ELSEVIER

Pharmacology Biochemistry and Behavior, Vol. 54, No. 3, pp. 581–585, 1996

0091-3057/96 $15.00 + .00

SSDI 0091-3057(95)02279-1

Nicotine Abstinence Syndrome Precipitated by an Analog of Neuropeptide FF

DAVID H. MALIN,*[1] J. RONALD LAKE,* PAUL E. SHORT,* J. BRANDON BLOSSMAN,* BRENT A. LAWLESS,* CHRISTINA K. SCHOPEN,* ELIZABETH E. SAILER,* KEVIN BURGESS† AND OWEN B. WILSON‡

*University of Houston-Clear Lake, Houston, TX 77058
†Texas A&M University, College Station, TX 77843
‡Baylor College of Medicine, Houston, TX 77030

Received 7 July 1995; Revised 30 October 1995; Accepted 15 November 1995

MALIN, D. H., J. R. LAKE, P. E. SHORT, J. B. BLOSSMAN, B. A. LAWLESS, C. K. SCHOPEN, E. E. SAILER, K. BURGESS AND O. B. WILSON. *Nicotine abstinence syndrome precipitated by an analog of neuropeptide FF.* PHARMACOL BIOCHEM BEHAV **54**(3) 581–585, 1996.—In a recently introduced rodent model of nicotine abstinence syndrome the observed behavioral signs closely resembled those typical of rat opiate abstinence syndrome. Nicotine-induced release of endogenous opioids may contribute to nicotine dependence; morphine potently reverses nicotine abstinence signs, while naloxone precipitates abstinence signs and prevents nicotine from alleviating them. Considerable evidence suggests that neuropeptide FF, an endogenous antiopiate peptide, contributes to opiate dependence. Third ventricle injection of neuropeptide FF precipitates abstinence syndrome in morphine-dependent rats, as does SC injection of its lipophilic analogs, dansyl-PQRFamide and dansyl-RFamide. Might NPFF also play a role in nicotine dependence? In the present study, SC injection of 15 or 25 mg/kg dansyl-RFamide or vehicle alone dose dependently precipitated an abstinence syndrome in nicotine-dependent rats. There was a significant, $p < 0.01$, positive linear trend of abstinence signs as a function of dose. Categories of abstinence signs had the same rank ordering by frequency as observed in spontaneous nicotine abstinence. Injection of 25 mg/kg dansyl-RFamide SC had no significant effect in nondependent rats.

Neuropeptide FF F8Famide Antiopiate peptides Nicotine Nicotine dependence Nicotine abstinence Nicotine withdrawal Endogenous opioid peptides Dansyl compounds

A RODENT model of nicotine abstinence syndrome has been developed based on continuous subcutaneous (SC) infusion of nicotine tartrate; spontaneous behavioral signs were observed in rats following either termination of nicotine infusion (38) or injection of the nicotinic antagonist mecamylamine (34). The model met a number of validity criteria, including reversibility by injection of a low dose of nicotine and comparative lack of behavioral signs in saline-infused controls (38).

Nicotine abstinence signs resembled those observed in mild morphine abstinence when dependence was induced by continuous SC drug infusion via Alzet osmotic minipump (30,33,38–40). There is ample evidence (5,13,20,23,45–47) that nicotinic receptor stimulation induces release of endogenous opioid peptides (EOPs). It has been hypothesized (33) that nicotine-induced EOP release overstimulates opiate receptors, resulting in an opiate dependence-like state. Cessation of either nicotinic or opiate receptor stimulation would then result in an opiate abstinence-like state. Consistent with this hypothesis, morphine potently reversed nicotine abstinence syndrome (33). Conversely, the opiate antagonist naloxone precipitated an immediate abstinence syndrome in nicotine-dependent rats (33). Furthermore, pretreatment with naloxone prevented nicotine from relieving nicotine abstinence syndrome (39), suggesting that nicotine may alleviate nicotine abstinence, in part, through stimulating EOP release.

