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RFamide NEUROPEPTIDE ACTIONS ON THE MOLLUSCAN HEART*

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FMRFamide and the related tetrapeptide FLRFamide are highly excitatory in molluscan non-cardiac smooth muscle. They are also exceptionally excitatory in the atrium and internally perfused ventricle of *Busycon canaliculatum.* These two peptides, usually thought of as classic molluscan cardio-acceleratory agents are in fact simply two members of a large and ever growing superfamily, the RFamide family, whose phylogenetic distribution has been so elegantly mapped by Walker [16]. Members of this family, often with extended peptide chains (e.g. penta, hepta and decapeptides), stretch in their known distribution from the cnidaria to the chordates. The effects of some of the members of this superfamily (FMRFamide, FLRFamide, YMRFamide, TNRNFLRFamide, SDPFLRFamide, LMS) were examined. The neuropeptides were found to be very potent at very low concentrations (10^{-9} M) in the ventricle of both *Buccinum* and *Busycon.* Other neuropeptides (HFMRdFamide, SCPb, NLERFamide and pEGRFamide) were found to be without any effect. The Ca^{2+} dependency of these neuropeptides was also tested. The peptides appear to induce contraction of the ventricles by release of Ca^{2+} from internal pools. The neuropeptides appear to stimulate contraction in these cardiac muscles through a completely different pathway to Serotonin (the main excitatory neurotransmitter for the cardiac muscle). When the peptides were applied together with Serotonin an additive effect was observed clearly indicating the release of Ca^{2+} through different pathways. The nature of the RFamide receptor was also tested. It appears that the RFamide neuropeptides mobilize the 2nd messenger IP_3 (Inositol trisphosphate), since the IP_3 blocker Neomycin Sulphate inhibited the response of the neuropeptides.

Keywords: Buccinum undatum – Busycon canaliculatum – Ca^{2+} dependency – RFamide neuropeptides – serotonin

INTRODUCTION

There have been many studies on the ventricle of molluscs, particularly on those of the bivalves [7, 8]. Since the discovery of the cardio excitatory neuropeptide FMRFamide by Price and Greenberg [13], the complexity of the innervation of the cardiac muscle has been established [4]. Families of FMRFamide-like peptides have

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now been identified and a common feature appears to be the RFamide terminal, which appears to be crucial for biological function [3]. Similar results have been reported for the proboscis smooth muscle [5, 8]. In the present study a more comprehensive and analytical research on the peptides and their actions is attempted. Several peptides were tested and their ionotropic effects recorded. There has been little interest in the link and comparison of the effects of the RFamide neuropeptide superfamily on the cardiac muscle. As a result in this study we report the findings on the ventricle of *Buccinum undatum* and the ventricle and of *Busycon canaliculatum*. The two species are closely related and their physiology and pharmacology are very similar, so that a comparison can be made.

MATERIALS AND METHODS

Mature specimens of *Busycon canaliculatum* and *Buccinum undatum* were obtained from the Woods Hole Marine Biological Laboratory and the University Marine Station at Millport, Isle of Cumbrae, respectively. The animals were kept in large aerated aquaria at 16° and 12 °C. After removal of the shell an incision was made in the pericardium to expose the heart which was removed intact and placed in aerated sea water. Two types of preparations were studied in parallel. In *Busycon*, the atrium was removed and a fine cannula was inserted through the atrioventricular valve in the ventricle. In *Buccinum* similar procedure was followed. In certain occasions the ventricle was to small to be perfused and as a result it was cut to strips, ligated by fine cotton on both ends and mounted on aerated jacketed organ baths. The ventricle tips (aorta ends) were in all cases connected, via heart clips, to tension transducers to record spontaneous contractions of the preparations. Force was recorded isometrically from the preparationa using Grass FT 0.3 transducers and the outputs were interfaced to a Gould thermal array recorded (in the case of *Busycon*) or a Grass polygraph (in the case of *Buccinum*). For internal perfusion, we used constant head perfusion reservoirs set at an input pressure of 10 cm H_2O. Previous studies of cardiac performance and dynamics [14, 15] showed the importance of input in regulating heart rate. The 10 cm H_2O provided a steady acceptable rate of 10–15 min^{-1} in intact ventricles.

