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A Nicotine Conjugate Vaccine Reduces Nicotine Distribution to Brain and Attenuates Its Behavioral and Cardiovascular Effects in Rats

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PENTEL, P. R., D. H. MALIN, S. ENNIFAR, Y. HIEDA, D. E. KEYLER, J. R. LAKE, J. R. MILSTEIN, L. E. BASHAM, R T. COY, W. D. MOON, R. NASO AND A. FATTOM. *A nicotine conjugate vaccine reduces nicotine distribution to brain and attenuates its behavioral and cardiovascular effects in rats.* **65**(1) 191–198, 2000.—Vaccination of animals to elicit drug-specific antibodies, or the passive transfer of such antibodies from other animals, can reduce the behavioral effects of drugs such as cocaine and heroin. To study the potential application of this approach to treating nicotine dependence, IgG was isolated from rabbits immunized with a nicotine–protein conjugate vaccine. Anesthetized rats received immune IgG containing nicotine-specific antibodies (Nic-IgG) or control-IgG IV. Thirty minutes later, rats received nicotine at 0.03 mg/kg IV, equivalent on an mg/kg basis to the nicotine intake from two cigarettes by a smoker. Compared to control-IgG, Nic-IgG reduced the brain nicotine concentration in a dose-related manner (65% reduction at the highest IgG dose). Pretreatment with Nic-IgG also reduced the distribution to brain of five repeated doses of nicotine (equivalent to the nicotine intake from 10 cigarettes) administered over 80 min. To study blood pressure effects, rats received control-IgG or Nic-IgG 1 day prior to administering nicotine. Nicotine-induced systolic blood pressure increases were attenuated by Nic-IgG in a dose-related manner, and were almost completely blocked by the highest Nic-IgG dose. Pretreatment with Nic-IgG also completely prevented the nicotine-induced stimulation of locomotor activity observed in rats receiving control-IgG. Nic-IgG did not prevent locomotor activation from cocaine, demonstrating its specificity for nicotine. These data demonstrate that the administration of nicotine-specific antibodies can reduce or prevent some of the pharmacokinetic, cardiovascular, and behavioral consequences of nicotine in rats. Effects were observed at nicotine doses and nicotine serum concentrations equal to or exceeding those typically associated with nicotine exposure in cigarette smokers. A potential role for immunization in the treatment of nicotine dependence is suggested. © 1999 Elsevier Science Inc.

Nicotine Immunization Vaccine Antibody Pharmacokinetics Blood pressure Locomotor activity

ACTIVE immunization to elicit the production of drug-specific antibodies, or the administration of exogenous drug-specific antibodies (passive immunization), has been shown to reduce heroin self-administration in monkeys (6,20), and cocaine self-administration in rats (12). Drug-specific antibodies act in this context by binding drug and reducing drug distribution to the brain (12,31). Reduction of drug self-administration through the use of immunization has been observed at drug doses and dosing rates relevant to human drug abuse (6,12,20). These studies suggest a potential role for immunization in the treatment of drug abuse. Tobacco dependence is largely the result of nicotine addiction (5,16,22). Reducing nicotine distribution to brain by the use of drug-specific antibodies might, therefore, be of possible use as an adjunct to the treat-

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ment of tobacco dependence. Nicotine is a particularly good candidate for this approach because abused doses are substantially lower than those of heroin or cocaine, and smaller amounts of antibody should be needed to attenuate its effects (4,25).

Active immunization of rats with a 6-amino derivative of nicotine conjugated to carrier protein has been shown to elicit high titers of nicotine-specific antibodies in rats and substantially alter the pharmacokinetics of subsequently administered nicotine (14,15). In rats given a single 0.03-mg/kg dose of nicotine, equivalent to the nicotine dose absorbed from two cigarettes by a smoker, immunization reduced the distribution of nicotine to brain by 38%. Decreased nicotine distribution was evident as early as 30 s after nicotine administration. Thus, brain nicotine concentrations were decreased over the period of time most critical to its reinforcing effects.

