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Peptidergic control of egg-laying in the cephalopod *Sepia officinalis*: involvement of FMRFamide and FMRFamide-related peptides

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Abstract

The peptidergic control of egg-laying was investigated in *Sepia officinalis* by using a myotropic bioassay. Three myotropic high-performance liquid chromatography fractions were obtained from optic lobe extracts. In the first fraction, FMRFamide (FMRFa) and FLRFa were isolated and sequenced. FMRFa-related peptides then were sought by dotting immunobinding of optic lobes extracts. The four immunoreactive fractions detected revealed the occurrence of FMRFa, FLRFa, FIRFa, and ALSGDAFLRFa predicted by the precursor already cloned from the optic lobes of *S. officinalis* (J Exp Biol 200:1483–9;1997). These peptides clearly appeared to be involved in the regulation of oocyte transport through the oviduct: the tetrapeptides FMRFa and FLRFa stimulated the contractions, whereas FIRFa and ALSGDAFLRFa lowered the tonus, the frequency, and the amplitude of the contractions. The occurrence of FaRPs in the nervous endings of the accessory sex glands suggested that this peptide family is involved in the regulation of secretory processes of the egg capsule. Indeed, FMRFa modulates the contractions of the main nidamental glands in vitro and, thus, should induce mechanical release of the secretion in vivo during ovulation. These results show that the FaRPs could play an important role in the synchronization of ovulation and egg capsule coating.

Keywords: Egg-laying; Oviduct; Accessory; Sex glands; Myotropins; FMRFamide; *Sepia officinalis*

1. Introduction

In invertebrates, a great number of bioactive neuropeptides have been characterized by means of myotropic bioassays. In mollusks, many neuropeptides have been purified on the basis of their capacity to modulate the contractions of various muscles such as the anterior byssus retractor muscle of *Mytilus edulis* [7–10,13–15], the esophagus and the penis retractor muscle of *Lymnaea stagnalis* [17,28], and the oviduct of *Sepia officinalis* [12]. In insects, the contractions of the oviduct, the gut, or the heart of various species allowed the identification of ≈50 peptides initially called myotropins [26]. However, complementary experiments have now shown that these peptides also are involved in the control of a large variety of physiological mechanisms such as pheromone production, water balance, and reproduction [26]. Thus, myotropic bioassays provide an important source of potential new neurotransmitters, neuromodulators, and neurohormones that can target numerous organs. By using this experimental approach, we have investigated the peptidergic control of oviducal contractions in *S. officinalis*. In the mature females of this cephalopod mollusk, the oocytes are carried out from the genital coelome to the mantle cavity by the peristaltic contractions of a large oviduct. The accessory sex glands release secretory substances that coat the oocytes just before the fertilization by the spermatophores stored by the female. Thus, the contractile activity of the oviduct provides a valuable and reproducible bioassay to isolate and purify myotropins. The in vitro bioassay based on the recording of the oviduct contractions allowed the screening of the central nervous system (CNS) in a previous study. Prepurified extracts from the different parts of the CNS were tested on the oviduct. The optic lobe extract revealed a strong inhibitory activity showing a decrease of the tonus, the amplitude, and the frequency of contractions. The purification and the sequencing led to the identification of a C-terminal, amidated dipeptide: Gly Trp-NH_2 [12]. In this paper, we have performed further investigations of the same organ (optic lobe) by using the same myotropic bioassay to isolate and characterize other putative neuropeptides involved in the control of egg laying.

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2. Materials and methods

2.1. Experimental animals

All cuttlefish were captured in the western English Channel between January and June by trawling. They were kept in 250-l outflow tanks in the Marine Station of Luc-sur-mer (University of Caen, Caen, France).

2.2. Oviduct bioassay

The oviduct was excised from vitellogenic and mature females and suspended from a displacement tranducer (Phymep, Bionic Instruments, Paris, France) connected to a computer controlling the recorder and the DATAC (Dispositif d'Acquisition et de Traitement Automatique de la Contraction). The muscle chamber (2.5 ml) was perfused at a flow rate of 0.5 ml/min with synthetic filtered sea water (Instant Ocean, Sarrebourg, France) containing 1 mM glucose and maintained at 15°C in a cooling room. The high-performance liquid chromatography (HPLC) fractions were injected in the perfusing flow by using a three-way tap to avoid mechanical and thermal stress. The fractions were traced into the muscle chamber by addition of phenol red. The oviduct-containing chambers were washed for at least 2 h before the application of organ extracts, HPLC fractions, or synthetic peptides. In all cases, the experiments were performed when the activity had returned to basal levels.

2.3. Extraction

A total of 100 optic lobes from mature females of *S. officinalis* was excised and stored in liquid nitrogen until extracted. Optic lobes were crushed in liquid nitrogen and extracted in ice-cold methanol/H_2O/acetic acid (90:9:1) for 12 h at 4°C (1 g of fresh tissue for 10 ml). After sonication and centrifugation, the supernatants were pooled. The pellets were redissolved in extraction medium, and the procedure was repeated. The extract was loaded into a C18 Sep-Pak. The Sep-Pak eluates (Molsheim, France) (methanol 100%) were evaporated in a Speedvac concentrator and kept at −80°C until HPLC fractionation.

2.4. First-step purification in HPLC

HPLC analysis was performed with a VARIAN-4050 integrator connected to a VARIAN-9012 solvent delivery system and with a VARIAN-9050 wave-length UV-VIS detector set at 214 nm. All water and ACN solutions used in the HPLC analysis contained 0.1% trifluoroacetic acid (TFA). For each assay, the dry optic lobe extracts were resuspended in 200 μl of 0.1% TFA in water and eluted by a 45-min linear gradient of 1.33% of ACN per min from 0 to 60% of ACN on a Merck 100RP18 column (Merck, 4 × 250 mm, 5 μm). The fractions containing the myotropic activity were identified by the oviduct bioassay.

