Reprinted from Neuropeptides
Volume 897 of the *Annals of the New York Academy of Sciences*

The Kinin Peptide Family in Invertebrates

PIETER TORFS,[a,d] JULIE NIETO,[a,b] DIRK VEELAERT,[a] DORIS BOON,[a] GEERT VAN DE WATER,[a] ETIENNE WAELKENS,[c] RITA DERUA,[c] JORGE CALDERÓN,[b] ARNOLD DE LOOF,[a] AND LILIANE SCHOOFS[a]

[a]*Zoological Institute, Katholieke Universiteit Leuven, Naamsestraat 59, B-3000 Leuven, Belgium*

[b]*Centro Nacional de Acuicultura e Investigaciones Marinas, P.O. Box 09-01-4519, Guayaquil, Ecuador*

[c]*Laboratory of Biochemistry, Katholieke Universiteit Leuven, Herestraat 41, B-3000 Leuven, Belgium*

ABSTRACT: Kinins comprise a family of peptides that were first found in the central nervous system of insects and recently also in mollusks and crustaceans. After the isolation of the first members of the kinin family, the leukokinins from *Leucophaea madera,* leukokinin-related peptides were found in the cricket *Acheta domesticus* and the locust *Locusta* migratoria, all through their ability to induce *Leucophueu muderue* hindgut contraction. Subsequently, kinins were found in the mosquitoes *Culex salinarius* and *Aedes aegypti* and in the earworm *Helicoverpa zea.* The first noninsect member of this family was isolated from a mollusk, the pond snail *Lymnaea stagnalis.* Most recently our group has isolated the first kinins from crustaceans. Six kinins were isolated from the white shrimp *Penaeus vunnumei.* To date, 35 members of this family have been isolated. The first relatively small family of insect kinins has grown into an expanding and rather large family with members in insects, crustaceans, and mollusks. In this paper we discuss the kinin family in terms of method of isolation, structure, *in vitro* and *in* vivo activity, distribution, receptors, and signal transduction. We will compare the crustacean and insect members of the kinin family, using the data available on crustacea.

ISOLATION AND IDENTIFICATION OF KININS

The first kinins were isolated from the cockroach *Leucophaea maderae* through their ability to induce cockroach hindgut contraction.[1–4] Subsequently, leukokinin-related peptides were found in the cricket *Acheta domesticus*[5] and the locust *Locusta migratoria,*' all through the use of the myotropic bioassay in combination with high performance liquid chromatography (HPLC). In 1993, the isolation of leukokinin-like peptides from the mosquito *Culex salinarius* was reported.[7,8] These peptides were isolated on the basis of the myotropic *Leucophaea* hindgut assay and a transepithelial voltage assay that used isolated mosquito Malpighian tubules from *Aedes aegypti.* For the isolation of the aedeskinins, Veenstra used a competitive

[d]Corresponding author: Tel.: +32 16 32 42 60; fax: +32 16 32 39 02.
e-mail: Pieter.Torfs@bio.kuleuven.ac.be

ELISA based on an antiserum most sensitive to leukokinins IV and VI as a bioassay in the HPLC purification.[9] The use of an antiserum sensitive to leukokinins IV and VI was motivated by the fact that these particular leukokinins were most active on the Malpighian tubules of ***Aedes aegypti.*** The three kinins from the abdominal ventral nerve cord of ***Helicoverpa zea*** were isolated through their stimulatory effect of the secretion by the Malpighian tubules of ***Manduca sexta.***[10] This diuretic effect is, in addition to the myotropic effect, a second effect that members of the kinin family induce ***in vitro.*** HPLC fractions were screened for diuretic activity using a modification of the "Ramsay assay."" Eight kinins (perikinins I to V and three kinins identical to leukokinins VII and VIII of ***Leucophaea maderae*** and locustakinin of Locusta migratoria) isolated from the American cockroach ***Periplaneta*** americana were purified by means of HPLC and a bioassay very similar to the one used for the isolation of the leuco-, acheta- and locustakinins. The use of the hindgut of *Periplaneta* instead of ***Leucophaea*** was the major difference.[12] The only nonarthropod member of the kinin family known to date was isolated from the pond snail ***Lymnaea stagnalis*** (Mollusca).[13] Cox and colleagues cloned a G-protein-coupled neuropeptide receptor. After transfection and functional expression of this receptor in Chinese hamster ovary (CHO) cells, the receptor-expressing ce11 line was used to assay for ligands by monitoring HPLC fractions originating from ***Lymnaea*** central nervous system (CNS). The endogenous ligand was identified by looking for changes in the leve1 of intracellular Ca^{2+}. The peptide isolated belonged to the kinin family and was therefore designated as lymnokinin.

Most recently, six members of the kinin family were isolated from the white shrimp ***Penaeus vannamei,*** the first crustacean members of this family to date.[14,15] Central nervous systems of 3,500 adult female white shrimps (*Penaeus **vannamei)*** were dissected and extracted in a methanolic solution and prepurified on Megabond Elute cartridges. Further purification was performed by a combination of reversed- and normal-phase HPLC, and the various fractions containing myotropic compounds were identified by the cockroach hindgut contraction assay. The effect of the fractions on the spontaneous contractile activity of the isolated hindgut was monitored. A myotropic substance can have an effect on the tonus, frequency, and amplitude of the spontaneous contractions. The digestive tract of ***Leucophaea*** *maderae* was carefully removed from the cockroach, al1 adhering tissues such as fat body, trachea, and Malpighian tubules were trimmed off, the hindgut was tied at the junction to the midgut, and the latter plus the foregut was cut off. The posterior end of the rectum was tied with thread as well, and then the whole preparation was suspended in a muscle chamber (3-mL plastic disposable syringe barrel) filled with a saline solution. The preparation was attached to a transducer, which displayed the signa1 to a recorder. After one hour of equilibration, the pattern of spontaneous contractions was relatively constant and the preparation could be used the whole day. Fractions could be monitored by drying and adding them to the bioassay chamber after dissolving them into the saline and observing the response on the recorder.[16] After each chromatographic separation, the fractions were monitored by the ***Leucophaea*** hindgut muscle preparation as described above. Therefore, the same cockroach hindgut contraction assay as prepared for the isolation of leuco-, acheta- and locustakinin was used, indicating that this assay is very useful as a detection system for crustacean neuropeptides.

