

THE ACTIONS OF FMRFamide-LIKE PEPTIDES ON VISCERAL AND SOMATIC MUSCLES OF THE SNAIL *HELIX ASPERSA*

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Accepted 7 April 1987

SUMMARY

Phe-Met-Arg-Phe-NH₂ (FMRFamide) and pyroGlu-Asp-Pro-Phe-Leu-Arg-Phe-NH₂ (pQDPFLRFamide) occur in the ganglia and tissues of the snail, *Helix aspersa*. This report describes the effects of these two neuropeptides on five visceral organs or somatic muscles isolated from the snail (Table 1).

The epiphallus, as well as the rest of the male reproductive tract, was contracted by both FMRFamide and pQDPFLRFamide, and the threshold was usually below 5×10^{-9} mol l⁻¹ (Fig. 1). Both peptides also reduced the resting tone of the crop and decreased the force and frequency of its rhythmic activity; FMRFamide is about 10 times more potent (Fig. 4). In contrast, pQDPFLRFamide was about 100 times more potent than FMRFamide as a cardioexcitatory agent (Fig. 5). The actions of the peptides on the pharyngeal and tentacle retractor muscles were markedly different: FMRFamide primarily contracted these muscles; and pQDPFLRFamide usually had no effect alone, but relaxed or diminished contractions induced by FMRFamide and acetylcholine (ACh) (Figs 6, 8, 9). Other analogues of FMRFamide were tested, but none was as effective a relaxing agent as pQDPFLRFamide. The effects of FMRFamide and pQDPFLRFamide on all of the preparations could be distinguished from those produced by ACh and 5-hydroxytryptamine (5-HT); thus the actions of the neuropeptides were not mediated by cholinergic or serotonergic neurones.

The stimulation of the musculature in the male reproductive tract and the inhibition of motility of the digestive system by FMRFamide and pQDPFLRFamide implicate these peptides in the control of reproductive behaviour. The effectiveness of pQDPFLRFamide in relaxing the retractor muscles and as a cardioexcitatory agent led to the hypothesis that this heptapeptide and FMRFamide, acting at distinct receptors, cooperate to regulate the excitability and contractility of the snail's musculature between the extremes of aestivation and active locomotion.

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INTRODUCTION

FMRFamide and pQDPFLRFamide are the two identified members of the family of FMRFamide-like peptides in *Helix aspersa* (Price *et al.* 1985). Both peptides are widely, but unevenly, distributed in the tissues of the snail (Lehman & Price, 1987); but immunoreactive (ir-) FMRFamide is invariably localized in the fine nerves innervating muscle in these tissues. Thus either peptide could be functioning as a neuromuscular transmitter or modulator and should therefore affect the tension and rhythmicity of those tissues.

The effects of FMRFamide on the neurones, tentacle retractor muscle (TRM) and heart of *Helix* have previously been investigated. FMRFamide stimulates K^+ and Na^+ currents and a Ca^{2+} -dependent K^+ current in individual, identified neurones of the visceral, right parietal and cerebral ganglia (Cottrell, Davies & Green, 1984). Concentrations of FMRFamide lower than 10^{-7} mol l $^{-1}$ contract the TRM, whereas higher concentrations inhibit rhythmicity and tone (Cottrell, Schot & Dockray, 1983b). Finally, FMRFamide is a cardioexcitatory agent in *Helix*, as in other molluscs (Greenberg & Price, 1980). The multiple effects of FMRFamide on the neurones and the TRM, together with the multiplicity of FMRFamide-like peptides present in *Helix* (Price *et al.* 1985), suggested that the actions might be allocated amongst the peptides. Therefore, we decided to compare the actions of the two identified FMRFamide peptides on several muscular organs of *Helix*.

In this study, tissues representative of each visceral system, and containing different levels of endogenous peptides, were isolated and challenged with FMRFamide and pQDPFLRFamide. We also examined the pharmacologies of acetylcholine and 5-hydroxytryptamine (known neurotransmitters in *Helix*; Leake & Walker, 1980) to test the possibility that the peptides are acting indirectly *via* cholinergic or serotonergic mechanisms. The mechanical activity of each tissue was changed by a direct action of the peptides, but the responses were neither exclusively excitatory nor exclusively inhibitory, and the effects of each peptide were not the same on every tissue.

MATERIALS AND METHODS

Helix aspersa were supplied by Dr R. F. Koch of California State University, Fullerton. The snails arrived aestivating, they were maintained in this condition, and were used within 3 weeks.

The shell was removed, and the animal was pinned out while still contracted. All of the visceral organs were exposed by a dorsal midline incision (anatomy described by Leake, 1975; Hyman, 1967).

Five muscles or muscular organs were isolated from the snail. The tissues (except for heart) were simply ligated and then transected distal to the paired ties, which were located as follows. The epiphallus was tied at its junctions with the penis retractor muscle and the vas deferens. The crop was tied just posterior to the oesophagus and just anterior to the stomach at the opening to the digestive gland. The pharyngeal retractor muscle was tied just posterior to its insertion on the buccal

mass and at its site of origin from the columella muscle. The tentacle retractor muscle was tied close to its origin on the columella and distal to the eye spot. The ventricle was cannulated by way of the auricle and perfused with *Helix* saline. A small hook in the wall of the aorta provided for recording while allowing the perfusing stream of saline to flow freely.

The isolated muscles, including the cannulated heart, were suspended in a muscle bath containing saline (Kerkut & Meech, 1965). Tension was recorded with a force-displacement transducer connected to an inkwriting oscillograph (Grass Instrument Co.). Each of the mechanical records is representative of at least eight preparations.

