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Rodent Model of Nicotine Abstinence Syndrome

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MALIN, D. H., J. R. LAKE, P. NEWLIN-MAULTSBY, L. K. ROBERTS, J. G. LANIER, V. A. CARTER, J. S. CUNNINGHAM AND O. B. WILSON. *Rodent model of nicotine abstinence syndrome.* PHARMACOL BIOCHEM BEHAV **43**(3) 779–784, 1992.—Few animal models are currently in use for the recognized clinical problem of nicotine dependence and abstinence. This study introduces a rapid and convenient model using the rat. Sixteen male rats were rendered nicotine dependent by 7 days of continuous subcutaneous infusion of either 3 mg/kg/day (n = 8) or 9 mg/kg/day (n = 8) nicotine tartrate salt; 8 control rats were infused with saline alone. Rats were observed for 15 min before, during, and after the drug infusion period using a tally sheet modified from a standard checklist of opiate abstinence signs. There were few signs observed in any group at baseline and at the end of the infusion period. However, nicotine-infused rats showed a significant, dose-related increase over the control group at 16 h after the end of infusion, largely subsiding by 40 h. The most frequently observed signs during withdrawal included: teeth-chattering/chews, writhes/gasps, ptosis, tremors/shakes, and yawns. A significant drop in locomotor activity and increase in weight gain following termination of nicotine infusion provided additional evidence of an abstinence syndrome. This syndrome was alleviated by SC administration of 0.4 mg/kg nicotine tartrate.

Nicotine Nicotine abstinence Nicotine withdrawal Nicotine dependence Locomotor activity Rat

REPEATED consumption of nicotine has been shown to produce both tolerance and dependence in humans (18). Subsequent abstinence from nicotine results in a withdrawal syndrome with most abstinence signs reaching peak intensity within 24 h (6,9,16). This syndrome is characterized by a variety of symptoms, notably, irritability, anxiety, difficulty in concentrating, restlessness, and impatience. Excessive hunger, sleep disturbances, drowsiness, and craving for nicotine are also observed. The nicotine abstinence syndrome is considered a major cause of the high relapse rate observed during the first few days of smoking cessation (18).

Rodent models of nicotine abstinence are potentially useful for research on mechanisms of nicotine dependence and to screen proposed interventions to aid in smoking cessation. The few rat models that have been described rely upon changes in conditioned behavioral responses (2,3,7) or changes in body weight or food consumption (10) to measure withdrawal intensity. The model presented here relies primarily upon the frequency of spontaneous behavioral signs observed in nicotine-dependent rats after nicotine is removed. This is similar to widely used rat models of the opiate abstinence syndrome (5,12) and is more analogous to methods used to quantify nicotine abstinence in humans (6,16).

Pilot studies suggested that the symptoms of nicotine abstinence in the rat are remarkably similar to those observed during opiate abstinence (5,12), including teeth-chattering, chews, abdominal writhes, gasps, ptosis, wet shakes, and tremors. Therefore, this study employed a behavior frequency checklist similar to that used to quantify the opiate abstinence syndrome.

The present study determined whether an index composed of such abstinence signs is a valid measure of abstinence severity according to the following criteria. First, nicotine-abstinent rats at the peak of their abstinence phase should have more signs than during the prenicotine baseline, the nicotine administration period, and the subsequent recovery phase. Second, animals previously infused with nicotine should have more abstinence signs than control animals previously infused with saline alone. Third, the frequency of abstinence signs should vary according to the amount of previous nicotine exposure. Finally, abstinence signs should be attenuated by a small acute dose of nicotine.

Increased weight gain (13) and decreased cortical arousal (8,17) have been observed in smoking cessation. In the present study, locomotor activity and weight gain were monitored to detect any transient changes during nicotine abstinence. Such

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changes would further confirm the existence of an abstinence syndrome following termination of continuous nicotine exposure.

EXPERIMENT 1

This experiment determined the time course and dose dependence of an abstinence syndrome following 7 days of continuous infusion of nicotine tartrate.

METHOD

Animals

Subjects were 24 male Sprague-Dawley rats weighing 325–375 g and maintained on a 12 L : 12 D cycle with ad lib food and water. Each rat was implanted subcutaneously under halothane anesthesia with one Alzet osmotic minipump (Model 2ML1) filled with either nicotine tartrate, [−] isomer in saline or with saline alone. The concentration was adjusted to compensate for small differences in subject weights, but it approximated 4.38 or 13.13 mg/ml nicotine tartrate, depending upon desired infusion rate. Subjects were randomly assigned to three groups: eight rats were infused with 9 mg/kg/day nicotine tartrate (equivalent to 3.15 mg/kg/day nicotine base), eight rats were infused with 3 mg/kg/day nicotine tartrate (equivalent to 1.05 mg/kg/day base), and eight rats were infused with saline alone. The infusion rates and subsequent test intervals were selected on the basis of a small pilot experiment. After 7 days of infusion, the pumps were removed under halothane anesthesia. Animals were inspected for any local tissue damage resulting from the infusion of low pH nicotine tartrate solution; none was found.

