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Proctolin: A possible releasing factor in the corpus cardiacum/corpus allatum of the locust

Lisa Clark [a], *Jin Rui Zhang* [b], *Stephen Tobe* [b], *Angela B. Lange* [a,*]

[a] *Department of Biology, University of Toronto at Mississauga 3359, Mississauga Rd., Mississauga, Ont., Canada L5L 1C6*
[b] *Department of Zoology, University of Toronto, 25 Harbord St., Toronto, Ont., Canada M5S 3G5*

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ABSTRACT

The corpus cardiacum (CC) and corpus allatum (CA) of the locust, *Locusta migratoria*, contain intense proctolin-like immunoreactivity (PLI) within processes and varicosities. In contrast, in the cockroach, *Diploptera punctata*, although a similar staining pattern occurs within the CC, PLI appears absent within the CA. The possible role of proctolin as a releasing factor for adipokinetic hormone (AKH) and juvenile hormone (JH) was investigated in the locust. Proctolin caused a dose-dependent increase in AKH I release (determined by RP-HPLC) from the locust CC over a range of doses with threshold above 10^{-8} M and maximal release at about 10^{-7} M proctolin. Isolated glandular lobes of the CC released greater amounts of AKH I following treatment with proctolin and in these studies AKH II was also released. Confirmation of AKH I release was obtained by injecting perfusate from incubated CCs into locusts and measuring hemolymph lipid concentration. Perfusate from CC incubated in proctolin contained material with similar biological activity to AKH. Proctolin was also found to significantly increase the synthesis and release of JH from locust CA, with the increase being greatest from CAs that had a relatively low basal rate of JH biosynthesis (<35 pmol h^{-1} per CA). In contrast, proctolin did not alter the synthesis and release of JH from the cockroach CA. These results suggest that proctolin may act as a releasing factor for AKHs and JH in the locust but does not act as a releasing factor for JH in the cockroach.

1. Introduction

In 1975, Brown demonstrated that the pentapeptide proctolin modulated hindgut contractions of the American cockroach, *Periplaneta americana* [4]. Proctolin has since been found to have multiple functions in insects and crustaceans, where it is associated with the control of visceral, cardiac, and skeletal muscle [1,7,17,19,24,32] and to influence egg-laying in *Apis mellifera* [20]. In addition to the well-known role of proctolin as a myotropic agent, a secondary function of proctolin as a releasing factor within the corpus cardiacum/corpus allatum (CC/CA) of the locust has been suggested, based on the pattern of proctolin-like immunoreactivity found within the locust CC and CA [6]. We recently reported that the CC contained extensive proctolin-like immunoreactivity within neuronal processes, blebs, and varicosities, with proctolin-like immunoreactive axons also present within each nervus corporis allatum (NCA) projecting to the paired CA in which they branch [6]. The locust CC/CA is known to be a major neurohemal and endocrine complex, and is known to release a variety of neurohormones/hormones including the adipokinetic hormones (AKH) and juvenile hormone (JH) [3,11,12,23].

* *Corresponding author*. Tel.: +1 905 828 3972; fax: +1 905 828 3792.
E-mail address: alange@utm.utoronto.ca (A.B. Lange).
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Adipokinetic hormone is an important neuropeptide in insects that functions to provide fuel, in the form of lipid and carbohydrate, for long-distance flight [23]. There are three known adipokinetic hormones (AKH I, II, and III) that are synthesized and stored within the CC of the African migratory locust, *Locusta migratoria* [11,29]. The brain of the locust is connected to the CC by two pairs of nerves, the nervi corporis cardiaci I and II (NCC I and II), and axons within these nerves originate from bilateral groupings of cells located within the protocerebrum [15]. The lateral neurosecretory cells send axons within the NCC II to the glandular lobe, whereas cell bodies within the pars intercerebralis and the medial neurosecretory cells, send axons within the NCC I to the storage lobe of the CC [15,31]. The only natural stimulus known for AKH release is flight and the energy deficit resulting from long-duration flights is countered by the mobilization of lipid from the fat body, which is stimulated by the AKHs [9,38].

