available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/peptides

Structure-activity relationships for in vitro diuretic activity of CAP2b in the housefly

Ronald J. Nachman [a,*], *Geoffrey M. Coast* [b]

[a] *Areawide Pest Management Research, Southern Plains Agricultural Research Center, USDA, 2881 F/B Road, College Station, TX 77845, USA*

[b] *School of Biological and Chemical Sciences, Birkbeck (University of London), London WC1E 7HX, UK*

ARTICLE INFO

Article history:
Received 11 September 2006
Received in revised form
15 September 2006
Accepted 15 September 2006
Published on line 8 December 2006

Keywords:
CAPA gene
Periviscerokinin
CAP2b
Perisympathetic organs
Insect neuropeptide
Malpighian tubule

ABSTRACT

A series of truncated and Ala-replacement analogs of the peptide Manse-CAP2b (pELYAFPRV-NH_2) were assayed for diuretic activity on Malpighian tubules of the housefly *Musca domestica* (*M. domestica*). The C-terminal hexapeptide proved to be the active core, the minimum sequence required to retain significant diuretic activity. However, full activity required the C-terminal heptapeptide, which was equipotent with the most active of the native housefly CAP2b peptides. Replacement of Arg^7 and Val^8 with Ala led to inactivity and a large 70-fold drop in potency, respectively, indicating that these were critical residues. The Leu^2 was semicritical, where a six-fold loss in potency was observed. Conversely, the replacement of all other residues with Ala led to much smaller effects on potency and these positions were considered to be noncritical. This structure-activity relationship data can aid in the design of mimetic agonist/antagonist analogs of this diuretic peptide family with enhanced biostability and bioavailability, as tools for arthropod endocrinologists and as potential pest management agents capable of disrupting the water balance in pest flies.

1. Introduction

It is well established that neuropeptides are key factors in controlling primary urine production in Malpighian tubules (CRF-like peptides, insect kinins, CAPA peptides) and fluid reabsorption from the hindgut (ion transport peptide (ITP)) [1,29]. CAP2b (pELYAFPRVa), a CAPA peptide (also included in the literature as a member of the periviscerokinin (PVK) family), was first identified as a cardioacceleratory factor in the moth *Manduca sexta* [11]. CAPA peptides, including CAP2b, demonstrate diuretic effects on the Malpighian tubules of a number of flies, including the fruit fly *Drosophila melanogaster*, the housefly *Musca domestica* (*M. domestica*), and the stable fly *Stomoxys calcitrans* [1,2–5,9,10,15,18,21,22,24–26,28]. By contrast, bioassays on the bug *Rhodnius prolixus* with Manduca CAP2b [23,29] indicated that native CAPA peptides might reduce secretion by Malpighian tubules and thus show antidiuretic effects. These peptides are putative hormones typical of the neurosecretory system in the abdominal ventral nerve cord (VNC) of insects. The CAPA gene, which encodes for two or more CAP2b/PVKs and a single pyrokinin (PK), is known from a number of holometabolous insects, including *D. melanogaster* [12,13] and *M. sexta* [14]. Expressed in median neurosecretory neurons of abdominal ganglia, these putative peptide hormones may be released into the hemolymph via perisympathetic organs (PSOs) which are segmentally reiterated neurohemal organs of the abdominal ganglia [7,8]. Larval PSOs of cycloraphous Diptera, however, become incorporated into

* *Corresponding author*. Tel.: +1 979 260 9315; fax: +1 979 260 9377.
E-mail address: nachman@tamu.edu (R.J. Nachman).

0196-9781/$ – see front matter
doi:10.1016/j.peptides.2006.09.020

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
15 SEP 2006		**00-00-2006 to 00-00-2006**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Structure-activity relationships for in vitro diuretic activity of CAP2b in the housefly	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **U.S. Department of Agriculture,Areawide Pest Management Research,2881 F/B Road,College Station,TX,77845**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
see report

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**5**	

the dorsal ganglionic sheath [20,30,31] during metamorphosis. Direct analysis of abdominal dorsal sheath tissues via MALDI-TOF/TOF mass spectrometry led to the identification of CAP2b/PVK peptides native to the housefly *M. domestica* [18,26,32] and three other species of flies [18,19]. The housefly CAP2b/PVK sequences were recently determined to be AGGTSGLYAFPRVa (Musdo-CAP2b/PVK-1) and ASLFNAPRVa (Musdo-CAP2b/PVK-2) [18]. The octapeptide CAP2b shares a common C-terminal heptapeptide sequence (LYAFPRVa) with the most active housefly CAP2b sequence, Musdo-CAP2b-1, revealing the likely reason that the *Manduca* peptide demonstrates relatively potent diuretic activity in the heterologous housefly Malpighian tubule fluid secretion bioasssay.

