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Peripheral Injection of DNS-RFa, A FMRFa Agonist, Suppresses Morphine-Induced Analgesia in Rats

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BRUSSAARD, A. B., K. S. KITS, A. TER MAAT, A. H. MULDER AND A. N. M. SCHOFFELMEER. *Peripheral injection of DNS-RFa, a FMRFa agonist, suppresses morphine-induced analgesia in rats.* PEPTIDES **10**(4) 735–739, 1989.—The present results demonstrate an antagonistic effect of DNS-RFa on morphine-induced analgesia in rats. This confirms previous evidence presented by others on the effects of FMRFa-related peptides when applied centrally. Unlike these peptides, however, it is shown here that DNS-RFa is effective upon peripheral injection. The effects of DNS-RFa on morphine-induced analgesia were dose-dependent (ED_{50} = 0.5 mg/kg). DNS-RFa alone (5 mg/kg) did not affect the control level of nociception. Peripheral injection of FMRFa (5 mg/kg) did not affect morphine-induced analgesia. DNS-RFa defines the minimal configuration to activate neuronal FMRFa receptors in the pond snail (4). The present report suggests also that in vertebrates the Arg-Phe-NH_2 sequence is essential and that DNS-RFa readily penetrates the blood-brain barrier.

Analgesia DNS-RFa FMRFa Neuropeptide Opioid antagonism Pharmacology

FMRFa-like immunoreactivity has a broad phylogenetic distribution (2). Extensive FMRFa-like immunoreactivity has been reported in the central nervous system of rats, with highest concentrations present in the spinal cord and hypothalamus (5,15). Three mammalian FMRFa-like peptides have been chemically characterized: Leu-Pro-Leu-Arg-Phe-NH_2 from chicken brain (6) and Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2 (F-8-F-NH_2) and Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Trp-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe-NH_2 (A-18-F-NH_2) in dorsal spinal cord, periaqueductal grey and medulla pons (14,27). Although structurally more complex than FMRFa [(18), Fig. 1], the above mentioned peptides share the characteristic C-terminal dipeptide amide (RFa) and they display biological activity similar to that of the original tetrapeptide (23,27). Specific FMRF receptors have been demonstrated in various invertebrate species, especially in molluscs (4, 10, 16, 17, 19, 20). Recently we have reported on the structure-activity relationship of the FMRFa receptor type on the neurosecretory caudo dorsal cells (CDCs) of the pond snail *Lymnaea stagnalis* (4). FMRFa is one of the native peptides in *Lymnaea* (7) and FMRFa-like immunoreactivity is closely associated with the CDCs (3). FMRFa inhibits the CDCs via a dual membrane action (3), mediated by a single receptor type (4). Our analysis included a new synthetic FMRFa analog: Dansyl-protected Arg-Phe-NH_2 (DNS-RFa) [(4), Fig. 1]. This substance, having a large hydrophobic domain (naphthalene-group), mimicked all FMRFa effects in CDCs and showed a clear cross desensitization with the native peptide. It was concluded that DNS-RFa defines the minimal configuration required for activation of the neuronal FMRFa receptor. This parallels the results of a recent binding study of FMRFa receptors in the brain of *Helix aspersa* (17).

Evidence has been presented that FMRFa or a family of immunoreactive FMRFa-like peptides may function as endogenous peptides with antiopiate effects (physiological antagonists of the opioid peptides). Firstly, intracerebroventricular injections of FMRFa blocked morphine-induced analgesia in mice (12) while intrathecal injections of antibodies directed against mammalian FMRFa-like peptides produced long lasting analgesia in rats, which was reversible by naloxone (23,25). Secondly intracerebroventricular injection of FMRFa reduced immobilization-induced analgesia, as well as the opioid-mediated analgesic and ingestive responses arising from intraspecific aggressive interactions and defeat in mice (11,12). However, it is unlikely that the antagonistic effects of FMRFa and related peptides are due to a direct blockade of opioid receptors. Cross-desensitization between FMRFa and naloxone is not likely [cf. (9)]. Firstly, binding studies have revealed that FMRFa has very low affinity for opioid receptors (28) and secondly, Kavaliers and co-workers (13) recently reported that calcium channel antagonists significantly reduced the inhibitory effects of FMRFa, but had no effects on naloxone-mediated inhibition of either morphine- or immobilization-induced analgesia. These findings suggested that, in vertebrates too, the effects of FMRFa-related peptides are mediated

ABBREVIATIONS

BBB	Blood Brain Barrier
DNS-RFa	Dansyl-Arg-Phe-NH_2
FMRFa	Phe-Met-Arg-Phe-NH_2

through specific FMRFa recognition sites. To further classify these recognition sites, in the present study we investigated whether the minimal FMRFa agonist DNS-RFa has FMRFa-like antagonistic effects on morphine-induced analgesia in rats.

