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Nicotine Alleviation of Nicotine Abstinence Syndrome Is Naloxone-Reversible

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MALIN, D. H., J. R. LAKE, M. C. PAYNE, P. E. SHORT, V. A. CARTER, J. S. CUNNINGHAM AND O. B. WILSON. *Nicotine alleviation of nicotine abstinence syndrome is naloxone-reversible.* PHARMACOL BIOCHEM BEHAV **53**(1) 81–85, 1996. – In a recently introduced rodent model of nicotine abstinence syndrome, the observed signs closely resembled those typical of rat opiate abstinence syndrome. Signs were precipitated by naloxone and potently reversed by morphine as well as nicotine itself, suggesting that nicotine might relieve nicotine abstinence syndrome through releasing endogenous opioids. To test this hypothesis, rats were continuously infused subcutaneously (SC) for 7 days with 9 mg/kg per day nicotine tartrate. Each rat was observed for abstinence signs at 18 and 21 h after termination of infusion. Three minutes before the 21-h test, all rats received 0.35 mg/kg nicotine tartrate, SC; 5 min before the nicotine injection, subjects received 9 or 4.5 mg/kg naloxone or saline alone, SC. Abstinence reversal scores were calculated as signs at 21 h as a percentage of signs at 18 h. Naloxone prevented nicotine alleviation of nicotine abstinence in a dose-related manner. However, naloxone in the absence of a nicotine injection had no effect on abstinence severity in either highly dependent or moderately dependent rats (infused with 9 or 5 mg/kg per day nicotine tartrate, respectively). These results support the hypothesis that endogenous opioids play a role in nicotine dependence and abstinence.

Nicotine Nicotine dependence Nicotine abstinence Nicotine withdrawal Opiates Naloxone Opiate antagonists Endogenous opioid peptides

RECENTLY, a rodent model of nicotine abstinence syndrome has been developed based on continuous subcutaneous (SC) infusion of nicotine tartrate and observing the frequency of spontaneous behavioral signs following either termination of infusion (21), or injection of the nicotinic antagonist mecamylamine (18). 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 vehicle-infused rats (21). However, any possible role of tartrate in the effects of nicotine tartrate infusion remains to be evaluated by studies of rats chronically infused with tartrate alone.

The observed nicotine abstinence signs closely resembled those seen in opiate abstinence syndrome (7,20). This raised the possibility that the release of endogenous opioid peptides by nicotinic receptor stimulation (4,11,13,14,23–25) resulted in chronic overstimulation of opiate receptors; this might induce an opiate dependence-like state, contributing to nicotine dependence. Cessation of nicotinic receptor stimulation would then result in decreased opiate receptor stimulation and an opiate abstinence-like state. Consistent with this hypothesis, morphine potently reversed nicotine abstinence syndrome (19). Conversely, the opiate antagonist naloxone precipitated an immediate abstinence syndrome in nicotine-dependent rats (19). These results suggest the possibility that nicotine might relieve nicotine abstinence, in part, through inducing the release of endogenous opioid peptides, thus alleviating the hypothesized opiate abstinence-like state.

The present study tested this hypothesis by determining whether pretreatment with the opiate antagonist naloxone could prevent nicotine alleviation of nicotine abstinence syn-

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drome. As a control measure, the study also assessed the effect of naloxone on nicotine abstinence syndrome in the absence of a subsequent nicotine injection.

EXPERIMENT 1: DOSE-RELATED EFFECT OF NALOXONE ON NICOTINE ALLEVIATION OF NICOTINE ABSTINENCE SIGNS

This experiment determined the effect of pretreatment with saline alone and two different doses of naloxone HCl on the alleviation of nicotine abstinence signs by SC nicotine injection.

Method

Subjects. The subjects were 25 male Sprague–Dawley rats, weighing 324–434 g and maintained on ad lib food and water and a 12 L : 12 D cycle.

Dependence induction. Each rat was implanted SC under halothane anesthesia with one Alzet (Alza Corp., Palo Alto, CA) 2ML1 osmotic minipump filled with nicotine tartrate [−] isomer in saline. The concentration of nicotine tartrate was adjusted for differences in subject weights, but it approximated 13 mg/ml, resulting in continuous SC infusion at the rate of 9 mg/kg per day for approximately 7 days. The minipumps were removed 164 h after pump implantation. These parameters have previously resulted in a high degree of dependence, as indicated by large numbers of subsequent abstinence signs (18,19,21).