In this study, we use Manduca CAP2b as a model of Musdo-CAP2b-1 and evaluate a series of truncated and Ala-replacement CAP2b analogs in a housefly Malpighian tubule fluid secretion bioassay to identify the active core and those residues most critical to activity.

2. Materials and methods

2.1. Insects

Housefly (*M. domestica*) larvae were obtained from a commercial fish bait supplier. They were held in the laboratory until they pupated and were then moved to a constant temperature room (28 °C; 12 L:12 D light:dark cycle). Flies that emerged within 12 h of one another were kept as separate age cohorts and provided with water and sucrose ad libidum. All of the experiments described in this paper used 3–4 day-old adult female flies.

2.2. Peptide synthesis

The CAP2b analogs were synthesized via Fmoc methodology on Rink Amide resin (Novabiochem, San Diego, CA) using Fmoc protected amino acids (Advanced Chemtech, Louisville, KY) on an ABI 433A peptide synthesizer (Applied Biosystems, Foster City, CA) under previously described conditions [17]. Crude products were purified on a Waters C_{18} Sep Pak cartridge and a Delta Pak C_{18} reverse-phase column (8 mm × 100 mm, 15 μm particle size, 100 Å pore size) on a Waters 510 HPLC controlled with a Millennium 2010 chromatography manager system (Waters, Milford, MA) with detection at 214 nm at ambient temperature. Solvent A = 0.1% aqueous trifluoroacetic acid (TFA); Solvent B = 80% aqueous acetonitrile containing 0.1% TFA. Conditions: initial solvent consisting of 20% B was followed by the Waters linear program to 100% B over 40 min; flow rate, 2.0 ml/min. Delta-Pak C-18 retention times: Manse-CAP2b[2-8], 9.0 min; Manse-CAP2b[3-8], 7.5 min; Manse-CAP2b[4-8], 5.0 min; Manse-CAP2b[5-8], 4.5 min; Manse-CAP2b[Ala^1], 10.5 min; Manse-CAP2b[Ala^2], 9.0 min; Manse-CAP2b[Ala^3], 4.5 min; Manse-CAP2b[Ala^5], 7.5 min; Manse-CAP2b[Ala^6], 12.5 min; Manse-CAP2b[Ala^7], 10.5 min; Manse-CAP2b[Ala^8], 9.0 min. The peptides were further purified on a Waters Protein Pak I125 column (7.8 mm × 300 mm)(Milligen Corp., Milford, MA). Conditions: flow rate: 2.0 ml/min; isocratic with solvent consisting of 80% acetonitrile made to 0.01% TFA. WatPro retention times: Manse-CAP2b[2-8], 7.0 min; Manse-CAP2b[3-8], 8.0 min; Manse-CAP2b[4-8], 11.5 min; Manse-CAP2b[5-8], 10.5 min; Manse-CAP2b[Ala^1], 15.5 min; Manse-CAP2b[Ala^2], 15.0 min; Manse-CAP2b[Ala^3], 13.5 min; Manse-CAP2b[Ala^5], 16.5 min; Manse-CAP2b[Ala^6], 11.0 min; Manse-CAP2b[Ala^7], 5.5 min; Manse-CAP2b[Ala^8], 15.0 min. Amino acid analysis was carried out under previously reported conditions [17] and used to quantify the peptide and to confirm identity, leading to the following analyses: Manse-CAP2b[2-8]: A[1.0], F[1.0], L[1.0], P[1.0], R[1.0], Y[1.0], V[1.0]; Manse-CAP2b[3-8]: A[1.0], F[1.0], P[1.1], R[1.0], Y[1.0], V[1.0]; Manse-CAP2b[4-8]: A[0.8], F[1.0], P[1.0], R[1.0], V[0.9]; Manse-CAP2b[5-8]: F[1.0], P[1.0], R[1.0], V[1.0]; Manse-CAP2b[Ala^1]: A[1.9], F[1.0], L[1.0], P[1.0], R[1.0], Y[1.1], V[0.9]; Manse-CAP2b[Ala^2]: A[2.0], E[0.9], F[1.0], P[0.9], R[1.0], Y[1.1], V[1.0]; Manse-CAP2b[Ala^3]: A[2.0], E[0.9], F[1.0], L[1.0], P[1.0], R[1.0], V[0.9]; Manse-CAP2b[Ala^5]: A[2.0], E[0.8], L[1.0], P[0.9], R[1.0], Y[1.2], V[0.9]; Manse-CAP2b[Ala^6]: A[2.0], E[1.0], F[1.0], L[0.9], R[1.0], Y[1.0], V[1.0]; Manse-CAP2b[Ala^7]: A[2.1], E[0.7], F[1.0], L[1.1], P[1.1], Y[0.8], V[1.0]; Manse-CAP2-b[Ala^8]: A[2.0], E[1.0], F[1.0], L[1.0], P[0.9], R[1.0], Y[1.0]. The identities of the peptide analogs were confirmed via MALDI-TOF-MS on a Kratos Kompact Probe MALDI-TOF MS machine (Kratos Analytical, Ltd., Manchester, UK) with the presence of the following molecular ions ($M + H^+$): Manse-CAP2b[2-8], 864.9 [$M + H^+$]; Manse-CAP2b[3-8], 751.5 [$M + H^+$]; Manse-CAP2b[4-8], 588.6 [$M + H^+$]; Manse-CAP2b[5-8], 517.6 [$M + H^+$]; Manse-CAP2-b[Ala^1], 936.8 [$M + H^+$]; Manse-CAP2b[Ala^2], 933.8 [$M + H^+$]; Manse-CAP2b[Ala^3], 883.9 [$M + H^+$]; Manse-CAP2b[Ala^5], 899.5 [$M + H^+$]; Manse-CAP2b[Ala^6], 949.3 [$M + H^+$]; Manse-CAP2-b[Ala^7], 890.6 [$M + H^+$]; Manse-CAP2b[Ala^8], 947.7 [$M + H^+$].