Previous studies have shown that the release of the AKHs from the CC is regulated by neuropeptides and the amine, octopamine [9,25,42]. Nässel et al. [21] were the first to demonstrate a role for locustatachykinin I as a releasing factor for AKH. It has since been shown that another locustatachykinin, Lom-TK II, induces the release of AKH from the CC in a dose-dependent manner and elevates the content of the second messenger cAMP within the glandular portion of the CC [22]. Another releasing factor for AKH is crustacean cardioactive peptide (CCAP), which induces a dose-dependent release of AKH from the CC [8,39], whereas SchistoFLRFamide and sugars have been shown to inhibit the release of AKH from the CC [8,41].

Juvenile hormone plays many roles in insects, from regulating growth, metamorphosis and vitellogenesis, to regulating polymorphisms in social insects [12]. Juvenile hormone biosynthesis and release occurs in cells of the CA, an endocrine organ associated with the CC [42]. The CA is innerved in part by neurosecretory cells (NSC) originating in the brain and CC by paired NCA I, and from the hypocerebral ganglion by paired cardiostomatogastric nerves or NCA II [12,15]. Changes in hemolymph JH titer direct the growth and development of immature insects as well as reproduction in adults [12]. This change in JH titer is modulated by neuropeptides that are secreted by NSC within the brain that project to each CA [13,37]. Two neuropeptide families that have been implicated in the control of JH biosynthesis, allatotropins and allatostatins, stimulate and inhibit JH production, respectively in selected species [2,10,14,16,43]. It recently has been shown that allatostatin-like immunoreactivity within the lateral NSC of the brain and within the CA colocalizes with FMRFamide-like immunoreactivity in the cockroach, *Diploptera punctata*, and that JH release is stimulated by FMRFamide-related peptides in females at the end of vitellogenesis [34].

The present paper examines the possibility that proctolin is a releasing factor within the CC/CA of locusts, by testing the ability to influence the release of AKH I or JH. In addition, since there is considerable information on the control of production of JH in *D. punctata*, we examined the proctolin-like immunoreactivity in the cockroach CC/CA and the influence of proctolin on JH release in this model insect.

2. Materials and methods

2.1. Animals

L. migratoria were reared in a colony at the University of Toronto at Mississauga, Canada under crowded conditions at a constant temperature of 30 °C on a 12-h photoperiod. The animals were fed wheat seedlings and bran. *D. punctata* were obtained from a colony maintained at the University of Toronto, Canada. The colony was fed rat chow and water ad libitum and was kept at 27 °C.

2.2. Chemicals

Synthetic adipokinetic hormone I and II were purchased from Peninsula Laboratories (Belmont, California) and reconstituted in 1 M glacial acetic acid to yield a stock solution containing 1 μg AKH I or AKH II/10 μl. These stocks were then divided into 10 μl aliquots and frozen at −20 °C. HPLC grade acetonitrile and water were purchased from Fisher Scientific Canada (Ottawa, Ont.). Trifluoroacetic acid (TFA) and trichloroacetic acid (TCA) were obtained from Sigma (Oakville, Ont., Canada). 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. Working dilutions of proctolin were made in saline from the frozen aliquots each day. Sigmacote (Sigma, Oakville, Ont., Canada) was used to prevent peptide adherence to the wells of the 96-well culture plates used for the incubations, as well as the Eppendorf tips used for collection of the incubation media.

2.3. Immunohistochemistry

Adult male and female *L. migratoria* and *D. punctata* brains with the corpora cardiaca and corpora allata attached were dissected in saline (150 mM NaCl, 10 nM 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 the immunohistochemistry protocol was followed as previously reported [6]. Corpora cardiaca and CA were used from both locusts and cockroaches (n = 10 females, n = 10 males).

2.4. AKH release bioassay

The corpora cardiaca of five female locusts, 3 weeks after their final molt, were excised and pooled in a culture plate well in 50 μl locust saline buffer (150 mM NaCl, 10 mM KCl, 4 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM Hepes). After rinsing three times with 50 μl saline, the pooled CCs were incubated for 1 h at room temperature on a shaker. The perfusate was collected in an Eppendorf and diluted 1:1 with 2.5 M acetic acid. The CC were then rinsed three times with 50 μl saline and incubated in 50 μl of proctolin (10^{-5} to 10^{-9} M) for 1 h at room temperature on a shaker. The incubation media was then collected in an Eppendorf containing 50 μl 2.5 M acetic acid. In another experiment, the storage lobes and glandular lobes of the CC were separated and incubated with proctolin as described for intact CCs.