Acetylcholine chloride (ACh) and 5-hydroxytryptamine (5-HT) were purchased from Sigma, and FMRFamide was purchased from Peninsula Laboratories. Several FMRFamide analogues were kindly supplied by Dr J. P. Riehm of the University of West Florida.

Drugs delivered to the cannulated heart were dissolved in saline and injected into the perfusing stream. For other muscles, drug solutions were added directly to the organ bath, and the doses were expressed as the final molar concentration (in mol l^{-1}) in the bath.

RESULTS

Survey of responses

Five muscles or muscular organs were tested. In each case, the response and sensitivity to FMRFamide and pQDPFLRFamide were characterized and compared with those to ACh and 5-HT, other known transmitters in *Helix*. The results are described below and summarized in Table 1.

Table 1. A summary of the responses of five *Helix* tissues

Tissue	FMRFamide	pQDPFLRFamide	Acetylcholine	5-HT
Epiphallus	(+) $1.5-15 \times 10^{-10}$	(+) $5-50 \times 10^{-10}$	(-) $1.5-5 \times 10^{-7}$ (+) $1.5-5 \times 10^{-6}$	(+) $1.5-15 \times 10^{-8}$
Crop	(-) $1.5-15 \times 10^{-9}$	(-) $1.5-15 \times 10^{-10}$	(+) $1.5-5 \times 10^{-8}$	(±) $1.5-5 \times 10^{-8}$
Ventricle	(+) 40 pmol*	(+) 0.4 pmol*	(-) 2 pmol*	(+) 0.1 pmol*
Tentacle retractor muscle	(+) $1.5-50 \times 10^{-9}$	(-) $1.5-5 \times 10^{-7}$	(+) $1.5-5 \times 10^{-6}$	(-) $1.5-5 \times 10^{-8}$
Pharyngeal retractor muscle	(+) $1.5-50 \times 10^{-8}$	(-) $1.5-5 \times 10^{-6}$	(+) $1.5-3 \times 10^{-6}$	(-) $1.5-5 \times 10^{-8}$

A summary of the responses of five *Helix* tissues to FMRFamide, pQDPFLRFamide, acetylcholine and 5-hydroxytryptamine (5-HT).

A (+) denotes a contracting response whereas a (−) denotes a relaxing response. The doses represent the threshold range for at least eight muscles and are the final concentration (in mol l^{-1}) in the bath, except in the case of the ventricle (*), in which the drugs were delivered into the perfusing stream (see text).

The responses of the ventricle to acetylcholine and 5-HT were taken from Lloyd, 1978.

Epiphallus

The freshly isolated organ was typically quiescent and shortened; in the course of 1 h it would slowly relax to a stable length. The response of the relaxed epiphallus to either FMRFamide or pQDPFLRFamide was a slowly developing contracture (Fig. 1A,B). FMRFamide was slightly more potent as a contracting agent; its threshold was $1.5\text{--}15 \times 10^{-10} \text{ mol l}^{-1}$, whereas the threshold for pQDPFLRFamide was $5\text{--}50 \times 10^{-10} \text{ mol l}^{-1}$. Both peptides also contracted other regions of the male reproductive tract, including the penis, dart sac and flagellum (not shown).

The action of ACh on the epiphallus was biphasic (Fig. 1C). At the low end of its effective concentration range, ACh relaxed contractures produced by treatment with pQDPFLRFamide or FMRFamide. The threshold for this effect of ACh was $1.5\text{--}5 \times 10^{-7} \text{ mol l}^{-1}$. These low doses of ACh had no effect on an untreated, relaxed epiphallus, but at higher doses ($1.5\text{--}5 \times 10^{-6} \text{ mol l}^{-1}$) ACh caused a slowly developing sustained contracture indistinguishable from the effects of FMRFamide or pQDPFLRFamide, except that the peptides were much more potent.

Both of the ACh effects, relaxation and contraction, were reduced or blocked by the molluscan anticholinergic agent, benzoquinonium chloride (BQ; $10^{-5} \text{ mol l}^{-1}$) (Fig. 2). The contracting effects of FMRFamide and pQDPFLRFamide were not reduced by BQ, but were slightly potentiated. Two other anticholinergic drugs, *d*-tubocurarine chloride and atropine ($10^{-5} \text{ mol l}^{-1}$), were ineffective as ACh antagonists on the epiphallus.

5-hydroxytryptamine (5-HT) induced phasic, arrhythmic contractions of the epiphallus (Fig. 1D). The threshold was $1.5\text{--}15 \times 10^{-8} \text{ mol l}^{-1}$, and higher