Behavioral observations. All rats were habituated to a 45 × 45 × 30-cm clear plastic observation chamber at 24 h before termination of nicotine infusion and at 4 h after termination. To assess the effects of naloxone and nicotine injections on abstinence signs, each rat was observed at 18 h following termination of nicotine infusion (preinjection) and at 21 h (postinjection). These intervals were chosen because previous studies (19,21) have observed high numbers of nicotine abstinence signs from 16–24 h following termination of nicotine infusion. All observations were performed under "blind" conditions for 15 min. Observers counted the frequency of signs on a checklist of nicotine abstinence signs developed and validated by Malin et al. (21). This checklist had been slightly modified from standard checklists of opiate abstinence signs (7,20). Signs included instances of teeth chatters/chews, writhes/gasps, ptosis, shakes/tremors, and miscellaneous less frequent signs.

Each rat's abstinence alleviation score was its number of postinjection abstinence signs as a percentage of its preinjection signs. Thus, a score of 100% would indicate no alleviation, whereas a score of 0% would indicate complete alleviation.

Injections. Three minutes before the second (21-h) observation, all subjects were injected with 0.35 mg/kg nicotine tartrate, SC. A similar dose has been shown largely to reverse nicotine abstinence syndrome (21). Five minutes before the nicotine injection, rats were pretreated with an SC injection of 9 mg/kg naloxone in saline (n = 8), 4.5 mg/kg naloxone in saline (n = 9), or saline alone (n = 8). The dose of 4.5 mg/kg naloxone has previously been shown to precipitate nicotine abstinence signs in rats gradually and continuously infused with nicotine (19). It seemed possible that the acutely administered nicotine in the present study might induce more intense endorphinergic activity. Therefore, a twofold dose increase of naloxone was also explored. High doses of naloxone (up to 10 mg/kg) have been required to disrupt certain actions mediated by endogenously released opioid peptides, such as stress-induced analgesia (1,2,17), as opposed to actions of exogenously administered opiates. In none of these studies did the high doses of naloxone precipitate an abstinence-like syndrome in nondependent rats.

Results

Figure 1 displays numbers of postinjection abstinence signs as a percentage of preinjection signs in rats receiving SC injections of saline alone, 4.5 mg/kg naloxone, or 9 mg/kg naloxone, followed in all cases by 0.35 mg/kg nicotine tartrate. Linear trend analysis revealed a significant positive trend of percentage scores (less abstinence alleviation) as a function of naloxone dose [$F(1, 22) = 16.45$, $p < 0.01$]. Departure from linearity was not significant [$F(1, 22) = 1.42$, NS]. Posthoc comparisons (Tukey's HSD) revealed that the 9-mg/kg naloxone group was significantly different from the saline group ($p < 0.01$) and the 4.5-mg/kg naloxone group ($p < 0.05$). These results were not distorted by major differences in preinjection abstinence scores, which were 25.8 ± 3.4 (mean ± SEM) in rats later receiving saline alone, 25.4 ± 4.6 in rats later receiving 4.5 mg/kg naloxone, and 26.4 ± 5.8 in rats later receiving 9 mg/kg naloxone. One-way analysis of variance (ANOVA) revealed no significant baseline differences among groups [$F(2, 22) = 0.01$, NS].

Figure 2 shows numbers of postinjection signs grouped by individual categories. (Abstinence alleviation scores were not used to avoid dividing by zero when an individual rat had a zero preinjection score in one category.) All types of signs, except for shakes and tremors, showed a dose-related increase. According to Dunnett's procedure for multiple post hoc comparisons with a single control group, the 9-mg/kg naloxone group was significantly different from saline controls in teeth chatters/chews ($p < 0.01$), writhes/gasps ($p < 0.05$), and ptosis ($p < 0.01$).

