



Involvement of adenosine cyclic-3',5'-monophosphate in the signal transduction pathway of mandibular organ-inhibiting hormone of the edible crab, *Cancer pagurus*

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Abstract

The juvenoid, methyl farnesoate (MF), is synthesized in the mandibular organs (MOs) of crustaceans, under the control of mandibular organ-inhibiting hormone (MO-IH). Using an in vitro assay to measure synthesis of MF by MOs, the effect of a variety of agents that affect signal transduction pathways was investigated. Of the compounds tested, only agents which affect cAMP (forskolin and 8-bromoadenosine cyclic-3',5'-monophosphate) levels were found to mimic the inhibitory action of MO-IH on MF synthesis. To further support these findings, the effect of MO-IH-1 on production of cAMP was investigated. The results demonstrated that MO-IH stimulated a dose-dependent increase in cAMP levels. Furthermore, a maximal 2-fold increase in cAMP was detected after a 5-min exposure of MO membranes to 100 nM MO-IH-1, falling to basal levels thereafter. The results presented strongly support a role for cAMP in the signal transduction mechanism of MO-IH that leads to inhibition of MF synthesis in MOs. © 1999 Published by Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Juvenile hormone; Mandibular organ-inhibiting hormone; Methyl farnesoate

1. Introduction

The isoprenoid, methyl farnesoate (MF), is synthesized and secreted from the mandibular organs (MOs) of crustaceans (Borst et al. 1987; Laufer et al. 1987). In view of the structural similarity of MF with insect juvenile hormone III and the established roles of juvenile hormone in insects (Riddiford, 1994), there has been considerable interest in the possible role of MF in growth and reproductive development in crustaceans. Positive correlation of increase of haemolymph titre of MF with ovarian development has been observed in the spider crab, *Libinia emarginata* (Laufer et al., 1987), and the freshwater prawn, *Macrobrachium rosenbergii* (Wilder et al., 1995). More recently, it was reported that MF levels in the haemolymph of the female edible crab, *Cancer pagurus*, were maximal at the beginning of

vitellogenesis, falling to basal levels thereafter (Wainwright et al., 1996a). Additionally, it has been shown that physiological doses of MF can stimulate an increase in oocyte diameter when cultured in vitro (Tsukimura and Kamemoto, 1991) and that feeding MF to female penaeid shrimps may increase fecundity and larval quality (Laufer, 1992). These foregoing reports clearly support a role for MF in the reproductive development of female crustaceans.

In order to gain a more complete understanding of the role of MF in crustaceans, considerable effort has been invested towards identifying factors which regulate the biosynthesis of MF in MOs. To this end, it was determined that factors contained within a neurosecretory complex of the crustacean eyestalk, the X-organ–sinus gland complex (XO-SG), were responsible, at least in part, for the negative regulation of MF biosynthesis in crustacean MOs. In recent reports, such factors, named mandibular organ-inhibiting hormones (MO-IHs), were isolated and characterized from the female edible crab, *Cancer pagurus* (Wainwright et al.,

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1996b) and in the spider crab, *Libinia emarginata* (Liu et al., 1997). MO-IH-1 and -2 of *Cancer pagurus* are 78-amino-acid peptides which inhibit MF biosynthesis in MOs in both a dose- and ovary stage-dependent manner. MO-IH-1 and -2 differ from each other by one amino acid and are members of the CHH/MIH/VIH family of crustacean neuropeptides (Keller, 1992), exhibiting significant sequence identity with the MIH group of peptides. In *Libinia emarginata*, three peptides exhibiting MO-IH activity were isolated. All three were 72-amino-acid peptides, blocked at the N- and C-termini, and possessed high sequence identity with CHH peptides of the CHH/MIH/VIH family of peptides. Clearly, within the CHH/MIH/VIH family of peptides there exist different types of MO-IHs dependent upon the species of crustacean examined.

