

SHORT COMMUNICATION

THREE ANTHOZOAN NEUROPEPTIDES, Antho-RFamide AND Antho-RWamides I AND II, MODULATE SPONTANEOUS TENTACLE CONTRACTIONS IN SEA ANEMONES

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Accepted 31 August 1990

Behavioural coordination in sea anemones involves both excitation and inhibition. Examples include reciprocal inhibition between longitudinal and circular muscles (Batham and Pantin, 1954), inhibitory actions during peristalsis (Ewer, 1960; McFarlane, 1974a), joint excitatory and inhibitory innervation of sphincter muscle (Lawn, 1976) and inhibition of neural pacemakers (McFarlane, 1974b).

We recently showed that three naturally occurring anthozoan neuropeptides (Antho-RFamide, Antho-RWamide I and II) have excitatory actions on endodermal muscles of *Calliactis parasitica* (Couch), and we proposed that they are neuromodulators or neurotransmitters (McFarlane *et al.* 1987, 1990). We know little, however, about possible inhibitory transmitters. Here we report the actions of these three neuropeptides on spontaneous contractions of isolated tentacles. For the first time we have identified peptide transmitter candidates with an inhibitory action in sea anemones.

Actinia equina (L.) were collected at Cayton Bay, North Yorkshire. They were kept in artificial sea water (ASW: Instant Ocean) at 15–20°C and were fed weekly. Antho-RFamide and the Antho-RWamides (Grimmelikhuijzen and Graff, 1986; Graff and Grimmelikhuijzen, 1988a,b) were synthesised by Bachem, Bubendorf, Switzerland. A tentacle was cut from an unanaesthetized animal and transferred to a Petri dish of ASW. A thread was tied round each end. The tentacle was suspended vertically in a 5 ml bath of ASW and attached to a light (150 mg) isotonic lever on a Washington Bioscience transducer. Gentle aeration (with a fine air stream) was used. Preparations were left for 30 min before experiments began. Isolated tentacles remained in good condition for several hours. They were not normally used for more than 2 h or for more than two applications of neuropeptide: there was some evidence for reduced responsiveness with repeated trials.

Neuropeptide solutions (in ASW) were pipetted into the bath. Control doses of

Key words: sea anemone, neuropeptides, neurotransmitters, *Actinia equina*.

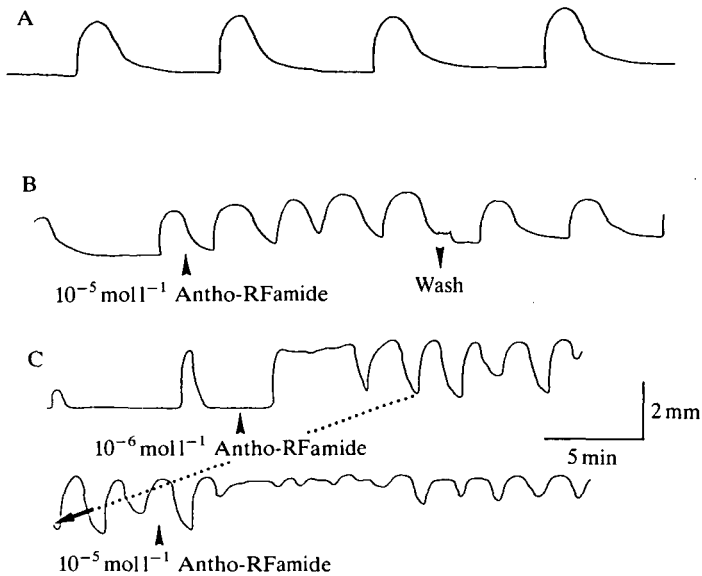


Fig. 1. Isolated tentacles of *Actinia equina* show spontaneous contractions. Contraction frequency is increased by Antho-RFamide. (A) Typical record from a tentacle, 30 min after removal, showing rhythmic contractions. (B) $10^{-5} \text{ mol l}^{-1}$ Antho-RFamide causes a marked increase in contraction frequency that is easily reversed by washing. This preparation was comparatively insensitive to the peptide. (C) A more responsive preparation, showing effects of a $10^{-6} \text{ mol l}^{-1}$ dose, followed by a $10^{-5} \text{ mol l}^{-1}$ dose.

ASW had no effect. The peptide was distributed by gentle aeration with the pipette; all concentrations given are the final bath concentration, assuming even

mixing. The thresholds quoted are the lowest found, and represent the levels seen in about 50% of preparations.

