J. exp. Biol. **164**, 295–299 (1992)
Printed in Great Britain © *The Company of Biologists Limited 1992*

SHORT COMMUNICATION

OPPOSITE ACTIONS OF THE ANTHOZOAN NEUROPEPTIDE Antho-RNamide ON ANTAGONISTIC MUSCLE GROUPS IN SEA ANEMONES

BY IAN D. MCFARLANE

Department of Applied Biology, University of Hull, Hull HU6 7RX, UK

RAINER K. REINSCHEID AND CORNELIS J. P. GRIMMELIKHUIJZEN

Centre for Molecular Neurobiology, University of Hamburg, Martinistrasse 52, 2000 Hamburg 20, Germany

Accepted 15 November 1991

Coelenterates are generally assumed to be primitive animals with simple nervous systems. We believe, however, that in at least one group, the sea anemones, the nervous system is in reality rather complex, a view supported by the growing number of neuropeptides recently extracted from these animals (Grimmelikhuijzen *et al.* 1990*a*,*b*). Three of these peptides (Antho-RFamide and the Antho-RWamides I and II) have demonstrable physiological actions on sea-anemone muscle preparations (McFarlane *et al.* 1987, 1990; McFarlane and Grimmelikhuijzen, 1991) and, in the case of the Antho-RWamides, on isolated muscle cells (McFarlane *et al.* 1991). Here we consider a fourth neuropeptide from sea anemones, Antho-RNamide (L-3-phenyllactyl-Leu-Arg-Asn-NH_2) (Grimmelikhuijzen *et al.* 1990), and show that it has opposite actions on adjacent antagonistic muscles in sea anemones.

Isolation and sequencing of Antho-RNamide are described elsewhere (Grimmelikhuijzen *et al.* 1990), as are supply of animals [*Calliactis parasitica* (Couch), *Actinia equina* (L.) and *Urticina felina* (L.)], methods of making isolated preparations, and neurophysiological techniques (McFarlane *et al.* 1987, 1991; McFarlane and Grimmelikhuijzen, 1991). The only novel preparations were circular rings (2 mm wide) cut from the particularly stout tentacles of *U. felina*. Threads were tied to the rings, and contractions were monitored with an isotonic transducer. Synthetic Antho-RNamide was a custom synthesis carried out by Bachem (Bubendorf, Switzerland). Quoted peptide concentrations assume even mixing. In the experiments with intact animals, Antho-RNamide was injected into the coelenteron through a fine plastic tube inserted into the lower column.

Antho-RNamide stimulates contractions of longitudinal muscle preparations. For example, isolated tentacle preparations of *Actinia equina* shorten due to contraction of ectodermal longitudinal muscles (McFarlane and Grimmelikhuijzen, 1991). Some tentacle preparations were quiescent, others spontaneously

Key words: sea anemones, neuropeptides, muscle contraction, excitation, inhibition.