Previous studies have addressed only the pharmacokinetic effects of immunization against nicotine. In the current study, antibody effects on nicotine distribution to brain, nicotine-induced increases in blood pressure, and nicotine-induced stimulation of locomotor activity were evaluated. Passive immunization, in which rats received nicotine-specific IgG purified from rabbit antiserum, allowed the investigation of antibody dose–response relationships. In contrast to the active immunization studies discussed above, the antibodies used in the current study were induced by a different immunogen that may have advantages as a potential therapeutic agent.

GENERAL METHODS

Drugs and Reagents

(−)-Nicotine bitartrate, (−)-cotinine, (−)-methyl-^{3}H-nicotine 81 Ci/mmol, and goat anti-IgG-peroxidase conjugate were obtained from Sigma Chemical Co. (St. Louis, MO). Internal standards for the nicotine/cotinine assay were a gift form Dr. Peyton Jacob. (−)-Nicotine-N-oxide was a gift from Professor John Gorrod. Nicotine was administered to rats as nicotine bitartrate, but all doses and measured concentrations are expressed as the base.

Synthesis of Immunogen

The hapten *trans*-3′-aminomethylnicotine was prepared by modification of *trans*-3′-hydroxymethylnicotine alcohol at the 3′ position and conjugation via the modified linker arm to recombinant *Pseudomonas aeruginosa* exoprotein A (rEPA) (11). The *trans*-3′-hydroxymethylnicotine alcohol was synthesized as described by Cushman and Castagnoli (9). The alcohol was treated with *p*-toluenesulfonyl chloride in dichloromethane in the presence of triethylamine to obtain the tosylate. The tosylate was converted to the azide by treatment with sodium azide in dimethylformamide at 80°C. Reduction of the azide with lithium aluminum hydride in tetrahydrofuran provided the hapten as a yellow oil. Hapten was conjugated to rEPA through a succinic acid linker using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl to activate the linker's carboxylic group. The obtained nicotine conjugate was purified by size-exclusion chromatography. The mean incorporation of nicotine hapten was 12 mol per mol of rEPA, calculated by determining the increase in the UV absorbance at 260 nm after incorporation of the hapten relative to the absorbance at 280 nm. Protein concentration was determined by colorimetric BCA assay (Pierce, Rockford, IL).

Production of Antibody

For production of nicotine-specific IgG, New Zealand white rabbits were immunized with 100 μg IM of nicotine immunogen in complete Freund's adjuvant on day 0, and boosted with 100 μg IM of immunogen in incomplete Freund's adjuvant on days 21 and 42. Rabbits were bled weekly to obtain serum, and boosted as needed to restore antibody levels. For active immunization studies, rats were immunized with nicotine immunogen 25 μg IP in complete Freund's adjuvant on day 0, and boosted with 25 μg IP of immunogen in incomplete Freund's adjuvant on days 21 and 35.

Immune rabbit serum was purified on a Protein G Sepharose 4 Fast Flow column (Pharmacia, Piscataway, NJ) equilibrated with PBS. Purified 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 and concentrated on a Pellicon XL polyethersulfone membrane (Millipore, Bedford, MA) with a 50-kDa cutoff. The solution was brought to a concentration of 50 mg/ml total IgG by addition of PBS. Control-IgG was similarly prepared from nonimmunized rabbits.

Characterization of Antibody

Serum IgG titers were measured by ELISA as previously described (14), but using a polyglutamate–hapten conjugate as the coating antigen. Crossreactivity of immune serum was calculated as the ratio of 50% inhibitory concentrations for various ligands compared to that of (−)-nicotine. Antibody affinity and binding capacity for nicotine were measured by soluble radioimmunoassay (24). The percentage of nicotine-specific IgG in the administered IgG, and serum antibody concentrations, were calculated from the binding capacity data.

