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## Expression and Functional Characterization of a *Drosophila* Neuropeptide Precursor with Homology to Mammalian Preprotachykinin A\*

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Peptides structurally related to mammalian tachykinins have recently been isolated from the brain and intestine of several insect species, where they are believed to function as both neuromodulators and hormones. Further evidence for the signaling role of insect tachykinin-related peptides was provided by the cloning and characterization of cDNAs for two tachykinin receptors from Drosophila melanogaster. However, no endogenous ligand has been isolated for the Drosophila tachykinin receptors to date. Analysis of the Drosophila genome allowed us to identify a putative tachykininrelated peptide prohormone (prepro-DTK) gene. A 1.5kilobase pair cDNA amplified from a Drosophila head cDNA library contained an 870-base pair open reading frame, which encodes five novel Drosophila tachykininrelated peptides (called DTK peptides) with conserved C-terminal FXGXR-amide motifs common to other insect tachykinin-related peptides. The tachykinin-related peptide prohormone gene (Dtk) is both expressed and post-translationally processed in larval and adult midgut endocrine cells and in the central nervous system, with midgut expression starting at stage 17 of embryogenesis. The predicted Drosophila tachykinin peptides have potent stimulatory effects on the contractions of insect gut. These data provide additional evidence for the conservation of both the structure and function of the tachykinin peptides in the brain and gut during the course of evolution.

Substance P was the first peptide signaling molecule to be identified by virtue of its effects upon blood pressure and smooth muscle contraction (1) and is the archetypal member of the tachykinin family of peptides. Vertebrate tachykinins represent a large family of peptides that elicit a wide range of both central and peripheral responses (2–5). Although these peptides are structurally diverse, all contain a conserved C-terminal FXGLM-amide motif. Like other biologically active peptides, substance P is derived from a larger prohormone polypeptide (preprotachykinin A (PPT-A)<sup>1</sup>) that also allows the production of several other biologically active peptides (neurokinin A, neuropeptide K, and neuropeptide  $\gamma$ ) (6). Three different isoforms of preprotachykinin can be produced as a result of alternative splicing of the PPT-A mRNA, which, in conjunction with alternative post-translational processing of the prohormone, allows the production of these peptides in a tissue-specific manner (7–9). A fifth mammalian tachykinin, neurokinin B, is derived from a separate gene product, preprotachykinin B (10).

The tachykinin family is not confined to vertebrates, and a large number of tachykinins have now been isolated from a variety of invertebrate species such as the cockroach Leucophaea maderae (11, 12), the mosquito Culex salinarius (13), and the echiuroid worm Urechis unicinctus (14). In contrast to the vertebrate tachykinins, almost all of the invertebrate tachykinins contain a conserved C-terminal FXGXR-amide motif and, for this reason, have been termed tachykinin-related peptides (TRPs). Notable exceptions are eledoisin, sialokinin I, and sialokinin II, which were isolated from the salivary glands of the cephalopod *Eledone moschata* and the mosquito Aedes aegypti, respectively, and which contain a C-terminal FXGLMamide like the vertebrate tachykinins (2, 16, 17). Antisera to insect TRPs display immunoreactivity in both the gut and the nervous system of a variety of insect species (18, 19), suggesting that, like the mammalian tachykinins, these peptides function as both neuromodulators and endocrine signaling molecules. A tachykinin receptor (STKR) has recently been cloned and characterized from the stable fly (20), and two tachykinin receptors have been characterized from Drosophila melanogaster: NKD and DTKR (21-23). Although expressed NKD and DTKR show responses to the locust peptide LomTK-II and to substance P and physalaemin, respectively, to date, no tachykinins or tachykinin-related peptides have been isolated from D. melanogaster. As a consequence, the endogenous ligands for NKD and DTKR are not known. A tachykinin precursor cDNA

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank<sup>TM</sup>/EBI Data Bank with accession number(s) AF248040.  $\ddagger$  To whom correspondence should be addressed. Tel.: 44-1132-332890; Fax: 44-1132-332835; E-mail: r.e.isaac@leeds.ac.uk.

<sup>&</sup>lt;sup>1</sup> The abbreviations used are: PPT-A, preprotachykinin A; TRP, tachykinin-related peptide; DTK, *Drosophila* tachykinin-related peptide; BAC, bacterial artificial chromosome; RACE, rapid amplification of cDNA ends; bp, base pair(s); LemTRP, <u>Leucophaea maderae tachykinin-r</u>elated peptide; LomTk-II, *Locusta migratoria* tachykinin-II; TAP, tachykinin-associated peptide; LTK-LI, <u>Locusta tachykinin-like immunoreactive</u>.

has recently been cloned from the mosquito A. aegypti that contains only a single tachykinin-like peptide, sialokinin I (24). Sialokinins are thought to be released into mosquito saliva and thus enter the host at a blood meal. However, as described above, the sialokinins are atypical compared with other invertebrate TRPs and may be the result of convergent evolution to exploit vertebrate tachykinin receptors (17). It therefore remains unclear whether other insect TRPs are synthesized from precursor molecules containing single or multiple copies of TRPs. Here we demonstrate for the first time the existence of an insect TRP prohormone gene (Dtk) that, like the mammalian PPT-A gene, codes for multiple copies of novel  $\underline{D}rosophila$ tachykinin-related peptides referred to as DTK peptides. In addition, we show that this gene is expressed in a restricted number of cell bodies in the brain and ventral nerve cord and in midgut endocrine cells, a site at which we demonstrate potent biological activity using the mature peptides. Sequence alignment showed that Dtk displays 31% sequence identity to human PTT-A in its substance P and neurokinin A progenitor sequences.

#### EXPERIMENTAL PROCEDURES

Materials—Unless otherwise stated, reagents were of molecular biology grade and purchased from Sigma. DTK peptides were synthesized via Fmoc (N-(9-fluorenyl)methoxycarbonyl) solid-phase methodology on an Applied Biosystems 433A peptide synthesizer according to previously described conditions (25) and were quantified by amino acid analysis.

Identification of a Tachykinin-like Prohormone in the D. melanogaster Genome—A TBLASTN search (26) of D. melanogaster genomic sequence (Berkley Drosophila Genome Project, Release Date September 1999) was carried out using the human PPT-A amino acid sequence (Swiss-Prot accession number P20366). Several BACs were identified that appeared to contain sequence coding for a tachykinin-like progenitor, and *ab initio* gene prediction was performed for a 5-kilobase pair region around these sequences using a *Drosophila*-specific version of FGENESH. Signal peptide prediction was performed using SignalP Version 1.1 (27).

cDNA Cloning and Sequencing-Using the FGENESH-predicted coding sequence of Dtk, 5'- and 3'-RACE primers were designed to amplify two overlapping fragments of the Dtk cDNA from an adult head cDNA library (Berkley Drosophila Genome Project GH Library): 5'-RACE primers, 5'-AATTAACCCTCACTAAAGGG-3' and 5'-AGCTTG-CTCATGATCGTCAC-3'; and 3'-RACE primers, 5'-GTAATACGACTC-ACTATAGGGC-3' and 5'-ACGTCTCCCACCAGCACTAC-3'. Touchdown polymerase chain reaction was performed using the following program: one cycle of 5 min at 95 °C; two cycles of 30 s at 95 °C, 1 min at 66 °C, and 2 min at 72 °C; two cycles of 30 s at 95 °C, 1 min at 64 °C. and 2 min at 72 °C; two cycles of 30 s at 95 °C, 1 min at 62 °C, and 2 min at 72 °C; two cycles of 30 s at 95 °C, 1 min at 60 °C, and 2 min 72 °C; two cycles of 30 s at 95 °C, 1 min at 58 °C, and 2 min at 72 °C; two cycles of 30 s at 95 °C, 1 min at 56 °C, and 2 min at 72 °C; 30 cycles of 30 s at 95 °C, 1 min at 55 °C, and 2 min at 72 °C; one cycle of 10 min at 72 °C. The largest polymerase chain reaction products from each reaction were cloned into a TA vector (Promega pGemTeasy TA vector) and sequenced.

