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Review

Tachykinin-related peptides and their receptors in invertebrates: A current view

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ABSTRACT

Members of the tachykinin peptide family have been well conserved during evolution and are mainly expressed in the central nervous system and in the intestine of both vertebrates and invertebrates. In these animals, they act as multifunctional messengers that exert their biological effects by specifically interacting with a subfamily of structurally related G protein-coupled receptors. Despite the identification of multiple tachykinin-related peptides (TKRPs) in species belonging to the insects, crustaceans, mollusks and echiuroid worms, only five invertebrate receptors harboring profound sequence similarities to mammalian receptors for tachykinins have been functionally characterized to date. Three of these have been cloned from dipteran insect species, *i.e.* NKD (neurokinin receptor from *Drosophila*), DTKR (*Drosophila* tachykinin receptor) and STKR (tachykinin-related peptide receptor from the stable fly, *Stomoxys calcitrans*). In addition, two receptors from non-insect species, present in echiuroid worms and mollusks, respectively have been identified as well. In this brief review, we will survey some recent findings and insights into the signaling properties of invertebrate tachykinin-related peptides via their respective receptors. In this context, we will also point out the necessity to take into account differences in signaling mechanisms induced by distinct TKRP isoforms in insects.

Contents

1. The tachykinin peptide family

Tachykinins (TKs), also known as neurokinins (NKs), form an evolutionarily well-conserved group of multifunctional brain/gut peptides that play important roles in neurotransmission and/or as neuromodulators in the central and peripheral nervous system [17,21,23,27,40]. Functioning as para- or autocrine factors or as endocrine messengers, tachykinins are also involved in the regulation of various other physiological processes such as intestinal motility [35], smooth muscle contraction and cardiovascular function [43]. This multimode action of tachykinins explains why they have been associated with diverse pathological conditions [14]. The archetypal mammalian tachykinin family member is substance P (SP). Together with neurokinin A (NKA, also termed substance K) and neurokinin B (NKB, also neuromedin K), SP shares the conserved C-terminal pentapeptide core motif, -FXGLM-NH_2 (with X being a variable amino acid residue), typically with an amidated C-terminal methionine. More recently, endokinins and hemokinins were identified as additional vertebrate members of the tachykinin peptide family [15,28,45].

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2. Tachykinin-related peptides are of ancient origin

In insects, immunoreactivity against vertebrate tachykinins has been demonstrated multiple times, both in neuronal and intestinal tissues [18,24,42]. The first biochemical evidence for the existence of insect tachykinin-related peptides (also designated as "*insecta*tachykinins") came with the identification of locustatachykinins I–IV (*Lom* TKs I–IV) [33,34]. These four neuropeptides were purified from brain–*corpora cardiaca*–*corpora allata*–suboesophageal ganglion extracts of the migratory locust, *Locusta migratoria,* and share limited (about 30%) sequence similarity to their vertebrate counterparts thereby appearing more similar to fish and amphibian tachykinins (up to 45%) than to mammalian family members [33,34]. Analogous to tachykinin-induced motility of mammalian smooth muscles, *Lom* TKs exert myotropic activity on cockroach hindgut as well as on *Locusta* foregut and oviduct preparations [33,34]. Since this initial discovery, and much enhanced by the still growing number of genome and EST (expressed sequence tag)-sequencing programs, tachykinin-related peptides have been identified in a broad range of invertebrates such as insects [39,41], crustaceans [3,26], mollusks [4,9], echiuroid worms[7,12,13] and nematodes[25]. In these species, TKRPs generally originate from larger precursor polypeptides via enzymatic cleavage and modification pathways.

