Pharmacology Biochemistry and Behavior, Vol. 58, No. 3, pp. 695–699, 1997

0091-3057/97 $17.00 + .00

ELSEVIER

PII S0091-3057(97)00006-3

Nicotine Abstinence Syndrome Precipitated by Central But Not Peripheral Hexamethonium

DAVID H. MALIN, J. RONALD LAKE, CHRISTINA K. SCHOPEN, JOHN W. KIRK, ELIZABETH E. SAILER, BRENT A. LAWLESS, TIMOTHY P. UPCHURCH, MERLE SHENOI AND NANCY RAJAN

University of Houston–Clear Lake, Houston, TX 77058

Received 3 September 1996; Revised 24 January 1997; Accepted 24 January 1997

MALIN, D. H., J. R. LAKE, C. K. SCHOPEN, J. W. KIRK, E. E. SAILER, B. A. LAWLESS, T. P. UPCHURCH, M. SHENOI AND N. RAJAN. *Nicotine abstinence syndrome precipitated by central but not peripheral hexamethonium.* PHARMACOL BIOCHEM BEHAV **58**(3) 695–699, 1997.—A rodent model of nicotine dependence has been developed based on continuous subcutaneous (SC) infusion of nicotine tartrate. Nicotine abstinence syndrome was precipitated by SC injection of the nicotinic antagonist mecamylamine, which freely crosses the blood–brain barrier. In contrast, the nicotinic antagonist hexamethonium crosses the blood–brain barrier very poorly. This study determined whether central or peripheral administration of hexamethonium could precipitate nicotine abstinence. In the first experiment, 26 nicotine-dependent rats were injected SC with 0.5, 5 or 10 mg/kg hexamethonium dichloride or saline alone and observed for 20 min. Few abstinence signs were observed in any group; there was no significant drug effect. In the second experiment, 18 rats were cannulated in the third ventricle and rendered nicotine dependent. One week later, rats were injected through the cannula with 12 or 18 ng hexamethonium or saline alone and observed for 20 min. Both dose groups differed significantly from the saline-injected group, and there was a significant positive linear trend of signs as a function of dose. The high dose had no significant effect in 14 nondependent rats. We conclude that hexamethonium is much more potent by the central route, and there is a major central nervous system component in nicotine dependence. © 1997 Elsevier Science Inc.

Nicotine Nicotine dependence Nicotine abstinence Hexamethonium Nicotinic receptor antagonists Rat

IN a rodent model of nicotine abstinence syndrome (34), nicotine dependence is induced by 7 days of continuous subcutaneous (SC) infusion of nicotine tartrate in the rat. Behavioral abstinence signs develop gradually following termination of nicotine infusion (34); abstinence signs are precipitated immediately by SC injection of the nicotinic antagonist mecamylamine (29). The model has met a number of validity criteria, including potent reversibility of abstinence signs by nicotine injection and comparative lack of signs in saline-infused rats (34). Recently, there has been independent confirmation of several phenomena connected with this model: spontaneous nicotine abstinence syndrome, nicotine alleviation of nicotine abstinence, and mecamylamine precipitation of nicotine abstinence (20).

The existence of a laboratory model of nicotine dependence and abstinence allows exploration of the underlying mechanisms of these phenomena. Such explorations in our laboratory have been guided by the hypothesis of common mechanisms underlying opiate and nicotine dependence (36). Nicotinic receptor stimulation induces release of endogenous opioid peptides (10,14,19,22,44,46,50). Nicotine abstinence syndrome in the rat involves many of the same behavioral signs as morphine abstinence syndrome (30,34,35,37). Nicotine abstinence signs are potently morphine reversible and can be precipitated by SC naloxone (30). Naloxone also prevents nicotine alleviation of nicotine abstinence (35).

