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Direct Activation of Guinea Pig Vagal Afferent Neurons by FMRFamide

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Abstract

Vagus nerve is composed of distinct two kinds of nerves, nodose and jugular ganglionic nerves. We tested pharmacological difference between two vagal nerves in the responsiveness to FMRFamide. The response probability to FMRFamide was significantly higher in nodose than jugular nerves in intracellular calcium measurement. Nodose nerves also depolarized membrane potential to FMRFamide more than jugular nerves did in patch clamp recording. But the probability of action potential discharge was same in both nerves. The inward current induced by FMRFamide was characterized as mixed cations. These results suggest that FMRFamide may act as an activator and modulator of vagal sensory nerves for treating symptoms in visceral diseases.

Keywords

FMRFamide; Vagus Nerve; Nodose Ganglion; Jugular Ganglion; Patch-Clamp Techniques; Intracellular Calcium Measurement

Introduction

FMRFamide is a member of a family of neuropeptides first discovered in mollusks but is known to be present across the animal phyla [1]. In mammals a gene has been described that encodes to RF-amides, namely neuropeptide FF and neuropeptide AF [2]. We recently found that mast cells express this neuropeptide FF gene, and may be source of RF-amides during allergic reactions [3]. RF-amides can effectively stimulate several mas-related gene protein receptors (Mrgprs), a family of G-protein coupled receptors selectively expressed on small diameter nociceptive sensory neurons [4]. Considered together, these observations led to the hypothesis that RF-amides may be mediators in mast cell-sensory nerve interactions associated with allergic disease.

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There has been little attention given however to the effect of RF-amides on primary afferent neurons in general and vagal afferent neurons in particular. That RF-amides may directly modulate primary afferent neuronal activity is supported by the recent observation that RF-amides, including FMRFamide and neuropeptide FF, can enhance the acid-induced activation of ASIC channels in isolated dorsal root ganglion neurons [5]. RF-amides also evoke strong action potential discharge in an *ex vivo* skin-afferent nerve preparation, most notably in nociceptive C-fibers [6]. This effect, however, does not appear to be secondary to ASIC channel activation.

Subcutaneous injection of FMRFamide into rat paws leads to nociceptive or algogenic behaviors, and intravenous injection has profound cardio-respiratory effects [6–8]. The algogenic behavior to peripheral RF-amide treatment is likely explained by activation of DRG C-fibers. The increase in blood pressure may be due to stimulation of naloxone sensitive receptors in the central nervous system [8]. In addition, we hypothesize some of the cardiopulmonary effects may be secondary to similar activity in the vagal sensory system. For example, RF-amides, including FMRF-amide causes apnea followed by a depression in respiratory rate; an effect commonly associated with stimulation of certain vagal C-fiber afferent neurons [9].

Vagal afferent neurons are situated in the nodose and jugular ganglia. These ganglia are anatomically, embryologically, and functionally distinguished from each other and are inferred to contribute distinctly to pathophysiology of diseases [10]. In this study, we aimed to see the direct effect of RFamide on vagal afferent nodose and jugular neurons.

Methods

The Hartley guinea pigs (300–400 g) were used and killed using CO₂. The jugular and nodose ganglia were harvested and incubated in the enzyme buffer (2 mg/ml collagenase and 2 mg/ml dispase II dissolved in Ca²⁺-, Mg²⁺-free Hanks' balanced salt solution) for 30 min at 37°C. The cells were gently dissociated by trituration with glass fire-polished Pasteur pipette. Two additional enzymatic digestions were repeated at 37°C for 20 min. The cells were washed by centrifugation (three times at 1000 g for 2 min) and suspended in L-15 medium containing 10% fetal bovine serum (FBS). The cell suspension was transferred onto poly D-lysine (0.1 mg/ml)-coated cover slips. The neurons were studied within 24 h of dissociation.

For intracellular calcium measurements, the neurons were loaded with Fura-2 AM (Invitrogen) and then, 20 min before experiment, superfused by infusion pump (8 ml/min) with 35°C Locke buffer (mM): NaCl, 136; KCl, 5.6; MgCl₂, 1.2; CaCl₂, 2.2; NaH₂PO₄, 1.2; NaHCO₃, 14.3; dextrose, 10 (pH 7.3–7.4). Intracellular calcium measurements were performed under a microscope equipped for epifluorescence. A field of cells was monitored by sequential dual excitation (352 and 380 nm) and ratios of the images were converted to calcium concentration according to methods and parameters presented previously [11]. The ratio images were acquired every 6 s. The neurons were considered to have responded to FMRFamide (1 μM, for 1 min) if the drug-induced peak increase was greater than 3 × standard deviation of baseline intracellular Ca²⁺ concentration. To check capsaicin sensitivity of tested neurons, capsaicin (1 μM, for 20 s) was applied to the neurons in the end of each experiment.