In *Cancer pagurus*, action of MO-IH on MOs action ultimately inhibits activity of a cytosolic *S*-adenosyl-L-methionine:farnesoic acid methyltransferase enzyme, thereby reducing MF synthesis (Wainwright et al., 1998). To further characterize the regulation of MF biosynthesis in MOs of *Cancer pagurus*, the signal transduction pathway involved in MO-IH action leading to inhibition of MF biosynthesis was investigated.

2. Materials and methods

2.1. Animals

Female edible crabs, *Cancer pagurus*, were obtained locally from fishermen and maintained in a re-circulating sea water system under ambient light and temperature conditions. Crabs were bilaterally eyestalk-ablated 24 h prior to mandibular organ dissection, unless stated otherwise.

2.2. Chemicals

A23187, thapsigargin, LY83583, RO31-8220, forskolin, 1,9-dideoxyforskolin, bisindolylmaleimide and phorbol-12-myristate 13-acetate were purchased from Calbiochem (Nottingham, UK). 8-Bromo-adenosine cyclic-3',5'-monophosphate (8Br-cAMP), 2'-*O*-dibutyryl guanosine cyclic 3',5'-monophosphate (diBu-cGMP) and 3-isobutyl-1-methylxanthine (IBMX) were purchased from Sigma (Poole, UK). Solvents for high-performance liquid chromatography (HPLC) were purchased from BDH (Leicester, UK).

2.3. Purification of mandibular organ-inhibiting hormone

Mandibular organ-inhibiting hormone (MO-IH) was purified from an acetic acid extract of sinus glands using a two-step HPLC procedure and quantified by

amino acid analysis as described previously (Wainwright et al., 1996b). In the present study, the more abundant MO-IH-1 was used.

2.4. Assay of methyl farnesoate synthesis

Mandibular organs were dissected from ice-anaesthetized crabs and left and right MOs cultured individually in 200 μ l of a modified medium 199 (Brody and Chang, 1989), supplemented with 37 kBq L-[methyl-³H]methionine, at 12°C for 24 h, unless stated otherwise. We have previously demonstrated that individual MOs synthesize MF for this time period in a hyperbolic manner (Wainwright et al., 1996a). For tests of the effect of second messenger pathway effector compounds on MF synthesis, one MO from an animal was used for treatment, with the other MO being an untreated control. The use of MOs from a single animal in this manner has previously been validated (Wainwright et al., 1996b). Such compounds were added to culture medium in an appropriate solvent vehicle (ethanol or dimethylsulphoxide) at a final concentration of less than 1% by volume. An equivalent volume of the same solvent was added to control incubations. Following incubation, individual MOs were frozen rapidly in liquid nitrogen. To extract radiolabelled MF, MOs were homogenized in 700 μ l of CH₃CN–4% NaCl_(aq) (5:2, v/v) and the homogenate was extracted with 500 μ l of hexane; the hexane layer was retained. The homogenate was extracted a further two times with hexane and the hexane layers combined, dried under a stream of nitrogen and the residue resuspended in 200 μ l of methanol prior to analysis by HPLC with on-line radioactivity detection. The results for these experiments were expressed as an inhibition index (Wainwright et al., 1996b) which is defined as one minus the ratio of MF detected in the treated MOs to that detected in control MOs.

2.5. High-performance liquid chromatography with on-line radioactivity detection

Radiolabelled MF in samples was quantified by HPLC with on-line radioactivity detection (A500; Flo-Beta, Canberra Packard, UK) using a Waters reversed-phase Novapak C₁₈ cartridge eluted isocratically with 80% methanol in water at a flow rate of 2 ml/min. The amount of radiolabelled MF synthesized was determined using proprietary integration software and the results were expressed as an inhibition index, as described previously (Wainwright et al., 1996b).

