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Regulation of methyl farnesoate production by mandibular organs in the crayfish, *Procambarus clarkii*: A possible role for allatostatins

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Abstract

Decapod crustaceans do not appear to produce juvenile hormone, but rather its immediate precursor, methyl farnesoate (MF). Both MF and its immediate precursor, farnesoic acid (FA) are produced by the mandibular organs (MO) in crustaceans. The MO are homologous to the insect corpora allata (CA), the site of juvenile hormone biosynthesis. However, the FGLamide allatostatin (ASTs) peptides, of which there are about 60 distinct forms reported from crustaceans, have previously been found to have no effect on MO activity in crustaceans. We have identified by immunocytochemistry the presence of FGLamide-like AST immunoreactivity in neurosecretory cells throughout the CNS as well as in neurohaemal structures such as the sinus gland and pericardial organs. The ASTs are likely delivered to the MO hormonally and/or by local neurohaemal release. Using MO from adult males, we have found wide variability between animals in the in vitro rates of MF and FA biosynthesis. Treatment with Dippu-ASTs has a statistically significant stimulatory effect on MF synthesis, but only in MO that are initially producing MF at lower rates. No effect on FA production was observed, suggesting that the FGLamide ASTs exert their effect on the o-methyl transferase, the enzyme responsible for the conversion of FA to MF.

Keywords: Crayfish; Methyl farnesoate; Farnesoic acid; Mandibular organ; Allatostatin

1. Introduction

The mandibular organs (MO) of crustaceans as first described by Le Roux (1968) are paired, vascularised, ductless glands situated at the base of the mandibular tendon. The MO are considered to be the crustacean equivalent of the insect corpora allata (CA) based on embryological (Le Roux, 1968) and ultrastructural evidence (Byard et al., 1975; Yudin et al., 1980), as well as on the similarity in their biosynthetic products (Laufer et al., 1987a; Borst et al., 1987; Ding and Tobe 1991; Tobe et al., 1989a, b). Both the crustacean MO and the insect CA produce sequiterpenoid compounds utilising the farnesyl diphosphate/isopentenoid biosynthetic pathway (Tobe and Bendena, 1999). In crustaceans, the end product of this pathway is methyl farnesoate (MF), which in the insect CA is the immediate precursor of juvenile hormone III (JH III), the most prevalent form of the insect JHs (Schooley et al., 1973; Schooley and Baker, 1985). The structures of MF and JH III differ only in the addition of an epoxide group added by the enzymatic epoxidation of MF.

In insects, the JHs are involved in the regulation of moulting and reproduction (Riddiford, 1994; Wyatt and Davey, 1996; Gade et al., 1997) and there is evidence that MF may act as the crustacean JH (for recent reviews see Homola and Chang, 1997; Laufer and Biggers, 2001). It has therefore been suggested that sequiterpenoid compounds may serve an evolutionarily conserved role as regulators or modulators of moulting and reproduction in arthropods (Tobe and Bendena, 1999).

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Although the role of MF in crustacean reproduction is not as well established as the role of JH in insects, evidence for its effects on crustacean moulting and reproduction has been accumulating since its discovery in the spider crab, *Libinia emarginata* (Laufer et al., 1987a; Borst et al., 1987). The presence of MF has since been identified in over 30 crustacean species and in some species, the biosynthesis and release of MF by the MO has been characterised (for examples see Laufer and Biggers, 2001). In addition to MF, the biosynthesis and in vitro release of FA from the MO has also been documented in the crayfish, *Procambarus clarkii*, and the crab, *Scylla serrata* (Cusson et al., 1991; Ding and Tobe 1991; Tobe et al., 1989b). Compared to MF, the possible role of FA in regulating moulting and reproduction has largely been overlooked, despite the observation that the amount of FA released by the MO can be far greater than the amount of MF released (Tobe et al., 1989b; Cusson et al., 1991). Although these authors did not detect FA in the haemolymph, other evidence suggests that FA is still a viable candidate for a crustacean hormone (Cusson et al., 1991; Ding and Tobe 1991; Tobe et al., 1989b). It was recently demonstrated that FA can stimulate vitellogenin gene expression in the hepatopancreas of the crab *Charybdis feriatus* (Mak et al., 2005).

