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Short communication

The neuropeptide Phe-Met-Arg-Phe-NH_2 (FMRFamide) can activate a ligand-gated ion channel in *Helix* neurones

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Abstract.

This report presents evidence that the molluscan neuropeptide FMRFamide can directly activate a ligand-gated ion channel in *Helix* neurones. Using the patch-clamp technique we have observed unitary currents activated by the application of FMRFamide onto outside-out patches. As for the whole-cell response, Na^+ ions are the main charge carriers. We conclude that FMRFamide may act as a fast depolarising neurotransmitter in the *Helix* nervous system.

Keywords: FMRFamide, single-channel, Na^+ ions, depolarisation

Introduction.

Many transmitter receptors are coupled by various biochemical processes to ion channels that are separate molecules. Some, however, incorporate a transmembrane ion channel, i.e. they are ligand-gated ion channels. Examples of these include nicotinic ACh receptors, GABAA, glycine and various glutamate receptors, and also ligand-gated ion channels which respond to 5-HT (Derkach, et al., 1989) and histamine (Hardie, 1989). Here we report evidence that FMRFamide, a naturally occurring neuropeptide, can also directly activate a ligand-gated ion channel. FMRFamide exerts several different actions on *Helix aspersa* neurones including a relatively fast Na-dependent depolarization (Cottrell, 1983), which resembles an ACh-induced, nicotinic-type, response observed in many *Helix* neurones. ACh directly activates unitary inward currents with a conductance of about 10pS in such neurones (Green, et al., 1989). We have now made further experiments to determine whether the Na-dependent FMRFamide response also results from the direct activation of a ligand-gated ion channel.

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Methods.

Experiments were made on both the F2 and C2 neurones of *Helix aspersa*, but patch recordings were made solely on the C2 neurone because it is more readily identifiable. Some experiments were made on patches taken from isolated C2 neurones maintained in culture for up to 7 days (see Green et al., 1990 for methods). Patch clamp and whole cell current-clamp recordings were made with a List Electronics L/M EPC 7 amplifier using established techniques (Hamill et al., 1981). Some whole cell and intracellular voltage clamp recordings were made with a Dagan 8100 Single Electrode System. Data were stored on video tape using a modified Sony PCM and subsequently filtered at 300Hz. Patch electrodes were made from Clark Electromedical GC150T-15 glass, coated with Sylgard and fire polished. They were routinely filled with solution comprising (mM): NaCl 3, KCl 90, MgCl2 10, CaCl2 2.6, EGTA 5 (free Ca^{2+} 0.1μM), HEPES 20 and adjusted to pH7.4 with KOH (i.e. intracellular solution). The extracellular solution comprised (mM): NaCl 90 (NaCl 85 for isolated neurones in culture), KCl 5, MgCl2 5, CaCl2 7, HEPES 20 and adjusted to pH7.4 with NaOH. FMRFamide (Peninsula Laboratories) was applied locally by pressure ejection from micropipettes containing 50uM (or 1mM for experiments on the F2 neurone) peptide in saline, or via diffusion from a wide tip pipette.

Results.

Both the C2 and F2 neurones were depolarised by FMRFamide, repeated application of which led to a desensitisation of the response (Fig.1A). Under voltage clamp the response was seen as an inward current and the reversal potential, which could not be determined, was positive to 0mV, as has also been seen in *Aplysia* neurones (Ruben et al., 1986). Impaling neurones with a CsCl containing recording electrode markedly

