School of Doctoral Studies in Biological Sciences

University of South Bohemia in České Budějovice
Faculty of Science

Anti-oxidative stress response in *Drosophila melanogaster* The role of adipokinetic hormone and adenosine

Ph.D. Thesis

RNDr. Milada Zemanová

Supervisor: Prof. RNDr. Dalibor Kodrík, CSc.
Faculty of Science, University of South Bohemia in České Budějovice
Institute of Entomology, Biology Centre of the CAS, České Budějovice

České Budějovice 2016

This thesis should be cited as:

Zemanová M. (2016): Anti-oxidative stress response in *Drosophila melanogaster* – The role of adipokinetic hormone and adenosine. Ph.D. Thesis Series, No. 18. University of South Bohemia, Faculty of Science, School of Doctoral Studies in Biological Sciences, České Budějovice, Czech Republic, 143 pp.

Annotation

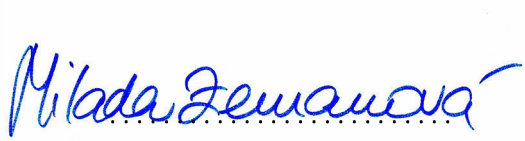
In this thesis, the phenomena of the oxidative stress (OS) and anti-oxidative stress responses in insects are described in a comprehensive review, and the outcomes of the experimental work are presented. The focus of the work was on defence reactions and their putative control by the adipokinetic hormone (AKH) and adenosine in the *Drosophila melanogaster* model. For this purpose, we studied the effect of the paraquat (oxidative stressor) treatment on adult flies and larvae carrying mutations in *Akh* (*Akh*^{1}) and *adenosine receptor* (*AdoR*^{1}) genes, and in both these genes together (*Akh* ^{1}*AdoR*^{1} double mutant). The initial mortality tests revealed the double mutant *Akh*^{1} *AdoR*^{1} was more sensitive to OS than either of the single mutants. The AKH synthesis under the OS condition seems to be out of the gene expression control, since the increase of an AKH amount in CNS was not linked with the stimulation of *Akh* gene expression after a paraquat treatment. Further, the gene expression of the antioxidant enzyme *glutathione S-transferase D1* (*GstD1*) increased rapidly with OS, though the enzyme activity increased negligibly regardless of both the OS and mutations. Interestingly, the relative expression of *GstD1* gene was minimal in the double *Akh*^{1} *AdoR*^{1} mutant; thus, it was concluded that both AKH and adenosine are employed in the *GstD1* gene expression control. Similarly, AKH and adenosine seem to act in tandem in glutathione (GSH) regeneration, since the GSH level was significantly lower in all untreated deficient flies with the maximal effect in the *Akh*^{1} *AdoR*^{1} double mutant; accordingly, the reduction in the GSH level was enhanced by paraquat treatment. Altogether, the important roles of both AKH and adenosine in the anti-oxidative stress response in *D. melanogaster* were demonstrated.

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracovala samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

Mirramu, NSW, 29. 11. 2016



This thesis originated from a partnership of the Faculty of Science, University of South Bohemia, and the Institute of Entomology, Biology Centre of the CAS, supporting doctoral studies in the Immunology and Developmental Biology study program.

Přírodovědecká fakulta
Faculty of Science

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

BIOLOGY CENTRE CAS

Entomologický ústav
Institute of Entomology

Financial support

This work was supported by grant No. 14-07172S (DK) from the Czech Science Foundation; by project RVO 60077344 from the Institute of Entomology, funded by the CAS; and by the Postdoc BIOGLOBE CZ.1.07/2.3.00/30.0032 project, co-financed by the European Social Fund and the state budget of the Czech Republic.

Acknowledgements [in Czech]

Dizertační práce před Vámi by nevznikla a veškeré mé úsilí by nevedlo k žádnému, zdaleka ne zdárnému, konci bez odborného vedení mého školitele, který mě přivedl k výzkumu adipokinetického hormonu hmyzu, představil problematiku oxidativního stresu a jakožto studentku neznalou laboratorní praxe trpělivě naučil základním laboratorním metodám a experimentálním přístupům. I když směr mého výzkumu, doktorandského studia a mého odhodlání nebyl vždy jednoznačný, vždy se mi dostalo plné podpory, patřičné motivace a někdy až nadměrné dávky optimismu, za což jsem svému školiteli velice vděčná. Děkuji svému školiteli, prof. RNDr. Daliborovi Kodríkovi, CSc., za vše, co mě naučil, za pomoc a rady při řešení každého vědeckého zádrhele, za nejednu šanci a nemalé úsilí, které věnoval mé práci a v neposlední řadě za jeho lidský a kamarádský přístup, kterého si vážím.

Děkuji svým kolegům ze začátků, kteří mě vřele přijali do týmu, za pomoc s osvojením laboratorních dovedností a skvělou atmosféru, Janče Kotyzové (Zralé), Ivě Bártů, Pavlu Jedličkovi. Pepovi Večeřovi za uvedení do experimentu s drosofilou a oxidativním stresem. Děkuji tehdejším členům laboratoře doc. Tomáše Doležala za pomoc a umožnění prvotních pokusů s drosofilou.

Děkuji svým kolegům finální etapy, Terce Staškové za velký pokrok s drosofilími pokusy, za který rovněž děkuji členům laboratoře prof. Michala Žurovce, Aničce Žaloudíkové, Romanu Sidorovi a Yu-Hsienovi za jeho optimistický vhled do výsledků mého bádání. Thank you, Yu-Hsien Lin, for your optimistic view of my results. Děkuji Danče Hlávkové, která se mnou zpracovala nezměrné množství vzorků. Děkuji Zdeňce Svobodové nejen za IT podporu. Děkuji Helče Štěrbové, Míše Mochanové, Markétě Hejníkové, Jitce Pflegerové, Tomáši Krůčkovi, Honzovi Černému, Kostjovi Vinokurovi, Emadovi a Umeshovi, že byli skvělá parta. Thank you, Ibrahim Emad and Gautam Umesh Kumar, for being a team. Můj veliký dík patří mé kolegyni, spoulautorce a kamarádce Áje Bednářové, která mi ukázala, že je to možné; za její nemalou pomoc v laboratoři, za naše nekonečné přínosné hovory, za její velký podíl na vzniku této práce, a hlavně za její nadšení pro věc, které je tak nakažlivé.

Děkuji Pauline McGuire za obsáhlou jazykovou korekturu. My special thanks to Pauline McGuire for her extensive language corrections.

Děkuji všem svým spolužákům a budějovickým kamarádům, že v tom byli se mnou, po dobu studia, po dobu badatelských nesnází a úspěchů, za to že České Budějovice jsou pro mě druhým domovem.

Děkuji všem kamarádům po celém světě, co mě kdy podpořili, vyslechli, uvařili mi nebo se třeba taktně neptali vůbec.

Děkuji svým rodičům, že mi věřili. Za jejich všestrannou podporu během všech stupňů mého studia.

List of papers and author's contribution

The thesis is based on the following papers and a supplemental manuscript (listed chronologically):

I. Kodrík D, Bednářová A, Zemanová M, Krishnan N (2015) Hormonal regulation of response to oxidative stress in insects – an update. *International Journal of Molecular Science*, **16**, 25788–25816, IF = 3.257

MZ was responsible for preparing chapters (a) Role of other insect hormones in oxidative stress, and (b) Drosophila model.

II. Zemanová M, Kodrík D (2016) The anti-oxidative stress response in *Drosophila melanogaster*. Involvement of adipokinetic hormone and adenosine. In: *Animal Physiology 2016 – Proceedings of International Scientific Conference* (eds Pavlík A, Sláma P, Škarpa P), pp. 317–323. Mendel University, Brno, IF = not defined

MZ was responsible for the experiments and preparing the manuscript.

III. Zemanová M, Stašková T, Kodrík D (2016) Role of adipokinetic hormone and adenosine in the anti-stress response in *Drosophila melanogaster*. *Journal of Insect Physiology*, **91-92**, 39–47, IF = 2.267

MZ was responsible for the experiments and preparation of the manuscript.

IV. Bednářová A, Zemanová M, Rakshit K, Kodrík D, Krishnan N. Effect of disruption of adipokinetic hormone signaling on homeostasis and aging characteristics in *Drosophila melanogaster. Physilogy & Behavior*, unpublished manuscript

MZ co-operated with the RING experiments and preparation of the manuscript.

Co-author agreement

The senior and corresponding authors of the manuscripts included in this thesis hereby confirm that MZ contributed significantly to these publications, according to the statement above.

.....

Prof. RNDr. Dalibor Kodrík CSc.

.....

RNDr. Andrea Bednářová, PhD.

Content

1 Introduction

1.1 The environment as a stress factor

The environment could be considered as a sum of all external conditions affecting the life, development, and survival of an organism (Johnson *et al.*, 1997). Every property of the environment varies with time and location. And, since animals have a worldwide spread and have colonized niches of biomes from tropical to arctic areas, they have to face up to the various challenges of their environment.

Because the success of an individual or species in itself depends on its ability to cope with that, every species has developed multifarious adaptations to the conditions that affect it during its lifetime. Nevertheless, there is no one environmental characteristic that can be consistent, and only a minimal change could affect the entire balance and effectively bring down the whole ecosystem with its inhabitants. The degree of an animal's adaptation differs in rank from generalist species, when organisms are highly adaptive to any changes, to specialist species, when only a minor perturbation can be fatal to organisms (Davies *et al.*, 2012). Environmental conditions are expressed by certain characteristics – e.g. ambient temperature, humidity, sun radiation, and food, water, shelter source etc.

For any particular environmental characteristic, when the level of the characteristic varies between the boundaries ideal for biological processes, such as growth and development of organisms, this is designated an optimum range, which is usually specific for individual species or populations (Putman & Wratten, 1984). When an environmental characteristic exceeds the lower or upper limit of an optimum range, which the species is adapted to, a stress situation arises. The stress results from either changes in the external environment (e.g. temperature, danger etc.) or internal milieu (injury, disease, malnutrition etc.) (Storey, 2004) and may upset a functional homeostasis of the whole organism (Ivanović & Janković-Hladni, 1991).

1.1.1 The anti-stress reaction of organisms

Animals exposed to unsuitable conditions produce a characteristic response, which is often referred to as a generalized stress response. In other words, stress can be understood as a complex of reactions of an organism to the

conditions outside the optimal range. Stress has played an important role in evolution and, from an ethologist's point of view, the reaction of animals to a stress situation is designated the 'fight or flight' response. Similarly, in physiological research we can observe the pattern of 'flight' – avoidance – or 'fight'– conformity and regulation (Cannon, 1915).

Generally, animals can effectively avoid adverse conditions in space or time. For the former, an option is migration. In response to their environmental conditions, animals can perform a seasonal migration, dispersion or vertical migration in soil, water etc. If there is no way to escape, organisms can introduce dormancy and survive in this state until hostile conditions abate. A state of dormancy can be either consequential or predictive. The initiation of consequential dormancy results from adverse conditions and it is terminated immediately the conditions are back in the optimal range. Consequential dormancy is quiescence, which is documented in many invertebrate species (e.g. cold immobility). Predictive dormancy, on the other hand, does not initiate and terminate immediately external conditions shift. Predictive dormancy represents a complex physiological process, which is endogenously regulated by hormones and synchronized by an internal biorhythm with photoperiodic calendar, and it depends on many factors. The predictive type of dormancy is diapause in ectotherms and hibernation in endotherms. It includes changes of the gene expression and proteome, cell cycle arrest, metabolism suppression, body temperature decrease, general stress tolerance increase and other physiological modifications. In addition, animals can survive unfavourable conditions in a state that is primarily immobile; e.g. an egg or pupa in invertebrates or embryo in mammals.

Those organisms that are not able to avoid adverse conditions in either space or in time employ the 'conformity and regulation' strategy. They can tolerate or compensate for deficiencies in their environment by activating physiological anti-stress reactions.

1.1.2 Physiological anti-stress reactions

Animals respond to unfavourable conditions by activating a wide array of behavioural and physiological responses, which are referred to as the stress response. The physiological stress response has been observed in organisms subjected to imminent life-threatening danger from a predator or competitor

and in those subjected to hostile environmental conditions – for example, thermal imbalance, such as hypothermia or hyperthermia; starvation conditions; the lack of a water source; osmotic stress conditions, such as hypersaline water; or oxygen stress, such as a hypoxic or hyperoxic condition.

In the case of imminent life-threatening danger, alarm reactions prepare animals to 'fight or flight'. In mammals, the pulse, blood pressure and muscle tonus increase. At the same time, the rate of blood flow in muscles increases and simultaneously decreases in parts of the body where activity can be suppressed, such as the body surface or digestive system. Moreover, oxygen consumption is elevated and liver store polysaccharide glycogen is cleaved. Thus, the stored energy can be released and redirected to impacted body parts to eliminate an imminent stress (Tsigos *et al.*, 2000). Nevertheless, in a persistent stress situation this could lead to energy store depletion, followed by the termination of any actions that are not essential (feeding, reproduction) and, in the final stage, to damage to the whole organism and its death.

1.2 Regulation of anti-stress reactions

Under uncomfortable conditions, organisms attempt to maintain a homeostasis of their inner environment. Its organization is realised on a molecular, cellular and physiological – organismal – level (Hightower, 1991).

1.2.1 The stress response on an organismal level

Since the anti-stress response includes a wide repertoire of actions of a behavioural and physiological character, it is to be expected that all those miscellaneous mechanisms need to be under the control of the superordinate system. An organismal stress response, which allows an organism to adapt to perturbations of homeostasis, is a reaction under the control of the nervous and endocrine systems. Successful adaptations require the ability not only to respond to the stress but also to control the stress response appropriately.

In mammals, the nervous stress response is mediated by brain stem noradrenergic neurons, sympathetic andrenomedullary circuits, and parasympathetic systems (Jansen *et al.*, 1995; Smith & Vale, 2006). Simultaneously, hormonal control is initiated at the hypothalamic-pituitary-adrenal axis. Firstly, the information about a stress

situation leads to the activation of a sympathetic nervous system that stimulates adrenalin (epinephrine) release from the adrenal medulla and glucagon release from pancreatic islet α-cells. The sympathetic nervous system control mediated via adrenalin activity in target tissues results in the elevation of heart and gas exchange, an increase in metabolic rate and blood flow redistribution. A glucagon effect is hyperglycaemic, since it triggers the cleavage of liver store glycogen and lipid and gluconeogenesis. Besides, the stress activation of a hypothalamus triggers a cascade of reactions. Firstly, corticotropin-releasing hormone is secreted from the hypothalamus, which leads to an adrenocorticotropic hormone release from the anterior pituitary gland, and adrenocorticotropic hormone stimulates a glucocorticoid secretion in the adrenal cortex. A glucocorticoids effect is pleiotropic, mainly hyperglycaemic. Altogether, physiological stress responses end in energy mobilisation and redirection to an imminent stress elimination (Everly & Lating, 2013).

In insects, similarly, the stress defence via biochemical and physiological reactions is controlled predominantly by adipokinetic hormones (AKHs), with minor participation of other neuropeptides.