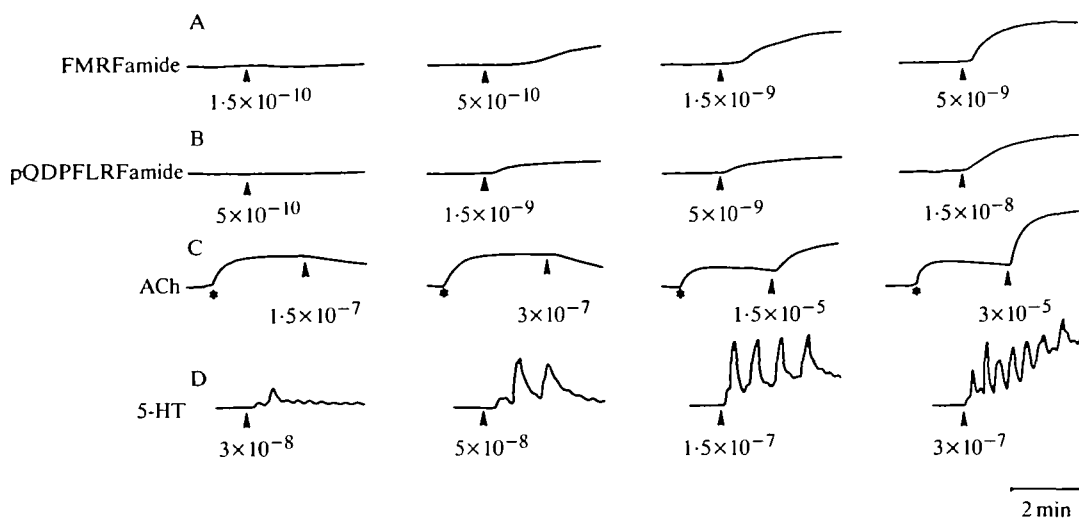


Fig. 1. Mechanical responses of the *Helix* epiphallus to increasing doses of (A) FMRFamide, (B) pQDPFLRFamide, (C) acetylcholine (ACh) and (D) 5-hydroxytryptamine (5-HT). Drugs were added to the bath at the arrows; doses are the final concentrations in the bath (in mol l^{-1}). *FMRFamide ($1.5 \times 10^{-8} \text{ mol l}^{-1}$) added; the resulting contracture revealed the biphasic effects of ACh.

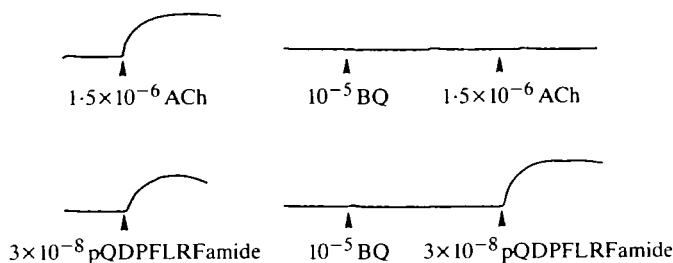


Fig. 2. Benzoquinonium chloride (BQ) blocks the mechanical response of the *Helix* epiphallus to acetylcholine (ACh) but not that to pQDPFLRFamide. After control responses had been obtained, the bath was washed out, the muscles were exposed to BQ, and the control dose was repeated. Drugs were added at the arrows; doses are final concentrations in the bath (in mol l^{-1}).

concentrations increased the frequency of the contractions and raised the tone of the preparation. These effects were clearly distinguishable from the slow, sustained contractures produced by ACh, FMRFamide and pQDPFLRFamide.

5-HT-induced contractures were not reduced by methysergide or 2-bromo-*d*-lysergic acid diethylamide (2-bromo-LSD), which acted as weak agonists. However, prolonged exposure to $10^{-5} \text{ mol l}^{-1}$ 5-HT caused the epiphallus to become tachyphylactic to the amine, but the response of each desensitized muscle to FMRFamide and pQDPFLRFamide was undiminished (Fig. 3).

Crop

The spontaneous activity of the freshly isolated crop was irregular, but within 1 h the contractions become much more regular, and the pharmacology was tested from this time onward. At doses close to threshold ($1.5\text{--}15 \times 10^{-9} \text{ mol l}^{-1}$ FMRFamide and $1.5\text{--}15 \times 10^{-8} \text{ mol l}^{-1}$ pQDPFLRFamide), both peptides reduced the tone of the preparation and the force and frequency of its contractions (Fig. 4A,B). Higher concentrations completely abolished the spontaneous activity of the crop. These effects were reversible; spontaneous activity reappeared after washing, and sensitivity to a repeated dose was not impaired.

ACh increased the frequency of spontaneous contractions of the crop; the threshold was $1.5\text{--}5 \times 10^{-8} \text{ mol l}^{-1}$ (Fig. 4C). As the dose was increased, the resting



Fig. 3. An isolated epiphallus of *Helix* is desensitized by repeated high doses of 5-hydroxytryptamine (5-HT). The control response of the muscle to pQDPFLRFamide is not reduced by 5-HT desensitization. Drugs were added at the arrows; doses are the final concentrations in the bath (in mol l^{-1}); the muscle was washed between doses.

tone also rose, and high concentrations produced a sustained contracture with superimposed small, phasic contractions. BQ, atropine and *d*-tubocurarine were all ineffective as ACh antagonists on the crop.

In contrast to ACh, 5-HT reduced the tone of the crop, while increasing the frequency of its spontaneous contractions. The threshold range was $1.5\text{--}5 \times 10^{-8}$ mol l⁻¹ (Fig. 4D). At higher doses ($>10^{-6}$ mol l⁻¹), 5-HT reduced the tension and the frequency of contractions. The actions of 5-HT were not blocked by methysergide or 2-bromo-LSD.

Ventricle

The ventricle responded to moderate doses of FMRFamide with a rapid increase in amplitude (Fig. 5A). The actions of pQDPFLRFamide were similar (Fig. 5B), but the heptapeptide was about 100 times more potent than FMRFamide, i.e. 4 pmol of pQDPFLRFamide and 350 pmol of FMRFamide produce similar responses.

Since the actions of ACh and 5-HT on the isolated *Helix* ventricle have been thoroughly studied (S.-Rozsa & Perenyi, 1966; Lloyd, 1978), we did not retest them. Most recently, Lloyd (1978) has shown that ACh decreases and 5-HT increases the amplitude of the ventricular beat; the thresholds are 2 and 0.1 pmol, respectively.

