of treatment) at the 5% level evaluated by Student's t-test are indicated by asterisks. The numbers above the columns represent fold-difference of glucose level after infection by *S. carpocapsae* as compared with the controls: however those differences were significant just in mutants with AdoR deficient function.
Further, no difference in glucose level were recorded among all untreated experimental groups, nevertheless, after the EPN treatment slight significant decrease 1.2 fold (AKH-def) and increase 1.2 fold (AdoR-def) were recorded. In the Double-def mutant both those effects were cancelled each other out. Those indicate a certain role of both AKH (stimulation) and adenosine (inhibition) on the glucose level in *Drosophila* haemolymph - although those effects are not dramatic.

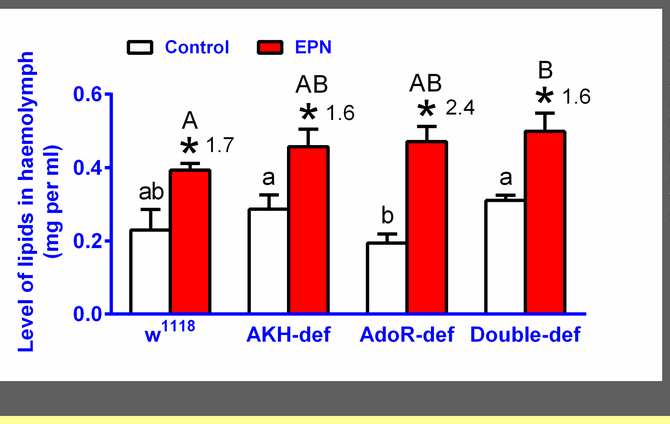


Fig. 5. The effect of EPN *S. carpocapsae* on lipid level in haemolymph of various *D. melanogaster* mutants 24 hours upon the infection. Statistically significant differences among the mutants at the 5% level evaluated by one-way ANOVA with Tukey's post-test are indicated by different letters (effect of mutation); differences between the experimental groups within the mutant (effect of treatment) at the 5% level evaluated by Student's t-test are indicated by asterisks. The numbers above the columns represent fold-difference of lipid level after infection by *S. carpocapsae* as compared with the controls: those differences were a bit higher in the mutant with AdoR-def function than those with AdoR normal one, however, it seem that *Drosophila* lipid metabolism is not substantially affected either by AKH nor adenosine.

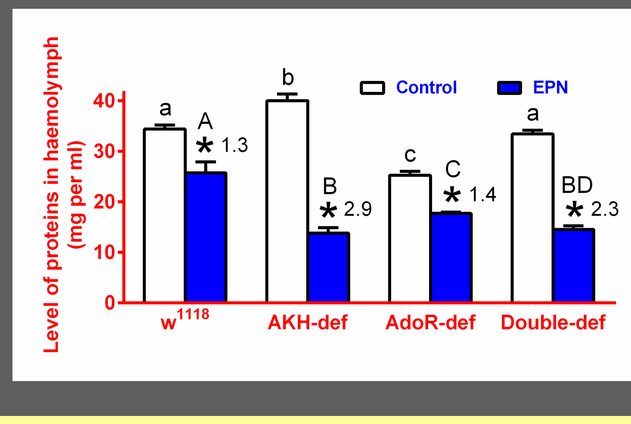


Fig. 6. The effect of EPN *S. carpocapsae* on protein level in haemolymph of various *D. melanogaster* mutants 24 hours upon the infection. Statistically significant differences among the mutants at the 5% level evaluated by one-way ANOVA with Tukey's post-test are indicated by different letters (effect of mutation); differences between the experimental groups within the mutant (effect of treatment) at the 5% level evaluated by Student's t-test are indicated by asterisks. The numbers above the columns represent fold-difference of protein level after infection by *S. carpocapsae* as compared with the controls: those data revealed significant decrease of protein level in all *Drosophila* mutants after the EPN infection. We can speculate that the EPN and their symbiotic bacteria curtail general release of proteins into the haemolymph to reduce production of defence proteins. It is possible that both AKH and adenosine affect this process, but their role is not clear at present.

SUMMARY AND CONCLUSIONS:

1. **Both Drome-AKH and adenosine affect metabolic pathways controlling vitality and mortality in *Drosophila* under the EPN infection.**
2. **Drome-AKH shows stimulatory role and adenosine minor modulatory role in regulation of trehalose level in *Drosophila* haemolymph; effect of both agents on glucose level is small.**
3. **Effect of both Drome-AKH and adenosine on haemolymph lipid metabolism in *Drosophila* is negligible.**
4. **EPN infection reduces a level of proteins in *Drosophila* haemolymph maybe by general reduction of protein release (including defence proteins) into the haemolymph; effect of Drome-AKH and adenosine is not clear.**

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