2.3. Isolated housefly Malpighian tubule preparations

Fluid secretion from isolated housefly Malpighian tubules was measured using the ''Ramsay assay'' as previously described [10]. Tubules were removed from 3 to 4 day post-emergent adult female flies. Flies were dissected under *Musca* saline [10] and both anterior and posterior tubules were transferred to small (10 μl) drops of bathing fluid (a 1:1 mixture of saline and Schneider's *Drosophila* medium) beneath water-saturated liquid paraffin in a Sylgard™ lined Petri dish. The tubules were allowed to equilibrate for 1 h before being challenged with test peptides. Urine escaped from the cut end of the tubule, which was pulled out into the liquid paraffin. Drops of urine were collected at 15 min intervals and their diameter (d) measured as they rested on the Sylgard base of the Petri dish using a Wild digital (MMS235) eyepiece micrometer. Urine volume was calculated as $\pi d^3/6$ and the rate of secretion obtained by dividing the secreted volume by the collection period. Data were normalized by expressing the increase in fluid secretion as a percentage of the response to 10 nM Musdo-K [16], which was added to all tubules at the end of each experiment. Dose-response curves were prepared using the computer program GraphPad Prism version 4.02 (GraphPad Software, San Diego, CA).

3. Results

A list of the fluid secretion activity of the two CAP2b/PVK peptides native to the housefly, Manse-CAP2b, Manse-CAP2b

Table 1 – Fluid secretion activity of CAP2b/PVK peptides and analogs on housefly (*M. domestica*) Malpighian tubules

Peptide	Sequence	Fluid secretion[a]	
		EC_{50} (nM) (95% CL)	Maximal response (%)
Musdo-CAP2b/PVK-1	AGGTSGLYAFPRVa	9 (7.2–11.1) [6]	100
Musdo-CAP2b/PVK-2	ASLFNAPRVa	102 (96–109) [6]	100
Manse-CAP2b	pELYAFPRVa	53 (39–72) [6]	
Manse-CAP2b[2-8]	LYAFPRVa	8 (3–22)	100
Manse-CAP2b[3-8]	YAFPRVa	428 (298–613)	100
Manse-CAP2b[4-8]	AFPRVa	*Tr*	
Manse-CAP2b[5-8]	FPRVa	*Tr*	
Manse-CAP2b[Ala^1]	**A**LYAFPRVa	23 (11–46)	100
Manse-CAP2b[Ala^2]	pE**A**YAFPRVa	373 (255–544)	80
Manse-CAP2b[Ala^3]	pEL**A**AFPRVa	33 (10–105)	100
Manse-CAP2b[Ala^5]	pELYA**A**PRVa	115 (27–485)	70
Manse-CAP2b[Ala^6]	pELYAF**A**RVa	89 (62–129)	100
Manse-CAP2b[Ala^7]	pELYAFP**A**Va	Inactive	
Manse-CAP2b[Ala^8]	pELYAFPR**A**a	3500 (2250–5500)	45