2.5. Isolation and identification of M2

The active fraction eluted between 25 and 28 min was labeled M2 and was concentrated and separated on a Vydac C18 column (3.9 × 250 mm, 5 μm) with a linear gradient of 0.6% of ACN per min from 12 to 24% of acetonitrile (ACN). The resulting active fractions, called M2α and M2β, were concentrated and sequenced by using an Applied Biosystems model 473A pulse liquid protein sequencer (Merck, France). A fraction containing M2α and M2β was analyzed by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) to state the occurrence of C-terminal amidation and to identify M2β.

2.6. Bioactivity of FMRFa on the perfused oviduct

Aliquots of synthetic peptides from 10^{-8} M to 10^{-5} M were tested on the oviduct bioassay to specify the threshold of activity and to investigate a possible dose-dependent response.

2.7. Immunohistochemistry

The tissues were fixed for 48 h in saline formaline, dehydrated, and embedded in paraffin. Sections of 7 μm were rehydrated in phosphate-buffered saline, pH 7.4, and successively incubated for 1 h at 20°C in 5% skim milk, 12 h at 4°C in rabbit FMRFa (Sigma, St. Louis, MO, USA) antiserum diluted 1:1000 (Peninsula), and 1 h at 20°C in swine anti-rabbit secondary antibody coupled with a peroxidase and diluted 1:500 (Sigma). Primary and secondary antibodies were suspended in 10 mM phosphate-buffered saline containing 0.05% Triton X-100 and 1% skim milk. Cells and fibers containing immunoreactive material were revealed by using diaminobenzidine tetrachloride as the chromogen. Four controls were performed as follows to check the specificity of the immunologic study: 1,2) tissue sections were alternatively incubated without primary antibody or secondary antibody; 3) the primary antibody was exposed to the original immunogen (FMRFa) in excess to remove all antibodies able to bind FMRFa and FMRFa-related peptides; and 4) all of the tissue sections were incubated for 30 min in methanol/H_2O_2 to inhibit putative active peroxidases.

2.8. Dotting immunobinding screening with FMRFa antiserum

2.8.1. Optic lobes

A dry optic lobe extract was eluted by a 45-min linear gradient of 1.33% of ACN per min from 0 to 60% of ACN on a Macherey-Nagel RP18ec column (4 × 250 mm, 5 μm). The 1-min fractions were evaporated and resuspended in methanol/H_2O (90:10) and transferred onto a poly(vinylidene difluoride) membrane with a slotdot concentrator. Fractions containing FMRFa-related peptides (FaRPs) were

identified as described for immunochemistry and using the same antibody.

2.8.2. Accessory sex glands and hemolymph of mature females

Extracts of oviducal gland (OG), main nidamental glands (MNG), accessory nidamental glands (ANG), and hemolymph were prepared as described above for optic lobes, as well as the separation and the detection of the FaRPs. The hemolymph was collected from 75 mature females at the level of the vena cava neurohemal area to obtain a total volume of 1200 ml. A volume of 20 ml was used to perform the HPLC followed by the dotting immunobinding.

2.9. MALDI-TOF analysis of F2, F3, and F4

The dry pellets of the fractions containing FaRPs immunoreactivity were concentrated and resuspended in 1:1 acetonitrile/01% TFA solution. One microliter of each sample was mixed with an equal volume of the MALDI matrix prepared as follows: 10 mg of α-cyano-4-hydroxy cinnamic acid dissolved in 1 ml of a 1:1 acetonitrile/0.1% TFA solution. Samples (1 μl) were spotted into the wells of the MALDI and air-dried in a vacuum chamber prior to being submitted to multiple shots from the nitrogen laser (337 nm). The neuropeptides of interest were identified in the spectrum by their monoprotonated form $[M+H]^+$.

2.10. Bioactivity of FMRFa on the incorporation of 3H leucine and 3H glucose in the proteins and the polysaccharides of the MNG

Dissociated nidamental cells were incubated with 3H leucine (Sigma) and 3H glucose (Sigma) as well as increasing FMRFa concentrations from 10^{-5} to 10^{-10} M.

2.10.1. Incorporation measurements in proteins

After incubation, the cells were precipitated overnight at 4°C with 500 μl of ice-cold 10% trichloroacetic acid (TCA). After centrifugation (20 min, 650 $\times$ *g*), the supernatants were collected for polysaccharide extraction. The protein pellets were resupended and washed three times in 500 μl of 10% TCA. Pellets were dissolved in 500 μl of 0.3 N KOH at 40°C. This solution (200 μl) was added to 4.5 ml of scintillation liquid for radioactivity determination.

2.10.2. Incorporation measurements in polysaccharides

TCA supernatant (400 μl) was mixed with 1.5 ml of 30% KOH and incubated for 20 min at 60°C. The polysaccharides were precipited overnight at 4°C in 3 ml of 95% ethanol and 200 μl of saturated sodium sulfate. After centrifugation (1 000 $\times$ *g*, 5 min), the pellets were resuspended and washed three times in 3 ml of 95% ethanol. Pellets were dried at 60°C and resuspended in 500 μl of distilled water. This solution (200 μl) was mixed with 4.5 ml of scintillation fluid for radioactivity determination.

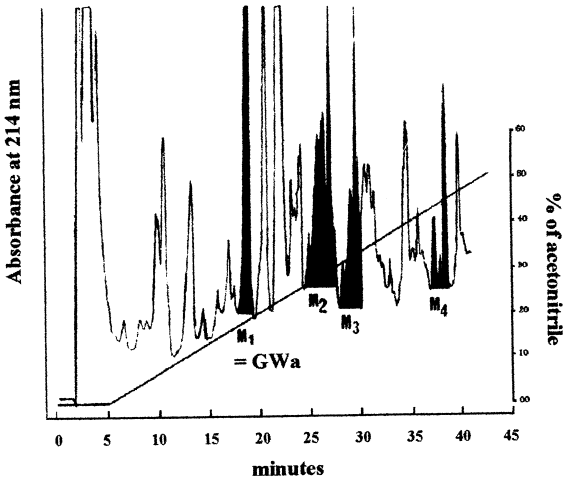

Fig. 1. First step of purification on a Merck 100RP18 column. One of the 10 C18 runs of C18 Sep-Pak eluates of 100 optic lobes. Four myotropic fractions called M1, M2, M3, and M4 are detected by the bioassay. M1 is the dipeptide GWamide already characterized in a previous study.