Behavioral Signs

Behavioral observations were performed at 9:00 a.m. on each of the following days: a baseline day, the last day of nicotine infusion, and at 16 and 40 h after termination of nicotine infusion. Each rat was observed under "blind" conditions for 15 min in a clear plastic observation chamber. Observers counted frequency of signs on a standard checklist of opiate abstinence signs (5,12) modified on the basis of a small pilot experiment with nicotine-abstinent rats. Categories included teeth-chattering/chews, writhes/gasps, shakes/tremors, ptosis (not counted more frequently than once per minute), and cumulated miscellaneous less frequent signs (yawns, dyspnea, and seminal ejaculation).

Activity

Rats were housed individually in plastic cages placed on a sensor of a Stoelting automated activity monitor (Model 31408). Activity counts were cumulated from 1:00–4:00 a.m. on a baseline day, the final day of nicotine infusion, and the first 2 days of nicotine abstinence (8–11 and 32–35 h after termination of nicotine infusion). The period from 1:00–4:00 a.m. has been shown to be a time of peak locomotor activity in the rat (15).

Weight

Subjects were weighed each day at 9:00 p.m. Weight change from the previous day was determined for a baseline day, each day of nicotine infusion, and the first 2 days of abstinence. The 9:00 p.m. time was selected because it allowed for the assessment of weight changes during the first complete day of withdrawal.

RESULTS

Behavioral Signs

As shown in Fig. 1, nicotine-abstinent rats exhibited a sharp, dose-related elevation of signs 16 h after termination

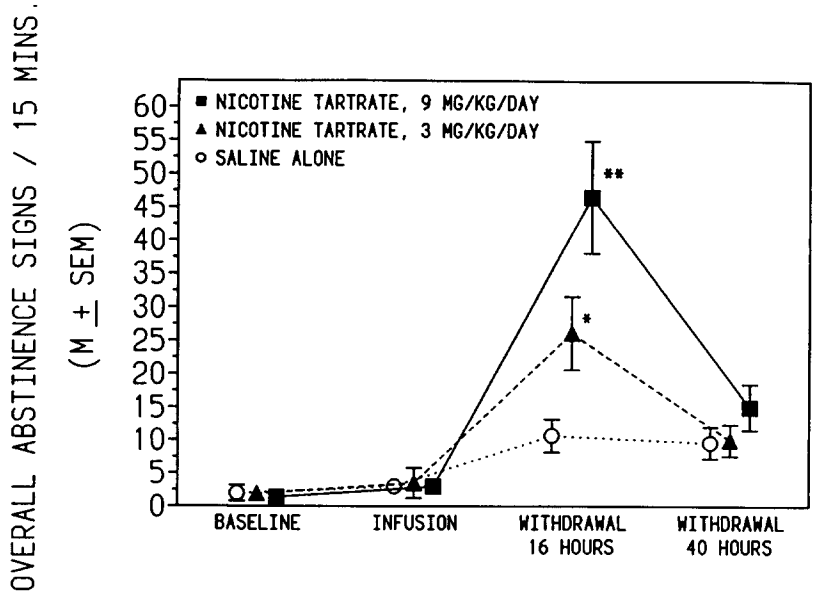

FIG. 1. Abstinence signs over 15 min in rats infused SC for 7 days with 9 mg/kg/day nicotine tartrate (■), 3 mg/kg/day nicotine tartrate (▲), or saline alone (○). Observations were made under blind conditions prior to infusion (baseline), at the end of the infusion period, and at 16 and 40 h after termination of infusion. $^{**}p < 0.01$ vs. both other groups, $^{*}p < 0.05$ vs. saline-infused group.

