Invertebrate Neuropeptide Conference

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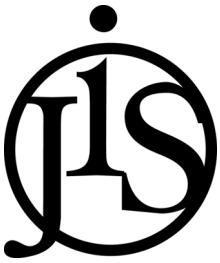

Invertebrate Neuropeptide Conference
January 9–13, 2005, Chiang Mai, Thailand

Held under the auspices of The International Neuropeptide Society

Organizers

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Image 1. Participants at the Invertebrate Neuropeptide Conference

Abstracts are listed in alphabetical order by the last name of the senior author.

Identification and expression of *Drosophila* farnesoic acid o-methyltransferases (FaMeT): an enzyme potentially regulated by neuropeptides

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Juvenile hormone plays a central role in the metamorphosis and reproduction of most insect species. The biosynthesis of juvenile hormone within the corpora allata of insects is regulated by neuropeptides. Allatostatin neuropeptides are known to act as inhibitors of juvenile hormone biosynthesis Allatotropin may, in certain insects, act to stimulate JH biosynthesis.

These neuropeptides act on a membrane receptor(s) of the corpora allata that activates signal transduction pathway(s). This activation ultimately serves to regulate enzymes in the biosynthetic pathway that converts acetyl CoA to the sesquiterpenoids.

Farnesoic acid o-methyltransferase (FAMeT) catalyzes the S-adenosylmethionine dependent conversion of Farnesoic acid to methylfarnesoic acid. It is thought that FAMeT may play a rate-limiting role in juvenile hormone biosynthesis in insects. FAMeT has been identified in the crustaceans, *Metapenaeus ensis* (shrimp) and *Homarus americanus* (Lobster). A database search based on sequence identity with crustacean FAMeT has revealed a putative gene product in *Drosophila melanogaster*. In order to characterize the putative *Drosophila* FAMeT ortholog's role in juvenile hormone biosynthesis we have analyzed the protein distribution, activity and in vivo expression. This work was supported by Natural

Sciences and Engineering Research Council of Canada.

Role of diuretic and antidiuretic peptides in extracellular fluid homeostasis in insects

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Analogous to the function of the vertebrate kidney, Malpighian tubules of insects help regulate the volume and composition of the extracellular fluid compartment (hemolymph). Prompt and precise regulation of extracellular fluid volume is particularly important for small animals in desiccating habitats where volume loss can lead to circulatory collapse. Prompt and precise regulation also defends against osmotic water loading in insects developing in fresh water, and it eliminates excess solute and water of gorging meals in hematophagous as well as phytophagous insects. Vertebrate kidneys and insect Malpighian tubules are the executors of extracellular fluid homeostasis, holding or getting rid of solute and water depending on physiological need. Circulating neuropeptides provide the instructions. Antidiuretic peptides request the conservation of extracellular fluid during periods of dehydration, and diuretic peptides call for the elimination of water in the case of ove hydration. The functional dynamic range of Malpighian tubules spans 1000-fold changes in transport activity as tubules respond to diuretic and antidiuretic agents. Both transcellular and paracellular transport pathways are modulated. For example, CRF-like diuretic peptides target transcellular transport pathways by stimulating active, electrogenic transport of cations through cells. In contrast, insect kinins affect the paracellular pathway, as in Malpighian tubules of the yellow fever mosquito *Aedes aegypti*. In particular, leucokinin increases the Cl- permeability of the paracellular pathway. The on/off effects of leucokinin proceed with switch-like speed suggesting channel-like properties of septate junctions. Synergistic effects of CRF- and kinin-like diuretic peptides document amplifying interactions between transcellular and paracellular transport pathways. So far, antidiuretic neuropeptides have been shown to affect only transcellular transport pathways. In Malpighian tubules of *Aedes aegypti* they inhibit electroneutral transport systems in epithelial cells. Although a decrease in paracellular permeability would be potently antidiuretic, such an effect on septate junctions has not yet been reported.

Signal transduction of the CRF-like *Manduca sexta* diuretic hormone studied by proteomic techniques

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Previous studies have shown that the diuretic hormone of *Manduca sexta* (Manse-DH) activates a Na+-K+-2Cl- cotransporter in the Malpighian tubules, and that this process is stimulated by a rise in intracellular cyclic AMP. In other systems CRF-like DH have been implicated in increasing the activity of the vacuolar ATPase, which is the driving force for salt, and hence fluid, excretion. We utilized proteomic analysis to determine directly which proteins are affected by treatment of Malpighian tubules of larval *Manduca sexta* with 10 nM Manse-DH. Tubules from 300 animals were maintained in aerated saline for 10 min, homogenized, and subcellular fractions collected. These were run on 2 dimensional SDS-PAGE gels. Control tubules were treated in an identical manner but without inclusion of DH in the medium. Analysis of the cytosolic fraction of tubules treated with Manse-DH shows over 200 protein spots that differ in either abundance, or mobility, between gels from control vs. treated tubules. Over 30 proteins found in control tubules are "missing" in treated tubules, possibly reflecting phosphorylation. Protein spots of interest were excised from the gel, digested with trypsin, and the tryptic digests analyzed by MALDI-TOF-TOF mass spectrometry. The results of mass spectral analysis of the proteins affected by Manse-DH treatment will be discussed. This research was supported by NIH (Grant GM48172 and BRIN 5P20RR16464), and the Nevada Agricultural Experiment Station.

The distribution and physiological roles of proctolin in the locust, *Locusta migratoria*

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Proctolin is a pentapeptide first isolated from the cockroach *Periplaneta Americana* where it was proposed to function as a neurotransmitter, with myotropic properties. Proctolin has since been shown to be widely distributed within insects but a comprehensive map of its distribution has not been undertaken for the African migratory locust, *Locusta migratoria*. Using immunohistochemistry, we found that proctolin-like immunoreactive neurons and processes are widely distributed throughout the central nervous system, stomatogastric nervous system and peripheral tissues, such as the oviducts and alimentary canal. Of note, are proctolin-like immunoreactive lateral neurosecretory cells in the brain that project processes to the corpus cardiacum and corpora allata. With this latter distribution in mind, we examined the possible involvement of proctolin as a releasing factor associated with the corpus cardiacum and corpora allata. Thus, proctolin is capable of stimulating the release of adipokinetic hormone from the corpus cardiacum and of stimulating juvenile hormone production from the corpora allata. This work was supported by NSERC.