1.2.1.1 The hormonal system in insects

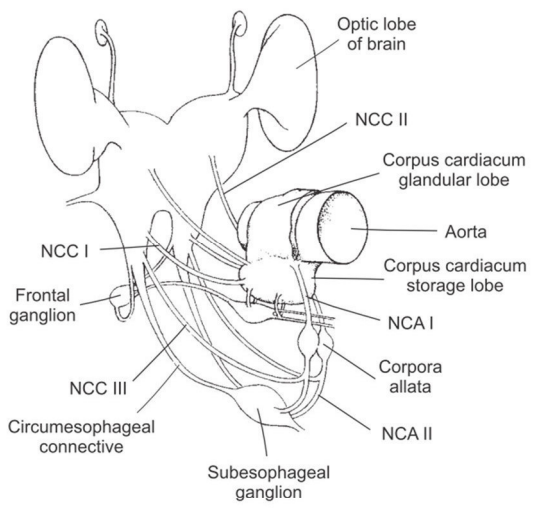


Fig. 1. The central nervous system of the locust. The *corpus cardiacum* and *corpora allata*, and their innervation – *nervi corporis allati* I (NCA I), *nervi corporis cardiaci* I, II, and III (NCC I, II, and III) (Klowden, 2008).

The disposition and function of the hormonal system of invertebrates, including insects, is comparable to that of mammals, with the extension to all vertebrates. The structure, and even effect, of some hormones has been defined to be common for both mammals and insects. On the other hand, the hormone pool of insects constitutes a majority of

neurohormones, hormones synthesised by neurosecretory cells, in contrast to only two mammalian neurohormones (oxytocin and antidiuretic hormone) (Gäde *et al.*, 1997).

The main role in the insect endocrine system is played by the brain-*corpora cardiaca-corpora allata* axis. The neurosecretory cells of the brain are the origin of many neurohormones, which control all aspects of insect life – reproduction, development, metabolism, metamorphosis and moulting, behaviour etc. The brain is neurally linked to *corpora cardiaca* (CC) (Fig. 1), a pair of neuroglandular bodies that are attached to the aorta; thus they serve as a neurohaemal organ. Other brain neurons are connected to *corpora allata* (CA) (Fig. 1), a pair organ synthesising terpenoids (juvenile hormones), of which the main function is a preservation of juvenile stadia from premature metamorphosis. The next important endocrine centrum is represented by prothoracic glands, which secret ecdysteroids – hormones controlling insect moulting and metamorphosis, and reproduction. In higher Diptera species, e.g. the fruit fly (*Drosophila melanogaster)*, the CC, CA and prothoracic glands have fused into one organ – the ring gland (Fig. 2) (King *et al.*, 1966). Apart from those, additional endocrine cells have been described in gonads, gut, neural ganglia of the ventral nerve cord, and others (Gäde *et al.*, 1997; Gullan & Cranston, 2005; Triplehorn & Johnson, 2005).

The function of every single hormone is complex and very often pleiotropic. Additionally, most physiological events are controlled by more than one hormone. For example, juvenile hormone and ecdysone in various ratios of its titre trigger larval moulting, pupariation or imago hatching but also regulate reproduction cycles, egg laying, metabolism, diapause (Chippendale, 1977) or caste

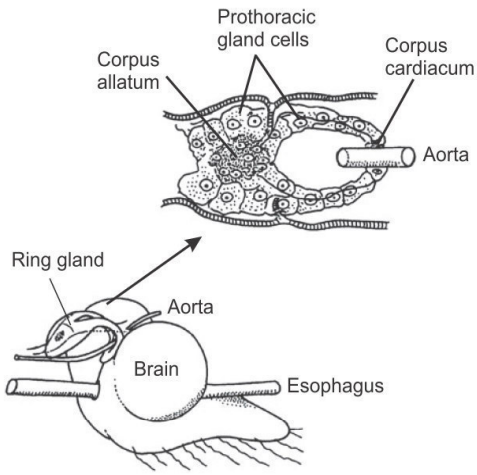


Fig. 2. The ring gland of higher dipterans, consisting of *corpus cardiacum*, *corpus allatum*, and the prothoracic gland (Klowden, 2008).

polymorphism in social species (Gilbert *et al.*, 2005).

As mentioned above, the most abundant group of insect hormones, neurohormones, affect practically all events during the insect's lifetime. Neurohormones are mostly small peptides secreted by neurosecretory cells in the central nervous system (CNS), CC, and in the ganglia of the ventral nerve cord. The action of these hormones is greatly pleiotropic, overlapping with other hormones, and often also glandotropic, i.e. regulating secretion of the other endocrine organs. The primary effect could be considered, according to the nature of the consequence of their actions, as gonadotropic, morfogenic, chromatotropic, metabolic, myotropic, or etotropic (Gäde *et al.*, 1997).

There are a number of insect neuropeptides with potential roles in the maintenance of a homeostasis; for instance, adipokinetic hormones (for details see below), insulin-like peptides with numerous functions in insects (Baker & Thummel, 2007); a glucagon-like peptide whose role in insect metabolism is not completely known and which may play a role in the oxidative stress defence (Alquicer *et al.*, 2009; Bednářová *et al.*, 2013a); and corazonin, with multiple activities including its originally described function, modulation of heart contractions (Veenstra, 1989) etc. (for review see Gäde *et* al. 1997; Nässel and Winther 2010).

So far, the research of neurohormones has revealed hundreds of different structures of these hormones, including those with a structural and functional similarity to vertebrate hormones. They are typically encompassed with the suffix '-like' (i.e. insulin-like hormones).

1.2.1.1.1 The adipokinetic hormone of insects

The most important homeostatic insect neurohormone is the adipokinetic hormone (AKH). Like other functional parallels between vertebrates and insect hormones, a group of AKHs has been considered as analogous to mammalian glucagon (Goldsworthy, 1994).

Up to the present time, more than sixty structures of AKH have been isolated and described in the insect kingdom (Gäde *et al.*, 2015). Although the AKH structure varies in different species, a lot of the AKHs have been reported to work in heterologous assay (i.e. in host insect species with different own AKH) (Socha *et al.*, 2004). Furthermore, the effect of an AKH application has been proven even in humans (Schacter & Schacter, 2005).

These small peptides belong to the adipokinetic hormone/red pigment concentrating hormone family (AKH/RPCH). The AKH common structure has been determinated as a chain length of eight, nine or ten amino acids with N-terminus blocked by pyroglutamate, and C-terminus amidated, and with aromatic amino acids at positions 4 (phenylalanine or tyrosine) and 8 (tryptophan) (Fig. 3) (Gäde & Goldsworthy, 2003; Gäde, 2009). For example, in *D. melanogaster* the sequence of the mature AKH octapeptide is pGlu-Leu-Thr-Phe-Ser-Pro-Asp-Trp-NH_2, where pGlu is pyroglutamic acid, and Trp-NH_2 is tryptophan carboxyamide (Schaffer *et al.*, 1990; Noyes *et al.*, 1995; Sajwan *et al.*, 2015).

The AKHs are synthesised and stored in the CC, alternatively in the corresponding CC cells of dipteran ring glands (Lee & Park, 2004; Isabel *et al.*, 2005). Moreover, a small amount of AKH has also been detected in the insect brain (Schooneveld *et al.*, 1985; Kodrík *et al.*, 2015a). Since the CC or ring gland are in an immediate connection with the aorta, the AKH can be quickly released into the haemolymph when necessary.

The mode of subsequent actions of AKH is typical of stress hormones and can be compared to the activity of the hypothalamic-pituitary-adrenal axis in mammals (Gäde & Goldsworthy, 2003; Kodrík, 2008). Similarly, the AKH stimulation triggers a reaction cascade that leads to the initiation of catabolic reactions (for example, activation of lipase (Spencer & Candy, 1976) and glycogen phosphorylase (van Marrewijk *et al.*, 1980)); and to metabolism increase (for example, lipoprotein transporters elevation (Kanost *et al.*, 1990)). Simultaneously, the actions which are not essential are inhibited – RNA (Kodrík & Goldsworthy, 1995), protein (Carlisle & Loughton, 1979) and lipid (Gokuldas *et al.*, 1988) syntheses. That altogether results in an energy release, mobilisation and redistribution (Gäde *et al.*, 1997; Gäde & Auerswald, 2003).

Apart of this initial stress alarm function, the AKH peptides are known for their pleiotropic effect, with a number of actions that boost their main roles in the stress energy metabolism (Kodrík, 2008). Among other functions, the AKHs stimulate heartbeat (Scarborough *et al.*, 1984; Noyes *et al.*, 1995), muscle tonus (O'Shea *et al.*, 1984), and general locomotion (Socha *et al.*, 1999; Kodrík *et al.*, 2000); enhance immune responses (Goldsworthy *et al.*, 2002, 2003); regulate starvation-induced foraging behaviour of *Drosophila* (Lee & Park, 2004); participate in the activation of the antioxidant mechanisms (Kodrík *et al.*, 2007, 2015b, Večeřa *et al.*, 2007, 2012; Velki *et al.*,

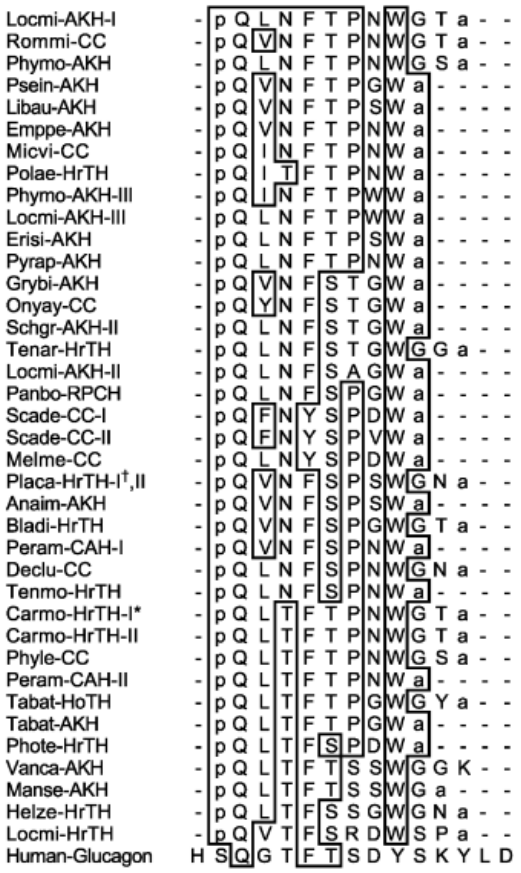


Fig. 3. Alignment of sequences of the main known adipokinetic and hypertrehalosemic hormones (AKH/HrTH) in insects. Peptide sequences were compiled largely from (Gäde *et al.*, 1997, 2003; Gäde & Kellner, 1999; Siegert, 1999; Kodrík *et al.*, 2000; Köllisch *et al.*, 2000; Siegert *et al.*, 2000; Lorenz *et al.*, 2001). The sequence of one peptide from *Carausius morosus* (Carmo-HrTHI*) contains a hexose modification on the Trp8 residue (Gäde *et al.*, 1992), and an additional peptide from *Platypleura capensis* (Placa-HrTHI†) contains a modification that has not been characterized yet (Gäde & Janssens, 1994). Anaim, *Anax imperator*; Bladi, *Blaberus discoidalis*; Declu, *Decapotoma lunata*; Emppe, *Empusa pennata*; Erysi, *Erythemis simplicicollis*; Grybi, *Gryllus bimaculatus*; Helze, *Heliothis zea*; Libau, *Libellula auripennis*; Locmi, *Locusta migratoria*; Manse, *Manduca sexta*; Melme, *Melolontha melolontha*; Micvi, *Microhodotermes viator*; Onyay, *Onymacris aygulus*; Panbo, *Pandalus borealis*; Peram, *Periplaneta americana*; Phote, *Phormia terranova*; Phymo, *Phymateus morbillosus*; Phyle, *Phymateus leprosus*; Polae, *Polyphaga aegyptiaca*; Psein, *Pseudagrion inconspicuum*; Pyrap, *Pyrrhocoris apterus*; Rommi, *Romalea microptera*; Scade, *Scarabaeus deludens*; Schgr, *Schistocerca gregaria*; Tabat, *Tabanus atratus*; Tenar, *Tenthredo arcuata*; Vanca, *Vanessa cardui*. The 15 N-terminal residues of glucagon are shown (16–29 deleted); Gln3 is conserved in all AKH and the motif Thr-Phe-Thr from glucagon is 100% conserved in nine sequences, and Ser8 and Asp9 of glucagons are conserved in certain AKH sequences and are conservative substitutions in others. Tyr10 is a conservative substitution for the completely conserved Trp (Schooley *et al.*, 2005).

2011; Krishnan & Kodrík, 2012; Bednářová *et al.*, 2013b); and also enhance a food intake and digestive process in the insect gut (Kodrík *et al.*, 2012). In accordance with synthesis suppression, the egg development has been found to be inhibited by AKHs activity (Lorenz, 2003).

In detail, the action of AKH has been described in the fat body and the signal transduction has been well documented at subcellular level (Gäde & Auerswald, 2003). The peptidic hormones, including AKHs, are not able to penetrate freely the cell membrane, thus their signal is transduced via a specific membrane receptor, called the AKH receptor (AKHR) (Park *et al.*, 2002; Staubli *et al.*, 2002). The AKHR, which is structurally related to the receptor of the vertebrate gonadotropin releasing hormone (GnRH), has been cloned or deduced from genomic sequences of several insect species (Park *et al.*, 2002; Staubli *et al.*, 2002). The AKHR is a G-protein coupled receptor (GPCR), and the linkage of AKH to AKHR triggers corresponding biochemical pathways leading to the release of energy-rich substrates – the adenylate cyclase (AC) and/or phospholipase C (PLC) (Vroemen *et al.*, 1997) pathways.

Comprehensively, the AKHR receptor is bound to the heterotrimeric GTP-binding protein (G-protein). The link of peptidic hormones to AKHR consecutively results in a conformal change of the receptor. When the alpha subunit of G-protein (G_α) is activated, GDP is replaced by GTP, which triggers the dissociation of G_α subunit from the receptor and the rest of G-protein trimer (beta and gamma subunits; $G_{\beta\gamma}$). Subsequently, the G_α subunit activates an enzyme adenylate cyclase, which generates cyclic AMP (cAMP), which serves as a secondary messenger molecule. High cAMP concentration leads to a cascade of proteinkinase A (PKA) activity, which consecutively triggers an activation of relevant enzymes (Fig. 4). In some insect species, the $G_{\beta\gamma}$ subunits activate phospholipase C enzyme, which catalyses the hydrolysis of the membrane phospholipid, phosphatidylinositol 4,5-bisphophate (PIP_2), resulting in the production of second messenger molecules diacyl glycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). Successively, IP_3s elicit Ca^{2+} release from the endoplasmic reticulum, while DAGs diffuse along the plasmatic membrane where, in conditions of high concentration intracellular Ca^{2+}, the membrane protein kinase C (PKC) is activated and consecutively triggers relevant enzyme activations (Gäde & Auerswald, 2003).

Specifically, in the insect fat body, which is a main target organ for AKH nutrient mobilisation, the linkage of AKH to AKHR on the cell membrane activates lipases via the AC signalling pathway. Lipases catalyse a cleavage of

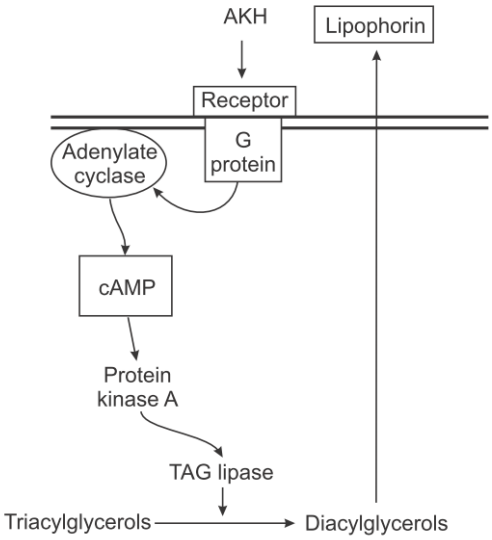


Fig. 4. AKH activates the enzyme triacylglycerol (TAG) lipase that hydrolyzes stored triacylglycerols to diacylglycerols. The diacylglycerols are transported to target tissues by lipophorin (Klowden, 2008).

triacylglycerols into diacylglycerols (Fig. 4). The diacylglycerols serve as a lipid transport form, and are directed to places of utilization (e.g. muscles), where fatty acids are broken down in a beta oxidation process (Van der Horst *et al.*, 2001). Further, metabolism of sugars is regulated by the PKC signalling pathway. The activated PKC triggers a cascade of protein kinase activity, which sequentially activates glycogen phosphorylase, which catalyses the breakdown of glycogen into glucose. From the glucose monosaccharides, the treholose disaccharide – insect glycide transport form – is synthesised. In muscles, trehalose is converted back into glucose and utilized as a source of energy (Arrese *et al.*, 1999; Gäde & Auerswald, 2003).

