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Functions, Receptors, and Mechanisms of the FMRFamide-Related Peptides

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Introduction

Since the discovery of FMRFamide in the ganglia of a bivalve mollusc (*Macrocallista nimbosa*), FMRFamide and its related peptides (FaRPs) have proven to be ubiquitous in the Mollusca and to play physiological roles in various tissues, including cardiac and non-cardiac muscles, nerves, and glands (*e.g.*, Greenberg *et al.*, 1983; Lehman and Price, 1987; Kobayashi, 1987; Bulloch *et al.*, 1988; Raffa, 1988; Cottrell and Bewick, 1989). The actions of FMRFamide and other FaRPs on these tissues and cells are varied and complex. Furthermore, polyneuronal innervation and the co-localization of one peptide with other peptides or non-peptide neuroeffectors makes the assignment of function to particular agents especially complicated (*e.g.*, Weiss *et al.*, 1986; Lloyd *et al.*, 1987; Sossin *et al.*, 1987; Cropper *et al.*, 1987a, b, 1988). Therefore, rather than redescribing all of the disparate data, we have concentrated on results from a limited number of representative preparations, including heart, somatic muscle, and nerve. We ask, finally, whether any generalities are applicable to the actions of FMRFamide and its relatives.

Heart of *Rapana thomasiana*

FMRFamide is cardioexcitatory in many molluscs, but in some species it also has inhibitory effects (Painter and Greenberg, 1982; other references in Kobayashi, 1987). In the prosobranch *Rapana*, both serotonin and FMRFamide enhanced the amplitude and frequency of heart beat, with FMRFamide having the lower threshold and producing the greater enhancement (Kawakami and Kobayashi, 1984). The excitatory effects of serotonin were blocked by methysergide, a potent antagonist of serotonin receptors in molluscan hearts. On the contrary, the effects of FMRFamide were not affected by methysergide, showing that the receptors for serotonin and for FMRFamide are different.

The amplitude of heart beat was also augmented when the *r*ight or *l*eft *c*ardiac *n*erves, RCN 3a, RCN 4, or LCN 1 were electrically stimulated. The excitatory effects of nerve stimulation were not affected by the application of methysergide for 60 min or more. Thus, serotonin is probably not involved in the neurally induced excitation of this heart.

Recently, we have found, by staining with an antiserum to FMRFamide, that immunoreactive cell bodies and fibers are distributed throughout the visceral ganglia, and that such fibers also occur in the atrium (Kobayashi, 1987). Therefore, although the mechanism of action of FMRFamide is still not yet clarified, this peptide may well play a physiological role as a cardioactive agent in *Rapana*.

We have studied the structure-activity relations (SAR) of FMRFamide on the isolated *Rapana* heart (Kobayashi and Muneoka, 1986), and have shown that:

(1) The C-terminal RFamide is critical for activity; potency is markedly diminished by substitution with D-amino acids and is abolished upon removal of the amide.

(2) The N-terminal phenylalanine and the methionine could be replaced by other residues, but a total length of at least four residues is important for activity.

(3) N-terminal elongation may have little effect.

(4) FMRFamide was the most potent of 14 peptides tested.

These features are common, but not inevitable, characteristics of FMRFamide-receptor interactions. In particular, N-terminal elongation enhances the action on some pulmonate preparations, and in such cases, FMRFamide may be a relatively weak agonist (see be-

low). However, in general, the contribution to potency of the various residues in each position along a peptide needs to be examined in much greater detail.

Heart of *Achatina fulica*

The mode of action of FMRFamide on this pulmonate heart was different from that observed in *Rapana*. So far, we have identified nine neurons in the central nervous system of *Achatina* that are involved in regulating the heart beat (Furukawa and Kobayashi, 1987a, b). One giant neuron, designated the '*P*eriodically *O*scillating *N*euron' (PON), was the most potent heart excitor. Both the cardioexcitation produced by PON, and the excitatory effects of serotonin application, were depressed by methysergide, suggesting that the transmitter between the PON neuron and the heart is serotonin.

The effects of several FaRPs were tested on the atrium of *Achatina* (Hori *et al.*, unpub.). The preparation (comprising the atrium with most of the ventricle cut away, the intestinal nerve, and the central ganglia) was isolated in a bath to which the peptides were added. Of the peptides studied, only FMRFamide showed conspicuous excitatory effects. Although the threshold for the direct effects of FMRFamide on the heart was quite high (*i.e.*, 10^{-5} *M* or more), lower concentrations of FMRFamide enhanced the cardioexcitatory actions of both PON stimulation and serotonin application. The sites of action of FMRFamide (*i.e.*, pre- or post-synaptic) have not been established, but in any case, these effects were in contrast to those of SCP_B which depressed the cardioexcitatory actions. Therefore, as one of its roles in *Achatina*, FMRFamide appears to be modulating the excitatory action of a transmitter to the heart. Neither the FMRFamide- nor the SCP-containing neurons in *Achatina* have been identified.