2.6. Preparation of mandibular organ membranes

Groups of 20–40 MOs were homogenized using a Potter-Elvehjem homogenizer in 600 μ l of ice-cold

HEPES buffer (0.037 M, containing 0.3 M sucrose, 0.01 M KF) pH 7.5 (Chen et al., 1994). The homogenate was centrifuged (1100 × g, 5 min, 4°C) and the supernatant re-centrifuged (12,000 × g, 10 min, 4°C). The resulting supernatant was removed, the pellet washed in the same buffer and re-centrifuged (12,000 × g, 10 min, 4°C) to obtain the mitochondrial fraction, and the original supernatant was centrifuged (150,000 × g, 90 min, 4°C) to obtain a microsomal pellet (contains plasma membranes) and cytosolic supernatant. The microsomal pellet was resuspended in 100 µl of hypotonic HEPES buffer (0.037 M, containing 0.01 M KF) pH 7.5, centrifuged (150,000 × g, 90 min, 4°C), and the microsomal pellet resuspended in hypotonic HEPES buffer (0.037 M, containing 0.01 M KF, 2 mM ATP, 10 mM MgCl₂·6H₂O, 450 µM IBMX, 1% (w/v) bovine serum albumin) pH 7.5 to give a final concentration of 1 MO equivalent per 20 µl.

2.7. Preparation of cAMP-[¹²⁵I]iodotyrosyl methyl ester for radioimmunoassay

cAMP-tyrosyl methyl ester (2 µg in 5 µl of 10 mM phosphate buffer pH 7.2) was added to 18.5 MBq of carrier-free Na¹²⁵I (Amersham, UK) in a 400-µl microfuge tube. Iodination of the tyrosyl group was initiated by addition of 10 µl of a 0.01% (w/v) solution of chloramine T in 10 mM phosphate buffer pH 7.2. The reaction was incubated at room temperature for 30 s and terminated by addition of 200 µl of 1 M acetic acid. The reaction mixture was applied to the top of a PD-10 gel filtration column (Pharmacia) and the products were eluted with 50 mM acetate buffer pH 4.6 (30 ml). Fractions of 1 ml were collected and a portion of each fraction (2 µl) was assayed for radioactivity. Fractions 17–21 containing cAMP-[¹²⁵I]iodotyrosyl methyl ester were pooled and diluted 2-fold with 50 mM acetate buffer pH 4.6 containing 0.5% (w/v) bovine serum albumin and stored at 4°C prior to use.

2.8. Estimation of cAMP production by MO membranes

Samples of membrane fractions (typically 50 µg of protein) were incubated in hypotonic HEPES buffer (0.037 M, containing 0.01 M KF, 2 mM ATP, 10 mM MgCl₂·6H₂O, 450 µM IBMX, 1% (w/v) bovine serum albumin) pH 7.5 for various times in the presence (treated) or absence (control) of MO-IH-1 (amounts indicated in Section 3) in a final volume of 50 µl at room temperature. Incubations were terminated by addition of 300 µl of acidified ethanol (1% (v/v) concentrated HCl in ethanol) and stored for 2 h at –20°C. Samples were centrifuged, the supernatants removed and evaporated to dryness. The residue was resuspended in 50 mM acetate buffer pH 4.6 and a portion

(25%) analysed for cAMP content by radioimmunoassay.

Estimation of cAMP was carried out by radioimmunoassay using a commercially available anti-cAMP serum (Calbiochem) with cAMP as standard, competing for binding with cAMP-[¹²⁵I]iodotyrosyl methyl ester. To enhance the sensitivity of detection of cAMP, both samples and standards were acetylated prior to radioimmunoassay (RIA; Brooker et al., 1979). For this, 20 µl of triethylamine and 10 µl of acetic anhydride were added simultaneously to each of the samples and vigorously mixed. Separation of bound and unbound cAMP was achieved by incubation of the RIA samples with goat anti-rabbit IgG bound to microparticulate cellulose (Immunodiagnostic Systems Ltd, UK) followed by centrifugation (2000 × g, 4 min). The supernatant was discarded and the pellet radioassayed. In general, the limit of detection of cAMP by this method was approximately 1 fmol cAMP per assay tube.