The impact of AKH on the regulation of energy metabolism is species specific. For example, in the firebug, *Pyrrhocoris apterus*, the injection of AKH significantly increases the level of haemolymph lipids (Kodrík *et al.*, 2000). Analogously, in *D. melanogaster* the ablation of AKH secreting cells results in a profound decrease in circulating carbohydrate levels (Isabel *et al.*, 2005).

1.2.2 The cellular stress response – adenosine as a main player

The mechanisms involved in the stress responses on a cellular level seem to be more evolutionarily conserved across species of invertebrates and vertebrates, insects and mammals included. In these processes an important role is played by adenosine, which serves as a paracrine signal within tissues. Adenosine is known as a key and conservative endogenous molecule, present in the body of every organism. As a crucial metabolite it mediates a main signal of the homeostasis, whether of metabolism, oxygen or energy.

1.2.2.1 Adenosine

Adenosine, a purine nucleoside, is composed of one molecule of an adenine, a purine derivate, which is attached to the sugar molecule ribose by a glycosidic bond (Fig. 5). The half-life time of this molecule, which can be found in the intracellular and extracellular matrix of any tissue, is rather short (Jacobson & Gao, 2009; Fredholm, 2010). An adenosine concentration is controlled by its conversion into inosine, or it can be transported into the cytoplasm, phosphorylated and bonded onto one, two or three phosphate groups, comprising AMP, ADP and ATP (adenosine mono-, di-, tri-phosphate). Cyclic AMP (cAMP), produced by an adenylate cyclase, previously mentioned, serves as a second messenger molecule. ATP is utilized in cellular metabolism as a molecule transferring the chemical energy between reactions in the form of macroergic bonds between adenosine and phosphate residue. As well, adenosine is one of the structural units of nucleic acids, both RNA and DNA, when attached to deoxyribose (Jacobson & Gao, 2009).

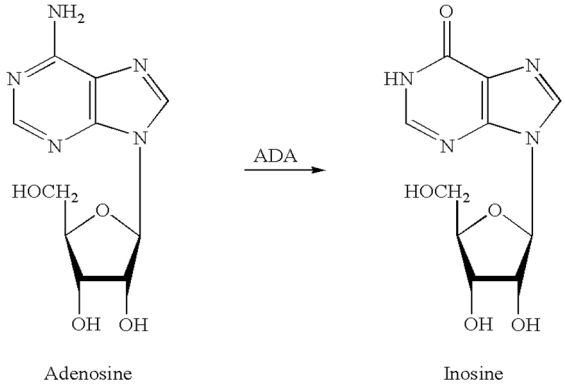


Fig. 5. Structure of adenosine molecule and scheme of conversion into inosine molecule catalysed by adenosine deaminase enzyme (ADA).

Adenosine is produced by the dephosphorylation of AMP (Fig. 6) (Conway & Cooke, 1939; Masino & Boison, 2013). In hydrolysis, which is catalysed by 5'-nucleotidase, the phosphoric ester bond of ribonucleoside and phosphate is cleaved (Sträter, 2006). There are two known nucleotidases that form adenosine. Cytosolic 5'-nucleotidase is responsible for the main formation of adenosine. For mammalian ventricle cells it has been reported that over 70% of adenosine is the product of cytosolic 5'-nucleotidase activity (Darvish *et al.*, 1996). Another membrane enzyme, ecto-5'-nucleotidase, increases the extracellular adenosine level by hydrolysis of extracellular adenine (nucleotide) to adenosine (nucleoside) in close proximity to the cell membrane; thus, adenosine can be rapidly transported into the cells before being degraded by other extracellular enzymes. Adenosine is also produced by S-adenosyl-L-homocysteine hydrolase, the function and structure of which

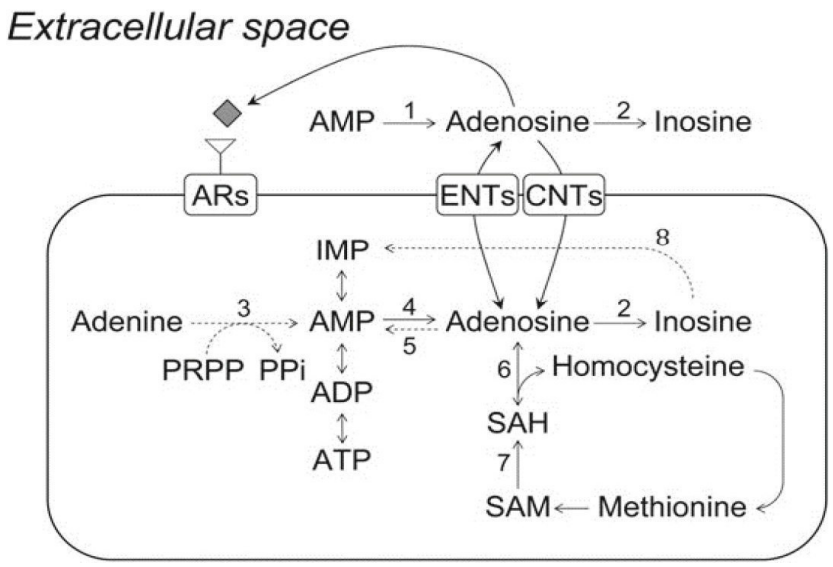


Fig. 6. Overview of adenosine transportation and metabolism. 1, ecto-5'-nucleotidase (5'-NT); 2, adenosine deaminase (ADA); 3, adenine phosphoribosyl transferase (APRTase); 4,5'-nucleotidase (cN-I); 5, adenosine kinase (ADK); 6, S-adenosyl-homocysteine hydrolase; 7, various methyltransferases 8, inosine-guanosine kinase. AR adenosine receptor, ENT equilibrative nucleoside transporter, CNT concentrative nucleoside transporter, PRPP 5-phosphoribose-1-pyrophosphate, PPi inorganic pyrophosphate, SAH S-adenosyl-homocysteine, SAM S-adenosyl-methionine (Masino and Boison, 2013).

have been identified in rat livers (De La Haba & Cantoni, 1959; Hu *et al.*, 1999). S-adenosyl-L-homocysteine hydrolase catalyses hydration of S-adenosyl-L-homocysteine into adenosine and homocysteine, which is reversible and dependent on the local concentration of homocysteine and adenosine to regulate the direction of enzyme activation (Loncar *et al.*, 1997).

Since adenosine serves as a homeostatic signal, its concentration needs to be meticulously equilibrated. The enzymes of adenosine degradation are adenosine deaminase and adenosine kinase (Fig. 6) (Jacobson & Gao, 2009). The former catalyses the hydrolytic deamination of adenosine or 2'-deoxyadenosine to produce inosine or 2'-deoxyinosine respectively (Frick *et al.*, 1987; Sideraki *et al.*, 1996). The latter enzyme, adenosine kinase, catalyses the adenosine phosphorylation to produce AMP. The activity of this enzyme is considered to be the major factor in the regulation of adenosine levels in the rat hippocampus (Pak *et al.*, 1994). AMP is further phosphorylated into ADP by adenylate kinase, and ADP into ATP by creatine

kinase (Lloyd & Fredholm, 1995). In this way, the activity of adenosine kinase influences energy homeostasis by the regulation of cellular AMP, ADP, ATP and intracellular and extracellular adenosine levels.

In addition to its metabolism, adenosine concentration is also modulated by its distribution. Being hydrophilic, nucleosides – adenosine included – cannot simply diffuse across the plasma membrane. Their transport is mediated by specialized membrane proteins – transporters. Two nucleoside transporter families have been identified: equilibrative and concentrative (Rose & Coe, 2008; Molina-Arcas *et al.*, 2009). Equilibrative nucleoside transporters enable the movement of nucleosides and nucleoside analogues down their concentration gradients across cell membranes. They act as bidirectional carriers, responsible for the influx and efflux of substrates. Concentrative nucleoside transporters, on the other hand, are unidirectional and act as Na^+-dependent symporters, which mediate only the influx of nucleosides powered by the transmembrane sodium gradient (Baldwin *et al.*, 2004; Gray *et al.*, 2004).

Apart from the transporters, the extracellular adenosine reacts with the membrane adenosine receptor (AdoR). The AdoR is a form of G-protein coupled receptor (GPCR); its mode of action is a typical reaction cascade running via AC or PLC pathways, with cAMP, DAG, IP_3 and Ca^{2+} as second messengers (as described above for AKH). In mammals, four subtypes of receptors have been described: A_1, A_{2A}, A_{2B} and A_3. Each subtype has different tissue distribution, G-protein binding preference and mode of action. A_1 and A_3 receptors couple to the G_i (inhibitory) proteins family, and their activation results in the inhibition of cAMP formation. Activation of A_{2A} and A_{2B} leads to increased cAMP levels via interaction with the G_s (stimulating) family protein. In *D. melanogaster* there is only a single AdoR; this receptor couples to the G_s subclass of G proteins (Kucerova *et al.*, 2012) and similarly to mammalian A_{2A} and A_{2B}. Its activation evokes a rise in cAMP concentration in the cells (Doleželová *et al.*, 2007; Kucerova *et al.*, 2012).

Because of similarities in its mode of action, adenosine also has been considered a stress 'hormone' (Haskó, 2002). Moreover, adenosine takes part in many metabolic pathways, and it has been found to regulate fat and glycide metabolism and to be involved in actions such as nervous or immune responses (Jacobson & Gao, 2009). As a retaliatory metabolite, adenosine is released extracellularly, via nucleoside membrane transport in imbalance, when disrupted by any factor like metabolic stress, hypoxia, parasitation or

cell damage processes (Jacobson & Gao, 2009; Fredholm, 2010; Csóka & Haskó, 2011). When it is then accumulated in extracellular matrix, adenosine affects surrounding cells. These changes in adenosine concentrations may interfere with cellular energy homeostasis (Fleischmannová *et al.*, 2012). Transportation of high levels of adenosine is followed by phosphorylation and ATP production as a part of adenosine conservation. ATP at higher concentrations may interfere with cellular homeostasis (Fleischmannová *et al.*, 2012). In equilibrium, adenosine concentration varies in range from 20 to 200 nM in cells and tissue. During stress its concentration increases up to 1000 times (Fredholm, 2010; Kucerova *et al.*, 2012).

In human medicine, adenosine is known as an immunomodulator (Haskó & Cronstein, 2004) with immunosuppressive effects. For example, the consequences of the excessive release of adenosine into the extracellular space may result in multiple organ failure in patients in surgical intensive care units (Haskó, 2002). Moreover, in *D. melanogaster*, adenosine/AdoR activity has been found to mediate the metabolic switch in the immune response. The adenosine release from immune cells leads to the suppression of the growth of wing discs and energy storage, which could retain more energy for the immune cells (Bajgar *et al.*, 2015).

1.2.3 Parallels between AKH and adenosine in stress response

The adenosine and AKH signalling seems to have an overlapping spectrum of actions. For example, on a cellular level both adenosine and AKH signals are mediated by GPCR, which modulates the production of cAMP (Park *et al.*, 2002; Jacobson & Gao, 2009). On a physiological level, both AKH and adenosine are involved in the regulation of energy metabolism, physiology of adipose tissue, immune response, oxygen metabolism and neural functions (Kodrík, 2008; Jacobson & Gao, 2009).

1.3 Oxidative stress

Mostly neglected, oxidative potential nevertheless is probably one of the most influential environmental factors. The oxidative potentiality of the environment is given by reactive oxygen species (ROS), which cause oxidative damage to live organisms. Oxidative stress (OS) refers to the cytotoxic subsequence caused by ROS. OS has significant effects mainly because of its

Tab. 1. Human disorders associated with oxidative stress (Armstrong, 1998).

Neurological
Alzheimers Disease
Down's Syndrome
Amyotrophic Lateral Sclerosis
Schizophrenia
Tardine Dyskinesis
Parkinson's Disease
Huntington's Chorea
Ataxia Telangectasia
Stroke

Ocular
Cataract
Age Related Macular Degeneration
Retinopathy of Prematurity
Light Damage

Endocrine
Diabetes
Infertility
Menopause
Thyroiditis
Neoplasia
Hyperthyroidism
Musculocutaneous
UV Injury
Xeroderma Pigmentosum
Duchenne Muscular Dystrophy

Vascular
Atherosclerosis
Coronary Artery Bypass Grafting
Circulatory Shock
Cardiac and Peripheral Disease

Hemolytic
RBC Fragility
Nephrotic Syndrome
Chronic Renal Failure
Membraneous Glomerulonephritis

Renal
Hemochromatosis
Anemia
Sepsis

Hepatic
Hepatitis
Fatty Necrosis
Fetal Alcohol Syndrome

Autoimmune
Rheumatoid Arthritis
HIV
Systemic Lupus Erythematosis

Pulmonary
Respiratory Distress Syndrome
Cystic Fibrosis
Emphysema
Sarcoid Alveolitis
Chronic Idiopathic Fibrosis

Gastrointestinal
Colitis
Acute Pancreatitis
Gastric Mucosal Erosion
Acute Cholecystitis

Other Conditions
Idiosyncratic Drug Reactions
Neoplasia
Trauma
Ischemic Reoxygenation Injury
Multiple Organ Dysfunction
Aging
Bloom's Syndrome
Transplantation Rejection
Air Pollution
Toxicity
Inflammation
Thermal Injury
Excessive Exercise
Apoptosis
Intermittant Claudication
Obesity
Pre-eclampsia

latent character. De facto, each oxygen-consuming organism is exposed to OS constantly throughout its lifespan. In human medicine, changes as a consequence of ROS activity are listed among the factors responsible for the development of neurodegenerative diseases, visceral diseases or cancer (Tab. 1). Therefore, better understanding of anti-oxidative stress mechanisms may be crucial to making progress in the therapeutic approach to OS-related diseases.

1.3.1 Source of oxidative stress

Under ordinary conditions, the rate of oxidant formation is balanced by the rate of oxidant elimination. A state of enhanced ROS production together with the simultaneous impairment of their scavenging systems results in OS, and the effects on cell function are called oxidative stress effects (Sies, 1991; Halliwell & Gutteridge, 2007; Lushchak, 2011).

Reactive oxygen species include radicals, which are unstable and highly reactive, containing one or more unpaired electrons – superoxide ($O_2^{\cdot -}$), hydroxyl ($OH^{\cdot -}$), peroxyl ($ROO\cdot$) alkoxyl ($RO\cdot$), hydroperoxyl ($HO_2^{\cdot -}$) – and non-radical components, which have no unpaired electrons: hydrogen peroxide (H_2O_2), hydrochlorus acid (HOCl), etc. (Tab. 2) (Rahman *et al.*, 2012). These toxic molecules are common products of life in an aerobic environment, and their toxicity varies from the highly reactive (hydroxyl) to the less toxic (superoxide radical).