Control of diuresis in the malarial mosquito, *Anopheles gambiae*

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Female mosquitoes imbibe a blood meal equivalent in volume to more than twice their unfed body weight, which restricts their maneuverability and makes them prone to predation. In addition, the meal represents a considerable NaCl and water load that threatens haemolymph homeostasis. Normally, they void little urine, but the blood meal signals the start of a pronounced diuresis commencing before the meal is completed. Work by Beyenbach's group with the yellow fever mosquito, *Aedes aegypti*, has shown that during the peak phase of diuresis Na+-rich urine is voided and about 40% of the volume load is excreted within 20 minutes. Subsequently, the rate of excretion diminishes and K+-rich urine hypo-osmotic to the haemolymph is voided, removing excess K+ derived from digested red blood cells. The initial diuresis and accompanying natriuresis are attributed to release of mosquito natriuretic peptide (MNP), which acts via cAMP to stimulate secretion of Na+-rich urine by the Malpighian tubules. This has been attributed to effects on MT principal cells, where cAMP opens a Na+ conductance in the basolateral membrane and activates Na+/K+/2Cl- cotransport. MNP has yet to be identified, but is thought to belong to the CRF-related family of diuretic hormones (DH). In support of this, Culsa-DP, a CRF-related DH from the saline water mosquito, Culex salinarius, stimulates cAMP production by *A. aegypti* Malpighian tubules and has diuretic and natriuretic activity, although both responses are limited compared with exogenous cAMP. BLAST searches of the malarial mosquito (*Anopheles gambiae*) genome identified homologues of peptides shown to have diuretic activity in other insects. These include CRF-related and calcitonin-like peptides (Anoga-DH_{44} and Anoga-DH_{31}, respectively), which are known to act via cAMP in stimulating secretion by fruit fly Malpighian tubules. Both were synthesised and tested in *An. gambiae* along with exogenous cAMP for effects on tubule electrophysiology and fluid secretion. Cyclic AMP mimicked effects previously reported in *A. aegypti* Malpighian tubules, namely accelerated secretion of Na+-rich urine and depolarisation of the principal cell basolateral membrane (V_{bl}) with an equivalent hyperpolarisation of the transepithelial potential (V_{tep}). The diuretic activity of Anoga-DH_{44} was about 50% that of cAMP and was not accompanied by a marked natriuresis. In contrast, Anoga-DH_{31} had diuretic and natriuretic activities indistinguishable from those of cAMP. Both peptides depolarised V_{bl} and hyperpolarised V_{tep}, but the response to Anoga-DH_{44} was short-lived even in the continued presence of the peptide. Based on these findings, the calcitonin-like peptide, Anoga-DH_{31}, is the more likely candidate for a mosquito natriuretic peptide. This work was supported by an NIH grant (GM 48172) to D.A.S.

A dipteran perspective on the phylogeny of short neuropeptide F

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Peptides of the neuropeptide F (NPF) and short NPF (sNPF) families apparently both have roles in feeding-related physiology of *Drosophila melanogaster*, according to recent reports. Although NPFs and sNPFs exhibit limited sequence similarity, they act through closely related G-protein coupled receptors. Within the immediate family of these receptors are those of vertebrates for neuropeptide Y, peptide YY, and pancreatic polypeptide, each of which resembles NPF. The relationships among sNPF family members have to date been less clear, even among diptera alone. The head peptides of the yellow fever mosquito, *Aedes aegypti*, have been thought to be related to sNPFs of *D. melanogaster* and of the African malaria mosquito, *Anopheles gambiae*. A physiological similarity is suggested by the finding that *A. aegypti* head peptides (HPs) alter host seeking behavior in females, wherein feeding is thus linked both to metabolism and to reproduction. A comparative approach to understanding the evolution of these seemingly related signaling systems may be viewed from several perspectives. Structural comparisons. For *D. melanogaster*, *A. gambiae*, and *A. aegypti*, sequence similarities among NPFs correspond to the relatedness of species. Comparisons of individual sNPF sequences are complicated by the occurrence of multiple peptides within a single prohormone, as typifies many other RF-amide-related peptides (FaRPs). Alignment of prohormone sequences suggests a general overall similarity of organization both for NPFs and for sNPFs/HPs. The organization of genes encoding these neuropeptides, however, exhibits some variations for which a simple phylogeny is not apparent. Information derived from the genome sequencing project for *A. aegypti* reveals that this species has separate transcripts for sNPFs and for HPs, suggesting an ancestral gene duplication event. Structure determinations. For adult *D. melanogaster*, extracts of body and hemolymph were purified by HPLC, immunoreactivity was monitored by RIA, and peptide structures were determined by mass spectrometry. Some sequences corresponding to sNPFs in the prohormone were evident, but none were HP-like. Occurrence of sNPFs in hemolymph suggests a possible endocrine role. Additional studies using PCR to examine the distributions of mRNA's for peptides and receptors are in progress. Structure-activity relations. Radioreceptor assays with a mammalian cell line stably expressing the *D. melanogaster* sNPFR were used to further profile the activities of *D. melanogaster*, *A. gambiae*, and *A. aegypti* NPFs, along with *A. aegypti* HPs. Select sNPFs exhibited IC_{50}'s in the sub-nanomolar range, with HPs substantially less active. NPFs are inactive when tested in the sNPFR receptor system, and sNPFs are correspondingly inactive in NPFR receptor assays. Occurrence of these peptides in hemolymph provides a basis for evolutionary pressures to reduce receptor cross-talk. Supported by NIH Grant AI33108 to M.R.B.

Water-borne protein pheromonal communication: attractin, enticin, temptin, and seductin act in concert to stimulate mate attraction in *Aplysia*

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The marine mollusk *Aplysia* releases the water-borne protein pheromone attractin during egg laying. This small protein has been characterized from six aplysiid species and stimulates the formation and maintenance of mating and egg-laying aggregations. Three additional water-borne protein pheromones that are released during egg laying (enticin, temptin, and seductin) have recently been isolated, characterized, cloned, expressed, tested in T-maze attraction assays, and shown to act in concert with attractin to stimulate mate attraction. We review the structure, function, localization, and behavioral aspects of attractin, enticin, temptin, seductin and an egg capsule structural protein (capsulin) that is highly expressed in the pheromone-secreting albumen gland, and report that water-borne animal-derived factors from non-laying animals act in concert with attractin, enticin, temptin, and seductin to stimulate mate attraction. Supported by National Science Foundation grant IBN-0314377 and John Sealy Memorial Endowment Fund grant #2579 to G.T.N.