Retractor muscles

The tentacle retractor muscle (TRM) was often spontaneously, if irregularly, active, whereas the pharyngeal retractor muscle (PRM) was usually quiescent. In both muscles, FMRFamide consistently produced a quickly developing tonic contraction; the higher doses also induced rhythmical activity (Fig. 6A, PRM; the

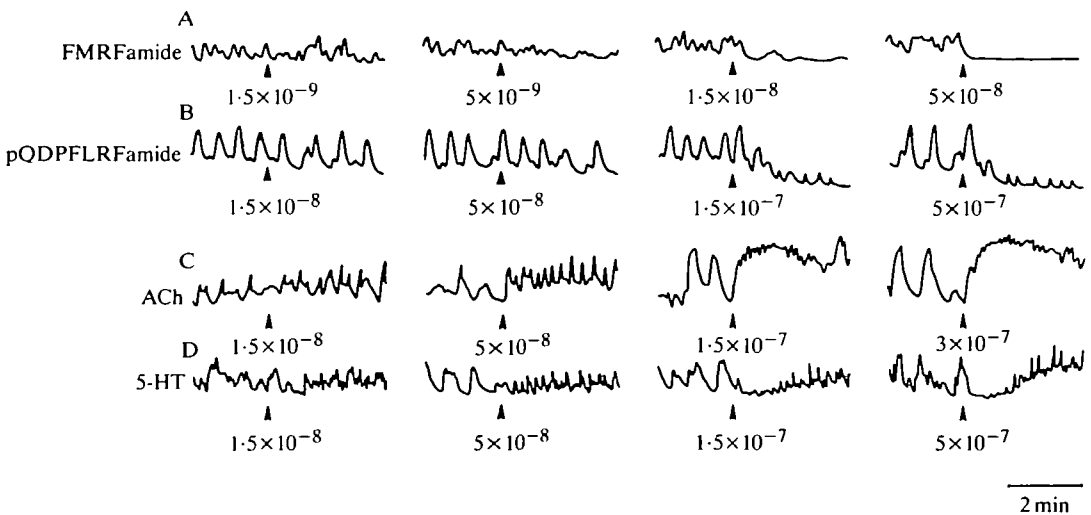


Fig. 4. Mechanical responses of the *Helix* crop to increasing doses of: (A) FMRFamide, (B) pQDPFLRFamide, (C) acetylcholine (ACh) and (D) 5-hydroxytryptamine (5-HT). Drugs were added to the bath at the arrows and are expressed as the final concentrations in the bath (in mol l⁻¹).

experiments with TRM were similar and are not illustrated). The thresholds of the two muscles differed: for the PRM it was $1.5\text{--}5 \times 10^{-8} \text{ mol l}^{-1}$; for the TRM it was $1.5\text{--}5 \times 10^{-9} \text{ mol l}^{-1}$. Infrequently at very high doses ($>10^{-5} \text{ mol l}^{-1}$), FMRFamide relaxed both muscles.

The effects of pQDPFLRFamide were in marked contrast to those of FMRFamide. The heptapeptide usually had no effects when tested on an untreated muscle; i.e. out of 34 preparations, only four muscles were relaxed and six were contracted. However, we could reliably demonstrate the effects of pQDPFLRFamide on muscles that had been contracted by a dose of ACh close to the EC_{50} ($1.5\text{--}5 \times 10^{-6} \text{ mol l}^{-1}$). pQDPFLRFamide consistently relaxed these muscles; the

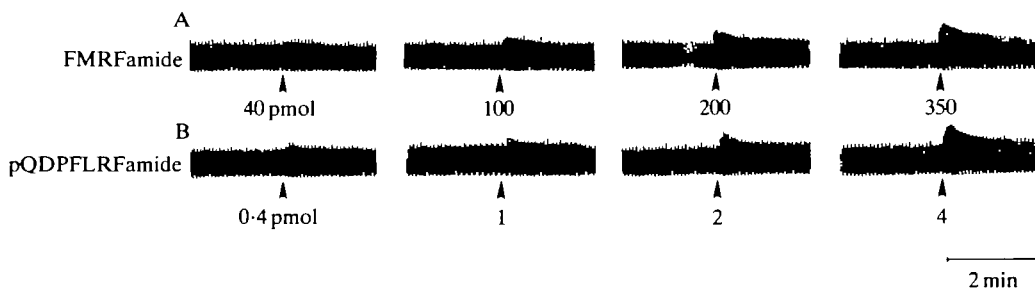


Fig. 5. Mechanical responses of the isolated, perfused *Helix* ventricle to increasing doses of: (A) FMRFamide, (B) pQDPFLRFamide, (C) acetylcholine (ACh) and (D) 5-hydroxytryptamine (5-HT). The peptides were injected into the perfusing stream at the arrows. Doses: 40 pmol is $40 \mu\text{l}$ of $10^{-7} \text{ mol l}^{-1}$ FMRFamide; 0.4 pmol is $400 \mu\text{l}$ of $10^{-9} \text{ mol l}^{-1}$ pQDPFLRFamide.

