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Proctolin-like immunoreactivity in the central and peripheral nervous systems of the locust, *Locusta migratoria*

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ABSTRACT

Proctolin-like immunoreactivity (PLI) was widely distributed in the locust, *Locusta migratoria*, within the central, peripheral and stomatogastric nervous systems, as well as the digestive system and retrocerebral complex. Proctolin-like immunoreactivity was observed in cells and processes of the brain and all ganglia of the ventral nerve cord. Of interest, PLI was found in the lateral neurosecretory cells, which send axons within the paired nervi corporis cardiaci II (NCC II) to the corpus cardiacum (CC). The CC contained extensive processes displaying PLI, which continued on within the paired nervi corporis allata (NCA) to the paired corpora allata (CA) where the axons entered and branched therein. The frontal and hypocerebral ganglia of the stomatogastric nervous system contained PLI within processes, resulting in a brightly staining neuropile. Each region of the gut contained PLI in axons and processes of varying patterns and densities. The paired ingluvial ganglia contained PLI, including an extensively stained neuropile and immunoreactive axons projecting through the nerves to the foregut. The hindgut contained PLI within longitudinal tracts, with lateral projections originating from the 8th abdominal ganglion via the proctodeal nerve. The midgut contained PLI in a regular latticework pattern with many varicosities and blebs. No difference in PLI in cells and processes of the central nervous system (CNS) was found between males and females.

1. Introduction

In 1975 Brown and Starratt isolated and sequenced the myotropic peptide proctolin (H-Arg-Tyr-Leu-Pro-Thr-OH) from the American cockroach, *Periplaneta americana* [7,8]. Since that time, proctolin-like immunoreactivity (PLI) has been found to be present in the central and peripheral nervous systems of arthropods, especially insects (see [18,30]). In light of immunological, biochemical, and bioassay data, proctolin has been suggested to act as a neurotransmitter, neuromodulator, and as a neurohormone [3,28,30]. In the central nervous system (CNS) of insects, crustaceans, arachnids, and some vertebrates, proctolin has been found in sensory, motor, and interneurons [6,13,33,18,28]. For example, in the cockroach, *P. americana*, neurons containing proctolin-like immunoreactivity have been found in the abdominal ganglia, and in particular the terminal abdominal ganglion where axonal processes project through the proctodeal nerve to innervate the hindgut, indicating a potential neuromodulator or neurotransmitter role for

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proctolin on the hindgut [3,31]. In *Locusta migratoria*, PLI is present in cells and processes in the seventh abdominal ganglion, with processes that project out of the sternal nerve and onto the oviducts [9,22,24].

Insect visceral muscle is modulated by proctolin, where it has been found to stimulate dose-dependent tonic contractions of the visceral muscle associated with the reproductive system, namely the oviduct and spermatheca, and the gut of the locust [5,20,24]. Proctolin has also been shown to be associated with skeletal muscle where it acts as a cotransmitter at the coxal depressor neuromuscular junction of *P. americana* as well as the ventral protractor muscle of *L. migratoria* [1,11]. Interestingly, in the Colorado potato beetle, *Leptinotarsa decemlineata*, proctolin-like immunoreactivity has also been shown to be associated with the corpus cardiacum (CC), a well-known neuroendocrine organ of insects [36], and in the lobster, *Homarus americanus*, within the pericardial organs, which are neurosecretory structures [33].

Despite the fact that proctolin was the first insect neuropeptide to be sequenced, it still has not been mapped completely in many insects and little is known of proctolin's physiological roles. In larval *Drosophila*, a map of PLI in the central nervous system has been completed [4] and more recently, the first proctolin receptor was cloned and characterized [10,17]. It was found by Northern blot analysis that the *Drosophila* proctolin receptor is differentially expressed between developing stages and the adult stage, where in the adult stage there is high expression in the head and low expression in the thorax and abdomen [10]. Recent work by Taylor et al. [35] has also identified the first proctolin precursor gene that encodes for a proctolin preprohormone.

With a re-interest brought about by the molecular work on the proctolin receptor and precursor gene, it is now time to revisit proctolin to determine its distribution within the insect, *L. migratoria*. This research serves as a comprehensive mapping of the presence and distribution of PLI within the central and peripheral nervous systems, and highlights target tissues for future physiological studies.