TABLE 1. Amino acid sequences of kinins isolated from different invertebrate species

Phylum/Classis	Species	Peptide name	Amino acid sequence
Arthropoda/Crustacea	*Penaeus vannamei*[14,15]	Pev-kinin 1	ASFSPWGa
		Pev-kinin 2	DFSAWAa
		Pev-kinin 3	PAFSPWGa
		Pev-kinin 4	VAFSPWGa
		Pev-kinin 5	pEAFSPWAa
		Pev-kinin 6	AFSPWAa
Arthropoda/Insecta	*Leucophaea maderae*[1-4]	Leucokinin 1	DPAFNSWGa
		Leucokinin II	DPGFSSWGa
		Leucokinin III	DQGFNS WGa
		Leucokinin IV	DASFHSWGa
		Leucokinin V	GSGFSSWGa
		Leucokinin VI	pESSFHSWGa
		Leucokinin VII	DPAFSSWGa
		Leucokinin VIII	GADFYSWGa
	Periplaneta americana"	Perikinin 1	RPSFASWGa
		Perikinin II	DASFSSWGa
		Perikinin III	DPSFNSWGa
		Perikinin IV	GAQFSSWGa
		Perikinin-V	SPAFNSWGa
	Locusta migratoria[6]	Locustakinin	AFSSWGa
	Acheta domesticus[5]	Achetakinin I	SGADFYPWGa
		Achetakinin II	AYFSPWGa
		Achetakinin III	ALPFSPWGa
		Achetakinin IV	NFKFNPWGa
		Achetakinin V	AFHSWGa
	Culex salinarius[7,8]	CDP 1	NPFHSWGa
		CDP II	NNANVFYPWGa
		CDP III	TKYVSKQFFSWGa
	Aedes aegypti[9]	Aedeskinin 1	NSKYVSKQKFYSWGa
		Aedeskinin II	NPFHAYFSAWGa
		Aedeskinin 111	NNPNVFYPWGa
	Helicoverpa zea[10]	Helicokinin 1	YFSPWGa
		Helicokinin II	VRFSPWGa
		Helicokinin III	KVKFSAWGa
Mollusca/Gastropoda	*Lymnaea stagnalis*[13]	Lymnokinin	PSFHSWSa

NOTE: Amina acids that are conserved throughout the family are in boldface.

STRUCTURAL FEATURES OF THE KININ FAMILY ON THE PROTEIN AND cDNA LEVEL

The insect kinins all share the C-terminal pentapeptide sequence FX^1X^2WG-amide where X^1 is F, H, N, S or Y and X^2 is A, P or S (TABLE 1). The core pentapeptide FYSWG-amide is as active as the parent molecule (leukokinin VIII) in cockroach hindgut myotropic[17] and cricket Malpighian tubule secretion[18] assays, so the core pentapeptide is all that is needed for activity. The negatively charged free acid

is inactive in the myotropic assay [19] and is 50,000-fold less potent in the cricket Malpighian tubule secretion assay compared with most amidated natural insect kinins.[20] The negatively charged C-terminal acid group prevents the ligand from interacting with the receptor sites, so the amidated carboxyl terminus seems to be required for activity.[21] Leukokinin VI is the only insect kinin that is blocked at the N-terminal by a pyroglutamyl residue. The blocked leukokinin exhibits similar *in vitro* activities in the myotropic *Leucophaea* hindgut assay[22] and the transepithelial voltage assay [23] as do other leukokinins, which indicates that the N-terminal pyroglutamyl has no obvious influence on activity. Structure-activity studies on leukokinin VIII revealed that when F^1 or W^4 of the core pentapeptide is replaced by A, the analogues are inactive in the *Leucophaea* hindgut assay. Analogues where W in position 4 is replaced by F of position 1, and vice versa, are as potent as the parent molecule; thus, aromatic groups are needed at these positions. [17] When interacting with receptors, the core sequence forms a β-turn conformation bringing together F and W.[24] A-analogues at other positions are reasonably active in the myotropic assay.

The observations in *Lymnaea stagnalis* indicate that the C-terminal residue is not that critica1 for binding to the *Lymnaea* kinin receptor.[13] The endogenous ligand for this receptor, lymnokinin, has a C-terminal amidated serine residue. A synthetic kinin analogue with the consensus C-terminal amidated glycine residue exhibits a similar potency as lymnokinin on the intracellular calcium concentration in CH0 cells expressing the *Lymnaea* kinin receptor.