Contractions of ectodermal longitudinal muscles were monitored in isolated tentacle preparations. Most tentacles showed spontaneous contractions (Fig. 1A), normally about 200–450 s apart (mean 295 ± 69 s, $N=100$, i.e. 10 intervals measured for 10 different preparations). Contractions reached 50% of maximum amplitude within approximately 5 s (mean 4.9 ± 2.3 s, $N=14$) and the peak was reached after approximately 23 s (mean 22.8 ± 6.9 s, $N=14$). Contractions were usually regular and rhythmic but some preparations showed irregular contractions or were completely quiescent. No explanation for this variability could be found; it was not obviously linked to the season when animals were collected, time of experimentation, nutritional state, or whether anaesthetic was used. Spontaneous contractions always showed a gradual decrease in frequency and regularity: we assume that this represents deterioration of the preparation, because by this time

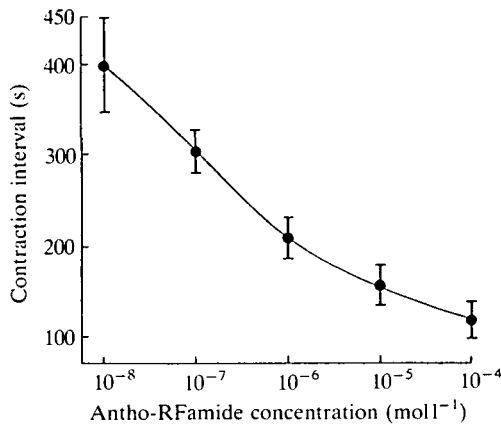


Fig. 2. Dose-response curve for the action of Antho-RFamide on tentacles of *Actinia equina*. Note that, because of the wide range of spontaneous contraction frequencies in different tentacles, the action of Antho-RFamide concentrations below $10^{-6} \text{ mol l}^{-1}$ can be shown clearly only on relatively quiescent preparations with contraction intervals greater than 350 s. The contraction interval was measured 5 min after addition of the peptide. For $10^{-6} \text{ mol l}^{-1}$ Antho-RFamide $N=12$, otherwise $N=8$. Bars show S.E.M.

contraction frequency (Fig. 1B). Contraction amplitude changed little, but contraction duration increased (e.g. in 10 trials, at an Antho-RFamide concentration of $10^{-5} \text{ mol l}^{-1}$, mean contraction duration increased by $45 \pm 11\%$). At high concentrations ($10^{-5} \text{ mol l}^{-1}$ and above) there was often a period of maintained contraction for up to 10 min following application, with only irregular periods of relaxation, before rhythmic activity reappeared (Fig. 1C). Increased concentrations led to an increase in the frequency of spontaneous contractions (Fig. 1C); this was maintained for several hours if the preparation was not washed. Antho-RFamide ($10^{-5} \text{ mol l}^{-1}$) always induced rhythmic contractions in quiescent preparations. Different preparations had different thresholds but the dose-response curve (Fig. 2), obtained from tentacles from 10 individuals, clearly shows a threshold below $10^{-7} \text{ mol l}^{-1}$ and near maximum action at around $10^{-4} \text{ mol l}^{-1}$.

In contrast to the excitatory action of the Antho-RWamides on endodermal muscles (McFarlane *et al.* 1990), we found an inhibitory action on isolated tentacles (Fig. 3). The duration of inhibition was dose-dependent. Below $10^{-5} \text{ mol l}^{-1}$ Antho-RWamide I, the contractions restarted when the preparation was not washed. Recovery took different forms: in about 50% of cases contractions restarted at the pre-test frequency (Fig. 3A), in 45% of cases contraction frequency was reduced (Fig. 3B) and remained low for at least 1 h. Rarely, the subsequent contractions were more frequent than before addition of peptide (Fig. 3C). Application of $5 \times 10^{-6} \text{ mol l}^{-1}$ Antho-RWamide I inhibited contractions for $425 \pm 90 \text{ s}$, range = 320–540 s (10 different tentacles tested). These intervals overlap the range of normal spontaneous contractions, so are only seen clearly when this concentration of Antho-RWamide I is applied to the more active