In Situ Hybridization-A 1.2-kilobase pair fragment of the Dtk cDNA, derived by 5'-RACE and containing the first two exons and 126 bp of the third exon, was cloned into a TA vector. This plasmid was linearized using either SalI (for the sense probe) or NcoI (for the antisense probe), and digoxigenin-labeled sense and antisense riboprobes were synthesized using T7 and SP6 RNA polymerases (Promega), respectively, and digoxigenin-labeled dNTPs (Roche Molecular Biochemicals). In situ hybridization was performed on wild-type (Oregon R strain) embryos, larvae, or adults using a modification of the method described previously (28). Dechorionization, devitellinization, and fixation of embryos were performed as described previously (29). Embryos were stored in ethanol at -20 °C until required. Prior to fixing, embryos were rinsed in 1:1 ethanol/xylene; soaked for 2 h in xylene; and then rinsed successively in 1:1 ethanol/xylene, ethanol, and methanol and 1:1 methanol/postfix (10 mM potassium phosphate buffer (pH 7.0) containing 140 mM NaCl, 0.1% Tween 20, and 5% formaldehyde). Adult or third instar larval tissues were dissected and stored in Drosophila saline (0.7% NaCl and 0.03% Triton X-100) for up to 30 min before use. Samples were fixed in postfix for 20 min at room temperature, followed by three washes with PBT (10 mM potassium phosphate buffer (pH 7.0) containing 140 mM NaCl and 0.1% Tween 20), and were incubated with proteinase K in PBT (4 µg/ml) for 3 min at room temperature. The reaction was stopped by two washes with PBT containing 2 mg/ml glycine, and the samples were washed twice with PBT before being incubated with postfix for a further 20 min at room temperature. The tissues were washed with five changes of PBT, followed by one wash with hybridization buffer (5 $\times$  SSC containing 50% formamide, 10 mM KPO<sub>4</sub>, 140 mM NaCl, 1 mg/ml glycogen, 0.2 mg/ml sheared salmon sperm DNA, and 0.1% Tween 20 (pH 7.0)) diluted 1:2 with PBT and a further wash with hybridization buffer prior to a 1-h preincubation with hybridization buffer at 55 °C. Samples were then incubated for 43 h at 55 °C with 100  $\mu$ l of hybridization buffer containing 17 ng of either the sense or antisense riboprobe. Following hybridization, the samples were washed four times with hybridization buffer at 55 °C, followed by a final wash overnight with hybridization buffer at 55 °C. Samples were washed once with 50% hybridization buffer and 50% PBT, followed by four washes with PBT, and then incubated for 2 h at room temperature with 1.0 ml of preadsorbed alkaline phosphataseconjugated anti-digoxigenin Fab fragment (Roche Molecular Biochemicals) diluted 1:2000 with PBT. Unbound antibody was removed by extensive washing with PBT, and bound antibody was visualized by incubating the samples with staining buffer  $(1 \times \text{phosphate-buffered})$ saline, 100 mM NaCl, 9.4% formaldehyde, 50 mM MgCl<sub>2</sub>, 50 mM EGTA, 100 mm Tris-HCl (pH 9.5), and 0.1% Tween 20) either for 1 h at room temperature or for 16 h at 4 °C with one change of staining buffer. Development was stopped by extensive washing with PBT containing 50 mm EDTA.

Immunocytochemistry—Mixed-stage embryos and tissues of larval (second and third instar) and adult wild-type Drosophila (Oregon R strain) were used for immunocytochemistry. Tissues were fixed in 4% paraformaldehyde in 0.1 M sodium phosphate buffer for 3–4 h. Central nervous systems and intestines were used for whole-mount immunocytochemistry as described (30) with an antiserum raised against the locust TRP LomTK-I (31) at a dilution of 1:1000. Either the peroxidase/ anti-peroxidase technique or immunofluorescence with a secondary an tiserum tagged with Cy3 was used. A number of adult brains and intestines were used for immunocytochemistry (peroxidase/anti-peroxidase method) of cryostat sections. Preabsorption controls were made with antiserum (at 1:1000) incubated with 50 nmol of LomTK-I. The specificity of the antiserum has been established previously (30, 31).

L. maderae Hindgut Contraction Assay—The DTK peptides were tested for their myostimulatory action in an *in vitro* hindgut muscle contraction assay utilizing the gut of the cockroach L. maderae (32, 33). The increases in amplitude of spontaneous contractions of the hindgut were monitored at different concentrations of peptides and plotted in percent of maximum contraction for each intestine. Dose-response curves were fitted to a four-parameter logistic equation (GraphPAD Prism Version 2.0). Each peptide was tested on three to five intestines, and the L. maderae peptide LemTRP-1 was used as a reference. Results are presented as mean  $\pm$  S.E.

Drosophila Bioassay for Myotropic Activity-DTK-1-5 were tested for myotropic activity on isolated midgut from adult insects. The gut was severed anterior to the crop and posterior to the midgut-hindgut junction and dissected free of tracheal connections and malpighian tubules. The entire midgut was then transferred to a small chamber  $(\sim 250 - \mu l \text{ volume})$  and perfused with saline of the following composition: 105 mм NaCl, 5 mм KNO<sub>3</sub>, 5 mм CaCl<sub>2</sub>, 3 mм MgSO<sub>4</sub>·7H<sub>2</sub>O, 10 mм L-histidine, and 28 mM glucose. The pH was adjusted to 6.8 with 0.1 M NaOH (34). Contractions were recorded using an optical transducer as described (35, 36). The chamber was perfused at  $\sim$ 800 µl/min, and peptides dissolved in saline were added to the perfusate using a 0.5-ml injection loop and rheodyne valve. Each peptide was tested on five to six midguts at concentrations from 0.1 nM to 1  $\mu$ M, preliminary studies having found no evidence for receptor desensitization. Data were normalized by expressing contraction height as a percentage of the maximum response obtained with the TRP under investigation, and doseresponse curves were fitted to a four-parameter logistic equation (GraphPAD Prism Version 3.00) with the maximum and minimum responses set at 100 and 0%, respectively.

#### RESULTS

Identification of a Tachykinin Prohormone Gene in D. melanogaster Genomic Sequence and Cloning and Sequencing of the cDNA—TBLASTN searches using the human PPT-A amino acid sequence identified several BACs (GenBank<sup>TM</sup>/EBI acces-