3. Results

3.1. Effect of various second messenger pathway-effector compounds on methyl farnesoate synthesis

To determine which signal transduction pathway(s) were involved in MO-IH action, experiments investigating the possible role(s) of Ca²⁺, protein kinase C, cGMP and cAMP were carried out. For these experiments, MOs were cultured in the presence (treated) or absence (control) of selected compounds known to affect signal transduction pathways. Following culture, the radiolabelled MF was extracted from each MO and analysed by HPLC with on-line radioactivity detection. The results (Table 1) clearly show that only compounds which affect intracellular levels of cAMP (forskolin), or mimic an increase in intracellular cAMP (8Br-cAMP), had any observed effect on MF synthesis.

3.2. Effects of forskolin and 8Br-cAMP on methyl farnesoate synthesis

Initial results using forskolin and 8Br-cAMP strongly supported a role for cAMP in MO-IH-mediated inhibition of MF synthesis (Table 1). Further experiments demonstrated that MF synthesis was inhibited by forskolin in a dose-dependent manner (Fig. 1a) and that incubation of MOs with 100 µM 1,9-dideoxy-forskolin (an inactive analogue of forskolin) showed no effect on MF synthesis (Table 1). Furthermore, the inhibitory effect of 5 µM forskolin could be enhanced by addition of 250 µM IBMX (inhibits phosphodiesterase activity and, therefore, the breakdown of cAMP; Fig. 1b).

To further strengthen the evidence for a role for cAMP in the signal transduction pathway of MO-IH leading to inhibition of MF synthesis, MOs were cultured in the presence (treated) or absence (control) of various concentrations of a membrane-permeable, non-hydrolysable analogue of cAMP, 8Br-cAMP. The results (Fig. 2) demonstrate a dose-dependent inhibition of MF synthesis in MO cultured in vitro.

3.3. Effect of MO-IH-1 on cAMP production

Initially, to establish the physiological relevance of a cAMP second messenger system activated by MO-IH-1 treatment, the time course of production of cAMP by MO membranes exposed to 100 nM MO-IH-1 was investigated. The production of cAMP in control MO membranes increased with time (Fig. 3).

Subsequently, to determine the effect of MO-IH on cAMP production by MO membranes, MO membranes were exposed to 100 nM MO-IH-1 for varying times and analysed for cAMP content. The results (Fig. 3) show that a maximum 2-fold change in cAMP levels occurs 5 min after addition of 100 nM MO-IH-1 to MO membranes, falling to basal levels thereafter.

To provide further evidence for the physiological relevance of MO-IH-stimulated production of cAMP, MO membranes were incubated for 5 min in the presence of various concentrations of MO-IH-1. The results

(Fig. 4a) clearly demonstrate a dose-dependent stimulation of cAMP production up to a maximum 2-fold change, which is in line with other results presented (Fig. 3).

To clarify whether the observed maximum 2-fold change in cAMP was physiologically relevant, whole MOs were incubated in the presence of 100 μ M forskolin (known to cause a sustained maximum inhibition of MF synthesis; Fig. 1a), the cAMP was extracted and estimated by radioimmunoassay (RIA). The results (Fig. 4b) showed that forskolin treatment caused a 2.4-fold increase in cAMP levels compared with untreated control samples.

4. Discussion

Initial results demonstrate that only compounds that affect intracellular cAMP levels were effective inhibitors of MF synthesis in MOs cultured in vitro. Both forskolin and 8Br-cAMP exhibited dose-dependent inhibition of MF synthesis in MOs cultured in vitro. Treatment of MOs with 1,9-dideoxyforskolin showed no such inhibitory effects, thus demonstrating that the inhibition of MF synthesis by forskolin was specific. Compounds known to affect signal transduction pathways involving Ca^{2+} , cGMP and protein kinase C had no effect on MF synthesis in MOs cultured in vitro (Table 1).