2.11. Myotropic activity of FMRFa on the main nidamental glands

The main nidamental glands were excised from vitellogenic females (stage IVa) and placed as described above for the recording of the contractions. For the first time, the contractions of this sexual gland were observed and recorded. Increasing concentrations of FMRFa (10^{-6}, 10^{-7}, and 10^{-8} M) were injected into the perfusing flow, and stimulations or alterations of the contractions were analyzed with the DATAC.

3. Results

3.1. Purification and identification of myotropins

The 10 C18 fractionations were pooled, and an amount corresponding to one animal equivalent (two optic lobes) was tested in the oviduct bioassay. Four active fractions called M1, M2, M3, and M4, which induced modifications of the contractions, were detected (Fig. 1): M1 eluted in the fractions 18 and 19 min corresponding to the GW-NH_2 showed an inhibitory activity [12], M2 eluted in the fractions 25, 26, and 27 min exhibited a strong increase of tonus and frequency, M3 eluted in the fractions 29 and 30 min decreased the tonus and frequency, and M4 eluted in the fractions 37 and 38 min decreased the frequency (Fig. 2). The second step of purification of M2 revealed two active fractions called M2α and M2β corresponding to two symmetrical peaks (Fig. 3 *a* and *b*). For M2α, Edman degradation revealed the primary sequence of Phe-Met-Arg-Phe and for M2β a signal too weak to be analyzed. MALDI-TOF analysis of the fraction M2 revealed the occurrence of oxidised Phe-Met-Arg-Phe-NH_2 ($[M+H]^+$ = 615.7) and Phe-Leu-Arg-Phe-NH_2 ($[M+H]^+$ = 581.9; Fig. 4). The

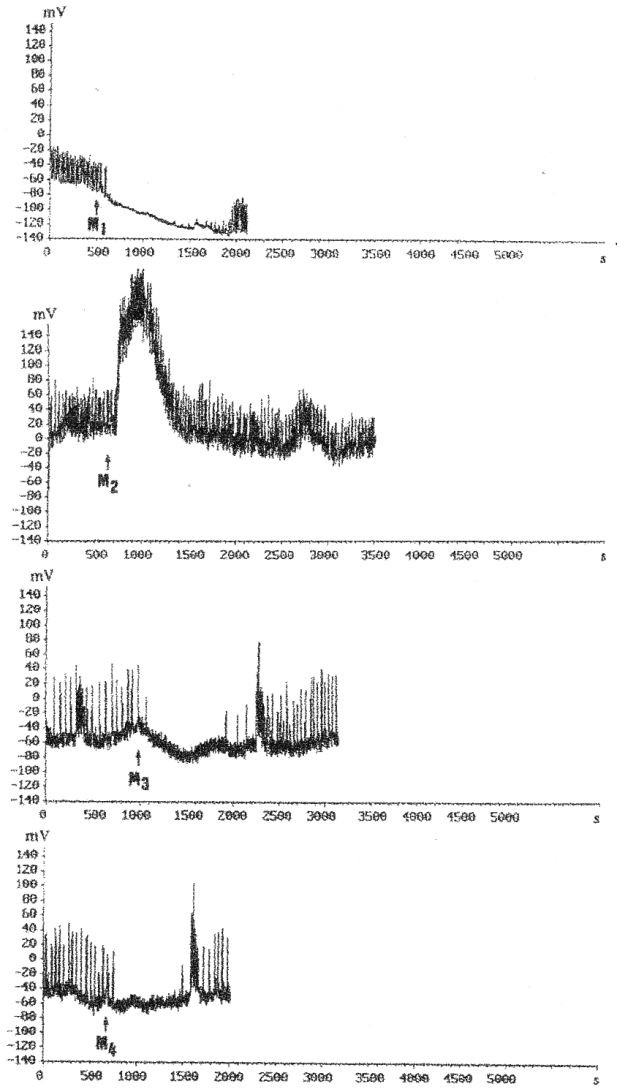

Fig. 2. Biologic activities of M1, M2, M3, and M4. The administration of M1 and M3 decrease the tonus, the amplitude, and the frequency. M2 increases the tonus and the frequency. M4 decreases the amplitude and the frequency.

comparison of retention times in the second step purification conditions confirmed that M2α was coeluted with synthetic FMRFa and M2β with synthetic FLRFa (Sigma).

3.2. Biologic activity on the oviducal contraction

To verify the occurrence of dose-related activity, increasing concentrations of synthetic FMRFa from 10^{-10} to 10^{-5} M were tested in the bioassay. A stimulation of contractions was observed at a threshold of $0.5.10^{-6}$ M (Fig. 5). Above the threshold concentration, the application of FMRFa on the oviduct induced an increase of the tonus, the frequency, and the amplitude of the contractions. The modification of the contraction was rapidly followed by return to basal activity levels.

3.3. Immunohistochemistry

By using polyclonal antibodies raised against C-terminal RFamide, we examined the distribution of FaRPs in the CNS and in the accessory sex glands. FMRFa-like immunoreactive material was observed in many fibers and neurones located in the neuropile of the optic lobes (Fig. 6A and 6B). Moreover, immunostaining fibers were found in the brain, in the optic tract connecting the optic lobes with the supraoesophagal mass (Fig. 6C), and in the muscular and conjunctive tissues surrounding the secretory epithelium of the accessory sex glands (Fig. 6D and 6E). Preincubation of the antisera in the presence of an excess of FMRFa or suppression of the incubation with primary or secondary antibody abolished immunolabeling.

