Antimicrobial Peptides in the Nervous System

Subjects: Immunology

Contributor: Sylwia Stączek , Małgorzata Cytryńska , Agnieszka Zdybicka-Barabas

Antimicrobial peptides (AMPs) are short, mainly positively charged, amphipathic molecules. AMPs are important effectors of the immune response in insects with a broad spectrum of antibacterial, antifungal, and antiparasitic activity. In addition to these well-known roles, AMPs exhibit many other, often unobvious, functions in the host.

antimicrobial peptides | insect immunity | aging | neurodegeneration

1. Introduction

Antimicrobial peptides (AMPs) are indispensable components of insect innate immunity. They exhibit antibacterial, antifungal, antiviral, and antiparasitic activity. Since the discovery of the first insect AMP, cecropin in *Hyalophora cecropia* hemolymph [1], many AMPs with different biochemical and antimicrobial properties have been described in species representing different insect orders. Several families of AMPs have been identified in *Drosophila melanogaster* (Diptera), e.g., cecropins, defensin, drosocin, drosomycin, diptericins, metchnikowin, and attacins [2]. In lepidopteran species, e.g., the greater wax moth *Galleria mellonella* or the mulberry silkworm *Bombyx mori*, cecropins, defensins, gloverins, moricins, proline-rich peptides, attacins, and anionic antimicrobial peptides have been characterized [3][4][5][6]. The Antimicrobial Peptide Database [7] (https://aps.unmc.edu/; accessed on 11 February 2023) collecting AMPs of natural origin contains 3569 peptides, including 367 insect-derived molecules.

2. AMPs in the Nervous System

Research conducted so far shows that there is a connection between alterations in immune signaling and neuronal health, communication, and activity in insects. Changes in neuroimmune communication and glial cell signaling may contribute to behavioral changes in the insect. In addition, the insect nervous system can mount a local immune response to infection, and activation of signaling pathways may lead to neurodegeneration, malfunctioning neurons, and altered behavior [8]. Moreover, the AMP expression pattern is affected by aging independently of infection, and it has been postulated that an increased level of some AMPs produced in non-neuronal tissues during aging can mediate a signal initiating neuronal aging [9].

AMPs are important for regulation of normal functions of the insect brain, e.g., sleep and non-associative learning in *Drosophila* [10][11]. Results reported by Dissel et al. showed that the transcript levels of *metchnikowin* (*Mtk*), *drosocin* (*Dro*), and *attacin* (*Att*) were differentially increased in glia, neurons, and the head fat body, respectively, in sleep-deprived flies. They further demonstrated that the expression of *Mtk* in glia but not in neurons and the expression of *Dro* in neurons but not in glia had a negative effect on memory but modulated sleep in an opposite

way. These AMPs were considered as candidates for conferring resilience/vulnerability to sleep deprivation [12]. A further link between sleep regulation and immune response in *Drosophila* was found by Toda et al. in their research on sleep mutants and the role of *nemuri*–a gene involved in sleep induction. *Nemuri* overexpression increased the sleep length and depth in *Drosophila*. Interestingly, *nemuri* encodes an AMP that can be secreted ectopically to drive prolonged sleep and to promote survival after infection. This peptide acts non-cell-autonomously to promote sleep. The nemuri peptide contains 64 amino acids, has sequence similarity to a Greenland cod (*Gadus ogac*) cathelicidin, and kills bacteria in vitro (*Serratia marcescens*, *Escherichia coli*) and in vivo. *Drosophila* adults overexpressing *nemuri* in neurons and infected with *S. marcescens* or *Streptococcus pneumoniae* survived longer, had a longer amount of sleep, and contained a lower bacterial load than control insects [13]. In another study, a *Drosophila* non-associative long-term memory (LTM) paradigm involving a natural predator of *Drosophila*, i.e., the endoparasitoid wasp *Leptopilina heterotoma*, was used for identification of novel memory genes during and after memory formation. The examination of transcriptional changes in the fly brain revealed that *cecropin A1* (*CecA1*), *CecA2*, *Dro*, *AttA*, *diptericin* (*Dpt*), and *DptB* were differentially regulated at various time points. Specifically, *CecA1*, *Dpt*, and *Dro* were downregulated and upregulated in 7-h and 4-day exposed individuals, respectively [14]. Evidence that AMPs can directly affect brain function was also presented by Barajas-Azpeleta et al., who found 10-12-fold upregulation of *DptB* expression in the adult fly head following behavioral training. Moreover, using *DptB* null flies, they demonstrated that *DptB* was required for modulation of long-term memory in *Drosophila*. Interestingly, only silencing the *DptB* expression in the head fat body affected long-lasting memory, clearly indicating the head fat body as the only relevant source of *DptB* for behavioral modification [15].