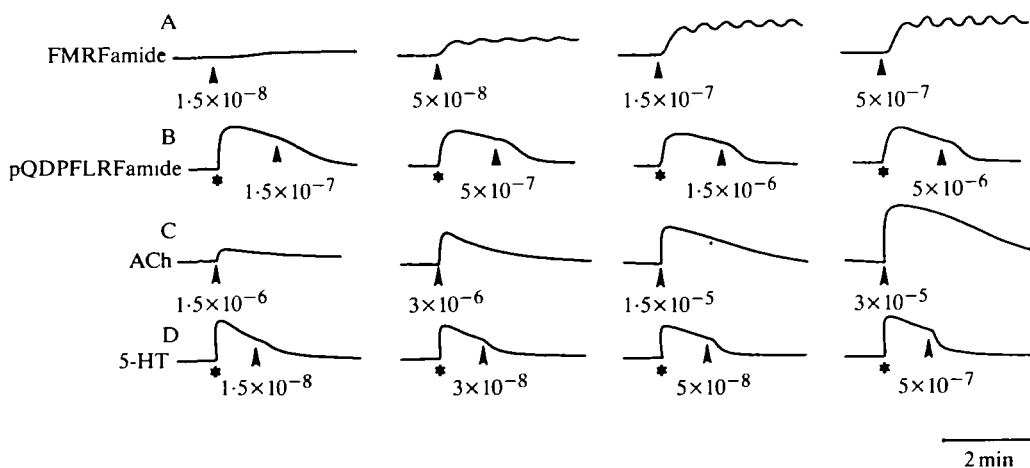


Fig. 6. Mechanical responses of the *Helix* pharyngeal retractor muscle to increasing doses of: (A) FMRFamide, (B) pQDPFLRFamide, (C) acetylcholine (ACh) and (D) 5-hydroxytryptamine (5-HT). All drugs were added at the arrows and doses are the final concentrations in the bath (in mol l^{-1}). The relaxing effects of pQDPFLRFamide and 5-HT were tested after the muscles had been contracted with ACh ($3 \times 10^{-6} \text{ mol l}^{-1}$) applied at the asterisk.

threshold concentration for both the TRM and PRM was $1.5\text{--}5 \times 10^{-7} \text{ mol l}^{-1}$ (Fig. 6B). At very high doses ($>10^{-5} \text{ mol l}^{-1}$), pQDPFLRFamide produced a further tone increase on top of the ACh-induced contraction (not shown).

The effects of ACh on the TRM and PRM were distinguishable from the contractions caused by FMRFamide. First, the rapidly developing ACh-induced contractures were usually not as well maintained as those in response to FMRFamide; tension declined within 5 min. Second, ACh never induced rhythmical contractions in the muscles (Fig. 6C). Finally, ACh-induced contractions were reduced or blocked in the presence of $10^{-5} \text{ mol l}^{-1}$ BQ, whereas those induced by FMRFamide were unaffected.

The relaxing effects of 5-HT and pQDPFLRFamide on these retractor muscles were very similar; both agents increased the rate of relaxation of an ACh-induced contracture (Fig. 6D). The relaxing effects of the two agonists could not be distinguished with 5-HT antagonists, and attempts to desensitize the muscles to 5-HT were unsuccessful.

pQDPFLRFamide as a modulator

Since pQDPFLRFamide has little effect of its own on retractor muscles, but is a relaxing agent, the relative effectiveness of the heptapeptide as a modulator of FMRFamide- and ACh-induced contractions of the PRM was examined. FMRFamide-induced contractions were much the more sensitive to pQDPFLRFamide. For example, equipotent doses of FMRFamide ($3 \times 10^{-8} \text{ mol l}^{-1}$) and ACh ($5 \times 10^{-7} \text{ mol l}^{-1}$) were reduced by about 100% and 50%, respectively, after the muscle had been pretreated with pQDPFLRFamide ($3 \times 10^{-8} \text{ mol l}^{-1}$) (Fig. 7).

Other aspects of the modulatory effect of pQDPFLRFamide are illustrated by logdose-response curves for FMRFamide and ACh generated in the presence of

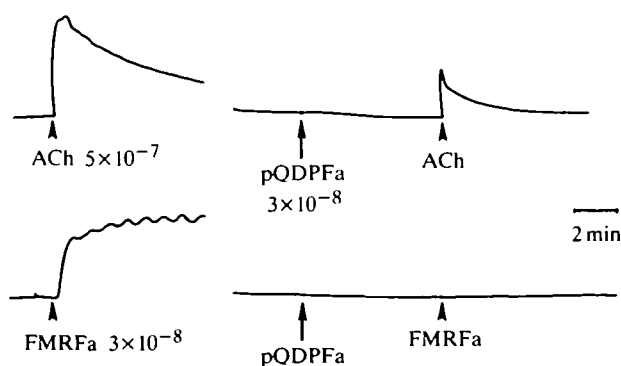


Fig. 7. The mechanical responses of *Helix* pharyngeal retractor muscles to acetylcholine (ACh) and FMRFamide are reduced by pQDPFLRFamide. After equipotent control responses had been obtained, the muscles were treated with $3 \times 10^{-8} \text{ mol l}^{-1}$ pQDPFLRFamide and the control dose of agonist was repeated. The response to ACh was diminished by about 50%, whereas that to FMRFamide was completely abolished. Drugs were added at the arrows and are expressed as the final concentration in the bath (in mol l^{-1}).

