Cell. Mol. Life Sci. 63 (2006) 2477–2490
1420-682X/06/212477-14
DOI 10.1007/s00018-006-6187-3

Cellular and Molecular Life Sciences

Review

Biology of the CAPA peptides in insects

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Received 28 April 2006; received after revision 5 June 2006; accepted 4 July 2006
Online First 4 September 2006

Abstract. CAPA peptides have been isolated from a broad range of insect species as well as an arachnid, and can be grouped into the periviscerokinin and pyrokinin peptide families. In insects, CAPA peptides are the characteristic and most abundant neuropeptides in the abdominal neurohemal system. In many species, CAPA peptides exert potent myotropic effects on different muscles such as the heart. In others, including blood-sucking insects able to transmit serious diseases, CAPA peptides have strong diuretic or anti-diuretic effects and thus are potentially of medical importance. CAPA peptides undergo cell-type-specific sorting and packaging, and are the first insect neuropeptides shown to be differentially processed. In this review, we discuss the current knowledge on the structure, distribution, receptors and physiological actions of the CAPA peptides.

Keywords. Neuropeptides, hormones, *Drosophila*, vesicle sorting, peptide processing, diuresis, Chelicerata, prohormone convertase.

Introduction

The endocrine system of insects is largely based on neurohormones, most of which are neuropeptides synthesized by neurosecretory cells in the central nervous system (CNS). These peptidergic neurons send projections to specialized neurohemal organs or to peripheral neurohemal zones, which are located along nerves or muscles. The major neurohemal organs of the insect CNS are the retrocerebral complex located behind the brain, and the thoracic and abdominal perisympathetic organs (PSOs) associated with the median and transverse nerves of the thoracic and abdominal ganglia. The peptidome of these neurohemal organs is tagma-specific, *i.e.* it differs between the head, thorax, and abdomen.
CAPA peptides are neuropeptides typical of the neurosecretory system in the abdominal ventral nerve cord (VNC) of insects. Synthesized in median neurosecretory neurons of abdominal ganglia, CAPA peptide hormones are likely to be released as hormones from the abdominal PSOs into the hemolymph. In some insects, CAPA peptides also occur in interneurons, from which they may be released within the CNS. In this review, we comprehensively summarize the current knowledge on the distribution, genes, processing and function of CAPA peptides in insects.

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A short history of the isolation and designation of CAPA peptides

As is the case for many insect neuropeptide families, the first CAPA peptide was identified from a cockroach. Using an extract of abdominal PSOs of *Periplaneta americana*, a peptide designated periviscerokinin-1 (PVK-1; derived from the term perivisceral organ, an alternative designation for PSO) was purified by means of a visceral muscle bioassay to monitor bioactivity during the puri-

fication procedure [1]. PVK-1 was the first neuropeptide to be identified from an insect PSO. Its isolation in 1995 was the first indication that abdominal ganglia may produce neurohormones different to those occurring in other neurohemal release sites such as the well-studied retrocerebral complex. A second PVK was later isolated from the same source [2], followed by the identification of a pyrokinin [3]. From our current knowledge, these peptides can all be regarded as orthologs of *Drosophila melanogaster* peptides encoded on the gene *capability* (*capa*) [4]. The designation '*capability'* originates from the gene's ability to encode for neuropeptides related to one of the cardioacceleratory peptides (CAPs) in the VNC/PSOs of the tobacco hawk moth, *Manduca sexta*. Starting in the early 1980s, the CAPs were extensively studied over many years, but without having their sequences elucidated. Initially, two CAPs were separated by gel filtration and identified as CAP_1 and CAP_2. Both CAPs were found to be accumulated in abdominal PSOs and the VNC of *M. sexta* [5]. Subsequent experiments pointed to a cellular origin of these CAPs in median neurosecretory neurons that appear during pupal metamorphosis, a finding that fitted with the lack of bioactive material in the larval VNC [6]. Later, a monoclonal antibody raised against partially purified PSO peptides stained these median neurons and, additionally, two posterior median cells as well as lateral secretory neurons in the abdominal ganglia. These additional cells were found to be already present in the larva [7]. The neurites of the two posterior median cells exited the abdominal ganglia via the ventral nerve. In accordance with these findings, CAP activity was later on also detected in the larval CNS [8]. The number of potential CAPs then increased from two to five ($CAP_{1a,b}$; $CAP_{2a,b,c}$) [9], before the first two peptides, CAP_{2a} and CAP_{2b}, were finally sequenced [10, 11]. CAP_{2a} turned out to be identical with the crustacean cardioactive peptide (CCAP), and CAP_{2b} can be grouped together with the PVKs into a single peptide family based on sequence similarities (Table 1). The expression pattern of CAP_{2b} ([12], and see below), however, has nothing in common with the cellular pattern described for the CAPs in the numerous studies preceding the sequence elucidation of CAP_{2b}. Accordingly, the median neurosecretory neurons expressing CAP_{2b} [12, 13] were never mentioned as CAP neurons in the earlier publications. Furthermore, the expression pattern of CAP_{2a} (CCAP) in the neurosecretory neurons of the abdominal ganglia is also different from that of the earlier described CAP neurons as already noted by Davis et al. [14]. Thus, neither CCAP nor CAP_{2b} seems to be related with the 'original' CAPs, although designations might suggest so. Therefore, publications on CAPs in *M. sexta* prior to the sequence elucidation of CAP_{2b} are not included in this review.

A potential source of confusion arises from the historical development of designations for the CAPA peptides. Numerous PVKs were identified in the 1990s from various insects (see Table 1), but the first gene encoding such peptides was characterized 2002 in *D. melanogaster* and was named *capa* ([4], and see above). In addition to two PVK orthologs, *capa* encodes a single pyrokinin in the fruit fly. This pyrokinin was subsequently detected in all *capa* genes sequenced from other insects, and received species-specific incoherent designations, such as CAPA-3 (*D. melanogaster*) [4], pyrokinin-5 (*P. americana*) [3], myotropin-1, or pyrokinin(-1) (*D. melanogaster, M. sexta*) [12, 15–17]. Unfortunately, a designation like pyrokinin-1 does not reveal whether this pyrokinin is encoded by a *capa* or by another pyrokinin-encoding gene such as *hugin* in *D. melanogaster* [18]. The *capa*-encoded pyrokinin-5 in *P. americana*, for example, simply obtained its name because it was the fifth pyrokinin isolated from this species [3]. On the other hand, a designation such as CAPA-3 [4] does not distinguish between PVKs and pyrokinins. Thus, to unambiguously refer to the respective peptides and genes, and to unify the confusing diversity of names that conceals evolutionary relationships, we will use the following nomenclature rules: for species with a known *capa* gene, each PVK/CAP_{2b}-related peptide as well as the respective pyrokinin (PK) carries the prefix CAPA, followed by the term PVK or PK and a number referring to the position in the prepropeptide when multiple peptide forms are present. For insect species without identified *capa* genes, the same rule applies, with the consequence that the numbering of multiple peptide forms may reflect the isolation history rather than the position in the prepropeptide. This is combined with the three- or five-letter designation for the species [19]. For example: the three *capa*-encoded peptides in *D. melanogaster* are named Drm-CAPA-PVK-1, Drm-CAPA-PVK-2, and Drm-CAPA-PK; CAP_{2b} is Mas-CAPA-PVK-2, and PK-5 of *P. americana* is Pea-CAPA-PK.

