

Presence of *Locusta* diuretic hormone in endocrine cells of the ampullae of locust Malpighian tubules

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Abstract. This is an investigation of an endocrine cell type in the midgut of the migratory locust *Locusta migratoria*. This cell type is found in the posterior region of the midgut and is especially common in the ampullae through which Malpighian tubules drain into the gut at the midgut-hindgut junction. Strong *Locusta* diuretic hormone-like immunoreactivity in these cells was colocalized with FMRFamide- and substance P-like immunoreactivities. At the ultrastructural level, immunoreactivity for *Locusta* diuretic hormone was found in spherical granules (mean diameter of 450 nm), the contents of which showed variable electron density. Fractionation of a methanolic extract of the ampullae by reversed-phase high performance liquid chromatography revealed the presence of two peaks of *Locusta* diuretic hormone-like immunoreactive material, both of which stimulate cyclic AMP production by isolated Malpighian tubules. The more hydrophobic material is most likely *Locusta* diuretic hormone, which has the same retention time when chromatographed under identical conditions.

Key words: Diuretic hormone – Endocrine cells – Midgut – Malpighian tubules – Bioassay – Immunocytochemistry – *Locusta migratoria* (Insecta)

Introduction

In recent years, diuretic peptides implicated in the regulation of Malpighian tubule function have been identified from several insect species (Coast 1995). They belong to two distinct families, namely the corticotropin-releasing factor (CRF)-like peptides, which have sequence and structural similarities to CRF, and the insect

myokinin, originally identified on the basis of their myotropic activity. In the migratory locust *Locusta migratoria*, both a CRF-like peptide [*Locusta* diuretic hormone (*Locusta*-DH)] and a myokinin (locustakinin) have been described (Kay et al. 1991; Schoofs et al. 1992). *Locusta*-DH is a potent stimulant of fluid secretion and cyclic AMP production by locust Malpighian tubules (Kay et al. 1991; Patel et al. 1995) and has been shown to function as a neurohormone in the control of post-feeding diuresis (Patel et al. 1995). Locustakinin, which acts via a cyclic AMP-independent mechanism (Thompson et al. 1995), is not as efficacious as *Locusta*-DH but, at low concentrations (<0.1 nM), potentiates the diuretic activity of the CRF-related peptide, and there is clear evidence of synergism between the two stimulants (Coast 1995). Other peptides with reported diuretic activity in locusts are an arginine vasopressin (AVP)-related peptide (Proux et al. 1987) and an adrenocorticotrophic hormone (ACTH)-like factor (Rafaeli 1993). However, the ACTH-like peptide has not been characterized, and the diuretic activity of the AVP-like peptide has recently been questioned (Coast et al. 1993).

All of the diuretic peptides that have been described are the products of neurosecretory cells in the insect central nervous system. *Locusta*-DH is synthesized by neurosecretory cells in the pars intercerebralis and is stored in the corpora cardiaca from where it can be released into the hemolymph (Patel et al. 1994). Likewise, the CRF-like diuretic hormone (*Manduca*-DH) of the tobacco hornworm *Manduca sexta* is found in neurosecretory cells of the pars intercerebralis and in the corpora cardiaca (Veenstra and Hagedorn 1991). *Locusta*-DH-like immunoreactive neurons are found also in the subesophageal ganglia and in the ventral nerve cord, as well as in regions of the brain that lie outside the pars intercerebralis (Patel et al. 1994). The presence of this peptide in a variety of neurons suggests a broader functional role, possibly as a neurotransmitter or neuromodulator, a view supported by the presence of immunoreactive axons within the connectives of the ventral nerve cord (Patel et al. 1994). Posterolateral clusters of 2–3 cells each in the

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