To the extent that nicotine dependence, like opiate dependence, results from overstimulation of opiate receptors, some of the same neurochemical mechanisms might be involved in both phenomena. One candidate for a common mechanism might be activation of the endogenous antiopiate peptide, neuropeptide FF (NPFF). NPFF, isolated from bovine brain by Yang et al. (54), has a sequence of FLFQPQRFamide. It has

[1] Requests for reprints should be addressed to D. H. Malin, UH-CL Box 237, 2700 Bay Area Blvd., Houston, TX 77058.

also been referred to as F8Famide (26), FMRFamide-like mammalian peptide (35), and morphine modulating peptide (42).

NPFF has been shown to counteract various actions of opiate drugs and EOPs (7,12,25,30,51,54). These antiopiate actions are mediated through specific NPFF receptors rather than through opiate receptors (2). A number of reports suggest that NPFF might play a role in opiate tolerance, dependence, and subsequent abstinence syndrome (28,29,35-37,48). Third ventricular injection of NPFF precipitated morphine abstinence syndrome more potently than naloxone itself (35). While there have been no reports of behavioral actions of NPFF via peripheral administration, SC injection of the lipophilic NPFF analogs dansyl-RFamide and dansyl-PQRFamide precipitated an abstinence syndrome in morphine-dependent rats (19,32). The present study determined whether SC injection of dansyl-RFamide would likewise precipitate an abstinence syndrome in nicotine-dependent rats. An additional experiment determined whether a similar dose of dansyl-RFamide would induce an abstinence-like syndrome in nondependent rats. These experiments provide an initial test of the hypothesis that NPFF is one common mechanism in opiate and nicotine dependence.

METHOD

Synthesis of Dansyl-RFamide

FMOC-Phe, FMOC-Arg, BOP, HOBt, and Rink's amide resin were purchased from Advanced ChemTech (Louisville, KY); dansyl (5-dimethylaminonapthalene-1-sulfonyl) chloride and *N*-methylmorpholine were obtained from Aldrich Chemical Co. (Milwaukee, WI). Dansyl-Arg-Phe-amide (dansyl-RFamide) was produced by conventional solid-phase synthesis (50) using FMOC *N*-protecting groups (3), Rink's amide resin, and BOP/HOBt/*N*-methylmorpholine in DMF for the coupling steps (8). The dansyl residue was added to the resin bound peptide using dansyl chloride (3 equivalents), *N*-methylmorpholine (4.5 equivalents) and catalytic DMAP in DMF. The peptide was cleaved using a mixture of crystalline phenol (0.75 g), 1,2-ethanedithiol (0.25 ml), thioanisole (0.5 ml), deionized water (0.5 ml), and TFA (10 ml). The peptide was purified via preparative HPLC using a C18 column. Satisfactory amino acid analysis and FABMS [glycerol, m/e 554.3 (M + 1)] were obtained.

Animals

Thirty male Sprague-Dawley rats weighing 134-190 g were maintained on ad lib food and water and a 12 L : 12 D cycle. Rats of this size were used to conserve the limited available amount of dansyl-RFamide.

Animal Treatments

All animals were implanted subcutaneously under halothane anesthesia with one Alzet 2 ML1 osmotic minipump. In the first experiment, 18 rats were rendered dependent by seven days continuous infusion of 9 mg/kg/day nicotine tartrate [−] isomer in saline. In a second experiment, 12 rats were infused with saline alone.

On day 7 of infusion (164 h after pump implantation), each subject was challenged by a SC injection; minipumps were not removed prior to the SC challenge. In the first experiment, nicotine-dependent rats received either 25 mg/kg dansyl-RFamide dissolved in a vehicle of 20% ethanol : distilled water (n = 6), 15 mg/kg dansyl-RFamide in vehicle (n = 6), or vehicle alone (n = 6). In the second experiment, saline-infused rats received either 25 mg/kg dansyl-RFamide (n = 6) or vehicle alone (n = 6).