Gramicidin-perforated whole cell patch clamp recordings were done in jugular and nodose neurons. A pipette (1.5–3 MΩ) was filled with pipette solution composed of the following (mM): KCl, 140; CaCl₂, 1; MgCl₂, 2; ethyleneglycol-*bis*-(β-aminoethyl ether)-*N,N'*-tetraacetic acid, 11; dextrose, 10; titrated to pH 7.3 with KOH; 304 mOsm. Gramicidin was

dissolved in dimethyl sulfoxide and mixed into the pipette solution to a final concentration of 1 $\mu\text{g/ml}$ just before each recording. During the experiments, the cells were continuously superfused (6 ml/min) by gravity with Locke solution. All recordings were done at 35°C. The membrane potential of the cells was held at -60 mV.

FMRFamide (Sigma) and capsaicin (Sigma) were dissolved in distilled water and ethanol at the concentration of 10 mM, respectively. Drugs were diluted with Locke solution at final concentration of 1 μM before each experiment. Data were expressed as means $\pm$ SEM. A student *t* test and χ^2 test were used when appropriate.

Results

Intracellular calcium measurement

We evaluated 128 vagal sensory neurons isolated from nodose and jugular ganglia. RFamide caused a brisk elevation in intracellular calcium in both nodose and jugular neurons which usually started within 30 s of FMRFamide application and almost returned to baseline within 5 min. There was, however, significant difference in the propensity of FMRFamide to stimulate nodose versus jugular neurons (Table 1, $p < 0.05$). FMRFamide (1 μM) stimulated the vast majority (85%) of nodose neurons ($n=73$). By contrast it stimulated calcium increases in only 40% of the jugular neurons ($n=55$).

FMRFamide responsiveness did not differentiate between capsaicin-sensitive and insensitive neurons. FMRFamide stimulated 49 of 71 (69%) of capsaicin-sensitive neurons and 35 of 57 (61%) of capsaicin insensitive neurons (Table 2.).

Patch clamp recordings

When vagal afferent neurons were studied in current-clamp mode, FMRFamide evoked a membrane depolarization. In concordance with better responsiveness of nodose neurons in intracellular calcium assay, the amount of membrane depolarization observed in nodose neurons was greater than that observed in jugular neurons. The membrane depolarizations averaged 10.3 ± 2.6 mV (ranged from 0 mV to 21.0 mV) and 4.6 ± 1.7 mV (ranged from -5.6 mV to 15.3 mV) in nodose and jugular ganglionic neurons, respectively. Nevertheless, in 50% of both the nodose and jugular neurons the membrane depolarization reached the threshold for action potential discharge (Fig 1A and Fig 1B, filled circles).

To get some idea of the nature of the currents leading to the membrane depolarization, we measured changes in membrane current passing through the cellular membrane in voltage clamp mode. We found the inward current induced by FMRFamide was associated with an increase in membrane conductance (Fig 2A). If there was no current rectification, the calculated reversal potential was about +33.3 mV (Fig 2B).

Discussion

The results demonstrate that FMRFamide can directly cause membrane depolarization and action potential discharge in vagal afferent neurons. The gap between resting membrane potential and the threshold for action potential seemed to be bigger in nodose neurons than the one in jugular neurons so that nodose neurons might need more depolarization than jugular neurons did to discharge action potential. In this study, the probabilities of action potential discharge by FMRFamide application were same in both nodose and jugular neurons in spite that nodose neurons depolarized more than jugular neurons did. The membrane depolarization was associated with the opening of undetermined cation channels. The overt activation and action potential discharge evoked by FMRFamide may explain some of the physiological effects of RF-amides in mammals. For example, RF-amides such

as FMRFamide can cause apneas followed by a decrease in the rate and increase in the depth of respiration. This is precisely the pattern observed upon activation of guinea pig jugular C-fibers in the lungs [9].

The nodose and jugular neurons have distinct pharmacological profiles. Nodose neurons have been known to be more sensitive to several inflammatory mediators in tissue such as histamine, serotonin, ATP and adenosine than jugular neurons [10,12–14]. In this paper nodose neurons were also more sensitive to FMRFamide. Together with the probability of action potential discharge the nodose neurons may have more receptors responding to FMRFamide but quite number of nodose neurons failed to make action potential discharge due to relatively big gap between resting membrane potential and threshold for action potential. It was possible that the neurons which failed to discharge action potential were sensitized to respond to other stimuli.