Factors responsible for inhibiting the activity of the MO have been localised to the eyestalks, based on observations that eyestalk ablation (ESA) resulted in hypertrophy of the MO (Le Roux, 1968; Hinsch, 1977), ESA elevated MF haemolymph titres (eg. Laufer et al., 1987a; Tsukimura and Borst, 1992) and inhibition of MF synthesis by eyestalk or sinus gland extracts (eg. Laufer et al., 1987b; Wainwright et al., 1996a, b). These factors are now known to be the mandibular organ inhibiting hormones (MOIHs) (Liu and Laufer, 1996; Wainwright et al., 1996b; Webster, 1998), peptides that share a high degree of sequence identity with the crustacean hyperglycemic hormone (CHH) peptide family that also includes the moult inhibiting hormones (MIH) and gonad inhibiting hormones (GIH) (Lacombe et al., 1999; Chan et al., 2003).

The MOIHs have clearly been shown to inhibit MO activity in vitro (Borst et al., 2002; Liu and Laufer, 1996; Wainwright et al., 1996b) and are assumed to function as MO inhibitors in vivo, even though their effects in vivo have not yet been clearly demonstrated (Borst et al., 2001, 2002). Still another unknown peptide with inhibitory effects on the MO in vivo was discovered during the purification of the MOIHs from *Cancer pagurus* (Borst et al., 2002). These authors found fractions, containing a peptide(s) with inhibitory activity, that were distinct from the fractions containing the MOIHs.

Few other peptides have been found that affect the activity of the MO. Pigment dispersing hormone (PDH) was found to stimulate MF synthesis in vitro in the crayfish *P. clarkii*, whereas red pigment concentrating hormone (RPCH) had an inhibitory effect (Landau et al., 1989). Whether these peptides function in vivo remains to be demonstrated, and their effects may be limited to *P. clarkii* since similar results have not been obtained in other crustaceans (Wainwright et al., 1996b; Borst et al., 2001).

Of the identified crustacean peptides that have been shown to affect MO activity, none resemble any of the insect peptides that regulate the CA. In insects, there are three peptide families with allatostatic activity and one peptide family with allatotropic activity that have been characterised to date (Bendena et al., 1999; Tobe, 1998; Elekonich and Horodyski, 2003). The first family of allatostatic peptides, initially discovered in the cockroach, *Diploptera punctata* (Woodhead et al., 1989) share the common carboxyl-terminal sequence Y/F-X-F-G-L/I-amide, and are commonly referred to as the FGLamide allatostatins (AST) or cockroach-type AST to distinguish them from the other peptides that have allatostatic activity (for reviews see Stay et al., 1994; Bendena et al., 1999, Stay, 2000).

ASTs of the FGLamide type have been sequenced from three species of crustacean: 20 from the crab, *Cancer maenas* (Duve et al., 1997a), 3 from the crayfish, *Orconectes limosus* (Dircksen et al., 1999), and over 40 FGLamide ASTs from the shrimp, *Penaeus monodon* (Duve et al., 2002). Although the FGLamide ASTs have not been sequenced from *P. clarkii*, the distribution of the FGLamide ASTs in the stomatogastric nervous system (STNS) and pericardial organs (PO) has been mapped in detail (Skiebe, 1999). It is therefore of interest to determine if the FGLamide ASTs function as inhibitors of MF biosynthesis by the MO, as one might hypothesise based on their function in insects in general, or whether they may stimulate MF biosynthesis in a manner similar to the early embryonic CA of *D. punctata* (Stay et al., 2002). In this study, we use immunocytochemical techniques to identify putative routes of transmission, from AST-immunoreactive (AST-IR) neurosecretory somata to the MO. This study also characterises the effects of the FGLamide ASTs on FA and MF production by the MO using a sensitive radiochemical assay (Pratt and Tobe, 1974; Tobe and Pratt, 1974).

2. Materials and methods

2.1. Animals

The crayfish used in this study were males purchased from Atchafalaya (LA, USA) or Boreal Biological Supply (St. Catharines, ON, Canada) and maintained in the animal care facilities of the Department of