reconstituted from detergent extracts in an active form. The ability to extract the transporters from their native membrane environment without loss of activity is an important initial step towards their characterization at the molecular level and their purification.

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FMRFamide and related peptides modulate the actions of 5-hydroxytryptamine and proctolin on the foregut of the locust, *Schistocerca gregaria*

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Introduction

The cardioexcitatory molluscan neuropeptide FMRFamide (Phe-Met-Arg-Phe-NH₂) [1] and some of the structurally related family of FMRFamide-like peptides, discovered subsequently in a range of species, have modulatory effects on invertebrate skeletal and visceral muscle. For example, FMRFamide has been shown to cause naloxone-sensitive potentiation of acetylcholine-induced contraction of smooth muscle from *Mytilus edulis* [2]. Furthermore, FMRFamide and related peptides have been shown to exert modulatory effects, described as both presynaptic and postsynaptic [3, 4] on the extensor tibiae preparation of *Schistocerca gregaria*. FMRFamide-like peptides have also been shown to be related structurally to the enkephalins [5] and, significantly, the indirect effects of FMRFamide on muscle contraction/relaxation were inhibited by the opiate antagonist naloxone.

The isolated foregut of the locust *S. gregaria* is contracted by the pentapeptide proctolin and relaxed by 5-hydroxytryptamine (5-HT) in a dose-dependent manner [6]. This report describes the naloxone-sensitive modulatory effects of FMRFamide, YGGFMRFamide (Tyr-Gly-Gly-Phe-Met-Arg-Phe-NH₂) and FLRFamide (Phe-Leu-Arg-Phe-NH₂) on proctolin-induced contraction and 5-HT-induced relaxation of the isolated locust foregut.

Materials and methods

Foreguts (oesophagus to proventriculus) isolated from mature adults of both sexes of *S. gregaria* were incubated in Clarke Insect Ringer solution at 18 ± 2 °C. All drug solutions were made up in Clarke Insect Ringer solution. Using a 6 min cycle with two washes, alternate standard doses of proctolin (10⁻⁴m) and 5-HT (10⁻⁵m) were applied to maintain tissue tone. The direct agonist actions of FMRFamide, YGGFMRFamide and FLRFamide were studied over a range of concentrations (10⁻⁴m–10⁻⁷m). Simultaneous addition of a standard concentration of peptide (2 × 10⁻⁴m) with doses of proctolin or 5-HT, in the presence and absence of naloxone (10⁻⁵m), was also investigated. The percentage values quoted are the mean of four to eight replicates ± S.E.M.

5-HT dose–response curves were constructed in the presence and absence of FMRFamide (2 × 10⁻⁴m) and FMRFamide plus naloxone (10⁻⁵m). Tissues were incubated with naloxone for 20 min before re-testing the effects of the agonists. The statistical significance of drug-induced changes in effects caused by standard concentrations of proctolin and 5-HT were tested using Student’s t-test.

Results and discussion

FMRFamide and FLRFamide had no direct agonist action on the tissue at the concentrations tested. However, YGGFMRFamide caused slight contraction at low doses (10⁻⁷m–10⁻⁵m) and slight relaxation at doses of 2 × 10⁻⁴m–10⁻⁵m.

FMRFamide, FLRFamide and YGGFMRFamide increased the relaxation induced by a standard dose of 5-HT by an average of 35.8 ± 6.5%, 49.3 ± 7.4% and 21.5 ± 5.7%, respectively. Naloxone (10⁻⁵m) significantly reduced potentiation of 5-HT-induced relaxation caused by FMRFamide and FLRFamide, but was not tested against YGGFMRFamide.

5-HT caused dose-dependent relaxation at doses ranging from 5 × 10⁻⁴m to 10⁻³m with a mean ED₅₀ value of 1.8 ± 0.4 × 10⁻⁵m (n = 10). Simultaneous addition of FMRFamide shifted the 5-HT dose–response curve to the left and significantly reduced the ED₅₀ to 1.0 ± 0.2 × 10⁻⁵m (n = 10; P < 0.0005). The 5-HT dose–response curve constructed in the presence of FMRFamide plus naloxone was not significantly different from the original 5-HT curve, with an ED₅₀ value of 1.4 ± 0.2 × 10⁻⁵m (n = 8).

