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SHORT COMMUNICATION

THE MODULATORY EFFECT OF SCHISTOFLRFamide ON HEART AND SKELETAL MUSCLE IN THE LOCUST *SCHISTOCERCA GREGARIA*

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SchistoFLRFamide (PDVDHVFLRFamide) is one of the endogenous FMRFamide-like neuropeptides found in the nervous system of the locust *Schistocerca gregaria* (Robb *et al.* 1989; Robb and Evans, 1990). FMRFamide-like neuropeptides modulate the actions of a wide variety of both skeletal and visceral muscles in insects (Evans and Myers, 1986*a*; Schoofs *et al.* 1993*b*) and have been suggested to act both as circulatory hormones and as locally released neurotransmitters (see discussion in Robb and Evans, 1990). However, the behavioural context(s) in which such neuropeptides are released is at present unclear, although recent evidence suggests they may have a role in feeding behaviour (Elia *et al.* 1993). SchistoFLRFamide belongs to one of the subclasses of FMRFamide-like neuropeptides found in insects, which also includes leucomyosuppressin (pQDVDHVFLRFamide; Holman *et al.* 1986), ManducaFLRFamide (pQDVVHSFLRFamide; Kingan *et al.* 1990) and TDVDHVFLRFamide found in both *Drosophila melanogaster* (Nichols, 1992) and *Neobellieria bullata* (Fonagy *et al.* 1992). The sequence of SchistoFLRFamide has also been found in *Locusta migratoria* (Schoofs *et al.* 1993*a*).

SchistoFLRFamide was originally isolated from the nervous system of *Schistocerca gregaria* using a combination of radioimmunoassay, HPLC and bioassay (Robb *et al.* 1989; Robb and Evans, 1990). Preliminary data indicate that it inhibits spontaneous contractions of the locust heart and has a complex dose-dependent effect upon contractions induced in the extensor tibiae muscle by stimulation of the slow excitatory motoneurone (Robb *et al.* 1989). In addition, SchistoFLRFamide has recently been shown to decrease the amplitude and frequency of myogenic contractions and to reduce basal tension in locust oviduct muscle (Peeff *et al.* 1993).

The present paper provides a more detailed account of the modulatory effect of SchistoFLRFamide on heart and skeletal muscle in the locust *Schistocerca gregaria*. The

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effects of the synthetic peptide were measured on the semi-isolated locust heart (Cuthbert and Evans, 1989). The results are expressed as the percentage change in the amplitude of the heartbeat and its frequency with respect to measurements obtained immediately before the application of the peptide. Measurements of heart rate and contraction amplitude were averaged over a 1 min period, starting (a) 1 min before the beginning of the peptide pulse, (b) 1 min after the start of the peptide pulse, (c) 4 min after the start of the peptide pulse and (d) 1 min after the end of the peptide pulse. The bioactivity of synthetic SchistoFLRFamide was also assayed on another well-defined FMRFamide