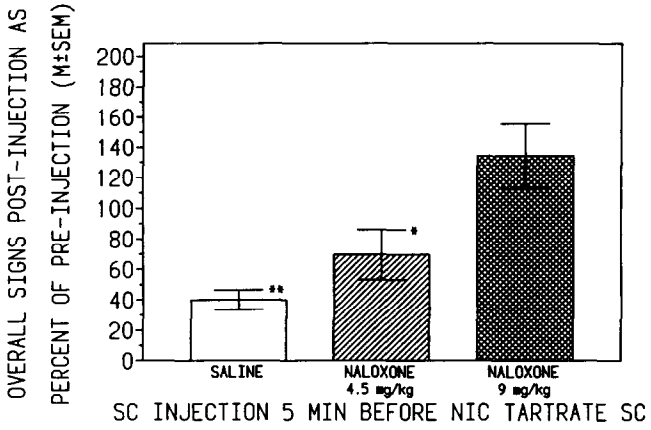


FIG. 1. Naloxone dose-dependently attenuates the alleviation of nicotine abstinence syndrome by an SC nicotine injection in highly dependent rats. Bars show overall nicotine abstinence signs at 21 h after termination of 7 days SC infusion of 9 mg/kg per day nicotine tartrate as a percentage of signs at 18 h after termination of infusion (mean ± SEM). Each rat was injected SC 3 min before the 21-h retest with 0.35 mg/kg nicotine tartrate. Five minutes before nicotine tartrate injection, rats were injected SC with saline alone (open bar), 4.5 mg/kg naloxone HCl (hatched bar), or 9 mg/kg naloxone HCl (cross-hatched bar). *$p < 0.05$ **$p < 0.01$ vs. 9-mg/kg naloxone group.

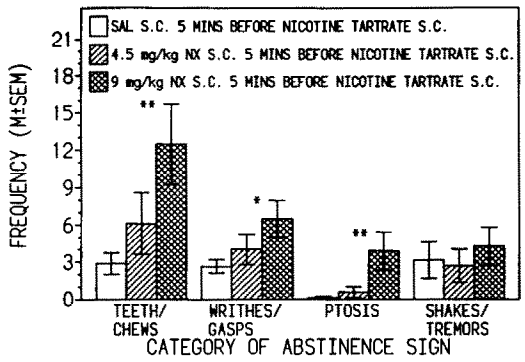


FIG. 2. Individual categories of abstinence signs (mean ± SEM) 21 h after termination of 7 days SC infusion of 9 mg/kg nicotine tartrate. Each rat was injected SC 3 min before the 21-h retest with 0.35 mg/kg nicotine tartrate. Five minutes before nicotine tartrate injection, rats were injected SC with saline alone (open bar), 4.5 mg/kg naloxone HCl (hatched bar), or 9 mg/kg naloxone HCl (cross-hatched bar). $*p < 0.05$, $**p < 0.01$ vs. saline group.

EXPERIMENT 2: NALOXONE ALONE FAILS TO ALTER NICOTINE ABSTINENCE IN HIGHLY DEPENDENT RATS

The results of Experiment 1 are susceptible to several interpretations. Naloxone may interfere with the abstinence-alleviating actions of nicotine-induced endorphin release. Alternatively, naloxone may independently intensify nicotine abstinence syndrome, compensating for attenuation of the syndrome by nicotine. This experiment determined whether the higher dose of naloxone intensifies nicotine abstinence syndrome in the absence of a SC nicotine injection.

Method

The methods were the same as in Experiment 1, with the following exceptions. The subjects were 14 male Sprague–Dawley rats infused as above with 9 mg/kg per day nicotine tartrate for 7 days. All rats received a SC injection of saline alone 3 min before the postinjection observation; no rats received a nicotine injection. Five minutes before the saline injection, rats were pretreated with SC injections of 9 mg/kg naloxone in saline or saline alone.

Results

Figure 3 displays postinjection abstinence signs as a percentage of preinjection signs in highly dependent rats receiving SC injections of 9 mg/kg naloxone or saline alone, followed in both cases by an injection of saline. In the absence of nicotine, there was no significant difference in abstinence alleviation scores between the naloxone and saline groups [$t(12) = 0.38$, NS]. Again, the preinjection abstinence signs were similar in the two groups: 28.4 ± 6.3 in rats later injected with saline and 26.7 ± 8.38 in rats later injected with naloxone.

EXPERIMENT 3: NALOXONE ALONE FAILS TO ALTER NICOTINE ABSTINENCE IN MODERATELY DEPENDENT RATS

It is possible that naloxone may have failed to intensify nicotine abstinence syndrome in Experiment 2 because of a "ceiling effect": the abstinence signs may have been near-maximal even in the absence of naloxone. This experiment determined whether naloxone could intensify the submaximal nicotine abstinence syndrome resulting from a lower rate of chronic nicotine infusion.