Recently, the expression of two AMPs belonging to defensins, i.e., galiomicin and gallerimycin, has been detected in the brains of *G. mellonella* larvae after injection of *Habrobracon hebetor* venom or topical application of the entomopathogenic nematode *Steinernema carpocapse*. However, the role of these peptides in the *G. mellonella* nervous system has not been investigated yet [16]. In this study, the upregulation of the *sericotropin* gene and peptide was also detected in the brains of challenged larvae. Sericotropin functions in lepidopteran insects as a stimulator of silk production. It has been demonstrated that synthetic peptides derived from the N- and C-terminal parts of sericotropin exhibited antibacterial activity against *Xenorhabdus* spp. bacteria, i.e., symbionts of *S. carpocapse*, suggesting an antimicrobial role of sericotropin expressed in the *G. mellonella* brain [16]. In the brains of the honeybee *A. meliffera* infected with deformed wing virus (DWV), transcriptomic and metabolomic analyses revealed overexpression of genes encoding proline-rich AMPs, abaecin and apidaecin, as well as hymenoptaecin and an increased level of these peptides [17]. Results obtained in a study on the response of the central nervous system (CNS) of *Locusta migratoria manilensis* infected with the fungus *Metarhizium acridum* also indicated an important cross-talk between the CNS and the immune system. The expression patterns in the CNS responded rapidly to the infection and changed as the infection proceeded. Many differentially expressed genes, directly involved in immune function and regulation, were identified, including genes encoding defensin and diptericin, which were up-regulated in the CNS of *M. acridum*-challenged locusts [18].

The use of *Drosophila* models of Alzheimer's disease (AD), traumatic brain injury (TBI), and ataxia-telangiectasia (AT) revealed a role of the Toll and Imd signaling pathways in neurodegeneration [19][20][21][22][23]. Although Toll signaling is engaged in proper development of the brain in *Drosophila*, it was demonstrated that suppression of Toll

signaling protected against neurodegeneration. Moreover, *Drosophila* mutants in the negative regulator of the Imd pathway, *dnr1* (defense repressor 1), had increased expression of AMP genes (*Cec*, *Dpt*, *Att*, *drosomycin* (*Drs*), *Mtk*), which was correlated with progressive age-dependent neuropathological changes and a shortened lifespan. It has also been demonstrated that neurodegeneration is dependent on the transcription factor Relish, and bacterial infection in the brain can trigger neurodegeneration through the neurotoxic effects of AMPs. On the other hand, *Relish* mutations inhibited upregulation of innate immune response genes and neurodegeneration in AT *Drosophila* mutants, whereas overexpression of active Relish in glial cells resulted in neurodegeneration [20][24]. Similar effects as those caused by mutations in *dnr1* were observed in flies with mutations in other negative regulators of the Imd signaling pathway: *zfh1* (transcription factor Zn finger homeodomain 1), *Pirk* (poor Imd response upon knock-in), *trbd* (deubiquitinase Trabid), and *tg* (Transglutaminase), strengthening the evidence for the negative role of AMPs in the development of diseases [25][26]. Moreover, the overexpression of *Dro*, *AttC*, and *CecA1* in neurons or glia was accompanied not only by a shortened lifespan but also by early appearance of locomotor defects in comparison with controls, suggesting a causative relationship between high levels of AMPs and neurodegeneration in *Drosophila* [26]. In turn, Barati et al. studied the effect of amyloid-β 42 (Aβ42) and tau on the Imd pathway and neuroinflammation gene expression in a *Drosophila* model. They showed that the expression of genes involved in the Imd pathway, such as *Relish* and AMPs (*AttA*, *DptB*), increased with age in the W^{1118} control flies and in the embryonic nervous system of AD transgenic flies Aβ42, tau^{WT}, or tau^{R406W}, but the level of AMPs in glia remarkably decreased compared to W^{1118}. The decline was higher in both tau flies compared to Aβ42 transgenic flies. The overexpression of AMPs in *Drosophila* leads to brain neurodegeneration and neuroinflammation [27]. Another example connecting overexpression of AMPs with neurodegeneration was provided by research on the role of the Yorkie transcription factor in polyglutamine (PolyQ)-mediated neurotoxicity (involved in Huntington's disease in humans) in *Drosophila*. It was found that PolyQ expansion increased the expression of *CecA*, *Att*, *Dpt*, *Dro*, and *Drs*. It was postulated that upregulation of AMPs can participate in PolyQ-mediated neurotoxicity in *Drosophila* [28].