2. Materials and methods

2.1. Animals

Male and female locusts of *L. migratoria* were obtained from a colony at the University of Toronto at Mississauga, Canada, raised on a 12 h light/12 h dark cycle at 30 °C under crowded conditions. The colony was fed on fresh wheat seedlings supplemented with bran.

2.2. Chemicals

Affinity purified goat anti-rabbit antibody conjugated to Cy3 was purchased from Sigma (Oakville, Ont., Canada) and was used at a dilution of 1:600. Proctolin was purchased from Bachem (Torrance, CA, USA) and was reconstituted in double distilled water to yield a stock solution of 10^{-3} M, which was divided into 10 μl aliquots and frozen at −20 °C.

2.3. Immunohistochemistry

The preparations consisted of the brain and ventral nerve cord, including the retrocerebral complex, as well as the oviducts and digestive tract (foregut, midgut, and hindgut). The gut was cut longitudinally so that all contents could be removed and the tissue could be flattened. Preparations were dissected in locust saline (150 mM NaCl, 10 mM KCl, 4 mM $CaCl_2$, 2 mM $MgCl_2$, 4 mM $NaHCO_3$, 5 mM HEPES (pH 7.2), 90 mM sucrose, 5 mM trehalose) and then fixed in 4% paraformaldehyde in Millonigs buffer (pH 7.3–7.4, 1 g NaCl, 2.9 g $Na_2PO_42H_2O$, 0.524 g $Na_2H_2PO_4H_2O$, 8 g paraformaldehyde, made up to 200 ml with ddH_2O) overnight at room temperature. The preparations were cleaned and washed in Tris–HCl buffer (pH 7.4, 6.05 g Trizma base, 8.5 g NaCl, made up to 1 L with ddH_2O) for 48 h at 4 °C on a shaker and then were run through a forward and reverse alcohol series (30, 50, 70, 90, and 100%) for 15 min each. The washed tissue was then incubated in rabbit anti-proctolin IgG fraction purified polyclonal antibody diluted 1:1000 in a solution of Tris–HCl buffer containing 2% normal goat serum, 0.25% Triton-X, 3% skim milk powder, and 0.25% bovine serum albumin on a shaker at 4 °C for 48 h. Tissues were washed extensively and left overnight at 4 °C on a shaker followed by incubation in affinity purified goat anti-rabbit antibody conjugated to Cy3 (Sigma, Oakville Ont., Canada) for 48 h at 4 °C on a shaker, containing 2% normal goat serum in a solution of Tris–HCl buffer containing 0.25% Triton-X, 3% skim milk powder, and 0.25% bovine serum albumin. The preparations were then run through a forward glycerol series (20, 40, 60, and 80%) for fifteen minutes each and then mounted in 100% glycerol and left overnight at 4 °C before viewing.

The anti-proctolin antiserum, generated against proctolin coupled to gluteraldehyde/polylysine (1:4), was tested for cross-reactivity using an ELISA. No cross-reactivity was observed against 10 μg/ml of gluteraldehyde/polylysine conjugates of perisulfakinin, locustatachykinin II, FMRFamide, crustacean cardioactive peptide, adipokinetic hormone I, leucomyosuppressin, corazonin and the allatostatins, Dip-AST 2, Dip-AST 7, and Dip-AST 8 [2].

Controls were performed in which the proctolin antiserum was pre-incubated for 24 h with 10^{-3} or 10^{-5} M synthetic proctolin. In total, 23 gut preparations (10 male, 13 female) were examined, as well as 46 central nervous system preparations (31 male, 15 female), 21 stomatogastric nervous system/retrocerebral complex samples (10 male, 11 female), and five oviduct preparations. Eleven control experiments were performed: five central nervous system preparations, two gut preparations, and two stomatogastric nervous system preparations.

Preparations were viewed with a Nikon Optiphot-2 Epiflourescence Microscope (Nikon Corporation, Tokyo, Japan) and drawn using a camera lucida attachment. Confocal images were taken using a Zeiss LSM 510 Confocal Laser Microscope (Carl Zeiss, Jena, Germany).

3. Results

3.1. Brain and ventral nerve cord

Proctolin-like immunoreactivity was found in the brain and in all ganglia of the ventral nerve cord. Composite camera lucida