The properties of the DNS-group enabled us to address a second question with respect to the passage of the blood-brain barrier (BBB). The degree to which peptides penetrate the BBB determines whether a peptide can affect central nervous system functions after peripheral administration and therefore bears directly on the therapeutic use of peptides. Only a few peptides have been shown to cross the BBB (1). An important factor in determining the blood to brain passage of peptides is their lipophilicity (1). DNS-RFa, unlike FMRFa itself, is a highly lipophilic substance. Thus, by administering DNS-RFa peripherally, the hypothesis that increasing the lipophilicity of a peptide enhances the passage through the BBB could be tested, using the antagonism of morphine-induced analgesia as an assay.

METHOD

Animals

Male Wistar rats (210–230 g, F. Winkelmann, Versuch-Tier Zucht, GmbH & Co. K. G., Borchen, West Germany) were used. Food and water were available ad lib. After a 7 day period of handling (2 min each day) animals were used once for an experiment.

Drugs

Before each experiment drugs were freshly dissolved to appropriate concentrations and kept on ice until 20 min before injection. Morphine sulphate was purchased from Sigma (St. Louis, MO); FMRFa (Phe-Met-Arg-Phe-NH_2, Fig. 1) was from Bachem (Bubendorf, Switzerland). These products readily dissolve in normal saline. Dansyl-RFa (DNS-Arg-Phe-NH_2, Fig. 1; DNS = 1-dimethylaminonaphthalene-5-sulfonyl) was firstly synthesized and purified in our laboratory as described previously (4). For this study it was purchased in larger amounts from UCB Bioproducts (Brain-l'Alleud, Belgium). The final peptide content of this batch was 80.98% (as measured by amino acid analysis and optical density measurements). DNS-RFa was dissolved and diluted in normal saline supplemented with 10% DMSO (dimethyl sulfoxide, Sigma, St. Louis, MO; final concentration after injection <0.05%). DNS-RFa is susceptible to light, hence it was kept in a dark place until the last minute before injection.

Experimental Procedure

In each experiment, groups of twelve rats received two intraperitoneal injections of 1 ml in a time-scaled procedure. Animals for peptide treatment were injected with DNS-RFa or FMRFa at t = 0 min in the left side of the abdomen given in a single injection unless stated otherwise, whereas control and 'morphine alone' animals received sham injection with saline at t = 0. The second injection at t = 10 min in the right side was saline in control and 'DNS-RF alone' animals and morphine in all other groups. Thirty min after the first injection the nociceptive threshold of the animals was assessed using a hot-plate technique. Individual rats were placed on the heated surface (Technilab Instruments Incorporated, Pequannods, NJ) and the latency to a paw-licking response was recorded, after which the rat was quickly removed from the surface and returned to the home cage. The temperature of the hot plate in these experiments was 52.5 ± 0.5°C. Noninjected rats displayed a nociceptive latency of 32.8 ± 9.1 sec at this temperature (compared to 70.2 ± 14.0 sec at 50.0 ± 0.5°C and 14.1 ± 5.0 sec at 55.0 ± 0.5°C). Few rats (<2%) did not show the stereotyped paw-licking response within 200 sec upon placement on the hot plate, hence they were not included in the calculations. Paw-licking behavior was not observed in control experiments with the plate set at a temperature of 20.0 ± 0.5°C. The effect of peptide injections was determined by comparing latencies of peptide-pretreated animals with those of both control

