Pharmacology Biochemistry and Behavior, Vol. 58, No. 2, pp. 559–563, 1997

0091-3057/97 $17.00 + .00

ELSEVIER

PII S0091-3057(97)00249-9

Hypothermic Effects of Neuropeptide FF Analogues in Mice

C. DESPRAT AND J.-M. ZAJAC[1]

Institut de Pharmacologie et de Biologie Structurale, CNRS, 205 route de Narbonne, 31 077 Toulouse, France

Received 8 August 1996; Revised 11 December 1996; Accepted 11 December 1996

DESPRAT, C. AND J. -M. ZAJAC. *Hypothermic effects of neuropeptide FF analogues in mice.* PHARMACOL BIOCHEM BEHAV **58**(2) 559–563, 1997.—The effects of neuropeptide FF (NPFF) and its analogues on mouse body temperature were examined. In a thermoneutral environment, administration of NPFF (Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2), 1DMe ([D.Tyr[1], (N.Me)Phe[3]] NPFF), and 3D ([D.Tyr[1], D.Leu[2], D.Phe[3]] NPFF) in the third ventricle produced marked hypothermia. The effect of 1DMe was dose-dependent, and 45 nmol decreased body temperature by 5.6°C. This effect was more pronounced when mice were placed at 4°C. Hypothermia was not reversed by naloxone, an opioid antagonist, and was not modified by morphine. After 5 days of chronic treatment with 1DMe, mice did not became tolerant to the hypothermic effect. These results indicate that central NPFF receptors may control body temperature independently from opioid functions. © 1997 Elsevier Science Inc.

Neuropeptide FF Body temperature Hypothermia Mice Opioid

NEUROPEPTIDE FF (NPFF) was first identified in bovine brain (31), and several studies have revealed the presence of NPFF immunoreactivity in mammals and in the human central nervous system (CNS) (22). NPFF produces its physiological effects by interacting with high-affinity receptors present in the CNS of several species (2,4,8,9).

The notion that NPFF may act as a neurotransmitter or neuromodulator has prompted experiments aimed at revealing the pharmacological and behavioral effects of this peptide. NPFF was first described as an "anti-opioid" peptide because it decreases opioid analgesia in rats (25,27,31) and mice (7,8, 13,19). Similarly, NPFF has been associated with opioid tolerance and dependence, because injection of NPFF into the rat third ventricle induced a morphine withdrawal-like behavioral syndrome (23). In addition, IgG purified from antiserum recognizing NPFF reversed naloxone-induced withdrawal in morphine-dependent rats (24).

However, NPFF also exhibits opioid-like effects, because after intrathecal injection in rats, NPFF induces analgesia (16,17). In the same manner, NPFF analogues inhibit intestinal transit after intracerebroventricular (ICV) injection (12). NPFF and opioid systems could constitute two physiologically coupled systems, each controlling the activity of the other, because NPFF does not bind to opioid receptors in the CNS (28).

NPFF immunoreactivity is localized in different CNS sites involved in temperature regulation, including the spinal cord, trigeminal nucleus, and medial preoptic area (4,11). The presence of NPFF and NPFF receptors in these structures suggests that one of the physiological functions of this peptide could be to participate in thermoregulation. Thus, we investigated in mice the putative thermoregulatory effects of NPFF administered into the third ventricle.

MATERIALS AND METHODS

Chemicals

Morphine hydrochloride was obtained from Francopia and naloxone from Sigma. NPFF (Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2), 1DMe ([D.Tyr[1], (N.Me)Phe[3]] NPFF), and 3D ([D.Tyr[1], D.Leu[2], D.Phe[3]] NPFF) were synthesized as previously described (13).

Animals

Animals were tested according to the recommendations of the International Association for the Study of Pain (IASP). Male CDF1 mice (25–35 g) were maintained at 22°C on a normal dark/light cycle with access to food and water ad lib. CDF1 mice were obtained by interbreeding DBA2 males and BALB/C females.