Behavioral Observations

Each subject was observed in a clear, rectangular chamber for 25 min immediately following the SC injection. All observations were performed under blind conditions. Observers counted the frequency of behavioral signs based on a checklist of nicotine abstinence signs developed and validated by Malin et al. (38). Categories included gasps/writhes, teeth chatter/chews, shakes/tremors, and miscellaneous less frequent signs (scratches, ptosis, yawns, dyspnea, seminal ejaculation/genital lick, foot lick, and chromodacryorrhea). Ptosis was counted no more than once per minute, dyspnea was scored as separate episodes of wheezing, and chromodacryorrhea was counted only once per subject.

RESULTS

Figure 1 shows the number of overall abstinence signs (cumulated across all categories) precipitated in nicotine-dependent rats by 15 and 25 mg/kg dansyl-RFamide SC and by vehicle alone. Linear trend analysis revealed a significant positive trend of abstinence signs as a function of dansyl-RFamide dose, $F(1, 15) = 9.84$, $p < 0.01$. Post hoc analysis (Dunn's/Bonferroni Procedure) revealed that the high dose group exhibited significantly more nicotine abstinence signs than the low dose group, $p < 0.05$, and the low dose group exhibited significantly more signs than the vehicle group, $p < 0.05$.

Figure 2 shows numbers of abstinence signs grouped by individual categories. Linear trend analysis revealed significant positive trends as a function of dose for gasps/writhes, $F(1, 15) = 10.79$, $p < 0.01$, and teeth chatter/chews, $F(1, 15) = 7.51$, $p < 0.05$. The positive trend in miscellaneous signs approached significance, $F(1, 15) = 3.78$, $0.05 < p < 0.10$. The positive trend in shakes/tremors was not significant, $F(1, 15) = 1.78$, NS. There were no significant deviations from linearity for overall signs or any category of signs.

In nicotine-naive, saline-infused rats, 25 mg/kg dansyl-

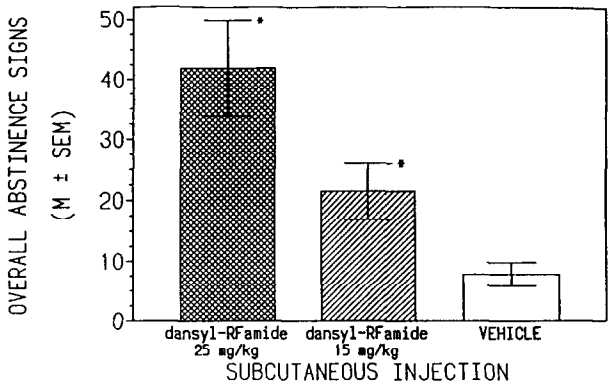

FIG. 1. Overall nicotine abstinence signs (means ± SEM) over 25 min precipitated by SC injection of 25 mg/kg dansyl-RFamide, 15 mg/kg dansyl-RFamide, or ethanol : distilled water vehicle alone. All rats were chronically infused for 7 days with 9 mg/kg/day nicotine tartrate. *$p < 0.05$, 25 mg/kg vs. 15 mg/kg and 15 mg/kg vs. vehicle (Dunn's procedure).