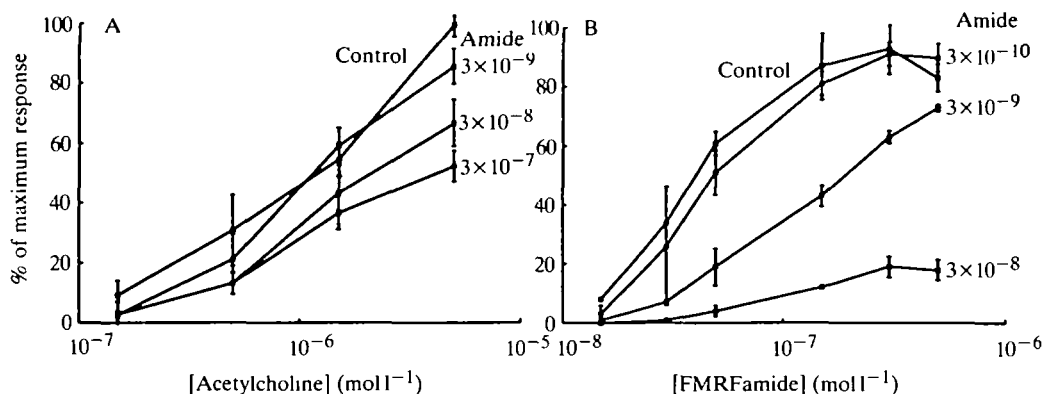


Fig. 8. Dose-response curves for (A) acetylcholine and (B) FMRFamide on pharyngeal retractor muscles of *Helix* illustrating the inhibitory effect of pQDPFLRFamide (Amide). Each point is the mean response (expressed as a percentage of maximum response) \pm standard error from at least four preparations. Open squares, control doses of agonist; closed squares, various concentrations of pQDPFLRFamide added 5 min before agonist (see Fig. 7). Amide concentrations are given in mol l^{-1} .

different concentrations of pQDPFLRFamide (Fig. 8). First, the contractions are reduced by pretreating the muscle with doses 100–1000 times smaller than those required to relax an already developed contracture. For example, $3 \times 10^{-9} \text{ mol l}^{-1}$ pQDPFLRFamide was the threshold for reducing an FMRFamide-stimulated contracture, whereas the dose required for relaxation was $1.5 \times 10^{-7} \text{ mol l}^{-1}$ (compare Fig. 8B with Fig. 6B). Second, both the slope and the maximal response of the FMRFamide dose-response curve were markedly depressed by increased concentrations of pQDPFLRFamide; the maximal FMRFamide-induced contracture was reduced 90 % by $3 \times 10^{-8} \text{ mol l}^{-1}$ pQDPFLRFamide. Thus, the antagonism cannot be competitive. Finally, the set of curves describing the reduction of ACh-induced contractions by pQDPFLRFamide (Fig. 8A) is markedly different from the FMRFamide set (Fig. 8B). For example, relatively high doses of pQDPFLRFamide could only reduce the ACh effects by about 50 %.

The modulatory effects of pQDPFLRFamide were distinguishable from those of 5-HT on the PRM. Whereas pQDPFLRFamide reduced contractions produced by both FMRFamide and ACh, 5-HT only inhibited FMRFamide-induced contractions; the ACh effect was enhanced (Figs 9, 10). The effect of 5-HT on the dose-response curve of FMRFamide is similar to that on the pQDPFLRFamide curve, but less potent. $3 \times 10^{-7} \text{ mol l}^{-1}$ 5-HT still abolished all FMRFamide activity (Fig. 10B). The modulation by 5-HT of the effects of ACh on the PRM have been examined in detail by Lloyd (1980*a,b*).

DISCUSSION

FMRFamide and pQDPFLRFamide, putative transmitters in *Helix* (Lehman & Price, 1986), had potent effects on five selected peripheral tissues of the snail. The results are summarized and compared with those for ACh and 5-HT in Table 1. In

every preparation either ACh or 5-HT mimicked the action of one or both of the peptides. However, the effects of the amines could usually be blocked by specific inhibitors or by tachyphylaxis, or could be distinguished by further experimentation. Thus, the peptides cannot be acting by releasing endogenous stores of 5-HT and ACh.

The actions of FMRFamide and pQDPFLRFamide range from contraction of the epiphallus and excitation of the ventricle, to biphasic effects on the retractor muscle and inhibition of the crop rhythm. Such varied effects are consistent with earlier

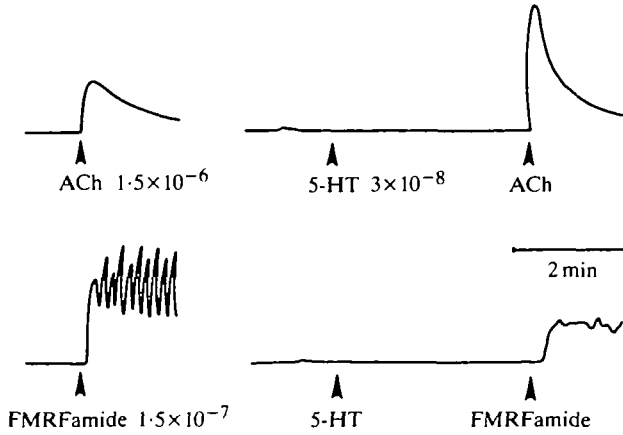


Fig. 9. The effects of 5-hydroxytryptamine (5-HT) on the mechanical responses of *Helix* pharyngeal retractor muscles to acetylcholine (ACh) and FMRFamide. After control responses had been obtained, the muscles were treated with $3 \times 10^{-8} \text{ mol l}^{-1}$ 5-HT and the dose was repeated. The response to ACh was augmented but relaxation was faster; that to FMRFamide was reduced by about 50 %. Doses were added at the arrows and are the final concentration in the bath (in mol l^{-1}).