#### {CCTGTTTCGTTTCGGCCTTATCCT

TCTG	CCGC	TTT	TAT	AAG	STCC	GTTG	TCG	CTTC	GAT	TGT	GCI	CAG	ITGI	TGC	ATAC	TTCI	CGG	CGCG	TTC }	
gqt	acq	cga	gca	ttt	gga	caa	aca	igtt	aga	tag	aac	tga	aag	gtga	aag	gata	att	ccc	caag	60
cqa	atī	qqc	- ttt	act	tca	qcg	cat	cga	ttt	cca	cgo	ccc	JCCt	aaa	ago	steg	geea	acat	cca	120
atq	cqc	cct	ctq	agc	qqt	ttg	ata	igco	ictg	gcg	ctc	gcto	gcto	gete	gct	cct	getg	gac	ggca	180
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cca	tcqa	aqt	qcq	qcq	gac	acg	gac	acc	gag	tcc	tco	ggc	ago	cae	gct	gaco	geed	cggo	cgcc	240
P	ธ	ŝ	A	A	D	Т	Е	т	Е	S	s	G	S	P	L	т	Ρ	G	А	
qaa	qaq	ccq	cgt	cgg	gtg	gta	aac	lcgc	gcg	ccc	acc	gtcc	ago	tto	at	tggg	gato	gego	cggc	300
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aaq	aag	gac	gag	gag	cac	gac	acc	tcg	gag	ggt	aac	tgg	gete	laa	atco	ggga	acco	cgat	ccg	360
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ctq	gati	tac	gcc	gac	gag	gag	gee	ggac	ago	ago	tac	gcg	ggag	yaad	ggg	gegg	gega	acto	gaag	420
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aaq	qca	ccq	ctq	gcc	ttt	gtt	ggo	ccto	jcgt	ggc	aac	jaaa	atto	cato	ccca	aato	caad	caad	ccgc	480
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ctq	tcc	qat	qtc	ctq	caq	age	cto	gac	igag	gag	cgo	ct	jcgg	ggat	age	cct	gct	gca	ggac	540
L	s	D	ั้ง	L	Q	ŝ	г_	E	E	E	R	L	R	D	S	L	L	Q	D	
ttc	ttc	qat	cqc	ata	qct	qqq	cqt	gat	qqc	tcc	geo	gto	iddo	caaq	jaga	age	geed	cac	cggg	600
F	F	D	Ŕ	ั้ง	Ā	Ğ	Ŕ	D	G	S	Ā	v	G	ĸ	R	A	P	т	G	
ttc	acq	qqc	atq	cqa	qqt	aaq	cqa	acco	gac	ctq	cto	gqco	ggg	ggg	gai	tgad	cgc	gga	ggcg	660
F	Т	G	м	R	G	ĸ	R	P	Ā	Ъ	L	A	G	G	D	D	A	Е	A	
gac	aaa	acc	acq	gag	cta	Icaa	caa	aaac	acdo	aca	ICCC	ato	caat	tco	tt	tqto	adda	gato	acac	720
gac D	gag E	gcc A	acg T	igag E	ctg L	caa 0	caa Q	aaq K	jegg R	Igcg A	P	ggto V	caat N	tco S	tti F	tgto V	9999 G	gato M	gcgc R	720
gac D qqc	gag E aag	gcc A aaq	acg T Igac	gag E atc	ctg L tcc	Q Cac	caa Q cac	aaag <b>K</b> acad	gegg R stac	Igcg <u>A</u> aao	ICCO P	ygto V .qca	caat <u>N</u> aqct	stcc s	Etti F	tgto V cqat	ggg G ttc	gato <u>M</u> ctai	gcgc R tqac	720 780
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gac D ggc G ttg L aag K gtg V	gag E aag aga R aag K cat H	gcc A aag gga G agc S cca P	acg T gac D aaa K gat D tgg W	gag E gtc V .cag .cag .ctg .ctg L Ictc	ctg L tcc S cag gag E tac Y	Caa Q Cac H Cgc R gggc G Ctg L	caa Q Q ttc F aac N tgc	aaag K gcac H cgcc A cgga G ggga G	gcgg R tac Y cgac D agtc V agaa E	Igeg A aag K ttc F G G aag <b>K</b>	P JCGt R aac N Jatt I CGa R	ygto V agca S agcu G agco A	A A Caaa K Caaa K Caaa C Caaa C C C C C C C	stee S L L E Caat N Caao N	F F S C C C C C C C C C C C C C C C C C	tgtg Cgat D ggCg A tgag E atto	gggg G S ggtg V gcaa Q C C L	gato M Ctai Y gcgi R agci A gggi G	gcgc R tgac D tggc G tctg L L aatg M	720 780 840 900 960
gac D ggc G ttg L aag K gtg V cga	gag E aag aga R aag K cat H ggc	gcc A aag gga G G S cca P aaa	acg T gac D aaa K gat D tgg W	igag E gtc V .cag .ctg .ctg I ctc	ctg L tcc S cag Q gag E tac Y aca	Caa Q Cac H Cgc R gggc G Ctg Ctg	caa Q Q Q tto F aac N tgo W	K gcac H cgcc A cgga G ggga G cgac	gegg R Etac Y Egac D Agtc R Agaa E gtac	Igeg A aag K ttc F G aag K Igec	P P R aac N att I Cga R gato	V gca A s ggt G agco A agco A agco A agco	A A Caaa K Caaa K Caaa D CCC P A A	stee S L Attr F Caat N Caac N	F F S C C C C C C C C C C C C C C C C C	tgtg cgat D ggcg A tgag E atto	gggg G S ggtg V gcaa Q Q ccto L agai	gaty M ctai Y gcgi R agci A ggg G tcti	gcgc R tgac D tggc G tctg L aatg M tgat	720 780 840 900 960 1020
gac D ggc G ttg L aag K gtg V cga R	gag E aag aga R aag K cat H ggc G	gcc A aag gga G G C Ca C Ca A A K	acg T gac D .aaa K gat D utgg W ucgg	igag E gtc V cag .cag .ctg .ctg Ictc L Icca	ctg L tcc S cag gag E tac Y gca A	Caa Q H CgC R gggC G Ctg L L L	cac Q Q tto F aac Tgo tco	K JCac H C G C G G G G G G G G G G G G G G G G	regg R Stac Y Sgac D Sgtc Y Sgaa E gtag	Igeg A aag K tto F G G G G K Igeg	ICCO P ICCO R R ICCO ICCO ICCO ICCO R ICCO ICCO	ygto V agca S agco A gtto	N Aget A Caaa K Cgao P Geeo P	stcc s tctc L attt F caat N caac N caac	F S S C S S S S S S S S S S S S S S S S	tgto V cgat D ggco A tgao E atto F gata	gggg G S ggtg V gcaa Q C C L agai	gaty M ctai Y gcgf R agcf A ggg G tcti	gcgc R tgac D tggc G tctg L tctg L aatg M tgat	720 780 840 900 960 1020
gac D ggc G ttg L aag K gtg V cga R gga	gag E aag aga R aag K cat H ggc aqt	gcc A aag gga G G C C a agc S c C a a a t C C a a a t C C	acg T gac D aaa K gat U gat W ucgg R	gag Egtc V cag .ctg .ctg Ictc L Icca P :tag	ctg L tcc S Q gag E tac Y aqc A	caa Q Cac H Cgc R ggc G Ctg L Ctt L ctt	caa Q Q tto F aav tgo tco s aaa	kaag gcac H cgcc A cgga G ggga G cgag E aact	regg R tac Y gac D agtc y agaa E gtag x	IGCS A Saag K Stto F G G G J G G K J G C G A A A A A A A A A A A A A A A A A	P Cgt R aac N Jatt I Cga R Jato	ygto V agca S agco A agco A gtto gtto	A aget A caaa K cgac D geec P gago	Etco S L Attt F Caat N Caao N Caao	F S S C S C S C S C S C S C S C S C S C	tgto V cgat D ggco A tgao E atto gata	gggg G S ggtg Q gcaa Q C c t agai	gatg M Ctai Y gcgf R agcf A gggg G tcti	gcgc R tgac D tggc G tctg L aatg L aatg tgat	720 780 840 900 960 1020
gac D ggc L ggc L g g g g g g g g g g g g g g	gag E aag aga R aga K cat H ggc agt	gcc A aag gga G G C C C C C A agc S C C A C C A C C A C C A C C A C C C C	acg T D aaa K gat D tgg W cgg R tct	gag gtc gtc V Q ctg L ctc Icca Icca Icca	ctg Lcc cag gag Ec y agc agc agc agc	Caa Q Cac H CgC R ggC G Ctg L Ctt L Ctt L Ctt	caa Q Q Q ttc F aac N tgc V tcc S aaa gaa	Raag R GCAC A CGCC A CGCC A CGCC A CGCC CGCC	regg R tac Y cgac D gtc V agaa F gtag x agg	Igeg A aag K tto F G G G G G G G G G G G G G C G G C C G C	P P Cogt R R Saac N Jatt I Jat <u>c</u> R Jat <u>c</u> R	ygto V gga ago A ggt G ggt ggt gga ago	A A Caaa K Caaa K Caaa C C C C C C C C C C	Etco S L Attt F Caat N Caat N Caat	F F S S S S S S S S S S S S S S S S S S	tgto v cgat D ggco A tgao E atto gata	gggg G S ggtg V gcaa Q Q cctg L agai	gaty M Ctai Y gcgi R agci G G tcti gggg	gcgc R tgac D tggc G tctg L tctg L tgat gacc gggc	720 780 840 900 960 1020 1080 1140
gac D ggc G ttg L aag V cga ggg ggg ggg tgg	gag E aag aga R cat ggc agt aga aga	gcc Aagg gga gga gga cca s cca kcc gca tcc gca	acg T gac D aaaa K gat D gat W cgg R cttt	gag gtc Vg Cag ctg ctg Lc c Lc c Lc c Lc c Lc c c c c c c c c	ctg L cag gag E tac y gag A agc aga aga	Caa Q Cac H Cgc R G G Ctg Ctg L Ctt L Ctt L Ctt	caa Q Q Q tto F aac W tco S aaa gaaa aca	K gcac H cgcc A cgcc A cgcc G ggga G G ggga Cgac E aact aaat	regg R stac y cgac D ggtc ggtc ggtag gtag ggga aaa	Igeg A Raag F G G G G G G G G C G G C G G C C C C C	P P Cgt R R aaco N Jatt I JCGa R Jat <u>c</u> Cat	ygto V cgca A cggt G ggt G ggt cgaa cgaa cgaa	A A Caaaa K Caaaa Caaaa Caaaa C Caaaa C Caaaa C D C C C C	stcc S L Attt F Caat N Caat N Caat S Caat	F F S S S S S S S S S S S S S S S S S S	tgtg cgat D ggcg A tgag E atto gata ataa	gggg G S ggtg V ggta Q gcat L aga acac	gati M Y gegg R age G G tet	gcgc R tggc D tggc G tctg L tggc L tggt ggc ggc tttg	720 780 840 900 960 1020 1080 1140 1200
gac D G G L G C L a A G V C G A G C C C C C C C C C C C C C C C C	gag E aag aga R aag K Cat G G C ag a aga aca	gcc A ag gga gga gg cca s a cca tcca gca tca a	acg T ggac D aaa K ggat V gg t t gg R t c t c t t t t t t t t t t t t t t t	gag Egtc V Cag Ctg Ctg L Ctag Ctag Ctag Ctag Ctag Ctag Ctag Ctag	ctg L Cag gag E tac y gag gag a agc agc agc agc agc agc	caa Q Cacc H Cgc R ggc G Ctg C Ctg L taca acagg	caa Q Q ttc F aa V tc S aaa gaa aa aa	A A A A A A A A A A A A A A A A A A A	JCGG R Etac Y CGC D JGC V AGGAS E JGGS CGGS CGGS C CGGS C CGGS C CGGS C CGGS C C C C	Igeg A Saag K Sttc F G G G G G G G G G G G G G G G G G G	P P C C C C C C C C C C C C C C C C C C	ygto V cgca A cago S cggt G ago G A gtto cgaa cgaa cgaa cgaa	Laat Nagot A Caaaa K Cgao P Jgcoo P Jggot Aagt Jggot	S S CCCCC L Attt F CCAAT N CCAAT CCAAT CCAAT CCAAT CCAAT CCAAT CCAAT CCAAT	$\frac{F}{F}$	tgt <u>y</u> V D gggc <u>y</u> A tgag E attgag gata gata ttt <u>t</u>	G G S S G G C C C C C C C C C C C C C C	gati M Y gcgg R agcc A gggg G tct gggg cctg gaa	gcgc R tgac D tggc G tctg L ggc L tctg ttgat ggcgc ttta	720 780 840 900 960 1020 1080 1140 1200 1260
