Cholecystokinin-like peptide (DSK) in
Drosophila, not only for satiety signaling

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Cholecystokinin (CCK) signaling appears well conserved over evolution. In Drosophila, the
CCK-like sulfakinins (DSKs) regulate aspects of gut function, satiety and food ingestion,
hyperactivity and aggression, as well as escape-related locomotion and synaptic plasticity
during neuromuscular junction development. Activity in the DSK-producing neurons is regulated
by octopamine. We discuss mechanisms behind CCK function in satiety, aggression, and
locomotion in some detail and highlight similarities to mammalian CCK signaling.

Keywords: neuropeptide, peptide hormone, aggression, feeding, intestinal function, locomotion

INTRODUCTION

Many neuropeptide signaling pathways are well conserved over evolution (1–3). One example is
the cholecystokinin (CCK) signaling that regulates satiety and food intake in nematode worms,
insects, and mammals (4–8). The first CCK-like peptide of insects was isolated from the cockroach,
Leucophaea maderae, and designated leucosulfakinin (9). In Drosophila, two sulfakinins (DSK1 and
DSK2; encoded by CG18090) and two DSK receptors (CCKLR1 and CCKLR2; encoded by CG6881
and CG6857) have been identified (10–12). The DSKs display strong sequence similarities to vertebrate
gastrin/CCKs and sulfakinins (SKs) of other invertebrates (Table 1) and also their G-protein coupled
receptors (GPCRs) are well conserved, suggesting a ligand–receptor coevolution (13). Actually, the
amino acid sequences of the two DSKs are identical in the nine residues of the carboxy terminus, and
DSK-II is N-terminally extended by five residues compared to DSK-I (12) (Table 1). The tyrosine
residues of mammalian CCK, and many of the insect sulfakinins, are sulfated, a modification essential
for proper activation of their receptors.

In mammals, CCK secreted from the intestine acts on receptors in the nucleus of the solitary
tract of the brain to signal satiety and thus inhibit feeding (7, 14). In Drosophila, DSKs released
from insulin-producing cells (IPCs) of the brain appear sufficient to induce satiety (8, 15). As with
many neuropeptides and peptide hormones, CCK is multifunctional and can also act locally in
the intestine to decrease gastrointestinal motility, stimulate secretion of pepsinogen, inhibit gastric
acid secretion, stimulate gallbladder contraction, and trigger secretion of hormones in the pancreas
(13, 16). Furthermore, CCK released from brain neuroendocrine cells has regulatory functions
in nociception, memory and learning processes, panic, and anxiety (17). To what extent are these

KEY CONCEPT 1 | Neuropeptides and peptide hormones
Neuropeptides and peptide hormones typically consist of 5–80 amino acids linked by peptide bonds. They act on
G-protein-coupled receptors (GPCRs) or in some cases receptor tyrosine kinases. In Drosophila, about 50 genes
have been identified that encode precursors of neuropeptides or peptide hormones, and more than 45 GPCRs are
known.

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Table 1 | Sequences of CCK-like peptides.

| Peptide   | Species                  | Sequence               |
|-----------|--------------------------|------------------------|
| DSK-I     | Drosophila melanogaster  | FDDYGHMRFamide         |
| DSK-II    | Drosophila melanogaster  | GGDDOFDDYGHMRFamide    |
| DSK 0     | Drosophila melanogaster  | NQKTMSFamide           |
| LSK       | Leucophaea maderae       | pQSDDYSGHMRFamide      |
| CCK-1     | Aplysia californica      | SYGDDYGGGRFamide       |
| CCK-2     | Aplysia californica      | QGAWSYDGLGGGRFamide    |
| NPL12a    | Caenorhabditis elegans   | DYPRLQFamide           |
| NPL12b    | Caenorhabditis elegans   | DGYRLQFamide           |
| CCK8      | Mammals                  | DYMGMVMDFamide         |

*Sequences from Ref. (2, 6, 9, 12). Bold tyrosine (Y) residues are sulfated.

**KEY CONCEPT 2** | Neuroendocrine cells
Neuropeptides and neuroendocrine cells are produced by a variety of neurons, and neuroendocrine cells in the central and peripheral nervous system as well as in glandular cells in other tissues, including the intestinal tract. Within the CNS, peptidergic neurons form a large variety of modulatory circuits. Peptide hormones are typically released into the circulation.

CCK functions subserved by DSKs in Drosophila and SKs of other insects? We provide a brief update on DSK signaling in Drosophila and show that, in addition to inducing satiety, several functions are conserved over evolution.

**MULTIPLE ROLES OF SULFAKININS IN INTESTINAL FUNCTION IN INSECTS**
In several insects, it was shown that SKs modulate spontaneous activity of the foregut and hindgut muscle, as well as heart contractions [see Ref. (3, 9, 13, 18)]. In some insect species, SKs were also shown to induce secretion of the digestive enzyme α-amylase (19). In Drosophila, the enteroneuroendocrine cells of the gut do not produce DSKs, and there is no direct innervation of the posterior intestine by DSK-expressing neurons (20, 21). Thus, actions of DSKs on the more posterior intestine are likely to be hormonal, via release from median neurosecretory cells (MNCs) that also produce insulin-like peptides and have axon terminations in neurohemal regions of the corpora cardiaca and anterior aorta, crop duct, and anterior midgut (8, 22). The DSK action on the heart, crop, and anterior intestine could, however, be by means of direct release onto these structures by axon terminations of the same neurosecretory cells. Taken together, these findings indicate that insect SKs and mammalian CCKs have some conserved actions in relation to intestinal function.