Sequences and differential processing of CAPA peptides

The first *capa* gene (CG15520) was identified after the completion of the *Drosophila melanogaster* genome project and contains two introns [4, 15, 16]. CAPA-PVK-1 is encoded on exon 2, and CAPA-PVK-2 plus CAPA-PK is encoded on exon 3 [4]. The cleavage of the predicted bioactive peptides from the 151 amino acid CAPA precursor was verified by mass spectrometric profiling of nervous tissues of both larvae [20, 21] and adults (Fig. 1) [17]. Besides the CAPA-PVKs and -PK, a so-called CAPA precursor peptide B (CPPB) has been detected by direct peptide profiling of the neurohemal organs of *D. melanogaster* [21]. This peptide links CAPA-PVK-2 and CAPA-PK within the precursor. Although CPPB is present in

Table 1. Selected sequences of CAPA peptides from arthropods and similar mollusc peptides.

Species	Insect order[a]	CAPA-PVK-1	CAPA-PVK-2	CAPA-PVK-3	CAPA-PVK-4	CAPA-PK	Gene	References
Drosophila melanogaster	1	GANMGLYAFPRVa	ASGLVAFPRVa	-	-	TGPSASSGLWFGPRLa	x	[4, 17, 20, 21]
Drosophila virilis	1	GANMGLYTFPRVa	ASLVPFPRVa	-	-	TGPSASSGMWFGPRLa	x	FlyBase BLAST
Anopheles gambiae	1	GPTVGLFAFPRVa	pQGLVPFPRVa	-	-	AGGTGANSAMWFGPRLa	x	[66, 69]
Aedes aegypti	1	GPTVGLFAFPRVa	pQGLVPFPRVa	-	-	AGNSGANSGMWFGPRLa	x	FlyBase BLAST
Musca domestica	1	AGGTSGLYAFPRVa	ASLFNAPRVa	-	-	AGPSATTGVWFGPRLa		[24]
Neobellieria bullata	1	NGGTSGLFAFPRVa	AGLLVYPRLa	-	-	AGPSATTGVWFGPRLa		[24]
Stomoxys calcitrans	1	AGGASGLYAFPRVa	NAKLYPVPRVa	-	-			[33]
Haematobia irritans	1	AGGTSGLYAFPRVa	NAKLYPMPRVa	-	-			[33]
Manduca sexta	2	DGVLNLYPFPRVa	pQLYAFPRVa	-	-	TEGPGMWFGPRLa	x	[12, 13]
Bombyx mori	2	PDGVLNLYPFPRVa	QLYAFPRVa	-	-	??PGMWFGPRLa	x	FlyBase BLAST
Nezara viridula	3	DQLFPFPRVa	EQLIPFPRVa	-	-	NGSAGNGGLWFGPRLa		Predel, unpubl.
Locusta migratoria	4	AAGLFQFPRVa	GLLAFPRVa	TSSLFPHPRLa[b]	-	DGGEPAAPLWFGPRVa[c]		[40, 70]
Schistocerca gregaria	4	AAGLFQFPRVa	GLLAFPRVa	TSSLFPHPRLa[b]	-	DGAETPGAAASLWFGPRVa[c]		[40, 70]
Mantophasma zephyra	5	AEAAGLIPFPRVa			-	NSGEGSGMWFGPRLa		Predel, unpubl.
Periplaneta americana	6	GASGLIPVMRNa	GSSGLISMPRVa	GSSSGLISMPRVa	-	GGGGSGETSGMWFGPRLa		[1-3]
Blatta orientalis	6	GASGLIPVMRNa	GSSGLISMPRVa	GSSSGLISMPRVa	-	GGGGSGETSGMWFGPRLa		[68]
Leucophaea maderae	6	GSSGLIPFGRTa	GSSGLISMPRVa	GSSGMIPFPRVa	-	FGETSGETKGMWFGPRLa		[37], Predel unpubl.
Blaberus craniifer	6	GSSGLIPFGRTa	GSSGLISMPRVa	GSSGMIPFPRVa	-	AGESSNEAKGMWFGPRLa		[37], Predel unpubl.
Gromphadorhina port.	6	GSSGLIPFGRTa	GSSGLISMPRVa	GSSGMIPFPRVa	GVGGSPGLIPFGRPa	FGETSGETKGMWFGPRLa		Predel, unpubl.
Aptera fusca	6	GSSGLIPFGRTa	GSSGLISMPRVa	GSSGIIPFPRVa	GVGSSGLISMPRVa	SGDTSSQAKGMWFGPRLa		Predel, unpubl.
Diploptera punctata	6	GSSGLIPFGRTa	GSSGLISMPRVa	GSSGMIPFPRVa	-	SGETSGEGNGMWFGPRLa		Predel, unpubl.
Ixodes ricinus	Acari	PALIPFPRVa			-			[28]
Aplysia californica	mollusc	PGYLAFPRMa	MNYLAFPRMa					[29]

[a] 1, Diptera; 2, Lepidoptera; 3, Heteroptera; 4, Caelifera; 5, Mantophasmatodea; 6, Blattodea.
[b] Described as MT-2.
[c] Described as PVK-3.

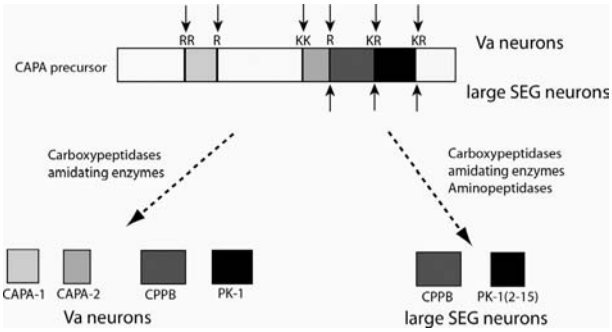

Figure 1. Model of the differential processing of the CAPA precursor in the Va and SEG neurons. Arrows indicate mono- and dibasic prohormone convertase cleavage sites. Note that the precursor contains the rare dibasic lysine (KK) cleavage site upstream of CAPA-PVK-2. The high abundance of Drm-CAPA-PVK-2 in the Va neurons clearly demonstrates that KK sites can be cleaved quantitatively. Figure from [21], with permission of the publisher (Blackwell Publishing).

neurohemal organs and might thus be co-released, it is unclear whether it has a physiological function.