Table 1
Modulation of methyl farnesoate synthesis by second messenger pathway effector compounds

Treatment ^a	Action of compounds	Effect on MF synthesis or MO-IH-1 action in vitro ^b
Up to 10 μ M A23187	Transports divalent cations (Ca^{2+}) across membranes	None
50 nM thapsigargin	Activates release of Ca^{2+} from intracellular stores	None
250 and 500 μ M dibutyryl-cGMP	Membrane-permeable, non-hydrolysable analogue of cGMP	None
20 μ M LY83583	Inhibits soluble guanylate cyclase activity	None
20 μ M LY83583 + 20 nM MO-IH-1		None
200 nM RO31-8220	Inhibits protein kinase C activity	None
200 nM RO31-8220 + 20 nM MO-IH-1		None
10 nM bisindolymaleimide	Selective inhibitor of protein kinase C isoforms	None
10 nM bisindolymaleimide + 20 nM MO-IH-1		None
50 nM phorbol-12-myristate-13-acetate	Stimulates protein kinase C activity	None
500 μ M 8-bromo-cAMP	Membrane-permeable, non-hydrolysable analogue of cAMP	Inhibition of MF synthesis
100 μ M forskolin	Activates adenylate cyclase	Inhibition of MF synthesis
100 μ M 1,9-dideoxyforskolin	Inactive analogue of forskolin	None
250 μ M 3-isobutyl-1-methylxanthine	Inhibits phosphodiesterase activity	Minimal inhibitory effect

^a For all experiments, appropriate control incubations were carried out to compare the effect of the individual treatments listed on MF synthesis in mandibular organs cultured in vitro.

^b In experiments where compounds were tested in conjunction with MO-IH, the results refer to the effect of the compound on MO-IH-1-mediated inhibition of MF synthesis in mandibular organs cultured in vitro. To assess whether MF synthesis in control and treated groups of MOs was statistically different, a paired *t*-test was carried out using a confidence limit of $P < 0.05$ to determine significance.

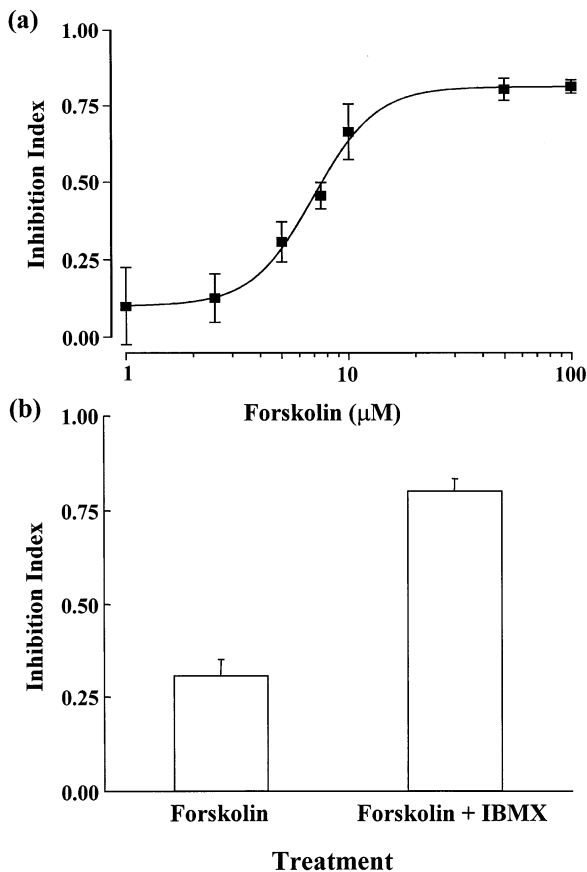


Fig. 1. Inhibition of methyl farnesoate synthesis by forskolin. Freshly dissected mandibular organs from female *Cancer pagurus* were cultured in a modified medium 199, supplemented with [*methyl*- ^3H]methionine, in the presence (treated) or absence (control) of forskolin. Following culture, radiolabelled methyl farnesoate was extracted from MOs and analysed by reversed-phase HPLC with on-line radioactivity detection. Results are expressed as an inhibition index (see Section 2). (a) Effect of forskolin on methyl farnesoate synthesis in mandibular organs cultured in vitro. (b) Effect of 5 μM forskolin and 5 μM forskolin together with 250 μM 3-isobutyl-1-methylxanthine (IBMX) on methyl farnesoate synthesis in mandibular organs cultured in vitro. Values are the mean \pm S.E. for $n = 5$ –15 pairs of MOs.