The Pev-kinins 1, 3, and 4 confirm the C-terminal consensus sequence.[14,15] Achetakinins II and III and helicokinin I display the strongest sequence similarities towards these Pev-kinins. In Pev-kinins 2, 5, and 6, however, the C-terminal amino acid is an alanine residue instead of the typical glycine. The threshold concentration for myotropic activity on the hindgut of the synthetic peptides is in the same range for the kinins ending in A-amide as for the kinins ending in the typical G-amide. Both Pev-kinins 1 and 2 are active on fluid secretion in the Malpighian tubule of crickets,[14] providing evidence that the C-terminal amino acid can be replaced by an alanine without substantial loss of activity. Substitution of alanine in this position is possible with a single base mutation. Because al1 the other arthropod kinins have the typical WG-amide carboxyterminal sequence, Pev-kinins 2, 5, and 6 make up a subfamily of the kinin superfamily. Pev-kinins 5 and 6 are identical except for the pyroglutamyl residue in Pev-kinin 5, which is blocking the N-terminus. Together with leukokinin VI, Pev-kinin 5 is the only N-terminally blocked member of the kinin family. Pev-kinins 5 and 6 display similar threshold concentrations on the *Leucophaea* hindgut assay; hence the N-terminal pyroglutamyl has no influence on myotropic activity. The pyroglutamyl residue would render these peptides more resistant to proteolytic degradation.

On the cDNA level, only the sequence encoding the *Aedes* kinins is known.[25] A cDNA encoding Aedes preprokinin was cloned from a cDNA library of the mosquito *Aedes aegypti*. This preprokinin encodes a putative signa1 peptide of 18 amino acid residues and a 210-amino acid residue prokinin. The latter encodes one copy each of the *Aedes* kinins 1, II, and III. No other possible products of this preprokinin have significant sequence similarity with any described protein or peptide. No other cDNA sequences for kinins are known in any other invertebrate species.

ACTIVITY OF THE ARTHROPOD KININ FAMILY *IN VITRO* AND IN *VIVO*

The insect members of this peptide family are associated with myotropic and diuretic activity. Leukokinins stimulate the visceral muscles of the hindgut of *Leucophaea* most potently.[22,26] Their effects on the foregut and oviduct are significantly lower, and the heart does not respond to any of the leukokinins. These leukokinins also depolarize the transepithelial voltage and stimulate fluid secretion in isolated Malpighian tubules of the mosquito *Aedes aegypti*.[23] Evidence exists that leukokinins increase the Cl^- permeability of the tubule wall, which results in an increase of the availability of Cl^- for secretion with Na+, K^+, and water. Locustakinin increases fluid secretion in Malpighian tubules and stimulates water reabsorption by the rectum of *Locusta migratoria*.[27] Locustakinin does not have a myotropic effect on the hindgut and oviduct of *Locusta migratoria*,[6] however, it stimulates hindgut muscle contractions in *Leucophaea maderae* displaying a threshold concentration of 9×10^{-12} M. In *L. migratoria*, locustakinin has a stimulatory *in vitro* effect on the spontaneous contractile activity of visceral muscle fibers associated with Malpighian tubules.[28] Locustakinin exerts its effect at concentrations that increase urine production; however, this muscle activity is not essential for diuresis. When the effects of achetakinins on the Malpighian tubules of *Acheta domesticus* are investigated, two effects occur: (1) an increase in the frequency of tubule writhing movements and (2) a stimulation of fluid secretion.[28] These two actions are thought to be independent. Other diuretic non-kinin peptides stimulate adenylate cyclase activity, whereas achetakinins appear to act independent of cyclic AMP (cAMP) as an intracellular second messenger. The effect of cAMP and achetakinins on tubule fluid secretion is synergistic, so they seem to stimulate different components of the secretion mechanism. The concentration necessary for threshold diuretic responses ranges from 4×10^{-12} to 7.2×10^{-11} M.[18] The culekinin depolarizing peptides (CDP) both stimulate depolarization of the transepithelial potential in Malpighian tubules of the mosquito with a threshold concentration of 8×10^{-11} M and increase the contraction rate of the *Leucophaea* hindgut.[8] Kinins from *Aedes aegypti* all depolarize the transepithelial voltage of the Malpighian tubules.[9] They also increase the rate of fluid secretion by the Malpighian tubules except for *Aedes* kinin II.[25] For the *Aedes* kinins strong depolarizing activity is not correlated with strong effects on fluid secretion. *Aedes* kinin I is a potent depolarizer but has little effect on fluid secretion of Malpighian tubules, whereas *Aedes* kinin III is a limited depolarizer and a potent inducer of fluid secretion. *Aedes* kinins have a stimulatory effect on contractions of *Aedes aegypti* hindgut. They mainly have an effect on the frequency of the contractions, and this effect proved to be reversible with a threshold concentration of 10^{-8} M. The helicokinins are potent stimulators of secretion by the Malpighian tubules of *Manduca sexta*.[10] All helicokinins stimulate fluid secretion from *Manduca* tubules at concentrations at or below 6.7×10^{-12} M. This makes them more potent stimulators of *Manduca sexta* Malpighian tubule secretion than the endogenous diuretic peptides Mas-DH and Mas-DP II. The perikinins of *Periplaneta americana* display threshold activity on the *Periplaneta* hindgut at concentrations between 5×10^{-10} M and 8×10^{-10} M.[12]

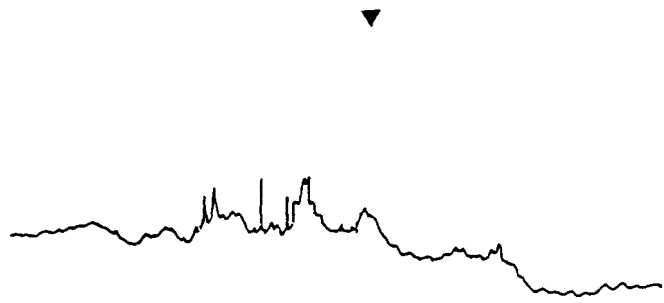

FIGURE 1. Response of the hindgut of *Penaeus vannamei* to Pev-kinin 1 at a concentration of 10^{-6} M. *Arrow* indicates the application of the sample. *Arrowhead* indicates rinsing the gut by changing the saline in the test chamber. Horizontal axis: 1 cm = 1 min.