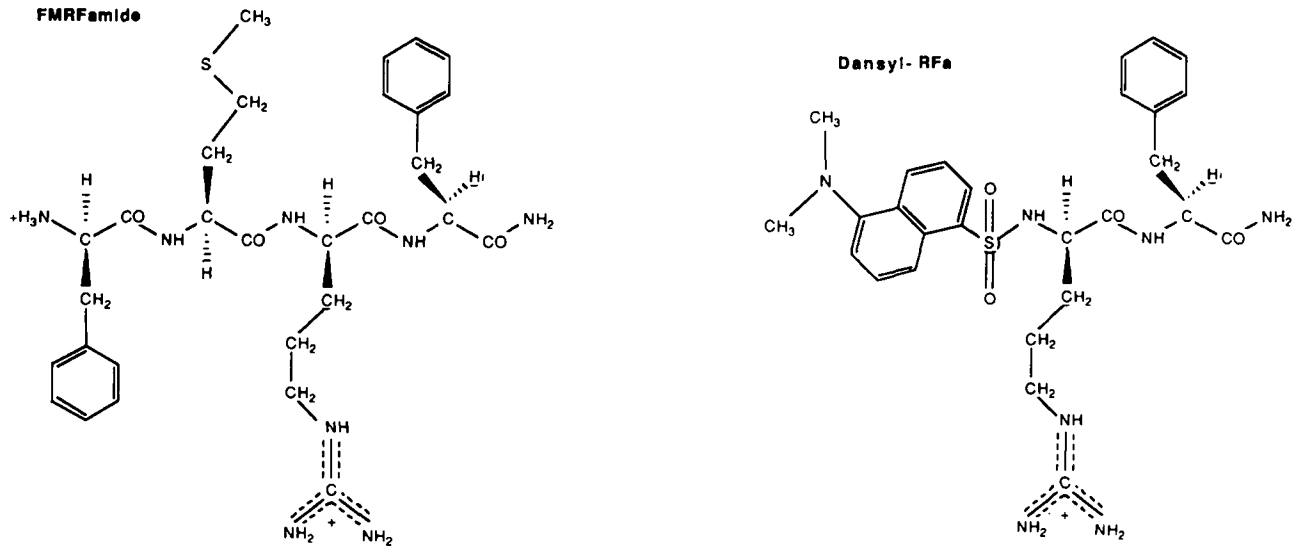

FIG. 1. Two dimensional representation of the structures of FMRFa (Phe-Met-Arg-Phe-NH_2) and DNS-RFa (Dansyl-Arg-Phe-NH_2).

animals and animals that received morphine alone. Data were expressed on a linear scale as the mean latency ± standard deviation (S.D.) in sec.

Statistical Analysis

Prior to statistical analysis a data transformation was necessary because of a strong relationship between means and variances. Data were transformed as log(x+1), which rendered variances homogeneous. The data thus obtained were analyzed using a one-way analysis of variance (single classification ANOVA for unequal sample sizes). As this showed for the data in Fig. 2 that $F(5,111) = 13.102$, $p<0.001$, and for the data in Fig. 3 that $F(3,42) = 32.658$, $p<0.001$, indicating that there were significant differences among the means of the respective groups, this allowed us to do an a posteriori test for multiple comparisons among pairs of means based on unequal sample sizes. Since we had rather unequal sample sizes both the GT2 method and the Tukey-Kramer method have been used (24). As the latter method gave the lowest critical or MSD values (MSD = minimum significant difference) in individual experiments, in the Results section we refer to significant differences according to the Tukey-Kramer method.

RESULTS

The time to paw-licking behavior was scored to determine the nociceptive latency upon placement of the rat on a hot plate. Paw-licking latencies of noninjected animals (32.8 ± 9 sec, n = 10) were not significantly different from the latencies of animals that were sham-pretreated with either one or two injections of saline (27.5 ± 12 sec, n = 10 and 30.5 ± 11 sec, n = 12, respectively). Administration of morphine sulphate in different concentrations showed that a dose of 7.5 mg/kg doubled the latency of the paw-licking response (control: 30.2 ± 11.2 sec, n = 36 versus morphine: 55.3 ± 15.3 sec, n = 36; significantly different, $\alpha<0.01$). Hence this dose of morphine was used to induce analgesia in further experiments.