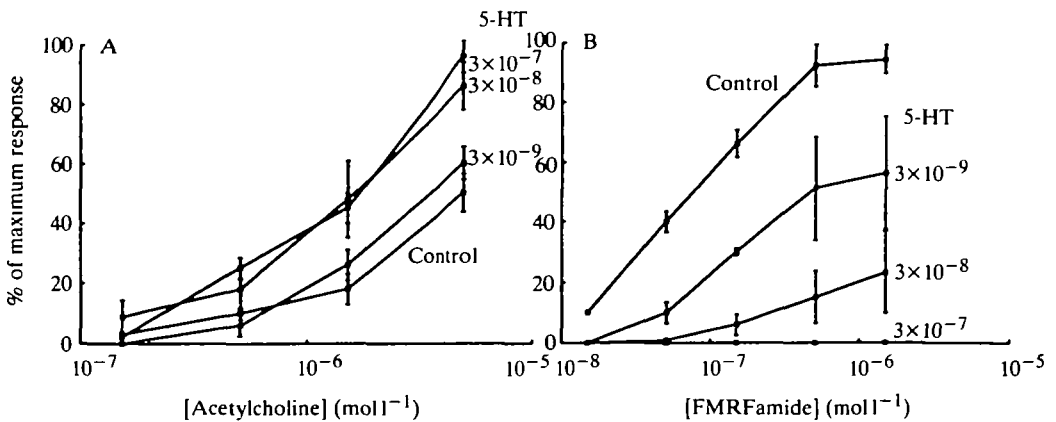


Fig. 10. Dose-response curves for (A) acetylcholine (ACh) and (B) FMRFamide on pharyngeal retractor muscles of *Helix* illustrating the actions of 5-hydroxytryptamine (5-HT). Each point is the mean response \pm standard error from at least four preparations. Open squares, control doses of agonist; closed squares, various concentrations of 5-HT (in mol l^{-1}) added 5 min before agonist (see Fig. 9).

reports about the actions of FMRFamide and its analogues on individual, identified neurones in *Helix* (Cottrell *et al.* 1984) and on the TRM (Cottrell, Greenberg & Price, 1983a). The multiplicity of actions of FMRFamide on *Helix* neurones, clam hearts and other preparations has already been noted (Painter & Greenberg, 1982; Greenberg *et al.* 1983). The question now is whether these diverse actions are suggestive of some specific physiological roles for these two peptides.

Visceral motility

The high concentrations of FMRFamide and pQDPFLRFamide, and the associated rich FMRFamidergic innervation of muscle in the male reproductive and digestive systems, suggest that these visceral organs may be targets of peptide action (Lehman & Price, 1987). This notion is supported by the potent effects of FMRFamide and pQDPFLRFamide: contraction of the entire musculature of the male reproductive tract, including the epiphallus, penis, dark sac and flagellum; and suppression of crop rhythmicity and tone.

These results lead us to speculate that the peptides participate in the control of reproductive behaviour in *Helix*, stimulating the male reproductive tract to transport sperm and associated secretions and, concomitantly, inhibiting peristalsis of the digestive system. A few scattered observations suggest that this function may be widespread in molluscs: ir-FMRFamide is highly concentrated in the male reproductive system of other gastropods (*Aplysia* and *Lymnaea*) (H. K. Lehman, unpublished observations), and FMRFamide has potent inhibitory effects on the anterior gizzard of *Aplysia* and *Navanax* (Austin, Weiss & Lukowiak, 1983) and, centrally, on the feeding motor programme of terrestrial slugs (Cooke, Delaney & Gelperin, 1985; D. J. Prior, personal communication) and of *Helisoma trivolvis* (Murphy, Lukowiak & Stell, 1985).

Somatic muscle

The effects of moderate concentrations of FMRFamide and pQDPFLRFamide on the tentacle and pharyngeal retractor muscles were markedly dissimilar. Whereas FMRFamide contracted the muscles, pQDPFLRFamide was usually ineffective. But the heptapeptide rapidly reduced the force of FMRFamide- and ACh-stimulated contractures.

The effect of pQDPFLRFamide on the retractor muscle of *Helix* is not due to competitive blockade of the FMRFamide receptor. First, the heptapeptide relaxes contractions produced not only by FMRFamide but also by ACh and other agonists. Moreover, pQDPFLRFamide reduces the maximal response to FMRFamide, shifting the dose-response curve downwards and thus indicating a non-competitive interaction. However, pQDPFLRFamide blocked FMRFamide-induced contractions more effectively than those induced by ACh and other agents, implying that pQDPFLRFamide may have the specific role of modulating the excitation of somatic muscle by FMRFamide.

The dissimilar effects of FMRFamide and pQDPFLRFamide are consistent with the notion that there are two FMRFamidergic receptors in *Helix* somatic muscle, one

mediating contraction, the primary response of FMRFamide, and the other mediating the relaxing action of pQDPFLRFamide. Since the two peptides have very similar sequences, they should each cross-react with the other's preferred receptor. Thus the relaxation produced by high concentrations of FMRFamide and the contraction produced by high doses of pQDPFLRFamide are not surprising.

The retractor muscles of *Helix* operate in two clearly defined states. First, while the animal is withdrawn into its shell, the muscles are contracted and may remain so for long periods, particularly during aestivation. Second, while the snail is actively crawling, the muscles are relaxed but capable of phasic contractions. The condition of the retractor muscles is coordinated with that of the heart, since it is the heart that develops the hydrostatic pressure in the cephalopodal sinus, the fluid skeleton against which the retractors work (Dale, 1974). The opposing actions of pQDPFLRFamide and FMRFamide on these muscles, and the 100-fold greater potency of pQDPFLRFamide as a cardioexcitatory agent, suggest that the two peptides may play a central role in converting a withdrawn snail into an active one, and *vice versa*. Our working hypothesis, described below, is modelled in Fig. 11.