Most invertebrate tachykinin-related peptides possess a characteristic C-terminal -FX_1GX_2R-NH_2 consensus sequence that, to our knowledge, has never been found to exist in vertebrates. Only few notable exceptions to this rule exist. Sialokinin I and II, two peptides isolated from the salivary glands of the yellow fever mosquito, *Aedes aegypti*, harbor the vertebrate-type tachykinin consensus signature [2]. Since mosquitoes feed on vertebrates, it can be hypothesized that sialokinins act as vasodilators, working through vertebrate tachykinin receptors present in their host. Also eledoisin and *Oct*-TK I–II, tachykinin-related peptides present in the salivary glands of the cephalopod mollusks, *Eledone moschata* and *Octopus vulgaris*, respectively, contain the vertebrate-type C-terminal core sequence. In addition, TKRPs isolated from the stable fly, *Stomoxys calcitrans*, the bivalve mollusk, *Anodonta cygnea*, and tachykinin-related peptide 6 from *Drosophila melanogaster* contain an Ala-residue instead of the otherwise preserved Gly-residue in the C-terminal core sequence (-FX_1**A**X_2R-NH_2). This seemingly small structural change bears significant consequences for *in vitro* receptor signaling as will be discussed further.

3. Receptors for tachykinin-related peptides in insects

Despite a long history of identifying and isolating invertebrate neuropeptides, which led to a plethora of known invertebrate tachykinin-related peptides, only five corresponding G protein-coupled receptors have been properly characterized to date. The first insect GPCR capable of sensing tachykinin-related peptides was cloned from the fruit fly and is termed DTKR (*Drosophila* tachykinin receptor, encoded by the gene *CG7887*) [16]. Within its transmembrane regions DTKR shows about 40–48% amino acid identity to vertebrate receptors for tachykinins. In humans, at least three distinct tachykinin receptors exist, *i.e.* NK1, NK2 and NK3, respectively [19]. When DTKR was functionally expressed in *Xenopus* oocytes, it responded to micromolar amounts of both substance P and physalaemin (an amphibian tachykinin), but not to any other vertebrate tachykinin tested. Treatment of the oocytes with pertussis toxin abolished these responses, demonstrating that DTKR intracellularly can interact with the vertebrate Gi/o-type of G_α-subunits [16]. Recently, two independent studies unambiguously demonstrated that fruit fly tachykinin-related peptides are the endogenous ligands for DTKR [1,31]. As mentioned earlier, in the fruit fly, tachykinin-related peptides [also referred to as *Drosophila* tachykinin-related peptides (DTKs)] are encoded by a single precursor gene that gives rise to six distinct peptides (DTKs 1–6) [36,39], which are probably produced in equimolar amounts. Without exception, all DTKs dose-dependently increased the intracellular calcium (Ca^{2+})-concentration, as well as cyclic AMP levels, when applied on DTKR-expressing HEK293 or *Drosophila* Schneider 2 (S2) cells. In both reports, DTK-1 appeared to be the most potent DTKR agonist [1,31]. Apart from the fruit fly, a cDNA fragment from the cockroach, *Leucophaea maderae*, encoding (a portion of) a putative DTKR ortholog has been cloned from brain tissue [8]. In addition, *in silico* database analysis clearly demonstrated that other DTKR orthologs have most likely been preserved in multiple insect species (Fig. 1).