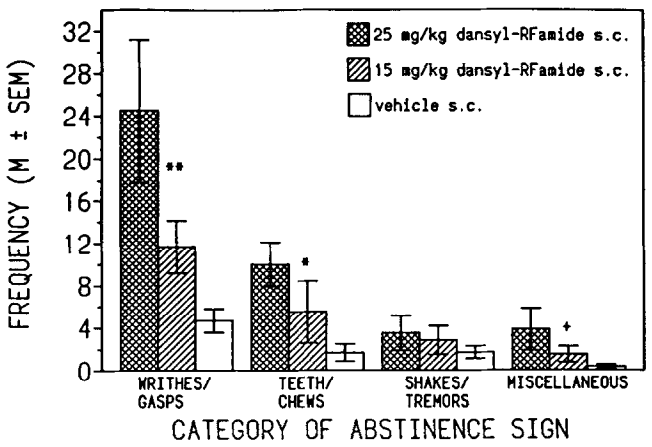

FIG. 2. Occurrences of various categories of nicotine abstinence signs (means ± SEM) over 25 min precipitated by SC injection of 25 mg/kg dansyl-RFamide, 15 mg/kg dansyl-RFamide, or ethanol: distilled water vehicle alone. All rats were chronically infused for 7 days with 9 mg/kg/day nicotine tartrate. $^{**}p < 0.01$, $^{*}p < 0.05$, $+$ $0.05 < p < 0.10$ positive linear trend for a given category of sign as a function of dansyl-RFamide dose.

RFamide precipitated only 16.0 ± 5.6 (means ± SEM) overall abstinence-like signs, while vehicle alone precipitated 11.2 ± 2.5 signs. There was no significant difference between these groups, $t(10) = 0.79$, NS.

GENERAL DISCUSSION

Subcutaneous injection of the NPFF analog dansyl-RFamide dose dependently precipitated an abstinence syndrome in nicotine-dependent rats, while it had no significant effect in nicotine-naive rats. It remains possible that dansyl-RFamide might induce a significant withdrawal-like syndrome in nicotine-naive rats at a higher dose. However, dansyl-RFamide clearly precipitates abstinence signs more readily in nicotine-dependent than in nicotine-naive rats.

The frequency profile of dansyl-RFamide-precipitated nicotine abstinence signs generally resembled those observed in spontaneous (38), mecamylamine-precipitated (34) and naloxone-precipitated (33) nicotine abstinence. In each case, the rank ordering was gasps/writhes > teeth chatter/chews > miscellaneous signs (including ptosis) > shakes/tremors. This profile also resembled the frequency profile of abstinence signs precipitated by dansyl-RFamide (19) or dansyl-PQRFamide (32) in morphine-dependent rats (gradually infused with morphine SC for 7 days via Alzet osmotic minipump). The rank order of morphine abstinence signs was the same as that of nicotine abstinence signs, except that shakes/tremors outnumbered miscellaneous signs (19,32). Every type of miscellaneous sign precipitated by dansyl-RFamide (19) or dansyl-PQRFamide (32) in morphine-dependent rats (scratches, ptosis, seminal ejaculation/genital lick and foot lick) was also observed in nicotine-dependent rats injected with dansyl-RFamide. In turn, the profile of morphine abstinence signs precipitated by the NPFF-analogs resembled that precipitated by 0.125 mg/kg naloxone SC (36) or 10 μg naloxone ICV (37) in similarly morphine-infused rats. It remains possible, however, that different methods of dependence induction (concentrated morphine pellets or injection series with escalating doses) would result in a different degree of morphine or nicotine dependence and a different profile of abstinence signs.

The similar effects of NPFF analogs in nicotine-dependent and morphine-dependent rats are consistent with the hypothesis of an endogenous opioid component in nicotine dependence. Nicotine increased plasma beta-endorphin-like immunoreactivity in the rat (5,23) and in smokers (13). Conversely, termination of chronic nicotine exposure in mice resulted in decreased hypothalamic beta-endorphin-like immunoreactivity (47). Similarly, nicotine induced increased plasma levels of enkephalins in the guinea pig (20) and in smokers (45,46). Nicotine also increased the release of enkephalins in the rat brain (6,44). In addition, a number of respiratory (52), neuroendocrine (10,11,21,49), and behaviorally reinforcing (17,24) actions of nicotine appear to be mediated by EOP release, since they are reversible by opiate antagonists.