3.4. Dotting immunobinding assay

3.4.1. Optic lobes

The immunologic screening of 1-min HPLC fractions revealed the occurrence of at least four immunoreactive factors: F1 eluted in the 24-, 25-, and 26-min fractions, F2 in the 29- and 30-min fractions, F3 in the 34-, 35-, 36-, and 37-min fractions, and F4 in the 39-, 40-, and 41-min fractions (Fig. 7).

3.4.2. Accessory sex glands

The immunologic screening of 1-min HPLC fractions revealed the same immunoreactive fractions first observed in the screening of the optic lobes (Fig. 8a–c).

3.4.3. Hemolymph

The immunologic screening of 1-min HPLC fractions did not reveal any immunoreactive fraction except for the controls with synthetic FMRFa.

3.5. MALDI-TOF analysis of F3 and F4

MALDI-TOF analysis revealed in F3 the occurrence of the tetrapeptide FIRFa ($[M+H]^+$ = 581.3) and the hexapeptide GNLFRFa ($[M+H]^+$ = 752.3) (Fig. 9a) and the presence in F4 of the decapeptide ALSGDAFLRFa ($[M+H]^+$ = 1095.3; Fig. 9b). ALSGDAFLRFa and FIRFa are the FaRPs predicted by the cDNA [21].

3.6. Biologic activity on the synthesis of polysaccharides and glycoproteins in the main nidamental glands

The incubation of dissociated nidamental cells with 3H leucine and 3H glucose did not show any activity of the FMRFa. The incorporation rate of 3H glucose in the polysaccharidic fraction was 655 ($\pm$92) dpm/10^6 cells and 153 ($\pm$35) dpm/10^6 cells for 3H leucine in the proteic fraction

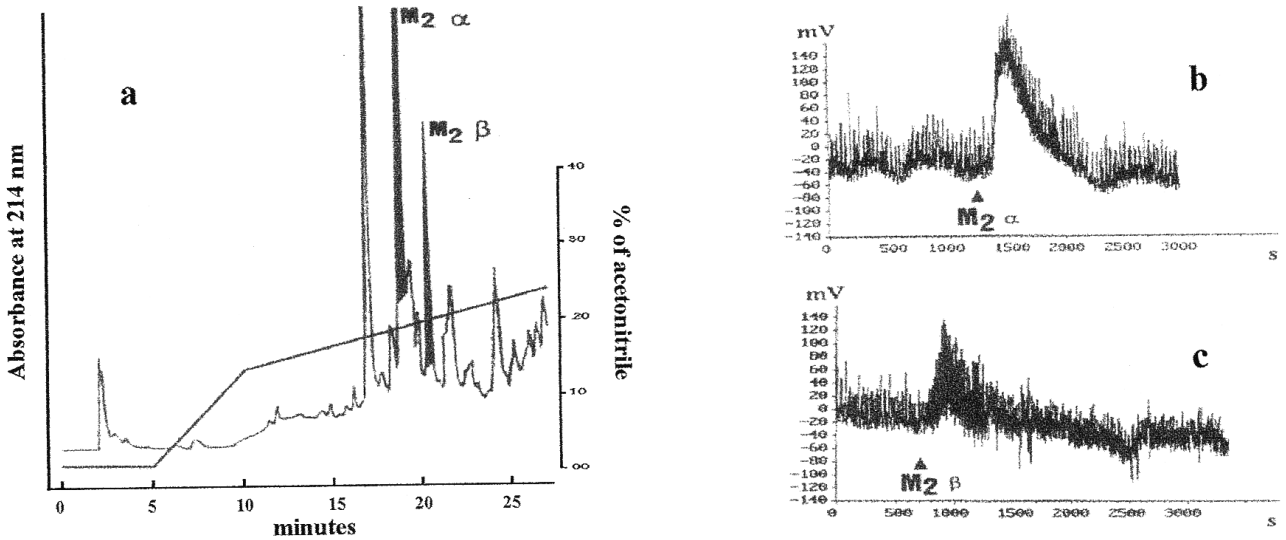

Fig. 3. (a) Second purification step of M2 on a Vydac C18 column. (b and c) Biologic activities of the myotropic fractions M2α and M2β.

(values counted in the controls). For concentrations of FMRFa ranging from 10^{-5} M to 10^{-10} M, the incorporation rate was not significantly different from the controls (Student's *t*-test, $n = 6$ replicates).

3.7. Myotropic activity of FMRFa on the contractions of the main nidamental glands

Increasing concentrations of FMRFa, 10^{-8}, 10^{-7}, and 10^{-6} M were injected into the perfusing flow. For the concentrations of 10^{-7} and 10^{-8} M, the contractions were inhibited showing a decrease of the tonus and the amplitude (Fig. 10b). Surprisingly, at 10^{-6} M of FMRFa, the contractions were stimulated strongly with an increase of the tonus and the amplitude (Fig. 10a) suggesting a dose-dependent alternative response.

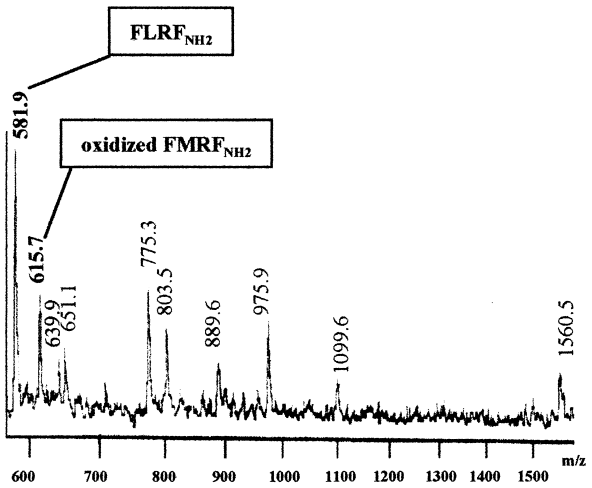

Fig. 4. MALDI-TOF spectrum of M2. The myotropic fraction contains the tetrapeptide FLRFamide ($[M+H]^+ = 581.9$) and the oxidized FMRFamide ($[M+H]^+ = 615.7$).