TABLE 1

FREQUENCIES OF INDIVIDUAL CATEGORIES OF ABSTINENCE SIGNS (MEAN ± SEM) OBSERVED DURING 15 min, 16 h AFTER END OF NICOTINE INFUSION

	Prior Infusion Rate of Nicotine Tartrate (mg/kg/day)		
Category	0	3	9
Teeth-chattering/chews	2.6 ± 1.5	7.0 ± 2.5	15.9 ± 4.9*
Gasps/writhes	3.2 ± 1.4	7.2 ± 1.4	17.1 ± 4.6*
Ptosis	0.6 ± 0.4	1.4 ± 0.5	6.5 ± 1.2*
Shakes/tremors	2.0 ± 0.6	7.0 ± 1.8†	4.1 ± 1.7
Misc. (yawns, dyspnea, seminal ejaculation)	0.1 ± 0.1	0.8 ± 0.3	1.3 ± 0.7‡

*$p < 0.01$ vs. saline-infused controls (Dunnett's t-test).
†$p < 0.05$ vs. saline-infused controls (Dunnett's t-test).
‡$0.05 < p < 0.10$ vs. saline-infused controls (Dunnett's t-test).

of nicotine infusion, with a return toward baseline at 40 h. Two-way analysis of variance (ANOVA) with one repeated-measures variable (time) revealed a significant main effect of nicotine dose, $F(2, 21) = 4.64$, $p < 0.05$, a significant time effect, $F(3, 63) = 52.13$, $p < 0.01$, and a significant dose × time interaction effect, $F(6, 63) = 9.18$, $p < 0.01$. Posthoc analysis [Tukey's honestly significant difference (HSD)] revealed significant differences among dose groups only at 16 h after termination of nicotine infusion: high- vs. low-dose group, $p < 0.01$; high-dose group vs. saline-infused control, $p < 0.01$; low-dose group vs. saline-infused controls, $p < 0.05$. Both the high- and low-dose groups had significantly more signs ($p < 0.01$) at 16 h after the end of nicotine infusion compared with their respective signs during baseline, infusion, and 40 h after the end of nicotine infusion. There were no significant differences over time within the saline-infused control group.

Table 1 shows the frequency of individual categories of behavioral symptoms recorded 16 h after termination of nicotine infusion for each dose group. The high-dose group differed significantly (Dunnett's t-test) from saline-infused controls in teeth-chattering/chews, writhes/gasps, and ptosis, while approaching significance in miscellaneous, less frequent signs. The low-dose group differed significantly from controls in shakes/tremors.

Activity

Figure 2 shows activity on the last day of infusion and the first 2 days of abstinence as percentage change from baseline. The high-dose group dropped well below baseline at 8–11 h after termination of nicotine infusion. Two-way ANOVA with one repeated-measures variable (time) revealed a significant time effect, $F(2, 40) = 7.59$, $p < 0.01$, as well as a significant

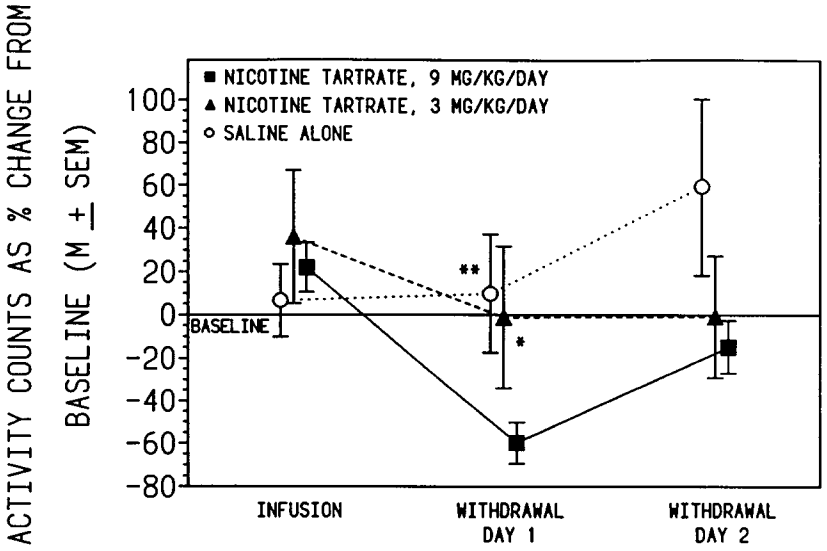

FIG. 2. Activity counts as percentage change from baseline in rats continuously infused SC for 7 days with 9 mg/kg/day nicotine tartrate (■), 3 mg/kg/day nicotine tartrate (▲), or saline alone (○). Observations were accumulated from 1:00–4:00 a.m. on the final infusion day and first 2 days of the withdrawal period (8–11 and 32–35 h after termination of nicotine infusion). **$p < 0.01$, *$p < 0.05$ vs. 9-mg/kg/day group.