Although we show that RF-amides can directly activate vagal afferent neurons, the mechanism underlying this effect is not known. FMRFamide is an effective agonist at several Mrgprs, and Mrgprs are expressed in vagal afferent neurons. Mrgprs may be G_q -coupled receptors, and G_q -coupled receptors are known to be capable of gating TRPV1 channels. It is unlikely, however, that TRPV1 is obligatory for the FMRFamide-induced responses, as FMRFamide was effective in stimulating many capsaicin-insensitive neurons. G_q -coupled receptors can also excite isolated sensory neurons via an inhibition of Kv7 M-currents [15]. It is unlikely that FMRFamide-induced membrane depolarization was due to inhibition of a resting potassium current, as it was associated with an increase in conductance of ions with a reversal potential of $\sim +30$ mV. In addition to Mrgprs, RF-amides are agonist of at least two other G-protein coupled receptors, termed FF1 and FF2 [16]. Although vagal ganglia were not studied, these investigators identified mRNA expression for both FF1 and FF2 in DRG neurons. At present there are no useful pharmacologic tools with which to evaluate the potential roles of FF1, FF2 or certain Mrgprs in the guinea pig.

We used the concentration of 1 μ M of FMRFamide in this study because Mrgprs [4], FF1 and FF2 [16] have fully responded to it in this concentration. In higher concentration, FMRFamide could affect the neurons in some uncertain way. In addition to GPCR mechanisms, RFamides may lead to stimulation of afferent neurons independently of GPCRs via a sensitization of certain acid-sensing ion channels in isolated DRG neurons but FMRFamide did not evoke an overt inward current at normal pH in that study [5]. It is unlikely that the inward currents and membrane depolarizations we observed in vagal afferent neurons at neutral pH is explained by sensitizing effects on acid-sensing channels.

Activation of mast cells leads to symptoms and behaviors consistent with afferent nerve activation, including itching, sneezing, coughing, and reflex effects in the respiratory and gastrointestinal tracts [17]. Recently, we have reported that Mrgpr C11 receptors were activated by a product of IgE-dependent stimulation of mast cells. Although the Mrgpr C11 activating product was not definitively identified, circumstantial evidence favored an RF-amide as one likely candidate [3]. The observation here with vagal afferent neurons, along with observations showing RF-amide can stimulate action potential discharge in DRG C-fibers innervating the skin, support the hypothesis that RF-amides may be relevant mediators in mast cell-sensory nerve activation in both the somatosensory and vagal-visceral systems.

Conclusion

We showed that FMRFamide directly activated the vagal sensory nerves by opening cation channels. The increase of intracellular calcium and the amount of membrane depolarization

responded to FMRFamide were higher in nodose ganglionic vagal nerves than jugular ones. But probabilities of action potential discharge were same in both nerves.