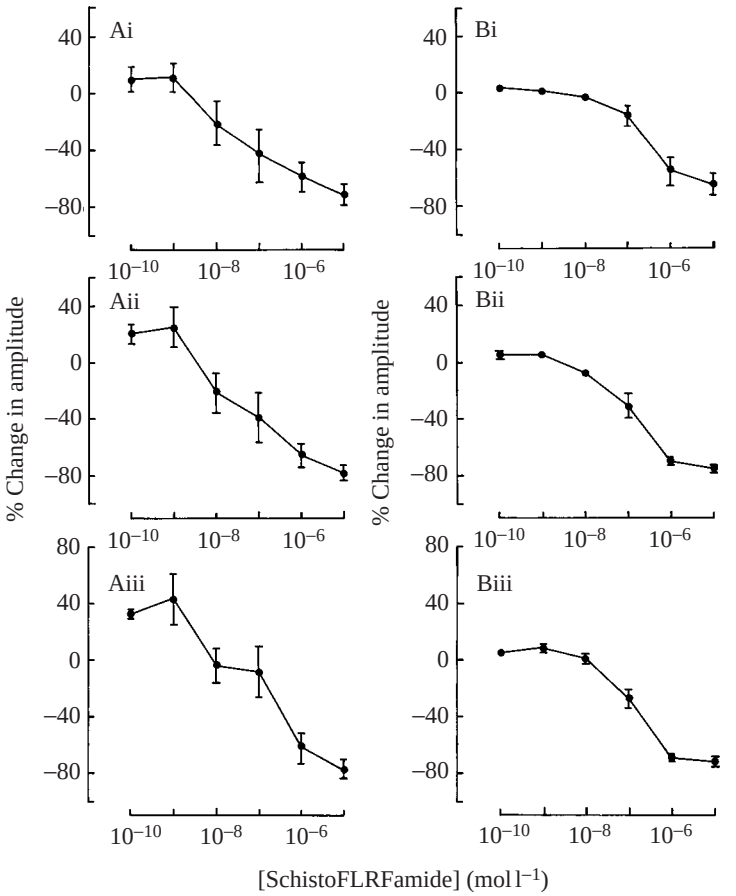

Fig. 1. Dose–response curves for the actions of synthetic SchistoFLRFamide on (A) the contraction amplitude and (B) the contraction frequency of the semi-isolated locust heart preparation during (i) the second minute of a 5 min exposure to the peptide, (ii) the final minute of a 5 min exposure to the peptide and (iii) the second minute after the end of a 5 min pulse of this peptide. The error bars represent the standard error of the mean, N=3. The frequency and amplitude of spontaneous heart contractions were measured almost isometrically by attaching the heart to a force transducer (see Cuthbert and Evans, 1989). The drug was superfused over the preparation in isotonic saline at a rate of 1 ml min^{-1} and each heart was maintained in a volume of 200 μl of saline.

bioassay preparation from the locust. The effects were measured on the twitch tension evoked in the extensor tibiae muscle of the hindleg of the locust by stimulating the slow excitatory motoneurone to this muscle at 1 Hz (Evans and Myers, 1986*b*). The results are expressed as the maximal effects on the twitch amplitude.

SchistoFLRFamide had a potent dose-dependent cardioinhibitory effect on both the amplitude and frequency of spontaneous contractions in the semi-isolated locust heart, with a threshold for an observable effect occurring between 10^{-9} and 10^{-8} mol l^{-1} (Fig. 1). At low concentrations (10^{-8} mol l^{-1}) SchistoFLRFamide had a slight cardioinhibitory effect, which lasted only while the heart was exposed to the peptide, and the heart rate returned to pre-exposure levels within 2 min of peptide removal (Figs 1Aiii, 2A). At higher concentrations (10^{-8} to 10^{-5} mol l^{-1}), SchistoFLRFamide had potent cardioinhibitory effects on both the amplitude and frequency of heart contraction (Figs 1, 2B). These effects were long-lasting and in many preparations were still visible more than 2 min after peptide removal from the superfusate (Fig. 1Aiii, Biii). Fig. 2B shows a preparation in which a 5 min pulse of 10^{-7} mol l^{-1} SchistoFLRFamide initially reduced both the frequency and amplitude of spontaneous contractions. The effect on the frequency of spontaneous contractions lasted for about 2 min after the removal of the peptide from the superfusate but, in this preparation, the contraction amplitude returned to control levels before the end of the pulse and was even potentiated by 35 s after the end of the pulse. In general, the effects of the peptide were observed for longer in the wash-out period when higher concentrations were applied (see Robb *et al.* 1989). At 10^{-6} mol l^{-1} SchistoFLRFamide these effects were more pronounced (see Robb *et al.* 1989). Heart contractions were rapidly abolished, an effect that persisted for several minutes after the peptide had been removed from the superfusate. As heartbeat frequency began to recover during the wash-out, contraction amplitude was always potentiated for several minutes. These potentiating effects of high doses of SchistoFLRFamide are not visible in the dose–response curves (Fig. 1); they did not occur until several minutes after the peptide had been removed from the superfusate.

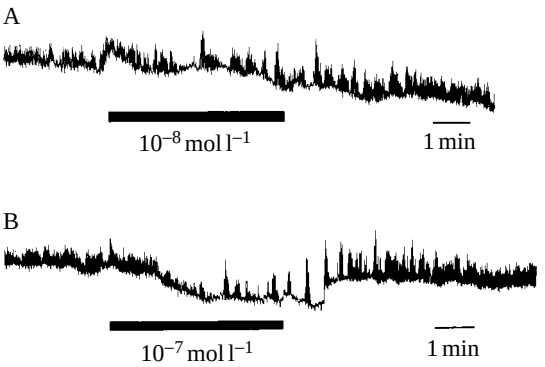

Fig. 2. The effect of 5 min pulses of various concentrations of synthetic SchistoFLRFamide (black bars) on the spontaneous contractions of the semi-isolated locust heart preparation. (A) The effect of a 5 min pulse of 10^{-8} mol l^{-1} SchistoFLRFamide; (B) the effect of a 5 min pulse of 10^{-7} mol l^{-1} SchistoFLRFamide.