Method

The methods were identical to those of Experiment 2, except that the 14 subjects were infused for 7 days with 5 mg/kg per day nicotine tartrate to induce a lesser degree of dependence and lower numbers of subsequent abstinence signs.

Results

As expected, there were far fewer preinjection abstinence signs following the 5 mg/kg per day nicotine tartrate infusion rate than in previous experiments employing the 9 mg/kg per day infusion rate. There were only 12.9 ± 2.2 signs in rats following the low nicotine infusion rate, compared with 27.6 ± 5.0 signs in rats following the high nicotine infusion rate in Experiment 2. This difference was significant [$t(26) = 2.67$, $p < 0.01$].

Figure 4 shows postinjection abstinence signs as a percentage of preinjection signs in moderately dependent rats receiving SC injections of 9 mg/kg naloxone or saline alone, followed in both cases by an injection of saline. In the absence of a nicotine injection, there was no significant difference in abstinence alleviation scores between the naloxone and saline groups [$t(12) = 0.12$, NS]. Once more, the preinjection signs were similar in the two injection groups: 13.6 ± 3.3 in rats later injected with saline and 12.1 ± 3.1 in rats later injected with naloxone.

GENERAL DISCUSSION

As in a previous study (21), SC injection of nicotine alleviated nicotine abstinence signs. In the present study, pre-

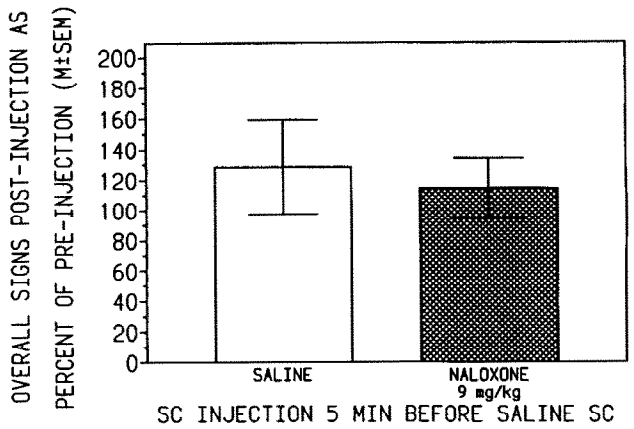


FIG. 3. Naloxone by itself failed to alter nicotine abstinence syndrome in highly dependent rats. Bars show overall nicotine abstinence signs at 21 h after termination of 7 days SC infusion of 9 mg/kg per day nicotine tartrate as a percentage of signs at 18 h after termination of infusion (mean ± SEM). Each rat was injected SC 3 min before the 21-h retest with saline alone. Five minutes before the saline injection, rats were injected SC with saline alone (open bar) or 9 mg/kg naloxone HCl (cross-hatched bar). Note that two saline injections (open bar) had virtually the same effect as nicotine tartrate in addition to the higher dose of naloxone (Fig. 1, cross-hatched bar).

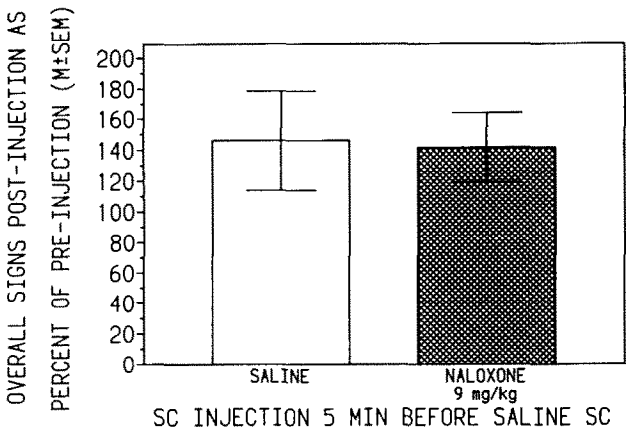


FIG. 4. Naloxone by itself failed to alter nicotine abstinence syndrome in moderately dependent rats. Bars show overall nicotine abstinence signs at 21 h after termination of 7 days SC infusion of 5 mg/kg per day nicotine tartrate as a percentage of signs at 18 h after termination of infusion (mean ± SEM). Each rat was injected SC 3 min before the 21-h retest with saline alone. Five minutes before the saline injection, rats were injected SC with saline alone (open bar) or 9 mg/kg naloxone HCl (cross-hatched bar).

treatment with the opiate antagonist naloxone interfered in a dose-dependent manner with nicotine's reversal of nicotine abstinence syndrome. The high dose of naloxone totally reversed the abstinence-alleviating action of 0.35 mg/kg nicotine; these rats (cross-hatched bar in Fig. 1) had virtually the same percentage change in signs as rats receiving two injections of saline alone (open bar in Fig. 3).