A truncated form of Drm-CAPA-PK (CAPA-PK$^{2–15}$) was detected in neurosecretory cells of the subesophageal ganglion (SEG) and in the retrocerebral complex [17], as well as the larval ring gland [21]. The truncated form was only accompanied by CPPB, and not by the CAPA-PVKs. This is, to our knowledge, the only biochemically substantiated case for a differential peptide processing in insects (Fig. 1) [21]. What is the mechanism behind this differential processing? The typical cleavage signals for peptide precursor processing by prohormone convertases are arginine-monobasic (R) sites or arginine/lysine-dibasic (RR, KR) sites flanking the N and C termini of the active peptides [22, 23]. In the precursor, CAPA-PK is flanked by dibasic cleavage sites, whereas the CAPA-PVKs have upstream dibasic cleavage sites and downstream monobasic signals (Fig. 1). In principle, the differential processing of the CAPA precursor in *D. melanogaster* might be caused by the presence of different sets of prohormone convertases in CAPA-expressing cells. Some CAPA-expressing neurons might express prohormone convertases only able to cleave dibasic sites, whereas other cells express prohormone convertases that cleave both mono- and dibasic sites. Alternatively, similar sets of prohormone convertase may be present in the CAPA-expressing neurons, but the pH or other qualities of the intravesicular environment differ, leading to different cleavage specificity of the convertases co-packaged with the peptides into large vesicles.

To find out if the cleavage sequence pattern is evolutionary conserved, we searched for *capa* genes in the literature, and performed a tblastn search in genome databanks of insect species whose genome has been fully sequenced (11 *Drosophila* species, the mosquitoes *Aedes aegypti* and *Anopheles gambiae,* the moth *Manduca sexta* and the honey bee *Apis mellifera*). The putative *capa* homologs of these species each encoded two CAPA-PVKs and one CAPA-PK, with exception of the honey bee where only the CAPA-PK could be detected. In all but one of these species, CAPA-PK is flanked by dibasic cleavage sites both up- and downstream; the CAPA-PVKs are flanked by an upstream dibasic cleavage sites and a downstream monobasic cleavage site. The exception is the *capa* gene of *M. sexta*, where all CAPA peptides are flanked by upstream dibasic cleavage sites and downstream monobasic cleavage sites. Concurrently, all of the CAPA expressing neurons of the abdominal VNC and the SEG contain the full set of Mas-CAPA peptides (Neupert and Predel; unpublished).

The CAPA precursor of *D. melanogaster* contains the rare dibasic lysine (KK) cleavage sequence upstream of CAPA-PVK-2 (Fig. 1). Processing of such KK sites in insects was ambiguous [22], but the high abundance of Drm-CAPA-PVK-2 in the Va neurons [17, 21] clearly demonstrates that KK sites can be cleaved quantitatively.

In addition to the holometabolous insects with known *capa* genes, CAPA-PVKs and CAPA-PKs are known from many other insects; a summary of known CAPA peptide sequences is given in Table 1. With four paralogs, the highest number of CAPA-PVKs was found in certain cockroach species, whereas only a single CAPA-PK seems to be expressed in the insects studied so far. Interestingly, sequence comparison of CAPA peptides from more than 50 cockroach species revealed that not a single deletion event occurred in any of the species investigated (all orthologs with an identical number of amino acids), although the sequences differed remarkably (Predel and Roth, unpublished). A comparison of CAPA-PVK sequences throughout the insects reveals a great variety of sequence modifications, but leucine at position 7 from the C terminus and the C-terminal PRVamide usually occur in at least one of the PVKs of a given species. Like other insect neuropeptides, CAPA-PVKs require a C-terminal amidation to become bioactive [1]. Interestingly, the replacement of the conservative Leu with Ile at position 7 from the C terminus yielded a peptide more potent than the native peptide in a diuretic assay [24].

In contrast to the PVKs, the CAPA-PKs have a highly conserved C-terminal WFGPRLa (in locusts: WFGPRVa, see Table 1), containing the pentapeptide FXPRLamide typical of all pyrokinins with X=G. The C-terminal WFGPRLamide is also known from diapause hormones encoded on the pheromone biosynthesis-activating neuropeptide (PBAN) gene of Lepidoptera [25] and from the pyrokinins PK-6,7 of the American cockroach, which all are expressed in neurosecretory neurons of the SEG [26, 27]. Recently, the first PVK from ticks (Acari) was identified from neurons in the abdominal portion of the synganglion. The tick PVK possesses all features typical