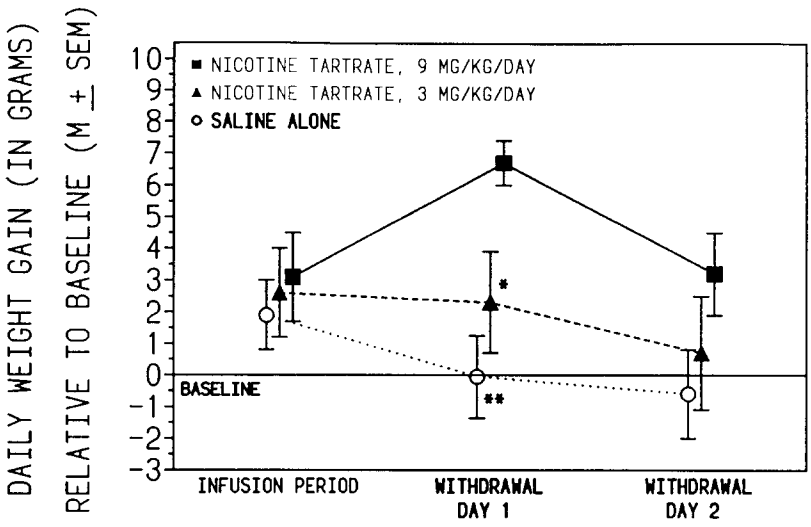

FIG. 3. Twenty-four hour weight gain as the difference in grams from weight gain on a baseline day in rats continuously infused SC for 7 days with 9 mg/kg/day nicotine tartrate (■), 3 mg/kg/day nicotine tartrate (▲), or saline alone (○). Weight change was averaged over the infusion period and measured from 4-28 h and 28-52 h after termination of nicotine infusion. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. 9-mg/kg/day group.

interaction effect (dose × time), $F(4, 40) = 5.21$, $p < 0.01$. The overall nicotine dose effect (including both infusion and abstinence periods) was not significant, $F(2, 20) = 0.97$, NS.

Posthoc analysis (Tukey's HSD) revealed significant differences between groups at 8–11 h of abstinence: high-dose group vs. saline-infused controls, $p < 0.01$; high- vs. low-dose group, $p < 0.05$. The low-dose group was not significantly different from saline-infused controls. Within the high-dose group, there was a significant 51% decrease in activity from the last day of the infusion period to 8–11 h after the end of nicotine infusion, ($p < 0.01$). Activity within the high-dose group at 32–35 h postwithdrawal did not differ significantly from activity on the last day of infusion.

Weight

Figure 3 shows daily weight gain during nicotine infusion and abstinence periods as the difference in grams from baseline weight gain. The high-dose group exhibited an abrupt

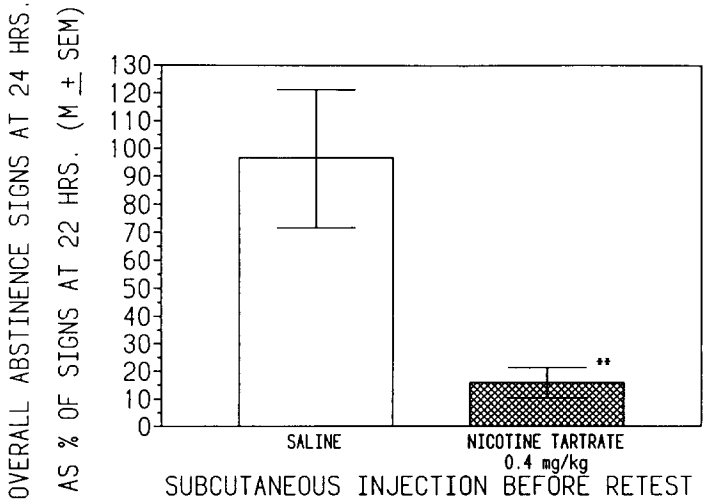

FIG. 4. Abstinence signs after injection of nicotine tartrate (0.4 mg/kg) or saline SC as a percentage of abstinence signs before injection. All rats had previously been infused SC for 7 days with 9 mg/kg/day nicotine tartrate. Observations were made at 22 h after termination of infusion (preinjection) and at 24 h (postinjection). $^{**}p < 0.01$ vs. saline-injected rats.

weight increase on the first but not the second day of nicotine abstinence. Two-way ANOVA with one repeated-measures variable (time) revealed a significant time effect, $F(2, 42) = 4.04$, $p < 0.05$, and interaction effect (dose × time), $F(4, 42) = 2.83$, $p < 0.05$. The overall nicotine dose effect (including both infusion and abstinence periods) approached significance, $F(2, 21) = 2.75$, $0.05 < p < 0.10$.