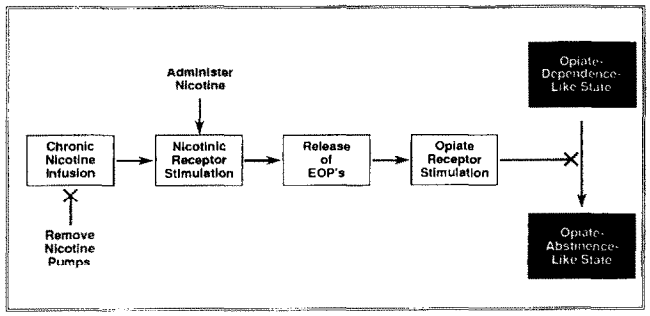
Did naloxone act by preventing the effects of the nicotine injection, or by independently intensifying nicotine abstinence, thereby offsetting the abstinence-alleviating effect of nicotine? Naloxone, in the absence of a subsequent nicotine injection, did not exacerbate nicotine abstinence syndrome in highly dependent rats (previously infused for 7 days with 9 mg/kg per day nicotine tartrate). This result cannot be attributed to a "ceiling effect," as the same result was seen in less dependent rats (previously infused for 7 days with 5 mg/kg per day nicotine tartrate) exhibiting far fewer preinjection abstinence signs. Therefore, naloxone appears to act primarily by interfering with an opioid effect of the nicotine injection.

These results are consistent with the hypothesis (Fig. 5) that nicotine might relieve nicotine abstinence, in part, through inducing the release of endogenous opioid peptides (EOPs). Nicotinic activation of endogenous opioid mechanisms is a well-established phenomenon. Nicotine increases plasma β-endorphin-like immunoreactivity in the rat (3,14) and in smokers (8). Conversely, termination of chronic nicotine exposure results in decreased hypothalamic β-endorphin-like immunoreactivity (26). Similarly, nicotine induces increased plasma levels of enkephalins in the guinea pig (11) and in smokers (24,25). Nicotine also increases the release of enkephalins in the rat brain (4,23).

A number of respiratory (28) and neuroendocrine (5,6, 12,27) actions of nicotine appear to be mediated by EOP release, because they are reversible by opiate antagonists. The reinforcing effects of nicotine may also be partially mediated by EOPs, as opiate antagonists have been reported to interfere with smoking behavior (9,15). However, Nemeth-Coslett and Griffiths (22) failed to find such an effect.

In view of these findings, it seems plausible that chronic nicotine exposure results in chronic release of EOPs with resulting overstimulation of brain opiate receptors. Might such overstimulation induce an opiate dependence-like state, which would then result in an opiate abstinence-like state following cessation of stimulation? Several results are at least consistent with this hypothesis. Houdi et al. (13) found that nicotine altered enkephalin release in many of the same brain regions (including the nucleus accumbens, periaquaductal grey, amygdala, locus coeruleus, and raphé nuclei) that are involved in opiate dependence and abstinence syndrome (16). It has been reported that opiate addicts and heavy cigarette smokers display parallel emotional profiles during abstinence from their respective habits (10). In rats, the spontaneous behavioral signs of nicotine abstinence bear a strong resemblance to those of mild to moderate morphine abstinence (21). Like morphine