**SATIETY SIGNALING**
Endocrinological studies demonstrated that SKs inhibit food intake in several insects, such as blowfly, locust, cricket, cockroach, and the beetle Tribolium castaneum (5, 13, 23–26). In Drosophila, targeted genetic interference with expression of DSKs also revealed that these peptides are important for satiety signaling (8, 15). In the adult Drosophila CNS, there is a small number of DSK-producing neurons: four very large interneurons posteriorly in the brain, about eight smaller interneurons dorsolaterally and ventrally in the brain, and a varying number of MNCs (8, 15, 20). The DSK-expressing MNCs are a subpopulation of the 14 IPCs (8). Thus, most of the IPCs produce both the insulin-like peptides DILP2, 3, and 5 and the DSKs.

**KEY CONCEPT 4** | Satiety signaling
Hunger and satiety can be determined either by willingness to approach food, or by amount of food ingested. Food intake in adult Drosophila can be monitored by a capillary feeding (CAFE) assay: flies feed from a calibrated capillary, and the amount ingested is calculated from the diminished level in the capillary. In some cases colored food is used and ingestion is determined by spectrophotometry.

There are several sets of neurons in the brain known to regulate feeding. Among these are the so-called hugin neurons that produce a neuropeptide of pyrokinin type, whose branches are known to superimpose those of the IPCs (27). Functional interactions between the brain IPCs and the hugin neurons were demonstrated recently (28). The IPCs could signal to the hugin neurons by both central or peripheral. The DSK receptor localization and targets of the peptide are yet to be identified, and it remains possible that the action could be either central or peripheral.
(DA), neuropeptide F, short neuropeptide F, allatostatin A, leucokinin, and hugin [see Ref. (3, 29)]. These sets of neurons are shown schematically in Figure 1.

It was demonstrated that CCK signaling through the CCK B receptor (CCKBR) within the rodent brain induces hyperactivity and aggression (30, 31). In support of this, CCKergic neuronal projections were identified within the limbic system, the brainstem, and the cerebral cortex, many of which overlap with neuronal pathways considered to be significant for the modulation of fear, anxiety, and aggression [for review, see Ref. (32)]. Furthermore, overexpression of CCKBR in the mouse brain increased aggressive behavior, while mice lacking CCKBR displayed increased exploratory behavior and reduced anxiety (31, 33).

In Drosophila, while DA and serotonin are involved in the modulation of aggressive behavior, the central regulator of aggression is the noradrenaline analog, octopamine (34–37). Recently, it was reported that Drosophila homologs of the human obesity-linked genes TFAP2B and KCTD15 [TfAP-2 and Tiwaz (Twz)] regulate at least two genes involved in the production and secretion of octopamine within the brain, Tyramine β hydroxylase (Tbh) and Vesicular monoamine transporter (Vmat) (Figure 2). Octopamine then regulates aggression, mating, and activity in Drosophila by controlling the expression of Dsk in the IPCs (36) (Figure 2).

Overexpressing TfAP-2 in octopaminergic neurons was sufficient to induce the expression of Dsk. This Dsk induction was blocked by feeding males an octopamine antagonist, indicating that TfAP-2 and Twz induce Dsk expression via octopamine signaling (Figure 2). Furthermore, Dsk overexpression in the IPCs was itself sufficient to induce hyperactivity and aggressive behavior. Interestingly, TfAP-2-induced aggressive behavior was...
blocked by feeding flies, a CCK antagonist. This suggests that octopamine-induced aggression is due to an increase in DSK signaling (Figure 2).

**DEVELOPMENT OF THE NEUROMUSCULAR JUNCTION AND MODULATION OF LOCOMOTION**

Similar to mammals, the *Drosophila* genome encodes two different Dsk receptors, CCKLR1 (CCKLR-17D1) and CCKLR2 (CCKLR-17D3). In *Drosophila* larvae, CCKLR-17D1 signaling was reported to be necessary for body-wall muscle contractions involved in stress-induced escape behavior (38). Moreover, it was demonstrated that Dsk and CCKLR-17D1 are required for proper neuromuscular junction (NMJ) formation in larvae (39). Interestingly, another study reported that octopamine regulates synaptic plasticity in the NMJ during development, as well as under starvation conditions. By activating OctB2R receptors in octopaminergic neurons, octopamine initiates signaling events that induce the development of new synaptic boutons at larval NMJs (40, 41). This lends itself to the hypothesis that, similar to what was reported in the *Drosophila* brain, octopamine and Dsk interact at NMJs to regulate their development, as well as plasticity under condition of increased locomotor behavior.

**CONCLUSION AND OUTLOOK**

The CCK-like peptides, DSKs, of *Drosophila* and SKs of other insects regulate gut function, satiety/food ingestion, hyperactivity, and aggression, as well as escape-related locomotion and synaptic plasticity during NMJ development. Thus, many of the functional roles of CCK signaling known in mammals are present also in insects. Recent studies have shown that the neurons controlling DSKs are under regulatory control by octopaminergic neurons (36) and more specifically the IPCs that co-express DILPs and DSKs are regulated by the octopamine receptor OAMB (42). An important question for the future is to determine the targets of DSK signaling within the brain and at peripheral sites that regulate the different aspects of behavior and physiology.

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