It is plausible that chronic nicotine-induced EOP release induces an opiate dependence-like state; cessation of nicotine-induced EOP release would then result in an opiate abstinence-like state. Consistent with this hypothesis, nicotine abstinence signs in the rat generally resemble morphine abstinence signs (38), are morphine reversible (33), and are precipitated by naloxone (33); nicotine alleviation of nicotine abstinence signs is naloxone reversible (39). Houdi et al. (22) found that nicotine altered enkephalin release in many of the same brain regions (including the nucleus accumbens, periaqueductal grey, amygdala, locus coeruleus and raphé nuclei) involved in opiate dependence and abstinence syndrome (27). Opiate addicts and heavy cigarette smokers display parallel emotional profiles during abstinence from their respective habits (18). Clonidine, a drug that potently reduces central noradrenergic activity and narcotic abstinence symptoms (1,9,16,53), has also been reported to alleviate discomfort during smoking cessation (14,15,41).

A variety of evidence suggests that NPFF plays a role in opiate dependence and abstinence. NPFF levels in CSF are increased in opiate-tolerant/dependent rats (36). A low dose (2 μg) of NPFF precipitated an opiate abstinence syndrome when injected into the third ventricle of morphine-dependent rats, while a higher dose (15 μg) induced a morphine-reversible quasi-morphine abstinence syndrome in opiate-naive rats (35). Intraventricular injection of IgG from NPFF antiserum reversed morphine dependence, as indicated by prevention of subsequent naloxone-precipitated abstinence syndrome (36, 48); it also reversed morphine tolerance (28). Intraventricular injection of daY8Ramide, a putative NPFF antagonist, produced a similar reversal of morphine dependence (37) and tolerance (29).

The role of NPFF can most conveniently be studied by analogs that cross the blood–brain barrier. Brussaard et al. (4) used the addition of dansyl to increase the lipophilicity of Arg-Phe-amide (the *C*-terminal dipeptide fragment essential to the bioactivity of NPFF and the related invertebrate neuropeptide FMRFamide). Subcutaneous injection of dansyl-RFamide antagonized morphine analgesia (4). Dansyl-RFamide binds specifically to NPFF receptors; it displaced specific binding of the labelled NPFF ligand [^{125}I]-Y8Famide to rat spinal cord membranes in a dose-dependent manner with a K_i of 73 nM (43).

The ability of a dansylated NPFF analog to precipitate nicotine abstinence syndrome as well as morphine abstinence syndrome (19,32) raises the possibility that activation of NPFF could be one mechanism common to opiate and nicotine dependence. Inactivation of NPFF by the related systemically active NPFF antagonist dansyl-PQRamide attenuates

opiate dependence (30). It will be of interest to determine whether inactivation of NPFF will likewise attenuate nicotine dependence.

ACKNOWLEDGEMENTS

This work was supported by NIDA DA08260 (D.H.M., P.I.) and DA06554 (K.B., P.I.), and UHCL Faculty Research Fund.

