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Passive immunization against nicotine prevents nicotine alleviation of nicotine abstinence syndrome

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Abstract

Passive immunization against nicotine interferes with its locomotor and pressor effects. The current study determined whether immunization could prevent another nicotine action: the reversal of nicotine abstinence syndrome. IgG containing 4.4–5.6% nicotine-specific antibody was isolated from rabbits immunized with 3′-amino-methyl-nicotine conjugated to a carrier protein. Twenty rats were rendered dependent by 7 days of subcutaneous infusion of 3.15 mg/kg/day nicotine (expressed as the base). Upon termination of nicotine infusion, each rat was injected intraperitoneally with 150 mg of IgG from normal serum ($n=13$) or from nicotine antiserum ($n=7$). Twenty-two and one-half hours later, all rats were observed over 15 min for baseline nicotine abstinence signs. Two and one-half hours after baseline observations, seven of the 13 rats pretreated with control IgG and all seven rats pretreated with nicotine-specific IgG were then challenged by 0.12 mg/kg (sc) nicotine. The remaining six rats pretreated with control IgG were challenged with saline alone. All rats were then observed again for abstinence signs. Nicotine injection caused significantly less reduction of abstinence signs in the immunized rats. The nicotine effect in immunized rats was comparable to the saline effect in nonimmunized rats. Immunization also significantly reduced free serum nicotine concentration and nicotine distribution to the brain. These results raise the possibility that immunization might prevent nicotine consumption from relieving the discomforts of smoking cessation.

Keywords: Nicotine; Nicotine dependence; Nicotine abstinence; Immunization; Antibodies; Vaccine; Rat

Active or passive immunization has been reported to reduce heroin (Bonese et al., 1974; Killian et al., 1978) and cocaine (Fox et al., 1996) self-administration. Active immunization against nicotine with the nicotine immunogen CMUNic (a 6-amino derivative of nicotine conjugated to a carrier protein) elicited high titers of nicotine-specific antibodies and reduced both plasma nicotine concentrations (Hieda et al., 1997) and nicotine distribution to the brain (Hieda et al., 1999). Passive immunization with IgG isolated from rabbits immunized with 3′-amino-methyl-nicotine conjugated to a carrier protein (Nabi NicVax) reduced nicotine distribution to the brain by up to 65% (Pentel et al., 2000). It continued to reduce nicotine distribution to the brain even after five repeated doses of nicotine administered over 80 min (Pentel et al., 2000). Passive immunization with nicotine-specific IgG prevented locomotor activation by nicotine, but had no effect on cocaine induced locomotor stimulation (Pentel et al., 2000). Passive immunization also dose-dependently reduced the pressor effect of nicotine (Pentel et al., 2000) and interfered with nicotine's stimulus properties (Alvarado et al., 1999). These results raise the possibility that passive immunization against nicotine might interfere with other nicotine actions, including those that help sustain the smoking habit.

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Tobacco smoking is maintained by both positive and negative reinforcement (Thompson and Hunter, 1998). Behaviors that lessen or remove an aversive condition are negatively reinforced. For example, smoking a single cigarette may relieve discomfort and craving resulting from smoking cessation, often leading to reinstatement of the smoking behavior. In fact, suffering from tobacco withdrawal is cited as the most common cause of relapse in smoking cessation programs (US Department of Health and Human Services, 1988). Immunization against nicotine may prevent reinstatement of the smoking habit by preventing nicotine from relieving the discomforts of tobacco withdrawal.

A rodent model of nicotine dependence has been developed and validated by (Malin et al., 1992; Malin et al., 1994; Malin et al., 1997; Malin et al., 1998b). In this model, nicotine dependence is induced in the rat by 7 days of continuous subcutaneous infusion of nicotine tartrate (Carboni et al., 1996; Hildebrand et al., 1997; Malin et al., 1992). Behavioral abstinence signs are precipitated immediately in dependent rats by injection of various nicotinic antagonists (Carboni et al., 1996; Hildebrand et al., 1997; Malin et al., 1994; Malin et al., 1997; Malin et al., 1998b). Spontaneous abstinence signs develop gradually following termination of nicotine infusion (Hildebrand et al., 1997; Malin et al., 1992), and are greatly reduced by subcutaneous injection of 0.12 or 0.14 mg/kg nicotine (Malin et al., 1992; Malin et al., 1996). The current study investigated whether passive immunization with nicotine-specific IgG would prevent the abstinence-alleviating action of a subcutaneous injection of nicotine in the rat. A parallel experiment determined the effect of immunization on protein binding and distribution to the brain of the same dose of nicotine.