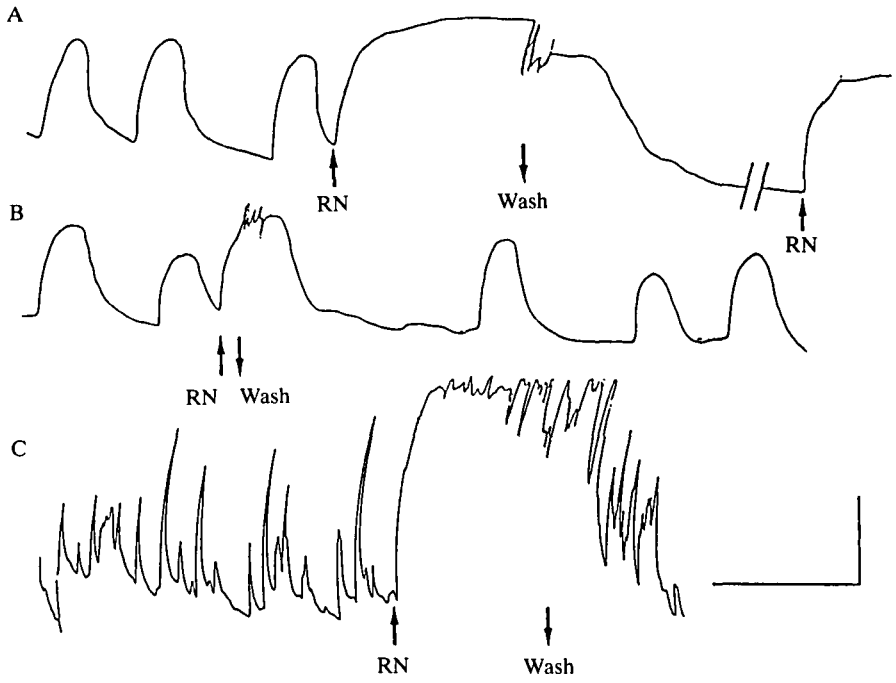

Fig. 1. Action of Antho-RNamide (RN) on ectodermal and endodermal longitudinal muscles of sea anemones. (A) Antho-RNamide (10^{-5} mol l^{-1}) evoked contraction of ectodermal longitudinal muscles in isolated tentacles of *Actinia equina*. The break in the record is a 5 min period without change in tentacle length. (B) Action of a 30 s pulse of Antho-RNamide (10^{-5} mol l^{-1}) on an isolated tentacle. (C) Antho-RNamide (5×10^{-5} mol l^{-1}) induced contraction of a column strip preparation of *Calliactis parasitica*. Upward-pointing arrows, addition of peptide; downward-pointing arrows, wash. Time scale: A,B 3 min, C 60 min. Amplitude scale: A,B 2 mm, C 5 mm.

active, but Antho-RNamide (threshold 10^{-6} mol l^{-1}) caused contraction in all cases (N=30). Maximal contraction was evoked at 5×10^{-5} mol l^{-1} (Fig. 1A) and, if the preparation was not washed, was maintained for over an hour. With rhythmically active tentacles, a short (30 s) pulse of Antho-RNamide (10^{-5} mol l^{-1}) caused a contraction that was quickly followed by a period of relaxation and, then, resumption of spontaneous activity (Fig. 1B).

After longer exposure (>60 s) to the peptide, however, the spontaneous activity usually failed to restart, no matter how often the preparation was washed. Although the spontaneous contractions were inhibited, the contractile system was still functional since a further dose of Antho-RNamide (10^{-5} mol l^{-1}) again evoked contraction (Fig. 1A). Antho-RNamide also evoked contraction of 5 mm wide longitudinal column strips in *Calliactis parasitica* (Fig. 1C). Mesenteries were trimmed away, so observed spontaneous contractions were of endodermal parietal muscles (the body wall has no ectodermal muscles). Threshold was around 10^{-6} mol l^{-1}, and maximal contraction was reached at 10^{-5} mol l^{-1} (N=15). These