In an initial experiment it was found that a pretreatment of two successive injections (to circumvent possible enzymatic degradation) of 5 mg/kg DNS-RFa (given 10 min and immediately before the morphine injection, respectively) totally suppressed the morphine-induced analgesia (morphine alone: 55.6 ± 10.0 sec, n = 12 versus DNS-RFa prior to morphine: 29.3 ± 12.4 sec, n = 15; significantly different, $\alpha<0.01$ and not different from the control level of: 33.0 ± 14.8 sec, n = 15). Since two successive injections of DNS-RFa produced such a large effect, in all further experiments discussed below, DNS-RFa has been given in a single injection at lower concentrations. The inhibitory effect of DNS-RFa on morphine-induced analgesia occurred in a dose-dependent manner (Fig. 2). DNS-RFa given at a concentration of 5 mg/kg totally suppressed the morphine-induced analgesia. A dose of 1 mg/kg DNS-RFa was somewhat less effective; the latency of this group still was significantly different from the morphine-induced latency at $\alpha<0.01$. The nociceptive threshold in the group pretreated with 0.5 mg/kg DNS-RFa was about halfway between the control latency and the level of morphine-induced analgesia (significantly different from morphine, $\alpha<0.05$). Animals which received the lowest dose (0.1 mg/kg) of DNS-RFa as a pretreatment and animals which were not pretreated (morphine only) displayed latencies which were not significantly different. Figure 2 also shows the data of animals which received DNS-RFa alone. A dose of 5 mg/kg DNS-RFa alone, which was maximally effective in suppressing morphine-induced analgesia, did not significantly affect the control level of nociception.

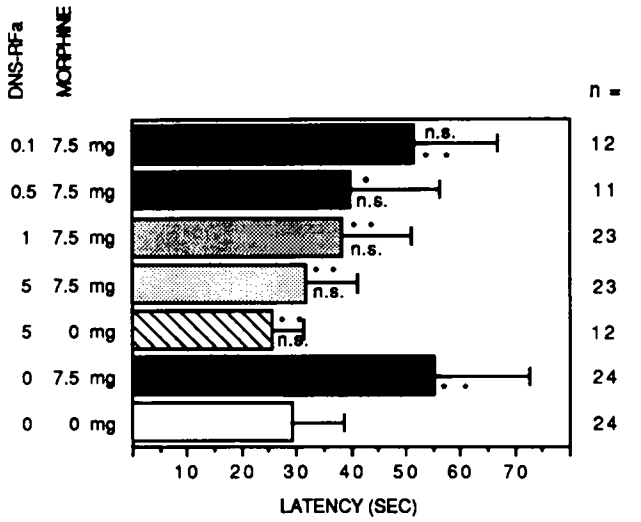

FIG. 2. The effects of intraperitoneal injections of DNS-RFa on morphine-induced analgesia. The latency of paw licking behavior on the hot plate is given in seconds on the X-axis. For this experiment DNS-RFa and morphine were given in separate injections, as indicated at the left side of the figure (mg/kg); at the right side the number of animals per group are indicated. ●● = $\alpha<0.01$, ● = $\alpha<0.05$ and "n.s." (above the standard deviations) = not significant as compared to the morphine-induced level; ** = $\alpha<0.01$, * = α0.05 and "n.s." (below the standard deviations) = not significant as compared to the control level.

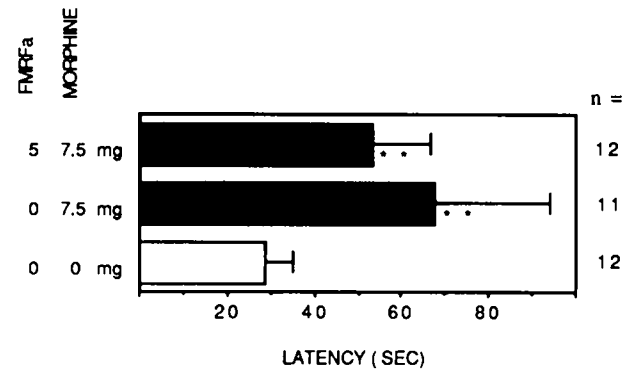

FIG. 3. The effect of IP injection of 5 mg/kg FMRFa on morphine (7.5 mg/kg)-induced analgesia (the numbers of animals per group are indicated at the right side of the figure and ** = $\alpha<0.01$ as compared to the control level of paw licking). Although the mean latency of FMRFa-pretreated animals seems somewhat reduced as compared to morphine alone, this difference was not significant.