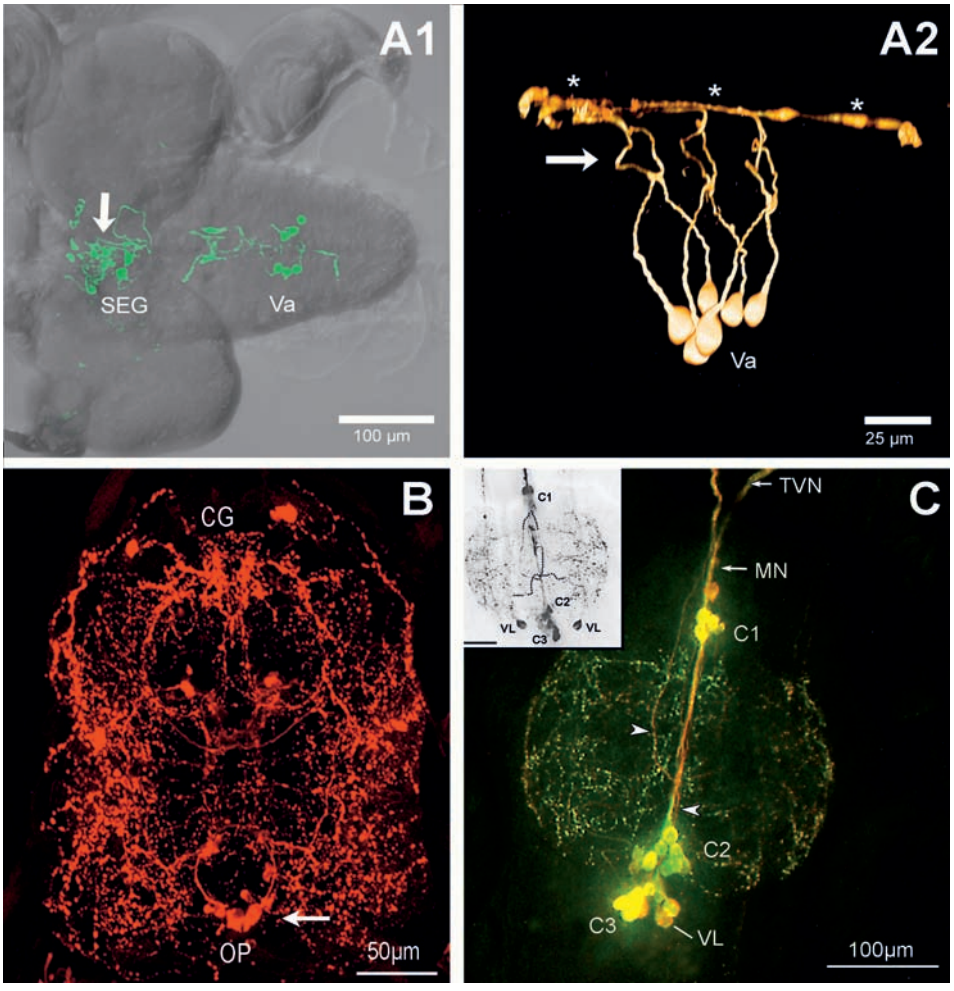

Figure 2. Wholemount immunofluorescence labeling using an antiserum raised against Pea-CAPA-PVK-2. (*A*) CNS of larval *Drosophila virilis*. (*A1*) overview of the staining in the CNS depicting the position of the Va neurons in the first three abdominal neuromeres, and the cell pair in the SEG. Arrow points to varicose endings in the ring gland; (*A2*) Voltex 3D reconstruction of the Va neurons in the abdominal neuromeres. The characteristic neurite loops are indicated by an arrow. The immunolabeled transverse nerves are indicated by asterisks. (*B*) CNS of the European hard tick, *Ixodes ricinus*. The two large neurons (arrow) in the ophistosomal ganglion (OP) were used to identify the first CAPA peptide from arachnids [28]. In contrast to the CAPA neurons from the abdominal VNC of insects, these cells possess neurites with extensive arborizations within the CNS. (*C*) CAPA-expressing neurons in the second abdominal ganglion of the stick insect *Eurycantha calcaratum* (Phasmatodea). The arborizations in the ganglion originate from descending fibers. As in cockroaches, three cell cluster (C1–3) and an additional pair of CAPA peptide expressing neurons (VL) are detectable. The loop-like processes (arrows) originate from the contralateral VL neurons, which are occasionally located lateral of the C2/C3 cell cluster (inset). MN, median nerve. TVN, transverse nerve. Previously unpublished micrographs.

of insect PVKs (see Table 1), and demonstrates that the occurrence of CAPA peptides is not restricted to insects (Fig. 2) [28]. The most sequence-related peptides outside the arthropods are the small cardioactive peptides (SCPs) of molluscs (Table 1) [29].

Localization and projections of CAPA neurons in the CNS

The localization and projection of CAPA neurons was mainly studied in *D. melanogaster*, *M. sexta*, and cockroaches as reviewed below. Further immunocytochemical work has been carried out for the bug *Rhodnius prolixus* [30] and ticks (Fig. 2) [28]. So far, CAPA peptides were found to be expressed only in the CNS, and not in the intestine, stomatogastric nervous system or any other tissue. The differences and common features in the expression pattern of CAPA peptides in *D. melanogaster, P. americana*, and *M. sexta*, are summarized in Figure 3.

Drosophila melanogaster

The simplest expression pattern of CAPA peptides occurs in *D. melanogaster* (Figs. 2, 3), which may reflect a reduction process in this insect with a conservation of

the most important part of this peptidergic system, the neuroendocrine cells in the VNC. In the fruit fly, CAPA peptides are expressed in a single pair of ventral median neurons in each of the first three abdominal neuromeres of the fused VNC [4, 31]. In the larva, these so-called Va neurons send axons dorsally that project into the respective median and proximal transverse nerves that show swellings indicative for a PSO. During metamorphosis, the median/transverse nerves become completely reduced and the Va neurons show a distinct neurite pruning [31]. Subsequent neuritic outgrowth in later pupal stages leads to a network of CAPA peptide-containing fibers just below the dorsal ganglionic sheath. This network is built by neurites of the two anterior Va neuron pairs. The Va neurons of the third abdominal neuromere project into the adult median abdominal nerve [31].

Abundant varicosities in the abdominal PSOs of larvae as well as in the arborizations below the dorsal ganglionic sheath of adults suggest a release of the CAPA peptides into the hemolymph [31]. Direct mass spectrometric profiling in larvae and adults supported this assumption by demonstrating the high abundance of the CAPA peptides in these neurohemal areas [17, 21]. Dorsal ganglionic sheath preparations of other adult cyclorraphous flies such as *Neobellieria bullata*, *Musca domestica*, *Stomoxys calcitrans*, and *Haematobia irritans* showed a similar accumulation of CAPA peptides [32, 33]. The peptidome of the putative release sites does not change during postembryonic development in *D. melanogaster* [17, 21]. Interestingly, no other prominent ion signals besides the CAPA peptides were found in mass spectra of abdominal release sites, indicating that the Va neurons are solely responsible for their peptide profile. Thus, the function of abdominal PSOs/release sites and CAPA peptides cannot be separated from each other, a feature that is typical of other insects as well.

In addition to the neurons in the abdominal VNC, a single *capa* expressing cell pair was detected in the SEG [4]. These SEG neurons are located in the labial neuromere and send neurites into the larval ring gland, and via the nervus corporis cardiaci into the adult retrocerebral complex. As mentioned in the '*Sequences and differential processing of CAPA peptides*' section, mass spectra from the ring gland and retrocerebral complex showed that the CAPA propeptide is differentially processed into CAPA-PK-1$^{2-15}$ and CPPB in the SEG cells, whereas CAPA-PVKs, the full CAPA-PK and CPPB are produced by the Va neurons.

Periplaneta americana

In the hemimetabolous American cockroach, the first ventrolateral CAPA peptide-expressing neurons (VL neurons) become detectable in the abdominal VNC around

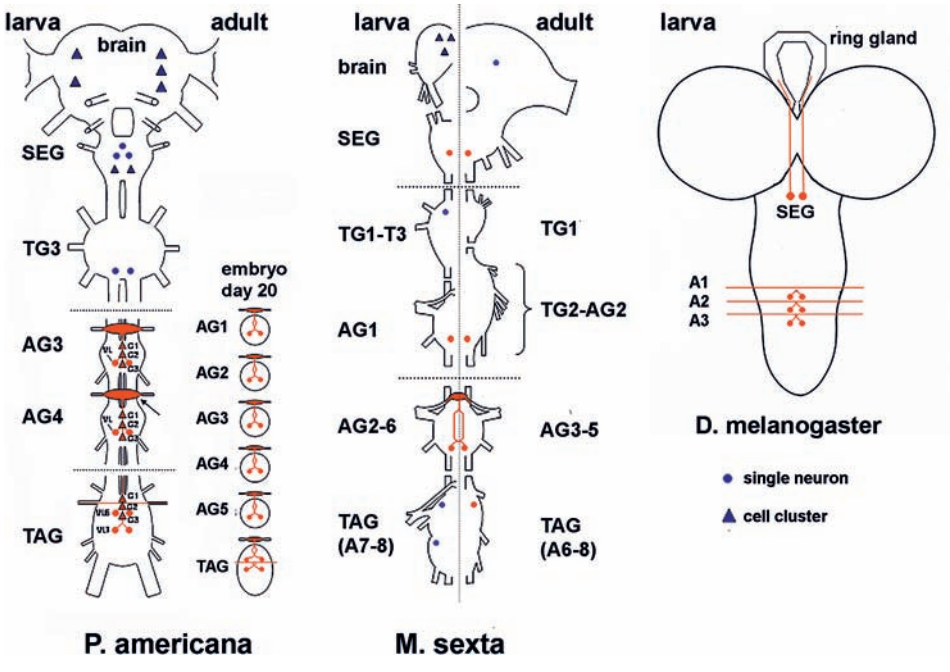

Figure 3. Schematic drawings of the distribution of CAPA peptides in the CNS of *P. americana*, *M. sexta*, and *D. melanogaster*. Neurosecretory neurons (red) persist throughout the postembryonal development in all species. The number of interneurons (blue) increases during postembryonal development of *P. americana* (M. Eckert, pers. commun.), but decreases in *M. sexta* [12]. Interneurons are absent in *D. melanogaster*. Designation of neurons or cell clusters follows [14, 31, 34]. The inset for *P. americana* shows the expression pattern of CAPA peptides around day 20 of embryonal development. This pattern is very similar to that of adult holometabolous insects such as *M. sexta* and *D. melanogaster*. SEG, subesophageal ganglion. TG, thoracic ganglion. AG, abdominal ganglion. TermG, terminal ganglion. TVN, transverse nerve. aPSO, abdominal perisympathetic organ.