[1]To whom requests for reprints should be addressed.

Intraventricular Injection

The procedure for ICV injection was adapted from Haley and McCormick (18). Briefly, drug was loaded into a 10-μl syringe. The mouse was hand-held and gently restrained, the skull was punctured perpendicular to the dorsal surface, and 5 μl of drug was injected into the third ventricle over a period of about 5 s. The stereotaxic coordinates for the third ventricle were 2 mm anterior to the interaural line, on the interhemispheric line, to a depth of 2.5 mm. For the chronic treatment, 45 nmol of 1DMe was injected ICV at 0900 h each day for 5 days.

Temperature Measurement

The animals were restrained in an aluminum device as described by Rosow et al. (29), with their tails loosely taped to horizontal posts. The animals could not huddle together, but the restraint did not otherwise affect convective heat exchange or thermoregulatory behavior. The ambient temperature was regulated to 21 ± 1°C. The colonic temperature was measured with a thermistor probe (Ellab Instrument, Copenhagen, Denmark) inserted to a depth of 2.5 cm in the rectum. Body temperature was recorded immediately before injection and then at 20-min intervals for 180 min. Each animal was used only once.

Statistics

For the dose–response curve (1DMe and morphine) and for chronic treatment, data were subjected to analysis of variance (ANOVA), and Dunnett's *t* was used for the post hoc pairwise comparison between means. For the effect of 1DMe in a cold environment and for the opioid antagonist effect, Student's *t*-test was used for comparison between means.

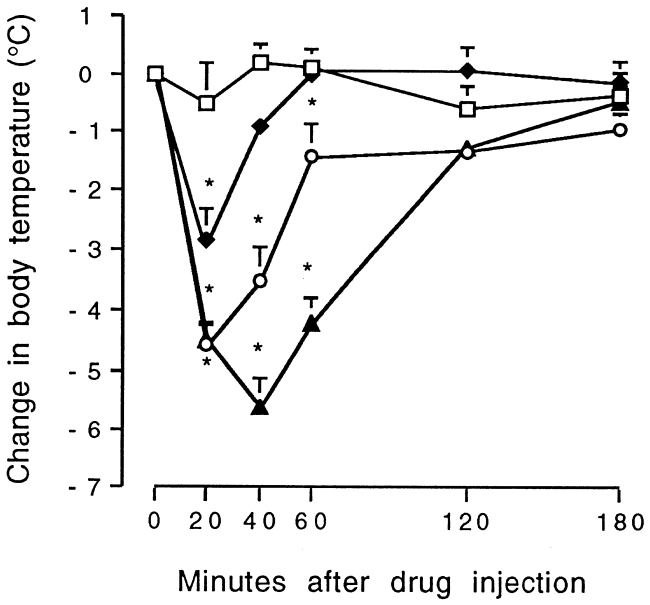

FIG. 1. Time course of the change in body temperature of mice treated ICV with 2 nmol (□), 10 nmol (◆), 20 nmol (○), or 45 nmol (▲) 1DMe. Rectal temperature was recorded 20, 40, 60, 120, and 180 min after drug administration. Each point represents the mean ± SEM of the change in body temperature of seven animals. *$p < 0.05$ vs. NaCl-treated group (Dunnett's *t*-test).

