Insect Biochemistry and Molecular Biology 39 (2009) 491–507

Contents lists available at ScienceDirect

Insect Biochemistry and Molecular Biology

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

Peptidomic survey of the locust neuroendocrine system

Elke Clynen*, Liliane Schoofs

Research Group Functional Genomics and Proteomics, K.U. Leuven, Naamsestraat 59, 3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 20 June 2007
Received in revised form
2 June 2009
Accepted 4 June 2009

Keywords:
Insect
Locusta migratoria
Mass spectrometry
Neuropeptides
Neuropeptidomics
Schistocerca gregaria

ABSTRACT

Neuropeptides are important controlling agents in animal physiology. In order to understand their role and the ways in which neuropeptides behave and interact with one another, information on their time and sites of expression is required. We here used a combination of MALDI-TOF and ESI-Q-TOF mass spectrometry to make an inventory of the peptidome of different parts (ganglia and nerves) of the central nervous system from the desert locust *Schistocerca gregaria* and the African migratory locust *Locusta migratoria*. This way, we analysed the brain, suboesophageal ganglion, retrocerebral complex, stomatogastric nervous system, thoracic ganglia, abdominal ganglia and abdominal neurohemal organs. The result is an overview of the distribution of sixteen neuropeptide families, *i.e.* pyrokinins, pyrokinin-like peptides, periviscerokinins, tachykinins, allatotropin, accessory gland myotropin, FLRFamide, (short) neuropeptide F, allatostatins, insulin-related peptide co-peptide, ion-transport peptide co-peptide, corazonin, sulfakinin, orcokinin, hypertrehalosaemic hormone and adipokinetic hormones (joining peptides) throughout the locust neuroendocrine system.

1. Introduction

Neuronal cells of the brain communicate with each other and with peripheral organs by the use of chemical messengers, *i.e.* neurotransmitters and neuropeptides. These signalling messengers and their (G-protein coupled) receptors occupy the highest hierarchic position in the physiology of animals and orchestrate many biological processes and behaviours including reproduction, metamorphosis, sugar and fat metabolism, regulation of body pigmentation, water balance, food intake, digestion, locomotion, *etc.* In comparison to vertebrates, the insect's nervous system is far more de-centralised. Most behaviour (feeding, locomotion, mating, *etc.*) is integrated and controlled by segmental ganglia instead of the brain. This explains the multitude of neuropeptides present in the different ganglia. In some cases, the brain may stimulate or inhibit activity in the segmental ganglia.

We performed a neuropeptidomic study on the desert locust (*Schistocerca gregaria*) and the African migratory locust (*Locusta migratoria*), two notorious pest insects. Plagues of these species have threatened agricultural production in Africa, the Middle East and Asia for centuries. During the most recent plague of 2004 the world's attention was again drawn to the severe consequences of locust swarms for the environment, economy and human health, causing famine and asthma epidemics. Both species show the interesting phenomenon of phase polymorphism, meaning that they can occur in different phases, ranging from harmless solitary individuals to a gregarious form that has the tendency to group together leading to swarm formation. The underlying mechanisms of this phase transition are still poorly understood, however, there are several indications that neuropeptides play an important role (Ayali et al., 1996; Clynen et al., 2002; Hoste et al., 2002).

In both *L. migratoria* and *S. gregaria* approximately 50 (neuro)-peptides have been biochemically characterised (Table 1). For most of these peptides there are little or no indications towards their *in vivo* role. In general, compared to mammals, the biological significance of invertebrate (neuro)peptides is little understood. Most of the structurally characterised neuropeptides have been isolated based on their effect on visceral muscle contraction *in vitro*, which not necessarily means that these peptides are involved in the regulation of

Abbreviations: a, amide; AG-MT, accessory gland myotropin; AKH, adipokinetic hormone; APRP, AKH-precursor related peptide; AST, allatostatin; AT, allatotropin; capa, capability; CCAP, crustacean cardioactive peptide; CNS, central nervous system; CRZ, corazonin; Da, Dalton; ESI-Q-TOF, electrospray ionisation quadrupole time of flight; EST, expressed sequence tag; FA, formic acid; HrTH, hypertrehalosaemic hormone; IRP, insulin-related peptide; ITP(L), ion-transport peptide (like); inv-TK, invertebrate tachykinin; JP, joining peptide; Lom, *Locusta migratoria*; M, molecular weight; MALDI-TOF, matrix-assisted laser desorption ionisation time of flight; MIP, myoinhibiting peptide; MS, mass spectrometry; MS–MS, tandem mass spectrometry; *m/z*, mass to charge ratio; NPY, neuropeptide tyrosine; OK, orcokinin; PK, pyrokinin; PKL, pyrokinin-like peptide; pQ, pyroglutamic acid; PVK, periviscerokinin; Scg, *Schistocerca gregaria*; SK, sulfakinin; sNPF, short neuropeptide phenylalanine; TFA, trifluoroacetic acid; TK, tachykinin; TKRP, tachykinin-related peptide.

* Corresponding author at: Zoological Institute, Naamsestraat 59, 3000 Leuven, Belgium. Tel.: +32 16 324260; fax: +32 16 323902.
E-mail address: elke.clynen@bio.kuleuven.be (E. Clynen).