gac D G G G G C G G C G G G C G C G C C C C	gag E aag R aag R aag K C A G C A G C a g G C a g a a a a a a a a a a a a a A B C A B C C A C A C A C A C A C A C A	gcc Aag gga gg gg gg c P aa K c c aa K c c aa K c c aa K c c aa S c P aa K ag c C P aa S c P aa S c P aa S c P aa S c P aa S c P aa S c P aa S c P a S c S c P a S c S c P a S c S c P a S c S c S c S c S c S c S c S c S c S	acg T gac D aaa K gat D gat W gat W cgg R ctcttttaat	gag Egtc V Cag Ctg Ctg Ct Cag Ctag Ctag Ctag Ctag	ctg L tcc S Q gag E tac Y gaa A gaa agc agaa agaa	Caa Q Cac H CGC R GGC CL L CLL L CLL L CLL CLL CLL CLL CL	caa Q Q Q tto F a N C W C S a a a c a c Q tto F a C N C C Q C Q C C Q C C Q C C Q C C Q C C C Q C C C Q C C C Q C	A A A A A A A A A A A A A A A A A A A	JCGG R Stac VCG D JCG JCC JCG JCC JCG JCC JCC JCC JCC JCC	Igeg A Saag K Sttc F G G G G G G G G G G G G G G G G G G	P P R R R R R R R R R R R R R R R R R R	ygto V gca A cago S cggt G gtto gato cgaa cgaa cgaa cgaa cgaa	A A Caaaa K Cgaaa D Ggcco P Gggco Aagt Gggco Cgaaa	S S CCCC L Attt F CCAAC N CCAAC N CCAAC CCAAC CCAAC CCAAC CCAAC CCAAC CCAAC CCAAC CC	F F S S S S S S S S S S S S S S S S S S	tgt <u>y</u> Cgat D ggcy A tgay E atto gat gat ttty tat	G G G C C C C C C C C C C C C C C C C C	gatg M Y gcgg R agcc G G tct gggg gaa ggaa gca	gcgc R tgac D tggc G tctg tctg tctg tctg tctg tctg tc	720 780 840 900 960 1020 1080 1140 1200 1320
gac D ggc G G L t g C L g g g g g g g g g g g g g g g g g	gag E aag R aag K Cat H ggc G t aga aga aga aga gaa gaa	gcc Aag ggG ggG ggC cP a K ccaa taa tcaattaa	acg T gac D aaa S D S C D C C C C C C C C C C C C C C C	gag gtc Vg caq ctc cc cc cc cc cc cc cc cc cc cc cc cc	ctg L S Q g g E C Q g g E C Y g ca S Q g g E C Y a g ca S Q g g E C Y a g ca S S C Q g g E C S S C A S S S C A S S S S S S S S S S	Caa Q H Cac R G G G G C C C C C C C C C C C C C C C	caa Q Q Q T F a C Q T F a C Q T F a C Q C Q C Q C C Q C C Q C C Q C C Q C C Q C C Q C C Q C C C Q C C Q C C C Q C C C Q C C C Q C	A a a g g c a c g c a c g g c c g g g g g g g g g g g g a a c g g g g g g g g g a a c t a a a c g g g a c g g g g a c g g g g g a c g g g g g a c g g g g g g g g g g g g g g g g g g g	JC99 R Stac D JCD JC JC JC JC JC JC JC JC JC JC JC JC JC	Igeg <u>A</u> aag Ktt Ggeg Jaaa ggeg Jaaa ggeg Jaaa ggeg Jaaa ggeg Jaaa Jaa	P P Cgt R aac N Jatt I Jatg R Jatg L L C Jatt L C C C C C C C C C C C C C C C C C C	ygto V cgca A cago S cggt G ago C gaa cgaa cgaa cgaa cgaa cgaa cgaa cga	N Aget A caaa K cgaa D ggco P ggco Agggo gggo cgaa	S Control	F F S S S S S S S S S S S S S S S S S S	tgt <u>g</u> Cgat D ggc <u>g</u> A tga <u>g</u> E attc F gata ttt <u>g</u> tati	gggg G S ggt Q gcat Q gcat Q C C C L agat	gatg M Ctai ggg R agc G G Ctct ggg G Ctct ggg gaa aaa	gcgc R tgac D tggc G tctg tctg tctg tctg tctg tctg tc	720 780 840 900 960 1020 1080 1140 1200 1320 1380
gac D G G L G G C C C C C C C C C C C C C C C	gag E aaga R aaga R aaga C A C A C A C A C A C A C A C A C A C	gcc Aag gG c gG c Paa tccaataa ataaa	acg T gac D aaa K gD C R t t g C R t t t t t t t t t t t t t t t t t t	gag EC V g U C C C C C C C C C C C C C C C C C C C	ctg Lcc Qg gEc Qg Ec Qg Cag agaa agaa agaa agaa agaa agaa ag	caa Q R ggc C C C C C C C C C C C C C C C C C C	caa Q cac Q tto F a C T T C S a a a a a a a a a a a a a a a a a a a	A a a g R g c a c a c a c a c a c a c a c a c a c	JC99 R Stac Stac Stac Stac Stac Stac Stac Stac	Igeg A aag Ktt Gg G G G G G G G G G G G G G G G G G	personal products of the product of	ygto V gca A cago S cggt A ggt C gaa cgaa cgaa cgaa cgaa cgaa cgaa cga	A aget A caaa K cgac D ccc P gccc P ggct A agt ggct a agt ggct a agt a agt a agt a agt a agt a agt a agt a agt b caaa A caaaa A caaa A caaa A caaa A caaa A caaa A caaa A caaa A caaa A caaa A	Etco S L L Caattr F Caaat N Caaao N Caaao N Caaao L Caaao L Caaat L Caaat L Caaat L Caaat L Caaat L Caaat L Caaat L Caaat L C Caaat L C C C C C C C C C C C C C C C C C C	Etti F Etti S S S S S S S S S S S S S S S S S S	tgt <u>g</u> cgat D ggcg A tgag attgag gata gata gata tttt tati	gggg G G G G G G G G G G G G G	gatg M Ctai Ctai gcgi R Agg G G Ctg gaa A G G G Ctg gaa aaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa	gcgc R tgac D tggc G t t t ggg t t t gggt t t t a c c c gggt t t a c c c gggt t ggg t c t ggc t t t t	720 780 840 900 960 1020 1080 1140 1200 1320 1380 1440
gac D ggc G ttg G ttg V a K gV ggggggggt tttaaa ata	gag E gag R aga R a C aga A a C aga aga aga aga ggt t a C	g A g g g g c P a K c c a a a c P a K c c a a a c P a K c c a a a t g t c a t a a t a t a t a t a t a t a t a	acg T gac D aaa K gat D gac R tt gat t gat t gat t gac R t t t t t t t t t t t t t t t t t t	g ag E c V g C L c C P g g G L c L c C P g g g a c L c C L c C P g g g a c L c L c C L c C L c L c L c L c L c L	ctg Lcc Qg EcQ Qg ECQ EQ EQ EQ EQ EQ ECQ ECQ ECQ ECQ ECQ	caa Q R R G G C C C C C C C C C C C C C C C C	caa Q cac Q tt F a N ge t S aaaaa a a t co a a a a a a a a a a a a a a a a a a a	Raag gcac H cgcc Ggga Ggga E Ggga Cgag Cgag Cgag Cgag Cgag Cgag Cgag	JC99 R Stac Y C90 V JC J JC J JC J JC J JC J JC J JC J J	Igeg <u>A</u> iaag itto iaag igeg iaaag igeg iaaag igeg iaaag igeg iaaag igeg iaaag igeg iaaag igeg iaaag iaag iaag igeg iaaag igeg iaaag igeg iaaag igeg iaaag igeg iaaag igeg iaaag igeg iaaag igeg iaaag igeg iaaag igeg iaaag igeg iaaag igeg iaaag igeg iaaag igeg	P P P P P P P P P P P P P P	ygto V gcago A cago S cggt A ggt G ggt cgaa cgaa cgaa cgaa cgaa cgaa cgaa	A agot A caase K cgac P ggg a agt gg	S S L Attt N Caat Caat Caat Caat Caat Caat Caat	F F S S S C C C C C C C C C C C C C C C	tgt <u>g</u> cgat D ggcg A tgag gat gat gat gat attt ctat aagi	gggg G G G G G G G G G G G G G	gatg M Ctai Ctai SCG R A G G G G C G G C C G G C C C G G C	gcgc R tggac D gc G g C L G g t L tgac ttac tac tgac ttac tac tac tac tac tac tac	720 780 840 900 960 1020 1080 1140 1260 1320 1380 1380
gac D ggc G ttg L a gg gg ggg gggg tttta a ta c tta a ta c ggg ggggggt tta a ta a	gag E gag a K a a R g C H C G taagad g g g a c a d g g g a c a a g g g a c a t f c c c c a c a a g g g a c a c c c c c c	g A g g g g c P a K c c a a t a a t a a t a a t a t a t a a t t a t a t t a t t a t t a t t a t t a t t a t t a t t a t t	acg T gac D aaa K t g D g C R t t aaat S C R t t t aaa C R t t aaa C R t t g g D g ac A C D g a C D aaa K t D g g C D aaa K t D g g C D aaa K t D g g C D aaa K t D g g C D aaa K t D g g C D aaa K t D g g C D aaa K t D g g C D aaa K t D g g C D aaa K t D g g C D aaa K t D g g C D aaa K t D g g C D aaa K t D g g C D aaa K t D g g C D aaa K t D g g C D aaa K t C D g g C D S C S C D S C S C S C S S C S S S S	g ag g E c V g C L c C P g g G C L c C P g g G C L t c L a c c P g g G C L t c L t t t t t a a t a c a c a c a c a c a c	ctg Lcc S gag E ac gag E ac gaa agaa agaa agaa agaa agaa agaa ag	caa Q H CAC B G G G G C L L L C C C L L L C C C C L L C	caa Q Q Q Q T F a N 99 T S aa a	A a a g g c a c g g c a c g g c a c g g g a c g g g a c g g g a c g g g a c g	JC 99 R C T AC C 9 D C 19 C 19 C 19 C 19 C 19 C 19 C 19 C 19	Igeg A A Control of the second A Control of the second	P P P P P P P P P P P P P P	ygto V cgca A cago S cggt G ggt cgaa cgaa cgaa cgaa cgaa cgaa cgaa	a a a a a a a a a a a a a a a a a a a	S S L Attt Caat Caat Caat Caat Caat Caat Caat	F F S S S C C C C C C C C C C C C C C C	tgt <u>c</u> cgat D ggc <u>c</u> A tga <u>c</u> E ctaa F gata sattt ctai	ggg G G S ggt V gcaa acae acae acae ggt tttt tttt taaa	gatg M Y gcgl R gcgl R G G G C C C ggg a a a a a a a a a a a a a a a	gege R tgac D G G tetg G tetg tet	720 780 840 900 960 1020 1080 1140 1200 1320 1320 1320 1440 1558