Primarily, ROS are generated as by-products of the mitochondrial respiratory chain, in which energy activation and electron reduction are employed (Halliwell & Gutteridge, 2007) (Fig. 7, 8). The superoxide molecule

Tab. 2. Reactive oxygen species (Rahman *et al.*, 2012).

Radicals	Non-radicals
Superoxide: O_2^-	Hydrogen peroxide: H_2O_2
Hydroxyl: OH^-	Hypochlorus acid: HOCL
Peroxyl: RO_2^-	Hypobrromus acid: HOBr
Alkoxyl: RO^-	Ozone: O_3
Hydroperoxyl: HO_2^-	Singlet oxygen: Δg

is usually formed as a consequence of electron leakage from mitochondrial electron carriers (complex I and III) (Farooqui & Farooqui, 2009). Briefly, a single electron originating from mitochondria is accepted by molecular oxygen, creating a superoxide radical ($O_2 + e^- \rightarrow O_2^{\cdot -}$) (Valko *et al.*, 2004), which is the primary radical and can further react with other biomolecules to form secondary radicals. Mitochondrial electron leakage may be extensively increased under disturbed or diseased conditions, resulting in more $O_2^{\cdot -}$ formation (Chance *et al.*, 1979; Beckman & Ames, 2000). The molecule of $O_2^{\cdot -}$ is a short-lived anion and it gets immediately dismutased by the enzyme superoxide dismutase (SOD) into the less reactive hydrogen peroxide and molecular oxygen ($2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$). Consecutively, O_2 can be reused to form new $O_2^{\cdot -}$, and highly diffusible H_2O_2 can be further converted into the highly reactive $OH^{\cdot}$.

Other ROS are created in the process of lipid peroxidation, which polyunsaturated fatty acids (RH) with unpaired electrons undergo. The reaction may be considered in

a. Homolysis of covalent bonds

$$A : B \rightarrow A^{\bullet} + B^{\bullet}$$

b. Addition of a single electron to a neutral atom

$$A + e \rightarrow A^{-\bullet}$$

c. Loss of a single electron from a neutral atom

$$A \rightarrow A^{+\bullet} + e$$

Fig. 7. General scheme of possible radical generation (Krishnan & Kodrík, 2012).

Superoxide anion generation:

$$O_2 + e^- \rightarrow O_2^{\bullet -}$$

$$H_2O_2 \rightarrow HO_2^{\bullet} + e^- + H^+$$

$$O_2 + NADPH \rightarrow O_2^{\bullet -} + NADP + H^+$$

Hydroxyl radical generation:

$$O_2^{\bullet -} + H_2O_2 \rightarrow {}^{\bullet}OH + OH^- + O_2 [\text{Haber–Weiss reaction}]$$

$$H_2O_2 + Fe^{2+}/Cu^+ \rightarrow {}^{\bullet}OH + OH^- + Fe^{3+}/Cu^{2+} [\text{Fenton reaction}]$$

Hydrogen peroxide generation:

$$O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$$

$$O_2^{\bullet -} + e^- + 2H^+ \rightarrow H_2O_2$$

$$O_2 + 2e^- + 2H^+ \rightarrow H_2O_2$$

Fig. 8. Generation of main reactive oxygen species (Krishnan & Kodrík, 2012).

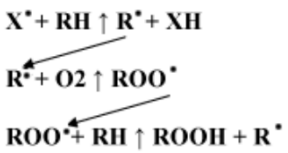


Fig. 9. Scheme of chain reaction of lipid peroxidation. X· = free radical, R· = lipid radical, ROO· = lipid peroxide, ROOH = lipid hydroperoxide (Barber & Bernheim, 1967).

three steps: initiation, propagation and termination. Firstly, in presence of a hydroxyl radical a hydrogen atom is abstracted from the lipid backbone, forming the water molecule and a carbon-centred lipid radical ($RH + OH^{\cdot -} \rightarrow H_2O + R\cdot$). In the propagation phase, this lipid radical reacts with molecular oxygen and forms a lipid peroxyl radical ($R\cdot + O_2 \rightarrow ROO\cdot$). This newly formed peroxy-radical can capture hydrogen atoms from the adjacent fatty acids to form a lipid hydroxyperoxide and another carbon-centred lipid radical ($ROO\cdot + RH \rightarrow ROOH + R\cdot$). Thus, one hydroxyl radical may start the cycle of initiating and propagating reactions, leading to massive lipid peroxidation in the cell (Fig. 9). The peroxidation reactions in membrane lipids are terminated when lipid radicals cross-link to form non-radical products ($R\cdot + R\cdot \rightarrow RR$, or $R\cdot + ROO\cdot \rightarrow ROOR$, or $ROO\cdot + ROO\cdot \rightarrow ROOR + O_2$) (Barber & Bernheim, 1967; Richter, 1987).

Moreover, the presence of redox-active metals, such as iron or copper, also contributes to ROS generation (Fig. 8). When metal ion homeostasis is disrupted, the level of free or unbound metals is elevated. Subsequently, free metals, the concentration of which is very low under physiological conditions, can be utilized in Fenton reaction – iron catalysed decomposition of hydrogen peroxide ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$) (Eq. 1) (Valko *et al.*, 2005; Farooqui & Farooqui, 2009). Produced hydroxyl radical ($HO\cdot$) is one of the most reactive radicals and can hereafter react with other biomolecules. Apart from through Fenton reaction, the hydroxyl radical also can be generated through Haber-Weiss reaction in the presence of catalytic amounts of iron ($H_2O_2 + Fe^{2+}/Cu^+ \rightarrow OH\cdot + OH^- + Fe^{3+}/Cu^{2+}$) (Eq. 2,3) (Liochev & Fridovich, 2002).

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$	Fenton reaction	Eq. 1
$Fe^{3+} + O_2\cdot^- \rightarrow Fe^{2+} + O_2$	iron reduction	Eq. 2
$O_2\cdot^- + H_2O_2 \rightarrow O_2 + \cdot OH + OH^-$	Haber-Weiss reaction	Eq. 3

Furthermore, ROS can be created in the process of special cycle systems such as xanthine/xanthine oxidase or NADPH oxidase activity, or by the activity of enzymes like myeloperoxidase, cytochrome p450, cyclooxygenase, lipoxygenase and nitric oxide synthase. Moreover, cell organelles such as microsomes and peroxisomes are usual sources of H_2O_2.

Under physiological conditions, the balance between ROS generation and elimination ensures the proper maintenance of cells. Under pathological conditions, however, not only ROS but also reactive nitrogen species (RNS) can be produced at increased rates (Halliwell & Gutteridge, 2007). Reactive nitrogen species are various nitric-oxide-derived compounds, either radicals, including nitric oxide ($NO\cdot$) and nitrous acid (HNO_2); or non-radicals, including nitrosyl anion (NO^-), nitrosyl cation (NO^+) and higher oxides of nitrogen etc. (Tab. 3) (Martínez & Andriantsitohaina, 2009; Rahman *et al.*, 2012). Nitric oxide is considered to be an important molecule in organisms because it is a signal of physiological processes of blood pressure regulation, smooth muscle relaxation, immune system regulation or neurotransmission (Alderton *et al.*, 2001). Nitric oxide and superoxide can react together to form peroxynitrite ($NO\cdot + O_2^{\cdot -} \rightarrow ONOO^-$), which causes lipid oxidation and DNA fragmentation.

Furthermore, both ROS and RNS are created not only endogenously but also by the agency of many exogenous factors. Various chemical (environmental pollutants, pesticides, drugs, metals, smog, abnormal oxygen concentration etc.), physical (ionizing radiation, temperature, noise, vibration etc.) and physiological (diseases, injury, aging, inflammation etc.) factors

Tab. 3. Reactive nitrogen species (Rahman *et al.*, 2012).

Radicals	Non-radicals
Nitric oxide: NO^-	Nitrogen dioxide: NO_2
Nitrous acid: HNO_2	Nitrosyl cation: NO
	Nitrosyl anion: NO^- NO^-
	Dinitrogen tetroxide: N_2O_4
	Dinitrogen trioxide : N_2O_3
	Peroxynitrite: $ONOO^-$
	Peroxinitrous acid: ONOOH
	Alkylperoxynitrites: ROONO

create a stress situation and may upset the functional homeostasis of organisms (Ivanović & Janković-Hladni, 1991).

1.3.2 Biological effect of ROS

ROS generated within the organism or taken from the environment are highly reactive with various biomolecules. On the one hand, this activity impairs diverse cell functions, but it also can be utilized by the organism itself (Halliwell & Gutteridge, 2007). For instance, it is known that low levels of ROS induce minor changes in enzyme activity, cell cycles, levels of Ca^{2+}, transcription factors and ion transporters, which support and maintain normal cell function through the tight regulation of a diverse intracellular signalling network. However, moderate or high ROS levels can inflict damage to all subcellular organelles, eventually leading to cell death (Adler *et al.*, 1999).

1.3.2.1 Harmful impact of ROS

Oxidative stress effects lead to DNA damage, mitochondrial malfunction, enzymatic inactivation or cell membrane damage as a consequence of ROS reaction with the main biomolecules – proteins, lipids, nucleic acids (Sies, 1991; Dröge, 2002; Halliwell & Gutteridge, 2007).

Oxidative damage to proteins may induce alterations (1) in the structure, like site-specific amino acid modifications, (2) in fragmentation of the peptide chain or (3) in the electrical charge, with subsequent loss of physiological cell functions. This, likewise, ends in cell death. Modifications of protein molecules include reversible oxidation of amino acids containing sulphur, while disulphide bridges (cysteine residues) or sulphoxide derivates (methionine residues) are formed. Further, the oxidation of arginine, lysine and histidine residues causes irreversible protein carbonylation. In cases where the modified amino acid is the key residue for the enzymatic function, the consequence of such a minor modification can be tremendous. Furthermore, such an inactivation of antioxidant enzymes may lead to the collapse of the entire antioxidant defence system (Fridovich & Freeman, 1986).

Generally, macromolecules of polyunsaturated fatty acids or lipids are very susceptible to oxidative damage. When the cell membrane is comprised of the phospholipids of polyunsaturated fatty acids, the lipid peroxidation

(described above), producing vast amount of lipid radicals, may result in loss of membrane integrity and function. For example, membrane fluidity, electrical resistance, membrane protein mobility, and the activity of ion pumps can be greatly impaired due to the loss of membrane lipids' character (Richter, 1987). In neural cells, the ROS cause changes in the physico-chemical attributes of membranes (microviscosity and fluidity), resulting in (1) phospholipid flip between the two halves of the lipid bilayer, (2) alteration of the orientation of optimal domains of receptors, enzymes, and ion channels, (3) changes in the number of receptors and their affinity for neurotransmitters, and (4) inhibition of ion pump performance and the entry of K^+ and Ca^{2+} into neural cells (Farooqui, 2012).

Reactions of ROS with nucleic acids produce single-strand breaks, and a base and sugar lesions that can be cytotoxic or mutagenic and, as a result, fatal to organisms. Both structural compounds of DNA, the sugar and base, are susceptible to oxidation, causing base degradation, breakage, or cross-linking with protein (Imlay & Linn, 1988). For instance, 8-hydroxy guanine is one of the most common lesions caused by ROS activity. The formation of 8-hydroxy guanine may lead to erroneous transversions of guanine-cytosine into an adenine-thymine base pair during DNA replication, resulting in the risk of mutagenesis. Other ROS modified bases include 5-hydroxyuracil, 5-hydroxycytosine, and 8-hydroxyadenine, which are known as premutagenic factors (Dizdaroglu & Bergtold, 1986; Wallace, 1998).

In humans, the oxidative damage that occurs to DNA may play a role in aging, carcinogenesis, mutagenesis, and neurodegenerative diseases, like dementia type diseases Parkinson's and Alzheimer's (Gabbita et al., 1998; Markesbery & Lovell, 2006). Compared to the rest of the body, the brain consumes large quantities of oxygen relative to its contribution to total body mass. This, accompanied by low levels of antioxidant and low levels of activity of antioxidant enzymes, places the brain at risk of oxidative damage (Farooqui, 2009).

1.3.2.2 Beneficial impacts of ROS

Beneficial effects of ROS occur under rather low or moderate physiological concentration and maintain important physiological processes such as infection defence or activation of various signalling pathways. In immune cells, such as neutrophils or macrophages, the invading microorganisms are

eliminated by oxygen dependent mechanisms, which utilize H_2O_2, originated from peroxisome and microsome organelles.

Furthermore, the tight regulation of ROS generation and removal makes fluctuations in their concentration transient, and thus they can serve as second messenger molecules. ROS can modulate the activity of a discrete set of biochemical reactions, which contribute to cell proliferation, migration and survival (Landriscina *et al.*, 2009). Hydrogen peroxide has been shown to be involved in cell signalling, most likely as a second messenger (Forman, 2007). And definite H_2O_2 concentrations affect the suppression of various genes involved in the T cell immune response, mitochondrial function, growth arrest of the cell, or iron metabolism (Morel & Barouki, 1999).

In neural cells, for instance, the pathways involved in an ROS-adaptive response may also play a critical role in the induction of an adaptive and survival response to the OS through the modulation of proliferation, synaptic plasticity, gene transcription, and neural excitability (Farooqui, 2012). In non-neural cells, ROS regulate many genes, including adhesion molecules and chemotactic factors, antioxidant enzymes, and vasoactive substances (Landriscina *et al.*, 2009). Some of these genes are associated with adaptive responses, which include the induction of SOD, catalase (CAT) and glutathione peroxidase (GSH-Px) by H_2O_2 (Lu *et al.*, 1993; Crawford & Davies, 1994).

1.3.3 Anti-oxidative stress response

A complex intracellular redox buffering network has been developed to adapt and protect cells against the dangerous effects of oxidative stress. When the OS is evolved and the ROS or RNS concentrations exceed physiological levels, the toxic radicals are converted into less reactive compounds in the antioxidant system of organisms. The radical scavenging system utilises various lines of defence (Fig. 10), which is performed by the antioxidant enzymes and low molecular weight antioxidants.

The small antioxidants (Fig. 11) are molecules that act to prevent excessive formation of ROS, eliminate them, or inhibit their interaction with biological molecules. Their structural properties allow them to donate an electron to a free radical and neutralize it. Molecules known for their antioxidant capacity are tocopherols (vitamin E), carotenes (vitamin A precursors), ascorbate (vitamin C), glutathione (GSH), lipoic acid etc. (Halliwell and Gutteridge 2007; Lushchak 2011). The mode of interaction of low

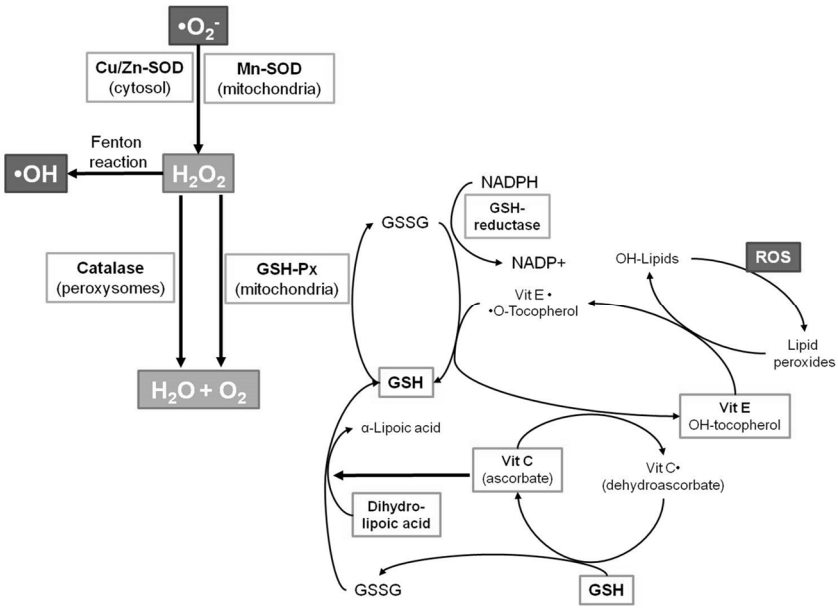


Fig. 10. Antioxidant defence in the organism (Lazo-de-la-Vega-Monroy & Fernández-Mejía, 2013).

molecular weight antioxidants and radicals has been described in detail; for example, vitamins E and C terminate lipid chain reactions, which can prevent further ROS build-up. Analogously to the process of pH regulation, biological systems tightly regulate the cell redox state. The redox state of cells is maintained by the equilibrium of redox pairs. For example, ascorbate is utilized by ascorbate peroxidase to scavenge hydrogen peroxide (ascorbic acid + H_2O_2 → dehydroascorbic acid + H_2O) (Mathews *et al.*, 1997; Jomova & Valko, 2012).