Fig. 3. Typical examples of the effects of 5 min pulses of various concentrations of SchistoFLRFamide on the amplitude of twitch tension induced in the hindleg extensor tibiae muscle by stimulation of the slow motor neurone to this muscle at 1 Hz in three different preparations (i–iii). (A) The effect of a 10^{-8} mol l^{-1} pulse. (B) The effect of a 10^{-7} mol l^{-1} pulse. (C) The effect of a 10^{-6} mol l^{-1} pulse. (D) The effect of a 10^{-5} mol l^{-1} pulse. Preparation i gave purely excitatory responses to all concentrations of peptide above threshold, whilst preparation ii gave excitatory responses at all concentrations above threshold except for the 10^{-6} mol l^{-1} pulse, which produced a biphasic effect with a transient inhibition preceding the main excitatory effect. In contrast, preparation iii produced an excitatory response to the 10^{-8} mol l^{-1} pulse, an inhibitory responses to the 10^{-7} mol l^{-1} and 10^{-6} mol l^{-1} pulses and a biphasic effect in response to the 10^{-5} mol l^{-1} pulse. The trace shown for preparation ii at 10^{-6} mol $^{-1}$ has been modified from Robb *et al.* (1989).

SchistoFLRFamide also produced a complex dose-dependent pattern of potentiation and inhibition on both the amplitude and relaxation rate of slow-motoneurone-induced twitch tension in the extensor tibiae muscle of the locust hindleg. At concentrations between 10^{-12} and 10^{-8} mol l^{-1}, SchistoFLRFamide produced a small but variable increase of 10–20 % in the amplitude of twitch tension. At higher concentrations, between 10^{-7} and 10^{-5} mol l^{-1}, the amplitude responses were complex and varied between preparations. In most preparations (six out of seven) the responses were excitatory (e.g. Fig. 3i), but in one preparation the amplitude of twitch tension was reduced at all concentrations tested. Many of the excitatory responses were biphasic, with an initial transient inhibition preceding the main excitatory response. Fig. 3ii shows a preparation in which a biphasic response was induced at 10^{-6} mol l^{-1} but in which exposure to 10^{-5} mol l^{-1} peptide produced purely a large excitation, whilst Fig. 3iii shows a preparation in which excitation was produced at 10^{-8} mol l^{-1}, inhibition at 10^{-7} mol l^{-1}

and 10^{-6} mol l^{-1} and a biphasic effect at 10^{-5} mol l^{-1}. Possible reasons for the different degrees of inhibition and excitation shown by individual preparations are currently being investigated. Similar biphasic results were obtained for the effect of SchistoFLRFamide on the relaxation rate of slow-motoneurone-induced twitch tension (data not shown).

Previous physiological investigations have provided evidence for the existence of at least two receptors for FMRFamide-like peptides in locusts, one producing excitation and the other inhibition in a number of preparations, including the extensor tibiae muscle of the hindleg (Evans and Myers, 1986*a*,*b*; Cuthbert and Evans, 1989), the heart (Cuthbert and Evans, 1989) and the oviducts (Peeff *et al.* 1993). Concentration-dependent biphasic effects of FMRFamide have also been reported in studies on hearts from a number of bivalve molluscs (Painter and Greenberg, 1982). Evidence has also been presented for the existence of multiple receptor types for members of this peptide family on molluscan neurones (Cottrell *et al.* 1984; Ruben *et al.* 1986; Cottrell and Davies, 1987), skeletal muscle (Cottrell *et al.* 1983) and heart (Payza, 1987). In the present paper, the most likely explanation for our results with SchistoFLRFamide is that this neuropeptide is capable of activating both excitatory and inhibitory subtypes of receptors in both the locust heart and extensor tibiae muscle preparations. However, in the absence of specific antagonists for these receptors, it is not possible to rule out a mechanism whereby the native peptide is acting on only a single class of receptors, whose effects vary according to the level of receptor activation. This explanation seems to be unlikely, however, since the closely related neuropeptide leucomyosuppressin, in which a single N-terminal amino acid, pyroglutamate, is substituted for the proline of SchistoFLRFamide, interacts exclusively with the inhibitory subclass of FMRFamide-like receptors (Evans and Myers, 1986*a*,*b*; Cuthbert and Evans, 1989). The exact responses obtained by the application of SchistoFLRFamide in these systems depended both on the concentration of the peptide used and on the response characteristics of the individual preparation. Thus, a detailed characterization of the pharmacology and mode of action of SchistoFLRFamide in these tissues awaits either the development of specific antagonists for the receptor subtypes or their cloning.

References

COTTRELL, G. A. AND DAVIES, N. W. (1987). Multiple receptor sites for a molluscan peptide (FMRFamide) and related peptides of *Helix*. *J. Physiol., Lond.* **382**, 51–68.