4. Discussion

By using an in vitro bioassay, we clearly have demonstrated the occurrence in the optic lobes of neuropeptides able to modulate the oviducal contractions. Successive steps of purification followed by Edmann degradation and MALDI-TOF analysis allowed identification of two of these factors as the tetrapeptides FMRFa and FLRFa. When applied to the oviduct, FMRFa stimulated the contractions above a threshold concentration of 0.5 10^{-6} M, increasing the tonus, the frequency, and the amplitude. Immunochemical study of the CNS revealed a strong immunostaining of many fibers and neurones in the optic lobes. Brain sections showed only a few immunostained fibers, suggesting that FaRPs were mainly expressed in the optic lobes. These observations confirm those of a previous immunohistological study performed in *S. officinalis* [20] that revealed many FMRFa immunolabeling

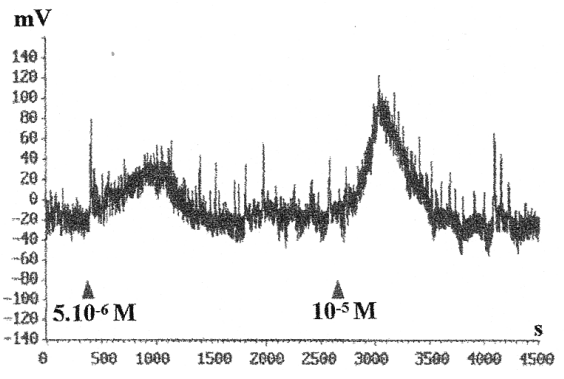

Fig. 5. Increase of tonus and frequency of oviduct contractions following administration of 5.10^{-6} and 10^{-5} M of synthetic FMRFamide. For a dose of 10^{-7} M, the contraction is not affected. The threshold of activity appeared to be between 5.10^{-6} and 10^{-7} M.