Glutathione (GSH), a tripeptide (Glu-Cys-Gly), in addition to being a cofactor of various antioxidant enzymes and the most abundant peptide in cells, performs many functions. Glutathione couple (GSH/GSSG) is one of the major cellular redox buffers (Wu et al. 2004). The antioxidant function of GSH is facilitated by the sulphydryl group of cysteine (Rennenberg, 1980). Glutathione can be converted to oxidized form, glutathione disulphide (GSSG), and thus the disulphide bounds of biomolecules caused by superoxide and hydroxyl radicals can be reduced. GSH is highly utilized in enzymatic reactions to eliminate peroxides, and in non-enzymatic reactions to convert

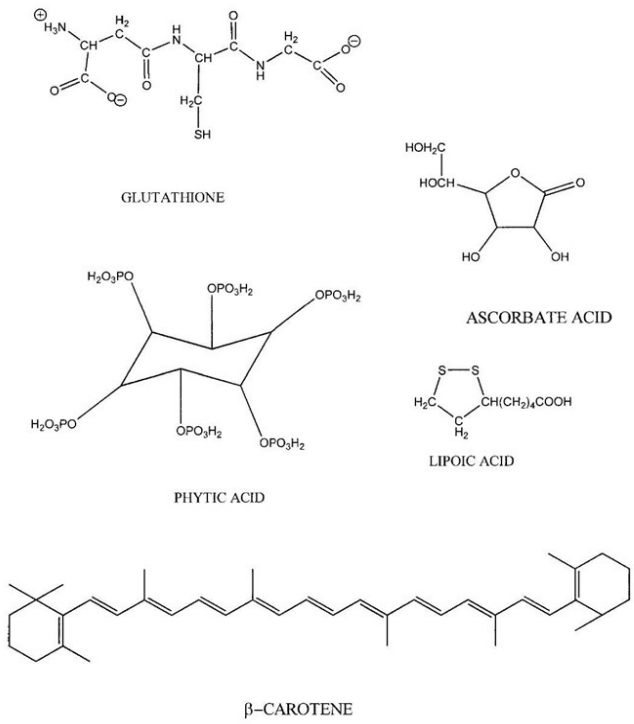


Fig. 11. Structure of main low molecular weight antioxidants.

ascorbate and tocopherol back into their reduced forms. The synthesis and degradation of GSH occurs continuously through the glutamyl cycle that has been well characterized in animals (Meister, 1988). The first step in GSH synthesis is the combination of glutamate and cysteine to form glutamylcysteine by the enzyme glutamylcysteine synthetase (Glu + Cys → Glu-Cys). The subsequent step involves the addition of glycine by the enzyme glutathione synthetase (Glu-Cys + Gly → Glu-Cys-Gly). The reduction of GSSG to GSH is catalysed by the enzyme glutathione reductase.

Among the main enzymes with antioxidant activity, the superoxide dismutase (SOD) is the first initiated in OS defence. SODs catalyse the dismutation of superoxide radicals to hydrogen peroxide and oxygen ($2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$). SODs can be found in all aerobic living organisms. They are dimeric or tetrameric metalloenzymes sized from 30kD to 150kD and, according to metal located at the active site, can be divided into the copper/zinc SODs (Cu/Zn-SODs) and the iron/manganese SODs (Fe/Mn-SODs). The Cu/Zn-SODs enzymes are mainly extracellularly located in the plasma and

cytosol of cells in tissues, and the Fe/Mn-SODs are active principally in mitochondria (Beckman & Ames, 2000; Suzuki *et al.*, 2000).

Subsequently, the hydrogen peroxide is decomposed into water and oxygen by enzyme catalase (CAT). The homotetrameric enzyme, a 24kD sized enzyme with a heme-iron active centre, is mainly located in peroxisomes, where the majority of intracellular H_2O_2 is generated. However, it also can be active in mitochondria where higher levels of ROS also can be produced.

Moreover, hydrogen peroxide can be converted into water by the selenium-dependent enzyme glutathione peroxidase (GSH-Px), an 85kD tetramer well distributed in cells, which utilizes the hydrogen atoms from reduced glutathione (GSH), forming oxidized glutathione (GSSG) described by the equation $2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$ (Farooqui, 2012). In the parallel transformation of GSH to GSSG, GSH-PXs compose various organic peroxides of large molecular weight and some lipid peroxides, in contrast to catalase, which tends to reduce rather small peroxides such as H_2O_2 but has no effect on larger molecules such as lipid hydroperoxides (Warner & Wispeand, 1997). In insects, peroxidase-like activity is represented by glutathione S-transferases (GSTs), which constitute a diverse family of detoxification enzymes (Konno & Shishido, 1992; Mittapalli *et al.*, 2007). Similarly to mammalian GSH-Pxs, GSTs are effective in targeting hydroperoxides ($ROOH + 2GSH \rightarrow ROH + H_2O + GSSG$), but are unreactive towards hydrogen peroxide.

Similarly to glutathione or ascorbate peroxidase/reductase, mentioned above, another antioxidant enzyme, thioredoxin reductase, controls the metabolism of the next important thiol containing antioxidant, thioredoxin. Thioredoxin is a multifunctional selenoprotein involving two redox-active cysteines and a conserved active site (Cys-Gly-Pro-Cys) (Lillig and Holmgren 2007).

1.3.4 Regulation of anti-oxidative stress reactions – hormonal vs paracrine signalling

Apart from the involvement of AKH and adenosine in the regulation of stress such as starvation, locomotor activity, injury or parasitism, it has been suggested that those signalling molecules also act in the anti-oxidative stress response of an organism.

The main results of the research into AKH's role in the anti-oxidative stress response in insects from the last decade have been reviewed by

Krishnan & Kodrík (2012) and Kodrík *et al.* (2015b). Firstly, the demonstration that AKH level in CNS or in haemolymph, or in both, has been significantly elevated under the OS conditions indicates the role of AKH in the anti-oxidative stress response of insects (Kodrík *et al.*, 2007; Večeřa *et al.*, 2007; Velki *et al.*, 2011; Bednářová *et al.*, 2013b). For instance, a treatment with an oxidative stressor paraquat (PQ) increased the AKH level in haemolymph about five times in *P. apterus* (Večeřa *et al.*, 2007), and 2.7 times in *Leptinotarsa decemlineata* (Kodrík *et al.*, 2007). Likewise, the other oxidative stressors, insecticides endosulfan and malathion, increased the AKH level in the haemolymph of treated bug *P. apterus* 2.5 times in 24 hours, whereas only a slight elevation of this neuropeptide was observed in its CNS (Velki *et al.*, 2011).

Further, the above-mentioned results suggesting the involvement of AKH in the anti-oxidative stress mechanism were supported by the other direct evidence of the crucial role of AKH in the regulation of OS in insects. The OS markers experimentally increased/decreased by oxidative stressors were reversed to the control level by the AKH co-application (Kodrík *et al.*, 2007; Večeřa *et al.*, 2007; Bednářová *et al.*, 2013b; Plavšin *et al.*, 2015). Thus, the restoration of the GSH level and suppression of protein carbonylation in haemolymph were observed when AKH was co-injected with oxidative stressors PQ, endosulfan or malathion in *P. apterus* or *L. decemlineata*, respectively (Kodrík *et al.*, 2007; Večeřa *et al.*, 2007; Velki *et al.*, 2011). Moreover, the CAT activity decrease and total antioxidant capacity of haemolymph increase was observed after the AKH co-injection with the stressors in *P. apterus*. Furthermore, the role of exogenous AKH in antioxidant response was also demonstrated in the midgut of *Spodoptera littoralis* larvae, where 5% tannic acid was used as the OS elicitor in larvae of the 6th (final) instar (Večeřa *et al.*, 2012). Decreased GST activity after the tannic acid feeding was restored to the control level after the AKH injection, while neither CAT nor SOD activities were affected by the hormonal treatment. The AKH application also elicited the suppression of protein carbonylation, which also suggests a retreat of OS. This regeneration of the cells reducing capability corresponded well with the rapid decrease in expression of CAT and SOD mRNAs after the AKH treatment. Although there are some indications that GSH may act as a cofactor of enzymatic reactions in the regulation of antioxidant response, the above listed results did not explicate a possible pathway of AKH operation in the mechanism of anti-oxidative stress control.

The mode of AKH action in the anti-oxidative stress response was afterwards described in *P. apterus* and *D. melanogaster* (Bednářová *et al.*, 2013c, 2015). The results revealed that both cAMP and protein kinase C pathways with the mobilisation of extra- and intracellular Ca^{2+} stores are employed. Those conserved signal transduction mechanisms of GPCR were also described for the AKH elicited mobilisation of nutrients; however, always as species-specific alternative pathways and never as constituents of a single anti-stress response.

The cytoprotective role of adenosine against oxidative damage has been proved in mammalian cells. During ischemia, the activity of A_3 receptor and G_i protein leads to a lower degree of lipid peroxidation in cells, to stimulation of antioxidant enzyme activity of SOD, CAT and GSH-Px, and also glutathione reductase (Maggirwar *et al.*, 1994). Moreover, the activity of these enzymes was attenuated by 8-phenyltheophylline, the antagonist of A_3 receptor (Maggirwar *et al.*, 1994). In addition, in rats and humans, adenosine has been shown to promote an increase in the gene expression, as well as the activity, of cardiovascular antioxidant enzymes, such as SOD, CAT and GSH-Px (Husain & Somani, 2005; Zhang *et al.*, 2005). Furthermore, in vivo testing of other adenosine receptors, a non-selective A_1/A_2 antagonist, 1,3-dipropyl-8-sulfophenylxanthine, showed a block of adenosine receptor results in OS in treated rats. These rats showed increased activity of NADPH oxidase, SOD, CAT and GSH-Px, a rise in H_2O_2 generation in mesenteric arteries and plasma lipid peroxidation, and decreased plasma antioxidant capacity (Sousa *et al.*, 2008).

1.4 Summary

Briefly summarized, the scope of AKH and that of adenosine activities seem to overlap. Firstly, both adenosine and AKH signals are mediated by GPCR, which modulates the production of cAMP (Park *et al.*, 2002; Jacobson & Gao, 2009). Both are involved in the regulation of energy metabolism, physiology of adipose tissue, immune response, oxygen metabolism and neural functions (Kodrík, 2008; Jacobson & Gao, 2009). Further, both have been revealed to be involved in the regulation of anti-oxidative stress response (Maggirwar *et al.*, 1994; Husain & Somani, 2005; Zhang *et al.*, 2005; Sousa *et al.*, 2008; Krishnan & Kodrík, 2012; Kodrík *et al.*, 2015b). However, evidence for the involvement of adenosine in the anti-oxidative stress response in insects has so far been

missing. The interaction of local homeostatic regulators and hormones in the control of stress response is not yet known, and adenosine, with its role in cellular signal transduction, could be a candidate for the key factor connecting both systems. The question also arises as to whether AKH and adenosine operate in parallel or in tandem in anti-oxidative stress regulation.

2 Objectives

The main aim of the study was to evaluate the possible involvement of both AKH and adenosine signalling in the anti-oxidative stress response in insect bodies using *D. melanogaster* as a model organism.

3 Results and Conclusion

The results of this thesis are included in three published papers and in an unpublished manuscript.

Paper I presents a comprehensive review in the insect endocrinology and oxidative stress field.

Firstly, the phenomenon of oxidative stress, its origin, and impact on living organisms is briefly summarized. Further, various means of antioxidant defence in insects are briefly overviewed and the role of hormones in their regulation is summarized. Furthermore, a complex characterization of AKH actions in the anti-stress reactions and trends in the recent research of the AKH-insecticide pest control are presented in detail.

Published in the *International Journal of Molecular Science* with impact factor 3.257, the review, with its substantial chapters illustrating the AKH involvement and plausible mechanism of its action in the anti-oxidative stress response and introducing the *Drosophila melanogaster* model in the anti-oxidative stress research, represents a sufficient theoretical base for our further research and manuscript preparation.

Paper II shows primary data of the examination of the AKH and adenosine involvement in the regulation of the anti-oxidative stress response in insects. The article is a part of *Animal Physiology 2016 – Proceedings of International Scientific Conference*.

Since the *Drosophila* model was successfully established, two available fly strains with a mutation in genes of *adipokinetic hormone* (*Akh*^{1}), and *adenosine receptor* (*AdoR*^{1}) were employed in the study. Besides, using those mutants a double mutant carrying a loss-of-function mutation in both the *Akh* and *AdoR* genes (*Akh*^{1} *AdoR*^{1}) was prepared. The anti-oxidative stress response in flies was proven, on the basis of an elevation of AKH titre and *glutathione S-transferase D1* (*GstD1*) gene expressions in the oxidative stress condition (paraquat treatment). However, the GST enzyme activity did not change with either the oxidative stress or fly mutations. Further, the actions of both AKH and adenosine were shown to be implicated in a control of the GST gene expression and important for the recovery of glutathione (GSH), a low molecular weight antioxidant.

In brief, the results clearly support AKH involvement in the antioxidant defence in *Drosophila*, which was shown to be a suitable model organism for

such studies. Although the presence of oxidative stress or functional AKH and adenosine did not impact the enzyme activity of GST, its gene expression dramatically increased following a paraquat administration, which yielded a requirement of further investigation.

Paper III comprises the most substantive results of our oxidative stress research and represents the core of the thesis presented here. The former experiments were extended and improved by tests of other oxidative stress markers. The final paper has been published in the *Journal of Insect Physiology* with an impact factor 2.267.

First of all, apart from previously used fly strains (*Akh*^{1}, *AdoR*^{1}, and *Akh*^{1} *AdoR*^{1} double mutant), a 'rescue' mutant fly which ectopically expresses *Akh* gene (*EE-Akh*) was included in order to evaluate the AKH effect in both directions – in its deficiency and its overproduction. An analogous 'rescue' *AdoR* mutant was not possible to prepare due to its lethality. Afterward, the initial mortality tests revealed a significant difference among all mutant flies. Further, in accordance with other insect species, the AKH titre in CNS of control flies (not carrying *Akh*^{1} mutation) increased with oxidative stress. However, the *Akh* gene expression stayed unchanged. Thus, it seems that regulation of the AKH synthesis is out of the gene expression control. Nevertheless, the study of the antioxidant enzyme GST revealed the opposite effect. Whereas the *GstD1* gene expression significantly increased with oxidative stress, the enzyme activity increase was negligible regardless of oxidative stress or mutations. Interestingly, the increase in the *GstD1* gene expression after the paraquat treatment was maximal in the double *Akh*^{1} *AdoR*^{1} mutant. Thus, it was concluded that both AKH and adenosine are employed in *GstD1* gene expression control. Last but not least, the maintenance of GSH was shown to be dependent on the presence of both AKH and adenosine, since the GSH level in all deficient flies was significantly lower than that in non-mutant flies under both the oxidative stress and control conditions. It seems that AKH and adenosine co-operate in GSH restoration, because the GSH level was the lowest one in the *Akh*^{1} *AdoR*^{1} double mutant. In addition, the *EE-Akh* 'rescue' flies seemed to be favoured in the matter of the anti-oxidative stress characteristics: GSH level in their bodies exceeded substantially that in control flies.

