

Insect neuroendocrinology in Bratislava, SVK

# Under Molecular Command

Arthropods use short peptides as brain hormones. How complex are their neuroendocrine systems? What are their biological functions? Dušan Žitňan and his group from the Slovak Academy of Sciences (SAV) in Bratislava gain a deep insight into ticks, flies and co.

Hormones govern a good deal of arthropod life. Those of them produced from amino acids in the tissues of the nervous system belong to the class of neuropeptides. They control processes like sexual behaviour, digestion, excretion and the development from larvae to adults. For the scramblers they are crucial: they keep their life cycle running. That is why an understanding of arthropod hormone systems might be important for humans, too. Chemical intervention could push pest management approaches of agricultural pathogens forward. Human diseases like malaria and borreliosis might be conquered once researchers know the molecules that control the lives of mosquitos and ticks. “And apart from that, basic molecular principles of arthropod neuroendocrinology are similar to the ones in humans,” says Dušan Žitňan, head of the Department of Molecular and Applied Zoology at the Institute of Zoology at the Slovak Academy of Sciences (SAV) in Bratislava, Slovakia. “Basic research can be done much more easily in these animals.”

## A personal breakthrough

Žitňan has been studying arthropod hormone systems since the late 1980s. At the beginning of his career he had been more interested in insect ecology but then his PhD advisor, Prof. František Sehnal convinced him that insect endocrinology might be more interesting. “At that time people did not know much about insect neuropeptides,” Žitňan remembers. “Then some researchers started to use antibodies against vertebrate molecules like vasopressin, oxytocin or insulin and found similar substances in flies, moths and many other insects.” During the following years of insect euphoria, scientists scanned a good deal of insect tissues. Žitňan concentrated on the nervous system and the endocrine organs of his model or-

ganisms – mostly moths (*Bombyx mori* and *Manduca sexta*) and *Drosophila*. During his PhD studies in Bratislava he used immunohistochemistry techniques and described neuropeptide-producing cells in the central and peripheral nervous systems and in the gut. “But then I started to ask for the functional aspects”, Žitňan says. “I wanted to know how nervous and peripheral endocrine tissues communicate and orchestrate complex behaviours by using hormones.” In 1996 *Science* published his personal breakthrough (vol. 271: 88-91).

At this time Žitňan worked as a postdoctoral investigator in the group led by Michael E. Adams at the University of California (Riverside, USA). Together with his colleagues, he found a new hormone which was crucial for the development of the tobacco hornworm *Manduca sexta*. De-

cheal glands. These organs were originally described and named in 1913 but had been forgotten for almost a century. Žitňan and his colleagues showed that the isolated central nervous system responds to ETH, but not to eclosion hormone, with patterned motor bursting corresponding to shedding behaviour. Based on that and other findings, they reasoned that ETH may be an immediate trigger for ecdysis through a direct action on the nervous system. “This paper was really important for us,” Žitňan says. “We didn’t only find new endocrine organs but also a new molecule which is crucial for the whole shedding behaviour.”

## Forever juvenile

In the years following Žitňan’s return to Slovakia, he and his team from the SAV in Bratislava conducted a lot of experiments on ETH. After the ETH receptor was identified by Yoonseong Park and Young-Joon Kim in the Adams’ lab, the Slovaks identified receptor-expressing neurons (target cells for ETH) in the insect brain and nerve cord. Furthermore, they showed that the expression of the ETH gene in the epitracheal glands and of the ETH receptor in the nervous system is triggered by ecdysone, the well known steroid hormone important for ecdysis. In the meantime, a lot of new experimental techniques have enriched their work. Nowadays, molecular biology and proteomics enable them to screen arthropods for new neuropeptides and neuropeptide receptors in a much more efficient

way. For example, in one of their newest publications, which they published together with a collaborating Japanese team they concentrate on the expression patterns of neuropeptide G-protein coupled receptors (GPCRs) in the silkworm *Bombyx mori* (*PLoS ONE* 3(8): e3048).