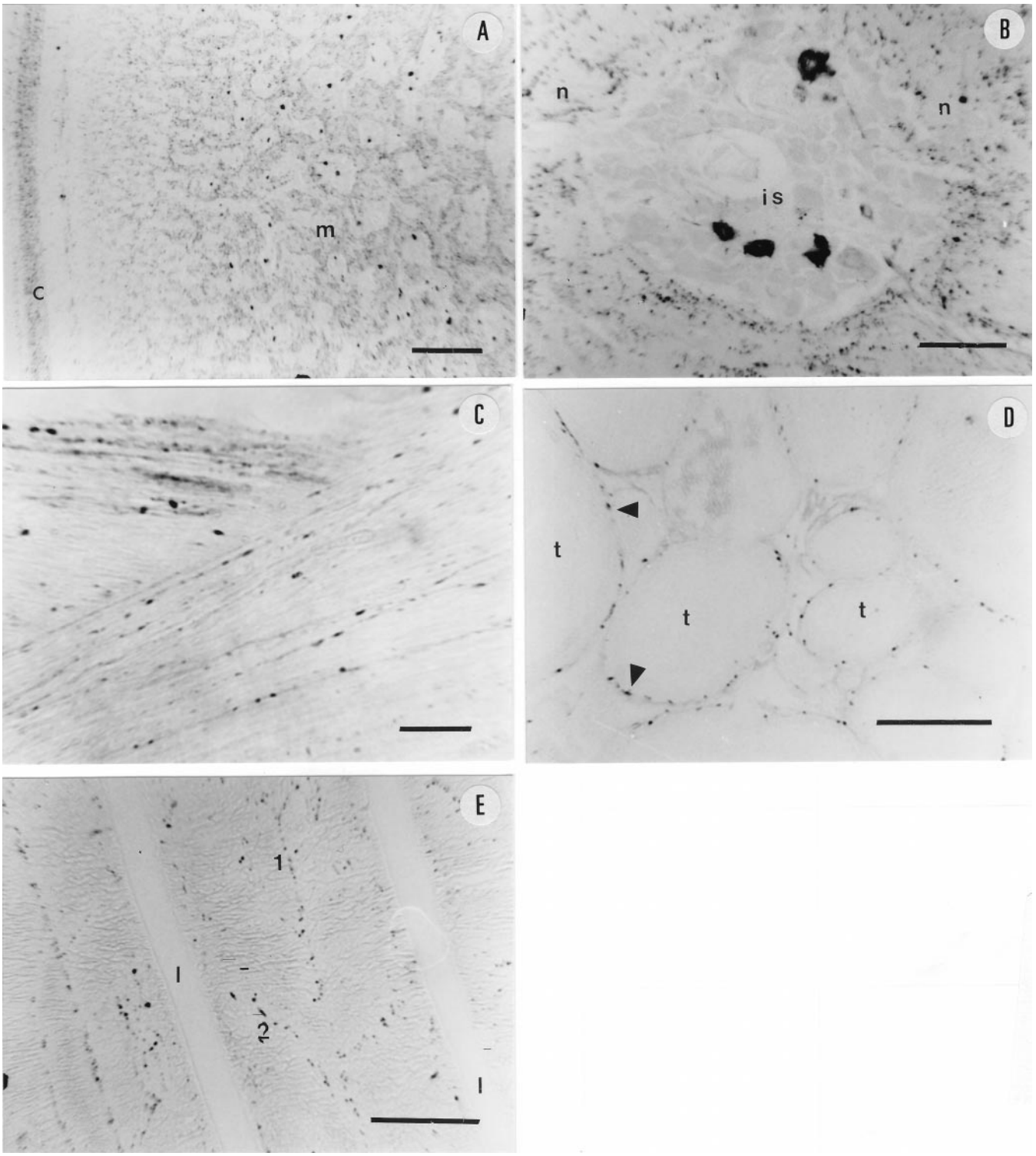

Fig. 6. (A) Longitudinal section of the optic lobe (scale bar 0.5 mm). FMRFamide immunostaining fibers are found in the cortex in the outer plexiform layer (c) and in the medulla (m) where immunostained cell bodies are also found. (B) Detail of the medulla of the optic lobe showing island of cell bodies (is), some of them strongly immunostained surrounded by neuropil (n) containing immunostained neural processes (scale bar 100 μm). (C) Immunostained nerve fibers in the optic tract (scale bar 100 μm). (D) Sections of the accessory nidamental glands showing immunostained nervous fibers (arrow) in the connective tissue surrounding the glandular tubule (t) (scale bar 200 μm). (E) Section of the leaflets of the oviducal gland. Immunostained nerve fibers are located within the thin network of connective tissue in the main axis of the leaflet (1) as in the secondary ramifications (2). l, lumen of the gland. Scale bar 100 μm.

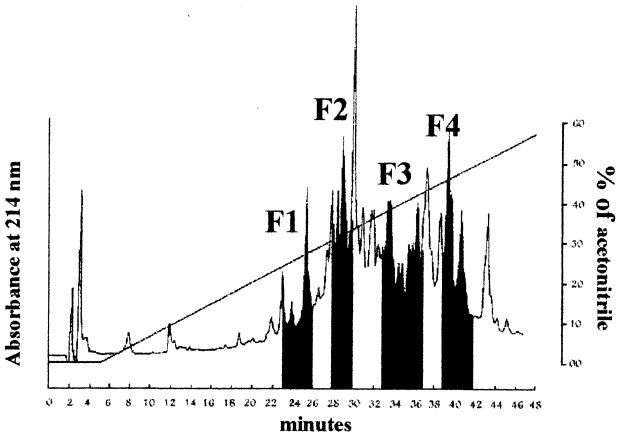

Fig. 7. Dotting immunobinding assay of 1-min optic lobes HPLC fractions separated onto MNRP18 column. F1 contained oxidized FMRFamide, F2 FLRFamide, and FMRFamide and F3/F4 unknown FMRFamide-related peptides.

fibers originating from the optic lobe and innervating the optic gland, a paraneural structure similar to the dorsal bodies of gastropods. Moreover, the myotropic activity of FMRFa on the systemic heart has been observed in *S. officinalis* and *Octopus vulgaris* [16,23,31]. Dotting immunobinding assay of optic lobes HPLC fractions by using polyclonal antibodies raised against C-terminal RFa allowed detection of at least two FaRPs called F3 and F4 and corresponding with the myotropic fractions M3 and M4 identified in the first purification step. These fractions had retention times different from those of F1 containing oxidized FMRFa (M2α) and F2 containing FMRFa and FLRFa (M2β). MALDI-TOF spectrum of F3 and F4 revealed the occurrence of the FaRPs predicted by the precursor [21], FIRFa and ALSGDAFLRFa. These results demonstrate that the FaRPs predicted by the cDNA are effectively expressed in the optic lobes. With regard to biologic activity, these neuropeptides induced opposite responses on the oviduct: the tetrapeptides FMRFa and FLRFa stimulated the contractions, whereas FIRFa and ALSGDAFLRFa inhibited the contractions with a decrease of the tonus, the frequency, and the amplitude. In *Lymnaea stagnalis*, similar observations have been reported about the control of the penis retractor muscle by FMRFa, FLRFa, and GDPFLRFa [27]. Thus, the N-terminal elongated FaRPs often exhibited different activities from those of FMRFa and FLRFa. On *Locusta migratoria* midgut, the elongated FaRPs inhibited the contractions [18] whereas other FaRPs did not have any activity [19]. The alternative modulation of target responses could be associated with the presence of two different receptors [25,29] and/or by the composition of the FaRPs cocktail released at the level of the target [30] and influenced by alternative splicing of mRNA [1] and/or by differential packaging as suggested by the structure of the precursor cloned in *Mytilus edulis* [6].