The Pev-kinins are stimulators of *Leucophaea* hindgut muscle contraction.[14,15] They alter frequency, amplitude, and tonus of the contraction. They display threshold concentrations of 5×10^{-7} to 5×10^{-10} M. To see whether these Pev-kinins had a myotropic effect in Penaeus itself, synthetic peptides were tested on the hindgut of shrimp in a preparation very similar to the *Leucophaea* hindgut preparation **(FIG. 1).** The hindgut was suspended in a 3-mL chamber filled with seawater. The Pev-kinins were able to stimulate *Penaeus* hindgut contraction at micromolar concentration. However, when these synthetic peptides were tested for potential myotropic effects on the hindgut of the crustacean *Astacus leptodactylus,* only Pev-kinin 2 was active at a concentration of 0.5 μM.[14] The Pev-kinins were also tested on Malpighian tubules of *Acheta domesticus*. They stimulated fluid secretion at a concentration of 1 μM. This stimulating diuretic activity was in the order of magnitude 1,000 times less potent than leukokinin 1, which was used as a control.

Achetakinins have effects on the concentration of lipid in the hemolymph and levels of protein synthesis in the fat body *in vivo* in crickets.[29] The kinins from *Helicoverpa zea* were not found to have any consistent *in vivo* diuretic activity using postinjection assays.[10] This indicates that other factors might be responsible for the diuretic activity, or that the helicokinins act together with other factors to produce the *in vivo* diuretic response as seen when a ventral nerve cord (VNC) extract is injected. In *Heliothis virescens* larvae, it has been demonstrated that oral application of helicokinins did not alter weight gain or further development of the larvae.[30] No other *in* vivo experiments were reported with members of the kinin family.

IMMUNOCYTOCHEMICAL LOCALIZATION

Antisera against leukokinin 1 have been applied to the nervous tissue of a range of insect species, including flies, cockroaches, locusts, and moths. One mollusk species and a cestode have also been found to contain leukokinin 1-like immunoreactive neurons. About 160 immunoreactive cell bodies have been counted in the brain of

the cockroach *Leucophaea maderae*.[31] These were al1 distributed in the protocerebrum and the optic lobes. Eight leukokinin 1 immunoreactive ce11 bodies were found in the subesophageal ganglion, 16 ce11 bodies in each of the three thoracic ganglia, and in each of the abdominal ganglia two pairs of strongly immunoreactive cells were found. In the brain of *Locusta migratoria,* a population of about 140 neurons was labeled with an antiserum directed against leukokinin I.[32] These ce11 bodies were distributed in the proto- and tritocerebrum. In the nervous system of some higher dipteran insects *(Drosophila melanogaster, Calliphora vomitoria,* and *Phormia terraenovae),* 14 abdominal leukokinin I immunoreactive neurons were found.[33] In addition, the moths *Agrotis segetum,*[34] *Spodoptera litura,*[35] the housefly *Musca domestica,*[36] and the *fly Sarcophaga bullata*[37] contained immunoreactive neurons. Leukokinin IV immunoreactive neurons were found in *Manduca sexta,*[38] the cockroach *Nauphoeta cinerea*, the cricket *Acheta domesticus*, the mosquito *Aedes aegypti,* the locust *Schistocerca americana,* and the honey bee *Apis mellifera*.[39] Leukokinin VIII immunoreactive neurons were found in the brain, frontal and subesophageal ganglion, al1 three thoracic ganglia, and the terminal ganglion of *Leucophaea maderae*.[40] In the brain, ce11 bodies were found in proto-, deuto-, and tritocerebrum, in the optic lobes and in the frontal ganglion.

Achetakinin-like immunoreactive material was found in the brain, subesophageal ganglia, the thoracic and abdominal ganglia, and the retrocerebral complex of *Acheta domesticus* by radioimmunoassay.[41] The achetakinin 1 antiserum was used for the localization of CDP II in *Culex salinarius*.[7] Immunoreactive neurons were detected in the mosquito head ganglia and the thoracic ganglia. In the abdominal ganglia of both *M. sexta* and *L. migratoria,* leukokinin immunoreactivity was colocalized with diuretic hormone immunoreactivity.[38,42] Locusta-diuretic peptide and locustakinin acted synergistically on Malpighian tubule fluid secretion.[42]

Neither the Malpighian tubules nor the hindgut is innervated by axons displaying leukokinin-like immunoreactivity in *Leucophaea*.[31] This suggests that the kinins have to be released into the circulation in order to reach their target organs. This is confirmed by the leukokinin immunoreactivity associated with neurohemal areas in *Leucophaea maderae,*[31] *Acheta domesticus,*[41] and *Periplaneta americana.* [12] Further evidence is provided by the fact that kinins have been detected in the hemolymph of *Leucophaea maderae*[43] and *Acheta domesticus.*[41]

A leukokinin IV antiserum labeled various fibers, but no cells were found to be immunoreactive in the brain of *Penaeus vannamei*.[14] A polyclonal antiserum raised against Pev-kinin 2 was used to immunostain sections of brain and thoracic and ventral ganglia. Neurosecretory cells were labeled in the thoracic ganglion (FIG. 2a and b). No staining was observed when the anti-Pev-kinin 2 serum was replaced by serum previously inactivated with synthetic Pev-kinin 2 or with control serum. One neurosecretory ce11 was labeled in the brain (FIG. 3a), and the control serum of the preimmunized rabbit showed no staining on an alternating section (FIG. 3b). Thus, in comparison with insects, fewer cells are stained in the central nervous system of *Penaeus*.