Neuropeptidergic control of *Octopus* oviducal gland

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The oviducal gland of the female of *Octopus vulgaris* lies about halfway along the oviduct. This gland can be morphologically divided into two regions: the former stretched from the proximal part of the oviduct to the central cavity constituted by an enlargement of the lumen of the oviduct. It contained the spermathecae embedded in compact connective tissue crossed by muscular fibres that were surrounded by a glandular compartment divided into an inner and an outer region, with respect to the lumen of the oviduct. The latter region stretched from the central cavity to the distal part of the oviduct. Progesterone and 17ß-estradiol receptors have been characterized and immunolocalized in the reproductive system of the female of *O. vulgaris*. Particularly in the oviducal gland, the nuclear localization of these receptors was present only in the cells of the glandular compartment of previtellogenic glands. We have evidence of FMRFamide-like and cGnRH-I-like immunoreactivity in the reproductive ducts of the female of *O. vulgaris*. Immunopositive fibres to both neuropeptides were localized in the fusiform ganglion from which the nerves that reach the female reproductive ducts arise. FMRFamide-like and cGnRH-I-like immunoreactive nerve endings were present in the oviducal gland branching around the alveoli in the outer glandular portion. No immunopositivity was observed in the inner glandular region of the gland. The nervous plexus surrounding the central region of the oviducal gland, showed both FMRFamide-like and cGnRH-I-like immunoreactivity. Based on these observations we suggested that FMRFamide and cGnRHI neuropeptides are involved in the nervous control of the activity of oviducal glands of *O. vulgaris*, possibly regulating the secretion of products such as mucus and mucilaginous substances. Moreover we have recently shown APGWamide immunoreactivity in the glandular cells of the inner part of the oviducal gland. No immunoreactive cell bodies or fibers were seen in the outer glandular part as well as in the central region. Here we report our studies on the effect that these neuropeptides could exert on the secretory activity of the oviducal gland. cAMP seems to be a possible second messenger involved in such process, although other pathways have been investigated. We discuss the findings of a neuropeptidergic action on the glandular cells of oviducal gland in a more complex frame of molecules, such as steroids, biogenic amines and neuromodulators, controlling the activity of the gland.

Partial characterization of a crustacean hyperglycemic hormone-like peptide in the tobacco hornworm, *Manduca sexta*

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The crustacean hyperglycemic hormones (CHH) comprise a major family of peptides that govern diverse physiological processes from reproduction to metabolism. Here we present an initial characterization of a CHH peptide family in the tobacco hornworm, *Manduca sexta*. Immunocytochemical analyses using two different crustacean antibodies (*Carcinas maenas* and *Cancer pagurus*) localized CHH-like peptides to neurosecretory cells in the pars intercerebralis of the brain, the corpora cardiaca, and lateral neurosecretory cells and transverse nerves of the ventral nerve cord. CHH-like peptides were detected in the hemolymph of 5 th stage larvae using ELISA, but specific crustacean CHH peptides were not detected by RIA. Moreover, staining was not fully blocked using the two specific crustacean CHH peptides. A conserved region of the CHH gene (79 base pairs) was amplified from genomic DNA and a cDNA library, using degenerate oligonucleotide primers drawn from a comparative analysis of crustacean and insect CHH coding sequences. Sequence data from *Manduca* cDNA showed a high degree of conservation with other insects and a lesser degree with crustacean species. Taken together, the above data suggest that the *Manduca* sequence(s) are related, but different from the crustacean peptides. Both immunocytochemical and molecular studies indicate the presence of CHH-like peptides at multiple developmental stages in larvae and pupae. These data will be used to study the influence of the CHH peptide family in regulating *Manduca* physiology, including ecdysis, and will add to a growing database of phylogenetic information on these multifaceted peptide hormones. This research was funded by a National Institutes of Health, MBRS SCORE Program-NIGMS Grant (2S06 GM52588-09), a National Center on Minority Health and Health Disparities grant (5P20-MD000262), a NIH Bridge to the future grant 92R25 FM48972-04) to A.D., and a

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The ecdysis-triggering and eclosion hormones act differentially on crustacean cardioactive peptide neurons during the initiation of ecdysis behaviors in the moth, *Manduca sexta*

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A model for the neural regulation of ecdysis behaviors in the moth, *Manduca sexta*, has implicated neuropeptides from peripheral gland cells, the brain, and the ventral nerve cord in this regulation. Crustacean cardioactive peptide (CCAP), released from homologous pairs of cells in the ventral nerve cord, has been suggested to be the direct trigger of ecdysis behaviors, by releasing the ecdysis motor program leading to the shedding of the old cuticle. Ecdysis-triggering hormone (ETH), originating from epitracheal glands lining the body wall, and eclosion hormone (EH), localized to two pairs of ventral medial neurons of the brain, are suggested to interact to elicit eventual CCAP release. ETH has been suggested to cross the blood brain barrier and induce EH release from the EH neurons, and the release of EH from the descending axons in the ventral nerve cord triggers release of CCAP. Release of CCAP is facilitated by increases in cGMP, and their activation is easily monitored using a cGMP-specific antiserum. It has also been suggested that ETH may act directly on the CCAP neurons to trigger ecdysis behaviors. We have looked at the roles of ETH and EH in activating the CCAP neurons, and in initiating ecdysis behaviors, by assessing (i) EH release from EH axons, (ii) elevation of cGMP in CCAP neurons, and (iii) timing of ecdysis behaviors under different experimental conditions both *in vivo* and *in vitro*. Our data suggests that ETH has a different mode of action in eliciting ecdysis behaviors independently of EH. This research was funded by a USDA research grant #693973 , a National Institutes of Health, MBRS SCORE Program-NIGMS grant (# 2S06 GM52588-09), a National Center on Minority Health and Health Disparities grant (#5P20-MD000262), and a MBRS-RISE fellowship to M.A.U. (#5R25GM59298-04).

Immunolocalization of an allatotropin in developmental stages of *Heliothis virescens* and *Apis mellifera*

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The biosynthesis of juvenile hormone by the corpora allata is partially regulated by stimulatory neuropeptides called allatotropins. We immunolocalized *Manduca sexta* allatotropin (Manse-AT)-like material in the larval central nervous systems of two species, the noctuid moth *Heliothis virescens* and the honeybee *Apis mellifera*. Patterns of Manse-AT containing cells in *H. virescens* persisted from one instar to the next. Immunoreactive cells were consistently observed most frequently in the lateral region of the protocerebrum and tritocerebrum of the brain, with 2-3 pairs of cells in the pars intercerebralis. The suboesophageal, abdominal, and terminal ganglia also showed consistent patterns of Manse-AT containing cells across instars. The number of immunoreactive cells in the brain and suboesophageal ganglion increased with the instar. The corpora allata/corpus cardiacum complex did not include any Manse-AT containing cells. In *A. mellifera*, Manse-AT containing cells were found only in a few brains of larvae late in the fifth instar (prepupae). Six to eight immunoreactive cells were present in the pars intercerebralis of these individuals. It is interesting to note that we did not find any Manse-AT-like material in the brains of larvae earlier in the fifth instar, whose corpora allata were shown to be more sensitive to in vitro stimulation by Manse-AT than prepupal corpora allata. These results will be discussed in the context of development and in honeybees, caste differentiation.