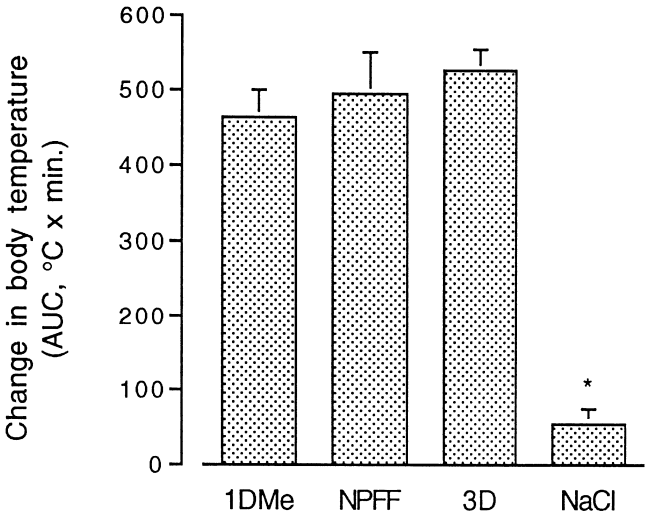

FIG. 2. Effects of several NPFF analogues on body temperature of mice treated ICV with NaCl or 45 nmol NPFF, 1DMe, or 3D. The ordinate represents the area under the curve (AUC) described by rectal temperature measured after ICV injection over a 3-h period (the AUC was computed by trapezoidal approximation). Each bar represents the mean ± SEM of the change in body temperature of seven animals. *$p < 0.05$ vs. NaCl-treated group [Dunnett's *t*-test; $F(3, 16) = 26.29$].

RESULTS

DMe Dose–Effect Relationship

DMe, an analogue of NPFF protected against enzymatic degradation, produced marked hypothermia after ICV administration (Fig. 1). The decrease in body temperature began 20 min after injection and was maximal at 20 or 40 min, depending on the dose. The colonic temperature of 1DMe-treated mice returned to the control level between 2 and 3 h after injection. The 1DMe-induced hypothermia was dose-dependent (Table 1). Only 2 nmol 1DMe did not significantly reduce body temperature compared with controls [$F(3, 17) = 14.36$, $p < 0.001$], whereas the temperature of mice reached 32°C after injection of 45 nmol 1DMe (Fig. 1). Because of this severe hypothermia, higher doses were not investigated. For the 120-min period following injection, 45 nmol 1DMe, NPFF, or 3D produced quantitatively the same hypothermic effect, expressed as area under the curve (Fig. 2).

Effect of 1DMe in a Cold Environment

To define the level of regulation modified by the stimulation of NPFF receptors, the effects of centrally administered 1DMe on body temperature were examined in a cold environment (4°C) (Fig. 3). A dose of 45 nmol 1DMe markedly increased the rate and magnitude of the decline in colonic temperature induced by 2 h of cold exposure. Two hours of cold exposure decreased body temperature by 5.7°C in naive mice and by 18.5°C in 1DMe-treated mice. This suggests that 1DMe reduced significantly tolerance to cold in mice.

Chronic Effects of 1DMe

During chronic treatment, animals received either 1DMe (45 nmol) or NaCl ICV once per day for 5 days (Fig. 4). The hypothermic effect of 1DMe decreased significantly during chronic treatment: on day 1 (J1), mouse body temperature was

33.8 ± 0.28°C (mean ± SEM) 20 min after injection, whereas on J5, body temperature was 35.8 ± 0.58°C [$F(4, 34) = 2.97$, $p < 0.05$]. The difference in mean body temperature between 1DMe- and NaCl-treated mice was not significantly different on J1 and J5. Furthermore on J5, 45 nmol 1DMe injected into mice previously chronically treated with NaCl decreased body temperature to the same extent as in 1DMe-treated animals. This result indicates that mice did not became tolerant to the hypothermic effects of 1DMe after 5 days of chronic treatment.

Opioid–NPFF Interaction

Opioid systems produce changes in body temperature [for review, see (1)], so the effects of third ventricle administration of morphine were investigated. In mice, after injection into the third ventricle, morphine (0.16–16 nmol) significantly reduced body temperature in a dose-dependent manner [$F(3, 12) = 77.01$, $p < 0.001$] (Table 1). The decrease in body temperature began 20 min after injection and reached maximal levels 40–60 min later. After treatment with 0.16 nmol morphine, mice recovered gradually and reached normal rectal temperature 3 h after injection; return to normal took longer at higher doses.