An important role of one of the AMPs, metchnikowin, in acute and chronic outcomes of TBI was demonstrated by Swanson et al. in a *Drosophila* TBI model. In an analysis of null mutations in 10 AMP genes (*AttA*, *AttB*, *AttC*, *AttD*, *defensin* (*Def*), *Dro*, *Drs*, *Mtk*, *DptA*, *DptB*), it was found that *Mtk* mutant flies exhibited reduced acute behavioral deficits and only the mutation in the *Mtk* gene protected flies from early mortality after TBI in different diet conditions. These results indicate that *Mtk* plays an infection-independent role in the nervous system and can promote neuropathological changes in the brain following TBI [29]. In another study conducted in a *Drosophila* Closed Head Injury (dCHI) model, an acute broad-spectrum immune response was detected in glia cells, where many AMP genes were up-regulated 24 h after TBI (*Att*, *Cec*, *Def*, *Dpt*, *Dro*, *Drs*, *Mtk*, *listericin*). It was found that the deletions of most of these AMPs shortened insect survival after TBI. In contrast, loss of *Def* extended survival, indicating that the *Drosophila* immune response to TBI, including the induction of AMP expression, may result in different effects. Moreover, in this research, a link between the immune response and sleep in flies was demonstrated, as loss of *Relish* protected against impaired control of sleep and movement after TBI [11].

Progress of neurodegenerative processes is correlated in time with an age-dependent increase in AMP expression in the head tissue of *Drosophila* [26]. Using a transgenic *Drosophila* model of AD, Wang et al. provided evidence for

distinct expression profiles of AMP genes in an aging model and in wild-type flies. Whole transcriptome profiles of wild-type *Drosophila* heads revealed upregulation of 12 AMP genes: *AttA*, *AttB*, *AttC*, *AttD*, *CecA2*, *CecC*, *DptA*, *DptB*, *Drs*, *Def*, *Dro*, and *Mtk*. A gradual increase in expression of these AMPs was observed in the wild-type flies during the normal aging process, whereas an initial decrease and a subsequent increase in the expression levels was found during aging of the AD flies. Interestingly, significant correlations between the abnormal amyloid β (Aβ) peptide concentration and abnormal expression of *AttB*, *AttC*, *CecA*, *Drs*, *Mtk*, and *LysS* were detected, suggesting contribution of AMP dysregulation to AD progression by inducing deposition of Aβ peptide aggregates [30]. However, in other studies, lysozyme was demonstrated to be beneficial in different *Drosophila* models of AD. For example, co-expression of human lysozyme rescued the rough eye phenotype indicative of toxicity in flies that expressed $A\beta_{1-42}$ in the eyes. Moreover, lysozyme binding with $A\beta_{1-42}$ in the *Drosophila* eye was detected, suggesting that such an interaction prevented from the toxic effects of $A\beta_{1-42}$ [31]. Interestingly, antimicrobial activity of the Aβ peptide was reported, suggesting that it is an AMP in the nervous system and that neurodegenerative Alzheimer's disease may be partially an infectious disease [32]. Increased activation of innate immunity mechanisms reflected by overexpression of AMP genes was also postulated as a cause of long-term deleterious effects leading to neurological deficits, persistent cell death in brains, and premature aging in a *Drosophila* model of radiation-induced neurotoxicity. In this study, upregulation of *Drs*, *DroA*, *Dpt*, *AttC*, *Cec*, and *Mtk* was detected in brains of 5-day- and 15-day-old adult flies after radiation exposure during larval development (late third larval instar), indicating contribution of persistent activation of immune signaling pathways to neurological deficits in adult flies [33].

The connection between immunity and nervous system is also evident at the level of neuropeptides. Among human neuropeptides, antimicrobial activity has been reported for enkelytin, substance P, neuropeptide Y, bombesin, adrenomedullin, vasoactive intestinal peptide, and pituitary adenylate cyclase activating polypeptide (PACAP) [34] [35]. Besides regulation of neurodevelopment, emotion, and certain stress responses, the PACAP neuropeptide acts as an antimicrobial agent. Although its antimicrobial function was demonstrated in mammals, the phylogenetic analysis predicted antimicrobial properties of eleven PACAP homologs, including PACAP from the cockroach *Periplaneta americana* [36].