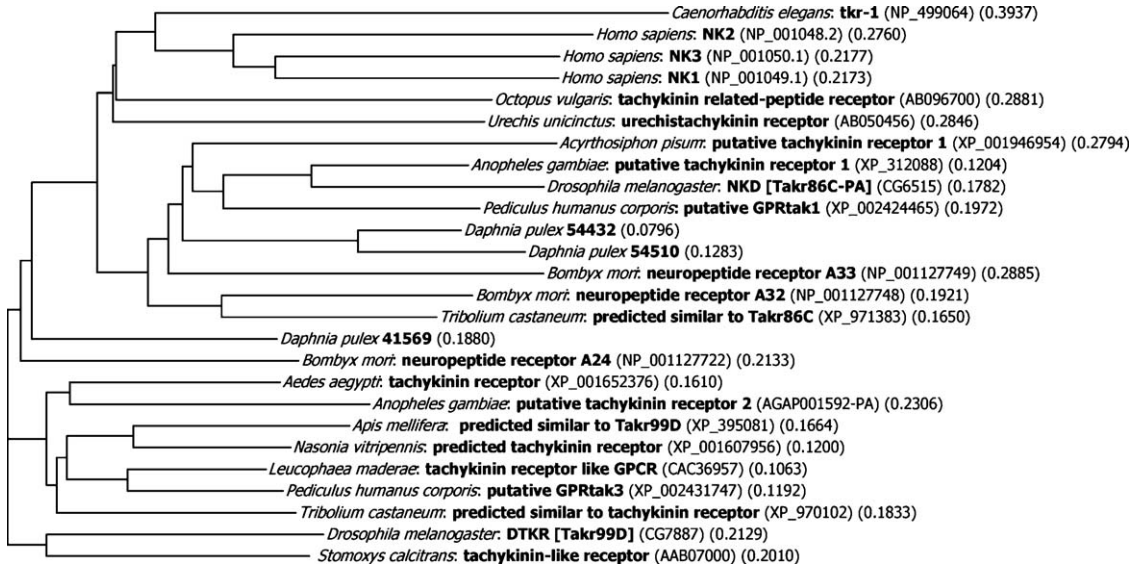

Fig. 1. Phylogenetic tree displaying G protein-coupled receptors from various invertebrates that show sequence similarity to vertebrate receptors for tachykinins. The amino acid sequences were first aligned using AlignX (Invitrogen). The tree was generated according to the Neighbor Joining method.

4. Do different isoforms of tachykinin-related peptides contribute to the complexity of the system?

A second fruit fly receptor for tachykinin-related peptides, termed NKD (neurokinin receptor from *Drosophila*), encoded by the gene *CG6515* was originally cloned by Monnier et al. [22]. Within their transmembrane regions, NKD and DTKR share approximately 50% sequence identity. Recently, by screening a synthetic library covering the vast majority of predicted *Drosophila* peptides in a cell-based luminescence assay, DTK-6 turned out to be the only known fly peptide with clear agonist activity on NKD-expressing cells [29]. As mentioned before, fruit fly DTK-6 possesses an Ala-substitution in the otherwise highly conserved C-terminal core motif of invertebrate tachykinin-related peptides (Table 1). Thus, these data suggested that NKD is able to discriminate between Ala- and Gly-containing isoforms of TKRPs, a feature that does not apply to DTKR. In the same study, the locust peptide *Lom* TK-II, that naturally contains a Gly-residue (Table 1), did not activate NKD-expressing *Drosophila* S2 cells [29], although earlier data reported an increased inositol trisphosphate (IP_3)-production in NKD-expressing mouse NIH-3T3 cells incubated with *Lom* TK-II [22]. Nevertheless, by replacement of the naturally occurring Gly-residue of *Lom*-TK-II (Ala-Pro-Leu-Ser-Gly-Phe-Tyr-**Gly**-Val-Arg-NH_2) with an Ala-residue, a synthetic peptide (*Lom*-TKII-Ala) was generated that yielded a much more efficacious NKD-ligand than *Lom* TK-II itself [29]. In addition, substitution of the Ala-residue of *Stc*-TK (Ala-Pro-Thr-Gly-Phe-Phe-**Ala**-Val-Arg-NH_2), the only tachykinin-related peptide so far isolated from the stable fly, with a Gly-residue completely abolished its activity on NKD-expressing cells [29].