To investigate the possible interaction between opioid and NPFF systems in the mediation of a hypothermic effect, mice were treated with opioid agonists and antagonists. As shown in Table 1, the hypothermic effect of morphine was completely prevented by intraperitoneal administration of naloxone (1 mg/kg), whereas 1DMe-induced effects were not modified significantly by naloxone.

A low dose of morphine (0.16 nmol) coinjected with an effective dose of 1DMe (20 nmol) did not increase the hypothermic effect of the NPFF analogue. Similarly, a low dose of 1DMe (2 nmol) coinjected with an effective dose of morphine (1.6 nmol) did not modify the hypothermic effect of morphine (Table 1).

DISCUSSION

In this study, NPFF and analogues of NPFF injected into the third ventricle induced marked hypothermia in mice. The hypothermic effect of 1DMe was dose-dependent, and all NPFF analogues tested induced quantitatively similar hypothermia.

Because no NPFF antagonist has been characterized, the dose–effect relationship is used in this study to define the specificity of the pharmacological effect. 1DMe is effective at very low doses (10–20 nmol) corresponding to doses able to decrease morphine analgesia in rats after ICV injection (10); these low 1DMe concentrations have also been found to reverse analgesia in mice (7,8,13). On a molar basis, NPFF appears to be as potent as 1DMe or 3D, although these two latter peptides are resistant to enzymatic degradation (13). However, the lack of difference in inducing hypothermia is probably due to the high dose used (45 nmol) and the weak

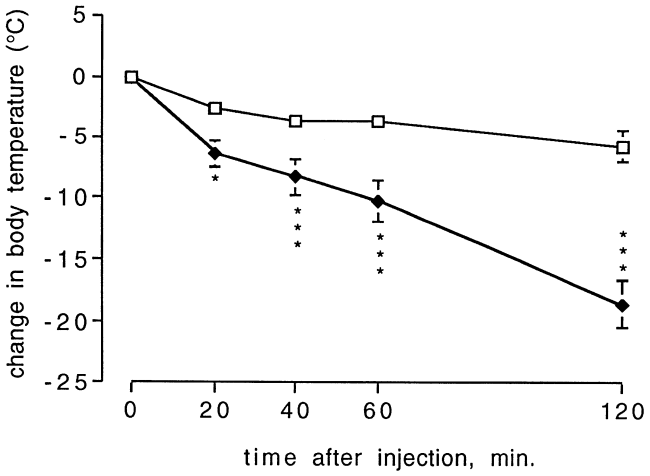

FIG. 3. Time course of the change in body temperature in a cold environment of mice treated ICV with NaCl (□) or 45 nmol 1DMe (◆). Mice were placed at 4°C, and rectal temperature was recorded 20, 40, 60, 120, and 180 min after injection. Each point represents the mean ± SEM of the change in body temperature of five animals. *$p < 0.05$ and **$p < 0.001$ vs. NaCl-treated group (Student's *t*-test).

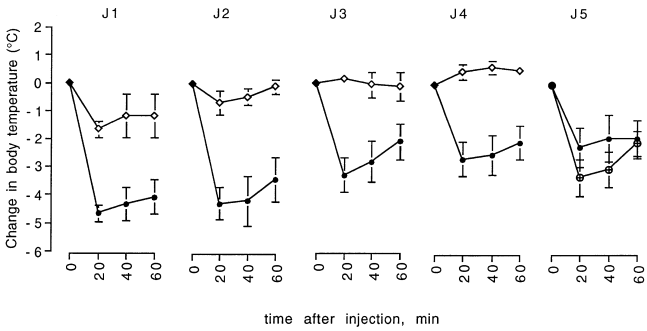

FIG. 4. Effects of chronic treatment with 1DMe on body temperature. Animals were treated with NaCl (◇) or 45 nmol 1DMe (●), one injection per day for 5 days. On day 5 (J5), mice treated chronically with NaCl were injected with 45 nmol 1DMe (⊕). Rectal temperature was recorded 20, 40, and 60 min after injection. Each point represents the mean ± SEM of the change in body temperature of seven animals.