These foregoing results strongly indicated a role for cAMP in MO-IH-mediated inhibition of MF synthesis. To further substantiate this, the effect of MO-IH on production of cAMP in MOs was investigated. Previous work has demonstrated that inhibition of MF synthesis in MOs by MO-IH-1 is entirely dependent upon the ovary stage of the MO donor animal, being most sensitive to MO-IH-1 at the beginning of vitellogenesis (Wainwright et al., 1996a). In view of this, and the fact that from a selection of crabs there is variability in ovary stages and, therefore, sensitivity of MOs to MO-IH, membranes from batches of MOs from animals at mostly early stages of ovarian development were used to test the effect of MO-IH on cAMP production. On the whole, the ovary stages of the MO donor animals used in these experiments indicated that they would be

sensitive to MO-IH treatment. Initially, when MO membranes were only subjected to one high-speed centrifugation ($150,000 \times g$; see Section 2) for assay of the effect of MO-IH-1 on cAMP production, variable results were obtained. Subsequently, when membranes were washed in hypotonic HEPES (0.037 M, containing 0.01 M KF) pH 7.5 and re-centrifuged, consistent results were obtained.

Treatment of MO membranes with MO-IH-1 caused a transient, 2-fold increase in cAMP after 5 min (Fig. 3). Using this assay system, the effect of MO-IH-1 on production of cAMP was shown to be dose-dependent (Fig. 4a). In previous work (Wainwright et al., 1998) we demonstrated that MOs taken from crabs in the early stages of vitellogenesis are more sensitive ($\text{ED}_{50} = 7$ nM) than those taken from crabs at later stages of vitellogenesis ($\text{ED}_{50} = 50$ nM). An approximate estimate of the ED_{50} from the current dose-response data would place this value close to the ED_{50} value for MOs from animals at early stages of vitellogenesis, which is entirely consistent with the observed stages of vitellogenesis of the animals used for these experiments. Using our current radiochemical assay to monitor MF synthesis in MOs cultured in vitro, only after 30–40 min is the amount of radiolabel incorporated into MF sufficiently large to show a statistically significant difference between control and MO-IH-treated MOs. Consequently, although cAMP levels rise to a maximum 5 min after MO-IH addition, our in vitro assays to measure MF synthesis are not sensitive enough to detect these rapid changes.

Treatment of MOs with 100 μM forskolin (shown to elicit maximum inhibition of MF synthesis in vitro; Fig. 1a) caused a 2.4-fold increase in cAMP levels compared with untreated control samples (Fig. 4b). Thus, the fold change of cAMP levels caused by forskolin treatment is

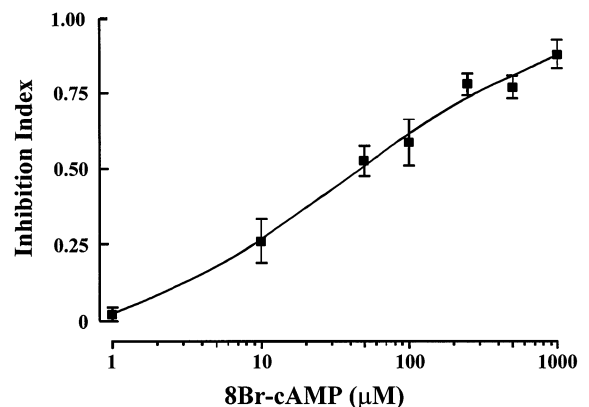


Fig. 2. Inhibition of methyl farnesoate synthesis by 8-Br-cAMP. Freshly dissected mandibular organs from female *Cancer pagurus* were cultured in the presence (treated) or absence (control) of various concentrations of 8Br-cAMP and radiolabelled methyl farnesoate extracted and assayed as described (see Section 2). Values are mean \pm S.E. for $n = 8$ –15 pairs of MOs.