Emergence from the shell would be initiated by the release of pQDPFLRFamide into the blood. The effects would be cardioexcitation, leading to an increased

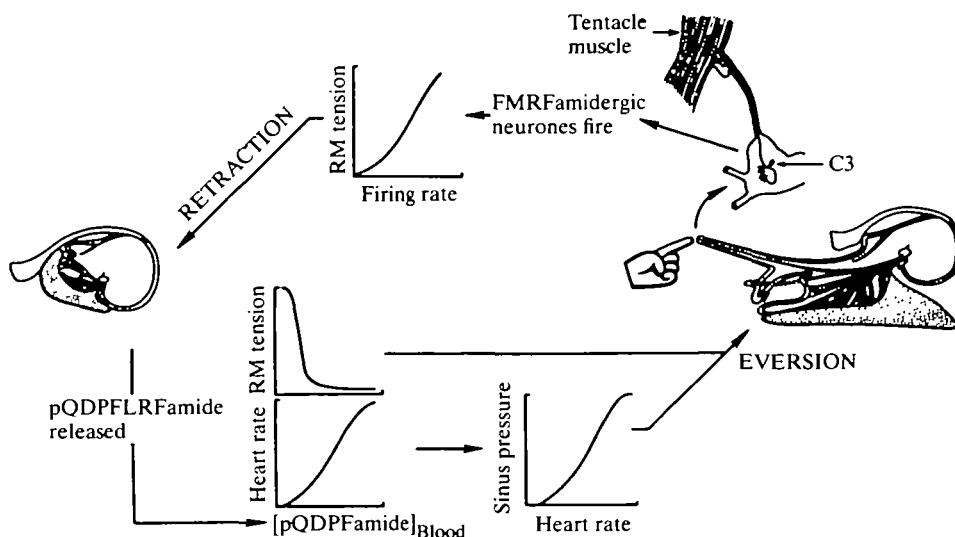


Fig. 11. A hypothetical model relating the opposed actions of FMRFamide and pQDPFLRFamide on the retractor muscles (RM) of *Helix* to the withdrawal of the snail into its shell and to its emergence. In the everted snail (right), the retractor muscles are relaxed. An appropriate stimulus causes FMRFamidergic motoneurons to fire, the retractor muscles to contract in proportion to the neuronal firing rate, and the snail to withdraw (left). The snail begins to emerge when the amount of pQDPFLRFamide delivered to the muscles and heart *via* the blood or neurones exceeds that of FMRFamide. As levels of pQDPFLRFamide increase, the retractor muscles relax and the rate and force of the heart beat increase, leading to higher sinus pressures and eversion of the snail's head and foot. The sketch of the innervated tentacle retractor muscle is redrawn from Cottrell, Schot & Dockray (1983b).

pressure in the cephalopedal sinus, and then filling of that sinus as the retractor muscles were relaxed. The result would be eversion of the head and active crawling. If an active animal were appropriately stimulated, FMRFamidergic motor neurones would fire (TRM only; Cottrell *et al.* 1983*b*) and FMRFamide would also be released into the blood. The retractors would then contract and the snail would withdraw.

Although the effects of FMRFamide and pQDPFLRFamide on the PRM and the TRM are similar, the peptides are delivered differently to the two muscles. The PRM, unlike the TRM, contains low levels of FMRFamide and pQDPFLRFamide, and immunocytochemical observations reveal no FMRFamidergic innervation (Lehman & Price, 1987). Nevertheless, the PRM *in vivo* (and the TRM as well) would be affected by the presence of peptide in the haemolymph, since both FMRFamide and pQDPFLRFamide are present in the blood in suprathreshold concentrations (Price *et al.* 1985). Moreover, the peptides must be acting on the PRM in parallel with at least two neural control systems. First, substantial evidence indicates that the PRM has a serotonergic innervation serving to relax the muscle and to increase its excitability (Lloyd, 1980*a,b*). [This is, of course, the typical modulatory effect of 5-HT on molluscan somatic muscle (reviewed by Muneoka & Twarog, 1983).] An excitatory innervation also occurs in the PRM, but the identity of the transmitter has not been resolved (reviewed by Lloyd, 1980*a*; Muneoka & Twarog, 1983).

The action of FMRFamide on the TRM would seem to reflect its role as an excitatory transmitter to that muscle. A single neurone in the cerebral ganglia of *Helix* was found to contain ir-FMRFamide; it innervates the TRM and, upon stimulation, contracts the muscle even in the presence of BQ (Cottrell *et al.* 1983*b*). The possibility that pQDPFLRFamide, or another transmitter, might also be released with FMRFamide from the same or different neurones has not been explored. Finally, although 5-HT relaxes the TRM, there is no evidence of a serotonergic innervation of this muscle. Indeed, Lloyd (1978) found very low levels of 5-HT in the tentacular nerve of *Helix*.

We thank Dr D. A. Price for critically reading the manuscript. This work was supported by NIH grant HL28440 to MJG; it is Contribution No. 261 from the Tallahassee, Sopchoppy and Gulf Coast Marine Biological Association. We gratefully acknowledge the help of Lynn Milstead in the preparation of this manuscript.

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