Expression of the allatotropin gene in *Manduca sexta*

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Manduca sexta allatotropin (Manse-AT) is a multifunctional peptide that was first isolated based on its

stimulatory activity on the adult female corpora allata. The gene is transcribed as three mRNAs that differ by alternative splicing to produce three different precursors. Each precursor contains the sequence for Manse-AT, and the two longer mRNAs contain peptide sequences related to Manse-AT, the allatotropin-like (ATL) peptides. Manse-AT and the ATL peptides are flanked by basic amino acid residues in the precursor, suggesting that they are produced following processing by the proprotein convertases. Remarkably, the presence of the three Manse-AT mRNAs is regulated in a stage- and tissue-specific manner. The availability of the cloned Manse-AT gene has provided the tools necessary to examine the spatial and temporal pattern of Manse-AT gene expression. We have used exon-specific probes in Northern blot analysis, *in situ* hybridization, and PCR to further characterize Manse-AT gene expression. The level of one of the alternatively spliced Manse-AT mRNAs is elevated in larvae that were starved, parasitized, or fed the ecdysteroid agonist RH-5992. In starved larvae, these elevated mRNA levels were seen exclusively in the terminal abdominal ganglion. The amount of Manse-AT mRNA in mated females were compared with those in virgin females. Although the corpora allata of mated *M. sexta* females exhibit an elevated rate of JH biosynthesis *in vitro*, the amount of Manse-AT mRNA was similar to that in virgin females when assayed by Northern blots. This suggests that the increased activity of the corpora allata in mated females is not simply due to elevated Manse-AT mRNA levels.

Purification of *Bombyx* neuropeptide showing summer-morph-producing-hormone (SMPH) activity in the Asian comma butterfly, *Polygonia c-aureum*

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The Asian comma butterfly, *Polygonia c-aureum* L., exhibits seasonal dimorphism (summer and autumn morphs), the development of which is determined by photoperiod and temperature during the larval stages. The physiological mechanism underlying the photoperiodic control of seasonal morph development involves a cerebral factor, named summer-morph- producing hormone (SMPH). A neuropeptide showing SMPH-activity was found to be extracted with 2% NaCl from pupal brains of *P. c-aureum*. SMPH-active peptide was found to exist in brain-extracts of the silkmoth, *Bombyx mori*. The *B. mori* SMPH-active peptide was demonstrated to have almost the same physicochemical characteristics as the SMPH of *P. c-aureum*. Seasonal morph development, once determined by photoperiod and temperature in the larval stages, was shown to be shifted toward summer morphs in *P. c-aureum* (or typical spring morphs in the small copper butterfly, *Lycaena phleas daimio*) by injecting a small amount of 20-hydroxyecdysone into abdomens of 0-day-old short-day pupae. The SMPH-active peptide of *B. mori* as well as that of *P. c-aureum* decreased dramatically the number of bristles distributed on the ventral side of their wings. In contrast, in summer morph butterflies developed from short-day pupae by the 20-hydroxyecdysone injection, the numbers of wing bristles were found to be decreased, but the effect of 20-hydroxyecdysone on the wing bristles is not so dramatic as compared to that of SMPH. In the present study, we attempted to extract the SMPH-active peptide from adult brain-suboesophageal ganglion complexes of *B. mori* and purified it by using a gel-filtration and 4 steps of reverse-phased HPLC. We are going to analyze amino acid sequence from the N-terminal of *B. mori* SMPH-active peptide. We will discuss about the roles of SMPH and ecdysone played in the regulation of seasonal morph development.

Neuropeptides regulating ecdysteroidogenesis in the prothoracic glands of the silkworm, *Bombyx mori*

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The insect brain regulates the activity of the prothoracic glands to secrete ecdysteroids, which affect molting and metamorphosis. We here report the identification of a novel prothoracicostatic factor and its receptor in the silkworm, *Bombyx mori*. The prothoracicostatic factor purified from pupal brains of *B. mori* is a decapeptide with the conserved structure of an insect myosuppressin, and thus named Bommo-myosuppressin (BMS). BMS dose-dependently suppressed the cAMP level and inhibited

ecdysteroidogenesis in the larval PGs at much lower concentrations than the prothoracicostatic peptide, the other prothoracicostatic factor reported previously. *In situ* hybridization and immunohistochemistry revealed the existence of BMS in the brain neurosecretory cells projecting to neurohemal organs, the corpus cardiacum, in which it is stored and from which it is released. We also identified and functionally characterized a specific receptor for BMS, and showed its high expression in the prothoracic glands. All these results suggest that BMS functions as a prothoracicostatic hormone and plays an important role in controlling insect development. We also present how ecdysteroidogenesis is regulated by multiple neuropeptides including prothoracicotropic hormone, BMS and prothoracicostatic peptide. Using *in vitro* prothoracic glands culture system, we revealed that these peptides affect the intracellular levels of the second messengers, Ca^{2+} and cAMP, prerequisite for ecdysteroidogenesis in the prothoracic glands. This work was supported by grants from Research for the Future Program of JSPS.

The biosynthesis of MF and FA by mandibular organs in *Penaeus monodon*: second messengers and possible regulation by allatostatins

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The sesquiterpenoids methyl farnesoate (MF) and its precursor (FA) appear to be involved in regulating aspects of crustacean reproduction and development. Therefore elucidating the regulation and actions of these two compounds within the Penaeid prawns is necessary to completely understand the regulation of reproduction in these economically important species. The biosynthesis of the compounds takes place in the mandibular organs (MO), and the *in vivo* release of MF as well as the *in vitro* release of MF and FA has been demonstrated in numerous crustacean species. We present data describing the biosynthesis and *in vitro* release of MF and FA by mandibular organs of the tiger prawn, *Penaeus monodon*, as well as evidence of the second messengers involved in the regulation of sesquiterpenoid biosynthetic activity. We also explore the possibility that the FGLamide type allatostatins are involved in regulating MF and FA biosynthesis/release by MO of *P. monodon*. By mapping the distribution of FGLamide AST-like immunoreactivity we have identified various routes by which the the ASTs may be delivered from neurosecretory cells to the MO. We have also quantified the amounts of AST-like material in the CNS, gut, and haemolymph of *P. monodon* to identify sources of the ASTs. The effects of the FGLamide ASTs on MF and FA biosynthesis will be characterized by radiochemical assay.