REFERENCES

1. Aghajanian, G. K. Tolerance of locus coeruleus neurones to morphine and suppression of withdrawal response by clonidine. Nature 276:186–188; 1978.
2. Allard, M.; Geoffe, 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. 500:167–176; 1989.
3. Atherton, E.; Sheppard, R. C. Solid phase peptide synthesis, a practical approach. Oxford: IRL Press; 1989.
4. Brussaard, A. J.; Kits, K. S.; Ter Matt, A.; Mudler, A. H.; Schoffelmeer, N. M. Peripheral injection of DNS-RFa, a FMRFa agonist, suppresses morphine-induced analgesia in rats. Peptides 9:735–739; 1989.
5. Conte-Devolx, B.; Oliver, C.; Giraud, P.; Gillioz, P.; Castanas, E.; Lissitsky, J. C.; Boudouresque, F.; Millet, Y. Effect of nicotine on in vivo secretion of melanocorticotropic hormones in the rat. Life Sci. 28:1067–1073; 1981.
6. Davenport, K. E.; Houdi, A. A.; VanLoon, G. R. Nicotine protects against mu-opioid receptor antagonism by beta-funaltrexamine: Evidence for nicotine-induced release of endogenous opioid. Neurosci. Lett. 113:40–46; 1990.
7. Demichel, P.; Rodriguez, J. C.; Roquebert, J.; Simonnet, G. NPFF, a FMRF-NH_2-like peptide, blocks opiate effects on ileum contractions. Peptides 14:1005–1009; 1993.
8. Felix, A. M.; Wang, C.-T.; Heimer, E. P.; Fournier, A. Applications of BOP reagent in solid phase synthesis II. Solid phase side-chain to side-chain cyclizations using BOP reagent. Int. J. Pept. Protein Res. 31:231–238; 1988.
9. Fielding, S.; Wilker, J.; Hynes, M.; Szewczak, M.; Novick, W. J.; Lal, H. A comparison of clonidine with morphine for antinociceptive and antiwithdrawal actions. J. Pharmacol. Exp. Ther. 207:899–905; 1978.
10. Flores, C. M.; Hulihan-Giblin, B. A.; Hornby, P. J.; Lumpkin, M. D.; Kellar, K. J. Partial Characterization of a neurotransmitter pathway regulating the in vivo release of prolactin. Neuroendocrinology 55:519–528; 1992.
11. Flores, C. M.; Hulihan-Giblin, B. A.; Kellar, K. J. Naltrexone blocks nicotine-induced prolactin release. Neuropharmacology 28:1287–1290; 1989.
12. 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.
13. Gilbert, D. G.; Meliska, C. J.; Williams, C. L.; Jensen, R. A. Subjective correlates of cigarette-induced elevations of β-endorphin and cortisol. Psychopharmacology (Berlin) 106:275–281; 1992.
14. Glassman, A. H.; Jackson, W. K.; Walsh, T.; Roose, S. P.; Rosenfeld, B. Cigarette craving, smoking withdrawal and clonidine. Science 226:864–866; 1984.
15. Glassman, A. H.; Stetner, F.; Walsh, B. T.; Raizman, P. S.; Fleiss, J. L.; Cooper, T. B.; Covey, L. S. Heavy smokers, smoking cessation, and clonidine: Results of a double-blind, randomized trial. JAMA 259:2863–2866; 1988.
16. Gold, M. S.; Redmond, D. E.; Kleber, H. D. Clonidine blocks acute opiate withdrawal symptoms. Lancet 2:599–602; 1978.
17. Gorelick, D. A.; Rose, J.; Jarvik, M. E. Effect of naloxone on cigarette smoking. J. Subst. Abuse 1:153–159; 1989.
18. Gossop, M.; Powell, J.; Grey, S.; Hajek, P. What do opiate addicts and cigarette smokers mean by "craving"? A pilot study. Drug Alcohol Depend. 26:85–87; 1990.
19. Hebert, K. M.; Malin, D. H.; Witherspoon, W. E.; Collins, M.; Harris, K.; Liu, T.-A.; Smith, D. A.; Avera, C.; Ho, K.-K.; Pal, B.; Burgess, K. Subcutaneous dansyl-RFamide, an analog of neuropeptide FF, precipitates morphine abstinence. Int. Behav. Neurosci. Soc. Abstr. 3:65; 1994.
20. Hexum, T. D.; Russett, L. R. Plasma enkephalin-like peptide response to chronic nicotine infusion in guinea pig. Brain Res. 406:370–372; 1987.