A third dipteran tachykinin receptor was cloned from the stable fly, *Stomoxys calcitrans* [5]. Overall amino acid sequence comparison indicates that STKR is highly similar to DTKR, *i.e.* they share about 80% identity in their transmembrane regions. When stably expressed in *Drosophila* S2 cells, *Lom* TKs elicited a transient increase of intracellular Ca^{2+}-levels and, in addition, an increase of the production of cyclic AMP, but this latter only when applied at higher concentrations. Vertebrate tachykinins did not show any activity on STKR at concentrations up to 10^{-5} M [38]. When the C-terminal methionine-residue (-Met-NH_2) of substance P and physalaemin was replaced by an arginine-residue (-Arg-NH_2), peptides with mixed mammalian and invertebrate tachykinin peptide sequences were generated. Intriguingly, these "chimeric" peptides behaved as potent agonists on STKR-expressing cells. Similarly, replacing the C-terminal arginine-residue (-Arg-NH_2) of *Lom* TKs with methionine significantly increased their activity on human neurokinin receptors. Their potency as agonists for human receptors was even further augmented if the penultimate amino acid residue was also synthetically replaced by its highly conserved human counterpart [37]. Clearly, these data indicate a major pharmacological difference between tachykinin-like peptides from vertebrates and invertebrates that mainly resides in the nature of the C-terminal amino acid (methionine *vs.* arginine, respectively) and is probably the result of ligand-receptor co-evolution. This postulation is in agreement with observed effects of tachykinins in *in vitro* gut contractility bioassays. For instance, a chimeric substance P molecule harboring an "invertebrate-type" C-terminal Arg-residue evoked increased activity in the cockroach hindgut bioassay, but became less potent on guinea pig ileum preparations [6].

In the stable fly, one single tachykinin-related peptide (*Stc*-TK) has been isolated so far. *Stc*-TK contains an Ala-residue (Ala-Pro-Thr-Gly-Phe-Phe-**Ala**-Val-Arg-NH_2) instead of the highly conserved Gly-residue that exists in the active core of most other invertebrate tachykinin-related peptides (Table 1). It has been elegantly shown that due to this naturally occurring Ala-substitution, *Stc*-TK behaves as a partial agonist on its endogenous receptor STKR. Of interest, this partial agonism only seems to affect intracellular Ca^{2+}-increases in *Drosophila* S2 cells, but not the receptor-mediated cyclic AMP responses [30]. Other Ala-containing tachykinin-related peptides, such as DTK-6, also behave as potent, but partial agonists for the STKR-induced Ca^{2+}-response [30]. Whether other *Stc*-TK isoforms exist and how they act on STKR remains to be elucidated. In any case, due to the *in vitro* signaling properties of STKR (*i.e.* partial agonism induced by distinct TKRP isoforms) and NKD (*i.e.* discrimination between Ala- and Gly-containing TKRP isoforms), it seems that distinct tachykinin-related peptides are able to stabilize multiple active receptor conformations, in accordance with the "dual nature" model for the vertebrate NK1 receptor [20]. As a consequence, these receptors may differ in their functional coupling to G-proteins and thus in their signal transduction cascades, dependent on the TK isoforms that occupy them. Evidently this phenomenon adds an additional layer of complexity to tachykinin signaling, at least *in vitro*. The *in vivo* significance of this model still requires investigation, but it favors the concept that distinct tachykinin-related peptides or their isoforms are likely to induce different physiological responses, possibly resulting in a "physiological fine-tuning", rather than being a group of fully redundant agonists.

Two non-insect GPCRs for tachykinin-related peptides have been characterized to date. The urechistachykinin receptor (UTKR) from the echiuroid worm, *Urechis unicinctus*, was originally cloned from ventral nervous tissue and shows a high degree of similarity

Table 1
Peptide sequences of tachykinin-related peptides mentioned throughout the text. Amino acid residues deviating from the consensus C-terminal core motif (-FX_1GX_2Ra) are depicted in bold and are underlined.