Posthoc analysis (Tukey's HSD) revealed significant differences between treatment groups only during the first day of nicotine abstinence: high- vs. low-dose group, $p < 0.05$; high-dose group vs. saline controls, $p < 0.01$.

EXPERIMENT 2

This experiment determined whether the nicotine abstinence signs observed above could be reversed by a small, acutely administered dose of nicotine tartrate.

METHOD

Thirteen male Sprague-Dawley rats, weighing 325–375 g, were implanted with an osmotic minipump (Alzet 2ML1) filled with nicotine tartrate in saline as in Experiment 1. Each rat was infused for 7 days at the rate of 9 mg/kg/day. The pumps were then removed and rats observed as before for behavioral abstinence signs. Two blind 10-min observations were performed, one at 22 h and the next at 24 h after abrupt termination of nicotine infusion. Three minutes before commencing the second observation, seven rats received 0.4 mg/kg nicotine tartrate in saline SC, while six rats received saline alone. In a small pilot experiment, this dose induced no rigidity or other observable disturbance in either nicotine-abstinent or nicotine-naive rats. Each rat's abstinence reversal score was its number of abstinence signs postinjection as a percentage of its number of signs preinjection.

RESULTS

Figure 4 shows the abstinence reversal scores for nicotine-abstinent rats receiving either 0.4 mg/kg nicotine tartrate or saline SC. There was an 84% decline in abstinence signs following nicotine injection. This contrasted with a decline of only 3.5 % following saline injection. This difference was significant, $t(11) = 3.43$, $p < 0.01$.

The greater change in abstinence signs seen in the nicotine-injected group does not seem attributable to differences between groups prior to injection. Rats that later received a saline injection had 21.0 ± 6.1 signs (mean ± SEM), while those that later received a nicotine tartrate injection had 23.3 ± 4.7 signs. This difference was not significant, $t(11) = 0.30$, NS.

GENERAL DISCUSSION

Overall behavioral signs (cumulated across the categories in Table 1) appeared to meet all of the stated criteria for a nicotine abstinence syndrome. Signs appeared far more frequently during the first day of abstinence than during baseline or infusion periods or during the second day of abstinence. The frequency of signs was dose related and far greater in previously nicotine-infused rats than in saline-infused controls. Abstinence signs were largely reversed by a small, acutely administered dose of nicotine tartrate. Sharp transient decreases in activity and increases in weight gain provide additional evidence for an abstinence syndrome of limited duration following nicotine infusion.

The amounts of nicotine infused did not appear to debilitate rats during the infusion period (based upon locomotor activity, weight gain, feeding patterns, and behavioral observations). The 3 mg/kg/day nicotine tartrate infusion rate corresponds to 1.05 mg/kg/day of nicotine base, comparable to the dose ingested by a 70-kg smoker who consumes 3 packs/day of average-yield (1.2 mg nicotine base) cigarettes (1). The 9 mg/kg/day infusion rate (3.15 mg/kg/day nicotine base) is comparable to the daily dose ingested by the heaviest (5 pack/day) smokers of high-yield (2.5 mg nicotine base) cigarettes (1). In comparing human and rat doses, one must consider the generally higher metabolic and drug clearance rates in the rat. Furthermore, nicotine was infused for only 1 week in this experiment, whereas a human smoker experiences years of chronic exposure. Of course, the specific behavioral abstinence signs differ between nicotine-abstinent rats and human beings, presumably because of species differences in behavioral expression of underlying irritability and anxiety.

One interesting aspect of the behavioral signs observed during nicotine abstinence is a close resemblance to the constellation of behavioral signs commonly observed in mild opiate abstinence syndrome (5,11). It has been reported that nicotinic cholinergic synapses in the hypothalamus control the release of metenkephalin (4,14). It is therefore possible that continuous nicotine exposure results in chronic overstimulation of opioid receptors in certain brain regions, inducing a state somewhat similar to opiate dependence. Alternatively, the resemblance between opiate and nicotine abstinence signs might simply reflect a limited repertoire of behaviors by which rats can demonstrate heightened irritability. It would be interesting to determine whether opiate receptor antagonists, such as naloxone, are able to precipitate abstinence signs in nicotine-dependent animals. Preliminary data from this laboratory indicate that naloxone does in fact precipitate abstinence signs in nicotine-dependent rats, suggesting a need for further investigation of opioid mechanisms in nicotine dependence.

It is hoped that the simple and rapid laboratory model of nicotine dependence presented here will be useful for studies probing the underlying mechanisms of nicotine dependence. It might also be useful for preliminary screening of proposed therapeutic interventions to alleviate the discomfort and irritability associated with smoking cessation.

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