21. Hodson, C. A.; Davenport, A.; Price, G.; Burden, H. W. Naltrexone treatment attenuates the inhibitory effect of nicotine on serum LH in rats. Life Sci. 53:839–846; 1993.
22. Houdi, A. A.; Pierzchala, K.; Marson, L.; Palkovits, M.; Van Loon, G. R. Nicotine-induced alteration in Tyr-Gly Gly and Met-enkephalin in discrete brain nuclei reflects altered enkephalin neuron activity. Peptides 12:161–166; 1991.
23. Jensen, R. A.; Gilbert, D. G.; Meliska, C. J.; Landrum, T. A. Characterization of a dose–response curve for nicotine-induced conditioned taste aversion in rats: Relationship to elevation of plasma β-endorphin concentration. Behav. Neural Biol. 53:428–440; 1990.
24. Karras, A.; Kane, J. M. Naloxone reduces cigarette smoking. Life Sci. 27:1541–1545; 1980.
25. Kavaliers, M. Inhibitory influences of mammalian FMRFamide (Phe-Met-Arg-Phe-amide)-related peptides on nociception and morphine- and stress-induced analgesia in mice. Neurosci. Lett. 115:307–312; 1990.
26. Kivipelto, L.; Rubenstein, J.; Yang, H.-Y.T.; Panula, P. Ontogeny of the F8Famide-like (morphine-modulating) peptides in the central nervous system of rats. J. Comp. Neurol. 304:2–21; 1991.
27. Koob, G. F.; Maldonado, R.; Stinus, L. Neural substrates of opiate withdrawal. Trends Neurosci. 15:186–191; 1992.
28. Lake, J. R.; Hammond, M. V.; Shaddox, R. C.; Hunsicker, L. M.; Yang, H.-Y. T.; Malin, D. H. IgG from neuropeptide FF antiserum reverses morphine tolerance in the rat. Neurosci. Lett. 132:29–32; 1991.
29. Lake, J. R.; Hebert, K. M.; Payza, K.; Deshotel, K. D.; Hausam, D. D.; Witherspoon, W. E.; Arcangeli, K. R.; Malin, D. H. Analog of neuropeptide FF attenuates morphine tolerance. Neurosci. Lett. 146:203–206; 1992.
30. Magnuson, D. S. K.; Sullivan, A. F.; Simonnet, G.; Roquest, B. P.; Dickenson, A. H. Differential interactions of cholecystokinin and FLFQPQRF-NH_2 with mu and delta opioid antinociception in the rat spinal cord. Neuropeptides 16:213–218; 1990.
31. Malin, D. H.; Lake, J. R.; Smith, D. A.; Jones, J. A.; Morel, J.; Claunch, A. E.; Stevens, P. A.; Payza, K.; Ho, K.-K.; Liu, J.; Ham, I.; Burgess, D. H. Subcutaneous injection of an analog of neuropeptide FF prevents naloxone-precipitated morphine abstinence syndrome. Drug Alcohol Depend. 40:37–42; 1995.
32. Malin, D. H.; Lake, J. R.; Arcangeli, K. R.; Deshotel, K. D.; Hausam, D. D.; Witherspoon, W. E.; Carter, V. A.; Yang, H.-Y. T.; Pal, B.; Burgess, K. Subcutaneous injection of an analog of neuropeptide FF precipitates morphine abstinence syndrome. Life Sci. 53:PL261–266; 1993.
33. Malin, D. H.; Lake, J. R.; Carter, V. A.; Cunningham, J. S.; Wilson, O. B. Naloxone precipitates nicotine abstinence syndrome in the rat. Psychopharmacology (Berlin) 112:339–342; 1993.
34. Malin, D. H.; Lake, J. R.; Carter, V. A.; Cunningham, J. S.; Wilson, O. B. The nicotine antagonist mecamylamine precipitates nicotine abstinence syndrome in the rat. Psychopharmacology (Berlin) 115:180–184; 1994.
35. 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-NH_2-like mammalian peptide precipitates opiate withdrawal syndrome in the rat. Peptides 11:277–280; 1990.
36. 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-NH_2-like mammalian octapeptide: Possible role in opiate dependence and abstinence. Peptides 11:969–972; 1990.
37. Malin, D. H.; Lake, J. R.; Leyva, J. E.; Hammond, M. V.;