Occurrence of progesterone and prostaglandin in polychaetes, *Perinereis sp*., and reproducing female black tiger shrimp, *Penaues monodon*

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Polychaetes are acknowledged to be the best maturation feed for shrimp broodstock due to their high nutritional value and some other unknown factors such as hormone-like substances. In crustaceans, vitellogenesis is controlled by numerous reproductive hormones, for example, eyestalk neuropeptides, biogenic amines, ecdysteroids and a juvenile hormone-like compound, methyl farnesoate. These hormonal factors therefore have been targeted verify their presence in polychaetes. Progesterone and prostaglandin were extracted from whole polychaetes and reproducing female *Penaues monodon* and measured by radioimmunassay, enzyme-immunoassay, and HPLC. The results showed that polychaetes contained progesterone, prostaglandin and a terpenoid hormone-like substance. Levels of each hormone in polychaetes varied according to sex, age, source and feed intake. Ovarian extracts, muscle and haemolymph of female shrimp also possessed significant amounts of progesterone and prostaglandin with varying level related to degree of maturation.

Novel excitatory pentadeca-peptides isolated from a rock-shell, *Thais clavigera*; The molluscan counterpart of the annelidan excitatory neuropeptides, GGNG-peptides

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The GGNG-peptides are 16-18 amino-acids peptides having an intramolecular disulfide-bond and C-terminal -GGNG-OH or $-GGN-NH_2$ structures as their common features. GGNG peptides have been identified from the three major annelidan orders, namely oligochaeta (earthworm), polychaeta (sandworm) and hirudinea (leech). All of them show an excitatory action on the digestive or reproductive systems in respective animals. Our preliminary data showing that the leech GGNG-peptide (LEP), but not other GGNG-peptides, had an excitatory action on molluscan tissues such as *Aplysia* esophagus is interesting to us, because LEP-like peptide may be functional in mollusks. However, no such peptides have been identified in mollusks so far. In 2003, we started a peptide-isolating project on a rock-shell, *Thais clavigera*, as the first step toward the understanding of peptidic mechanisms controlling the reproductive activity of the animal. One of the achievements of the project was the identification of two LEP-like peptides in a mollusk. Those peptides were isolated by the combination of fractionation with HPLC and the dot-blot assay using anti-LEP antibody. After the structural analysis by the automated Edman degradation and mass spectrometry with Q-Tof, the accuracy of the analysis was confirmed by the co-elution of synthetic and native peptides on reversed-phase and cation-exchange HPLC. The LEP-like peptides of *T. clavigera* are tentatively named as TEPs here. In TEPs, the position of disulfide bond and C-terminal $GGN-NH_2$ structure were conserved. But, the peptides have two tryptophans, while other GGNG-peptides have just one.

TEP increased the frequency and amplitude of the rhythmic contractions of esophagus of *T. clavigera*. It also induced the contractions of penis, prostate gland and female reproductive gland. We found that both of the tryptophans in TEPs are indispensable for the bioactivity of the peptides. It is possible to assume that interaction between the two tryptophans, as well as the intramolecular disulfide bond, contribute to the active conformation of the TEP. This work is supported by the grant-in-aid from the Japan Society for the Promotion of Science to F. M. and that from the Ministry of the Environment, Japan, to T. H.

Structural and conformational aspects of the interaction of insect kinin and pyrokinin-like neuropeptides on expressed receptors

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The recent explosion in the availability of cloned and expressed G protein coupled receptors has afforded an opportunity to identify neuropeptides that can serve as putative ligands and to undertake functional analyses. The interaction of two classes of insect neuropeptides with their respective expressed receptors is investigated from structural/conformational perspectives. The first class of neuropeptides are the insect kinin class of neuropeptides that stimulate Malpighian tubule fluid secretion in a number of insects including the cricket and housefly. A series of Ala-replacement and truncated analogs was evaluated on a G-coupled receptor from the Southern cattle fever tick, *Boophilus microplus*. These evaluations identify residues that are critical for receptor interaction. Evaluation of several restricted-conformation analogs

identifies the conformation associated with successful interaction with this tick receptor. An insect kinin analog containing (2S,4S)-4-aminoglutamate, a novel, *cis*-peptide bond, type VI beta turn motif, demonstrates significant diuretic activity. This provides confirmatory evidence for the active conformation, and a new scaffold with which to design biostable, mimetic agonist and antagonist analogs. Spectroscopic/molecular modeling investigations of insect kinin analogs with a tetrazole motif provide a structural and stereochemical basis for the observed transformations of insect kinin analogs from agonist to antagonist activity in an insect diuretic assay. The second class of neuropeptides is a broad class of pyrokinin-like insect neuropeptides that represent products of the capa-gene. Restricted conformation analogs provide evidence for a *trans* Pro, type I beta turn as the receptor interaction conformation with the PBAN receptor from *Heliothis virescens* and ETH receptor from *Manduca sexta*. A novel mimic of a *trans*Pro, consisting of a conformationally-locked *trans* alkenePro isostere, was incorporated into a pyrokinin sequence, and shown to demonstrate strong affinity for the *Heliothis* PBAN receptor. This provides compelling evidence for a *trans*Pro orientation and is fully consistent and supportive of a type I beta turn as the preferred conformation for successful receptor interaction. The analog provides another scaffold with which to design biostable, mimetic agosnist/antagonist analogs. Comparisons are made of the structural and conformational requirements for receptor interaction/activity of PBAN, ETH and CAP2b sub-members of the capa gene class. Finally, the search for antagonists of the ETH neuropeptide class will be discussed.