One of the most basic questions regarding nicotine dependence remains to be addressed: Is nicotine abstinence triggered by reduced nicotinic receptor stimulation in the central or peripheral nervous system? Various behavioral and physiological actions of nicotinic, such as modulation of feeding (26,47) and regulation of heart rate and blood pressure (21), may involve peripheral and central mechanisms. Nicotinic antagonists that do not readily cross the blood–brain barrier should be useful in differentiating central and peripheral

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

mechanisms of nicotine dependence. In contrast to mecamylamine, hexamethonium penetrates the blood–brain barrier very poorly (16). However, small amounts of hexamethonium may enter the cerebrospinal fluid or brain tissue following peripheral administration (3,27). Peripheral administration of mecamylamine, but not hexamethonium, has been reported to reverse many centrally mediated actions of nicotine, including tail tremor, locomotor hyperactivity, reductions in body weight and neuroendocrine changes (7,15,40,47,49). In contrast, central administration of hexamethonium antagonized the central actions of nicotine on blood pressure, heart rate, nociception, thermal regulation, salivation and modulation of motor reflexes (1,13,17,18,23).

The present experiments tested the relative ability of hexamethonium to precipitate nicotine abstinence syndrome by peripheral (SC) and central (third ventricle) administration. The specific brain regions responsible for the expression of nicotine dependence and abstinence in the rat have not been clearly identified. The third ventricle was chosen as the injection site based on the hypothetical relationship previously noted between nicotine and opiate abstinence syndromes. This ventricle is close to a number of brain regions believed to be critical for precipitation of morphine abstinence in the rat (24). Third ventricle injection of naloxone (33) or the antiopiate peptide NPFF (31) potently precipitated morphine abstinence sydrome. Third ventricle injection of naloxone precipitated the most rapid and numerous morphine abstinence signs when compared with other ventricular injection sites (32). In addition, ventricular injection allows the drug to spread to many brain regions adjacent to the ventricular system rather than being restricted to a specific brain site, as would be the case with a tissue injection.

EXPERIMENT 1: PERIPHERALLY ADMINISTERED HEXAMETHONIUM FAILS TO PRECIPITATE NICOTINE ABSTINENCE SYNDROME

This experiment determined whether nicotine abstinence syndrome would be precipitated by SC injection of hexamethonium. Doses of hexamethonium ranging from 0.5 to 10 mg/kg have frequently been used to evaluate the peripheral component of nicotine effects on behavior or neuroendocrine activity in rats (4,8,12,25,26,41,43,45,47–49). The present study tested three doses of hexamethonium within this range to evaluate the dose relatedness of any abstinence-precipitating effect.

Method

Animals. The subjects were 26 male Sprague–Dawley rats, weighing 330–377 g. All subjects were maintained on ad libitum food and water and a 12-h light–dark cycle.

Drug treatments. Animals were implanted SC under halothane anesthesia with one Alzet 2ML1 osmotic minipump and rendered dependent by 7 days of continuous infusion of 9 mg/kg/day nicotine tartrate (−)isomer in saline. On day 7 of infusion (approximately 164 h after pump implantation), subjects were randomly divided into four groups consisting of 6 or 7 rats each. Subjects were challenged with 0.5, 5 or 10 mg/kg hexamethonium dichloride (RBI, Natick, MA) SC or with saline alone.

Behavioral observations. Immediately following the SC injection, each subject was observed for 20 min in a clear plastic rectangular chamber measuring 48 × 38 × 20 cm. All observations were performed under "blind" conditions. Observers tallied each occurrence of behavioral signs based on a standard checklist of nicotine abstinence signs developed and validated by Malin et al. (34). Categories included gasps/abdominal writhes, teeth chatter/chews, wet shakes/tremors and miscellaneous less frequent signs (scratches, ptosis, yawns, seminal ejaculations/genital licks, escape jumps). Ptosis was counted no more than once per minute and continuous teeth chattering no more than once every 15 s. Each rat's overall abstinence score consisted of abstinence signs cumulated across all categories.

Results

Figure 1 shows the overall nicotine abstinence signs precipitated by 0.5, 5 or 10 mg/kg hexamethonium SC or by saline alone. There were few signs in all four treatment groups. One-way analysis of variance (ANOVA) indicated no significant effect of hexamethonium dose [$F(3, 22) = 1.91$, NS]. According to Dunnett's procedure for multiple post hoc comparisons with a single control group, none of the hexamethonium dose groups differed significantly from the saline-injected controls.

EXPERIMENT 2: CENTRALLY ADMINISTERED HEXAMETHONIUM PRECIPITATES NICOTINE ABSTINENCE SYNDROME

This experiment determined whether third ventricle injection of hexamethonium precipitates nicotine abstinence syndrome. It also determined whether centrally administered hexamethonium induces signs more readily in nicotine-dependent rats than in nondependent rats, as would be expected in a true nicotine abstinence syndrome.