Rogillio, R. B.; Arcangeli, K. R.; Ludgate, K.; Moore, G. M.; Payza, K. Analog of neuropeptide FF attenuates morphine abstinence syndrome. Peptides 12:1011–1014; 1991.
38. Malin, D. H.; Lake, J. R.; Newlin-Maultsby, P.; Roberts, L. K.; Lanier, J. G.; Carter, V. A.; Cunningham, J. S.; Wilson, O. B. A rodent model of nicotine abstinence syndrome. Pharmacol. Biochem. Behav. 43:779–784; 1992.
39. Malin, D. H.; Lake, J. R.; Payne, M. C.; Short, P. E.; Carter, V. A.; Cunningham, J. S.; Wilson, O. B. Nicotine alleviation of nicotine abstinence syndrome is naloxone-reversible. Pharmacol. Biochem. Behav. 53:81–85; 1996.
40. Malin, D. H.; Murray, J. B.; Crucian, G. P.; Schweitzer, F. C.; Cook, R. E.; Skolnick, M. H. Auricular microelectrostimulation: Naloxone reversible attenuation of opiate abstinence syndrome. Biol. Psychiatry 24:886–890; 1988.
41. Ornish, S. A.; Zisook, S.; McAdams, L. A. Effects of transdermal clonidine treatment on withdrawal symptoms associated with smoking cessation. Arch. Int. Med. 148:2027–2031; 1988.
42. Panula, P.; Kivipelto, L.; Nieminen, O.; Majane, E. A.; Yang, H.-Y. T. Neuroanatomy of morphine-modulating peptides. Med. Biol. 65:127–135; 1987.
43. Payza, K.; Akar, C. A.; Yang, H.-Y. T. Neuropeptide FF receptors: Structure-activity relationships and effect of morphine. J. Pharmacol. Exp. Ther. 267:88–94, 1993.
44. Pierzchala, K.; Houdi, A. A.; VanLoon, G. R. Nicotine-induced alterations in brain regional concentrations of native and cryptic Met- and Leu-enkephalin. Peptides 8:1035–1043; 1987.
45. Pomerleau, O. F.; Fertig, J. B.; Seyler, E.; Jaffe, J. Neuroendocrine reactivity to nicotine in smokers. Psychopharmacology (Berlin) 81:61–67; 1983.
46. Pomerleau, O. F. Nicotine and the central nervous system: Biobehavioral effects of cigarette smoking. Am. J. Med. 93(1A):2–8; 1992.
47. Rosecrans, J. A.; Hendry, J. S.; Hong, J. S. Biphasic effects of chronic nicotine treatment on hypothalamic immunoreactive beta-endorphin in the mouse. Pharmacol. Biochem. Behav. 23: 141–143; 1985.
48. Rothman, R. B.; Xu, H.; Yang, H.-Y. T.; Long, J. B. Anti-opioid peptides in morphine tolerance and dependence: Focus on NPFF. In: Hammer, R. P., ed. The neurobiology of opiates. Boca Raton: CRC Press; 1993:147–163.
49. Seckl, J. R.; Johnson, M.; Shakespear, C.; Lightman, S. L. Endogenous opioids inhibit oxytocin release during nicotine-stimulated secretion of vasopressin in man. Clin. Endocrinol. 28: 509–514; 1988.
50. Stewart, J. M.; D. Young. Solid phase peptide synthesis. Rockford, IL: Pierce Chemical Co.; 1984.
51. Tang, J.; Yang, H.-Y. T.; Costa, E. Inhibition of spontaneous and opiate-modified nociception by an endogenous neuropeptide with Phe-Met-Arg-Phe-NH_2-like immunoreactivity. Proc. Natl. Acad. Sci. USA 81:5002–5005; 1984.
52. Tobin, M. J.; Jenouri, G.; Sackner, M. A. Effect of naloxone on change in breathing pattern with smoking. Chest 82:530–537; 1982.
53. Tseng, L.; Loh, H. H.; Wei, E. T. Effects of clonidine on withdrawal signs in the rat. Eur. J. Pharmacol. 30:93–99; 1975.
54. 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.