Drosophila melanogaster allatostatin signaling pathways in heart and foregut

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Allatostatin (AST) peptides inhibit juvenile hormone production from the insect corpora allata. The multiple allatostatins present in insect species can be grouped into three families based on structure. The consensus structure for the AST A peptide family is -YXFGLamide, for the consensus structure for AST B is -WXXXXXXWamide, and the consensus structure for AST C is pEVR(F/Y)RQCYFNPISCF. The consensus structures for AST A and AST B peptides represent only the C-terminal amino acid residues; AST C represents the length of the peptide. The N-terminal extensions of AST A and AST B peptides vary in sequence and length; the overall structure of AST C is highly conserved. Additionally, AST C contains a cyclic N terminal amino acid, and its C terminus is not amidated. Another marked difference is AST A and AST B precursors encode polyproteins; AST C precursor contains a single copy of one peptide. Based on nucleotide sequence and peptide structure data the three AST peptide families are present in *Drosophila melanogaster*. In *Manduca sexta*, where AST C was first identified, the peptide demonstrates allatostatic activity. However, AST C does not inhibit juvenile hormone production in *D. melanogaster*. We previously reported *D. melanogaster* AST C decreases the frequency of contractions of the heart and the foregut or crop. To further investigate allatostatic peptide structure requirements for myotropic activity we tested AST A and AST B peptides on heart and foregut contractions. Our data support the conclusion there are multiple allatostatin signaling pathways in *D. melanogaster* heart and in foregut. This research was supported in part by a National Science Foundation grant and REU supplement to R.N.

The presence of gonadotropin releasing hormone-like peptides in *Procambarus clarkii*, *Cherax destructor*, and *Macrobrachium rosenbergii*

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Neuropeptides of the gonadotrophin releasing hormone (GnRH) family are traditionally considered to be present only in vertebrates. Recent evidence, however, indicates that GnRH may be an ancient molecule, which arose well before the emergence of the Phylum Chordata. GnRH-like peptides have been detected in a number of invertebrates, ranging from anthozoans to gastropods and cephalopods. Currently there is no

report on the existence of GnRH-like peptides in crustaceans. Using rabbit anti-mGnRH as a primary antibody, together with appropriate secondary antibodies, we demonstrated the presence of GnRH-like material in *Procambarus clarkii*, *Cherax destructor*, and *Macrobrachium rosenbergii*. GnRH-like immunoreactivity was observed as tracts in the sinus glands and clustered cells in the X-organs. On each side of the circumesophageal connective, GnRH-like immunoreactive axon tracts were found. Injection of an GnRH analogue, buserelin, into male *Macrobrachium rosenbergii* for 3, 5, 7, 9, 11, and 13 days resulted in increases in testicular index as well as sperm count. Immunocytochemical detection of phosphohistone in testicular cells revealed higher mitotic activity in the buserelin-injected group relative to the control group.

Signal transduction in the corpora allata of adult *Heliothis virescens*

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The coordinated action of juvenile hormone and neuropeptide hormones such as allatotropin is necessary for egg development in *Heliothis virescens*. Allatotropins presumably act on corpora allata cells by binding to membrane receptors, thereby stimulating intracellular signal transduction pathways. Our studies indicated that Ca^{2+} is an important regulator of JH biosynthesis in *H. virescens*. A Ca^{2+} ionophore, A23187, stimulated JH production, as did *Manduca sexta* allatotropin (Manse-AT). Thapsigargin, a drug which is known to increase intracellular calcium concentrations, significantly stimulated corpora allata activity. By incubating the corpora allata with a membrane-permeable Ca^{2+} chelator, BAPTA/AM, we could antagonize the stimulatory effects of thapsigargin and those of Manse-AT. This suggests that Manse-AT may indeed affect corpora allata activity by increasing intracellular Ca^{2+} concentration. The drug 2-APB, which inhibits IP_3-induced Ca^{2+} release from intracellular Ca^{2+} stores in some animal systems, had no effect on *H. virescens* corpora allata when applied alone. This drug also failed to reduce stimulatory Manse-AT effects when applied together with Manse-AT, indicating that 2-APB may not act as an efficient Ca^{2+} release blocker in *H. virescens*. Modification of intracellular Ca^{2+} concentration clearly affected corpora allata activity. However, corpora allata were insensitive to changes in extracellular Ca^{2+}, suggesting efficient Ca^{2+} homeostasis. The diacylglycerol (DAG)-kinase inhibitor R59022 had no effect on corpora allata activity, but it potentiated effects of Manse-AT. This indicates that DAG might serve as a second messenger. PDBu, the phorbolester activator of protein kinase C, did not affect corpora allata activity when applied alone or in combination with Manse-AT. This does not exclude protein kinase C involvement in corpora allata regulation. In most animals there are several, tissue-specific, protein kinase C isoforms present, which show different sensitivities towards inhibitory or stimulatory drugs, thus, requiring further experiments.

The distribution and effects of Dippu-allatostatin-like peptides in the blood-feeding bug, *Rhodnius prolixus*

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Polyclonal antisera generated against *Diploptera* allatostatin 7 (Dippu-AST 7) or 11 (Dippu-AST 11) were used to examine the distribution and content of allatostatin-like immunoreactive material in the blood-feeding bug, *Rhodnius prolixus*. Allatostatin-like immunoreactivity is distributed throughout the central nervous system (assessed by immunohistochemistry and RIA) and is present in apparent interneurons, neurosecretory neurons and neurohaemal sites, and in neurons projecting to the digestive system, dorsal vessel, tergo-sternal muscles and fat body tissue. Immunoreactive processes are seen over the posterior midgut and hindgut, and positively-stained endocrine cells are evident in the midgut. Positively-stained lateral neurosecretory cells in the brain project axons though the nervi corpori cardiaci II and along the dorsal vessel where they form neurohaemal-like terminals. An RIA has also been used to quantify the amount of allatostatin-like material throughout a variety of *Rhodnius* tissues, and the effects of *Diploptera* allatostatins on visceral and cardiac muscle contraction have been examined. This work was

supported by NSERC and NIH.

The neurosecretory components of the transverse nerve in *Drosophila melanogaster* appear to develop in a manner that is distinct from purely neural lineages