	Peptide name	Peptide sequence
Insecta		
Drosophila melanogaster	DTK-1	APTSSFIGMRa
	DTK-2	APLAFVGLRa
	DTK-3	APTGFTGMRa
	DTK-4	APVNSFVGMRa
	DTK-5	APNGFLGMRa
	DTK-6	pQRFADFNSKFV**A**VRa
Locusta migratoria	*Lom*-TK-I	GPSGFYGVRa
	Lom-TK-II	APLSGFYGVRa
	Lom-TK-III	APQAGFYGVRa
	Lom-TK-IV	APSLGFHGVRa
Aedes aegypti	Sialokinin I	NTGDKFYGL**M**a
	Sialokinin II	DTGDKFYGL**M**a
Stomoxys calcitrans	*Stc*-TK	APTGFF**A**VRa
Echiuroid worms		
Urechis unicinctus	*Uru*-TK-I	LRQSQFVGSRa
	Uru-TK-II	AAGMGFFGARa
	Uru-TK-III	AAPSGFFGARa
	Uru-TK-IV	AAYSGFFGARa
	Uru-TK-V	APSMGFFGARa
	Uru-TK-VII	APKMGFFGARa
Mollusca		
Eledone moschata	Eledoisin	pEPSKDAFIGL**M**a
Anodonta cygnea	*Anc*-TK	pEYGFH**A**VRa
Octopus vulgaris	*Oct*-TK-I	KPPSSSEFIGL**M**a
	Oct-TK-II	KPPSSSEFVGL**M**a
	Oct-TKRP-I	VNPYSFQGTRa
	Oct-TKRP-II	LNANSFMGSRa
	Oct-TKRP-III	TVSANAFLGSRa
	Oct-TKRP-IV	SDALAFV**P**TRa
	Oct-TKRP-V	MNSLSFG**P**P**K**a
	Oct-TKRP-VI	YSPLDFIGSRa
	Oct-TKRP-VII	ASLHNTHFI**P**SRa
		Vertebrate consensus motif: FXGLMa
Substance P	SP	RPKPQQFFGLMa
Physalaemin		pEADPNKFYGLMa

to other tachykinin receptor sequences [11]. The genomic composition of the UTKR encoding gene was found to partially coincide with that of the genes coding for DTKR, NKD and mammalian receptors for tachykinins, suggesting a common ancestral gene [32]. Via functional expression of UTKR in *Xenopus* oocytes, it was demonstrated that this receptor can be activated by urechistachykinins I–V and VII (*Uru*-TKs I–V and VII), isolated from *U. unicinctus* nervous tissue [7,12,13]. In contrast, substance P or synthetic *Uru*-TK analogs with a C-terminal Met-NH_2 moiety instead of the Arg-NH_2 motif did not evoke any response, again demonstrating the major importance of the C-terminal amino acid residue for receptor activation [11].

More recently, *Oct*-TKRPR, the first receptor for tachykinin-related peptides (TKRPs) from the mollusk, *Octopus vulgaris*, was functionally analyzed [10]. Previously it had been shown that in this species two types of TKRPs are present, *i.e.* (i) *Oct*-TKRP I-VII, peptides purified from brain tissue with a C-terminal Arg-residue (or Lys in case of *Oct*-TKRP V) and (ii) *Oct* TK-I and II that were isolated from the salivary gland and both display the consensus vertebrate C-terminal moiety [9,10]. Assaying *Oct*-TKRPR by functionally expressing it in *Xenopus* oocytes made it clear that *Oct*-TKRP I–VII are the endogenous ligands for this receptor. Of interest, *Oct* TK-I did not evoke any response on the receptor-expressing cells at all [10]. This is in full agreement with previously mentioned data demonstrating the major pharmacological importance of the C-terminal amino acid for signaling via receptors for tachykinins. Intriguingly, this evolutionary divergence seems to have even occurred within this single species.

5. Distribution and functions of tachykinin-related peptides in *Drosophila*

Most functional studies on insect tachykinin-related peptides and their receptors have been performed in the fruit fly. DTKR expression is already detectable at early embryonic stages and persists throughout all further developmental stages of the fly [16]. Recently, DTKR expression has been demonstrated in the hindgut of stage 16 embryos [1]. NKD is also present in early stage embryos and becomes highly expressed around stage 16 [22]. The combined expression profile of both fly receptors corresponds well with that of the DTK-precursor gene. This latter gene has strongly upregulated expression levels starting from stage 17 embryos [36]. At present, little is known about the precise function of tachykinin-related peptides during early fly development. As the appearance of this neuropeptide signaling system matches well with the onset of neuronal development, it is tempting to speculate that it might play a role herein. Other studies indicate that knocking down the *in vivo* expression-level of the DTK-precursor gene by means of RNAi leads to a nearly 100% lethality during the embryonic stage [44], suggesting a fundamental role in development.