TABLE 1

EFFECTS OF MORPHINE AND 1DMe ON BODY TEMPERATURE

Treatment (nmol)	Area under Curve
Morphine	
0.16	45.3 ± 18.1
1.6	471.2 ± 90.4
16	1,216.3 ± 23.9
1DMe	
2	88.8 ± 40.4
10	118.6 ± 15.7
20	293.2 ± 50.6
45	464.2 ± 73.5
Pretreatment with naloxone	
Morphine 1.6	157 ± 69.2***
1DMe 45	530.6 ± 73.5
Coinjection	
1DMe 2 + morphine 1.6	565.2 ± 23.7
1DMe 20 + morphine 0.16	352.6 ± 43.4

The area under the curve (±SEM) was calculated for the 3-h period following ICV injection. For pretreatment, naloxone (1 mg/kg) was administered intraperitoneally 10 min before ICV injection of morphine or 1DMe.

***$p < 0.001$ vs. morphine alone at the same dose (Student's *t*-test).

difference in affinity of these peptides towards NPFF receptors (13,14).

We described here a direct effect of NPFF on the central nervous system that did not require preliminary stimulation of opioid receptors. Several pharmacological pro-opioid effects of NPFF have been described that are dependent upon stimulation of opioid receptors, e.g., NPFF inhibition of intestinal transit in mice after ICV injection. This latter effect was reduced by a low dose of morphine, indicating that NPFF acted indirectly by modulation of the opioid system (12). In contrast, hypothermia induced by NPFF was not modified by naloxone, an opioid antagonist, or by morphine, an opioid agonist, suggesting that opioids and NPFF induce hypothermia by different mechanisms. Hypothermia is, therefore, the first central NPFF effect elucidated that is apparently independent of the activity of the opioid system.

Central thermoregulatory control involves a set of neuromodulators, neurotransmitters, and neural networks (6,15,21). Homeotherms maintain a constant internal temperature around the set point by balancing heat production (thermogenic response) and heat loss (thermolysis response). To change body temperature, a drug alters either the control system or one of the effector systems. Ambient temperature changes have long been known to alter profoundly the thermoregulatory responses to many drugs. Drugs that suppress the thermogenic response to cold reduce body temperature even more when ambient temperature is low. The decrease induced by 45 nmol 1DMe is more dramatic at 4°C that at 21°C. Thus, NPFF seems to be involved in thermogenic response and not in set point modification. To confirm this hypothesis, it would be interesting to use a thermocline to study alteration of behavioral thermoregulation. A modification of blood flux could induce heat gain or heat loss. Intravenous administration of NPFF has been reported to produce a dose-dependent increase in blood pressure and heart rate (3). Thus, these cardiovascular modifications could represent a part of heat loss mechanisms.

In the range of doses tested, 1DMe reduces opioid-induced analgesia in adult mice after ICV injection, but it seems unlikely that the hypothermic effect induced by 1DMe is related to its anti-opioid effect on analgesia. Indeed, opioid-induced hypothermia is not modified by 1DMe, and several studies have shown that antinociceptive tail-flick responses induced by different drugs are produced independently from changes in either tail-skin or body temperature (20,26). Thus, modulation of opioid analgesia by NPFF is probably independent of NPFF-induced hypothermia.

After ICV injection, NPFF analogs induced hypothermia (this study) and inhibited morphine antinociception in mice (8,13) and in rats (10,25). In contrast, at the spinal level, low doses (0.086–6.4 nmol) of NPFF are sufficient to produce an analgesic effect (16,17). Such differences in doses could be related to injection route (intracerebral vs. spinal), animal species, or pharmacological effect.

Different authors have suggested that the opioidergic and NPFFergic systems might be in homeostasic equilibrium in the central nervous system and that disruption of this equilibrium could be responsible for the states of tolerance and dependence of opioids (30). According to this model, the surexpression of the NPFF system could be partly responsible for the withdrawal syndrome. The abstinence syndrome in rats and mice is associated with hypothermia and thermoregulatory behavior such as teeth chattering, piloerection, and wet dog shakes (5). These behaviors could correspond to stimulation of NPFF receptors.