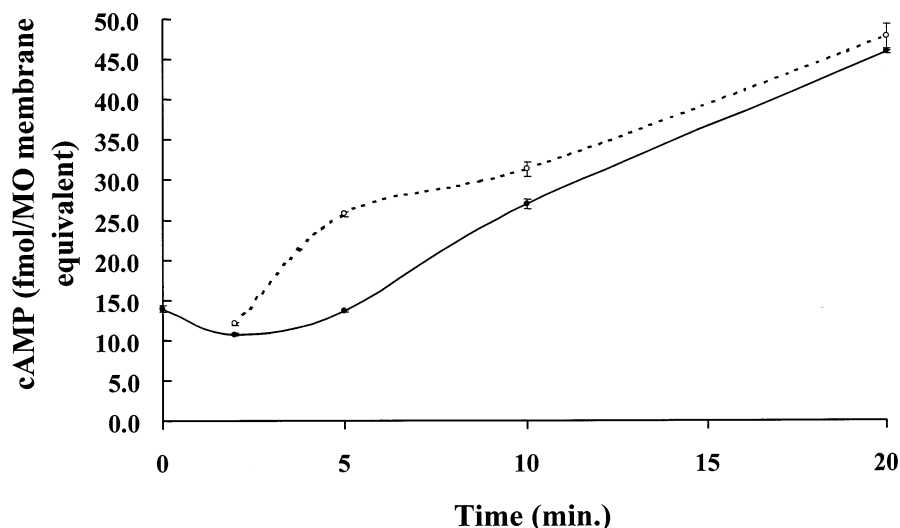


Fig. 3. Time course of stimulation of production of cAMP in mandibular organ membranes by MO-IH-1. Batches of 20–40 mandibular organs were dissected and membrane fractions prepared as outlined in Section 2. MO membranes were cultured in the presence (treated; open circles, dashed line) or absence (control; closed circles, solid line) of 100 nM MO-IH-1 for 5 min and the cAMP content estimated by RIA. Values represent the mean \pm S.E. for $n = 4$.

of the same order of magnitude as that produced by 100 nM MO-IH-1. This supports the physiological relevance of the MO-IH-stimulated production of cAMP.

An interesting observation derived from the use of cAMP analogues and forskolin to inhibit MF synthesis in MOs is that the degree of inhibition of a given dose of these compounds was independent of the vitellogenic stage of the MO donor animal (results not shown). The stage-independent effect of forskolin is in contrast to that observed when MF synthesis is inhibited by MO-IH, which clearly exhibits a vitellogenic stage-dependent effect (Wainwright et al., 1996b, 1998). This may suggest that the receptor for MO-IH on the MO cell surface undergoes developmental changes which lead to modulation of the inhibitory action of MO-IH and, thus, may be one mechanism by which haemolymph levels of MF are regulated throughout a cycle of ovarian development in *Cancer pagurus*.

With respect to the signal transduction mechanisms utilized by analogous systems, crude extracts of lobster sinus glands have been shown to inhibit MF biosynthesis in lobster MOs, the inhibition being mimicked by treatment of lobster MOs with diBu-cGMP, suggesting a requirement for guanylate cyclase activity in regulation of MF biosynthesis in lobster MOs (Tsukimura et al., 1993). An earlier report showed, by use of the Ca^{2+} ionophore A23187, that Ca^{2+} was involved in the signal transduction pathway of inhibition of MF synthesis in crayfish MOs (Landau et al., 1989). However, these experiments did not utilize purified MO-IH.

In crustaceans, molt-inhibiting hormone (MIH) is a structurally similar peptide that negatively regulates ecdysteroidogenesis in Y-organs. It has been shown