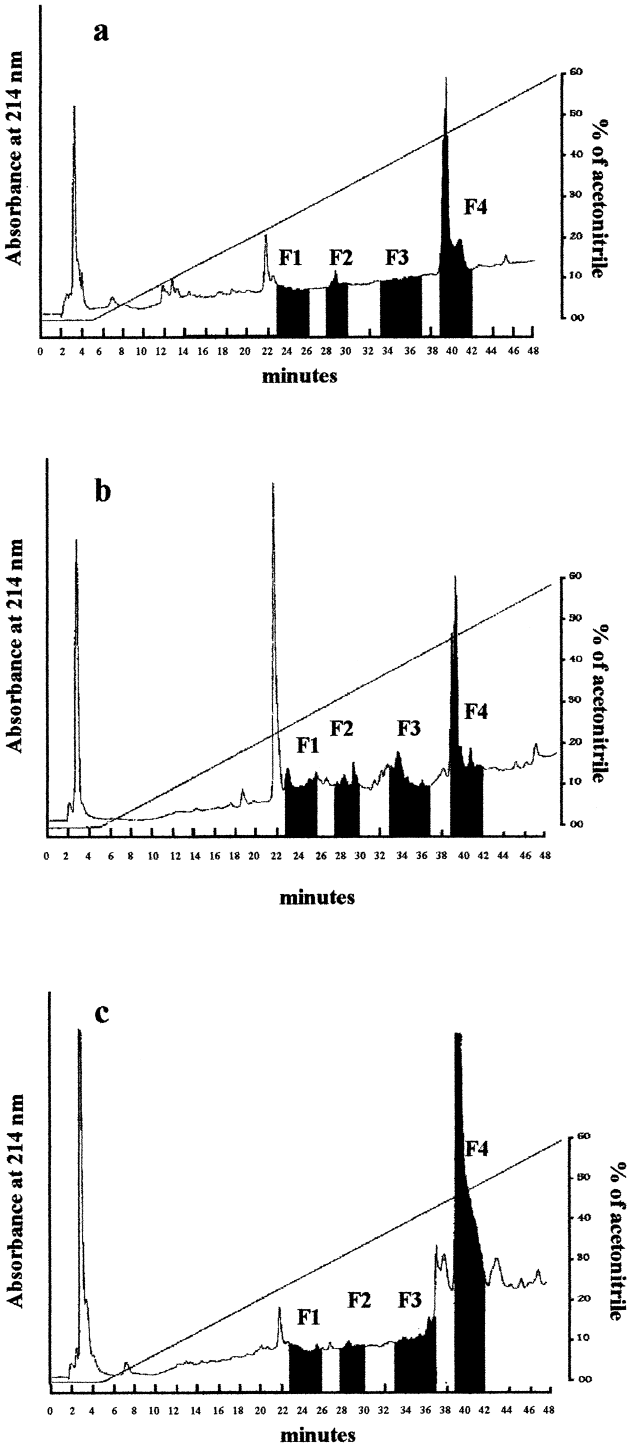

Fig. 8. Dotting immunobinding assay of 1-min accessory sex glands HPLC fractions separated onto MNRP18 column. (a) Accessory nidamental glands; (b) main nidamental glands; (c) oviducal gland. For each sexual gland, the screening reveals the occurrence of the same immunoreactive fractions observed in optic lobes.

Dotting immunobinding assay of accessory sex glands HPLC fractions revealed the presence of the same immunoreactive fractions identified in the optic lobes, suggesting that these glands, oviducal gland (OG), ANG, and

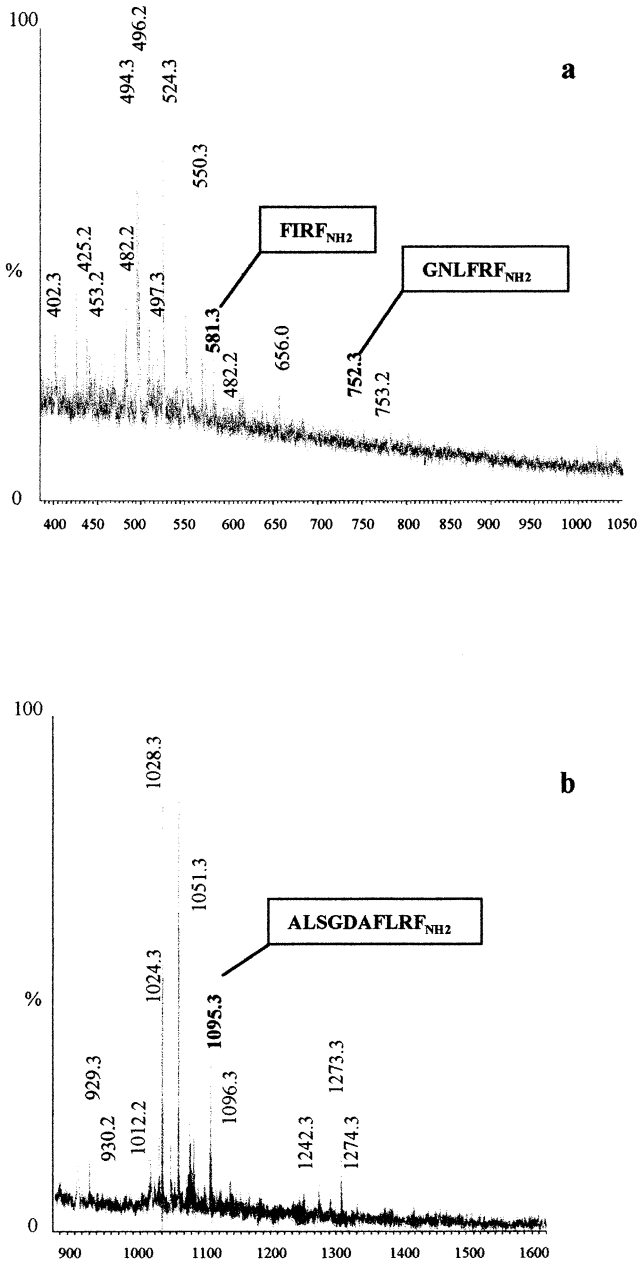

Fig. 9. (a) MALDI-TOF spectrum of the fraction F3 showing the occurrence of the peptides FIRFamide ($[M+H]^+ = 581.3$) and GNLFRFamide ($[M+H]^+ = 752.3$). (b) MALDI-TOF spectrum of the fraction F4 showing the occurrence of the decapeptide ALSGDAFLRFamide ($[M+H]^+ = 1095.3$).

MNG are targeted by the FaRPs expressed in the optic lobes. Putative biologic activity of FMRFa on these glands was investigated by using in vitro bioassays such as incorporation of 3H leucine and 3H glucose into proteins and polysaccharids. These are the main macromolecules forming the egg capsule that are secreted by these glands [2,3,11]. Increasing concentrations of FMRFa had no effect on the rate of incorporation of leucine or glucose. Nevertheless, the FMRFa was able to modulate the contractions of the main nidamental glands at concentrations ranging from 10^{-8} to 10^{-6} M. At 10^{-6} M, FMRFa stimulates the contractions with an increase of the tonus, the frequency, and the amplitude, whereas concentrations of 10^{-7} and 10^{-8} M inhibited the contractions. The FMRFa and probably the FaRPs are able to modulate the contractions of the main nidamental glands and, therefore, the coating of the oocytes. Moreover, the FaRPs could be closely involved in the release of the secretion into the lumen of the accessory sex glands. Such a secretagogue activity on α-amylase was found for FMRFa and for leucomyosuppressin, a N-terminal elongated FaRP, on *Pecten maximus* digestive gland [5] and on *Rhynchophorus ferrugineus* midgut [24]. Correlatively, many immunostained fibers in the thin connective and muscular wall of the glandular tubules revealed a large FaRPs innervation of these structures. These immunolabelled fibers form a plexus originating from the visceral nerve and innervating the accessory sex glands and the oviduct [32]. Moreover, dotting immunobinding assay of HPLC hemolymph fractions did not allow the detection of any immunoreactive fractions, suggesting that the FaRPs act as neurotransmitters on their targets.