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References

1. Walker RJ, Papaioannou S, Holden-Dye L. A review of FMRFamide- and RFamide-like peptides in metazoa. *Invert Neurosci.* 2009; 9:111–153. [PubMed: 20191373]
2. Perry SJ, Yi-Kung Huang E, Cronk D, et al. A human gene encoding morphine modulating peptides related to NPFF and FMRFamide. *FEBS Lett.* 1997; 409:426–430. [PubMed: 9224703]
3. Lee MG, Dong X, Liu Q, et al. Agonists of the MAS-related gene (Mrgs) orphan receptors as novel mediators of mast cell-sensory nerve interactions. *J Immunol.* 2008; 180:2251–2255. [PubMed: 18250432]
4. Han SK, Dong X, Hwang JI, Zylka MJ, Anderson DJ, Simon MI. Orphan G protein-coupled receptors MrgA1 and MrgC11 are distinctively activated by RF-amide-related peptides through the G α q/11 pathway. *Proc Natl Acad Sci U S A.* 2002; 99:14740–14745. [PubMed: 12397184]
5. Xie J, Price MP, Wemmie JA, Askwith CC, Welsh MJ. ASIC3 and ASIC1 mediate FMRFamide-related peptide enhancement of H⁺-gated currents in cultured dorsal root ganglion neurons. *J Neurophysiol.* 2003; 89:2459–2465. [PubMed: 12612000]
6. Yudin YK, Tamarova ZA, Ostrovskaya OI, Moroz LL, Krishtal OA. RFA-related peptides are algogenic: evidence in vitro and in vivo. *Eur J Neurosci.* 2004; 20:1419–1423. [PubMed: 15341614]
7. Mues G, Fuchs I, Wei ET, et al. Blood pressure elevation in rats by peripheral administration of Tyr-Gly-Gly-Phe-Met-Arg-Phe and the invertebrate neuropeptide, Phe-Met-Arg-Phe-NH₂. *Life Sci.* 1982; 31:2555–2561. [PubMed: 7154851]
8. Wong TM, Greenberg MJ, Tse SY. Cardiovascular effects of intraventricular injection of FMRFamide, Met-enkephalin and their common analogues in the rat. *Comp Biochem Physiol C.* 1985; 81:175–179. [PubMed: 2861046]
9. Chou YL, Scarupa MD, Mori N, Canning BJ. Differential effects of airway afferent nerve subtypes on cough and respiration in anesthetized guinea pigs. *Am J Physiol Regul Integr Comp Physiol.* 2008; 295:R1572–R1584. [PubMed: 18768768]
10. Undem BJ, Chuaychoo B, Lee MG, Weinreich D, Myers AC, Kollarik M. Subtypes of vagal afferent C-fibres in guinea-pig lungs. *J Physiol.* 2004; 556:905–917. [PubMed: 14978204]
11. Lee MG, Macglashan DW Jr, Undem BJ. Role of chloride channels in bradykinin-induced guinea pig airway vagal C-fibre activation. *J Physiol.* 2005; 566:205–212. [PubMed: 15860525]
12. Chuaychoo B, Lee MG, Kollarik M, Pullmann R Jr, Undem BJ. Evidence for both adenosine A1 and A2A receptors activating single vagal sensory C-fibres in guinea pig lungs. *J Physiol.* 2006; 575:481–490. [PubMed: 16793905]
13. Chuaychoo B, Lee MG, Kollarik M, Undem BJ. Effect of 5-hydroxytryptamine on vagal C-fiber subtypes in guinea pig lungs. *Pulm Pharmacol Ther.* 2005; 18:269–276. [PubMed: 15777609]
14. Lee MG, Kollarik M, Chuaychoo B, Undem BJ. Ionotropic and metabotropic receptor mediated airway sensory nerve activation. *Pulm Pharmacol Ther.* 2004; 17:355–360. [PubMed: 15564076]
15. Linley JE, Rose K, Patil M, Robertson B, Akopian AN, Gamper N. Inhibition of M current in sensory neurons by exogenous proteases: a signaling pathway mediating inflammatory nociception. *J Neurosci.* 2008; 28:11240–11249. [PubMed: 18971466]
16. Bonini JA, Jones KA, Adham N, et al. Identification and characterization of two G protein-coupled receptors for neuropeptide FF. *J Biol Chem.* 2000; 275:39324–39331. [PubMed: 11024015]
17. Undem BJ, Kwong K, Blackwell; 2008. The sensory and autonomic nervous system in asthma and rhinitis in allergy and allergic disease.

Figure 1.

Patch clamp recordings in nodose and jugular ganglia. Membrane depolarization and action potential discharge were induced by FMRFamide application in isolated neurons from jugular and nodose ganglia (A). The average of amplitude of membrane depolarization was bigger in nodose (n=10) than jugular (n=10) ganglionic neurons but induction probability of action potential was same 50% in both ganglionic neurons (B). Filled circle and opened rhombus indicate presence and absence of action potential discharge, respectively.

A)

B)

Figure 2.

Patch clamp recording in FMRFamide sensitive neuron of nodose ganglion. Incremental depolarizing steps (10 mV) from -80 mV to -40 mV (50 ms of duration) were applied to the nodose neuron before, during and after FMRFamide application at 35° . Membrane potential was clamped at -60 mV. Inward current with increasing membrane conductance (inset) was induced by FMRFamide application (A). The peak current evoked by each voltage step at resting ($\blacksquare$) and during FMRFamide application ($\circ$) was plotted in I - V relation graph. Calculated reversal potential was about 33.3 mV if there was no rectification (B).

Table 1

FMRamide sensitivity in nodose and jugular ganglia in intracellular calcium measurements

	Nodose ganglia (n=73)	Jugular ganglia (n=55)
FMRFa sensitive	62 (85%) *	22 (40%)
FMRFa insensitive	11 (15%)	33 (60%)

*
p<0.05, χ^2 test

Table 2

FMRFa sensitivity in capsaicin-sensitive and -insensitive neurons of vagal ganglia in intracellular calcium measurements

	Capsaicin-sensitive (n=71)	Capsaicin-insensitive (n=57)
FMRFa sensitive	49 (69%)	35 (61%)
FMRFa insensitive	22 (31%)	22 (39%)