Since in neurosecretory cells in the pond snail (3) FMRFa is about 100 times more effective than DNS-RFa, we also checked whether a relatively high dose of FMRFa (5 mg/kg), injected intraperitoneally in rats, could affect the state of analgesia induced by morphine. In the experiment shown in Fig. 3 this proved not to be the case, since the animals pretreated with the tetrapeptide (before injection with morphine) displayed latencies which were significantly longer than those of control animals ($\alpha<0.01$) and did not differ significantly from those of animals which received morphine alone. The latter results suggest that, unlike DNS-RFa, FMRFa does not readily penetrate the blood-brain barrier.

DISCUSSION

The present results demonstrate an antagonistic effect of the

minimal FMRFa agonist DNS-RFa on morphine-induced analgesia. This finding confirms previous evidence on effects of FMRFa-related peptides when injected either intrathecally or intracerebroventricularly (11–13, 25, 27). In contrast to the peptides used in the latter studies, however, DNS-RFa is effective when administered peripherally. The absence of significant effects of DNS-RFa on the control level of nociception is consistent with the findings of Kavaliers (11–13) who found that the central application of FMRFa only affects opioid-induced analgesia and not the control level of nociception. Moreover this minimizes the possibility that DNS-RFa has nonspecific effects. It is inferred that DNS-RFa readily penetrates the BBB in rats and that the inhibitory effect on morphine-induced analgesia of this substance results from specific activation of neuronal FMRFa-like receptors.

In mammals central application of FMRFa produces long lasting effects on morphine-induced analgesia at relatively low concentrations [ED_{50} values of about 0.15 μg intrathecally or intracerebroventricularly (13,25)]. In contrast our results show that peripheral injection of FMRFa of 5 mg/kg does not antagonize morphine-induced analgesia, indicating that FMRFa, unlike DNS-RFa, does not readily pass the BBB. This inference is in line with the results of Banks and Kastin (1) who found only low BBB penetration of a slightly lipophylic analog of FMRFa (i.e., iodinated, N-tyrosinated FMRFa), as estimated 5 seconds after injection into the carotid artery. The ED_{50} value of DNS-RFa-induced suppression of morphine analgesia in our study was found at 0.5 mg/kg (about 10^{-6} M blood concentration), whereas a peripheral injection of FMRFa at 5 mg/kg did not affect the analgesia (10^{-5} M in the blood). If ED_{50} values of central effects of FMRFa and DNS-RFa in rats are comparable to those of the central effects in the pond snail [$7 \cdot 10^{-9}$ M and $4 \cdot 10^{-7}$ M, respectively (4)], this suggests that DNS-RFa to a large extent penetrates the BBB. This implies that, regardless of the strong basic residue in Arg^3, DNS-RFa, having a N-terminal large hydrophobic domain (Fig. 1), displays the favorable lipophilicity to penetrate the BBB.

On neurosecretory cells in the brain of *Lymnaea*, the Arg^3-Phe^4-NH_2 sequence is essential for activation of FMRFa receptors, whereas N-terminal amino acids are involved in binding, and can be substituted for by the DNS group (4). The effects of DNS-RFa in rats described in this paper are in line with this idea. The structural requirements to activate typical FMRFa-like receptors both on the CDCs in *Lymnaea* (4) and in muscle preparations of other marine molluscs (16, 19, 20) are strikingly similar. They are also to a large extent consistent with the results of a recent binding study using *Helix* brains (17). It is therefore conceivable that the homology between the various members of the family of FMRFa-like peptides is paralleled by a homology between the various appropriate receptors to FMRFa peptides in different animals (10). The data presented in this paper indicate that as in *Lymnaea* (4) and in *Mytilus* (Muneoka, personal communication), DNS-RFa also activates a vertebrate FMRFa receptor.