Thus, in *S. officinalis*, immunohistological mapping as well as biologic activities demonstrated the involvement of the FaRPs in the regulation of physiological mechanisms related to egg laying. The co-regulation of the activity of the oviduct and MNG suggests a possible synchronization of ovulation and egg capsule secretion. In this cephalopod, egg laying is composed of successive well-stereotyped steps: 1) oocytes accumulate in the proximal oviduct until mating; 2) the deposit of spermatophores in the sub buccal pouch of the female triggers a cyclic contractile activity of the oviduct which allows the transport of the oocytes and their release in the mantle cavity where they are coated by accessory sex glands secretions; 3) the coated oocytes are fertilized with the spermatophores stored by the female; and 4) they are finally attached to a substrate located on the bottom. The frequency of egg laying is clearly related to the cyclic contractile activity observed in vitro: one egg each 3 or 4 min. The cyclic activity of the oviduct is induced by modifications of the neuropeptide cocktail in nervous endings and blood. Optic lobes appear to be an important source of peptidic neuromodulators (GW-NH_2 family [12] and FaRPs) involved in the control of egg laying.

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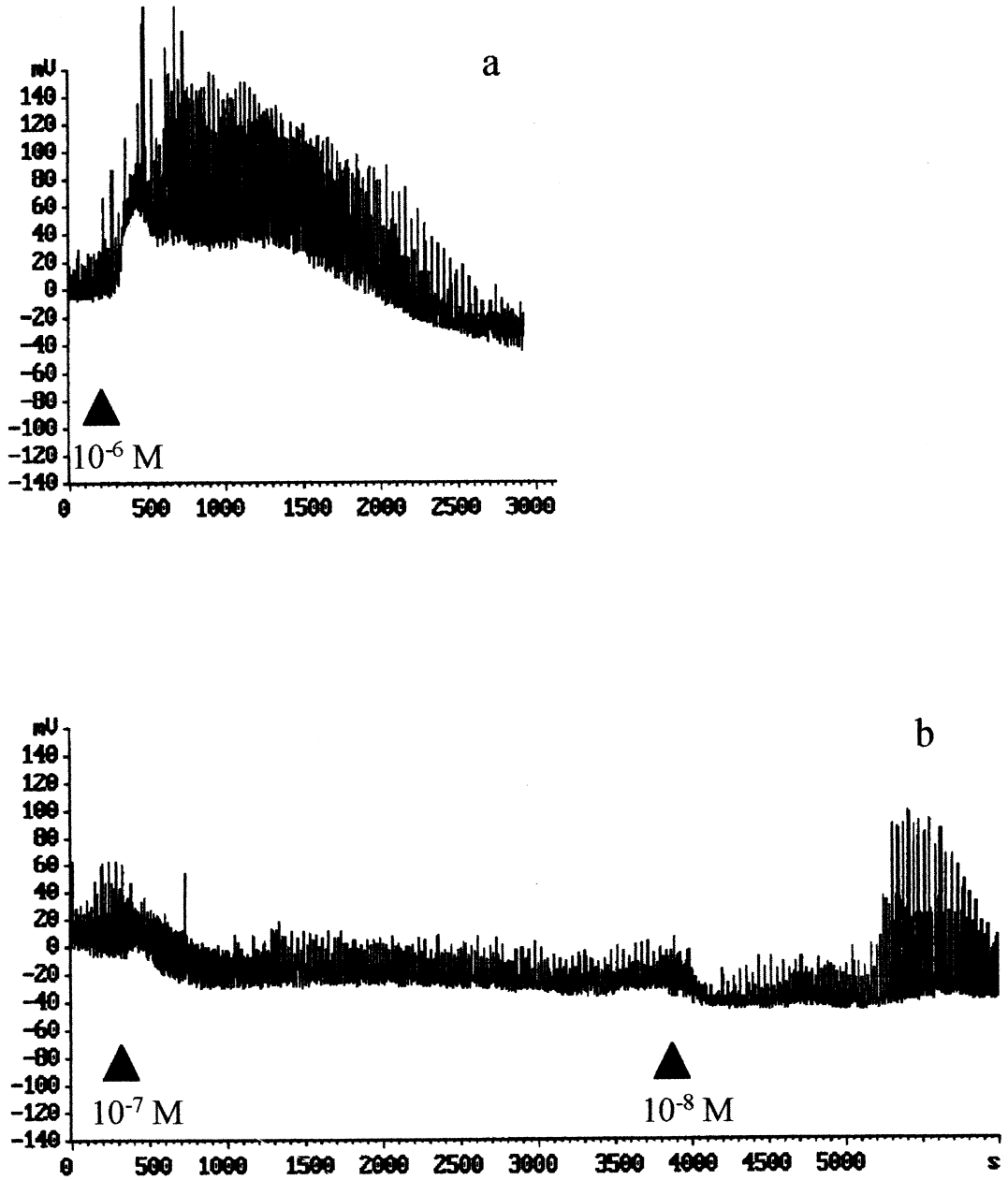

Fig. 10. Alternative modulation of the main nidamental glands contractions by increasing concentrations of FMRFa. A stimulation is observed for 10^{-6} M, whereas for 10^{-7} and 10^{-8} M, the tonus and the amplitude of the contractions are lowering.

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