Leukokinin-like immunoreativity was also found in the nervous tissue of the nematode *Ascaris suum,*[44] the snail *Helix pomatia,*[45] and the spider *Cupiennius salei,*[46] suggesting that (leuko)kinin-related peptides may have a wider phylogenetic distribution than has been thought.

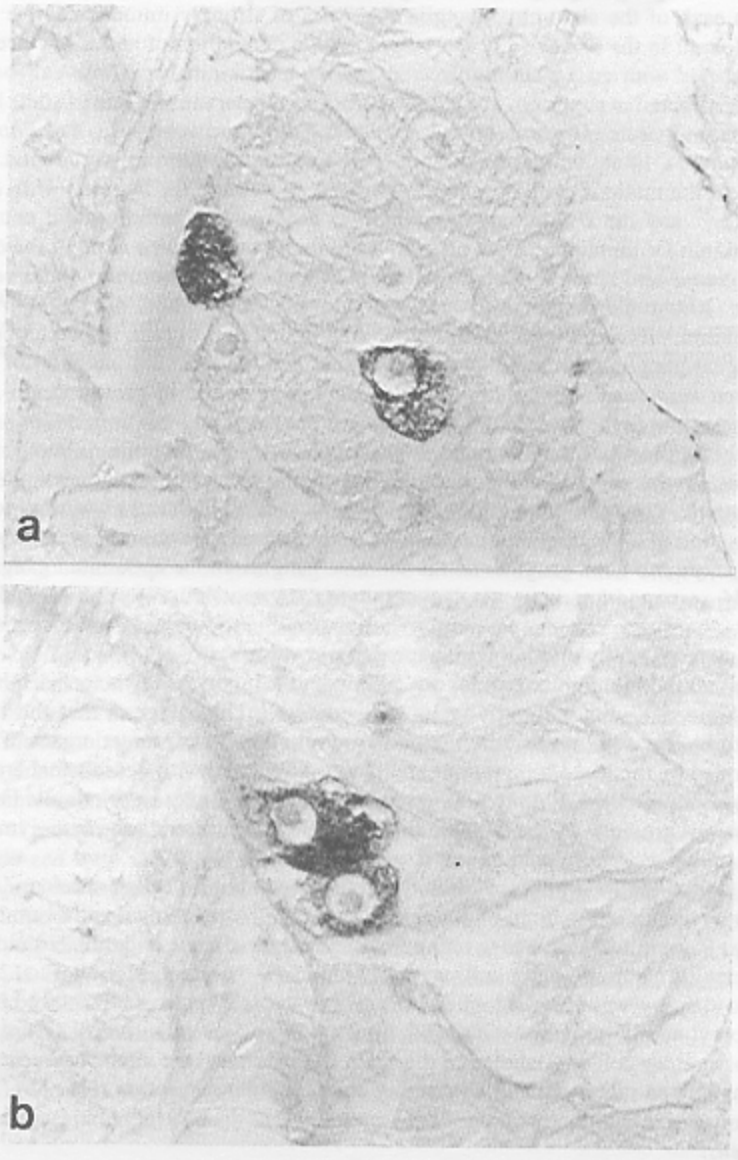

FIGURE 2. Neurosecretory cells in the thoracic ganglion of *Penaeus vannamei* containing Pev-kinin 2 immunoreactivity.

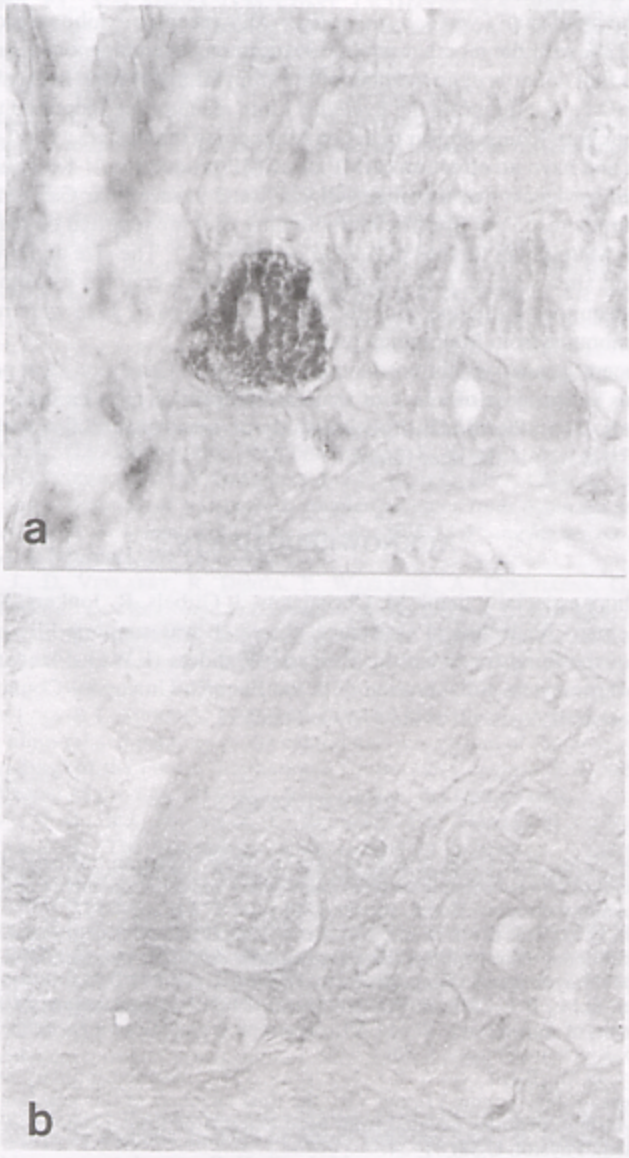

FIGURE 3. (a) A neurosecretory cell in the brain of *Penaeus vannamei* showing Pev-kinin 2 immunoreactivity. (b) An alternate section used as a control showing no immunoreactivity with the control serum of the preimmunized rabbit.