FIG. 1. Nucleotide and deduced amino acid sequences of prepro-DTK. A BAC genomic sequence (GenBank<sup>TM</sup>/ EBI accession number AC020418) upstream of the sequenced cDNA is included in *braces*. This region contains the predicted transcriptional start site (NNPP Version 1.2) shown in *large font* and a TATA box. The 870-bp Dtk open reading frame begins at nucleotide 121 of the sequenced cDNA and finishes at nucleotide 990 (including the stop codon). Predicted mature DTK peptides are underlined, and flanking pairs of basic residues representing processing sites are shown in boldface. The predicted N-terminal signal peptide is indicated in boldface italics. A consensus polyadenylation site is shown in *bold lower-case italics*, and the first stop codon is marked by an asterisk. Nucleotides 1-76, exon 1; nucleotides 77-771, exon 2; nucleotides 772-976, exon 3; nucleotides 977-1535, exon 4; and nucleotides 1536-1558, poly(A) tail.

sion numbers AC007692, AC007889, and AC007890) containing genomic sequence coding for part of a tachykinin-like peptide progenitor sequence that aligned with the neurokinin A progenitor sequence of human PPT-A. Subsequently, a fourth BAC was also identified (GenBank<sup>TM</sup>/EBI accession number AC020418). Ab initio gene prediction revealed a putative threeexon gene. The coding sequence of this predicted *Drosophila* tachykinin gene (*Dtk*) contains five copies of glycine-extended tachykinin-like peptides, each flanked by pairs of basic residues, and a predicted N-terminal signal peptide for targeting of the prohormone to the secretory pathway.

A 1558-bp cDNA was amplified by polymerase chain reaction from an adult Drosophila head cDNA library as described under "Experimental Procedures." The cDNA contains an 870-bp open reading frame, 120 bp of 5'-untranslated region, and a 568-bp 3'-untranslated region that includes a 23-bp-long poly(A) tail (Fig. 1). Translation of the open reading frame confirmed the prediction of sequences corresponding to five tachykinin-related peptides (DTK-1-5) (Table I) flanked by pairs of basic residues, which presumably serve as processing sites for prohormone convertases. In addition to the DTK peptides, an additional six possible peptide products, called tachykinin-associated peptides (TAP-1-6) (Table I), exist in the prohormone sequence. Three of these peptide sequences (TAP-1, TAP-2, and TAP-5) have a C-terminal Gly residue and therefore have the potential to be processed to amidated peptides, as shown in Table I. TAP-5 has a structure at the C terminus similar to a recently isolated tachykinin-related peptide from another dipteran, Sto-

#### TABLE I

#### Amino acid sequences of DTK-1–5 and TAP-1–6

Each of the DTK peptides contains a conserved  $FX_1GX_2R$ -amide motif common to other invertebrate tachykinin-related peptides. With the exception of DTK-2,  $X_2$  is a methionine residue. The conserved Nterminal AP motif in each DTK is likely to protect against degradation by aminopeptidases. Of the TAPs, TAP-5 possesses a C-terminal FVAVR-amide motif and is reminiscent of a tachykinin.

Peptide	Sequence
DTK-1	APTSSFIGMR-amide
DTK-2	APLAFVGLR-amide
DTK-3	APTGFTGMR-amide
DTK-4	APVNSFVGMR-amide
DTK-5	APNGFLGMR-amide
TAP-1	DEEHDTSEGNWLGSGPDPLDYADEEADSSYAEN-amide
TAP-2	FIPINNRLSDVLQSLEEERLRDSLLQDFFDRVAGRDGSAV-amide
TAP-3	PALLAGGDDAEADEATELQQ
TAP-4	DVSHQHY
TAP-5	AALSDSYDLRGKQQRFADFNSKFVAVR-amide
TAP-6	SDLEGNGVGIGDNHEQALVHPWLYLWGE

*moxys calcitrans* (37). All the other TAPs are novel sequences showing little homology to known bioactive peptides.

Genomic Organization of the Drosophila Prohormone Gene Dtk—BACs containing the Dtk sequence (GenBank<sup>TM</sup>/EBI accession numbers AC007692 and AC007889) map to region 87A4-87B2 of the right arm of chromosome 3. Dtk spans 11.2 kilobase pairs of genomic sequence and contains four exons and three introns, the first intron being 9292 bp in size (Fig. 2).



FIG. 2. Schematic diagram showing the genomic organization of the *Dtk* gene (not to scale). Exons are represented by *boxes*, with the first exon shown to the left. The position of the gene relative to the BAC genomic sequence (GenBank<sup>TM</sup>/EBI accession number AC020418) is shown on the *bar* below. The positions of each of the exons in relation to the BAC genomic sequence (AC020418) are as follows: exon 1, nucleotides 21561 to 21486; exon 2, nucleotides 12194 to 11500; exon 3, nucleotides 11296 to 11092; and exon 4, nucleotides 10883 to 10325. DTK progenitor sequences are labeled and indicated by *black boxes*.

Sequences coding for DTK-1-4 are contained in exon 2, whereas the DTK-5 progenitor sequence is contained in the third exon. The first exon contains entirely 5'-untranslated region. Analysis of the genomic sequence upstream of Dtk (NNPP Version 2.1 (38, 39)) revealed a predicted transcriptional start site 38 bp upstream of the 5'-end of the sequenced Dtk cDNA and a TATA box 30 bp farther upstream. The cDNA that we have sequenced therefore appears to be almost full-length.

Expression Pattern of the DTK Prohormone-The central nervous system and the intestines of dipteran insects are known to be rich sources of tachykinin-related peptides; and therefore, these tissues, along with 0-24-h embryos, were selected for in situ hybridization studies. Strong staining was observed in a group of 14 cells plus one isolated cell in the midgut of stage 17 embryos (Fig. 3). Staining in the midgut was not observed at earlier stages. In addition, at the same stage, Dtk was expressed in a pair of medially located unidentified cells, just posterior to the brain, and in two lateral groups of cells that may be associated with tracheae. Expression of Dtk in the larval gut was restricted to cells with endocrine cell-like morphology in the posterior midgut, just anterior to the malphigian tubules (Fig. 4, A and B). Expression in the adult female gut was also restricted to the midgut, with no Dtk transcript detected in the hindgut (Fig. 4C). However, in contrast to the third instar larval midgut, Dtk expressing-cells were detected throughout the length of the adult female midgut, with the exception of a region toward the middle of the midgut (one-third of the midgut length) in which we have not been able to detect *Dtk*-expressing cells in any of the samples examined (more than five specimens). The cells showing a hybridization signal in the female adult midgut also appeared to be endocrine cells; however, they were more numerous, smaller, and more elongated than those staining in the larval midgut (Fig. 4C). In the brain of third instar larvae, the Dtktranscript was detected in a restricted number of neuronal cell bodies (Figs. 4, *E* and *F*; and 6*A*). There were nine pairs of cell bodies displaying a strong hybridization signal in the brain and another four pairs displaying a weaker signal (12 well labeled central nervous systems were analyzed). In the ventral nerve cord, two pairs of cell bodies were detected in the metathoracic neuromere and eight pairs in abdominal neuromeres (Figs. 4Gand 6A).