This study demonstrates that NPFF analogues induce hypothermia unrelated to an interaction of NPFF with the opioid system. The exact mechanisms by which NPFF induces this reduction in body temperature remain to be established.

REFERENCES

1. Adler, M. W.; Geller, E. B.; Rosow, C.; Cochin, J.: The opioid system and temperature regulation. Annu. Rev. Pharmacol. Toxicol. 28:429–449; 1988.
2. Allard, M.; Geoffre, S.; Legendre, P.; Vincent, J. D.; Simonnet, G.: Characterization of rat spinal cord receptors to FLFQPQRFamide, a mammalian morphine modulating peptide: A binding study. Brain Res. 500:169–176; 1989.
3. Allard, M.; Labrouche, S.; Nosjean, A.; Laguzzi, R.: Mechanisms underlying the cardiovascular responses to peripheral administration of NPFF in the rat. J. Pharmacol. Exp. Ther. 274:577–583; 1995.
4. Allard, M.; Zajac, J.-M.; Simonnet, G.: Autoradiographic distribution of receptors to FLFQPQRFamide, a morphine-modulating peptide, in rat central nervous system. Neuroscience 49:101–116; 1992.
5. Belknap, J. K.: Components of the opioid withdrawal syndrome in mice are thermoregulatory response. Pharmacol. Biochem. Behav. 34:241–245; 1989.
6. Bligh, J.: The central neurology of mammalian thermoregulation. Neuroscience 4:1213–1236; 1979.
7. Desprat, C.; Roumy, M.; Zajac, J.-M.: Neuromodulatory effects of neuropeptide FF on opioid functions in mice. Regul. Pept. 54: 81–82; 1994.
8. Desprat, C.; Zajac, J.-M.: Ontogeny of neuropeptide FF pharmacology and receptors in mouse brain. Dev. Brain Res. 82:118–126; 1994.
9. Dupouy, V.; Puget, A.; Eschalier, A.; Zajac, J.-M.: Species differences in the localization of neuropeptide FF receptors in rodents and lagomorph brain and spinal cord. Peptides 17:399–405; 1996.
10. Dupouy, V.; Zajac, J.-M.: Effects of neuropeptide FF analogs on morphine analgesia in the nucleus raphe dorsalis. Regul. Pept. 59:349–356; 1995.
11. Dupouy, V.; Zajac, J.-M.: Neuropeptide FF receptors in rat brain: A quantitative light microscopic autoradiographic study using [^{125}I] [D.Tyr^{1}, (NMe)Phe^{3}] NPFF. Synapse 24:282–296; 1996.
12. Gicquel, S.; Fioramonti, J.; Bueno, L.; Zajac, J.-M.: Effects of F8Famide analogs on intestinal transit in mice. Peptides 14:749–753; 1993.
13. Gicquel, S.; Mazarguil, H.; Allard, M.; Simonnet, G.; Zajac, J.-M.: Analogues of F8Famide resistant to degradation, with high affinity and in vivo effects. Eur. J. Pharmacol. 222:61–67; 1992.
14. Gicquel, S.; Mazarguil, H.; Desprat, C.; Allard, M.; Devillers, J.-P.; Simonnet, G.; Zajac, J.-M.: Structure–activity study of neuropeptide FF: Contribution of N-terminal regions to affinity and activity. J. Med. Chem. 37:3477–3481; 1994.
15. Gordon, C. J.; Heath, J. E.: Integration and central processing in temperature regulation. Annu. Rev. Physiol. 48:595–612; 1986.
16. Gouardères, C.; Jhamandas, K.; Sutak, M.; Zajac, J.-M.: Role of opioid receptors in the spinal antinociceptive effects of neuropeptide FF analogues. Br. J. Pharmacol. 117:493–501; 1996.
17. Gouardères, C.; Sutak, M.; Zajac, J.-M.; Jhamandas, K.: Antinociceptive effects of intrathecally administered F8Famide and FMRFamide in the rat. Eur. J. Pharmacol. 237:73–81; 1993.
18. Haley, T. J.; McCormick, W. G.: Pharmacological effect produced by intra-cerebral injection of drugs in conscious mouse. Br. J. Pharmacol. 12:12–15; 1957.
19. Kavaliers, M.; Yang, H.-Y. T.: IgG from antiserum against endogenous mammalian FMRF-NH_2-related peptides augments morphine- and stress-induced analgesia in mice. Peptides 10:741–745; 1989.