RECEPTORS AND SIGNAL TRANSDUCTION

A G-protein-coupled kinin receptor has recently been isolated from a CNS cDNA library of the pond snail *Lymnaea stagnalis*.[13] This receptor exhibits a sequence homology of 32% with the rat neuropeptide Y receptor; its endogenous ligand is lymnokinin, the first nonarthropod member of the kinin family to date. Intracellular Ca^{2+} levels in CH0 cells expressing the *Lymnaea* kinin receptor are increased upon binding of lymnokinin. Kinins appear to act on Malpighian tubules via a Ca^{2+}-dependent mechanism and have no effect on cAMP or cGMP production.[47] Kinin activity can be mimicked by agents that mobilize cell Ca^{2+}, and intracellular Ca^{2+} chelators reduce the diuretic response. Specific kinin binding sites are reported on plasma membranes from Malpighian tubules of *Acheta domesticus*.[48] The size and properties of these binding sites suggest that it could be a G-protein-coupled receptor. Furthermore, in mosquitoes kinin-specific binding sites of 60 to 62 kDa were reported on plasma membranes from Malpighian tubules.[49]

Kinin receptors and signa1 transduction pathways in Crustacea have not been investigated as yet because of a lack of structural information on kinins in this group of arthropods. The present identification study on crustacean kinins will stimulate this type of research in the near future.

ACKNOWLEDGMENTS

The authors especially thank M. Christiaens, J. Gijbels, R. Jonkers, S. Kotanen, and J. Puttemans for technical assistance. Research was supported by project No. G0356.98 of the Fund for Scientitic Research-Flanders (F.W.O.-Flanders) and the Belgian govemment in collaboration with the Flemish University Council (VLIR). D.V. is a Research Assistant at the F.W.O.-Flanders.

REFERENCES

1. **HOLMAN,** G.M., B.J. COOK & R.J. NACHMAN. 1986. Isolation, primary structure and synthesis of two neuropeptides from ***Leucophaea maderae***: members Of a new family of cephalomyotronins. Comn. Biochem. Phvsiol. C 84: 205–211.
2. HOLMAN, G.M., B.J. **COOK** & R.J. NACHMAN. 1986. Primary structure and synthesis of two additional neuropeptides from ***Leucophaea maderae***: members of a new family of cephalomyotropins. Comp. Biochem. Physiol. C 84: 271-276.
3. **HOLMAN, G.M., B.J. COOK & R.J. NACHMAN.** 1987. Isolation, primary structure, and synthesis of leucokinins V and VI: myotropic peptides of ***Leucophaea maderae***. Comp. Biochem. Physiol. C 88: 27–30.
4. **HOLMAN, G.M., B.J. COOK & R.J. NACHMAN.** 1987. Isolation, primary structure and synthesis of leucokinins VII and VIII: the final members of this new family of cephalomyotropic peptides isolated from head extracts of *Leucophaea maderae*. Comp. Biochem. Physiol. C 88: 31–34.
5. **HOLMAN, G.M., R.J. NACHMAN** & M. WRIGHT. 1990. A strategy for the isolation and structural characterization of certain insect myotropic peptides that modify the spontaneous contractions of the isolated cockroach hindgut. *In* Chromatography and Isolation of Insect Hormones and Pheromones. A.R. McCafferv & I.D. Wilson. Eds.: 195–204. Plenum. New York.