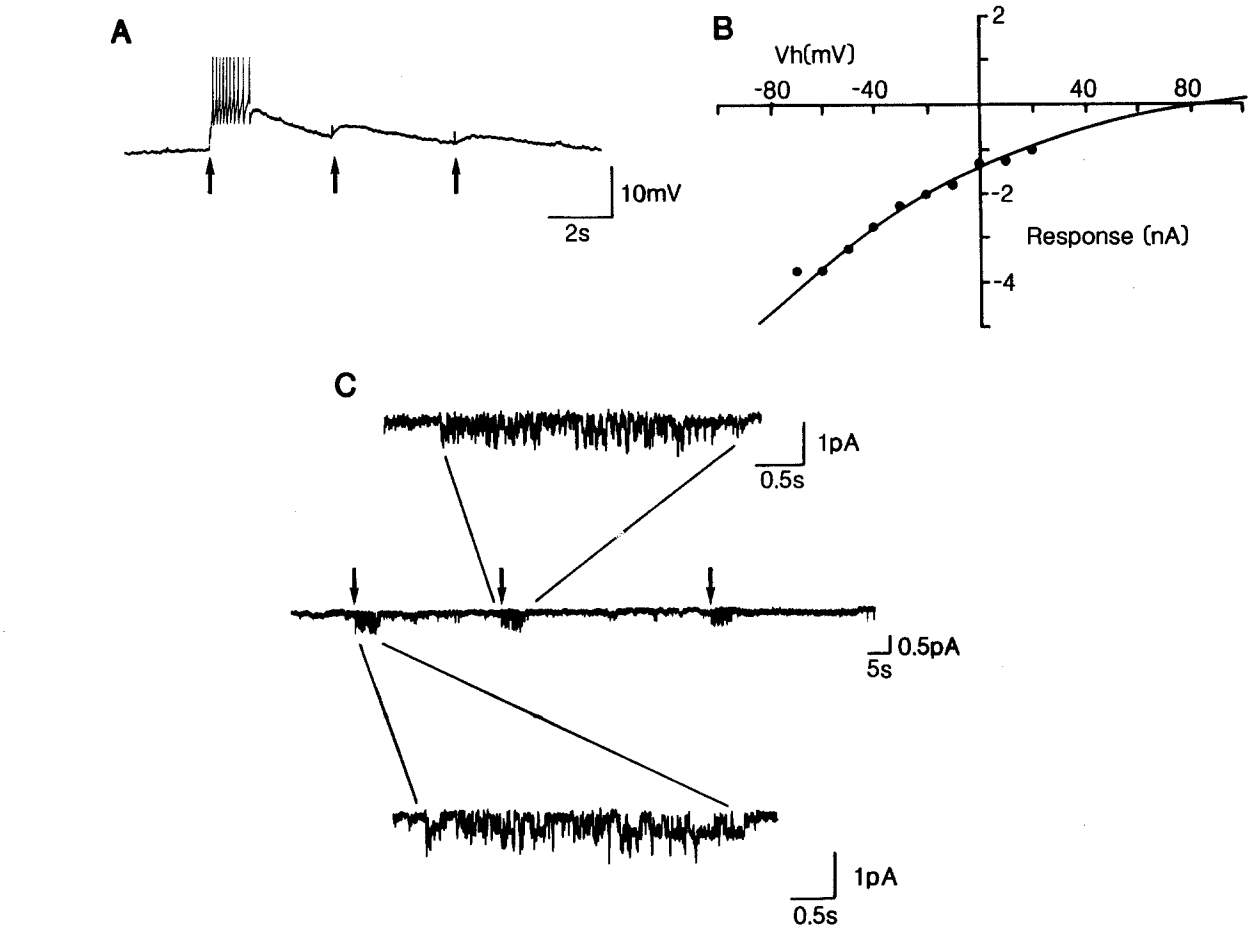

Fig.1 (A) Recording from a C2 neurone in whole-cell current-clamp mode, showing the depolarising FMRFamide response and desensitisation. The peptide was ejected from a micropipette for 80ms at 100kPa. (B) I-V curve of the FMRFamide response induced in an F2 neurone voltage-clamped with a CsCl containing electrode. Most of the background K^+ current was suppressed by the CsCl. The curve is a plot of the constant field equation for a Na^+ current with a permeability coefficient of 1.62×10^{-10} $cm^3 s^{-1}$. (C) Unitary current responses to FMRFamide (63kPa applied as indicated by the arrows in the middle trace) in an outside-out patch excised from an isolated C2 neurone and held at -70mV. (D) I-V curve of the FMRFamide activated channel in physiological solutions. The curve is that given by the constant field equation with a Na^+ permeability coefficient of 1.21×10^{-14} $cm^3 s^{-1}$. (E) I-V curve of these channels in symmetrical high Na^+ (ca. 100mM) solutions. The straight line gives a Na^+ permeability coefficient of 1.73×10^{-14} $cm^3 s^{-1}$, and gives a conductance, under these conditions, of 6.6pS.

reduced outward currents normally seen at depolarised potentials, thus enabling the cells to be held at more positive potentials. The I-V curve of the response in such a neurone is shown in Fig.1B, where the FMRFamide induced current is still inward at +20mV. The curve is a fit to the data of the constant field equation assuming the response is a pure increase in a Na^+ conductance (see legend). The FMRFamide induced response was unaffected by 100μM tubocurarine, which at this concentration effectively abolishes the fast depolarising responses to ACh, dopamine, histamine, aspartate and glutamate in molluscan neurones (Carpenter, et al., 1977).

Application of FMRFamide by pressure ejection or diffusion onto outside-out patches excised from either isolated or *in situ* C2 perikarya evoked small inward unitary currents at negative potentials (Fig.1C). Such currents were not observed in cell-attached patches when the peptide was added outwith the patch. In most active outside-out patches, currents from more than one channel were observed, suggesting that the FMRFamide receptors are often clustered (eg. Fig.1D). However, less than 10% of all patches tested responded, suggesting that the receptors are only sparsely distributed on the perikaryon. Since molluscan synapses are usually axo-axonic, the receptors on the C2 perikaryon are probably extra-synaptic. The unitary currents recorded at -55mV, close to the resting potential, were only about 0.25pA in physiological saline, giving a slope conductance at this potential of 3.8pS (see Fig.1D). The amplitude of the unitary currents were smaller at more depolarized potentials, and as for whole cell currents, the values agreed well with the prediction given by the constant field equation for a pure Na^+ conductance (Fig.1D). With symmetrical solutions containing approximately 100mM Na^+, the unitary currents had a conductance of 6.6pS and the reversal potential was essentially 0mV (Fig.1E). Unitary current responses were observed in some patches maintained in isolation for 20 minutes, indicating that a second messenger is not required for the response. Brezina's (1988) observation that the fast Na-dependent response to FMRFamide in *Aplysia* neurones is unaffected by GTP-gamma-S is in accord with our results.

As far as we are aware, this is the first report of a neuropeptide directly activating a ligand-gated ion-channel. The insensitivity of the response to tubocurarine makes it unlikely that FMRFamide is activating a receptor normally sensitive to low molecular weight transmitters. Our results suggest that FMRFamide can act as a fast transmitter in addition to having slower, modulatory-type effects, some of which clearly involve second messengers (Cottrell, et al., 1984; Brezina, 1988). It will be interesting to see whether peptide-activation of ligand-gated ion channels occurs in other systems.

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