In conclusion, the results showed the AKH and adenosine involvement in the anti-oxidative stress response in *Drosophila*.

Unpublished manuscript is prepared to be submitted in the journal *Physiology & Behavior*, with an impact factor 2.461. The influence of AKH signalling was studied on the senescence characteristics during aging in *Drosophila* in a sexually dimorphic manner.

The results showed that the longevity of flies was not affected by both the lack of AKH signaling or its overexpression. Similarly, locomotor activity rhythms were not affected by lack of undefected AKH. However, Akh^1 females were arrhythmic in old age, and over-expressing *Akh* (*Akh-oex*) females were also arrhythmic in old age. Further, negative geotaxis was significantly impaired in Akh^1 flies, while it was significantly enhanced in *Akh-oex* flies. Furthermore, the body mass did not change across genotypes or age in a particular sex, though young Akh^1 flies of both sexes did show significantly higher body mass compared to age matched flies of other genotypes. Finally, the differential expression of genes involved in the energy homeostasis and aging revealed that while *dTOR* and *Akt* expression were elevated in Akh^1 flies compared to other genotypes, *AMPK* and *dFoxO* expression levels were significantly impaired.

The results presented in this manuscript confirmed the impact of AKH signaling on aging and senescence related characteristics in *D. melanogaster*.

In conclusion, the results of this thesis demonstrated both the important roles of AKH and adenosine in the anti-stress response elicited by paraquat, and the significant role of AKH in aging in the *D. melanogaster* model. As far as we know, this study provides the first evidence for the involvement of adenosine in the anti-oxidative stress reaction in insects. Taken together, we believe that our contribution to the study of the regulation of an anti-oxidative stress response in insects will be inspiration for further oxidative stress research in fields beyond insect physiology. In consequence, such an outcome might affect both the insect pests control approach and also medical attitude to the treatment of human diseases like cancer and ischemic or neurodegenerative disorders.

4 References

Adler V, Yin Z, Tew KD, Ronai Z (1999) Role of redox potential and reactive oxygen species in stress signaling. *Oncogene*, **18**, 6104–6111.

Alderton WK, Cooper CE, Knowles RG (2001) Nitric oxide synthases: structure, function and inhibition. *The Biochemical Journal*, **357**, 593–615.

Alquicer G, Kodrík D, Krishnan N, Večeřa J, Socha R (2009) Activation of insect anti-oxidative mechanisms by mammalian glucagon. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, **152**, 226–233.

Armstrong D (1998) Free radical and antioxidant protocols. Introduction. *Methods in Molecular Biology (Clifton, N.J.)*, **108**, v–viii.

Arrese EL, Flowers MT, Gazard JL, Wells MA (1999) Calcium and cAMP are second messengers in the adipokinetic hormone-induced lipolysis of triacylglycerols in *Manduca sexta* fat body. *Journal of Lipid Research*, **40**, 556–564.

Attrill H, Falls K, Goodman JL, Millburn GH, Antonazzo G, Alix, Rey AJ, Marygold SJ, the FlyBase consortium (2016) FlyBase: establishing a Gene Group resource for *Drosophila melanogaster*. *Nucleic Acids Research*, **44**, D786-792.

Bajgar A, Kučerová K, Jonátová L, Tomčala A, Schneedorferová I, Okrouhlík J, Doležal T (2015) Extracellular adenosine mediates a systemic metabolic switch during immune response. *PLoS Biology*, **13**, e1002135.

Baker KD, Thummel CS (2007) Diabetic larvae and obese flies-emerging studies of metabolism in *Drosophila*. *Cell Metabolism*, **6**, 257–266.

Baldwin SA, Beal PR, Yao SYM, King AE, Cass CE, Young JD (2004) The equilibrative nucleoside transporter family, SLC29. *Pflügers Archiv: European Journal of Physiology*, **447**, 735–743.

Barber AA, Bernheim F (1967) Lipid peroxidation: its measurement, occurrence, and significance in animal tissues. *Advances in Gerontological Research*, **2**, 355–403.

Beckman KB, Ames BN (2000) Oxidants and aging. In: *Handbook of Oxidants and Antioxidants in Exercise* (eds Sen CK, Packer L, Hänninen O), pp. 755–796. Elsevier, Amsterdam.

Bednářová A, Kodrík D, Krishnan N (2013a) Unique roles of glucagon and glucagon-like peptides: Parallels in understanding the functions of adipokinetic hormones in stress responses in insects. *Comparative Biochemistry and Physiology, Part A*, **164**, 91–100.

Bednářová A, Krishnan N, Cheng I-C, Večeřa J, Lee H-J, Kodrík D (2013b) Adipokinetic hormone counteracts oxidative stress elicited in insects by hydrogen peroxide: in vivo and in vitro study. *Physiological Entomology*, **38**,

54–62.

Bednářová A, Kodrík D, Krishnan N (2013c) Adipokinetic hormone exerts its anti-oxidative effects using a conserved signal-transduction mechanism involving both PKC and cAMP by mobilizing extra- and intracellular Ca^{2+} stores. *Comparative Biochemistry and Physiology, Part C*, **158**, 142–149.

Bednářová A, Kodrík D, Krishnan N (2015) Knockdown of adipokinetic hormone synthesis increases susceptibility to oxidative stress in *Drosophila*–a role for dFoxO? *Comparative Biochemistry and Physiology, Part C*, **171**, 8–14.

Cannon WB (1915) *Bodily changes in pain, hunger, fear and rage, an account of recent researches into the function of emotional excitement*. D. Appleton and Co., New York and London, 334 pp.

Carlisle JA, Loughton BG (1979) Adipokinetic hormone inhibits protein synthesis in Locusta. *Nature*, **282**, 420–421.

Chance B, Sies H, Boveris A (1979) Hydroperoxide metabolism in mammalian organs. *Physiological Reviews*, **59**, 527–605.

Chippendale GM (1977) Hormonal regulation of larval diapause. *Annual Review of Entomology*, **22**, 121–138.

Conway EJ, Cooke R (1939) The deaminases of adenosine and adenylic acid in blood and tissues. *The Biochemical Journal*, **33**, 479–492.

Crawford DR, Davies KJ (1994) Adaptive response and oxidative stress. *Environmental Health Perspectives*, **102**, 25–28.

Csóka B, Haskó G (2011) Adenosine, inflammation pathways and therapeutic challenges. *Joint, Bone, Spine: Revue du Rhumatisme*, **78**, 4–6.

Darvish A, Pomerantz RW, Zografides PG, Metting PJ (1996) Contribution of cytosolic and membrane-bound 5'-nucleotidases to cardiac adenosine production. *The American Journal of Physiology*, **271**, H2162-2167.

Davies NB (Nicholas B., Krebs JR (John R., West SA (2012) *An introduction to behavioural ecology*. Wiley-Blackwell, 506 pp.

Dizdaroglu M, Bergtold DS (1986) Characterization of free radical-induced base damage in DNA at biologically relevant levels. *Analytical Biochemistry*, **156**, 182–188.

Doleželová E, Nothacker H-P, Civelli O, Bryant PJ, Žurovec M (2007) A *Drosophila* adenosine receptor activates cAMP and calcium signaling. *Insect Biochemistry and Molecular Biology*, **37**, 318–329.

Dröge W (2002) Free radicals in the physiological control of cell function. *Physiological Reviews*, **82**, 47–95.

Everly GS, Lating JM (2013) The anatomy and physiology of the human stress response. In: *A Clinical Guide to the Treatment of the Human Stress Response*, pp. 17–51. Springer New York, New York, NY.

Farooqui AA (2009) *Hot Topics in Neural Membrane Lipidology*. Springer Science & Business Media, 408 pp.

Farooqui AA (2012) Generation of reactive oxygen species in the brain: signaling for neural cell survival or suicide. In: *Oxidative Stress in Vertebrates and Invertebrates: Molecular Aspects of Cell Signaling* (eds Farooqui T, Farooqui AA), pp. 3–15. John Wiley & Sons, Inc., Hoboken, NJ, USA.

Farooqui T, Farooqui AA (2009) Aging: an important factor for the pathogenesis of neurodegenerative diseases. *Mechanisms of Ageing and Development*, **130**, 203–215.

Fleischmannová J, Kučerová L, Sandová K, Steinbauerová V, Brož V, Šimek P, Žurovec M (2012) Differential response of *Drosophila* cell lines to extracellular adenosine. *Insect Biochemistry and Molecular Biology*, **42**, 321–331.

Forman HJ (2007) Use and abuse of exogenous H_2O_2 in studies of signal transduction. *Free Radical Biology & Medicine*, **42**, 926–932.

Fredholm BB (2010) Adenosine receptors as drug targets. *Experimental Cell Research*, **316**, 1284–1288.

Frick L, Mac Neela JP, Wolfenden R (1987) Transition state stabilization by deaminases: Rates of nonenzymatic hydrolysis of adenosine and cytidine. *Bioorganic Chemistry*, **15**, 100–108.

Fridovich I, Freeman B (1986) Antioxidant defenses in the lung. *Annual Review of Physiology*, **48**, 693–702.

Gabbita SP, Lovell MA, Markesbery WR (1998) Increased nuclear DNA oxidation in the brain in Alzheimer's disease. *Journal of Neurochemistry*, **71**, 2034–2040.

Gäde G (2009) Peptides of the adipokinetic hormone/red pigment-concentrating hormone family: a new take on biodiversity. *Annals of the New York Academy of Sciences*, **1163**, 125–136.

Gäde G, Auerswald L (2003) Mode of action of neuropeptides from the adipokinetic hormone family. *General and Comparative Endocrinology*, **132**, 10–20.

Gäde G, Goldsworthy GJ (2003) Insect peptide hormones: a selective review of their physiology and potential application for pest control. *Pest Management Science*, **59**, 1063–1075.

Gäde G, Janssens MP (1994) Cicadas contain novel members of the AKH/RPCH family peptides with hypertrehalosaemic activity. *Biological Chemistry Hoppe-Seyler*, **375**, 803–809.

Gäde G, Kellner R (1999) Dragonfly *Erythemis simplicicollis* contains a novel adipokinetic neuropeptide. *Archives of Insect Biochemistry and Physiology*, **40**, 99–106.

Gäde G, Kellner R, Rinehart KL, Proefke ML (1992) A tryptophan-substituted member of the AKH/RPCH family isolated from a stick insect corpus cardiacum. *Biochemical and Biophysical Research Communications*, **189**, 1303–1309.

Gäde G, Hoffmann KH, Spring JH (1997) Hormonal regulation in insects: facts, gaps, and future directions. *Physiological Reviews*, **77**, 963–1032.

Gäde G, Auerswald L, Šimek P, Marco HG, Kodrík D (2003) Red pigment-concentrating hormone is not limited to crustaceans. *Biochemical and Biophysical Research Communications*, **309**, 967–973.

Gäde G, Šimek P, Marco HG (2015) Two novel tyrosine-containing peptides (Tyr4) of the adipokinetic hormone family in beetles of the families Coccinellidae and Silphidae. *Amino Acids*, **47**, 2323–2333.

Gilbert LI, Yatrou K, Gill SS (2005) *Comprehensive Molecular Insect Science*. Elsevier, Oxford, UK, 842 pp.

Gokuldas M, Hunt PA, Candy DJ (1988) The inhibition of lipid synthesis in vitro in the locust, *Schistocerca gregaria*, by factors from the *corpora cardiaca*. *Physiological Entomology*, **13**, 43–48.

Goldsworthy GJ (1994) Insect adipokinetic hormones: are they the insect glucagon? In: *Proceedings of XII. International Congress of Comparative Endocrinology.* (eds Davey KG, Peter RE, Tobe SS), pp. 486–492. National Research Council of Canada, Ottawa, Canada.

Goldsworthy G, Opoku-Ware K, Mullen L (2002) Adipokinetic hormone enhances laminarin and bacterial lipopolysaccharide-induced activation of the prophenoloxidase cascade in the African migratory locust, *Locusta migratoria*. *Journal of Insect Physiology*, **48**, 601–608.

Goldsworthy G, Chandrakant S, Opoku-Ware K (2003) Adipokinetic hormone enhances nodule formation and phenoloxidase activation in adult locusts injected with bacterial lipopolysaccharide. *Journal of Insect Physiology*, **49**, 795–803.

Gray JH, Owen RP, Giacomini KM (2004) The concentrative nucleoside transporter family, SLC28. *Pflügers Archiv : European Journal of Physiology*, **447**, 728–734.

Gullan PJ, Cranston PS (2005) *The insects : an outline of entomology*. Blackwell Pub, 505 pp.

Halliwell B, Gutteridge JMC (2007) *Free radicals in biology and medicine*, 4th edn. Oxford University Press, New York.

Haskó G (2002) Adenosine: a potential mediator of immunosuppression in multiple organ failure. *Current Opinion in Pharmacology*, **2**, 440–444.

Haskó G, Cronstein BN (2004) Adenosine: an endogenous regulator of innate immunity. *Trends in Immunology*, **25**, 33–39.

Hightower LE (1991) Heat shock, stress proteins, chaperones, and proteotoxicity. *Cell*, **66**, 191–197.

Van der Horst DJ, Van Marrewijk WJ, Diederen JH (2001) Adipokinetic hormones of insect: Release, signal transduction, and responses. *International Review of Cytology*, **211**, 179–240.

Hu Y, Komoto J, Huang Y, Huang Y, Gomi T, Ogawa H, Takata Y, Fujioka M, Takusagawa F (1999) Crystal structure of S-adenosylhomocysteine hydrolase from rat liver. *Biochemistry*, **38**, 8323–8333.

Husain K, Somani SM (2005) Interaction of exercise and adenosine receptor agonist and antagonist on rat heart antioxidant defense system. *Molecular and Cellular Biochemistry*, **270**, 209–214.

Imlay JA, Linn S (1988) DNA damage and oxygen radical toxicity. *Science (New York, N.Y.)*, **240**, 1302–1309.

Isabel G, Martin J-R, Chidami S, Veenstra JA, Rosay P (2005) AKH-producing neuroendocrine cell ablation decreases trehalose and induces behavioral changes in *Drosophila*. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, **288**, R531-538.

Ivanović J, Janković-Hladni M (eds.) (1991) *Hormones and metabolism in insect stress.* CRC Press Inc., Boca Raton, Florida, U.S.A., 184 pp.

Jacobson KA, Gao ZG (2009) Adenosine. In: *Intercellular Communication in the Nervous System* (ed Malenka RC), pp. 627–638. Elsevier, Boston, MA.

Jansen ASP, Nguyen X V., Karpitskiy V, Mettenleiter TC, Loewy AD (1995) Central command neurons of the sympathetic nervous system: Basis of the fight-or-flight response. *Science*, **270**, 644–646.

Johnson DL, Ambrose SH, Bassett TJ, Bowen ML, Crummey DE, Isaacson JS, Johnson DN, Lamb P, Saul M, Winter-Nelson AE (1997) Meanings of environmental terms. *Journal of Environment Quality*, **26**, 581.