6. SCHOOFS, L., G.M. HOLMAN, P. PROOST, J. VAN DAMME, T.K. HAYES & A. DE LOOF. 1992. Locustakinin, a novel myotropic peptide from *Locusta* migratoria, isolation, primary structure and synthesis. Regul. Pept. 37: 49-57.
7. CLOTTENS, F.L., S.M. MEOLA, G.M. COAST, T.K. HAYES, M.S. WRIGHT, R.J. NACHMAN & G.M. HOLMAN. 1993. Characterization of an antiserum against an achetakinin I-analog and its use for the localization of culekinin depolarizing peptide II in the mosquito, *Culex salinarius*. Regul. Pept. 49: 145-157.
8. HAYES, T.K., G.M. HOLMAN, T.L. PANNABECKER, MS. WRIGHT. A.A. STREY, R.J. NACHMAN, D.F. HOEL, J.K. OLSON & K.W. BEYENBACH. 1994. Culekinin depolarizing peptide: a mosquito leucokinin-like peptide that influences insect Malpighian tubule ion transport. Regul. Pept. 52: 235-248.
9. VEENSTRA, J.A. 1994. Isolation and identification of three leucokinins from the mosquito *Aedes aegypti*. Biochem. Biophys. Res. Commun. 202: 715-719.
10. BLACKBURN, M.B., R.M. WAGNER, J. SHABANOWITZ, J.P. KOCHANSKY, D.F. HUNT & A.K. RAINA. 1995. The isolation and identification of three diuretic kinins from the abdominal ventral nerve cord of adult *Helicoverpa zea*. J. Insect. Physiol. 41: 723-730.
11. RAMSAY, J.A. 1954. Active transport of water by the Malpighian tubules of the stick insect, *Dixippus morosus* (Orthoptera, Phasmidae). J. Exp. Biol. 31: 104-l 13.
12. PREDEL, R., R. KELLNER, J. RAPUS, H. PENZLIN & G. GADE. 1997. Isolation and structural elucidation of eight kinins from the retrocerebral complex of the American cockroach, *Periplaneta americana*. Regul. Pept. 71: 199–205.
13. COX, K.J.A., C.P. TENSEN, R.C. VAN DER SCHORS, K. WAN LI, H. VAN HEERIKHUIZEN, E. VREUGDENHIL, W.P.M. GERAERTS & J.F. BURKE. 1997. Cloning, characterization, and expression of a G-protein-coupled receptor from *Lymnaea stagnalis* and identification of a leucokinin-like peptide, PSFHSWSamide, as its endogenous ligand. J. Neurosci. 17: 1197–1205.
14. NIETO, J., D. VEELAERT, R. DERUA, E. WAELKENS, A. CERSTIAENS, G. COAST, B. DEVREESE, J. VAN BEEUMEN, J. CALDERON, A. DE LOOF & L. SCHOOFS. 1998. Identification of one tachykinin- and two kinin-related peptides in the brain of the white shrimp, *Penaeus vannamei*. Biochem. Biophys. Res. Commun. 248: 406–411.
15. NIETO, J., J. VEENSTRA, E. WAELKENS, J. CALDERÓN, G. BAGGERMAN, D. VEELAERT, A. DE LOOF & L. SCHOOFS. 1999. Isolation and identification of one tachykinin and three kinin-related peptides in the central nervous system of *Penaeus vannamei*. *In* Proceedings of the Fourth International Crustacean Congress. F.R. Schram & J.C. von Vaupel Klein, Eds. :95 l-960. Brill. Leiden, the Netherlands.
16. HOLMAN, G.M., R.J. NACHMAN, L. SCHOOFS, T.K. HAYES, M.S. WRIGHT & A. DELOOF. 1991. The *Leucophaea maderae* hindgut preparation: a rapid and sensitive bioassay tool for the isolation of insect myotropins of other insect species. Insect Biochem. 21: 107-l 12.
17. NACHMAN, R.J. & G.M. HOLMAN. 1991. Myotropic insect neuropeptide families from the cockroach *Leucophaea maderae*: structure-activity relationships. *In* Insect Neuropeptides: Chemistry, Biology and Action. J.J. Menn & E.P. Masler, Eds.: 194-214. American Chemical Society. Washington, DC.
18. COAST, G.M., G.M. HOLMAN & R.J. NACHMAN. 1990. The diuretic activity of a series of cephalomyotropic neuropeptides, the achetakinins, on isolated malpighian tubules of the house cricket, *Acheta domesticus*. J. Insect Physiol. 36: 481-488.
19. NACHMAN, R.J., J.W. TILLEY, T.K. HAYES, G.M. HOLMAN & R.C. BEIER. 1994. Pseudopeptide mimetic analogs of insect neuropeptides. In Natural and Derived Pest Management Agents. P. Hedin, J. Menn & R. Hollingsworth, Eds.: 210–229. American Chemical Society. Washington, DC.
20. NACHMAN, R.J., G.M. COAST, G.M. HOLMAN & R.C. BEIER. 1995. Diuretic activity of C-terminal group analogs of the insect kinins in *Acheta domesticus*. Peptides 16: 809–813.

21. NACHMAN, R.J., G.M. COAST, V.A. ROBERTS & G.M. HOLMAN. 1995. Incorporation of chemical/conformational components into mimetic analogs of insect neuropeptides. In Insects: Chemical, Physiological and Environmental Aspects 1994. D. Konopinska, Ed.: 51–60. University of Wroclaw. Wroclaw, Poland.

22. COOK, B.J., G.M. HOLMAN, R.M. WAGNER & R.J. NACHMAN. 1990. Comparative pharmacological actions of leucokinins V-VIII, on the Visceral Muscles of *Leucophaea maderae*. Comp. Biochem. Physiol. 95C: 19-24.

23. HAYES, T.K., T.L. PANNABECKER, D.J. **HINCKLEY,** G.M. **HOLMAN,** R.J. **NACHMAN,** D.H. **PETZEL & K.W. BEYENBACH.** 1989. Leucokinins, a new family of ion transport stimulators and inhibitors in insect Malpighian tubules. Life Sci. 44: 1259-1266.

24. ROBERTS, V.A., R.J. NACHMAN, G.M. COAST, M. HARIHARAN, J.S. CHUNG, G.M. **HOLMAN, H.** WILLIAMS & J.A. TAINER. 1997. Consensus chemistry and beta-turn conformation of the active core of the insect kinin neuropeptide family. Chem. Biol. 4: 105-l 17.

25. VEENSTRA, J.A., J.M. PATTILLO & D.H. PETZEL. 1997. A single cDNA encodes all three Aedes leucokinins, which stimulate both fluid secretion by the malpighian tubules and hindgut contractions. J. Biol. Chem. 272: 10402-10407.

26. COOK, B.J., G.M. HOLMAN, R.M. WAGNER & R.J. NACHMAN. 1989. Pharmacological actions of a new class of neuropeptides, the leucokinins I-IV, on the visceral muscles of *Leucaphaea maderae*. Comp. Biochem. Physiol. 93C: 257-262.

27. COAST, G.M. 1995. Synergism between diuretic peptides controlling ion and fluid transport in insect Malpighian tubules. Regul. Pept. 57: 283-296.

28. COAST, G.M. 1998. The influence of neuropeptides on Malpighian tubule writhing and its significance for excretion. Peptides 19: 469–480.

29. GOLDSWORTHY, H.J., G.M. COAST, H. WHEELER, 0. CUSINATO, I. KAY & B. KHAMBAY. 1992. The structural and functional activity of neuropeptides. In Roya1 Entomological Society Symposium on Insect Molecular Science. J.M. Crampton & P. Egglestone, Eds.: 205-225. Academic Press. London.