All the drugs used in this study were obtained from Sigma and American Peptide Company. They were all made in stock concentrates in distilled water. All experiments except the Ca^{2+} employed natural sea water. The Ca^{2+}-free salines were made up following the recipe of Brooks [1]. All experimental data are typical of at least 4 separate experiments.

RESULTS

Figure 1 shows the excitatory effect of 10^{-6} M FMRFamide on the ventricles of *Bucciunum undatum* and *Busycon canaliculatum*.

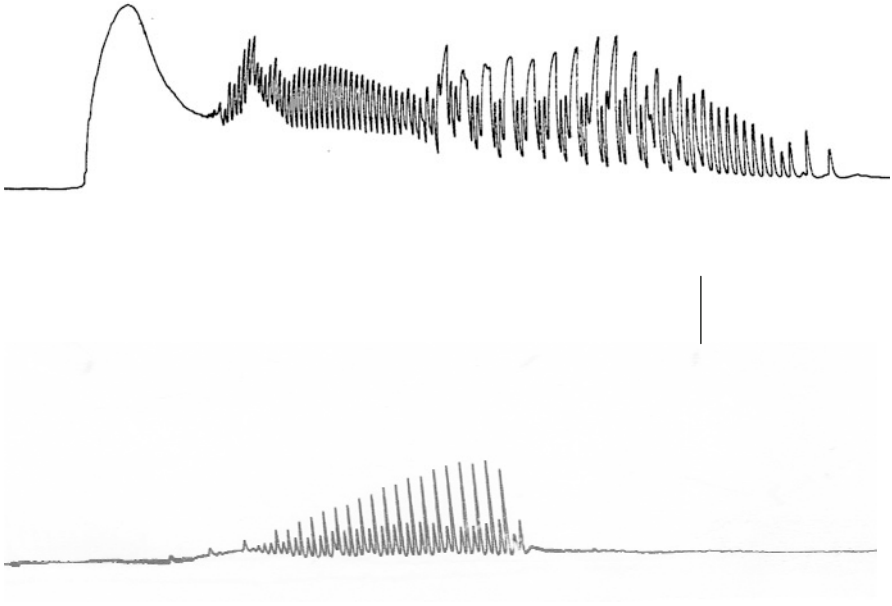

Fig. 1. This figure demonstrates the effect of 10^{-6} M FMRFamide on the ventricles of *Buccinum undatum* (top) and *Busycon canaliculatum* (bottom). Tension bar applies to all figures and is equal to 1 g

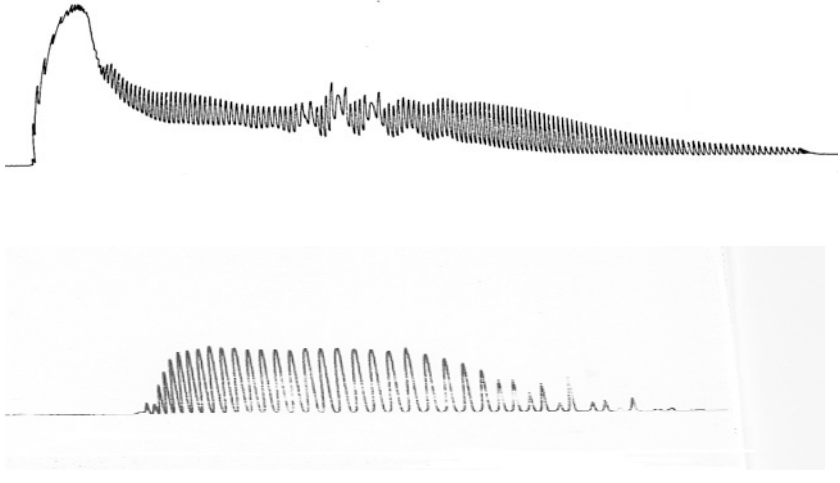

Fig. 2. This figure shows the effect of TNRNFLRFamide at 10^{-6} M in the ventricles of *Buccinum undatum* (top) and *Busycon canaliculatum* (bottom)