Jomova K, Valko M (2012) Free radicals, signal transduction, and human disease. In: *Oxidative Stress in Vertebrates and Invertebrates* (eds Farooqui T, Farooqui AA), pp. 17–32. John Wiley & Sons, Inc., Hoboken, NJ, USA.

Kanost MR, Kawooya JK, Law JH, Ryan RO, Heusden MC van, Ziegler R (1990) Insect haemolymph proteins. *Advances in Insect Physiology*, **22**, 299–396.

King RC, Aggarwal SK, Bodenstein D (1966) The comparative submicroscopic morphology of the ring gland of *Drosophila melanogaster* during the second and third larval instars. *Zeitschrift für Zellforschung und Mikroskopische Anatomie*, **73**, 272–285.

Klowden MJ (2008) *Physiological systems in insects*, 2nd edn. Elsevier, 1-688 pp.

Kodrík D (2008) Adipokinetic hormone functions that are not associated with insect flight. *Physiological Entomology*, **33**, 171–180.

Kodrík D, Goldsworthy GJ (1995) Inhibition of RNA synthesis by adipokinetic hormones and brain factor(s) in adult fat body of *Locusta migratoria*. *Journal of Insect Physiology*, **41**, 127–133.

Kodrík D, Socha R, Šimek P, Zemek R, Goldsworthy GJ (2000) A new member of the AKH/RPCH family that stimulates locomotory activity in the firebug, *Pyrrhocoris apterus* (Heteroptera). *Insect Biochemistry and Molecular Biology*, **30**, 489–498.

Kodrík D, Krishnan N, Habuštová O (2007) Is the titer of adipokinetic peptides in *Leptinotarsa decemlineata* fed on genetically modified potatoes increased by oxidative stress? *Peptides*, **28**, 974–980.

Kodrík D, Vinokurov K, Tomčala A, Socha R (2012) The effect of adipokinetic hormone on midgut characteristics in *Pyrrhocoris apterus* L. (Heteroptera). *Journal of Insect Physiology*, **58**, 194–204.

Kodrík D, Stašková T, Jedličková V, Weyda F, Závodská R, Pflegerová J (2015a) Molecular characterization, tissue distribution, and ultrastructural localization of adipokinetic hormones in the CNS of the firebug *Pyrrhocoris apterus* (Heteroptera, Insecta). *General and Comparative Endocrinology*, **210**, 1–11.

Kodrík D, Bednářová A, Zemanová M, Krishnan N (2015b) Hormonal regulation of response to oxidative stress in insects—an update. *International Journal of Molecular Sciences*, **16**, 25788–25817.

Köllisch G V, Lorenz MW, Kellner R, Verhaert PD, Hoffmann KH (2000) Structure elucidation and biological activity of an unusual adipokinetic hormone from *corpora cardiaca* of the butterfly, *Vanessa cardui*. *European Journal of Biochemistry / FEBS*, **267**, 5502–8.

Konno Y, Shishido T (1992) Distribution of glutathione S-transferase activity in insect tissues. *Applied Entomology and Zoology*, **27**, 391–397.

Krishnan N, Kodrík D (2012) Edocrine control of oxidative stress in insects. In: *Oxidative Stress in Vertebrates and Invertebrates: Molecular Aspects of Cell Signaling* (eds Farooqui T, Farooqui AA), pp. 261–270. Wiley-Blackwell, New Jersey.

Kucerova L, Broz V, Fleischmannova J, Santruckova E, Sidorov R, Dolezal V, Zurovec M (2012) Characterization of the *Drosophila* adenosine receptor: the effect of adenosine analogs on cAMP signaling in *Drosophila* cells and their utility for in vivo experiments. *Journal of Neurochemistry*, **121**, 383–395.

De La Haba G, Cantoni GL (1959) The enzymatic synthesis of S-adenosyl-L-homocysteine from adenosine and homocysteine. *The Journal of Biological Chemistry*, **234**, 603–608.

Landriscina M, Maddalena F, Laudiero G, Esposito F (2009) Adaptation to oxidative stress, chemoresistance, and cell survival. *Antioxidants & Redox Signaling*, **11**, 2701–2716.

Lazo-de-la-Vega-Monroy M-L, Fernández-Mejía C (2013) Oxidative stress in *diabetes mellitus* and the role of vitamins with antioxidant actions. In: *Oxidative Stress and Chronic Degenerative Diseases - A Role for Antioxidants* (ed Morales-Gonzalez JA), pp. 209–232. InTech.

Lee G, Park JH (2004) Hemolymph sugar homeostasis and starvation-induced hyperactivity affected by genetic manipulations of the adipokinetic hormone-encoding gene in *Drosophila melanogaster*. *Genetics*, **167**, 311–323.

Liochev SI, Fridovich I (2002) The Haber-Weiss cycle – 70 years later: an alternative view. *Redox Report : Communications in Free Radical Research*, **7**, 55–57.

Lloyd HG, Fredholm BB (1995) Involvement of adenosine deaminase and adenosine kinase in regulating extracellular adenosine concentration in rat hippocampal slices. *Neurochemistry International*, **26**, 387–395.

Loncar R, Flesche CW, Deussen A (1997) Determinants of the S-adenosylhomocysteine (SAH) technique for the local assessment of cardiac free cytosolic adenosine. *Journal of Molecular and Cellular Cardiology*, **29**, 1289–1305.

Lorenz MW (2003) Adipokinetic hormone inhibits the formation of energy stores and egg production in the cricket *Gryllus bimaculatus*. *Comparative Biochemistry and Physiology, Part B*, **136**, 197–206.

Lorenz MW, Kellner R, Völkl W, Hoffmann KH, Woodring J (2001) A comparative study on hypertrehalosaemic hormones in the Hymenoptera: sequence determination, physiological actions and biological significance. *Journal of Insect Physiology*, **47**, 563–571.

Lu D, Maulik N, Moraru II, Kreutzer DL, Das DK (1993) Molecular adaptation of vascular endothelial cells to oxidative stress. *The American Journal of Physiology*, **264**, C715-722.

Lushchak VI (2011) Adaptive response to oxidative stress: Bacteria, fungi, plants and animals. *Comparative Biochemistry and Physiology, Part C*, **153**, 175–190.

Maggirwar SB, Dhanraj DN, Somani SM, Ramkumar V (1994) Adenosine acts as an endogenous activator of the cellular antioxidant defense system. *Biochemical and Biophysical Research Communications*, **201**, 508–515.

Markesbery WR, Lovell MA (2006) DNA oxidation in Alzheimer's disease. *Antioxidants & Redox Signaling*, **8**, 2039–2045.

van Marrewijk WJA, van den Broek ATM, Beenakkers AMT (1980) Regulation of glycogenolysis in the locust fat body during flight. *Insect Biochemistry*, **10**, 675–679.

Martínez MC, Andriantsitohaina R (2009) Reactive nitrogen species: molecular mechanisms and potential significance in health and disease. *Antioxidants*

& Redox Signaling, **11**, 669–702.

Masino S, Boison D (2013) *Adenosine - A Key Link between Metabolism and Brain Activity*. Springer, New York.

Mathews MC, Summers CB, Felton GW (1997) Ascorbate peroxidase: A novel antioxidant enzyme in insects. *Archives of Insect Biochemistry and Physiology*, **34**, 57–68.

Meister A (1988) Glutathione metabolism and its selective modification. *The Journal of Biological Chemistry*, **263**, 17205–17208.

Mittapalli O, Neal JJ, Shukle RH (2007) Tissue and life stage specificity of glutathione S-transferase expression in the Hessian fly, *Mayetiola destructor*: implications for resistance to host allelochemicals. *Journal of Insect Science (Online)*, **7**, 1–13.

Molina-Arcas M, Casado FJ, Pastor-Anglada M (2009) Nucleoside transporter proteins. *Current Vascular Pharmacology*, **7**, 426–434.

Morel Y, Barouki R (1999) Repression of gene expression by oxidative stress. *The Biochemical Journal*, **342 Pt 3**, 481–496.

Nässel DR, Winther AME (2010) *Drosophila* neuropeptides in regulation of physiology and behavior. *Progress in Neurobiology*, **92**, 42–104.

Noyes BE, Katz FN, Schaffer MH (1995) Identification and expression of the *Drosophila* adipokinetic hormone gene. *Molecular and Cellular Endocrinology*, **109**, 133–141.

O'Shea M, Witten J, Schaffer M (1984) Isolation and characterization of two myoactive neuropeptides: further evidence of an invertebrate peptide family. *The Journal of Neuroscience : The Official Journal of The Society for Neuroscience*, **4**, 521–529.

Pak MA, Haas HL, Decking UKM, Schrader J (1994) Inhibition of adenosine kinase increases endogenous adenosine and depresses neuronal activity in hippocampal slices. *Neuropharmacology*, **33**, 1049–1053.

Park Y, Kim Y-J, Adams ME (2002) Identification of G protein-coupled receptors for *Drosophila* PRXamide peptides, CCAP, corazonin, and AKH supports a theory of ligand-receptor coevolution. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 11423–11428.

Plavšin I, Stašková T, Šerý M, Smýkal V, Hackenberger BK, Kodrík D (2015) Hormonal enhancement of insecticide efficacy in *Tribolium castaneum*: oxidative stress and metabolic aspects. *Comparative Biochemistry and Physiology, Part C*, **170**, 19–27.

Putman RJ, Wratten SD (1984) The organism and its environment. In: *Principles of Ecology*, pp. 15–42. Springer Netherlands, Dordrecht.

Rahman T, Hosen I, Islam MMT, Shekhar HU (2012) Oxidative stress and human

health. *Advances in Bioscience and Biotechnology*, **3**, 997–1019.

Rennenberg H (1980) Glutathione metabolism and possible biological roles in higher plants. *Phytochemistry*, **21**, 2771–2781.

Richter C (1987) Biophysical consequences of lipid peroxidation in membranes. *Chemistry and Physics of Lipids*, **44**, 175–189.

Rose JB, Coe IR (2008) Physiology of nucleoside transporters: back to the future... *Physiology*, **23**, 41–48.

Sajwan S, Sidorov R, Stašková T, Žaloudíková A, Takasu Y, Kodrík D, Zurovec M (2015) Targeted mutagenesis and functional analysis of adipokinetic hormone-encoding gene in *Drosophila*. *Insect Biochemistry and Molecular Biology*, **61**, 79–86.

Scarborough RM, Jamieson GC, Kalish F, Kramer SJ, McEnroe GA, Miller CA, Schooley DA (1984) Isolation and primary structure of two peptides with cardioacceleratory and hyperglycemic activity from the *corpora cardiaca* of *Periplaneta americana*. *Proceedings of the National Academy of Sciences*, **81**, 5575–5579.

Schacter B, Schacter LP (2005) Compositions and methods for promoting lipid mobilization in humans.

Schaffer MH, Noyes BE, Slaughter CA, Thorne GC, Gaskell SJ (1990) The fruitfly *Drosophila melanogaster* contains a novel charged adipokinetic-hormone-family peptide. *Biochemical Journal*, **269**, 315–320.

Schooley DA, Horodyski FM, Coast GM (2005) 3.10 – Hormones Controlling Homeostasis in Insects. In: *Comprehensive Molecular Insect Science, Volume 3: Endocrinology* (eds Gilbert LI, iatrou K, Gill SS), pp. 493–550. Elsevier.

Schooneveld H, Romberg-Privee HM, Veenstra JA (1985) Adipokinetic hormone-immunoreactive peptide in the endocrine and central nervous system of several insect species: A comparative immunocytochemical approach. *General and Comparative Endocrinology*, **57**, 184–194.

Sideraki V, Wilson DK, Kurz LC, Quiocho FA, Rudolph FB (1996) Site-directed mutagenesis of histidine 238 in mouse adenosine deaminase: substitution of histidine 238 does not impede hydroxylate formation. *Biochemistry*, **35**, 15019–15028.

Siegert KJ (1999) Locust *corpora cardiaca* contain an inactive adipokinetic hormone. *FEBS Letters*, **447**, 237–240.

Siegert KJ, Kellner R, Gäde G (2000) A third active AKH is present in the pyrgomorphid grasshoppers *Phymateus morbillosus* and *Dictyophorus spumans*. *Insect Biochemistry and Molecular Biology*, **30**, 1061–1067.

Sies H (1991) Oxidative stress: introduction. In: *Oxidative Stress: Oxidants and Antioxidants.* (ed Sies H), pp. xv–xxii. Academic Press, London.

Smith SM, Vale WW (2006) The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in Clinical Neuroscience*, **8**, 383–395.

Socha R, Kodrík D, Zemek R (1999) Adipokinetic hormone stimulates insect locomotor activity. *Naturwissenschaften*, **86**, 85–86.

Socha R, Kodrík D, Šimek P, Patočková M (2004) The kind of AKH-mobilized energy substrates in insects can be predicted without a knowledge of the hormone structure. *European Journal of Entomology*, **101**, 29–35.

Sousa T, Pinho D, Morato M, Marques-Lopes J, Fernandes E, Afonso J, Oliveira S, Carvalho F, Albino-Teixeira A (2008) Role of superoxide and hydrogen peroxide in hypertension induced by an antagonist of adenosine receptors. *European Journal of Pharmacology*, **588**, 267–276.

Spencer IM, Candy DJ (1976) Hormonal control of diacyl glycerol mobilization from fat body of the desert locust, *Schistocerca gregaria*. *Insect Biochemistry*, **6**, 289–296.

Staubli F, Jorgensen TJD, Cazzamali G, Williamson M, Lenz C, Sondergaard L, Roepstorff P, Grimmelikhuijzen CJP (2002) Molecular identification of the insect adipokinetic hormone receptors. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 3446–3451.

Storey KB (2004) Adventures in oxygen metabolism. *Comparative Biochemistry and Physiology, Part B*, **139**, 359–369.

Sträter N (2006) Ecto-5'-nucleotidase: Structure function relationships. *Purinergic Signalling*, **2**, 343–350.

Suzuki K, Ohno H, Oh-ishi S, Kizaki T, Ookawara T, Fujii J, Radák Z, Taniguchi N (2000) Superoxide dismutases in exercise and disease. In: *Handbook of Oxidants and Antioxidants in Exercise*, 1^{sth} edn (eds Sen CK, Packer L, Hänninen OOP), pp. 243–296. Elsevier, Amsterdam, The Netherlands.

Triplehorn C, Johnson N (2005) The anatomy, physiology, and development of insects. In: *Borror and DeLong's Introduction to the Study of Insects*, 7th edn, Vol. 18, p. vi. Thompson Brooks/Cole.

Tsigos C, Kyrou I, Kassi E, Chrousos G (2000) *Stress, Endocrine Physiology and Pathophysiology*. MDText.com, Inc.

Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J (2004) Role of oxygen radicals in DNA damage and cancer incidence. *Molecular and Cellular Biochemistry*, **266**, 37–56.

Valko M, Morris H, Cronin MTD (2005) Metals, toxicity and oxidative stress. *Current Medicinal Chemistry*, **12**, 1161–1208.

Večeřa J, Krishnan N, Alquicer G, Kodrík D, Socha R (2007) Adipokinetic hormone-induced enhancement of antioxidant capacity of *Pyrrhocoris apterus* hemolymph in response to oxidative stress. *Comparative Biochemistry and*

Physiology, Part C, **146**, 336–342.

Večeřa J, Krishnan N, Mithöfer A, Vogel H, Kodrík D (2012) Adipokinetic hormone-induced antioxidant response in *Spodoptera littoralis. Comparative Biochemistry and Physiology, Part C*, **155**, 389–395.

Veenstra JA (1989) Isolation and structure of corazonin, a cardioactive peptide from the American cockroach. *FEBS Letters*, **250**, 231–234.