1. Experiment 1. Effect of passive immunization on nicotine alleviation of nicotine abstinence syndrome

This experiment determined whether passive immunization against nicotine could prevent reversal of nicotine abstinence syndrome by a subcutaneous injection of nicotine. The basic design is summarized in Fig. 1.

1.1. Methods

1.1.1. Subjects

The subjects were 20 male Sprague–Dawley rats, weighing 419–468 g. The rats were maintained on ad lib. food and water and a 12-L/12-D cycle. All rats were implanted subcutaneously with one Alzet 2ML1 osmotic minipump under halothane anesthesia utilizing a custom-built halothane/oxygen respirator. They were rendered dependent by 7 days of continuous infusion of nicotine tartrate (−)isomer in saline. The nicotine infusion rate was 3.15 mg/kg/day, expressed as the base. One week later (approximately 164 h following implantation), pumps were removed under halothane anesthesia in order to abruptly terminate nicotine infusion.

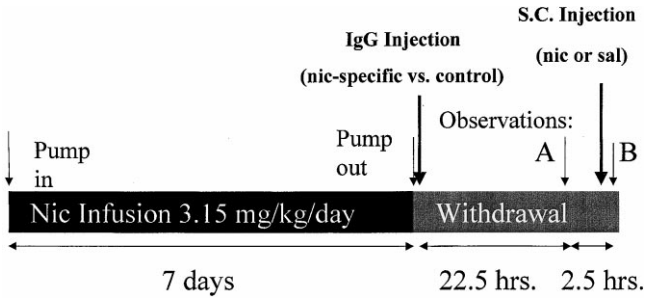

Fig. 1. The design of Experiment 1. The timeline is not drawn to scale. The nicotine infusion rate was 3.15 mg/kg/day, expressed as the base. Nicotine abstinence signs in immunized and nonimmunized rats were observed for 15 min at Time A (preinjection) and at Time B (starting 3 min after subcutaneous injection of saline or 0.12 mg/kg nicotine). Immunized rats received 150 mg of nicotine-specific IgG, while nonimmunized rats received the same dose of normal rabbit IgG. The ability of the subcutaneous injection to reverse the abstinence syndrome is indicated by postinjection signs (at Time B) as a % of preinjection signs (Time A). In Experiment 2, blood and brain samples were obtained at Time B.

1.1.2. Antibody

The hapten *trans*-3′-amino-methyl-nicotine (Cushman and Catagnoli, 1972) was prepared and conjugated to recombinant *Pseudomonas aeruginosa* exoprotein A (rEPA) as described in Pentel et al. (2000). Female New Zealand white rabbits were immunized with 100 μg (sc) nicotine immunogen in complete Freund's adjuvant on Day 0, and boosted with 100 μg (sc) immunogen in incomplete Freund's adjuvant on Days 21 and 42. Rabbits were bled weekly and boosted as needed to restore antibody levels. Immune rabbit IgG was purified using a Protein G Sepharose 4 Fast Flow column (Pharmacia, Piscataway, NJ) equilibrated with PBS. Rabbit IgG was eluted with 0.1 M glycine buffer at pH 2.7 and immediately neutralized with 1 M Tris buffer at pH 9. The IgG was diafiltered against PBS on a Pellicon XL polyethersulfone membrane (Millipore, Bedford, MA) with a 50-kDa cutoff. The purified IgG contained 4.4–5.6% nicotine-specific antibody. The solution was brought to a concentration of 65 or 85 mg/ml total IgG by addition of PBS. Control IgG was similarly prepared, but from nonimmunized rabbits.

Immediately after pump removal, while the subjects were still under anesthesia, seven rats were injected with 150 mg (ip) nicotine-specific IgG and 13 rats received 150 mg control (nonimmune) IgG. This dose of nicotine-specific IgG was selected because it almost totally prevented the pressor actions of nicotine and produced serum nicotine antibody concentrations similar to those achieved in rats by active immunization (Pentel et al., 2000). IgG was injected 25 h prior to subcutaneous nicotine or saline challenge in order to allow antibodies to reach maximum titer in the rats' blood (based on data from pilot studies).