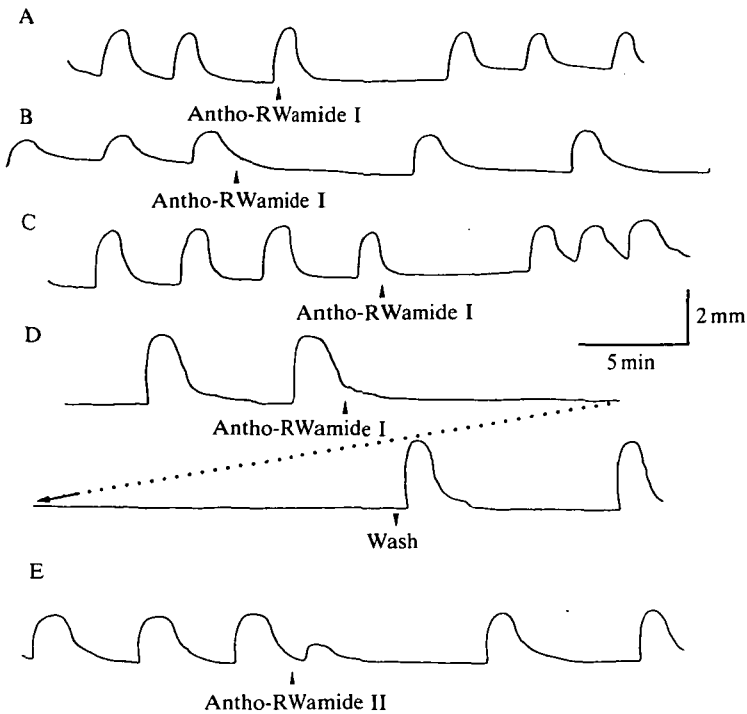


Fig. 3. Antho-RWamide I and II inhibit spontaneous contractions of isolated tentacles of *Actinia equina*. (A,B,C) Antho-RWamide I at $5 \times 10^{-6} \text{ mol l}^{-1}$, (D) Antho-RWamide I at $2 \times 10^{-5} \text{ mol l}^{-1}$, (E) Antho-RWamide II at $5 \times 10^{-6} \text{ mol l}^{-1}$.

preparations. At higher concentrations ($\geq 2 \times 10^{-5} \text{ mol l}^{-1}$), however, Antho-RWamide I completely abolished spontaneous activity (Fig. 3D). Activity returned after washing (even after 2 h of inhibition). Antho-RWamide II also inhibited spontaneous contractions (Fig. 3E), but inhibition was usually preceded by a small contraction immediately after addition of peptide. As with Antho-RWamide I, the threshold for inhibition was around $10^{-6} \text{ mol l}^{-1}$.

Excitatory and inhibitory peptides can interact. Preparations inhibited with $5 \times 10^{-6} \text{ mol l}^{-1}$ Antho-RWamide I or II will contract in $10^{-5} \text{ mol l}^{-1}$ Antho-RFamide. Conversely, preparations excited by $10^{-6} \text{ mol l}^{-1}$ Antho-RFamide can be inhibited by $10^{-5} \text{ mol l}^{-1}$ Antho-RWamide I or II. Below $10^{-5} \text{ mol l}^{-1}$ the action of Antho-RWamide I or II is temporary; above this concentration these neuropeptides can completely abolish Antho-RFamide-induced contractions.

The Antho-RWamides inhibit spontaneous contractions of ectodermal tentacle muscles in *Actinia equina*: this is the first time that naturally occurring substances have been shown to inhibit spontaneous contractions in sea anemones. Carlyle (1974) found that $10^{-3} \text{ mol l}^{-1}$ glutamic acid inhibited electrically evoked contractions of sphincter muscle of *A. equina*. However, the slow onset of inhibition, and the high concentration required, suggests to us that glutamic acid is not a natural inhibitory transmitter in *A. equina*. The dual action of the Antho-RWamides,

excitatory on some muscles (McFarlane *et al.* 1990) and inhibitory on others, is seen in other invertebrate neuropeptides. For example, FMRFamide induces contraction in *Helix aspersa* epiphallus, but reduces resting tone and rhythmic activity in the crop (Lehman and Greenberg, 1987).

Antho-RFamide and the Antho-RWamides might act directly on the ectodermal longitudinal muscles of the tentacles or on their nervous pacemakers; the present experiments do not distinguish between these possibilities. Quaglia and Grasso (1986) have described possible peptidergic neurosecretory cells in *A. equina* tentacles: these neurones synapse onto each other but there are also other release sites of the presumed peptide hormones near, but not in contact with, the subepithelial nerve plexus. Clearly such cells could modulate activity in the plexus, but the identity of the peptide is not yet known.

IDM was supported by grants from the University of Hull Research Fund and the Leverhulme Trust. CJPG obtained financial support from the Bundesministerium für Forschung und Technologie and the Deutsche Forschungsgemeinschaft. We thank Dr P. A. V. Anderson for his comments during the preparation of this paper.

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