Method

Eighteen male Sprague–Dawley rats, weighing 373–445 g, were stereotaxically cannulated in the third ventricle under equithesin anesthesia. The coordinates were 0.2 anterior, 0.0 lateral and 9.2 inferior to skull bregma. Cannula placements were confirmed subsequent to the experiment by injection of methylene blue dye. Under the same anesthesia, the rats were implanted SC with an Alzet 2ML1 osmotic minipump and rendered dependent by 7 days of continuous infusion of 9 mg/kg/day nico-

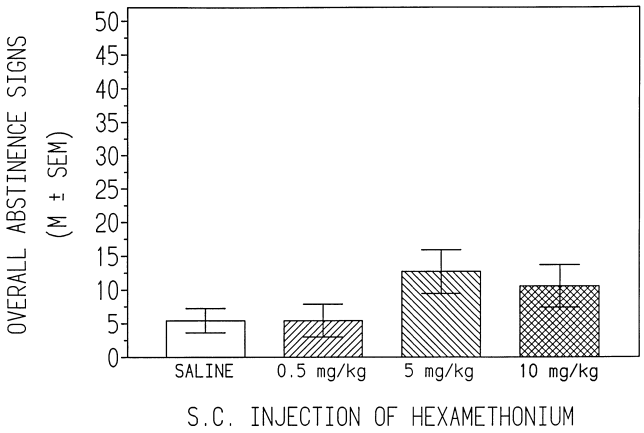

FIG. 1. Overall abstinence signs (mean ± SEM), cumulated across all categories, in nicotine-dependent rats injected SC with saline only or with 0.5, 5 or 10 mg/kg hexamethonium dichloride. There were no significant differences between any of the hexamethonium dose groups and those injected with saline alone.

tine tartrate (−)isomer in saline. Rats were housed individually following surgery.

After 7 days of nicotine infusion, the 18 rats were randomly divided into three equal groups. Subjects were challenged by third ventricle injection of either 12 or 18 ng hexamethonium dichloride in 20 μl saline or saline alone. These doses were selected on the basis of small pilot experiments. Injections were gradually infused via motorized syringe at the rate of 4 μl/min. The injection rate and volume were consistent with procedures for precipitating morphine abstinence syndrome by third ventricle infusion of opiate antagonists or antiopiate peptides (31). Subjects were observed for abstinence signs under "blind" conditions for 20 min, beginning with the onset of the injection.

To assess the effect of hexamethonium on nondependent subjects, 14 cannulated rats, weighing 386–447 g, were implanted SC with an Alzet osmotic minipump filled with saline alone. After 7 days of continuous saline infusion, 7 rats were challenged by third ventricle injection of 18 ng hexamethonium dichloride in 20 μl saline and 7 were challenged by saline alone. Each rat was then observed over 20 min for abstinence-like behavioral signs.

Results

Figure 2A shows overall abstinence signs precipitated by third ventricle injection of 12 or 18 ng hexamethonium or saline alone in nicotine-dependent rats. One-way ANOVA indicated a significant effect of hexamethonium dose [$F(2, 15) = 7.13$, $p < 0.01$]. Linear trend analysis indicated a significant positive trend of abstinence signs as a function of dose [$F(1, 15) = 14.24$, $p < 0.01$]. According to Dunnett's procedure for multiple post hoc comparisons with a single control group, the low-dose group differed significantly from the saline-injected group ($p < 0.05$), as did the high-dose group ($p < 0.01$).

Figure 3 shows the occurrence of various categories of abstinence signs. One-way ANOVA revealed a significant effect of hexamethonium dose on shakes/tremors [$F(2, 15) = 6.30$, $p < 0.05$]. According to Dunnett's procedure, only the high-dose group differed significantly from saline controls ($p < 0.01$). The effect of hexamethonium dose on chews/teeth chatter closely approached significance [$F(2, 15) = 3.25$, $0.05 < p < 0.10$]; only the high-dose group differed significantly from controls ($p < 0.05$). The effect of hexamethonium dose on gasps/writhes also approached significance [$F(2, 15) = 2.24$, $0.05 < p < 0.10$]. The difference between both the high- and low-dose groups and the control group approached significance ($0.05 < p < 0.10$). There was no significant effect of hexamethonium dose on miscellaneous less frequent signs [$F(2, 15) = 1.12$, NS].