COTTRELL, G. A., DAVIES, N. W. AND GREEN, K. A. (1984). Multiple actions of a molluscan cardioexcitatory neuropeptide and related peptides on identified *Helix* neurones. *J. Physiol., Lond.* **356**, 315–333.

COTTRELL, G. A., GREENBERG, M. J. AND PRICE, D. A. (1983). Differential effects of a molluscan neuropeptide FMRFamide and the related met-enkephalin derivative YGGFMRFamide on the *Helix* tentacle retractor muscle. *Comp. Biochem. Physiol.* **75**C, 373–375.

CUTHBERT, B. A. AND EVANS, P. D. (1989). A comparison of the effects of FMRFamide-like peptides on locust heart and skeletal muscle. *J. exp. Biol.* **144**, 395–415.

ELIA, A. J., TEBRUGGE, V. A. AND ORCHARD, I. (1993). The pulsatile appearance of FMRFamide-related peptides in the haemolymph and loss of FMRFamide-like immunoreactivity from neurohaemal areas of *Rhodnius prolixus* following a blood meal. *J. Insect Biol.* **39**, 459–469.

EVANS, P. D. AND MYERS, C. M. (1986*a*). Peptidergic and aminergic modulation of insect skeletal muscle. *J. exp. Biol.* **124**, 143–176.

EVANS, P. D. AND MYERS, C. M. (1986*b*). The modulatory actions of FMRFamide and related peptides on locust skeletal muscle. *J. exp. Biol.* **126**, 403–422.

FONAGY, A., SCHOOFS, L., PROOST, P., VANDAMME, J., BUEDS, H. AND DELOOF, A. (1992). Isolation, primary structure and synthesis of neomyosuppressin, a myoinhibiting neuropeptide from the grey fleshfly, *Neobellieria bullata. Comp. Biochem. Physiol.* **102**C, 239–245.

HOLMAN, G. M., COOK, B. J. AND NACHMAN, R. J. (1986). Isolation, primary structure and synthesis of leucomyosuppresion, an insect neuropeptide that inhibits spontaneous contractions of the cockroach hindgut. *Comp. Biochem. Physiol.* **85**C, 329–333.

KINGAN, T. G., TEPLOW, D. B., PHILLIPS, J. M., RIEHM, J. P., RAO, K. R., HILDEBRAND, J. G., HOMBERG, U., KAMMER, A. E., JARDINE, I., GRIFFIN, P. R. AND HUNT, D. F. (1990). A new peptide in the FMRFamide family isolated from the CNS of the hawkmoth, *Manduca sexta. Peptides* **11**, 849–856.

NICHOLS, R. (1992). Isolation and structural characterization of *Drosophila* TDVDHVFLRFamide and FMRFamide-containing neural peptides. *J. molec. Neurosci.* **3**, 213–218.

PAINTER, S. D. AND GREENBERG, M. J. (1982). A survey of the responses of bivalve hearts to the molluscan neuropeptide FMRFamide and to 5-hydroxytryptamine. *Biol. Bull. mar. biol. Lab., Woods Hole* **162**, 311–322.

PAYZA, K. (1987). FMRFamide receptors in *Helix aspersa*. *Peptides* **8**, 1065–1074.

PEEFF, N. M., ORCHARD, I. AND LANGE, A. B. (1993). The effects of FMRFamide-related peptides on an insect (*Locusta migratoria*) visceral muscle. *J. Insect Physiol.* **39**, 207–215.

ROBB, S. AND EVANS, P. D. (1990). FMRFamide-like peptides in the locust: distribution, partial characterisation and bioactivity. *J. exp. Biol.* **149**, 335–360.

ROBB, S., PACKMAN, L. C. AND EVANS, P. D. (1989). Isolation, primary structure and bioactivity of SchistoFLRFamide, a FMRFamide-like neuropeptide from the locust, *Schistocerca gregaria. Biochem. biophys. Res. Commun.* **160**, 850–856.

RUBEN, P., JOHNSON, J. W. AND THOMPSON, S. (1986). Analysis of FMRFamide effects on *Aplysia* bursting neurones. *J. Neurosci.* **6**, 252–259.

SCHOOFS, L., HOLMAN, G. M., PAEMEN, L., VEELAERT, D., AMELINEKY, M. AND DELOOF, A. (1993*a*). Isolation, identification and synthesis of PDVDHVFLRFamide (SchistoFLRFamide) in *Locusta migratoria* and its association with the male accessory glands, the salivary glands, the heart and the oviduct. *Peptides* **14**, 409–421.

SCHOOFS, L., VANDEN BROECK, J. AND DE LOOF, A. (1993*b*). The myotropic peptides of *Locusta migratoria*: Structures, distribution, functions and receptors. *Insect Biochem. molec. Biol.* **23**, 859–881.