References

1. Steiner, H.; Hultmark, D.; Engström, A.; Bennich, H.; Boman, H.G. Sequence and specificity of two antibacterial proteins involved in insect immunity. Nature 1981, 292, 246–248.
2. Hanson, M.A.; Lemaitre, B. New insights on Drosophila antimicrobial peptide function in host defense and beyond. Curr. Opin. Immunol. 2020, 62, 22–30.
3. Cytryńska, M.; Mak, P.; Zdybicka-Barabas, A.; Suder, P.; Jakubowicz, T. Purification and characterization of eight peptides from Galleria mellonella immune hemolymph. Peptides 2007, 28, 533–546.

4. Wojda, I. Immunity of the greater wax moth Galleria mellonella. Insect Sci. 2017, 24, 342–357.
5. Nesa, J.; Sadat, A.; Buccini, D.F.; Kati, A.; Mandal, A.K.; Franco, O.L. Antimicrobial peptides from Bombyx mori: A splendid immune defense response in silkworms. RSC Adv. 2020, 10, 512–523.
6. Wojda, I.; Cytryńska, M.; Zdybicka-Barabas, A.; Kordaczuk, J. Insect defense proteins and peptides. Subcell. Biochem. 2020, 94, 81–121.
7. Wang, G.; Li, X.; Wang, Z. APD3: The antimicrobial peptide database as a tool for research and education. Nucleic Acids Res. 2016, 44, D1087–D1093.
8. Mangold, C.A.; Hughes, D.P. Insect behavioral change and the potential contributions of neuroinflammation—A call for future research. Genes 2021, 12, 465.
9. Stuart, B.A.R.; Franitza, A.L.; Lezi, E. Regulatory roles of antimicrobial peptides in the nervous system: Implications for neuronal aging. Front. Cell Neurosci. 2022, 16, 843790.
10. Montanari, M.; Royet, J. Impact of microorganisms and parasites on neuronally controlled Drosophila behaviours. Cells 2021, 10, 2350.
11. van Alphen, B.; Stewart, S.; Iwanaszko, M.; Xu, F.; Li, K.; Rozenfeld, S.; Ramakrishnan, A.; Itoh, T.Q.; Sisobhan, S.; Qin, Z.; et al. Glial immune-related pathways mediate effects of closed head traumatic brain injury on behavior and lethality in Drosophila. PLoS Biol. 2022, 20, e3001456.
12. Dissel, S.; Seugnet, L.; Thimgan, M.S.; Silverman, N.; Angadi, V.; Thacher, P.V.; Burnham, M.M.; Shaw, P.J. Differential activation of immune factors in neurons and glia contribute to individual differences in resilience/vulnerability to sleep disruption. Brain Behav. Immun. 2015, 47, 75–85.
13. Toda, H.; Williams, J.A.; Gulledge, M.; Sehgal, A. A sleep-inducing gene, nemuri, links sleep and immune function in Drosophila. Science 2019, 363, 509–515.
14. Bozler, J.; Kacsoh, B.Z.; Chen, H.; Theurkauf, W.E.; Weng, Z.; Bosco, G. A systems level approach to temporal expression dynamics in Drosophila reveals clusters of long term memory genes. PLoS Genet. 2017, 13, e1007054.
15. Barajas-Azpeleta, R.; Wu, J.; Gill, J.; Welte, R.; Seidel, C.; McKinney, S.; Dissel, S.; Si, K. Antimicrobial peptides modulate long-term memory. PLoS Genet. 2018, 14, e1007440.
16. Shaik, H.A.; Mishra, A.; Sehadová, H.; Kodrík, D. Responses of sericotropin to toxic and pathogenic challenges: Possible role in defense of the wax moth Galleria mellonella. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 2020, 227, 108633.
17. Pizzorno, M.C.; Field, K.; Kobokovich, A.L.; Martin, P.L.; Gupta, R.A.; Mammone, R.; Rovnyak, D.; Capaldi, E.A. Transcriptomic responses of the honey bee brain to infection with deformed wing virus. Viruses 2021, 13, 287.