The nondependent rats challenged with centrally administered hexamethonium actually had fewer abstinence-like signs than the nondependent rats challenged with saline alone (Fig. 2B). This difference was not significant [$t(12) = 1.42$, NS].

DISCUSSION

Peripheral administration of hexamethonium over a wide dose range failed to precipitate a significant nicotine abstinence syndrome. This result does not totally eliminate the possibility of some partial peripheral component in nicotine dependence. Still higher SC doses of hexamethonium may have precipitated some abstinence signs. However, this possibility would be difficult to evaluate because much higher doses would largely inactivate the autonomic nervous system, resulting in gross physiological abnormalities (11). Subcutaneously administered hexamethonium also may have a gradual or delayed effect. However, continued observation of many of the subjects for an additional 10 min did not result in markedly altered ratios of overall signs among the saline and three hexamethonium dose groups.

In contrast, third ventricle injection of hexamethonium precipitated significant numbers of abstinence signs in a dose-related manner. The profile of abstinence signs generally resembled that seen in spontaneous (34) and mecamylamine-precipitated (29) nicotine abstinence syndromes. With hexamethonium, however, there were fewer instances of teeth chatter/chews and more shakes/tremors. Hexamethonium was extremely potent by the central route. The highly effective 18 ng central dose was 195,000 times smaller than the ineffective 10 mg/kg peripheral dose. Thus, although the data do not totally rule out a peripheral component in nicotine depen-

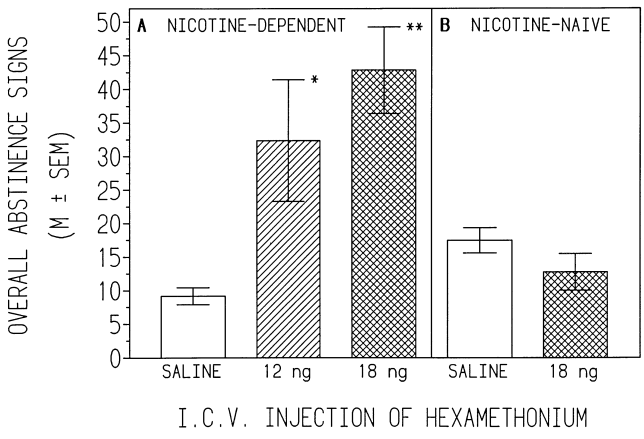

FIG. 2. A: Overall abstinence signs (mean ± SEM), cumulated across all categories, in nicotine-dependent rats injected in the third ventricle (i.c.v.) with saline only or with 12 or 18 ng hexamethonium dichloride. $*p < 0.05$, $**p < 0.01$ vs. saline (Dunnett's test). B: Overall abstinencelike signs in nondependent rats injected in the third ventricle with saline only or 18 ng hexamethonium dichloride.

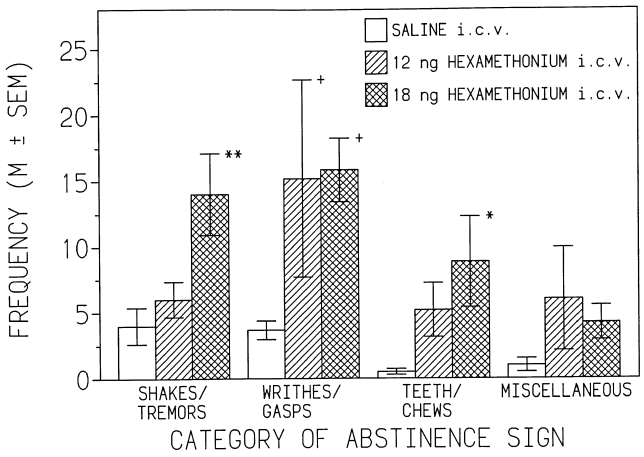

FIG. 3. Individual categories of nicotine abstinence signs precipitated by third ventricle (i.c.v.) injection of saline alone or 12 or 18 ng hexamethonium dichloride in nicotine-dependent rats. $+0.05 < p < 0.10$, $*p < 0.05$, $**p < 0.01$ vs. saline (Dunnett's test).

dence and abstinence, they strongly suggest a major central nervous system component.