It has been argued previously (8) that opiate peptide systems are likely to have their endogenous counterparts. Certain stressful environmental situations might raise the pain threshold by an opioid-mediated mechanism. The function of this may be to allow the animal to suppress pain-related behaviors, thus facilitating escape and avoidance. However this analgesia usually persists only a few minutes. This is conceivable, considering the idea that suppression of pain should not outlast its stimulus too long since it is believed that the function of pain is to alert the animal to danger [see (8)]. Candidates for an antiopiate system in the brain are two endogenous peptides with antiopiate effects, called MIF-1 and Tyr-MIF-1. [The C-terminal tripeptide of oxytocin has been designated MIF-1, and it has been shown that hypothalamic tissue contains an exopeptidase that stepwise degrades oxytocin to MIF-1. The structurally-related tetrapeptide Tyr-MIF-1 also occurs in the brain, though it is not part of oxytocin (8).] The molecular mechanism underlying their antiopiate effects is unclear at the moment, though Tyr-MIF-1 has been found to inhibit the binding of the milk opiate casomorphin to its binding sites and in this manner could act to modulate the strength or length of the pain experience. However, as Galina and Kastin (8) point out, it would be naive to think that a single endogenous peptide could be found with the properties of a receptor antagonist like naloxone, since a system capable of modulating the diverse behavioral effects of the endogenous opiates should itself contain many elements.

Several lines of evidence support the idea that the next to MIF-1/Tyr-MIF-1 involvement, FMRFa-like peptides are also involved in an endogenous antiopiate system [for review see (21)]. The data in this paper support this hypothesis. As in molluscs, their effects are mediated through specific FMRFa-like receptors which are directly or indirectly coupled to ionic channels through second messengers, rather than through the blockade of opioid (μ and κ) receptors (9, 13, and 28). As we have shown previously that DNS-RFa defines the minimal functional configuration for the activation of FMRFa-like receptors (4), it may be used as a tool for the design of specific FMRFa analogs, agonists and/or competitive antagonists.

If endogenous FMRFa-like peptides in mammals have one or more specific receptors, which are different from opioid receptors, one might argue that application of these peptides, apart from having antagonistic effects on opioid-mediated analgesia, should have significant effects of its own as well. In mice an increase in grooming-related activities has been shown to occur upon intrathecal injection of FMRFa (22). This behavior was not blocked by naloxone. Moreover, in an intriguing report by Telegdy and Bollók (26), effects have been described of intracerebroventricularly applied FMRFa on passive avoidance responding and electroshock-induced amnesia. In their study low doses of FMRFa had a negative effect on both the consolidation of learning and on the retrieval of memory, whereas similar doses in combination with an electroshock induced total amnesia. Because oxytocin has similar amnesia-producing effects and a block of opioid-mediated transmission by receptor antagonists both induces some amnesic effects and enhances oxytocin secretion, it has been argued that oxytocin might mediate some of the actions of FMRFa (21,26).

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REFERENCES

1. Banks, W. A.; Kastin, A. J. Peptides and the blood-brain barrier: Lipophilicity as a predictor of permeability. Brain Res. Bull 15: 287–292; 1985.
2. Boer, H. H.; Schot, L. P. C.; Veenstra, J. A.; Reichelt, D. Immunocytochemical identification of neuronal elements in the central nervous system of a snail, some insects, a fish and a mammal, with a antiserum to the molluscan cardioexcitatory tetrapeptide FMRFamide. Cell Tissue Res. 213:21–27; 1980.