[a] Values are derived from dose–response curves based on data points derived from six replicates.

truncated analogs, and a Manse-CAP2b Ala scan series of analogs on housefly Malpighian tubules is presented in Table 1. The fluid secretion activity of Manduca CAP2b (EC_{50} = 53 nM) lies midway between that of the native Musdo-CAP2b/PVK-1 (EC_{50} = 9 nM) and Musdo-CAP2b/PVK-2 (EC_{50} = 102 nM); and thus can serve as a reasonable model for a structure-activity relationship study of CAP2b/PVK peptides in the housefly. Interestingly, removal of the pE residue on the N-terminus of Manse-CAP2b leads to the C-terminal heptapeptide sequence common to both Manse-CAP2b and Musdo-CAP2b-1; and the diuretic activity of this fragment Manse-CAP2b[2-8] (EC_{50} = 8 nM) matches that of Musdo-CAP2b-1 (EC_{50} = 9 nM). The increase in activity observed with the loss of pGlu^1 likely arises from the fact that it now more closely matches the sequence of the native peptide. Removal of another amino acid residue from the N-terminus, as in Manse-CAP2b[3-8] leads to a large drop of an order of magnitude from the parent Manduca CAP2b peptide. Removals of a third (Manse-CAP2b[4-8]) and fourth (Manse-CAP2b[5-8]) residue lead to analogs that retain only trace activity. In the case of the Ala scan series, replacement of the Arg^7 (Manse-CAP2b[Ala^7]) led to an analog with no significant activity, indicating that Arg^7 is therefore the most critical of residues. Replacement of Val^8 (Manse-CAP2b[Ala^8]; EC_{50} = 3500 nM) led to a large drop in activity (70-fold) in comparison with the parent peptide, and demonstrated retention of only 45% of the maximal response of Manse-CAP2b and the native housefly peptides. Residues pGlu^1 (Manse-CAP2b[Ala^1]; EC_{50} = 23 nM), Tyr^3 (Manse-CAP2b[Ala^3]; EC_{50} = 33 nM), Phe^5 (Manse-CAP2b[Ala^5]; EC_{50} = 115 nM), and Pro^6 (Manse-CAP2b[Ala^6]; EC_{50} = 89 nM) would appear not to be critical for housefly diuretic activity as the resulting analogs were either a little more or a little less active. While Manse-CAP2b[Ala^5] demonstrated a maximal response of 70%, this value is not statistically different from that of the parent peptide. Leu^2 would appear to be a semicritical residue, as Manse-CAP2b[Ala^2] (EC_{50} = 373 nM), proved to be about six-fold less active.

4. Discussion

The evaluation of the CAP2b sequence from *M. sexta*, Manse-CAP2b, in heterologous Malpighian tubule fluid secretion assays in fruit flies and houseflies [3,5,18] established that this family of peptides demonstrated diuretic activity in addition to cardioacceleratory activity. Seven of the eight residues of Manse-CAP2b are identical with the most potent of the native housefly CAP2b/PVK peptides, Musdo-CAP2b/PVK-1. As can be seen in Table 1, the EC_{50} for housefly Malpighian tubule fluid secretion of Manse-CAP2b is midway between that of the two CAP2b/PVK peptides native to the housefly; and thus can serve as a relevant model sequence to determine a structure-activity relationship profile. The C-terminal fragment Manse-CAP2b[3-8] retains a significant portion of the Malpighian tubule fluid secretion activity of the parent peptide (Table 1). The active core, the minimum sequence required to retain significant diuretic activity in the housefly, is therefore the C-terminal hexapeptide. However, full activity requires the C-terminal heptapeptide fragment (Manse-CAP2b[2-8], which is more active than the parent Manse-CAP2b and equipotent with the native Musdo-CAP2b-1 (Table 1). The results of the Ala-replacement series identify two residues, Arg^7 and Val^8, as critical for diuretic activity. The residue Leu^2 is semicritical; whereas all others are not critical. Not surprisingly, the critical (R^7 and V^8) and semicritical (L^2) residues are among the most conserved among the CAP2b/PVK family throughout arthropods. The most conserved residues among the CAP2b/PVKs are the C-terminal PRVa-amide as well as a Leu at position 7 (equivalent to Leu^2 in Manse-CAP2b) from the C-terminus [27]. The structure-activity relationship profile determined in this study can aid in the design and development of peptidomimetic agonist/antagonist analogs of this diuretic peptide family with enhanced biostability and bioavailability as tools for arthropod endocrinologists and as potential pest management agents capable of disrupting the water balance in target insects.