Tissue Distribution of Drosophila Tachykinin-related Peptides—Immunocytochemistry was performed on Drosophila brain, midgut, and hindgut with an antiserum to the locust peptide LomTK-I. This antiserum is known to cross-react with peptides of the family of invertebrate TRPs, but with no other known peptides. In the larval central nervous system, 22 neurons were consistently labeled with this antiserum. Of these, 18 were in the brain and subesophageal ganglion (Figs. 5, A and B; and 6B). Two pairs of Locusta tachykinin-like immunoreactive (LTK-LI) neurons (DN 1 and DN 2) with cell bodies in the subesophageal ganglion formed varicose axons that descended throughout the ventral nerve cord (thoracic and abdominal ganglia) (Figs. 5A and 6B). Two pairs of tritocerebral neurons (Tr 1 and Tr 2) formed branches in tritocerebral neu-



FIG. 3. Dorsal view of a stage 17 embryo. Strong hybridization was seen in a group of 14 cells in the midgut (MG) plus an isolated cell, also apparently part of the midgut (arrow).



FIG. 4. Micrographs showing the Dtk mRNA transcript in Drosophila tissues detected by in situ hybridization. A, posterior midgut of third instar larvae. Strong hybridization signals are visible in large endocrine cells. Hybridization was also seen in a number of smaller cells. Malphigian tubules are labeled with an arrow. Bar = 50 $\mu$ m. B, detail of third instar larval midgut endocrine cells. Bar = 25  $\mu$ m. C, adult midgut where a larger number of smaller endocrine cells are seen to contain Dtk mRNA compared with the third instar larval midgut. Malphigian tubules are labeled by an arrow.  $Bar = 50 \ \mu m. D$ , detail of adult midgut endocrine cells expressing Dtk mRNA.  $Bar = 25 \ \mu m. E$ , distribution of Dtk mRNA in neuronal cell bodies in larval brain. Bar = 50 µm. F, detail of Dtk-expressing neuronal cell bodies. Pr, protocerebral cell bodies; Tr, tritocerebal cell bodies; DN 1 and DN 2, large descending neurons.  $Bar = 25 \ \mu m$ . G, distribution of Dtk mRNA in the ventral nerve cord of a third instar larva. The arrow indicates the metathoracic cell body. Segmentally distributed cell bodies are seen in abdominal ganglia (Abd g).  $Bar = 25 \ \mu m$ .

rophils (Fig. 5*B*). The remaining five pairs of neurons (*Pr* 1–5) formed processes locally in the protocerebrum (Figs. 5, *A* and *B*; and 6*B*). Two pairs of LTK-LI neuronal cell bodies were seen in the metathoracic part of the ventral nerve cord (Fig. 6*B*). Immunolabeling of cell bodies in the abdominal neuromeres was



FIG. 5. Micrographs of tachykinin immunoreactivity in Drosophila tissues (using antiserum to LomTK-I). The tissues were whole-mounted, and a Cy3-tagged secondary antiserum was used. A, LTK-LI neurons in the brain and anterior thoracic ganglia of a third instar larva. The anterior large descending neurons (DN 1) of the subesophageal ganglion are in focus, and one of their descending axons can be seen at the arrow. B, detail of LTK-LI cell bodies in the brain of a third instar larva. Focus is on arborizations in tritocerebral neurophils. DN 1 and DN 2 (large descending neurons), Pr (protocerebral cell bodies), and Tr (tritocerebal cell bodies) are identifiable neuronal cell bodies. C, LTK-LI neurons in the central nervous system of a second instar larva. In focus are DN 1 and a few protocerebral cell bodies. D, posterior midgut of a third instar larva (just anterior to the Malpighian tubules (MT)). Note a few large endocrine cells displaying immunoreactivity (e.g. at the arrow). E, posterior midgut of an adult fly. A larger number of smaller LTK-LI endocrine cells can be seen. F, detail of two endocrine cells in side view. The gut lumen is toward the bottom of figure, and the gut surface to the body cavity is at the top. Note the unstained nuclei toward the apical end of the cells.

inconsistent, but occasionally, segmentally arranged cell bodies could be seen. The same neurons were also present in the brain and ventral nerve cord of second instar larvae (Fig. 5*C*). In the adult brain of *Drosophila*, there are many more LTK-LI neurons, and they form complex arborizations in brain neuropils (18). The two pairs of descending neurons (*DN 1 and DN 2*) were also seen in adults. In the adult ventral nerve cord, the metathoracic cell bodies were still present, in addition to a pair of large cell bodies in the posterior part of the abdominal neuromeres (data not shown).

In the midgut of third instar larvae, there were ~20 LTK-LI cells with a morphology similar to that of endocrine cells (Fig. 5D). These were found only in the posterior region of the midgut, just anterior to the attachment of the Malpighian tubules. The cells were rather large, with a diameter of ~9.5 ± 0.27  $\mu$ m (mean ± S.E., n = 38; from three intestines). In the adult midgut, there were a much larger number of slightly smaller LTK-LI endocrine cells (Fig. 5*E*). These had an elongated shape and often spanned the gut epithelium (Fig. 5*F*). Their diameters were 4.4 ± 0.13  $\mu$ m (mean ± S.E., n = 36; from three intestines). As seen by *in situ* hybridization, the LTK-LI endocrine cells were restricted to the anterior and posterior thirds of the midgut.



FIG. 6. A, tracing of cell bodies expressing Dtk mRNA in the central nervous system of a third instar larva. Cell bodies displaying strong hybridization signals are shown in black, and weaker signal are depicted by unfilled cell bodies. A strong signal is seen in five pairs of smaller protocerebral cell bodies  $(Pr \ 1-5)$  and two pairs of larger cell bodies (DN 1 and 2) in the subesophageal ganglion as well as two pairs of tritocerebal cell bodies (Tr 1-2). In the ventral nerve cord, there are two pairs of cell bodies in the metathoracic ganglion (MT 1) and eight pairs in the abdominal ganglia (Abd gangl). B, tracing of LTK-LI neurons in a third instar larva (strongly labeled neurons shown as black and *unfilled* are weakly labeled). The axons are traced only for the large descending neurons (DN 1) with cell bodies in the subesophageal ganglion. The other pair of subesophageal descending neurons (DN2) have similar axons, running more laterally than those of DN 1. Five pairs of protocerebral (Pr 1-5) and two pairs of tritocerebral (Tr 1-2) LTK-LI cell bodies were seen in all specimens. In the metathoracic ganglion, two pairs of neurons were detected (MT 1). In abdominal ganglia, immunolabeling was variable; often only anterior located cell bodies were seen.

Biological Activity of the Drosophila Tachykinins-Three of the DTK peptides (DTK-1-3) and LemTRP-1 were tested in the L. maderae hindgut contraction assay. This was done since all of the other arthropod TRPs were isolated with the aid of this assay, alone or in combination with enzyme immunoassay, and dose-response curves have been constructed for all (19). Doseresponse curves for each of these peptides are shown in Fig. 7. DTK-1 and -3 were about equally bioactive. The concentrations yielding a half-maximum response (EC $_{50}$ ) were  ${\sim}3.6 imes10^{-8}$  M for DTK-1 and  $5.1 \times 10^{-8}$  M for DTK-3. DTK-2 was nearly an order of magnitude less bioactive, with an  $\mathrm{EC}_{50}$  of  $1.5 imes 10^{-7}$  M. In comparison, LemTRP-1 was almost an order of magnitude more bioactive than DTK-1 and -3 (EC\_{50} = 4.9  $\times$   $10^{-9}$   ${\rm {\tiny M}}).$  The differences in  $EC_{50}$  values between LemTRP-1 on one hand and DTK-1 and -3 on the other were statistically significant (p <0.01; analysis of variance), as was that between DTK-1 and -3 and DTK-2 (p < 0.05).

The myotropic activity of all five DTK peptides was tested on midguts from adult D. melanogaster. This tissue was selected because results from both immunocytochemistry and in situ hybridization had revealed high densities of Dtk-expressing midgut endocrine cells. The majority of midguts removed from adult flies were not spontaneously active, but responded to the addition of supra-threshold concentrations of DTK-1 to the perfusate with several phasic contractions that lasted for several minutes (Fig. 8). The peak height of the contractions was dose-dependent and was used to generate dose-response curves. Fitted curves are shown in Fig. 9, and  $EC_{50}$  values are listed in Table II. When tested on adult midgut, the rank order of potency was DTK-3 > DTK-5 > DTK-1 > DTK-2 = DTK-4. To compare their efficacy, each DTK peptide was tested at 1  $\mu$ M on the same preparation, and the peak height of contraction was expressed as a percentage of the maximum response. In general, this concentration is sufficient to evoke a maximum response (Fig. 9), although the dose-response curve for DTK-2

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FIG. 7. Dose-response curves for the myostimulatory activity of three of the DTK peptides on isolated *L. maderae* hindgut. As a reference, the *L. maderae* peptide LemTRP-1 was tested. Each point represents the mean  $\pm$  S.E. of three to five replicates. Note that LemTRP-1 is about an order of magnitude more bioactive than the two best DTK peptides.



FIG. 8. Representative trace showing the effect of 3-30 nm DTK-1 upon contraction of isolated *D. melanogster* adult midgut. *Scale bars* indicate amplitude of contraction (y axis) and time (x axis).