3. Brussaard, A. B.; Kits, K. S.; Ter Maat, A.; Van Minnen, J.; Moed, P. J. Dual inhibitory action of FMRFamide on neurosecretory cells controlling egg laying behavior in the pond snail. Brain Res. 447: 35–51; 1988.
4. Brussaard, A. B.; Kits, K. S.; Ter Maat, A. One receptor type mediates two independent effects of FMRFamide on neurosecretory cells in *Lymnaea*. Peptides 10(2):289–297; 1989.
5. Chronwall, B. M.; Olschowaka, J. A.; O'Donohue, T. L. Histochemical localization of FMRFamide-like immunoreactivity in the rat brain. Peptides 5:569–584; 1984.
6. Dockray, G. J.; Reeve, J. R., Jr.; Shively, J.; Gayton, R. J.; Barnard, C. S. A novel active pentapeptide from chicken brain identified by antibodies to FMRFamide. Nature 305:328–330; 1983.
7. Ebberink, R. H. M.; Price, D. A.; Van Loenhout, H.; Doble, K. E.; Riehm, J. P.; Gerearts, W. P. M.; Greenberg, M. J. The brain of *Lymnaea* contains a family of FMRFamide-like peptides. Neuropeptides 8:515–522; 1987.
8. Galina, Z. H.; Kastin, A. J. Existence of antiopiate systems as illustrated by MIF-1/Tyr-MIF-1. Life Sci. 39:2153–2159; 1986.
9. Gayton, R. J. Mammalian neuronal actions of FMRFa and the structurally related opioid Met-enkephalin-Arg6-Phe7. Nature 298: 275–276; 1982.
10. Greenberg, M. J.; Payza, K.; Nachman, R. J.; Holman, G. H.; Price, D. A. Relationships between FMRFa-related peptides and other peptide families. Peptides 9:125–135; 1988.
11. Kavaliers, M.; Hirst, M. FMRFamide, a putative endogenous opiate antagonist: evidence from suppression of defeat-induced analgesia and feeding in mice. Neuropeptides 6:485–494; 1985.
12. Kavaliers, M.; Hirst, M. Inhibitory influences of FMRFamide and PLG on stress-induced opioid analgesia and activity. Brain Res. 372:370–374; 1986.
13. Kavaliers, M. Calcium channel blockers inhibit the antagonistic effects of Phe-Met-Arg-Phe-amide (FMRFamide) on morphine- and stress-induced analgesia in mice. Brain Res. 415:380–384; 1987.
14. Majane, E. A.; Yang, H.-Y. T. Distribution and characterization of two putative endogenous opioid anatagonist peptides in bovine brain. Peptides 8:657–662; 1987.
15. O'Donohue, T. L.; Bishop, J. F.; Chronwall, B. M.; Groome, J.; Watson, W. H., III. Characterization and distribution of FMRFamide immunoreactivity in the rat central nervous system. Peptides 5: 563–568; 1984.
16. Painter, S. D.; Greenberg, M. J. A survey of the responses of bivalve hearts to the molluscan neuropeptide FMRFamide and to 5-hydroxytryptamine. Biol. Bull. 162:311–332; 1982.
17. Payza, K. FMRFa receptors in *Helix aspersa*. Peptides 8:1065–1074; 1987.
18. Price, D. A.; Greenberg, M. J. Purification and characterization of a cardioexcitatory neuropeptide from the central ganglia of a bivalve mollusc. Prep. Biochem. 7:261–281; 1977.
19. Price, D. A.; Greenberg, M. J. The pharmacology of the molluscan cardioexcitatory neuropeptide FMRFa. Comp. Pharmacol. 11:237–241; 1980.
20. Price, D. A. FMRFamide: Assays and artifacts. In: Lever, J.; Boer, H. H., eds. Molluscan neuroendocrinology. New York: North Holland Publishing Co.; 1983:184–190.
21. Raffa, R. B. The action of FMRFamide (Phe-Met-Arg-Phe-NH_2) and related peptides on mammals. Peptides 9:915–922; 1988.
22. Raffa, R. B.; Heyman, J.; Porreca, F. Intrathecal FMRFamide (Phe-Met- Arg-Phe-NH2) induces excessive grooming behavior in mice. Neurosci. Lett 65:94–98; 1986.
23. Roth, B. L.; Disimone, J.; Majane, E. A.; Yang, H.-Y. T. Elevation of arterial pressure in rats by two new vertebrate peptides FLFQPQRF-NH2 and AGEGLSSPFWSLAAPQRF-NH2 which are immunoreactive to FMRF-NH2 antiserum. Neuropeptides 10:37–42; 1987.
24. Sokal, R. R.; Rohlf, F. J. Comparisons among means: unplanned comparisons In: Sokol, R. R.; Rohlf, F. J., eds. Biometry, second edition. San Francisco: W. H. Freeman and Company; 1981:242–262.
25. Tang, J.; Yang, H.-Y. T.; Costa, E. Inhibition of spontaneous and opiate-modified nociception by an endogenous neuropeptide with Phe-Met-Arg-Phe-NH2-like immunoreactivity. Proc. Natl. Acad. Sci. USA 81:6680–6683; 1984.
26. Telegdy, G. T.; Bollók, I. Amnesic action of FMRFamide in rats. Neuropeptides 10:157–163; 1987.
27. Yang, H. Y. T.; Fratta, W.; Majane, E. A.; Costa, E. Isolation, sequencing, synthesis and pharmacological characterization of two brain neuropeptides that modulate the action of morphine. Proc. Natl. Acad. Sci. USA 82:7757–7761; 1985.
28. Zhu, X. Z.; Raffa, R. B. Low affinity inhibition of opioid receptor binding. Neuropeptides 8:55–61; 1986.