20. Lichtman, A. H.; Smith, F. L.; Martin, B. R.: Evidence that the antinociceptive tail-flick response is produced independently from changes in either tail-skin temperature or core temperature. Pain 55:283–295; 1993.
21. Lipton, J. M.; Clark, W. G.: Neurotransmitters in temperature control. Annu. Rev. Physiol. 48:613–623; 1986.
22. Majane, E. A.; Casanova, M. F.; Yang, H.-Y. T.: Biochemical characterization of $FMRFNH_2$-like peptide in spinal cord of various mammalian species using specific radioimmunoassays. Peptides 9:1137–1144; 1988.
23. Malin, D. H.; Lake, J. R.; Fowler, D. E.; Hammond, M. V.; Brown, S. L.; Leyva, J. E.; Prasco, P. E.; Dougherty, T. M.: $FMRF\text{-}NH_2$-like mammalian peptide precipitates opiate-withdrawal syndrome in the rat. Peptides 11:277–280; 1990.
24. Malin, D. H.; Lake, J. R.; Hammond, M. V.; Fowler, D. E.; Rogillio, R. B.; Brown, S. L.; Sims, J. L.; Leecraft, B. M.; Yang, H.-Y. T.: $FMRF\text{-}NH_2$-like mammalian octapeptide: Possible role in opiate dependence and abstinence. Peptides 11:969–972; 1990.
25. Millon, M.; Fioramonti, J.; Gicquel, S.; Zajac, J.-M.; Bueno, L.: Comparative action of Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2 analogs on intestinal motility and nociception in rats. J. Pharmacol. Exp. Ther. 265:96–102; 1993.
26. Nemeroff, C. B.; Osbahr, A. J.; Manberg, P. J.; Ervin G. N.; Prange, A. J.: Alterations in nociception and body temperature after intracisternal administration of neurotensin, β-endorphin, other endogenous peptides, and morphine. Proc. Natl. Acad. Sci. USA 76:5368–5371; 1979.
27. Oberling, P.; Stinus, L.; Moal, M. L.; Simonnet, G.: Biphasic effect on nociception and antiopiate activity of neuropeptide FF (FLFQPQRFamide) in the rat. Peptides 14:919–924; 1993.
28. Raffa, R. B.; Kim, A.; Rice, K. C.; Costa, B. R. D.; Codd, E. E.; Rothman, R. B.: Low affinity of FMRFamide and four FaRPs, F-8-Famide (NPFF) and A-18-Famide, for opioid μ, δ, κ_1, κ_{2a}, or κ_{2b} receptors. Br. J. Pharmacol. 117:493–501; 1994.
29. Rosow, C. E.; Miller, J. M.; Pelikan, E. W.; Cochin, J.: Opiates and thermoregulation in mice. I. Agonists. J. Pharmacol. Exp. Ther. 213:273–283; 1980.
30. Rothman, R. B.: A review of the role of anti-opioid peptides in morphine tolerance and dependence. Synapse 12:129–138; 1992.
31. Yang, H.-Y. T.; Fratta, W.; Majane, E. A.; Costa, E.: Isolation, sequencing, synthesis, and pharmacological characterization of the two brain neuropeptides that modulate the action of morphine. Proc. Natl. Acad. Sci. USA 82:7757–7761; 1985.