FIG. 9. Dose-response curves for the myostimulatory activity of each of the five DTK peptides on isolated *D. melanogaster* adult midgut. The curves presented are the mean results of experi-

ments performed on five to six individual midguts. does not appear to plateau. The experiment was repeated seven times, varying the order in which the peptides were added to the gut. The results are included in Table II. When tested at 1  $\mu$ M, the activities of DTK-1, -3, -4, and -5 did not differ significantly, whereas the response to DTK-2 was <50% maximal. The small amount of synthetic DTK-2 available precluded testing this peptide at higher concentrations. However, it is clear that DTK-2 has both low potency and low activity on the midgut of adult *D. melanogaster*. Similar EC<sub>50</sub> values were also obtained using larval midgut (data not shown).

#### DISCUSSION

A BLAST search using the human PPT-A amino acid sequence led to the identification of a *D. melanogaster* preprotachykinin gene (Dtk). Some minor errors in the *ab initio* prediction of the coding region from the genomic sequence were

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EC<sub>50</sub> values for DTK-1–5 were calculated from the dose-response curves shown in Fig 9. The effect of 1  $\mu$ M of each DTK upon adult midgut is shown in the third column. DTK-2 demonstrated both low potency and low activity compared with the other DTK peptides as judged by its higher EC<sub>50</sub> value and the poor response to the peptide when tested at 1  $\mu$ M.

Peptide	$\mathrm{EC}_{50}$	95% confidence limits	Maximum response $(\text{mean} \pm \text{S.E.} (n))$				
	пМ	пМ	%				
DTK-1	16.3	12.4 - 21.5	$84.5 \pm 6.2 \ (7)$				
DTK-2	72.4	51.2 - 102.3	$44.2 \pm 6.3  (7)$				
DTK-3	7.0	4.5 - 11.1	$86.9 \pm 4.6 (7)$				
DTK-4	79.1	65.9 - 95.0	$80.7 \pm 6.6  (7)$				
DTK-5	11.5	7.4 - 18.0	$86.8 \pm 4.2 \ (7)$				

evident from comparison with the deduced amino acid sequence of a Dtk cDNA amplified from a head cDNA library. The genomic region containing the initial exon was not included in the original prediction due to the large size (9.2 kilobase pairs) of the first intron. In addition, the predicted boundary between the second and third exons appears to have been predicted incorrectly.

Each of the tachykinin-like sequences in Dtk is present in a glycine-extended form flanked by pairs of basic residues. This is a common feature of many insect peptide hormone precursors, where the initial processing step involves hydrolysis of the peptide bond on the C-terminal side of a pair of basic amino acids (40). The presence of multiple members of a peptide family in a single prohormone is common in insects, and this prohormone structure is somewhat similar to that seen in a gene encoding tachykinins from the echiuroid worm *U. unicinctus* (41). This gene contains seven copies of TRPs, and in this respect, *Dtk* appears to be more similar to the echiuroid TRP gene than to the sialokinin gene from the mosquito *A. aegypti*, which encodes a single tachykinin peptide, exclusively expressed in mosquito salivary glands (24).

Tissue-specific splice variants of the mammalian tachykinin prohormone mRNA are known to occur (7), and it is therefore interesting to note that the genomic organization of the *Dtk* gene provides the potential for alternative mRNA splicing to produce all five DTK peptides or just DTK-1-4 since the DTK-5 progenitor sequence is coded for by a separate exon. Evidence for tissue-specific expression of insect tachykinins comes from analysis of the distribution of LemTRPs in the cockroach *L. maderae*, where LemTRP-3 and -4 have been isolated only from the midgut, and LemTRP-6-9 appear to be brain-specific (11, 12). However, preliminary reverse transcriptase-polymerase chain reaction experiments to detect an alternatively spliced *Dtk* transcript lacking the third exon in *Drosophila* adults and third instar larvae have not identified an alternative transcript.<sup>2</sup>

Conserved structural features of the DTK peptides include an N-terminal Ala-Pro sequence and the C-terminal  $FX_IGX_2R$ amide motif, where  $X_I$  is Ile, Leu, Val, or Thr, and  $X_2$  is Met or Leu. This FXGXR-amide motif is a common feature of almost all invertebrate tachykinins isolated to date (19), and the Argamide residue is required for their biological activity in the cockroach hindgut bioassay (42). The N-terminal Ala-Pro residues are likely to protect the peptides from proteolytic degradation by aminopeptidases since few peptidases are capable of hydrolyzing peptide bonds adjacent to prolyl residues due to the structural constraints imposed by the inclusion of this imino acid (43). This N-terminal motif is therefore common among invertebrate peptides (19), and a prolyl residue is also

<sup>2</sup> R. J. Siviter, unpublished results.

		*	*	*	*	***	*	* ** ***		
TKN1_Human	:	RRP	KPQ	QFI	GI	MGKR	DAD	SSIEKQVALLKALYGHGQISHKRHKTDSFVGLMGKR	:	54
preproDTK	:	KR-	APT	GF:	ſGM	RGKR	PAL	LAGDDDAEADEATELQQ <b>KRAPVNSFVGMRGKK</b>	:	49

FIG. 10. ClustalX alignment of prepro-DTK and human PPT-A amino acid sequences. The overall sequence identity was 8%. Only the region that aligns with the substance P and neurokinin A progenitor sequences is shown. In this region, sequence identity rises to 31%.

present at an equivalent position in a number of mammalian peptides. However, proline-specific dipeptidyl peptidase activity can selectively cleave the Xaa-Pro-containing TRPs and vertebrate tachykinins (44, 45).

Although we have presented DTK-1 and -2 as a decapeptide and nonapeptide, respectively (see Table I), and have used these synthetic peptides for assessing biological activity, the presence of an alternative N-terminal dibasic processing site in each case allows the possibility that N-terminally extended forms of both peptides may be produced. The sequences of the resulting peptides would be VVKRAPTSSFIGMR-amide and LKKAPLAFVGLR-amide, respectively. These sequences are reminiscent of the mammalian tachykinins neuropeptide K and neuropeptide  $\gamma$ , both of which are N-terminally extended forms of neurokinin A and contain an unprocessed Lys-Arg motif. Several examples of invertebrate N-terminally extended TRPs also exist, e.g. the Leucophaea TRPs LemTRP-2 and -3, which contain unprocessed KR and KK sites, respectively (11). Whether these Leucophaea extended TRPs represent processing intermediates or are final products in some tissues is not clear, but both are biologically active in a Leucophaea hindgut contraction bioassay, and LemTRP-1 and -3 show similar  $EC_{50}$ values using this assay (11, 33). It would therefore appear that if similar N-terminally extended DTK peptides exist, they would most likely be biologically active. The ability to produce N-terminally extended peptides therefore seems to be a conserved feature of both vertebrate and invertebrate tachykinin prohormones. Neurokinin A, neuropeptide K, and neuropeptide  $\gamma$  display different affinities for the mammalian NK2 tachykinin receptor (46), and it is possible that N-terminally extended DTK peptides would also show differing affinities for their endogenous receptors.

A number of additional amidated peptides are also potential products of processing of prepro-DTK at pairs of basic residues (see Table I). Of these, TAP-5 seems to bear the most resemblance to a TRP, although there is an alanine residue instead of the glycine in the FXGXR-amide motif, which represents a conservative change. In this respect, this peptide is similar to the recently characterized stomoxytachykinin from the stable fly *S. calcitrans* (APTGFFAVR-amide), which is capable of inducing contractions in the *Leucophaea* hindgut contraction bioassay (37). It is not clear whether any of the peptides shown in Table I have physiological roles, but the fact that three of them could be produced as amidated peptides suggests that they may possess biological activity.

Immunocytochemistry with an antiserum recognizing the conserved C-terminal pentapeptide of insect TRPs labeled a small number of neurons in the larval central nervous system of *Drosophila*. All of these correspond to neurons displaying the *Dtk* transcript as shown by *in situ* hybridization. The only prominent discrepancy between *Dtk* transcript distribution and TRP immunoreactivity is seen in the abdominal ganglia: segmentally distributed cell bodies always display the transcript, but immunolabeling is variable in these neurons. Two pairs of subesophageal neurons are descending neurons of the subesophageal ganglion with axons supplying varicose processes to all the neuromeres of the thoracic-abdominal nerve cord. The others appear to be local interneurons. By comparison, the blowfly displays a larger number of LTK-LI neurons both in the brain and ventral nerve cord (30). The distribution

of LTK-LI cells in the larval and adult midgut of *Drosophila* is very similar to that revealed in the blowfly *Calliphora* (47). In both fly species, the larval endocrine cells are fewer but substantially larger than their adult counterparts. The larval midgut is degraded during metamorphosis, and the adult midgut develops from special anlagen (15). Thus, the endocrine cells of the midgut do not persist during post-embryonic development, in contrast to neurons of the central nervous system. High levels of *Dtk* expression were observed in cells of the midgut from late embryogenesis to the adult stages, which indicates the functional importance of these gut peptides in all feeding stages. As was observed with the immunocytochemistry, *Dtk*expressing cells in the adult midgut were both smaller and more numerous than those seen in the midgut of third instar larvae.