18. Zhang, W.; Chen, J.; Keyhani, N.O.; Jin, K.; Wei, Q.; Xia, Y. Central nervous system responses of the oriental migratory, Locusta migratoria manilensis, to fungal infection. Sci. Rep. 2017, 7, 10340.
19. Katzenberger, R.J.; Loewen, C.A.; Wassarman, D.R.; Petersen, A.J.; Ganetzky, B.; Wassarman, D.A. A Drosophila model of closed head traumatic brain injury. Proc. Natl. Acad. Sci. USA 2013, 110, E4152–E4159.
20. Petersen, A.J.; Katzenberger, R.J.; Wassarman, D.A. The innate immune response transcription factor relish is necessary for neurodegeneration in a Drosophila model of ataxia-telangiectasia. Genetics 2013, 194, 133–142.
21. Bolus, H.; Crocker, K.; Boekhoff-Falk, G.; Chtarbanova, S. Modeling neurodegenerative disorders in Drosophila melanogaster. Int. J. Mol. Sci. 2020, 21, 3055.
22. Nayak, N.; Mishra, M. Drosophila melanogaster as a model to understand the mechanisms of infection mediated neuroinflammation in neurodegenerative diseases. J. Integr. Neurosci. 2022, 21, 66.
23. Yu, S.; Luo, F.; Xu, Y.; Zhang, Y.; Jin, L.H. Drosophila innate immunity involves multiple signaling pathways and coordinated communication between different tissues. Front. Immunol. 2022, 13, 905370.
24. Cao, Y.; Chtarbanova, S.; Petersen, A.J.; Ganetzky, B. Dnr1 mutations cause neurodegeneration in Drosophila by activating the innate immune response in the brain. Proc. Natl. Acad. Sci. USA 2013, 110, E1752–E1760.
25. Myllymäki, H.; Rämet, M. Transcription factor zfh1 downregulates Drosophila Imd pathway. Dev. Comp. Immunol. 2013, 39, 188–197.
26. Kounatidis, I.; Chtarbanova, S.; Cao, Y.; Hayne, M.; Jayanth, D.; Ganetzky, B.; Ligoxygakis, P. NF-κB immunity in the brain determines fly lifespan in healthy aging and age-related neurodegeneration. Cell Rep. 2017, 19, 836–848.
27. Barati, A.; Masoudi, R.; Yousefi, R.; Mosefi, M.; Mirshafiey, A. Tau and amyloid beta differentially affect the innate immune genes expression in Drosophila models of Alzheimer's disease and b-D monnuroic acid (M2000) modulates the dysregulation. Gene 2022, 808, 145972–145984.
28. Dubey, S.K.; Tapadia, M.G. Yorkie regulates neurodegeneration through canonical pathway and innate immune response. Mol. Neurobiol. 2018, 55, 1193–1207.
29. Swanson, L.C.; Rimkus, S.A.; Ganetzky, B.; Wassarman, D.A. Loss of the antimicrobial peptide metchnikowin protects against traumatic brain injury outcomes in Drosophila melanogaster. G3 Genes Genomes Genet. 2020, 10, 3109–3119.

30. Wang, M.; Peng, I.F.; Li, S.; Hu, X. Dysregulation of antimicrobial peptide expression distinguishes Alzheimer's disease from normal aging. Aging 2020, 12, 690–706.
31. Sandin, L.; Bergkvist, L.; Nath, S.; Kielkopf, C.; Janefjord, C.; Helmfors, L.; Zetterberg, H.; Blennow, K.; Li, H.; Nilsberth, C.; et al. Beneficial effects of increased lysozyme levels in Alzheimer's disease modelled in Drosophila melanogaster. FEBS J. 2016, 283, 3508–3522.
32. Vojtechova, I.; Machacek, T.; Kristofikova, Z.; Stuchlik, A.; Petrasek, T. Infectious origin of Alzheimer's disease: Amyloid beta as a component of brain antimicrobial immunity. PLoS Pathog. 2022, 18, e1010929.
33. Sudmeier, L.J.; Samudrala, S.S.; Howard, S.P.; Ganetzky, B. Persistent activation of the innate immune response in adult Drosophila following radiation exposure during larval development. G3 Genes Genomes Genet. 2015, 5, 2299–2306.
34. Schluesener, H.J.; Su, Y.; Ebrahimi, A.; Pouladsaz, D. Antimicrobial peptides in the brain: Neuropeptides and amyloid. Front. Biosci. 2012, 4, 1375–1380.
35. Augustyniak, D.; Kramarska, E.; Mackiewicz, P.; Orczyk-Pawiłowicz, M.; Lundy, F.T. Mammalian Neuropeptides as Modulators of Microbial Infections: Their dual role in defense versus virulence and pathogenesis. Int. J. Mol. Sci. 2021, 22, 3658.
36. Lee, E.Y.; Chan, L.C.; Wang, H.; Lieng, J.; Hung, M.; Srinivasan, Y.; Wang, J.; Waschek, J.A.; Ferguson, A.L.; Lee, K.F.; et al. PACAP is a pathogen-inducible resident antimicrobial neuropeptide affording rapid and contextual molecular host defense of the brain. Proc. Natl. Acad. Sci. USA 2021, 118, e1917623117.