day 18 of the second half of embryonic development [34]. Already 1 day later, immunoreactive fibers invade the intensely stained abdominal PSOs via the median/transverse nerves. On day 20, immunolabeled VL neurons appear in all unfused abdominal ganglia and the first two neuromeres of the terminal ganglion. These VL neurons are homolog to the Va neurons of *D. melanogaster*. Their neurites form a characteristic loop before they extend into the median nerve, a feature which is also found in the fruit fly Va neurons and their homologs in the other insect species investigated (Fig. 2) [13, 31, 34]. In addition, the VL neurons and their homologs in other insects possess no or only minor dendritic arborizations in the CNS. During the larval development of *P. americana*, additional CAPA peptide-producing neurons appear, which all project into the abdominal PSOs but lack the neuritic loop described above. Two median cell clusters (C1, C3) appear in the dorsal cortex of the five unfused abdominal ganglia and the first neuromere of the terminal ganglion. A third postero-median cluster (C2) is located ventrally, below C3. In adults, C1, C2, and C3 finally consist of six to ten cells each [35]. In the second neuromere of the terminal ganglion, only the two VL neurons express CAPA peptides. Immunocytochemical and mass spectrometric data confirmed that the abdominal CAPA neurons already contain the complete set of colocalized CAPA peptides (three CAPA-PVKs and the CAPA-PK) when they become first detectable by immunolabelings with any of the antisera against the different CAPA peptides [34].

Mass spectra from the abdominal PSOs showed that the CAPA peptides are the most abundant neuropeptides stored in these neurohemal release sites [36]. This was later found to be true for other insects as well (Fig. 4) [13, 17, 21, 32, 37–40]. A quantification of CAPA-PVK-1 by enzyme–linked immunosorbent assay substantiated the role of the abdominal PSOs as the storage sites for CAPA peptides. Whereas the different abdominal ganglia each contained between 100 and 300 fmol of CAPA-PVK-1, up to 2 pmol occurred in a single abdominal PSO [41].

Distal to the abdominal PSOs, immunoreactive branches of the transverse nerves form putative release sites on the surface of the hyperneural muscle (ventral diaphragm of cockroaches), as indicated by beaded arborizations. Immunolabeling in the transverse nerves distal to the hyperneural muscle is weak or absent [34].

In contrast to *D. melanogaster*, *capa*-expressing neurosecretory cells do not occur in the SEG of *P. americana*. However, a number of interneurons are described from the brain/SEG and exhibit extensive arborizations in these ganglia [35]. Some of these interneurons could be analyzed by mass spectrometry, which verified that they have a peptidome identical to that of the median neurosecretory neurons in the abdominal ganglia [42]. Among these interneurons, a single cell in the protocerebrum shows a distinct interganglionic projection by crossing all ganglia

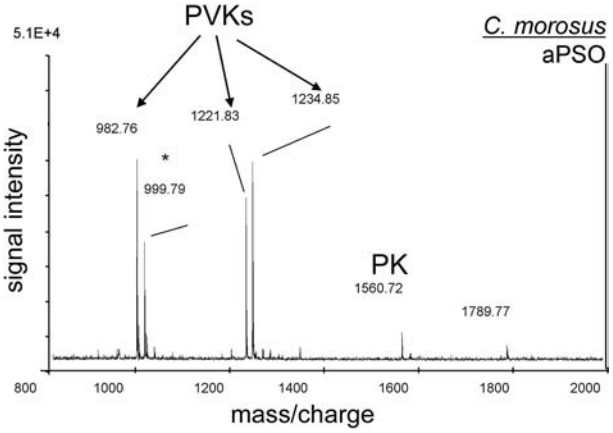

Figure 4. MALDI-TOF mass spectrum of a preparation of a single abdominal PSO of the stick insect *Carausius morosus* (Phasmatodea). As in other insects studied so far, the CAPA peptides are the most abundant peptides in these neurohemal organs, which indicates that the functions of CAPA peptides and of abdominal PSOs cannot be separated from each other. The ion signal marked by an asterisk represents the unblocked form of the neighboring CAPA-PVK without a N-terminal pyroglutamate. Previously unpublished data.

of the VNC to innervate the dorso-caudal neuropil on the most posterior edge of the terminal ganglion [35]. In the thoracic ganglia, only a single pair of median CAPA neurons exists in the first abdominal neuromere fused with the metathoracic ganglion and projects into the brain. The distribution of CAPA peptides was subsequently studied in a number of distantly related cockroaches and was very similar to that described for *P. americana* [43].

Manduca sexta

The expression of the CAPA peptides in the abdominal VNC of the holometabolous *M. sexta* is very similar to that in the holometabolous fruit fly. Only two neurosecretory neurons in a ventral, median position in the abdominal ganglia express CAPA peptides [12, 13]. These NS-M4 neurons [14] are homolog to the Va neurons of the fruit fly and the VL neurons in the cockroach. The neurites of the NS-M4 neurons show the characteristic loop in the ganglion before they enter the abdominal PSOs. In larvae, the PSOs of the first abdominal ganglion and the anterior neuromere of the terminal ganglion disappear during metamorphosis. In conjunction, these PSOs do not contain CAPA peptides [13]. All other unfused abdominal ganglia of larvae express the CAPA peptides, which are the only abundant neuropeptides in the abdominal PSOs. This is consistent with the situation in *P. americana* and *D. melanogaster*. Unlike in cockroaches and fruitflies, however, a number of additional neuropeptides appears in abdominal PSOs of adult moths, and it was shown that at least the CCAP becomes colocalized with the CAPA peptides within the NS-M4 neurons [13, 14].

As in *D. melanogaster*, two cells in the labial neuromere of the SEG express the *capa* gene in larval and adult *M. sexta* [12]. The products of the *capa* gene, however, are identical in the neurosecretory neurons of the SEG and abdominal ganglia of *M. sexta* (see section '*Sequences and differential processing of CAPA peptides*'). About 15 pairs of CAPA interneurons were detected by *in situ* hybridization in the brain of first instar larvae. Interestingly, their number decreases during postembryonic development: only one pair still expresses the *capa* gene in the adult brain [12].