Buccal Muscles of *Rapana*

The reciprocating movement of the radular rasp during feeding is produced in this prosobranch by the alternating contraction and relaxation of two pairs of opposing buccal muscles, the radula protractors and retractors. These muscles are innervated by the radula nerves which arise in the buccal ganglia (Furukawa and Kobayashi, 1985). In these radula muscles, the FaRPs seem to modulate the release of transmitters by acting presynaptically (Yanagawa *et al.*, 1988).

FMRFamide enhanced contractions of the radula protractor that were elicited by short pulses of electrical field stimulation (probably affecting nerve elements in the muscle), but the peptide had no such effect on the opposing muscle, the radula retractor. In contrast to FMRFamide, its close analog FLRFamide enhanced the contraction of the retractor but had no enhancing effect on the protractor. When neuromuscular transmission was blocked by application of 80 m*M* Mg^{++}, contraction of the protractor elicited by stimulation with long pulses (*i.e.*, direct muscle stimulation) was not enhanced by FMRFamide. Similarly, contraction of the retractor caused by muscle stimulation was not enhanced by FLRFamide (Yanagawa *et al.*, 1988).

Previously, we showed that the principal excitatory transmitter in the radula protractor is ACh, whereas that in the retractor is glutamate. Moreover, we know that serotonin acts to excite the protractor and to inhibit the retractor (Kobayashi and Muneoka, 1980; Muneoka and Kobayashi, 1980). Now we present the hypothesis that FMRFamide and FLRFamide act on presynaptic sites in the protractor and retractor, respectively, to enhance their contractions, possibly by increasing the release of transmitter.

Anterior Byssus Retractor Muscle (ABRM) of *Mytilus edulis*

FMRFamide also appears to have a presynaptic action on the ABRM of a bivalve mollusc (Muneoka and Matsuura, 1985). The ABRM of *Mytilus* can be set into a prolonged contracture by acetylcholine (ACh), and this catch tension is relaxed by serotonin released from the relaxing nerve; the serotonergic relaxation is blocked by mersalyl (references in Muneoka and Matsuura, 1985).

FMRFamide at low concentrations (10^{-8}–10^{-7} *M*) also relaxes ACh-induced catch tension. Moreover, this relaxation of the ABRM, like that of serotonin, was also blocked by mersalyl. However, when the muscle was denervated, ACh-induced catch tension was not relaxed by FMRFamide, although serotonin still relaxed it. These results are consistent with the notion that FMRFamide is acting on the relaxation inducing neurons in the muscle to release serotonin from their terminals.

FMRFamide showed various actions on the *Mytilus* ABRM, and catch relaxation is only one of them. The peptide also enhanced the contraction elicited by electrical stimulation of the muscle, or by application of ACh to it (Muneoka and Matsuura, 1985). The threshold for these effects was about 10^{-9} *M*. At higher concentrations (more than 10^{-7} *M*), FMRFamide caused its own contraction. These actions of FMRFamide are probably postsynaptic.

The structure-activity relations of FMRFamide for contraction of the ABRM were different from those for relaxation (Muneoka and Saitoh, 1986). Although the C-terminal RFamide was, as usual, critical for contraction, effective relaxation could still be produced when D-Arg and D-Phe (or other residues) were substituted for the C-terminal Arg and Phe, respectively. Even the C-terminal amide was not essential for relaxation. These results sug-