The distribution of LTK-LI material suggests multiple functions for DTK peptides as both neuroactive compounds and regulators of gut function (brain-gut peptides). In the central nervous system, the LTK-LI neurons are all interneurons, as in most other insects studied so far (19). The DTK peptides tested (DTK-1-3) were all able to stimulate muscle contractions in *L*. maderae hindgut, as has been shown for all known arthropod TRPs (19). Their bioactivities were, however, not as strong as that of LemTRP-1, which showed an  $EC_{50}$  value almost an order of magnitude lower than that observed for the best DTK peptides. We were also able to demonstrate biological activity for all five DTK peptides using a Drosophila midgut bioassay. In this assay, the biological activity of each of the peptides proved to be considerably better than that demonstrated using the Leucophaea hindgut assay. The calculated  $EC_{50}$  values ranged from 7 to almost 80 nM and, as such, are similar to those reported for LemTRPs in the Leucophaea assay (19). The relatively poor response of the Leucophaea hindgut preparation to the DTK peptides is therefore likely to be a consequence of the heterologous nature of the assay. Although we have demonstrated that each of the DTK peptides is capable of stimulating muscle contraction in the midgut of both larval and adult D. melanogaster, it will be interesting to investigate what other aspects of midgut physiology are controlled by these peptides. In addition, there is a possibility that the *Dtk*-expressing/ LTK-LI midgut endocrine cells might release DTK peptides to act in an endocrine manner at other sites in the organism.

The fact that we were able to identify a stretch of Drosophila genomic sequence containing a Dtk gene using the human PPT-A amino acid sequence points to the existence of a common evolutionary ancestor for the vertebrate and insect tachykinins. Although the overall sequence identity between human PPT-A and prepro-DTK is low (8%), the identity increases to 31% in the region of prepro-DTK that aligns with the substance P and neurokinin A portion of human PPT-A (Fig. 10). However, the gene structures of Dtk and mammalian tachykinin genes appear to be very divergent, e.g. six introns are present within the genomic sequence of the rat PPT-A gene (Tac1) (6), whereas only three are found in the *Dtk* genomic sequence, none of which are conserved between the two genes. In addition, Dtk codes for five copies of TRPs, whereas Tac1 contains only substance P and neurokinin A plus the two N-terminally extended forms of neurokinin A: neuropeptide K and neuropeptide  $\gamma$ . It therefore seems likely that an ancestral tachykinin prohormone gene contained either a single tachykinin-like sequence or two tachykinin-like sequences, which were not separated by an intron in the genomic sequence. In this hypothesis, duplications following the divergence of the arthropod and vertebrate lineages would have increased the number of TRP progenitor sequences present in the arthropod prohormone. TRPs therefore appear to be evolutionarily ancient. Further evidence for this is provided by the fact that NKD and DTKR, the two Drosophila tachykinin receptors characterized to date, show high homology to cloned mammalian tachykinin receptors, and expressed DTKR is able to respond to the vertebrate tachykinins substance P and physalaemin (21, 22). As we have demonstrated in this report, the neuronal and gut distribution of mammalian tachykinins and the ability to stimulate gut muscle contraction are also conserved in Drosophila. With the identification of Dtk, it appears that the entire tachykinin-mediated signaling system is functionally conserved in Drosophila.

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#### REFERENCES

- 1. Von Euler, U. S., and Gaddum, J. H. (1931) J. Physiol. (Lond.) 72, 74-86
- Erspamer, V. (1981) Trends Neurosci. 4, 267-273
- Otsuka, M., and Yoshioka, K. (1993) Physiol. Rev. 73, 229-308 3
- Holzer, P., and HolzerPetsche, U. (1997) Pharmacol. Ther. 73, 173-217
- Holzer, P., and HolzerPetsche, U. (1997) Pharmacol. Ther. 73, 219-263
- 6.
- Korater, M. S., and Krause, J. E. (1990) J. Neurosc. 10, 2203–2214
  Nawa, H., Kotani, H., and Nakanishi, S. (1984) Nature 312, 729–734
- 8. Kage, R., McGregor, G. P., Thim, L., and Conlon, J. M. (1988) J. Neurochem. **50,** 1412–1417
- Harmar, A. J., Hyde, V., and Chapman, K. (1990) FEBS Lett. 275, 22-24
- Kotani, H., Hoshimaru, M., Nawa, H., and Nakanishi, S. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 7074-7078
- 11. Muren, J. E., and Nässel, D. R. (1996) Regul. Pept. 65, 185–196
- Muren, J. E., and Nässel, D. R. (1997) Peptides (Elmsford) 18, 7–15
  Meola, S. M., Clottens, F. L., Holman, G. M., Nachman, R. J., Nichols, R., Schoofs, L., Wright, M. S., Olson, J. K., Hayes, T. K., and Pendleton, M. W. (1998) Neurochem. Res. 23, 189-202
- 14. Ikeda, T., Minakata, H., Nomoto, K., Kubota, I., and Muneoka, Y. (1993) Biochem. Biophys. Res. Commun. 192, 1-6
- 15. Bautz, A. M. (1979) Arch. Zool. Exp. Gen. 120, 183-194
- 16. Erspamer, V. (1962) Experientia (Basel) 18, 58-61
- 17. Champagne, D. E., and Ribeiro, J. M. C. (1994) Proc. Natl. Acad. Sci. U. S. A.

91, 138-142

- 18. Nässel, D. R., Lundquist, C. T., Muren, J. E., and Winther, Å. M. E. (1998) in Recent Advances in Arthropod Endoctinology (Coast, G. M., and Webster, S. G., eds) pp. 248-277, Cambridge University Press, Cambridge
- 19. Nässel, D. R. (1999) Peptides (Elmsford) 20, 141-158
- 20. Guerrero, F. D. (1997) Peptides (Elmsford) 18, 1-5
- 21. Li, X. J., Wolfgang, W., Wu, Y. N., North, R. A., and Forte, M. (1991) EMBO J. 10. 3221-3229
- Monnier, D., Colas, J. F., Rosay, P., Hen, R., Borrelli, E., and Maroteaux, L. (1992) J. Biol. Chem. 267, 1298–1302
- 23. Rosay, P., Colas, J. F., and Maroteaux, L. (1995) Mech. Dev. 51, 329-339 24. Beerntsen, B. T., Champagne, D. E., Coleman, J. L., Campos, Y. A., and James,
- A. A. (1999) Insect Mol. Biol. 8, 459-467 25. Nachman, R. J., Garside, C. S., and Tobe, S. S. (1999) Peptides (Elmsford) 20,
- 23 29
- 26. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403-410
- 27. Nielsen, H., Engelbrecht, J., Brunak, S., and von Heijne, G. (1997) Protein Eng. 10, 1–6
- 28. Tautz, D., and Pfeifle, C. (1989) Chromosoma (Berl.) 98, 81-85
- 29. Ashburner, M. (1989) Drosophila: A Laboratory Manual, pp. 122-123, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- 30. Lundquist, C. T., Clottens, F. L., Holman, G. M., Riehm, J. P., Bonkale, W., and Nässel, D. R. (1994) J. Comp. Neurol. 341, 225-240
- Nässel, D. R. (1993) *Cell Tissue Res.* 274, 27–40
  Holman, G. M., Nachman, R. J., Schoofs, L., Hayes, T. K., Wright, M. S., and De Loof, A. (1991) *Insect Biochem.* **21**, 107–112 Winther, Å. M. E., Muren, J. E., Lundquist, C. T., Osborne, R. H., and Nässel,
- 33. D. R. (1998) Peptides (Elmsford) 19, 445-458
- 34. Cook, B. J., Chen, A. C., and Pryor, N. W. (1997) J. Entomol. Sci. 32, 487-499
- Coast, G. M. (1998) Peptides (Elmsford) 19, 469-480 35.
- 36. Holman, G. M., Nachman, R. J., and Coast, G. M. (1999) Peptides (Elmsford) **20,** 1–10
- Nachman, R. J., Moyna, G., Williams, H. J., Zabrocki, J., Zadina, J. E., Coast, G. M., and Vanden Broeck, J. (1999) Neuropeptides 897, 388-400
  Reese, M. G., and Eeckman, F. H. (1995) The Seventh International Genome
- Sequencing and Analysis Conference, Hilton Head Island, SC
- 39. Reese, M. G., Kulp, D., Tammana, H., and Haussler, D. (2000) Genome Res. 10, 529-538
- 40. Veenstra, J. A. (2000) Arch. Insect Biochem. Physiol. 43, 49-63
- 41. Kawada, T., Satake, H., Minakata, H., Muneoka, Y., and Nomoto, K. (1999)
- Biochem. Biophys. Res. Commun. 263, 848-852
- Ikeda, T., Minakata, H., and Nomoto, K. (1999) FEBS Lett. 461, 201–204
  Yaron, A., and Naider, F. (1993) Crit. Rev. Biochem. Mol. Biol. 28, 31–81
- Mentlein, R. (1988) FEBS Lett. 234, 251-256 44.
- 45. Nässel, D. R., Mentlein, R., Bollner, T., and Karlsson, A. (2000) J. Comp.
- Neurol. 418, 81–92
- 46. Goldhill, J., and Angel, I. (1998) Eur. J. Pharmacol. 363, 161-168
- Kim, M. Y., Muren, J. E., Lundquist, C. T., and Nässel, D. R. (1997) Arch. Insect Biochem. Physiol. 34, 475–491

### Expression and Functional Characterization of a*Drosophila* Neuropeptide Precursor with Homology to Mammalian Preprotachykinin A

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