that cAMP and Ca^{2+} were involved in signal transduction of MIH of the crab, *Cancer antennarius* (Mattson and Spaziani, 1986). Furthermore, in both the crayfish, *Orconectes limosus*, and the crab, *Carcinus maenas*, treatment of Y-organs with MIH or sinus gland extract (containing MIH) caused a modest and rapid elevation of cAMP levels, followed by a larger and more prolonged increase of cGMP levels (Sedlmeier and Fenrich, 1993; Saïdi et al., 1994). Since crude extract of sinus glands which contain many different peptides, rather than pure MIH, was used in the crayfish study, it is not inconceivable that multiple signal transduction pathways were activated. However, in *Carcinus maenas*, the effect of purified MIH on Y-organs is rapid (5 min) and reversible (Saïdi et al., 1994), and thus it may be that, initially, cAMP is the second messenger leading to a rapid inactivation of enzymes involved in ecdysteroidogenesis through modulation of phosphorylation states, whilst the signal transduction system maintains the inhibited state of the MIH-treated Y-organ over a longer period of time (hours). Thus, a mechanism of (pulsatile) release of peptides from XO-SG may be supported by a longer-lasting response of the peptide target tissue mediated by a dual cAMP/cGMP second messenger system. With respect to the current work, it has been shown that MOs of *Cancer pagurus* treated with MO-IH-1 or -2 also exhibit a rise in cGMP levels following that of cAMP (Webster, unpublished), but, unlike MIH-mediated inhibition of ecdysteroidogenesis in Y-organs, cGMP analogues or substances which affect intracellular cGMP levels have no effect on MF synthesis (Table 1). The significance of the increase in cGMP in these systems is unclear at present, but it is

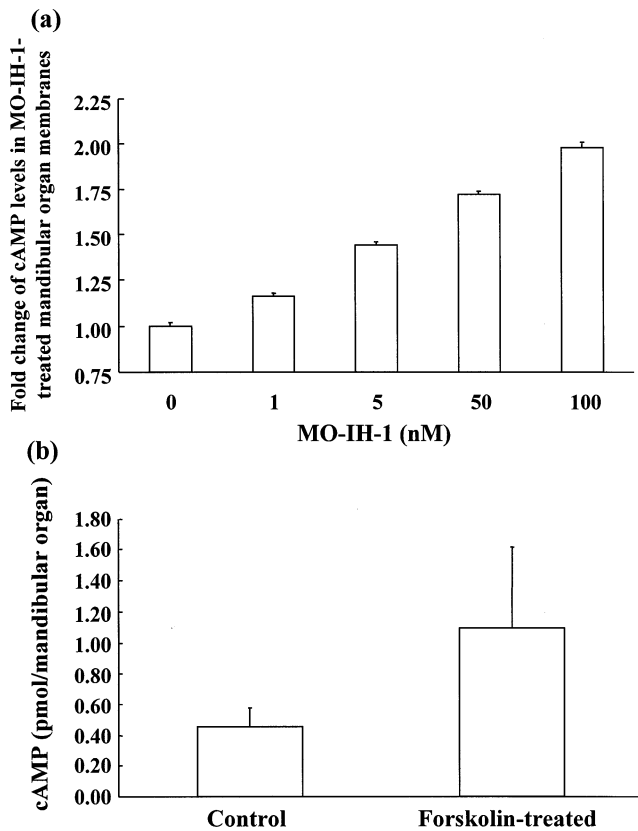


Fig. 4. Dose-dependent stimulation of production of cAMP by MO-IH-1. Batches of 20–40 mandibular organs were dissected and membrane fractions prepared as outlined in Section 2. (a) Dose-dependent stimulation of production of cAMP by MO-IH-1 in mandibular organ membranes incubated for 5 min. Values are expressed as a fold change in cAMP compared with the amount of cAMP produced by untreated mandibular organ membranes (15.9 ± 0.5 fmol per mandibular organ membrane equivalent). (b) Effect of forskolin on intracellular levels of cAMP in untreated control and 100 μ M forskolin-treated intact mandibular organs. Values are the mean \pm S.E. of $n = 4$ –8 samples.

apparent from the current study that cGMP does not play an obvious role in inhibition of MF synthesis. However, this does not preclude other roles for MO-IH in regulation of physiological processes in MOs which may utilize cGMP as a second messenger.

In summary, the results presented demonstrate that cAMP is involved in the signal transduction pathway of MF biosynthesis in MOs from the edible crab, *Cancer pagurus*.

Acknowledgements

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