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In *Manduca sexta*, the transverse nerve (TN) consists of 1 motoneuron and 8-10 neurosecretory cells in every abdominal hemisegment. Neural components can be classified into 3 groups: (1) a posterior, lateral cluster of 3 or 4 Bursicon+ and CCAP/CAP+ neurosecretory cells that constitute the well described 'B-cell pathway', (2) a medial neurosecretory cell (Va) that extends bifurcating processes into the TN, and (3) a motoneuron that projects posteriorly, bifucates to extend out of the central nervous system in the TN and innervates spiracle muscles in abdominal segments. In addition to the neural components, there are also glial 'strap cells' which are essential for pioneering the TN in *M. sexta*; the strap cells are joined by other migrating glia that together line the transervse nerve. Finally, there are mesodermally derived DM cells at the dorsal surface of the TN in both *Drosophila melanogaster* and *M. sexta*; these cells have both glial and neural characteristics. We have previously reported that all of the components of the TN in *D. melanogaster* appear to be analogous to those observed in *Manduca* embryos. (1) The B-cell pathway of neurosecretory cells is likely to be derived from NB 4-3 and NB 5-4, which generate the only cells in the *D. melanogaster* embryonic central nervous sytstem that have trajectories similar to the unique M. sexta B-cell like profiles. (2) NB 5-5 produces a horizontally bifurcating cell, one of only two in the central nervous system, which may be the homolog of the Va neurosecretory cell of *M. sexta*. (3) NB 4-1 produces the other horizontally bifurcating cell, which we believe likely to be the TN motoneuron; it extends posteriorly in the median nerve before dividing to send axons into both branches of the TN. Its muscle targets remain an open question, although several studies reveal a motoneuron matching this description that innervates abdominal muscle 25. (4) We have observed one case of putative TN glia arising from NB 1-3. (5) We have observed mesoderm precursors that produce the DM cells, as well as some or all of muscles 6, 7, 12 and 13. Interestingly, all NBs giving rise to the neurosecretory cells of the TN (NBs 4-3, 5-4 and 5-5) form at a similar time and position within the neuroectoderm, suggesting a common patterning mechanism may be involved in the generation of neurosecretory cell lineages. We have examined these lineages in a number of backgrounds that affect dorso-ventral patterning and molecular aspect of axonal pathfinding. We report here that molecules normally involved in axonal pathfinding of the intersegmental nerves, segmental nerves and the central nervous system do not affect the axon trajectories of transverse nerve neurosecretory components. Furthermore, genetic backgrounds that skew the dorso-ventral cell fate assignments of the neuroblast stem cells giving rise to these lineages, also do not appear to affect these neurosecretory components. Together these results suggest that the development of the transverse nerve (or perhaps neurosecretory cells) may be distinct from those described for more wholly neural lineages in *D. melanogaster* .

Role of juvenile hormone on changes in trehalase activity and bombxin A expression in the bamboo borer, Omphisa fuscidentalis

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Since trehalose metabolism depends on the trehalase activity that is present on the surface of the midgut, trehalase activities in midgut homogenates were measured through the larval diapause period and the pupal stage. The midgut homogenates exhibited low trehalase activity from December to April, showed a 4-fold increase in May and remained high in the pupal stage in July. After juvenile hormone analogue (JHA) application, trehalase activity began to increase after the G0 stage and attained the maximal level after pupation. The results indicated that JHA brings about an increase in the ecdysteroid titer, which causes the increase in the trehalase activity. In this study we examined the effects of 20-hydroxyecdysone on the change in the trehalase activity in the midgut. 20-hydroxyecdysone induced G0 morphology three

days after the injection, and trehalase activity increased from G0. *In vitro* culture of whole midgut in Grace's insect medium with 1µg/ml 20-hydroxyecdysone for 72 h increased the trehalase activity to a high level at 48 hours of culture and remained high thereafter. This clearly shows that 20-hydroxyecdysone is the factor causing the increase in the trehalase activity. Bombyxin has been reported to be involved in the regulation of carbohydrate metabolism and causes an elevation of the trehalase activity in the midgut and muscle of *Bombyx mori*. We examined the change in bombyxin A expression in the brain of the bamboo borer after 20-hydroxyecdysone injection. Bombyxin A expression in the brain increased gradually from G0 and remained high during G1-G3. These results suggest that JHA stimulates the increase in hemolymph ecdysteroids and then the increased ecdysteroids induced the increases in trehalase activity and may be involved in bombyxin mRNA expression in the brain.

Activity of DH31-like peptides in the blood-feeding bug, *Rhodnius prolixus*

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Diuretic hormone 31 (DH31)-like peptides, which stimulate increases in the rate of Malpighian tubule secretion, have been isolated from *Diploptera punctata* and predicted from genome sequences of *Drosophila melanogaster*. Previously, using an antibody raised against Dippu DH31, we have demonstrated the presence of DH31-like immunoreactivity in the central nervous system, associated neurohaemal sites as well as in processes over the dorsal hindgut, salivary glands and the anterior dorsal vessel of 5th instar *Rhodnius prolixus*. Recently, utilizing an enzyme linked immunosorbent assay (ELISA) we have quantified the DH31-like material in the central nervous system of *R. prolixus* and used high pressure liquid chromatography combined with ELISA to detect fractions containing DH31-like material. These data suggest the presence of a peptide or peptides related to the DH31 family of peptides in *R. prolixus*. We have shown previously that Dippu DH31, tested in *R. prolixus* Malpighian tubule secretion assays, stimulated only very low rates of Malpighian tubule secretion. We were interested in exploring whether the DH31 peptides may have roles on tissues other than the Malpighian tubules. Since DH31-like immunoreactivity was observed over the hindgut and anterior dorsal vessel, tissues which also play a role in post-feeding diuresis, we were interested in the activity of DH31-like peptides on these tissues. Hence we tested Dippu DH31-like peptide in both hindgut and dorsal vessel contraction assays and found it stimulated a dose-dependent increase in frequency of contraction on both tissues.

The identification of Fraenkel's pupariation factor in the grey flesh fly, *Neobellieria bullata*

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Thirty five years ago, Zdarek and Fraenkel demonstrated that nervous tissue extracts influenced development by accelerating pupariation in the grey flesh fly, *Neobellieria (Sarcophaga) bullata* . We identified this pupariation factor as SVQFKPRLamide, designated Neb-pyrokinin-2 (Neb-PK-2). The central nervous system of *N. bullata* wandering stage larvae, i.e. preceding pupariation, were dissected and extracted prior to HPLC separation. Chromatographic fractions were screened with a bioassay for pupariation accelerating activity. Only one fraction showed huge pupariation activity. Mass spectrometry revealed the presence of a pyrokinin, whose primary sequence could not be unequivocally determined by tandem mass spectrometry. However, this Neb-pyrokinin appeared to be very prominent in the ring gland from which it was subsequently purified and identified by Edman based sequencing. Synthetic Neb-PK-2 accelerates pupariation with a threshold dose of only 0.2 pmol and therefore, Neb-pyrokinin is considered to be the genuine pupariation factor. The immunohistochemical distribution pattern of Neb-PK-2 is very similar to that of *Drosophila* pyrokinin-2, from which it differs by only 1 amino acid residue. Hence, the recently identified G-protein coupled receptors (CG8784, CG8795) for *Drosophila* pyrokinin-2 might play

an important role in puparium formation.