Acknowledgments

This study was supported with a Collaborative Research Grant (#LST.CLG.979226) from the North Atlantic Treaty Organization (NATO) (RJN) and a Binational Agricultural Research and Development Grant (BARD #IS3356-02) (RJN), and a grant provided by the USDA/DOD DWFP Research Initiative (#0500-32000-001-01R) (RJN). In addition, we acknowledge the capable technical assistance of Allison Strey, Pawel Zubrzak and Nan Pryor of the USDA Areawide Pest Management Research Unit, Southern Plains Agricultural Research Center.

REFERENCES

[1] Beyenbach KW. Transport mechanisms of diuresis in *Malpighian tubules* of insects. J Exp Biol 2003;206:3845–56.
[2] Cazzamali G, Torp M, Hauser F, Williamson M, Grimmelikhuijzen CJP. The *Drosophila* gene CG9918 codes for a pyrokinin-1 receptor. Biochem Biophys Res Commun 2005;335:14–9.
[3] Coast GM. The neuroendocrine regulation of salt and water balance in insects. Zoology (ZACS) 2001;103:179–88.
[4] Coast GM, Orchard I, Phillips JE, Schooley DA. Insect diuretic and antidiuretic hormones. In: Evans PD, editor. Advances in Insect Physiology, London: Academic Press; 2002. p. 279–409.
[5] Davies SA, Huesmann GR, Maddrell SH, O'Donnell MJ, Skaer NJV, Dow JAT, et al. CAP2b, a cardioacceleratory peptide, is present in *Drosophila* and stimulates fluid secretion via cGMP. Am J Physiol 1995;269:R1321–6.
[6] Davis NT, Homberg U, Dircksen H, Levine RB, Hildebrand JG. Crustacean cardioactive peptide-immunoreactive neurons in the hawkmoth *Manduca sexta* and changes in their immunoreactivity during postembryonic development. J Comp Neurol 1993;338:612–27.
[7] Eckert M, Herbert Z, Pollák E, Molnár L, Predel R. Identical cellular distribution of all abundant neuropeptides in the major abdominal neurohemal system of an insect (*Periplaneta americana*). J Comp Neurol 2002;452: 264–75.
[8] Eckert M, Predel R, Gundel M. Periviscerokinin-like immunoreactivity in the nervous system of the American cockroach. Cell Tissue Res 1999;295:159–70.
[9] Gäde G, Auerswald L, Predel R, Marco HG. Substrate usage and its regulation during flight and swimming in the backswimmer, *Notonecta glauca*. Physiol Entomol 2004;29:84–93.
[10] Holman GM, Nachman RJ, Coast GM. Isolation, characterization and biological activity of a diuretic myokinin neuropeptide from the housefly, *Musca domestica*. Peptides 1999;20:1–10.
[11] Huesmann GR, Cheung CC, Loi PK, Lee TD, Swiderek K, Tublitz N. Amino acid sequence of CAP2b, an insect cardioacceleretory peptide from the tobacco hornworm *Manduca sexta*. FEBS Lett 1995;371:311–4.
[12] Iversen A, Cazzamali G, Williamson M, Hauser F, Grimmelikhuijzen CJP. Molecular cloning and functional expression of a *Drosophila* receptor for the neuropeptides CAPA-1 and -2. Biochem Biophys Res Commun 2002;299:628–33.
[13] Kean L, Cazenave W, Costes L, Boderick KE, Graham S, Pollock VP, et al. Two nitridergic peptides are encoded by the gene capability in *Drosophila melanogaster*. Am J Physiol Regul Integr Comp Physiol 2002;282: 1297–307.
[14] Loi PK, Tublitz NJ. Sequence and expression of the CAPA/CAP2b gene in the tobacco hawkmoth, *Manduca sexta*.. J Exp Biol 2004;207:3681–91.
[15] Meng X, Wahlström G, Immonen T, Kolmer M, Tirronen M, Predel R, et al. The *Drosophila hugin* gene codes for myostimulatory and ecdysis modifying neuropeptides. Mech Dev 2002;117:5–13.
[16] Nachman RJ, Coast GM, Tichy SE, Russell DH, Miller JA, Predel R. Occurrence of insect kinins in the flesh fly, stable fly and horn fly–mass spectrometric identification from single nerves and diuretic activity. Peptides 2002;23: 1885–94.