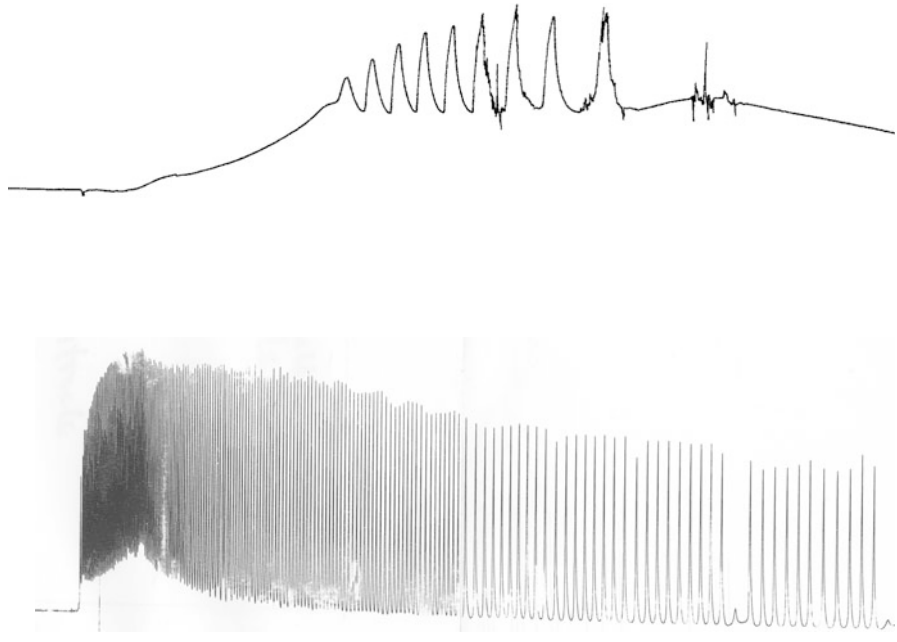

Fig. 3. A different peptide, namely LMS, was added at the same concentration, 10^{-6} M, on the ventricles of *Buccinum undatum* (top) and *Busycon canaliculatum* (bottom). Rhythmicity was generated in both preparations

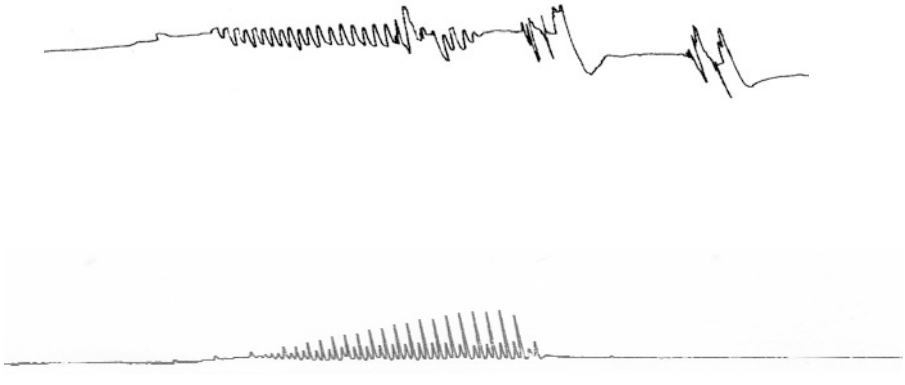

Fig. 4. In this figure, 10^{-6} M of FMRFamide were added in the two ventricles in Ca^{2+} free saline. The responses are considerably reduced, indicating that intracellular Calcium is mobilised for the generation of tension. Top trace is a ventricle from *Buccinum undatum* and bottom trace from *Busycon canaliculatum*

The excitatory effect of a different peptide, namely TNRNFLRFamide, at the same concentration can be seen in Figure 2. In Figure 3, the effect of LMS, at 10^{-6} M on the two species. It is clear from the above traces that the neuropeptides have similar actions on the ventricles of the two species. It appears that *Busycon canaliculatum* responds more rhythmically to the neuropeptides but this could also be due to the fact that the ventricles were a lot bigger.