Neuropeptides of the beetle *Tenebrio molitor* analysed by ELISA and MALDI-TOF mass spectrometry

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Analysis of the neuropeptide genes and associated G-protein-coupled receptor genes of *Drosophila melanogaster* and *Anopheles gambiae* indicates that dipteran insects may have no more than ~ 45 different peptide hormone 'types', with physiological function being governed primarily by the presence or absence of the appropriate receptor(s) in any given target organ or stage of development. Whether non-dipteran insects will be shown to have the same limited range of peptide hormones and a similar number of receptors remains to be seen. We are interested primarily in peptide hormones that regulate feeding and development in both lepidopteran and non-lepidopteran pests of agriculture. At least four different classes of neuropeptide hormone, three different forms of allatostatin and one type of allatotropin have been implicated in the regulation of juvenile hormone synthesis in different insect groups. These hormones are also myoactive, having effects on various tissues such as foregut, hindgut, antennal pulsatile organ, oviduct and heart. Some are also implicated in other functions, such as modulation of midgut enzyme activity and inhibition of vitellogenin production. The –Y/FXFGLamide (A-type) allatostatin / myoinhibitory peptide family has been shown to be present in all insect groups examined, and to have a wider presence in other invertebrates. In contrast, our knowledge of the distribution and significance of the pEVRF/YRQCYFNPISCF-OH (Manse-AS, Drome-AS, C-type) allatostatins is less complete. The occurrence and wider significance allatotropins (Manse-AT; Aedae-AT) is also less well understood. In the present study, we have used a combination of direct extraction and narrow-bore liquid chromatography, together with enzyme-linked immunoassays and matrix-assisted laser desorption-ionisation time of flight (MALDI-TOF) mass spectrometry to examine the presence and distribution of allatostatins and allatotropins in the beetle *T. molitor*. These findings are discussed in relation to the possible functions of these peptides in non-lepidopteran insects. This work was supported by Pesticides Safety Directorate, DEFRA, U.K.

Purification of orange-pupa-inducing factor (OPIF) from short-day larval ganglia complexes of the swallowtail butterfly, *Papilio xuthus*

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Pupae of *Papilio xuthus* show color polymorphism, and the development of pupal color is controlled by environmental and hormonal factors. Non-diapause pupae exhibit color dimorphism such as green and brown. The brown pupae are produced by the secretion from prothoracic ganglion of the pupal-cuticle-melanizing hormone that causes melanization of the pupal cuticle. On the other hand, diapausing pupae show color polymorphisms, including green, orange, and brownish-orange types. Recently, we investigated the role of the site of pupation on the induction of the development of orange types (including brownish-orange types), and the neuroendocrine mechanism underlying the control of color polymorphism in short-day pupae. All short-day larvae of the wandering stage developed into orange, or brownish-orange, type pupae when they were placed in rough-surfaced containers after gut-purge. Utilizing a pharate pupal ligation between the thorax and abdomen, the neuroendocrine mechanism underlying the control of color polymorphism was shown to involve a head-thorax factor (orange-pupa-inducing factor: OPIF) that induced orange types in short-day pupae. OPIF was bioassayed using the ligatured abdomens of short-day pharate pupae and extracted with a 2% NaCl solution from 5th-instar larval ganglia complexes following the mesothoracic complex ($TG_{2,3}$-$AG_{1\text{-}7}$). OPIF may not exist in the brains of day 0 pupae or in brain-subesophageal ganglion and prothoracic ganglion complexes of 5th-instar larvae. The action of OPIF on short-day pharate pupae is considered dose-dependent. In this study, we attempted to purify OPIF from the nerve cord of 5th-instar short-day larvae that induces orange-type pupae, and to analyze N-terminal amino acid sequence of OPIF.

Ecdysis-triggering hormone receptors and insect belly dancing

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Ecdysis-triggering hormone (ETH) produced by the inka cells act on the central nervous system to initiate the ecdysis sequence in insects. We identified a gene expressing two subtypes of the ETH receptor (ETHR-A and ETHR-B). *In situ* hybridization revealed differential expression of each receptor subtype in a variety of neurons in the central nervous system. Obvious ETHR staining of these neurons in pharate larvae (8-24 h prior to ecdysis) disappeared or remained very weak after ecdysis and during feeding stages, but became apparent after ecdysteroids reached the highest levels in the hemolymph. This indicates that ETHR expression and appearance of central nervous system sensitivity to ETH are controlled by elevated ecdysteroid levels prior to each ecdysis. Combined *in situ* hybridization and immunohistochemical staining showed that the ETHR neurons produce multiple neuropeptides, including eclosion hormone, diuretic hormones, kinins, statins and FMRFamide-related peptides. These neuropeptides induce different phases of pre-ecdysis and ecdysis behaviors when applied on the isolated central nervous system. Our recent data show that ETH action on specific central nervous system neurons results in coordinated release of multiple neuropeptides, which control the ecdysis sequence and associated physiological functions.

A venom peptide of the vermivorous snail *Conus austini*, from the Gulf of Mexico, with a novel arrangement of cysteine residues

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Cone snails are a group of venomous marine gastropods that are found in tropical and subtropical waters. Venoms contained in the venom ducts of *Conus* species are delivered by harpoon-like, hollow, radular teeth to rapidly inmobilize fish, molluscs or worms. Each species has its own distinct complement of venom peptides, and each cone snail hunts only one kind of prey, the only exception is *Conus californicus*, a generalist species. Most venoms so far examined have yielded an array of low molecular weight peptides, the conotoxins, which typically have 7-40 amino acids and are highly constrained by one to four disulfide bridges. Some conotoxins, however, do not have cysteine residues, but this kind of peptide is less frequent. Another common feature of conotoxins is the presence of a variety of post-translational modifications, such as amidation of the C-terminus and γ-carboxylation of glutamate, among others. Until now, more than 100 conotoxins have been purified and characterized at several levels. Most studies have been made with species from the shallow waters of the Indopacific region that prey on fish or molluscs, although worm-hunting cones represent more than 80% of the species whose feeding type is known. Here, we describe the isolation and primary structure determination of one conotoxin of a vermivorous cone -*Conus austini*- from the Gulf of Mexico, collected at depths of 80 m. This peptide has a monoisotopic mass of 2,644.12 Da (MALDI-MS) and 22 amino acid residues. It contains 6 cysteines arranged in a novel pattern and could represent a new family of conotoxins. This work was supported by Grants 41477-Q (CONACYT, México) and 204403 (PAPIIT-DGAPA-UNAM, México).