Velki M, Kodrík D, Večeřa J, Hackenberger BK, Socha R (2011) Oxidative stress elicited by insecticides: a role for the adipokinetic hormone. *General and Comparative Endocrinology*, **172**, 77–84.

Vroemen SF, Van Marrewijk WJA, De Meijer J, Van den Broek ATM, Van der Horst DJ (1997) Differential induction of inositol phosphate metabolism by three adipokinetic hormones. *Molecular and Cellular Endocrinology*, **130**, 131–139.

Wallace SS (1998) Enzymatic processing of radiation-induced free radical damage in DNA. *Radiation Research*, **150**, S60-79.

Warner BB, Wispeand JR (1997) Transgenic models for the study of lung injury and repair. In: *Oxygen, Gene Expression, and Cellular Function* (eds Clerch LB, Massaro DJ), pp. 75–97. Marcel Dekker, Inc., New York.

Zhang Y, Handy DE, Loscalzo J (2005) Adenosine-dependent induction of glutathione peroxidase 1 in human primary endothelial cells and protection against oxidative stress. *Circulation Research*, **96**, 831–837.

5 Papers

Paper I

Kodrík D, Bednářová A, Zemanová M, Krishnan N (2015) Hormonal regulation of response to oxidative stress in insects – an update. *International Journal of Molecular Science*, **16**, 25788-25816.

Abstract

Insects, like other organisms, must deal with a wide variety of potentially challenging environmental factors during the course of their life. An important example of such a challenge is the phenomenon of oxidative stress. This review summarizes the current knowledge on the role of adipokinetic hormones (AKH) as principal stress responsive hormones in insects involved in activation of anti-oxidative stress response pathways. Emphasis is placed on an analysis of oxidative stress experimentally induced by various stressors and monitored by suitable biomarkers, and on detailed characterization of AKH's role in the anti-stress reactions. These reactions are characterized by a significant increase of AKH levels in the insect body, and by effective reversal of the markers — disturbed by the stressors — after co-application of the stressor with AKH. A plausible mechanism of AKH action in the anti-oxidative stress response is discussed as well: this probably involves simultaneous employment of both protein kinase C and cyclic adenosine 3',5'-monophosphate pathways in the presence of extra and intra-cellular Ca^{2+} stores, with the possible involvement of the FoxO transcription factors. The role of other insect hormones in the anti-oxidative defense reactions is also discussed.

Paper II

Zemanová M, Kodrík D (2016) The anti-oxidative stress response in *Drosophila melanogaster*. Involvement of adipokinetic hormone and adenosine. In: *Animal Physiology 2016 – Proceedings of International Scientific Conference* (eds Pavlík A, Sláma P, Škarpa P), pp. 317–323. Mendel University, Brno.

Abstract

The anti-oxidative stress response was studied in *Drosophila melanogaster* larvae and adults with mutation in genes of *adipokinetic hormone* (*Akh*^{1}), and *adenosine receptor* (*AdoR*^{1}), and both these genes together (*Akh*^{1} *AdoR*^{1} double mutant). To elicit the oxidative stress we administered paraquat (PQ) in food. Mortality tests revealed the double mutant *Akh*^{1} *AdoR*^{1} was more sensitive to PQ toxicity than either of the single mutants. The PQ administration significantly increased the Drome-AKH hormone level in control *w*^{1118} and *AdoR*^{1} larvae. On the contrary, PQ significantly increased expression of *glutathione S-transferase D1* (*GstD1*) gene. It seems that both functional adenosine receptor and AKH itself are important for the proper control of the *GstD1* gene expression under oxidative stress. On the other hand, differences in glutathione S-transferase (GST) activity among the strains, and between untreated and PQ treated groups were minimal. Next, the glutathione (GSH) level was significantly lower in all untreated mutant groups as compared with untreated control *w*^{1118} flies and declined further when the flies were treated with PQ. Thus, we demonstrated the important role of AKH and adenosine in control of anti-stress response elicited by PQ in *D. melanogaster* model.

Paper III

Zemanová M, Stašková T, Kodrík D (2016) Role of adipokinetic hormone and adenosine in the anti-stress response in *Drosophila melanogaster*. *Journal of Insect Physiology*, **91-92**, 39–47.

Abstract

The role of adipokinetic hormone (AKH) and adenosine in the anti-stress response was studied in *Drosophila melanogaster* larvae and adults carrying a mutation in the *Akh* gene (Akh^1), the *adenosine receptor* gene ($AdoR^1$), or in both of these genes (Akh^1 $AdoR^1$ double mutant). Stress was induced by starvation or by the addition of an oxidative stressor paraquat (PQ) to food. Mortality tests revealed that the Akh^1 mutant was the most resistant to starvation, while the $AdoR^1$ mutant was the most sensitive. Conversely, the Akh^1 $AdoR^1$ double mutant was more sensitive to PQ toxicity than either of the single mutants. Administration of PQ significantly increased the Drome-AKH level in w^{1118} and $AdoR^1$ larvae; however, this was not accompanied by a simultaneous increase in *Akh* gene expression. In contrast, PQ significantly increased the expression of the *glutathione S-transferase D1* (*GstD1*) gene. The presence of both a functional adenosine receptor and AKH seem to be important for the proper control of *GstD1* gene expression under oxidative stress, however, the latter appears to play more dominant role. On the other hand, differences in glutathione S-transferase (GST) activity among the strains, and between untreated and PQ-treated groups were minimal. In addition, the glutathione level was significantly lower in all untreated AKH- or AdoR-deficient mutant flies as compared with the untreated control w^{1118} flies and further declined following treatment with PQ. All oxidative stress characteristics modified by mutations in *Akh* gene were restored or even improved by 'rescue' mutation in flies which ectopically express *Akh.* Thus, the results of the present study demonstrate the important roles of AKH

and adenosine in the anti-stress response elicited by PQ in a *D. melanogaster* model, and provide the first evidence for the involvement of adenosine in the anti-oxidative stress response in insects.

Unpublished manuscript

Bednářová A, Zemanová M, Rakshit K, Kodrík D, Krishnan N. Effect of disruption of adipokinetic hormone signaling on homeostasis and aging characteristics in *Drosophila melanogaster*. *Physilogy & Behavior*, unpublished manuscript

Abstract

The impact of disruption of AKH signaling was studied on the senescence characteristics during aging in *Drosophila* in a sexually dimorphic manner. In the study a mutant that produces a non-functional AKH peptide (Akh^{1}) was compared with isogenized wild-type controls (w^{1118}), *Akh*-rescue line with ectopic expression of *Akh* in mutated *Akh* background (*EE-Akh*), and also with flies overexpressing *Akh* (*Akh-oex*). Interestingly, lack of AKH signaling did not affect longevity of flies under ad libitum feeding conditions and overexpression of *Akh* did not have any beneficial effects either between sexes. Locomotor activity rhythms were unaffected in Akh^{1} mutants though a significant decline in strength of rhythm was recorded with age. Females of both Akh^{1} and *Akh-oex* flies were arrhythmic in old age. Negative geotaxis was significantly impaired in Akh^{1} flies while it was significantly enhanced in *Akh-oex* flies. No change in body mass was recorded across genotypes or age in a particular sex, though young Akh^{1} flies of both sexes did show significantly higher body mass compared to age matched flies of other genotypes. Differential expression of genes involved in energy homeostasis and aging revealed that while *dTOR* and *Akt* expression were elevated in Akh^{1} flies compared to other genotypes, *AMPK* and *dFoxO* expression levels were significantly impaired. Taken together, the results reveal a significant role for AKH in aging and senescence related characteristics but these would be more prominent in conditions when situations of stress exist.

6 Appendix

List of abbreviations

A_1	A_1 subtypes of AdoR
A_{2A}	A_{2A} subtypes of AdoR
A_{2B}	A_{2B} subtypes of AdoR
A_3	A_3 subtypes of AdoR
AC	adenylate cyclase
AdoR	adenosine receptor
AdoR	gene for AdoR
AdoR^1	mutation in AdoR1 gene
ADP	adenosine diphosphate
AKH	adipokinetic hormone
Akh	gene for AKH
AKH/RPCH	adipokinetic hormone/red pigment concentrating hormone family
Akh^1 *AdoR*^{1}	double mutant carrying both *Akh*^{1} and *AdoR*^{1} mutation
Akh^1	mutation in *Akh* gene
Akh-oex	flies over-expressing *Akh*
AKHR	AKH receptor
Akt/PI3	protein kinase B/phosphoinositide kinase 3
AMP	adenosine monophosphate
AMPK	5'-AMP-activated kinase
ATP	adenosine triphosphate
CA	*corpora allata*
cAMP	cyclic adenosine 3',5'-monophosphate
CAT	catalase
CC	*corpora cardiaca*
CNS	central nervous system
DAG	diacyl glycerol
dFoxO	Forkhead box class O transcription factors gene in *Drosophila*
Drome-AKH	*Drosophila melanogaster* adipokinetic hormone
dTOR	target of rapamycin proteins gene in *Drosophila*
EE-Akh	'rescue' mutant flies which ectopically express *Akh* gene
FoxO	Forkhead box class O transcription factors
GDP	guanosine 5'-diphosphate
G_i	inhibitory G-protein family

GnRH	gonadotropin releasing hormone
GPCR	G-protein coupled receptor
G-protein	GTP-binding protein
G_s	stimulating G-protein family
GSH	reduced glutathione
GSH-Px	glutathione peroxidase
GSSG	forming oxidized glutathione
GST	glutathione S-transferase
GstD1	gene for GST delta1
GTP	guanosine 5'-triphosphate
G_α	alpha subunit of G-protein trimer
$G_{\beta\gamma}$	beta and gamma subunits of G-protein trimer
H_2O_2	hydrogen peroxide
HNO_2	nitrous acid
HO·	hydroxyl radical
$HO_2\cdot^-$	hydroperoxyl
HOCl	hydrochlorus acid
IP_3	inositol 1,4,5-trisphosphate
NO^-	nitrosyl anion
NO^+	nitrosyl cation
NO·	nitric oxide and
$O_2\cdot^-$	superoxide
$OH\cdot^-$	hydroxyl
$ONOO^-$	peroxynitrite
OS	oxidative stress
PIP_2	phosphatidylinositol 4,5-bisphophate
PKA	proteinkinase A
PKC	protein kinase C
PLC	phospholipase
PQ	paraquat (N,N'-dimethyl-4,4'-bipyridinium dichloride)
R·	carbon-centred lipid radical
RH	polyunsaturated fatty acids
RNS	reactive nitrogen species
RO·	alkoxyl
ROO·	form lipid peroxyl radical
ROO·	peroxyl
ROOH	lipid hydroxyperoxide

ROS	reactive oxygen species
SOD	superoxide dismutase
TALEN	transcription activator-like effector nucleases
TOR	target of rapamycin proteins
Trx	thioredoxin

Curriculum vitae

Milada Zemanová

Personal details
born: 29th May 1984, Žatec, Czech Republic
email: milada.muj@gmail.com

Work experience
March 2010 – June 2016
Research assistant at the Institute of Entomology, Biology centre of Academy of Science of the Czech Republic, České Budějovice

November 2012 – October 2014
Working holiday experience in New Zealand and Australia

Education
PhD. studies since 2010 in Physiology and Developmental Biology, Department of Animal Physiology, Faculty of Science, University of South Bohemia in České Budějovice, Czech Republic

2012 RNDr. gained in Experimental biology, Department of Animal Physiology, Faculty of Science, University of South Bohemia in České Budějovice, Czech Republic
Thesis: Poikilothermic traits in Mashona mole-rat (*Fukomys darlingi*). Reality or myth?

2011 Graduated MSc. in Zoology, Department of Zoology, Faculty of Science, University of South Bohemia in České Budějovice, Czech Republic
Thesis: The thermoregulatory abilities in mole-rat *Fukomys darlingi* and its development in pups

2010 Graduated MSc. in Experimental biology, Department of Animal Physiology, Faculty of Science, University of South Bohemia in České Budějovice, Czech Republic
Thesis: see above

2007 Graduated BSc. in Biology, Faculty of Biology, University of South Bohemia in České Budějovice, Czech Republic
Thesis: Bioenergetics of reproduction and postnatal development of two species of social mole-rat of genus *Fukomys*

Teaching experience
Teaching assistant in practical course: Developmental Biology, Animal Physiology

Stay abroad
Institute of Neuroinformatics, University of Zurich, Switzerland, May 2012, Head of the Laboratory: Prof. Dr. Steven N. Fry, Supervisor: Dr. Jan Bartussek PhD.

Publications
Zemanová M, Stašková T, Kodrík D (2016) Role of adipokinetic hormone and adenosine in the anti-stress response in *Drosophila melanogaster*. *Journal of Insect Physiology*, **91-92**, 39–47.

Zemanová M, Kodrík D (2016) The anti-oxidative stress response in *Drosophila melanogaster*. Involvement of adipokinetic hormone and adenosine. In: *Animal Physiology 2016 – Proceedings of International Scientific Conference* (eds Pavlík A, Sláma P, Škarpa P), pp. 317–323. Mendel University, Brno.

Kodrík D, Bednářová A, Zemanová M, Krishnan N (2015) Hormonal regulation of response to oxidative stress in insects – an update. *International Journal of Molecular Science*, **16**, 25788-25816.

Zemanová M, Šumbera R, Okrouhlík J (2012) Poikilothermic traits in Mashona mole-rat (*Fukomys darlingi*). Reality or myth? *Journal of Thermal Biology*, **37**, 485–489.

Conference presentations
Zemanová M, Kodrík D (2016) The anti-oxidative stress response in *Drosophila melanogaster* – Involvement of adipokinetic hormone and adenosine (oral presentation). *12th Internatinal Scientific Conference Animal*

Physiology 2016, Bořetice, Czech Republic.

Zemanová M, Stašková T, Kodrík D (2016) Oxidační stres u hmyzu a jeho obranný mechanismus: úloha hormonální a buněčné signalizace (oral presentation). *92th annual meeting of Czech and Slovak Physiology Society*, *Physiological days*, České Budějovice, Czech Republic.

Zemanová M, Rodríguez-Illamola A, Stašková T, Sidorov R, Kodrík D (2015) Regulation of antioxidative stress response in *Drosophila melanogaster*: Involvement of the adipokinetic hormone and adenosine (poster). *Conference of Society for Experimental Biology Meeting*, Prague, Czech Republic.

Zemanová M, Večeřa J, Fencková M, Kodrík D (2012) Oxidative stress in *Drosophila melanogaster* and a role of adipokinetic hormone in its solution (poster). *26th Conference of European Comparative Endocrinologists*, Zurich, Switzerland.

Zemanová M, Večeřa J, Fencková M, Doležal T, Kodrík D (2012) Genové manipulace – nástroj pro studium hormonální regulace antioxidačních mechanismů u octomilky obecné *Drosophila melanogaster* (oral presentation). *88th annual meeting of Czech and Slovak Physiology Society*, *Physiological days*, Hradec Králové, Czech Republic.

Zemanová M (2011) Hormone regulation of metabolism in insects (poster). *2nd annual meeting of the European PhD Network in Insect Science*, Tours, France.

milada.muj@gmail.com

Anti-oxidative stress response in *Drosophila melanogaster* – The role of adipokinetic hormone and adenosine.
Ph.D. Thesis Series, 2016, No. 18

Printed in the Czech Republic by Typodesign
Edition of 20 copies

University of South Bohemia in České Budějovice
Faculty of Science
Branišovská 1760
CZ-37005 České Budějovice, Czech Republic

Phone: +420 387 776 201
www.prf.jcu.cz, e-mail: sekret-fpr@prf.jcu.cz