In the traces in Figure 4 the neuropeptides were added at 10^{-6} M in Ca^{2+}-free salines. Their effects are considerably smaller. Although there was no external Ca^{2+} in the salines the preparations responded, indicating the mobilisation of internal Ca^{2+} stores. Microscopic investigations [10] of these muscles have clearly demonstrated the presence of intrinsic Sarcoplasmic Reticulum, possibly the store area of the cells' internal calcium. There is a lot of speculation on the 2nd messenger used by the neuropeptides. Ellis [4] and Huddart et al. [7, 8] suggested the IP_3 pathway. Neomycin sulphate is a potent IP_3 blocker. When applied at a standard dose of 2.5 mM it reduced the effect of the neuropeptides (namely FMRFamide on this example) to a minimal response. This can be clearly observed in Figure 5.

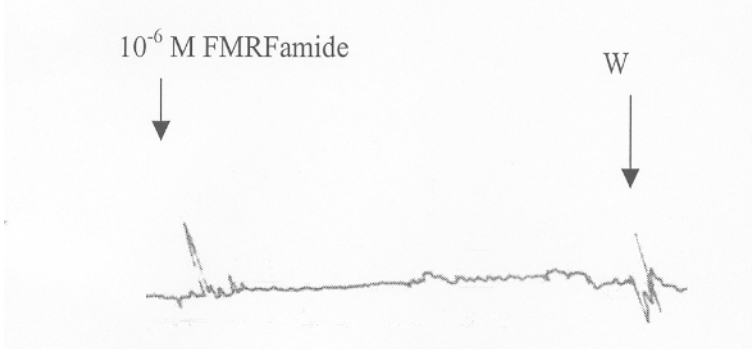

Fig. 5. The ventricle from *Buccinum undatum* was pre-treated with the potent IP_3 blocker Neomycin sulphate. The response to the peptide is minimal indicating that the peptide works via an IP_3 pathway

DISCUSSION AND FUTURE APPROACH

There have been a lot of studies [4, 7, 8] on the effect of neuropeptides and especially of the RFamide superfamily on the molluscan heart. Here we attempt a comparative approach on the effect of neuropeptides on the ventricles of *Buccinum undatum* and *Busycon canaliculatum*. It is evident that the neuropeptides appear to be very potent and even at minute concentrations (10^{-6} M) they excite the preparations. This agrees with findings by previous workers [7, 8, 15].

Calcium dependency seems to be of some importance in these preparations. The ventricles responded to the neuropeptides in Ca^{2+}-free conditions but the response was limited. Interestingly, when the preparations were re-introduced to the peptides

in Ca^{2+}-free conditions for the second time the responses recorded were even smaller. Possibly the internal Ca^{2+} stores were exhausted on the first occasion and as a result little or no contraction was observed the second time. Previous experiments with vertebrate Ca^{2+} antagonists gave confusing results (Nifedipine was excitatory, [6]), once again stressing the fundamental differences between invertebrate and vertebrate pharmacology.

Finally, Ellis [4] speculated that the neuropeptides act on a G-protein coupled receptor and utilize IP_3 as a second messenger. The very potent IP_3 blocker neomycin sulphate was employed in this study and it managed to diminish the effect of FMRFamide, indicating the use of the IP_3 pathway. We are currently working on mapping the structure of the neuropeptides and hence possibly identifying the molluscan receptor. Future work also includes the testing of a greater number of different peptides so that a pattern can emerge.

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