Cell-type-specific sorting and packaging of CAPA peptides

The intracellular sorting and packaging of CAPA peptides was extensively studied in the American cockroach with specific antisera differentially recognizing the individual peptides in the abdominal PSOs. An immunoelectron microscopic approach revealed a number of surprising results. Median neurosecretory cells of abdominal ganglia of *P. americana* express, in addition to the CAPA peptides, three other abundant peptides (YLSamide, SKNamide, VEA-OH) that have no sequence similarity to any known insect neuropeptide [44]. Immunoelectron microscopic double and triple labelings demonstrated that all these peptides from median neurosecretory cells of the abdominal ganglia are first individually packaged into separate dense vesicles when leaving the trans-Golgi network [42]. These vesicles then fuse with each other in the cytoplasm, and become translucent on their way to the axon. Within the axon, all six abundant neuropeptides of the CAPA neurons are co-localized in translucent vesicles that are subsequently transported to the abdominal PSOs [42]. Thus, a highly regulated heterotypic fusion process seems to occur during the passage of the premature vesicles to the axon, which is corroborated by a considerable loss of electron-dense material. This is unusual since neuropeptides are typically packaged and transported within dense vesicles (*e.g.* [45]). However, also the CAPA peptides in the Va neurons of *D. melanogaster* are stored in translucent vesicles [31].

In interneurons of the brain of *P. americana*, the CAPA peptides are separately packed into dense vesicles. These vesicles do not fuse with each other [42]. Thus, cell-type-specific sorting mechanisms occur that differ between CAPA-expressing neurosecretory cells and interneurons. This offers the opportunity of a differential release of CAPA peptides from interneurons but not from the abdominal PSOs. Nevertheless, the fusion process of the dense vesicles in the neurosecretory CAPA neurons of the abdominal ganglia appears to be an evolutionary derived process with an as yet unclear physiological function.

Physiological actions of CAPA peptides

The main function of CAPA-PVKs appears to be the hormonal regulation of diuresis and the modulation of muscle contractions (Table 2). However, a systematic screen for CAPA-PVK actions using different bioassays for a single species has still to be performed. Current data suggest that the diuretic and myomodulatory functions of CAPA-PVKs are mutually exclusive (see Table 2). CAPA-PVKs do regulate either muscle contractions or diuresis in any of the insect species studied. It is noteworthy that CAPA-PVKs act as diuretic hormones in blood-feeding Diptera and as anti-diuretic hormones in blood-feeding Heteroptera. These insects are important vectors for widespread serious diseases such as malaria, Dengue fever and Chagas disease. After a blood meal, it is highly critical for these blood-feeding insects to get rid of the temporary excess of water to minimize predation risk [46]. To understand and manipulate CAPA-PVK signaling is thus potentially of medical importance.

In contrast to the CAPA-PVKs, effects and roles for the CAPA-PKs have not been investigated in detail. In flies, a role in pupariation and puparial tanning seems possible [47, 48]. In *P. americana*, CAPA-PK has virtually no effect in visceral muscle assays sensitive to other pyrokinins naturally occurring in the American cockroach [49]. Furthermore, the role of all CAPA peptides in interneurons is completely unknown.

An interesting finding with as yet unclear significance comes from microarray analysis of circadian gene expression in *D. melanogaster* [50]. Although *capa* is a non-cycling gene, its mRNA was highly up-regulated in *Clock* (*Clk*) mutant flies *versus* wild-type. Since CLK is a transcription factor central to the molecular clock machinery, this finding suggests that the expression of *capa* might be directly or indirectly affected by a circadian clock.

CAPA receptors

Four orthologs (CG8784, CG8795, CG9918, CG14575) of the neuromedin U (NMU) receptor were identified in the *D. melanogaster* genome shortly after its publication [16]. The consensus sequence for the vertebrate NMU receptors is LXXPRXa [51], and it was speculated that the different *Drosophila* PRXamides (pyrokinins encoded by *hugin* and *capa*, the two ecdysis-triggering hormones (ETHs) and the CAPA-PVKs) might be agonists of these receptors [16, 52]. Functional expression studies later identified the agonists of the NMU receptor orthologs [53–56], and demonstrated that the CAPA-PVKs and the CAPA-PK each have their own specific G-protein coupled receptor. In the expression systems used, CG14575 was only activated by the CAPA-PVKs, and not by CAPA-PK or other PRXamides [54, 56]. CG9918 was activated by

Table 2. Physiological effects of CAPA peptides.

Target organ	Peptide	Effect (effective concentration)	Species	Reference
Dorsal vessel	Pea-CAPA-PVK-1	Cardioacceleratory ($\geq 10^{-9}$ M)	*Periplaneta americana*	[1]
	Pea-CAPA-PVK-2	Cardioacceleratory ($\geq 3 \times 10^{-10}$ M)	*P. americana*	[71]
	Mas-CAPA-PVK-2	Cardioacceleratory ($\geq 10^{-9}$ M)	*Manduca sexta*	[11]
		No cardioacceleratory effect	*Drosophila melanogaster*	[72]
Closer muscle of segmental vessels	Pea-CAPA-PVK-1	Myotropic ($\geq 10^{-9}$ M)	*P. americana*	[35]
Antennal heart	Pea-CAPA-PVK-1	Cardioacceleratory ($\geq 10^{-8}$ M)	*P. americana*	Predel, unpubl.
	Pea-CAPA-PVK-2	Cardioacceleratory ($\geq 10^{-10}$ M)	*P. americana*	[71]
	Lem-CAPA-PVKs	Cardioacceleratory ($\geq 10^{-8}$ M)	*Leucophaea maderae*	[37]
Hyperneural muscle	Pea-CAPA-PVK-1	Myotropic ($\geq 10^{-10}$ M)	Blattid cockroaches	[1, 43]
		No myotropic effect	Non-blattid cockroaches	[43]
	Pea-CAPA-PVK-2	Myotropic ($\geq 5 \times 10^{-11}$ M)	*P. americana*	[2]
		Potentiation of glutamate-induced contraction (at subthreshold concentration)	*P. americana*	[58]
	Lem-CAPA-PVKs	No myotropic effect	*L. maderae*	[37]
Malphighian tubules	Pea-CAPA-PVK-1	Weak diuretic effect (tested at 10^{-7} M)	*Acheta domestica*	GM Coast, see [52]
	Pea-CAPA-PVK-2	Moderate diuretic effect (tested at 3×10^{-8} M)	*A. domestica*	GM Coast, see [52]
		Moderate diuretic effect (tested at 3×10^{-8} M)	*Musca domestica*	GM Coast, see [52]
	Mas-CAPA-PVK-2	Diuretic (in nanomolar range)	*D. melanogaster*	[73]
		Maximally diuretic	*M. domestica*	[74]
	Drm-CAPA-PVK-1,2	Diuretic ($EC_{50} = 10^{-8}$–10^{-7} M)	*D. melanogaster, Mosquitos*	[4] [66]
	Ang-CAPA-PVKs	Diuretic ($\geq 10^{-8}$ M)	*D. melanogaster, Mosquitos*	[66]
	Mud-CAPA-PVKs	Diuretic ($EC_{50} \geq 10^{-8}$ M)	*Musca domestica*	[24]
	Stc-CAPA-PVKs	Diuretic ($EC_{50} = 5 \times 10^{-9}$ M)	*Stomoxys calcitrans*	[33]
	Mas-CAPA-PVK-2	Anti-diuretic (in nanomolar range)	*Rhodnius prolixus*	[30, 67]
		Anti-diuretic ($EC_{50} = 8.5 \times 10^{-8}$ M)	*Tenebrio molitor*	[75]
		No diuretic effect	*Locusta migratoria*	[74]
		No diuretic effect	*M. sexta*	[76]
	Drm-CAPA-PVK-1, Ang-CAPA-PVKs	No diuretic effect	*Schistocerca gregaria*	[66]
	Drm-CAPA-PVK-1	No diuretic effect	*P. americana*	[66]
	Drm-CAPA-PVK-1, Ang-CAPA-PVKs	Diuretic effect	*Glossina morsitans*	[66]
Foregut	Pea-CAPA-PVK-2	Myotropic ($\geq 10^{-9}$ M)	*P. americana*	[71]
Hindgut	Pea-CAPA-PVK-2	No myotropic effect	*P. americana*	[71]
Fat body	Pea-CAPA-PVK-2	No effect on trehalose production at 10^{-6} M	*P. americana*	C. Wegener, J. Liewald, G. Wegener, see [52]
		No effect on fructose 2,6bisphosphate content at 10^{-6} M		
Oviduct	Pea-CAPA-PVK-2	No effect (at up to 10^{-7} M)	*P. americana*	[71]
Pupal tissue	Pea-CAPA-PVK-2 Neb-CAPA-PVKs	No or marginal acceleration of pupariation behavior (≥25 pmol injected/larva)	*Neobellieria bullata*	[48, 77]
	Neb-CAPA-PK	Acceleration of pupariation behavior (≥1 pmol injected/larva)	*N. bullata*	[48]
	Pea-CAPA-PVK-2	Marginal acceleration of puparial tanning (≥25 pmol injected/larva)	*N. bullata*	[77]
	Drm-CAPA-PVK-1	No acceleration of puparial tanning (>300 pmol injected/larva)	*N. bullata*	[47]
	Drm-CAPA-PK	Acceleration of puparial tanning (threshold 1 pmol injected/larva)	*N. bullata*	[47]
	Pea-CAPA-PK	Acceleration of puparial tanning (threshold 0.1 pmol injected/larva)	*N. bullata*	[47]