Another issue regarding the rodent model of nicotine dependence and abstinence concerns the specificity of these phenomena to events at the nicotinic receptor. Mecamylamine is primarily a noncompetitive nicotinic antagonist (9,38,39,52). It interacts with calcium ion channels, some of which are associated with other types of receptors (42). Hexamethonium appears to act as both a competitive (28,51,53) and noncompetitive (2,5,6,28) antagonist at nicotinic receptors. Thus, it might be questioned whether abstinence signs precipitated by these two drugs are specific to interference with nicotinic receptors. However, the noncompetitive or allosteric binding site of hexamethonium on the nicotinic receptor complex is totally distinct from that of memcamylamine (28). Also, in preliminary studies in our laboratory, the purely competitive (9) nicotinic antagonist dihydro-β-erythroidine readily precipitated abstinence signs in nicotine-dependent rats. Thus, three distinct types of interference with nicotinic receptor function all precipitated abstinence syndrome preferentially in nicotine-dependent as opposed to nicotine-naive rats. In addition, termination of nicotine infusion resulted in a similar spontaneous abstinence syndrome (34). The most parsimonious interpretation of all four abstinence syndromes is that they result from chronic overstimulation of nicotinic receptors followed by reduced or impaired receptor activation.

Finally, although nicotine-infused rats had far more behavioral signs than saline-infused rats when challenged centrally with the nicotinic antagonist hexamethonium, they actually had 47% fewer signs than saline-infused rats when challenged with saline alone (Fig. 2). This finding raises the possibility that continuous nicotine infusion can, by some unknown mechanism, reduce the baseline level of irritability.

ACKNOWLEDGEMENTS

This work was supported by NIDA DA08260 (D. H. M., P. I.) and by the UH-CL Faculty Research Support Fund.