0965-1748/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ibmb.2009.06.001

Table 1
List of neuropeptides (<3000 Da) identified in *Locusta migratoria* and *Schistocerca gregaria*. Mono-isotopic masses (M) are given in dalton (Da); disulphide bridges are taken into account (one S–S = M – 2 Da); pQ = pyroglutamic acid, a = amide, X_1 = leucine or isoleucine, X_2 = lysine or glutamine.

***L. migratoria* neuropeptide**	**Amino acid sequence**	**M (Da)**	**References**
Tachykinins			
Lom-TK-1	GPSGFYGVRa	937.5	Schoofs et al., 1990d
Lom-TK-2	APLSGFYGVRa	1064.6	Schoofs et al., 1990d
Lom-TK-3	APQAGFYGVRa	1063.6	Schoofs et al., 1990b
Lom-TK-4	APSLGFHGVRa	1038.6	Schoofs et al., 1990b
Lom-TK-5	APMRGFQSVRa	1146.6	Clynen et al., 2006
Pyrokinins			
Lom-PK-1	DSGDEWPQQPFVPRLa	1768.9	Clynen et al., 2003a; Schoofs et al., 1991a
Lom-PK-2	pQSVPTFTPRLa	1126.6	Schoofs et al., 1993a
Lom-PK-3	GAVPAAQFSPRLa	1211.7	Clynen et al., 2003a; Schoofs et al., 1990e
Lom-PK-4	EGDFTPRLa	932.5	Clynen et al., 2003a; Schoofs et al., 1990c
Lom-PK-5	RQQPFVPRLa	1138.7	Clynen et al., 2003a; Schoofs et al., 1992a
Lom-PK-6	RLHQNGMPFSPRLa	1550.8	Clynen et al., 2003a; Schoofs et al., 1992a
Lom-PK-7	X_1HX_2NGMPFSPRX_1a	1394.7	Clynen et al., 2003a
Lom-PK-8	pQX_2PFVPRX_1a	965.5	Clynen et al., 2003a
Lom-PK-9	X_2PFVPRX_1a	854.5	Clynen et al., 2003a
Lom-PK-10	VX_1AGPFVPRX_1a	1066.7	Clynen et al., 2003a
Pyrokinin-like peptide			
Lom-PKL-1	TSSLFPHPRLa	1152.6	Clynen et al., 2003a,c
Sulfakinin			
Lom-SK	pQLASDDY(SO_3)GHMRFa	1500.6	Schoofs et al., 1990a
Allatotropin			
Lom-AT	GFKNVALSTARGFa	1365.8	Paemen et al., 1991b
Accessory gland myotropin			
Lom-AG-MT-2	AHRFAAEDFGALDTA	1590.7	Paemen et al., 1991a
Crustacean cardioactive peptide			
Lom-CCAP	PFCNAFTGCa	955.4	Veelaert et al., 1997a
Ecdysis-triggering hormones			
Lom-ETH-1	SDFFLKTAKSVPRIa	1606.9	Clynen et al., 2006
Lom-ETH-2	SDLFLKSAKSVPRIa	1558.9	Clynen et al., 2006
[His^7]-corazonin			
Lom-CRZ	pQTFQYSHGWTNa	1349.6	Tawfik et al., 1999
Proctolin			
Lom-PROC	RYLPT	648.4	Schoofs et al., 1993d
Periviscerokinins			
Lom-PVK-1	AAGLFQFPRVa	1103.6	Predel and Gäde, 2002
Lom-PVK-2	GLLAFPRVa	870.5	Clynen et al., 2003c
Lom-PVK-3	DGGEPAAPLWFGPRVa	1566.8	Clynen et al., 2003c
FLRFamides/myosuppressins			
Lom-FLRFa-1	PDVDHVFLRFa	1242.7	Schoofs et al., 1993b
Lom-FLRFa-2	ADVGHVFLRFa	1158.6	Peeff et al., 1994
Allatostatins-A			
Lom-AST-4	GPRTYSFGX$_1$a	995.5	This paper
Lom-AST-5	GRX$_1$YSFGX$_1$a	910.5	This paper
Lom-AST-9	PX$_1$YGGDRRFSFGX$_1$a	1482.8	This paper
Lom-AST-10	APAEHRFSFGX$_1$a	1229.6	This paper
Allatostatin B/myoinhibiting peptide			
Lom-MIP	AWQDLNAGWa	1058.5	Schoofs et al., 1991b
Allatostatins C			
Lom-AST-C	SYWKQCAFNAVSCFa	1649.7	Veenstra, 2009
Lom-AST-CC	(p)QLRYYRCYFNPISCF	1969.9/1952.9	Clynen et al., 2006; Veenstra, 2009
Kinin			
Lom-KIN	AFSSWGa	652.3	Schoofs et al., 1992b
Arginine vasopressin-like diuretic hormones (inotocins)			
Lom-F-1	CLITNCPRGa CLITNCPRGa CLITNCPRGa	972.5	Proux et al., 1987
Lom-F-2		1944.9	Schooley et al., 1987
Insulin-related peptide			
Lom-IRP co-peptide	pQSDLFLLSPK	1129.6	Clynen et al., 2003b