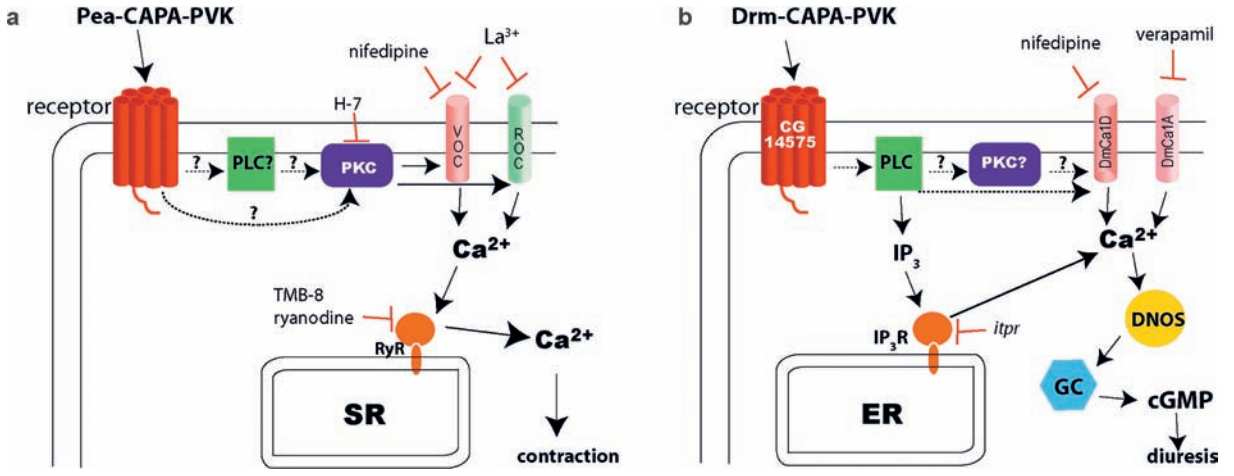

Figure 5. Mode of action of CAPA-PVKs on the cockroach hyperneural muscle (*a*), and the *Drosophila* Malpighian tubules (*b*). Similarities exist in the activation of Ca^{2+} influx through voltage-operated (L-type) Ca^{2+} channels in the plasma membrane (VOC; DmCa1D, DmCa1A), and the activation of Ca^{2+} release from intracellular stores. Ambiguous signal pathways are indicated by question marks. (*a*) Based on [58], (*b*) based on [62, 64]. Pharmacological blockers and mutations used to decipher the signaling pathways are shown in italics.

CAPA-PK and, about a potency less efficient, by the pyrokinins encoded by *hugin* and ETH-1. CAPA-PVKs and ETH-2 were inactive [53]. The other two NMU receptor orthologs CG8784 and CG8795 apparently are receptors for the pyrokinins encoded by *hugin* and could not be activated by CAPA peptides [55].

The finding of a *Drosophila* receptor specific for CAPA-PK and the low efficacy of this pyrokinin on other PRXa receptors of the fruit fly [54, 55, 57] indicate that CAPA-PK has function(s) distinct to other PRXamides such as the CAPA-PVKs, ETHs, and the *hugin* pyrokinins. A role of CAPA-PK distinct from that of the CAPA-PVKs in *Drosophila* is also suggested by the differential processing and localization of these peptides (see section '*Sequences and differential processing of CAPA peptides*').

The analysis of the tissue distribution of the CG14575 and CG9918 receptors, which remains an important task for the near future, will eventually unravel the full range of CAPA peptide target tissues in those species in which the receptor genes have been identified.

Myomodulatory mode of action of PVKs

The myomodulatory mode of action of CAPA-PVKs has been studied pharmacologically using the cockroach hyperneural muscle bioassay [58]. The direct activation of hyperneural muscle contractions by CAPA-PVKs are thought to enhance the distribution of the released CAPA peptides within the hemolymph. Muscle contractions induced by Pea-CAPA-PVK-2 were completely dependent on the influx of extracellular Ca^{2+}. Concomitantly, the main effect of CAPA-PVK-2 appeared to be the activation of a Ca^{2+} influx through both voltage-dependent nifedipine-sensitive and non-voltage (receptor)-operated Ca^{2+} channels in the sarcolemmal membrane. The resulting increase in the free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) triggered a Ca^{2+}-induced Ca^{2+} release via ryanodine receptors. The myotropic effect of CAPA-PVK-2 could be mimicked by TPA [a protein kinase C (PKC) activator and diacylglycerol mimetic], and partially inhibited by a PKC inhibitor. It might thus be possible that CAPA-PVK-2 activates phospholipase C or D, the enzymes responsible for the production of diacylglycerol. A contribution of cyclic nucleotides could be ruled out pharmacologically. A hypothetical model of the myotropic mode of action of CAPA-PVKs is shown in Figure 5.

Diuretic mode of action of CAPA-PVKs

A large body of literature exists on the actions of CAPA-PVKs on the Malpighian tubules (the insect 'kidneys'). Detailed work has been carried out for *D. melanogaster,* mostly by the group of Julian Dow. This group also demonstrated by a transcriptome analysis that the CAPA receptor CG14575 is up-regulated in the Malpighian tubules [59].