REFERENCES

1. Armitage, A. K.; Hall, G. H.: Effects of nicotine on the systemic blood pressure when injected into the cerebral ventricles of cats. Int. J. Neuropharmacol. 6:143–149; 1967.
2. Ascher, P.; Large, W. A.; Rang, H. P.: Studies on the mechanism of action of acetylcholine antagonists on rat parasympathetic ganglion cells. J. Physiol. 295:139–170; 1979.
3. Asghar, K.; Roth, L. J.: Entry and distribution of hexamethonium in the central nervous system. Biochem. Pharmacol. 20:2787–2795; 1971.
4. Benwell, M. E.; Balfour, D. J.; Birrell, C. E.: Desensitization of the nicotine-induced mesolimbic dopamine responses during constant infusion with nicotine. Br. J. Pharmacol. 114:454–460; 1995.
5. Bertrand, D.; Ballivet, M.; Rungger, D.: Activation and blocking of neuronal nicotinic acetylcholine receptors reconstituted in *Xenopus* oocytes. Proc. Natl. Acad. Sci. USA 87:1993–1997; 1990.
6. Blackman, J. G.: Dependence on membrane potential of the blocking action of hexamethonium at a sympathetic ganglionic synapse. Proc. Univ. Otago Med. Sch. 48:4–5; 1970.
7. Brazell, M. P.; Mitchell, S. N.; Gray, J. A.: Effect of acute administration of nicotine on in vivo release of noradrenaline in the hippocampus of freely moving rats: A dose response and antagonist study. Neuropharmacology 30:823–833; 1991.
8. Corrigall, W. A.; Coen, K. M.: Nicotine maintains robust self-administration in rats on a limited-access schedule. Psychopharmacology 99:473–478; 1989.
9. Damaj, M. I.; Martin, B. R.: Calcium agonists and antagonists of the dihydropyridine type: Effect on nicotine induced antinociception and hypomotility. Drug Alcohol Depend. 32:73–79; 1993.
10. Davenport, K. E.; Houdi, A. A.; Van Loon, 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.
11. Day, M. D.: Autonomic pharmacology: Experimental and clinical aspects. New York: Churchill Livingston; 1979.
12. Endoh, K.; Kao, J.; Baker, M.; Leung, F. W.: Intragastric nicotine protection against 40% ethanol injury in rat stomach: Role of ganglionic stimulation or blockade. Dig. Dis. Sci. 37:1840–1846; 1992.
13. Feldberg, W.; Guertzenstein, P. G.: Vasodepressor effects obtained by drugs acting on the ventral surface of the brain stem. J. Physiol. 258:337–355; 1976.
14. Gilbert, D. G.; Meliska, C. J.; Williams, C. L.; Jensen, R. A.: Subjective correlates of cigarette-smoking-induced elevations of peripheral beta-endorphin and cortisol. Psychopharmacology 106: 275–281; 1992.
15. Gomita, Y.; Suemaru, K., Furuno, K.; Araki, Y.: Nicotine- induced tail-tremor and drug effects. Pharmacol. Biochem. Behav. 34:817–821; 1988.
16. Gosling, J. A.; Lu, T. C.: Uptake and distribution of some quaternery ammonium compounds in the central nervous system of the rat. J. Pharmacol. Exper. Ther. 167:56–62; 1969
17. Hall, G. H.: Changes in body temperature produced by cholinomimetic substances injected into the cerebral ventricle of unanesthetized cats. Br. J. Pharmacol. 44:634–641; 1972.
18. Hall, G. H.; Reit, E.: Analysis of some central actions of nicotine injected into the cerebral ventricles of cats. J. Physiol. 185:400–417; 1966.
19. Hexum, T. D.; Rossett, L. R.: Plasma enkephalin-like peptide response to chronic nicotine infusion in guinea pig. Brain Res. 406:370–372; 1987.
20. Hildebrand, B. E.; Nomikos, G. G.; Bondjers, C.; Nisell, M.; Krahl, S. E.; Svensson, T. H.: Behavioral manifestations of the nicotine abstinence syndrome in the rat. Soc. Neurosci. Abstr. 21: 1471; 1995.
21. Houdi, A. A.; Dowell, R. T.; Diana, J. N.: Cardiovascular responses to cigarette smoke exposure in restrained conscious rats. J. Pharmacol. Exp. Ther. 275:646–653; 1995.
22. Jensen, R. A.; Gilbert, D. G.; Meliska, C. J.; Landrom, 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.
23. Khan, I.; Taylor, P.; Yaksh, T.: Cardiovascular and behavioral responses to nicotinic agents administered intrathecally. J. Pharmacol. Exp. Ther. 270:150–158; 1994.
24. Koob, G. F.; Maldonado, R.; Stinus, L.: Neural substrates of opiate withdrawal. Trends Neurosci. 15:186–191; 1992.
25. Ksir, C.; Hakan, R.; Hall, D. P.; Kellar, K. J.: Exposure to nicotine enhances the behavioral stimulant effect of nicotine and increases binding of [^{3}H]acetylcholine to nicotinic receptors. Neuropharmacology 24:527–531; 1985.
26. Levin, E. D.; Ellison, G. D.; Salem, C.; Jarvik, M.; Gritz, E.: Behavioral effects of acute hexamethonium in rats chronically intoxicated with nicotine. Physiol. Behav. 44:355–359; 1988.
27. Levine, R. R.: Presence of certain onium compounds in brain tissue following intravenous administration in rats. Nature 184: 1413–1414; 1959.
28. Loiacono, R.; Stephenson, J.; Stevenson, J.; Mitchelson, F.: Multiple binding sites for nicotine receptor antagonists in inhibiting [^{3}H](−)nicotine binding in rat cortex. Neuropharmacology 32: 847–853; 1993.
29. Malin, D. H.; Lake, J. R.; Carter, V. A.; Cunningham, J. S.; Hebert, K. M.; Conrad, D. L.; Wilson, O. B.: The nicotinic antagonist mecamylamine precipitates nicotine abstinence syndrome in the rat. Psychopharmacology 115:180–184; 1994.