FMRFamide, FLRFamide and YGGFMRFamide inhibited the contraction caused by a standard dose of proctolin by an average of 63.8 ± 6.5%, 26.6 ± 2.7% and 44.5 ± 10.2%, respectively. Naloxone (10⁻⁵m) significantly reduced this action of FMRFamide and FLRFamide, but was not tested against YGGFMRFamide.

It has been proposed that FMRFamide exerts its modulatory role in this tissue by activating naloxone-sensitive recep-
Characterization of novel post-synaptic-density-enriched glycoproteins gp130 and gp117 with a monoclonal antibody

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A major structural and functional component of chemically transmitting synapses is an electron-dense, proteinaceous body underlying the post-synaptic membrane, the post-synaptic density (psd). One important group of psd components are glycoproteins. These molecules are thought to play important roles in the structure, function and formation of the post-synaptic apparatus. Studies of isolated psds show that they possess a unique spectrum of glycoproteins which is distinct from that of the synaptic membrane (SM) [for a review see 1]. In particular, psds are enriched in a number of high-Mᵣ glycoproteins which bind to the lectin concanavalin A (con A) [2, 3]. Lectin overlay studies show that the major psd con-A-binding glycoproteins are of Mᵣ 180 000, 145 000, 116 000 (formerly designated gp130) and 110 000. However, progress in studying both psd and SM glycoproteins has been hindered by a lack of suitable specific probes. We are therefore raising a library of monoclonal antibody (mab) probes to characterize and study the function of these molecules. We have previously described antibodies which recognize major SM glycoproteins of Mᵣ 85 000, 55 000 and 50 000 and a psd-enriched glycoprotein of Mᵣ 65 000 [4–7]. In the present communication we describe the use of a mab, psd gp130, to characterize two novel psd-enriched glycoproteins of Mᵣ 130 000 and 117 000.

The psd gp130 mab was raised by immunization of Balb/C mice with SM con-A-binding material as previously described [5]. Three days after the final immunization the splenocytes were fused with myeloma P3X63.Ag8653 cells according to the procedures of Galfre & Milstein [8]. The resulting hybridoma cell lines were screened by enzyme-linked immunosorbent assay (ELISA) using SM and SM con-A-binding material as test antigens. Positive clones were subsequently screened by immunodevelopment of Western blots of either SM or SM con-A-binding material. Blots were developed with primary antibody followed either by biotinylated anti-mouse IgG and avidin cross-linked biotinylated peroxidase, i.e. Vectastain ABC reagents [5], or with 125I-labelled sheep anti-mouse Ig F(ab)₂ fragments [6]. Western blots of SM and SM con-A-binding material immunodeveloped with mab psd gp130 (Fig. 1a) show that the antibody specifically recognizes two glycoprotein bands and that the 5-HT receptor antagonist ketanserin reduced the action of both the indolealkylamine and FMRFamide. Consequently, it would appear that the actions of both substances are likely to be expressed by activation of the ketanserin-sensitive 5-HT receptor. The action of naloxone to prevent FMRFamide exerting its full effects on the 5-HT dose-response curve further supports this proposal. Similarly, naloxone had no effect on proctolin-induced contraction, but abolished FMRFamide-induced inhibition of the contractile effects of proctolin.

All three FMRFamide-related peptides are approximately equipotent at increasing relaxation induced by a standard dose of 5-HT. However, FLRFamide is less potent than either FMRFamide or YGGFMRFamide in inhibiting the contraction induced by a standard dose of proctolin. This result may reflect the sensitivity of the receptor for -Met-Arg-Phe-NH₂-type compounds.

Fig. 1 shows a model proposed to explain the interaction between FMRFamide-related peptides, proctolin and 5-HT.

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Abbreviations used: con A, concanavalin A; mab, monoclonal antibody; LM, light membranes; psd, post-synaptic density; SM, synaptic membranes; CNS, central nervous system; N-CAM, neural cell adhesion molecule.

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