In the fruit fly, it could be shown that Mas-CAPA-PVK-2 and the Drm-CAPA-PVKs activate tubule fluid secretion by increasing $[Ca^{2+}]_i$ in the principal cells via (i) activation of Ca^{2+} influx through nifedipine- and verapamil-sensitive L-type voltage-dependent Ca^{2+} channels in the plasma membrane [4, 60, 61], and (ii) intracellular production of inositoltrisphosphate (IP_3) and activation of IP_3 receptors (IP_3R), resulting in Ca^{2+} release from the endoplasmic reticulum into the cytoplasm [62]. The CAPA-PVK-induced rise of $[Ca^{2+}]_i$ and the diuretic action is fully dependent on the presence of extracellular Ca^{2+} [60]. A reduction of CAPA-PVK-induced fluid secretion

and rise in $[Ca^{2+}]_i$ in *trpl* mutants also suggest an involvement of transient receptor potential-like (TRPL) channels [63]. The CAPA-PVK-induced rise in $[Ca^{2+}]_i$ triggers the activity of the Ca^{2+}-sensitive isoform of the nitric oxide (NO) synthase DNOS. Once activated, DNOS produces the messenger molecule NO, which leads to an increase of cGMP via a soluble guanylate cyclase and subsequently to enhanced fluid secretion [64] (Fig. 5). The finding that the Mas-CAPA-PVK-1 and Drm-CAPA-PVKs induced primary $[Ca^{2+}]_i$ increase in principal cells is not affected by inhibition of NO/cGMP [4, 65] indicates that the peptide-induced Ca^{2+} influx is upstream of NO/cGMP signaling.

In *Anopheles stephensi*, *Aedes aegypti*, and *Glossina morsitans*, Drm-CAPA-PVK-1 and Ang-CAPA-PVKs also increase fluid secretion by activation of the NO/cGMP pathway. This suggests a conserved mode of diuretic action of CAPA-PVKs throughout the Diptera [66]. The anti-diuretic effect of Mas-CAPA-PVK-2 on the Malpighian tubules of *Rhodnius* also seems to be linked to an increase in cGMP [30, 67]. Whether this increase is brought about by a prior activation of a Ca^{2+} influx has to be investigated.

Evolution of CAPA functions

A comparison of the mode of action of CAPA-PVKs on visceral muscles and Malpighian tubules shows that the activation of the CAPA-PVK receptor obviously results in the same primary signaling mechanism in both tissues, *i.e.* the activation or up-regulation of Ca^{2+} entry through voltage-dependent nifedipine-sensitive (L-type) Ca^{2+} channels and mobilization of internal Ca^{2+} release from the endoplasmic reticulum. The resulting increase in $[Ca^{2+}]_i$ then triggers different signal transduction cascades, resulting in different actions (see Fig. 5). This, in combination with the different effects listed in Table 2, suggests that the peptide-receptor interactions and primary signaling events rather than the specific physiological function(s) are evolutionarily conserved. In other words, the CAPA-PVK function(s) in a given species apparently depends on the receptor distribution and the downstream signaling cascades activated by the CAPA-PVK-induced increase in $[Ca^{2+}]_i$.

Release of CAPA peptides

Direct evidence that CAPA peptides are released as neurohormones comes from the blood-sucking bug *Rhodnius prolixus* [30]. In this bug, there is one pair of ventral median cells in each of the abdominal neuromeres 2–4 that shows strong PRXa-immunoreactivity. These cells [which seem to be homolog to the Va neurons of *D. melanogaster*, the NS-M4 neurons of *M. sexta*, and the VL neurons of *P. americana* (see section '*Localization and projections of CAPA neurons in the CNS*')] project into the second to fourth. abdominal nerve where they form extensive neurohemal sites. At 3–5 h following a blood meal, a strong decrease of PRXa immunoreactivity could be observed at these neurohemal sites. This fits well with the anti-diuretic function of CAPA-PVKs in *R. prolixus*, which is thought to terminate post-feeding diuresis induced by serotonin and other peptidergic neurohormones [30, 67].

Evidence for a neurohormone release of CAPA-PVKs from the median/transverse nerve system comes from *in vitro* experiments with isolated ventral nerve cords of the American cockroach. Using a specific ELISA against Pea-CAPA-PVK-1, a high-K^+ induced release of CAPA-PVK-1 could be demonstrated [43]. Interestingly, a prerequisite for a measurable release was that not only the abdominal PSOs, but also transverse nerve branches of the more distally situated hyperneural muscle (the cockroach ventral diaphragm) had to be present. This suggests that distal transverse nerve branches on the hyperneural muscle, and not the abdominal PSOs, are the main release site for CAPA peptides in *P. americana*. This suggestion is strengthened by the finding of electron-microscopic release profiles in these nerve branches on the muscle, whereas the CAPA-containing fibers in the center of the abdominal PSOs were devoid of ultrastructural correlates of exocytotic events [34]. Furthermore, the CAPA-peptide containing vesicles within the abdominal PSOs are not situated in the outer rim but in the median part, which is surrounded by a distinct glial sheath [34]. This renders a direct release of the Pea-CAPA peptides from PSOs very unlikely. Beaded arborizations typical of hormone release sites were also detected in ramifications of the abdominal transverse nerves in the ventral diaphragm of *M. sexta* [13]. In *D. melanogaster*, however, CAPA-containing vesicles were abundant below the neural sheath of the proximal part of abdominal transverse nerves, where also release profiles of CAPA-containing vesicles could be found [31]. Thus, in the fruit fly, the abdominal PSO apparently subserve a function both as storage and release site.

Conclusions and future perspectives

Insect CAPA peptides are intimately linked to the neurohemal system of the abdominal nervous system and obviously act as diuretic or myotropic hormones. Whereas considerable knowledge about their chemical structure, distribution, receptors and intracellular signaling cascades has accumulated, the full range of CAPA peptide actions has still to be defined by analyzing the tissue distribution of CAPA receptors. The finding of a differential processing and packaging of the CAPA precursor provides a good opportunity to study the poorly understood

mechanisms underlying peptide packaging and sorting in the genetically amenable fruit fly. Further open questions regard the regulation of CAPA expression, which might be influenced by a circadian clock, as well as the regulation of CAPA peptide release. Since sequence information for the CAPA peptides exists from an unusual variety of different insect species spanning different taxa, CAPA peptides hold a great potential for the reconstruction of phylogenetic relationships and peptide sequence differentiation during evolution [68].

Acknowledgements. Recent work contributed by the laboratories of the authors was funded by the Deutsche Forschungsgemeinschaft (PR 595/1-4; We 2652/2) and the Fonds der Chemischen Industrie (Sachkosten-Zuschuss für den Hochschullehrer-Nachwuchs to C. W.). We thank Susanne Neupert, Sascha Platz (Jena) and Anton Gorbashov (Marburg) for supporting the review with unpublished immunolabelings, and Wolf Huetteroth (Marburg) for critical reading of the manuscript.

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