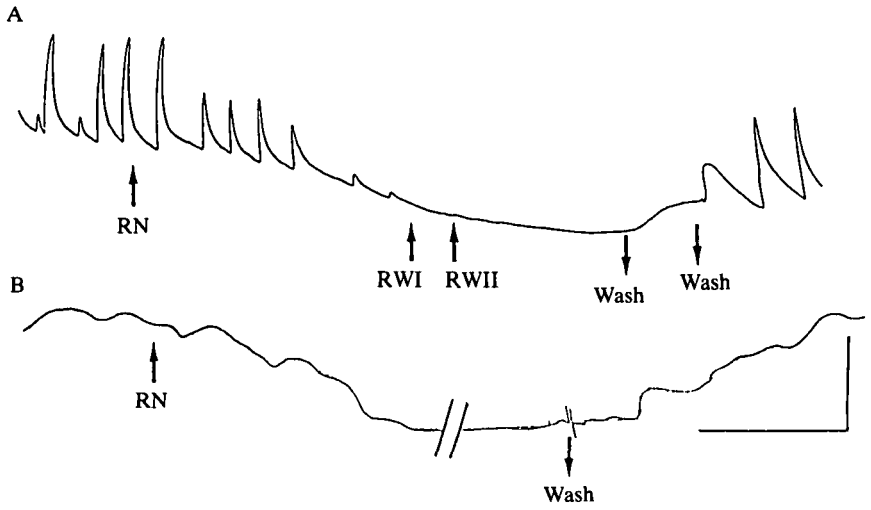

Fig. 2. Action of Antho-RNamide on circular muscles of sea anemones. (A) Inhibition of spontaneous contractions of sphincter muscle rings of *Calliactis parasitica* by 5×10^{-5} mol l^{-1} Antho-RNamide (RN). Subsequent applications of Antho-RWamides I and II (RWI and RWII, both 5×10^{-5} mol l^{-1}), natural excitants of sphincter muscle, were without effect. (B) Inhibition of spontaneous contractions of tentacle circular muscle of *Urticina felina* by 2×10^{-5} mol l^{-1} Antho-RNamide. The break in the record is a 5 min period without change in tentacle ring length. Upward-pointing arrows, addition of peptide; downward-pointing arrows, wash. Time scale: A 60 min, B 3 min. Amplitude scale: A 5 mm, B 2 mm.

column strips showed a rapid return to a normal spontaneous rhythm after washing.

Antho-RNamide inhibited the spontaneous activity of circular muscle preparations. This is shown in Fig. 2A for a mesogloeal sphincter muscle ring from *Calliactis parasitica* upper column. Threshold was around 10^{-6} mol l^{-1}. The action had a slow onset, shown by a gradual reduction in amplitude of spontaneous contractions. At 10^{-5} mol l^{-1}, the contractions were diminished rather than abolished, and recovery occurred within 60 min, even without washing ($N=10$). Contractions were abolished at 5×10^{-5} mol l^{-1} but normal spontaneous activity restarted after washing. In the presence of Antho-RNamide (5×10^{-5} mol l^{-1}) the sphincter failed to contract in response to subsequent addition of 5×10^{-5} mol l^{-1} Antho-RWamides I or II, two neuropeptides known to have a direct excitatory action on sphincter muscle (McFarlane *et al.* 1991) (Fig. 2A). Circular rings of *Calliactis parasitica* cut below the sphincter contain only endodermal circular muscle. They were also spontaneously active and the activity was completely inhibited by 5×10^{-5} mol l^{-1} peptide. Similar results were obtained with *Actinia equina* column rings and *Urticina felina* tentacle rings (Fig. 2B).

We were unable to show whether Antho-RNamide acts directly on muscle cells or indirectly on conducting systems that innervate the muscle. A direct excitatory

action has been proved in the case of Antho-RWamides I and II: they make isolated sphincter muscle cells contract (McFarlane *et al.* 1991). Unfortunately, we cannot test for a direct excitatory action of Antho-RNamide, because viable isolated cells have not yet been obtained from longitudinal muscles. We cannot use isolated sphincter muscle cells to test whether Antho-RNamide inhibition is direct, for these cells do not contract and relax spontaneously. An experiment designed to look for indirect neuronal actions of Antho-RNamide was unsuccessful because the anemones contracted and threw off the electrodes. The standard way of monitoring electrical activity in the three known conducting systems in sea anemones is to attach suction electrodes to tentacles of intact animals. Pulses are identified by applying electrical stimulation to the column (McFarlane *et al.* 1990). We could not, however, use this method because injection of Antho-RNamide into the coelenteron caused the tentacles to shorten immediately, thereby dislodging the electrodes. The anemone stayed open after Antho-RNamide injection (i.e. the sphincter muscle did not contract) and the tentacles and the column shortened to half their resting length. The tentacles became noticeably thicker as they shortened. These shape changes are predicted by the observed actions on isolated preparations. The final concentration of injected peptide was approximately 10^{-5} mol l^{-1} and recovery was complete within 90 min. Control injections of sea water had no effect.