30. 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.
31. 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.
32. Malin, D. H.; Lake, J. R.; Hammond, M. V.; Fowler, D. E.; Leyva, J. E.; Rogillio, R. B.; Sloan, J. B.: FMRF-NH_2-like mammalian octapeptide in opiate dependence and withdrawal. In: Harris, L. ed. Problems of drug dependence 1990: Proceedings of the 52nd annual scientific meeting. Rockville, MD: NIDA; 1991: 271–277.
33. 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.
34. 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.
35. 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.
36. Malin, D. H.; Lake, J. R.; Short, P. E.; Blossman, J. B.; Lawless, B. A.; Schopen, C. K.; Sailer, E. E.; Burgess, K.; Wilson, O. B.: Nicotine abstinence syndrome precipitated by an analog of neuropeptide FF. Pharmacol. Biochem. Behav. 54:581–585; 1996.
37. 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. Psychiatr. 24:886–890; 1988.
38. Martin, B. R.; Martin, T. J.; Fan, F.; Damaj, M. I.: Central actions of nicotine antagonists. Med. Chem. Res. 2:564–577; 1993.
39. Martin, B. R.; Onaivi, E. S.; Martin, T. J.: What is the nature of mecamylamine's antagonism of the central effects of nicotine? Biochem. Pharmacol. 38:3391–3397; 1989.
40. Matta, S. G.; Beyer, H. S.; McAllen, K. M.; Sharp, B. M.: Nicotine elevates rat plasma ACTH by central mechanism. J. Pharmacol Exp. Ther. 243:217–226; 1987.
41. Meltzer, L. T.; Rosecrans, J. A.: Nicotine and arecoline as discriminative stimuli: Involvement of a non-cholinergic mechanism for nicotine. Pharmacol. Biochem. Behav. 29:587–593; 1988.
42. O'Dell, T. J.; Christensen, B. N.: Mecamylamine is a selective noncompetitive antagonist of NMDA and ASP-induced currents in horizontal cells dissociated from the catfish retina. Neurosci. Lett. 94:93–98; 1988.
43. Okamoto, M.; Kita, T.; Okuda, H.; Nakashima, T.: Tolerance to the convulsions induced by daily nicotine treatment in rats. Jap. J. Pharmacol. 59:449–455; 1992.
44. Pomerleau, O. F.; Fertig, J. B.; Seyler, E.; Jaffe, J.: Neuroendocrine reactivity to nicotine in smokers. Psychopharmacology 81: 61–67; 1983.
45. Ren, T.; Sagar, S. M.: Induction of c-fos immunostaining in the rat brain after the systemic administration of nicotine. Brain Res. Bull. 29:589–597; 1992.
46. 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.
47. Schechter, M. D.; Cook, P. G.: Nicotine-induced weight loss in rats without an effect on appetite. Eur. J. Pharmacol. 38:63–69; 1976.
48. Sharp, B.; Matta, S.: Detection by *in vivo* micro-dialysis of nicotine-induced norepinephrine secretion from the hypothalamic paraventricular nucleus of freely moving rats: dose dependency and desensitization. Endocrinol. 133:11–19; 1993.
49. Suemaru, K.; Oishi, R.; Gomita, Y.: Characteristics of tail-tremor induced by nicotine in rats. Naunyn-Schmiedebergs Arch. Pharmacol. 350:153–157; 1994.
50. Suh, H. W.; Hudson, P. M.; McMillian, M. K.; Das, K. P.; Wilson, B. C.; Wu, G. C.; Hong, J. S.: Long-term stimulation of nicotinic receptors is required to increase proenkephalin A mRNA levels and the delayed secretion of [Met5]-enkephalin in bovine adrenal medullary chromaffin cells. J. Pharmacol. Exp. Ther. 275:1663–1670; 1995.
51. Takayama, H.; Majewska, M. D.; London, E. D.: Interactions of noncompetitive inhibitors with nicotinic receptors in the rat brain. J. Pharmacol. Exp. Ther. 253:1083–1089; 1989.
52. Varanda, W. A.; Aracava, Y.; Sherby, S. M.; Van Meter, W. G.; Eldefrawi, M. E.; Albuquerque, E. X.: The ACh receptor of the neuromuscular junction recognizes mecamylamine as a noncompetitive antagonist. Mol. Pharmacol. 28:128–137; 1985.
53. Yamada, S.; Kagawa, Y.; Takayanagi, N.; Nakayama, K.; Tsuji, K.; Kosuge, T.; Hayashi, E.; Okada, K.; Inoue, S.: Comparison of antinicotinic activity by neosurugatoxin and the structurally related compounds. J. Pharmacol. Exp. Ther. 243:1153–1158; 1987.