1.1.3. Observations

Rats were habituated to a clear plastic rectangular chamber measuring 48 × 38 × 20 cm for 15 min on each of the 2 days preceding the experiment and once again 17 h after nicotine pump removal. Twenty-two and one-half hours after pump removal, each rat was placed in the observation chamber for 15 min and baseline behavioral abstinence signs counted. Numbers of spontaneous nicotine abstinence signs are near maximal at 20–22 h following termination of nicotine infusion (Malin et al., 1996; Malin et al., 1998a). The abstinence signs were tallied based on a standard checklist of nicotine abstinence signs (Malin et al., 1992). Categories included gasps/abdominal writhes, teeth chatter/chews, shakes/tremors, ptosis, and miscellaneous less frequent signs (seminal ejaculation, scratches, and yawns). Ptosis was not counted more than once each minute and continuous teeth chattering no more than once every 15 s.

Two and one-half hours after baseline observations, rats were challenged with a subcutaneous injection of either saline or nicotine tartrate. The dose of nicotine was 0.12 mg/kg, expressed as the base. Six of the 13 rats pretreated with control IgG received a saline injection, while the remaining seven received nicotine. All seven rats pretreated with nicotine IgG were challenged with a nicotine injection. Three minutes after the subcutaneous injection, rats were again observed over 15 min for nicotine abstinence signs. All observations were carried out under "blind" conditions. Each rat's overall abstinence score was the total number of signs postinjection as a percentage of signs preinjection (baseline). For each individual category of sign, absolute postinjection scores were analyzed in order to avoid dividing by zero in cases where a rat had no preinjection signs in a category.

1.2. Results

The severity of abstinence syndrome prior to nicotine or saline injection was not markedly affected by the type of IgG administered. Rats receiving control IgG had an average of 31.6 ± 3.2 (mean ± S.E.M.) baseline signs in comparison to 37.2 ± 7.5 signs for rats receiving nicotine-specific IgG. This difference was not significant, $t(18) = 0.81$, NS. In contrast, only nicotine-specific IgG largely prevented nicotine reversal of abstinence signs. As shown in Fig. 2, nicotine injection resulted in a much more marked reduction of abstinence signs in nonimmunized rats (pretreated with control IgG) than in immunized rats (pretreated with nicotine-specific IgG). In fact, the effect of the nicotine injection in immunized rats differed little from the effect of a saline injection in nonimmunized rats.

A one-way ANOVA revealed a significant difference among the groups in percentage reduction of nicotine abstinence signs from baseline to postinjection observations, $F(2, 17) = 4.02$, $P < .05$. Post hoc analysis by

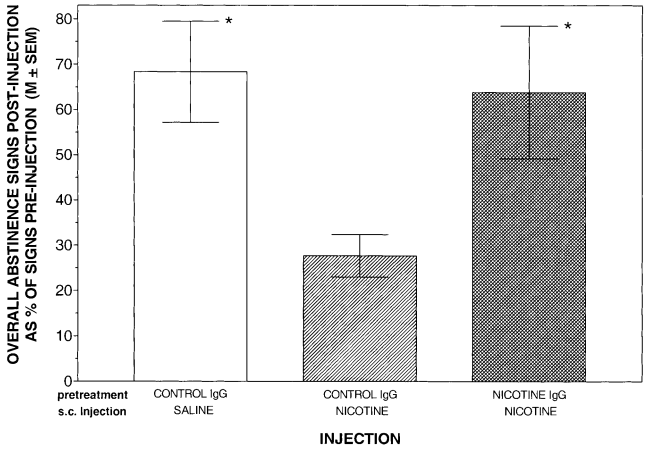

Fig. 2. Overall nicotine abstinence signs following a subcutaneous injection of saline or 0.12 mg/kg nicotine as a percentage of preinjection signs. Rats were pretreated 25 h earlier with 150 mg (ip) of either control IgG or nicotine-specific IgG. * $P < .05$ vs. control IgG + nicotine.

Fisher's least significant difference (LSD) test revealed that nicotine induced a significantly greater percentage reduction, $P < .05$, in nonimmunized rats than did either nicotine in immunized rats or saline in nonimmunized rats. There was no significant difference between the latter two groups. The difference in response to nicotine in the immunized and nonimmunized rats does not appear to be attributable to any difference in preinjection abstinence scores. The immunized rats subsequently challenged with nicotine had 37.2 ± 7.5 baseline abstinence signs (mean ± S.E.M.), while nonimmunized rats subsequently challenged with nicotine had 34.7 ± 5.4 baseline abstinence signs. This difference was not significant, $t(12) = 0.10$, NS.