In third instar *Drosophila* larvae tissue distribution of DTKR has been mapped by polyclonal antibodies generated against fragments of the receptor protein. Strong immunoreactivity was detected in a group of four anteriorly located neuronal cell bodies that arborize in the brain and form axons that cross the midline and descend further into the ventral nerve cord [1]. An additional weaker signal was also apparent in two pairs of more posteriorly situated cell bodies and in the Malpighian tubules. It has recently been established that NKD is more widely distributed throughout the larval brain than DTKR. NKD-immunoreactivity labels four pairs of putative median neurosecretory cells as well as four pairs of cell bodies in the suboesophageal ganglion (SOG), eight pairs of cells in the anterior part of the abdominal ganglion and three pairs located more posteriorly [29]. One pair of cells in the SOG likely gives rise to axons that protrude into the ventral nerve cord. Targeting the DTK precursor gene by RNAi suggested that tachykinin signaling mediates olfactory behavior in fruit fly larvae. Whereas wild type larvae tend to be repelled strongly by odorants such as benzaldehyde, RNAi-targeted larvae responded rather indifferently [44].

Both DTKR and NKD receptor proteins are expressed in the adult central nervous system in a mutually exclusive way, *i.e.* DTKR seems to be located in areas where NKD is absent and *vice versa* [29]. The most prominent DTKR presence was detected in the antennal lobes, the central body, the optic lobes and the neuropil ventral to the mushroom bodies [1]. This distribution matches well with that of DTK peptides in the central nervous system and indicates that DTK signaling likely modulates different neuronal circuits. In agreement with this, decreasing levels of DTK-production severely altered locomotor activity and olfactory perception [44]. NKD in the adult brain is present in three pairs of neuronal cell bodies in the protocerebrum and a cluster of cell bodies in the median neurosecretory cell group. The dorsolaterally located neuron cell bodies show axons that extend into the contralateral hemisphere and show arborizations into the dorsal and lateral protocerebrum. Furthermore, two pairs of cell bodies were labeled in the SOG as well as some small cell bodies in the optic lobe. The large SOG neurons extend axons to the oesophagus and form arborizations throughout the ventral SOG. Interestingly, unlike DTKR, NKD is expressed in endocrine cells and muscle fibers of the midgut [29]. As midgut endocrine cells also produce DTKs [36], it seems likely that these may act as autocrine or paracrine factors that regulate midgut function via NKD-signaling.

6. Conclusions and future directions

During the past decade it has become clear that the tachykinin family of peptides has been well preserved in a broad range of animal species belonging to different phylogenetic clades. However, there is a huge discrepancy between the efforts that have been performed to identify and isolate invertebrate tachykinin-related peptides and the detailed characterization of their corresponding receptors, a group of structurally related G protein-coupled receptors. Indeed, only five invertebrate receptors for TKRPs have been properly analyzed to date. Some of these receptors seem to display only moderate or no TK-like ligand specificity, while others can only be activated by specific peptide isoforms. Indeed, diverse *in vitro* cell-based signal transduction experiments have been employed to study signal transduction induced by distinct TKRPs. These studies also made clear that separate peptide isoforms are sometimes capable of inducing and/or stabilizing different receptor conformations upon binding that results in distinct receptor signaling properties. It would be fruitful in the future to extend these studies and to investigate whether these *in vitro* observations are also relevant for *in vivo* situations. Anyhow, all these above mentioned features add an extra level of complexity to the invertebrate tachykinin signaling system, contributing to the diverse physiological roles that TKRPs play in invertebrates.

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