[17] Nachman RJ, Isaac RE, Coast GM, Holman GM. Aib-containing analogues of the insect kinin neuropeptide family demonstrate resistance to an insect angiotensin-converting enzyme and potent diuretic activity. Peptides 1997;18:53–7.
[18] Nachman RJ, Russell WK, Coast GM, Russell DH, Predel R. Mass spectrometric assignment of Leu/Ile in neuropeptides from single neurohemal organ preparations of insects. Peptides 2005;26:2151–6.
[19] Nachman RJ, Russell WK, Coast GM, Russell DH, Miller JA, Predel R. Identification of PVK/CAP2b neuropeptides from single neurohemal organ preparations of the stable fly and horn fly via MALDI-TOF/TOF tandem mass spectrometry. Peptides 2006;27:521–6.
[20] Nassel DR, Ohlsson LG, Cantera R. Metamorphosis of identified neurons innervating thoracic neurohemal organs in the blowfly—transformation of cholecystokinin like immunoreactive neurons. J Comp Neurol 1988;267:343–56.
[21] Neupert S, Predel R, Russell WK, Davies R, Pietrantonio PV, Nachman RJ. Identification of tick periviscerokinin, the first neurohormone of Ixodidae: single cell analysis by means of MALDI-TOF/TOF mass spectrometry. Biochem Biophys Res Commun 2005;338:1860–4.
[22] O'Brien MA, Taghert PH. A peritracheal neuropeptide system in insects: release of myomodulin-like peptides and ecdysis. J Exp Biol 1998;201:193–209.
[23] Paluzzi J-P, Orchard I. Distribution, activity and evidence for the release of an anti-diuretic peptide in the kissing bug *Rhodnius prolixus*. J Exp Biol 2006;209:907–15.
[24] Park Y, Kim YJ, Adams ME. Identification of G protein-coupled receptors for Drosophila PRXamide peptides, CCAP, corazonin, and AKH supports a theory of ligand-receptor coevolution. Proc Natl Acad Sci USA 2002;99:11423–8.
[25] Predel R, Kellner R, Baggerman G, Steinmetzer T, Schoofs L. Identification of novel periviscerokinins from single neurohaemal release sites in insects MS/MS fragmentation complemented by Edman degradation. Eur J Biochem 2000;267:3869–74.
[26] Predel R, Russell WK, Tichy SE, Russell DH, Nachman RJ. Capa-gene products in *Musca domestica* and *Neobellieria bullata*. Peptides 2003;24:1487–91.
[27] Predel R, Wegener C. Periviscerokinins/CAPA gene related peptides in arthropods. CMLS 2006;63:2477–90.
[28] Predel R, Wegener C, Russell WK, Tichy SE, Russell DH, Nachman RJ. Peptidomics of CNS-associated neurohemal systems of adult *Drosophila melanogaster*: a mass spectrometric survey of peptides from individual flies. J Comp Neurol 2004;474:379–92.
[29] Quinlan MC, Tublitz NJ, O'Donnell MJ. Anti-diuresis in the blood-feeding insect *Rhodnius prolixus* Stål: the peptide CAP2b and cyclic GMP inhibit *Malpighian tubule* fluid secretion. J Exp Biol 1997;200:2363–7.
[30] Santos JG, Pollak E, Rexer KH, Molnar L, Wegener C. Morphology and metamorphosis of the peptidergic Va neurons and the median nerve system of the fruitfly, *Drosophila melanogaster*. Cell Tissue Res 2006;326:187–99.

[31] Sato Y, Oguchi M, Menjo N, Imai K, Saito H, Ikeda M, et al. Precursor polyprotein for multiple neuropeptides secreted from the suboesophageal ganglion of the silkworm *Bombyx mori*: characterization of the cDNA encoding the diapause hormone precursor and identification of additional peptides. Proc Natl Acad Sci USA 1993;90:3251–5.

[32] Wegener C, Reinl T, Jänsch L, Predel R. Direct mass spectrometric peptide profiling and fragmentation of larval peptide hormone release sites in *Drosophila melanogaster* reveals tagma-specific peptide expression and differential processing. J Neurochem 2006;96: 1362–74.