Following subcutaneous injection, the numbers of abstinence signs in most categories were lower in nonimmunized rats injected with nicotine than in immunized rats injected with nicotine or nonimmunized rats injected with saline (Fig. 3). Postinjection abstinence scores for the groups were compared using Fisher's LSD test following the appropriate one-way ANOVAs. Nonimmunized rats injected with nicotine had significantly fewer teeth chatter/chews and shakes/tremors than immunized rats injected with nicotine, $P < .01$ and $P < .05$, respectively. The difference between these groups approached significance for gasps/writhes, $P = .07$, and was not significant for ptosis and miscellaneous signs. Nonimmunized rats injected with nicotine had significantly fewer instances of gasps/writhes and ptosis than nonimmunized rats injected with saline, $P < .05$. There was no significant difference between these groups for teeth chatter/chews, shakes/tremors, or miscellaneous signs.

Lastly, there were no significant differences between immunized rats injected with nicotine and nonimmunized rats injected with saline in any category except teeth chatter/chews. In this category, immunized rats injected with

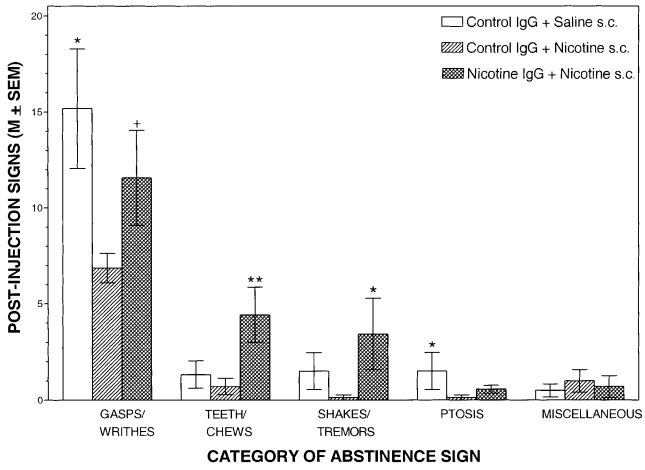

Fig. 3. Occurrences (means ± S.E.M.) of individual categories of abstinence signs following a subcutaneous injection of saline or 0.12 mg/kg nicotine in rats pretreated 25 h earlier with 150 mg (ip) of either control IgG or nicotine-specific IgG. $**P<.01$ vs. control IgG + nicotine and $P<.05$ vs. control IgG + saline. $*P<.05$ vs. control IgG + nicotine and $+P=.07$ vs. control IgG + nicotine.

nicotine actually had significantly more signs than nonimmunized rats injected with saline, $P<.05$.

2. Experiment 2. The effect of passive immunization on serum and brain distribution of nicotine

This experiment determined whether the same passive immunization treatment that prevented nicotine's abstinence alleviating action in Experiment 1 could also alter nicotine binding in serum or nicotine distribution to the brain.

2.1. Methods

2.1.1. Subjects

The design of this experiment was parallel to Experiment 1 with the primary exception that blood and brain samples were taken and nicotine levels were assessed rather than behavioral abstinence signs. The subjects were 18 male Sprague–Dawley rats, weighing 380–427 g, and implanted with Alzet 2ML1 osmotic minipumps under droperidol/fentanyl anesthesia. (Injectable anesthesia was employed by the Minneapolis laboratory in the absence of the custom-built halothane/oxygen respirator utilized in Experiment 1 by the Houston laboratory.) Each rat was rendered dependent by 7 days of continuous infusion of nicotine tartrate. The nicotine infusion rate was 3.15 mg/kg/day, expressed as the base. At the time of pump removal, six rats were injected intraperitoneally with 150 mg nicotine-specific IgG and 12 received 150 mg control IgG.

2.1.2. Collection of blood and brain samples

Twenty-four and one-half hours after pump removal, rats were again anesthetized and 1.5 ml blood samples were obtained from the tail vein. Immediately afterwards, rats received subcutaneous injections of either saline or nicotine tartrate. The dose of nicotine was 0.12 mg/kg, expressed as the base. The six rats pretreated with nicotine-specific IgG and six of the 12 rats pretreated with control IgG were injected with nicotine. The remaining six rats pretreated with control IgG were injected with saline alone. This third group served as a control to determine whether any major portion of detected nicotine was derived from the previous continuous infusion as opposed to the subsequent subcutaneous injection. At 10.5 min after the subcutaneous injection, rats were decapitated, trunk blood collected, and brains rapidly removed. This time interval corresponded to the approximate midpoint of the postinjection behavioral observations reported in Experiment 1. Trunk blood was collected to obtain large volumes for the assays below. Trunk blood has a similar nicotine concentration to blood from a femoral catheter at 1 min postnicotine injection, according to analysis of previous data (Hieda et al., 1999).