A. Hypothetical Mechanism for Nicotine Alleviation of Nicotine Abstinence Signs



B. Predicted Naloxone-Reversal of Nicotine Alleviation of Nicotine Abstinence Signs

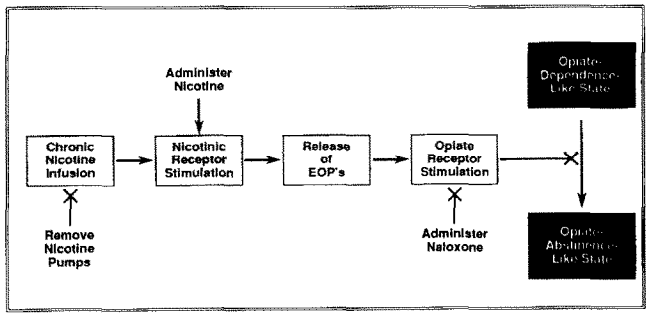


FIG. 5. (A) Hypothetical chain of events by which nicotine might reverse nicotine abstinence syndrome, in part through release of endogenous opioid peptides (EOPs). It is assumed that the organism has already received chronic nicotine exposure, resulting in chronic EOP release, chronic opiate receptor overstimulation, and an opiate-dependence-like state. The dependence state is expressed as an abstinence state unless renewed EOP release is induced by renewed nicotine administration. (B) By blocking opiate receptors, naloxone hypothetically disrupts the chain of events triggered by nicotine injection. This would explain the ability of naloxone to prevent nicotine alleviation of nicotine abstinence signs.

abstinence syndrome, nicotine abstinence signs in the rat can be precipitated by the opiate antagonist naloxone and potently alleviated by injection of morphine (19). The apparent involvement of endogenous opioids in nicotine's reversal of nicotine abstinence further suggests a critical role for endogenous opioids in nicotine dependence.

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REFERENCES

1. Amir, S.; Amit, Z. Endogenous opioid ligands may mediate stress-induced changes in the affective properties of pain related behavior in rats. Life Sci. 23:1143–1152; 1978.
2. Bodnar, R. J.; Kelly, D. D.; Spiaggia, A.; Ehrenberg, C.; Glusman, M. Dose-dependent reductions by naloxone of analgesia induced by cold-water stress. Pharmacol. Biochem. Behav. 8: 667–672; 1978.
3. 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.
4. 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.
5. 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.
6. Flores, C. M.; Hulihan-Giblin, B. A.; Kellar, K. J. Naltrexone blocks nicotine-induced prolactin release. Neuropharmacology 28:1287–1290; 1989.
7. Gianutsos, G.; Drawbaugh, R.; Hynes, M.; Lal, H. The narcotic withdrawal syndrome in the rat. In: Ehrenpreis, S.; Neidle, A., eds. Methods in narcotic research. New York: Marcel Dekker; 1975:293–309.
8. Gilbert, D. G.; Meliska, C. J.; Williams, C. L.; Jensen, R. A. Subjective correlates of cigarette-induced elevations of beta-endorphin and cortisol. Psychopharmacology 106:275–281; 1992.
9. Gorelick, D. A.; Rose, J.; Jarvik, M. E. Effect of naloxone on cigarette smoking. J. Subst. Abuse 1:153–159; 1989.
10. 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.
11. Hexum, T. D.; Russett, L. R. Plasma enkephalin-like peptide response to chronic nicotine infusion in guinea pig. Brain Res. 406:370–372; 1987.
12. 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.
13. 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.
14. 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.
15. Karras, A.; Kane, J. M. Naloxone reduces cigarette smoking. Life Sci. 27:1541–1545; 1980.
16. Koob, G. F.; Maldonado, R.; Stinus, L. Neural substrates of opiate withdrawal. Trends Neurosci. 15:186–191; 1992.
17. Lewis, J. W.; Cannon, J. T.; Liebeskind, J. C. Opioid and nonopioid mechanisms of stress analgesia. Science 208:623–625; 1980.
18. Malin, D. H.; Lake, J. R,; Carter, V. A.; Cunningham, J. S.; Wilson, O. B. Naloxone precipitates nicotine abstinence syndrome in the rat. Psychopharmacology 112:339–342; 1993.
19. 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 115:180–184; 1994.
20. 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.
21. 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.
22. Nemeth-Coslett, R.; Griffiths, R. R. Naloxone does not affect cigarette smoking. Psychopharmacology 89:261–264; 1986.
23. 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.
24. Pomerleau, O. F. Nicotine and the central nervous system: Biobehavioral effects of cigarette smoking. Am. J. Med. 93:2–8; 1992.
25. Pomerleau, O. F.; Fertig, J. B.; Seyler, E.; Jaffe, J. Neuroendocrine reactivity to nicotine in smokers. Psychopharmacology 81: 61–67; 1983.
26. 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.
27. 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.
28. Tobin, M. J.; Jenouri, G.; Sackner, M. A. Effect of naloxone on change in breathing pattern with smoking. Chest 82:530–537; 1982.