The actions of Antho-RNamide on isolated muscle preparations of sea anemones and intact animals can be summarized as follows: (i) it evokes contraction of longitudinal muscles in tentacles and body wall; and (ii) it inhibits endogenous contractions of circular muscles in tentacles and body wall. Although we do not know whether Antho-RNamide has direct or indirect actions, these results are important because the different actions of Antho-RNamide on longitudinal and circular muscles may have a functional significance. Sea anemones have antagonistically arranged muscle sheets: longitudinal muscles, found in tentacle ectoderm and body wall endoderm, are closely associated with circular muscles lying in tentacle and body wall endoderm. Coordination of paired antagonistic muscles during changes in body shape requires that, when one contracts, the other is inhibited. The opposing actions of Antho-RNamide are precisely those expected of a transmitter that coordinates antagonistic actions of longitudinal and circular muscles. Complete coordination requires an additional substance with actions opposite to those of Antho-RNamide. The Antho-RWamides fulfil this requirement; their effects on tentacle ectodermal muscles (inhibition) and endodermal circular muscles (excitation) are the mirror image of those of Antho-RNamide (McFarlane *et al.* 1991; McFarlane and Grimmelikhuijzen, 1991).

I.D.M. was supported by a Ciba-Geigy ACE award, C.J.P.G. by the Bundesministerium für Forschung und Technologie and the Deutsche Forschungsgemeinschaft (Gr 762/7–3).

References

GRIMMELIKHUIJZEN, C. J. P., GRAFF, D., KOIZUMI, O., WESTFALL, J. A. AND MCFARLANE, I. D. (1990*a*). Neurons and their peptide transmitters in coelenterates. In *Evolution of the First Nervous Systems* (ed. P. A. V. Anderson), pp. 95–109. New York: Plenum Press.

GRIMMELIKHUIJZEN, C. J. P., RINEHART, K. L., JACOB, E., GRAFF, D., REINSCHEID, R., NOTHACKER, H.-P. AND STALEY, A. L. (1990*b*). Isolation of L-3-phenyllactyl-Leu-Arg-Asn-NH_2 (Antho-RNamide), a sea-anemone neuropeptide containing an unusual amino-terminal blocking group. *Proc. natn. Acad. Sci. U.S.A.* **87**, 5410–5414.

MCFARLANE, I. D., ANDERSON, P. A. V. AND GRIMMELIKHUIJZEN, C. J. P. (1991). Effects of three anthozoan neuropeptides, Antho-RWamide I, Antho-RWamide II and Antho-RFamide, on slow muscles from sea anemones. *J. exp. Biol.* **156**, 419–431.

MCFARLANE, I. D., GRAFF, D. AND GRIMMELIKHUIJZEN, C. J. P. (1987). Excitatory actions of Antho-RFamide, an anthozoan neuropeptide, on muscles and conducting systems in the sea anemone *Calliactis parasitica*. *J. exp. Biol.* **133**, 157–168.

MCFARLANE, I. D., GRAFF, D. AND GRIMMELIKHUIJZEN, C. J. P. (1990). Evolution of conducting systems and neurotransmitters in the Anthozoa. In *Evolution of the First Nervous Systems* (ed. P. A. V. Anderson), pp. 111–127. New York: Plenum Press.

MCFARLANE, I. D. AND GRIMMELIKHUIJZEN, C. J. P. (1991). Three anthozoan neuropeptides, Antho-RFamide and Antho-RWamides I and II, modulate spontaneous tentacle contractions in sea anemones. *J. exp. Biol.* **155**, 669–673.