2.1.3. Nicotine assay

Serum nicotine concentrations were measured by gas chromatography (Jacob et al., 1981) and serum protein binding was measured by equilibrium dialysis (Hieda et al., 1997). Brain samples were digested in 5 vol of NaOH prior to extraction (Hieda et al., 1999). Nicotine concentrations in brain were also measured by gas chromatography (Jacob et al., 1981) and corrected for brain blood content as previously described (Hieda et al., 1997).

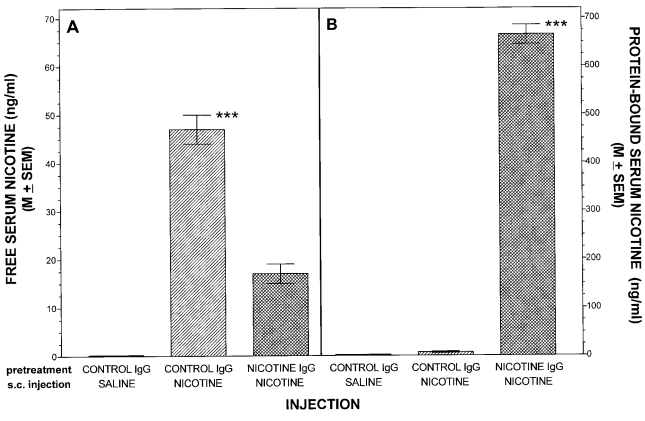

Fig. 4. (A) Free nicotine: serum concentrations (ng/ml) of free nicotine (means ± S.E.M.) 10.5 min following a subcutaneous injection of saline or 0.12 mg/kg nicotine in rats pretreated 25 h earlier with 150 mg (ip) of either control IgG or nicotine-specific IgG. $***P<.001$ vs. both other groups. (B) Protein-bound nicotine: serum concentrations (ng/ml) of protein-bound nicotine (means ± S.E.M.) 10.5 min following a subcutaneous injection of saline or 0.12 mg/kg nicotine in rats pretreated 25 h earlier with 150 mg (ip) of either control IgG or nicotine-specific IgG. $***P<.001$ vs. both other groups.

2.2. Results

Fig. 4A shows the concentration of free (unbound) nicotine in serum 10.5 min following injection of saline in rats pretreated with control IgG or injection of nicotine in rats pretreated with either control or nicotine-specific IgG. A one-way ANOVA revealed a significant difference among these three groups, $F(2, 15) = 184.27$, $P < .001$. Among nonimmunized rats, there was an average of 47 ng/ml free nicotine after the nicotine injection, but no detectable free nicotine after a saline injection. Based on post hoc analysis (Fisher's LSD test), this difference was significant, $P < .001$. This suggests that there was minimal free nicotine remaining from the previous nicotine infusion, terminated approximately 25 h earlier by pump removal. Comparing the two groups injected with nicotine, rats immunized against nicotine had only 36.1% of the free nicotine concentration found in nonimmunized rats. This difference was also significant, $P < .001$.

Protein-bound nicotine (shown in Fig. 4B) also differed significantly among the three treatment groups, $F(2, 15) = 1071.89$, $P < .001$. In nonimmunized rats injected with saline, there was no detectable protein-bound nicotine remaining from the previous nicotine infusion. In contrast with free serum nicotine, a comparison of the two groups injected with nicotine revealed that the immunized rats had 94.2 times the concentration of protein-bound nicotine found in rats pretreated with control IgG. This was also a significant difference, $P < .001$, according to Fisher's LSD test.

Brain nicotine levels 10.5 min after subcutaneous injection of either nicotine or saline are shown in Fig. 5. An ANOVA revealed significant differences among the three groups, $F(2, 15) = 109.73$, $P < .001$. Comparing the two nonimmunized groups, saline-injected rats had only 6.0% of the brain nicotine concentration found in nicotine injected rats. This significant difference, $P < .001$, shows that brain nicotine levels largely reflected the recent subcutaneous injection as opposed to the previous chronic nicotine infusion. Finally, the immunized rats injected with nicotine had only 53.2% of the brain nicotine concentration found after nicotine injection in nonimmunized rats. This difference was also significant, $P < .001$.