30. SEINSCHE, **A., H. DYKER, P.** LÖSEL, **J. SCHERKENBECK & A.G. BAYER.** 1998. Regulation of water balance in *Heliothis virescens:* influence of helicokinins and ACE-inhibitors [abstr.]. Abstracts of the 19th Conference of European Comparative Endocrinologists, Nijmegen, the Netherlands: 62.

3 1. NÄSSEL, D.R., R. CANTERA & A. KARLSSON. 1992. Neurons in the cockroach nervous system reacting with antisera to the neuropeptide leucokinin I. J. Comp. Neurol. 322: 45–67.

32. NASSEL, D.R. 1993. Insect myotropic peptides: differential distribution of locustatachykinin- and leucokinin-like immunoreactive neurons in the locust brain. Cell Tissue Res. 274: 27–40.

33. CANTERA, R. & D.R. NÄSSEL. 1992. Segmenta1 peptidergic innervation of abdominal targets in larval and adult dipteran insects revealed with an antiserum against leucokinin I. Cell Tissue Res. **269:** 459-471.

34. CANTERA, R., B.S. HANSSON, E. HALLBERG & D.R. NÄSSEL. 1992. Postembryonic development of leucokinin 1-immunoreactive neurons innervating a neurohemal organ in the turnip moth *Agrotis segetum*. Cell Tissue Res. 269: 65-77.

35. IEE, B.H., H. KANG, D. KWONG, C.I. PARK, W.K. KIM & M.Y. KIM. 1998. Postembryonic development of leucokinin-like immunoreactive neurons in the moth *Spodoptera litura*. Tissue Cell 30: 74-85.

36. IABONI, A., G.M. HOLMAN, R.J. NACHMAN, I. ORCHARD & G.M. COAST. 1998. Immunocytochemical localisation and biological activity of diuretic peptides in the housfly, *Musca domestica*. Cell Tissue Res. 294: 549–560.

37. SIVASUBRAMANIAN, P. 1994. Localization of leucokinin-like immunoreactive neurones and their metamorphic changes in the ventral ganglion of the fly, *Sarcophaga bullata*. Comp. Biochem. Physiol. A 109: 151–155.

38. CHEN, Y., J.A. VEENSTRA, H. HAGEDORN & N.T. DAVIS. 1994. Leucokinin and diuretic hormone immunoreactivity of neurons in the tobacco hornworm, *Manduca* sexta, and co-localization of this immunoreactivity in lateral neurosecretory cells of abdominal ganglia. Ce11 Tissue Res. 278: 493–507.

39. CHEN, Y., J.A. VEENSTRA, N.T. DAVIS & H.H. HAGEDORN. 1994. A comparative study of leucokinin-immunoreactive neurons in insects. Ce11 Tissue Res. 276: 69-83.

40. MEOLA, S.M., F.L. CLOTTENS, G.M. COAST & G.M. HOLMAN. 1994. Localization of leucokinin VIII in the cockroach, *Leucophaea maderae*, using an antiserum directed against an achetakinin-I analog. Neurochem. Res. 19: 805–814.

41. CHUNG, J.S., G.J. GOLDSWORTHY & G.M. COAST. 1994. Haemolymph and tissue titres of achetakinins in the house cricket *Acheta domesticus*: effect of starvation and dehydration. J. Exp. Biol. 193: 307-319.

42. THOMPSON, K.S., R.C. RAYNE, C.R. GIBBON, S.T. MAY, M. PATEL, G.M. COAST & J.P. BACON. 1995. Cellular colocalization of diuretic peptides in locusts: a potent control mechanism. Peptides 16: 95–104.

43. MUREN, E.J., T.C. LUNDQUIST & D.R. NÄSSEL. 1993. Quantative determination of myotropic neuropeptide in the nervous system of the cockroach *Leucophaea maderae*: distribution and release of leucokinins. J. Exp. Biol. 179: 289–300.

44. SMART, D., C.F. JOHNSTON, C. SHAW, D.W. HALTON & K.D. BUCHANAN. 1993. Use of specific antisera for the localisation and quantitation of leucokinin immunoreactivity in the nematode, *Ascaris suum*. Comp. Biochem. Physiol. C 106: 517–522.

45. ELEKES, K., L. HERNADI, J.E. MUREN & D.R. NÄSSEL. 1994. Peptidergic neurons in the snail *Helix pomatia:* distribution of neurons in the central and peripheral nervous systems that react with an antibody raised to the insect neuropeptide, leucokinin I. J. Comp. Neurol. 341: 257-272.

46. SCHMID, A. & C. BECHERER. 1996. Leucokinin-like immunoreactive neurones in the central nervous system of the spider *Cupiennius salei*. Ce11 Tissue Res. 284: 143–152.

47. O'DONNEL, M.J., J.A.T. Dow, G.R. HUESMANN, N.J. TUBLITZ & S.H.P. MADDRELL. 1996. Separate control of anion and cation transport in Malpighian tubules of *Drosophila melunogaster*. J. Exp. Biol. 199: 1163-l 175.

48. CHUNG, J.S., C.H. WHEELER, G.J. GOLDSWORTHY & G.M. COAST. 1995. Properties of achetakinin binding sites on malpighian tubule membranes from the house cricket, *Achetu domesticus*. Peptides 16: 375-382.

49. HAYES, T.K., A. STREY, S. BELK, G.M. HOLMAN, R.J. NACHMAN, D. PETZEL, J. READIO, R. MEOLA, T. PANNABECKER & K. BEYENBACH. 1997. Biochemical characterization of mosquito kinin and related receptors. Ann. N. Y. Acad. Sci. 814: 342-345.