In this study, the researchers scanned the newly sequenced genome of the silk-



Dušan Žitňan (2<sup>nd</sup> from right) and his “neuropeptide experts”

veloping insects repeatedly shed their cuticle by means of a stereotyped behaviour called ecdysis. This behaviour had been thought to be initiated by the brain peptide eclosion hormone. The new hormone, which the researchers called ecdysis triggering hormone (ETH), was produced by so called Inka cells contained in a segmentally distributed endocrine system of epitra-



worm for homologues of already known *Drosophila* neuropeptide GPCRs. They compared the amino acid sequences of the silkworm genes with the proteome of *Drosophila* and made sure that they coded for neuropeptide GPCRs. They determined the expression of all these receptors in several different organs and developmental stages of *Bombyx*. "This work was important because among the neuropeptide receptors of the silkworm we found the long sought after receptor for allatotropin," says Žitňan. Allatotropin (AT) is an important hormone which stimulates production of the juvenile hormone in a gland attached to the insect brain called corpora allata (CA). This latter molecule is not only crucial for processes like reproduction of adults but also for the development of larvae. It is another key player during ecdysis and prevents the metamorphosis from larvae to adults; it keeps the animals in a juvenile stadium. The receptor for AT was thought to be expressed in the CA where the juvenile hormone is produced. Instead, the researchers found its expression only in the neighbouring structure, corpora cardiaca (CC). "This finding showed that allatotropin cannot influence the biosynthesis of the juvenile hormone directly," states Žitňan.

### A novel network

In order to find other players in the CC-CA network, the researchers identified two other newly found GPCRs using a heterologous expression system. Sequence comparisons with *Drosophila* GPCRs showed that one of them was the receptor for the so called short neuropeptide F (sNPF). In situ hybridisation analysis revealed that it was mainly expressed in the CC. In cell culture experiments sNPF clearly inhibited the biosynthesis of the juvenile hormone. "These findings suggest that allatotropin might act only indirectly on the biosynthesis of the juvenile hormone," explains Žitňan. "Instead, it might influence the release or expression rate of the short neuropeptide F in the corpora cardiaca, which binds to its receptor in the corpora allata and, by this, inhibits the biosynthesis of the juvenile hormone." More work on this is needed but the model seems plausible, so far. And at the end of the day, another finding of the paper is even more important for Žitňan. Another GPCR he and his colleagues found in the CA system was revealed to be the receptor for an old friend, the ecdysis triggering hormone. "This is very interesting," Žitňan says. "This shows that ETH might have something to do with the control of the juvenile hormone

production." He and his colleagues plan to investigate this further.

### Help from the EU

Another recent pioneering work by the Žitňan group was a study of the neuroendocrine system of the hard tick *Rhipicephalus appendiculatus*, a species which has barely been investigated by neuroendocrinologists, to date (*Cell Tiss. Res.* 335: 639-55) "Ticks are important human disease vectors," Žitňan explains. "Understanding their hormone networks is valuable basic knowledge." Only a small number of tick neuropeptides were known before the study. The researchers used antibodies against 18 well known neuropeptides of insects and crustaceans as probes. Amongst them were familiar substances like the adipokinetic hormone (AKH), the diuretic hormone (DH), the eclosion hormone (EH) or the ecdysis triggering hormone (ETH). The screen revealed a whole bunch of hits. Fifteen out of 18 antibodies showed strong specific reactions in various cell types and axonal projections of the tick brain. Some of the antibodies reacted in peripheral nerves, in the salivary glands and in so-called lateral segmental organs (LSO) which are attached to the brain and whose functions are unclear, so far.

"One of the interesting findings of this study is that we describe a hitherto unknown unique system of neurons innervating the tick salivary glands and some neuropeptides which had not been found in these structures in insects or crustaceans," reveals Žitňan. "This might reflect an adaptation for the specialised function associated with the ectoparasitic life of ticks."

Salivary glands play a crucial role for the blood sucking arthropods as they are important for feeding and for the production of immunosuppressing factors in the saliva, which ticks inject into the blood of their hosts. The neuropeptides might modulate the release of immunomodulatory and cement proteins from specific secretory cells in salivary glands during feeding. "The unique innervation and the presence of distinct neuropeptides may be interesting for the pharma industry," says Žitňan. "Someday we might effectively disrupt these neuropeptide networks by using special vaccines." After this descriptive study, Žitňan and his group are planning functional and physiological experiments in order to push their ideas forward.

The money for these projects may come from the EU in future; in particular, some visions for applied studies on vaccines could be brought forward by European grants. Besides, the researchers still are collaborating with Japanese groups and with the Michael E. Adams group in California. Some of their projects are partly funded by the National Institutes of Health (NIH) in the USA. Another part of the money comes from one of the two Slovak fund organisations. All in all, the Žitňan group consists of about ten people: some of them work directly at the Institute of Zoology; others come from other SAV Institutes such as the Institute of Virology. "The financial situation for basic research in Slovakia is not very good," Žitňan explains. "But it is definitely better than ten years ago." The group has many new ideas. Some projects will have to wait; some of them are already in progress.

MATTHIAS NAWRAT

## ONE FINE DAY IN THE LAB...

BY LEONID SCHNEIDER