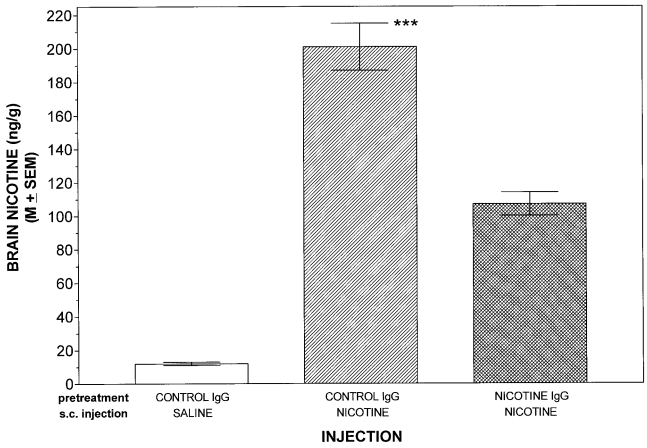

Fig. 5. Brain nicotine levels (means ± S.E.M.) 10.5 min following a subcutaneous injection of saline or 0.12 mg/kg nicotine in rats pretreated 25 h earlier with 150 mg (ip) of either control IgG or nicotine-specific IgG. *** $P < .001$ vs. both other groups.

2.3. Discussion

The results of the current study support the hypothesis that passive immunization with nicotine-specific antibody can greatly interfere with the pharmacological actions of nicotine. Immunization with 150 mg of nicotine-specific IgG reduced the abstinence alleviating effect of 0.12 mg/kg nicotine by 89%. While this nicotine dose may not have been completely absorbed by the time of observation, it did produce a serum nicotine level in nonimmunized controls of 46 ng/ml, which is at the high end of concentrations observed in smokers (Benowitz and Jacob, 1984). The reduction in abstinence signs after nicotine injection in immunized rats differed little from the reduction in signs after saline injection in nonimmunized rats. The 31.7% reduction of signs over the 2.5 h between observations in the saline-injected group presumably reflects the likelihood that nicotine abstinence syndrome is past its peak at the time of retest, 25 h after termination of nicotine infusion (Malin et al., 1992). The 25-h interval was necessary to allow full distribution of intraperitoneally administered antibody into blood (based on data from pilot experiments).

The differential effects of nicotine-specific and control IgG on abstinence reversal by subcutaneously injected nicotine do not appear to depend on baseline (preinjection) differences. Among the rats subsequently challenged with nicotine, those that were immunized displayed similar numbers of baseline signs to those that were not immunized. Since the nicotine-specific IgG by itself clearly had little effect on abstinence syndrome, it was not necessary to include an additional control group of nicotine immunized rats injected with saline.

Immunization resulted in the retention of more nicotine abstinence signs despite nicotine injection in every category except miscellaneous signs (a category with very few signs in any group). Therefore, the attenuation of nicotine's abstinence-alleviating effect was not limited to any one type of behavioral abstinence sign.

Consistent with its behavioral effects, immunization greatly altered nicotine distribution in both serum and brain. Following a nicotine injection, immunized rats had a significantly higher serum concentration of protein-bound nicotine and lower concentrations of free nicotine in both serum and brain. Consistent with earlier data (Pentel et al., 2000), these results support the proposed mechanism of action of immunization: sequestration of nicotine in serum, reduction of the free drug concentration (the form that is able to distribute to tissues), and reduced distribution of nicotine to the brain. In addition, the current data has implications for

the quantitative relationship between pharmacokinetic and behavioral effects of immunization. In this protocol, an approximately 50% reduction in brain nicotine concentration was sufficient to essentially completely prevent an important behavioral effect to nicotine. Thus, completely preventing nicotine distribution to the brain may not be necessary to substantially alter its behavioral effects.

The results of the present study suggest the possibility that immunization against nicotine might be useful in smoking cessation. A heavy smoker in the first few days of smoking cessation is likely to experience discomfort, craving, and dysphoria (Hughes et al., 1991). Under these conditions, the smoker is prone to relapse, smoke a cigarette and experience relief. This relief constitutes strong negative reinforcement and is likely to help reinstate the smoking habit. However, a smoker sufficiently immunized against nicotine might not experience relief. Immunization might thus prevent negative reinforcement from smoking, aiding the extinction of smoking behavior. The question might be raised: Would immunization itself intensify the aversiveness of nicotine abstinence? In the present experiment, immunization simultaneous to termination of nicotine exposure did not markedly intensify the abstinence syndrome seen at 22.5 h post-termination. However, it remains to be determined whether immunization commencing prior to smoking cessation would alter the onset or nature of a subsequent nicotine abstinence syndrome.

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