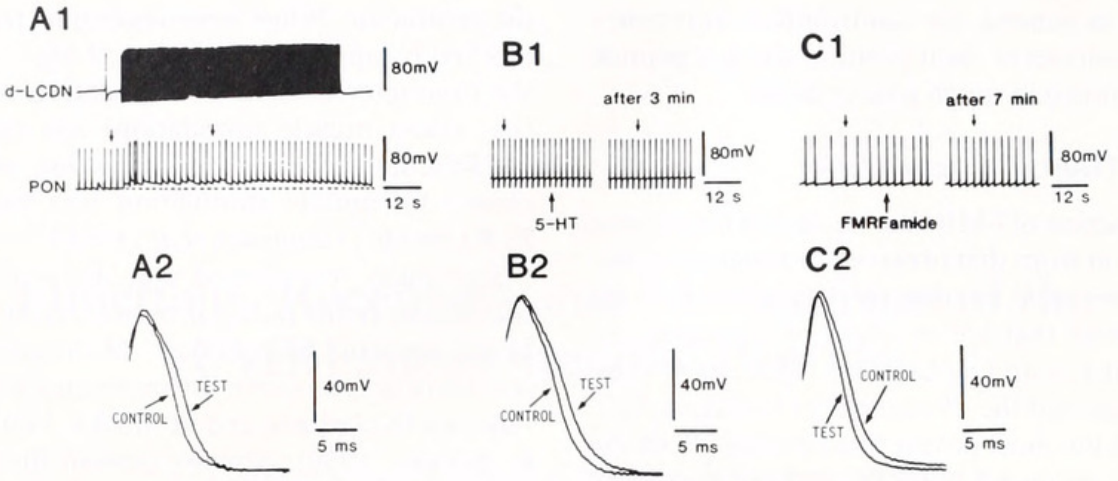

Figure 1. Duration of the action potential of the 'Periodically Oscillating Neuron' (PON) was increased by a burst of impulses in a cerebral neuron (d-LCDN) (A), and by an application of 5-HT (5×10^{-6} *M*) (B), but was decreased by FMRFamide (10^{-6} *M*) (C). In (A) and (B), PON was hyperpolarized to −50 mV (dotted line in A1) and driven to fire by a depolarizing current injection. In (C), spontaneous firings were recorded. Arrows in A1, B1, and C1 indicate selected spikes which are displayed at expanded time scale in A2, B2, and C2. (A and B; from Furukawa and Kobayashi, 1988).

gest that the *Mytilus* ABRM has at least two pharmacologically distinct classes of receptors which can be activated by FMRFamide.

Central Neurons and Synapses

Recently, the mechanisms underlying the actions of the FaRPs at central synapses have been intensively investigated (Cottrell *et al.*, 1984; Colombaioni *et al.*, 1985; Ruben *et al.*, 1986; Berladetti *et al.*, 1987; Brezina *et al.* 1987; Thompson and Ruben, 1988). In sensory neurons of *Aplysia*, the inhibitory responses to FMRFamide appear to be mediated by lipoxygenase metabolites of arachidonic acid, which open S-type K^+-channels (Piomelli *et al.*, 1987). This action of FMRFamide is in contrast to that of serotonin which closes these S-K^+-channels; *i.e.*, the effect of serotonin is mediated by cAMP by way of a different guanine nucleotide-binding protein than that coupled to the FMRFamide receptor (Volterra and Siegelbaum, 1988).

Similar results have also been obtained in the heart excitatory neuron, PON, of *Achatina*. FMRFamide increased background K^+-conductance (*i.e.*, it increased K^+-current through S-channels), and it also decreased inward Ca^{++}-current and, in turn, reduced Ca^{++}-dependent K^+-current. Moreover, these actions of FMRFamide were, again, opposite to those of serotonin. As a result, the duration of the action potential of a PON was decreased by FMRFamide, but was increased by serotonin (Fig. 1).

Serotonin is considered to act as an excitatory neurotransmitter at the synapse between a pair of command neurons in the cerebral ganglia and the PON (Furukawa and Kobayashi, 1988). Now we have found that FMRFamide is acting counter to serotonin at the same synapse (Hori *et al.*, unpub.), although the FMRFamide containing neuron has yet to be identified.

The actions of the tetra- and heptapeptide FaRPs have been examined recently on identified neurons of a pulmonate snail, *Helix aspersa* (Cottrell and Davies, 1987). The tetrapeptides were more potent than the heptapeptides at producing a slow increase in potassium conductance (gK). In addition, the tetrapeptides produced an increase in gNa, a conductance change not seen at all in response to the heptapeptides. On the other hand, the heptapeptides produced a fast increase in gK which was not observed when tetrapeptides were applied.

The exclusive actions of the tetrapeptides and heptapeptides on different ionic currents strongly suggest that multiple receptors are present. Recently, a receptor was demonstrated in *Helix* heart and brain that is specific for the tetrapeptides; *i.e.*, the heptapeptides were very ineffective at displacing radioligand bound to membranes from these tissues (Payza, 1987).

Summary

We have surveyed the functions, receptors, and mechanisms of the FMRFamide-related peptides by focussing primarily on preparations we have studied. Even these few examples clearly illustrate the versatility of the FaRPs: acting as neurotransmitters, they can directly excite or inhibit target cells; or they can potentiate or oppose the actions of a variety of other neuroeffector molecules. In the end, there are no general characteristics that can be assigned to the effects of FMRFamide or its analogs. Rather, the results show that the FaRPs exhibit multiple actions on various tissues, reflecting the struc-

tural variation, not only of the peptides, but also of their receptors.

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