Immune serum showed 2.7% crossreactivity with the nicotine metabolite cotinine, and <1% crossreactivity with the nicotine metabolite nicotine-N-oxide or the nicotinic receptor ligand acetylcholine. The affinity of Nic-IgG for (−)-nicotine, measured from an aliquot of the Nic-IgG doses administered to rats, was $1.0 \pm 0.2 \times 10^8\ M^{-1}$ (mean ± SE). The mean nicotine-specific IgG content of each 50 mg Nic-IgG dose was or 3.1 mg, or 6.2% of total IgG.

Nicotine Assay

Concentrations of nicotine in serum and brain were measured by gas chromatography with nitrogen-phosphorus detection (14,17). Nicotine concentrations are expressed as the base. Brain nicotine concentration is expressed as ng/g wet weight, corrected for brain blood content (15). Limits of quantitation are 2 ng/ml nicotine and 10 ng/ml cotinine. ^{3}H-nicotine was measured by adding 0.1 ml of serum or brain homogenate to scintillation fluid.

EXPERIMENTS

Experiment 1. Effects of Nic–IgG (Passive Immunization) on the Distribution of a Single Dose of Nicotine

The ability of pretreatment with Nic-IgG to bind nicotine in serum and reduce nicotine distribution to the brain was evaluated.

Methods. Sprague–Dawley rats weighing 242 to 347 g were anesthetized with droperidol/fentanyl IM, and PE50 catheters placed in the right jugular and left femoral veins (14). Groups of six rats were administered via the femoral vein catheter 12.5, 25, or 50 mg of Nic-IgG or 50 mg control-IgG. Thirty minutes later, serum was obtained for measurement of pretreatment antibody titers. Rats then received nicotine bitartrate 0.03 mg/kg (of the base), equivalent to the nicotine dose

absorbed from two cigarettes in a human, infused over 10 s (4). This nicotine dose, route, and infusion rate were used because they 1) produce serum nicotine concentrations in the 10–40-ng/ml range observed in regular cigarette smokers; 2) produce initial arterial nicotine concentration several times higher than concurrent venous concentrations, similar to the arterial–venous concentration difference observed with cigarette smoking in humans (14); and 3) represent a behaviorally active nicotine dose in both humans and rats (3,4,8). Three minutes after the nicotine infusion, rats were decapitated, trunk blood collected, and brain removed and stored at −20°C for analysis. Trunk blood can be used for this purpose because differences in nicotine concentrations between arterial and venous blood at this time interval after nicotine infusion are negligible (15). Two additional groups had a femoral vein catheter placed, received 50 mg control-IgG or Nic-IgG, and were allowed to recover from anesthesia. These groups were reanesthetized 24 h later, a jugular vein catheter was placed, and nicotine administered as above. The purpose of these groups was to determine whether a longer interval between Nic-IgG and nicotine, to allow more extensive distribution of IgG out of serum, would diminish the effects of the Nic-IgG. Differences in brain and serum nicotine concentrations were compared using one-way ANOVA with Dunnett's test for individual post hoc comparisons, and linear trend analysis.

Results. Although this protocol was not designed to specifically address toxicity, no detrimental effects of vaccination were observed in immunized rats. Four groups of rats received nicotine 30 min after IgG. In these rats, the mean serum nicotine concentration (Fig. 1, top left panel), measured 3 min after the nicotine dose, was significantly increased by each dose of Nic-IgG ($p < 0.001$) in a dose-related manner according to linear trend analysis ($p < 0.001$). Serum nicotine concentrations were 6.2, 10.6, and 18.4 times that of controls for the 12.5, 25, and 50 mg Nic-IgG doses, respectively. The mean brain nicotine concentration (Fig. 1, bottom left panel) was significantly reduced by all doses of Nic-IgG ($p < 0.001$) in a dose-related manner ($p < 0.001$). Brain nicotine concentrations were reduced by 33, 52, and 65% compared to controls for the 12.5, 25, and 50 mg doses, respectively.

Two additional groups of rats received nicotine 24 h after Nic-IgG (Fig. 1, right panel). The mean serum nicotine concentration, measured 3 min after the nicotine dose, was significantly increased in rats receiving Nic-IgG compared to controls ($p < 0.001$) and was 12.3 times that of controls. The mean brain nicotine concentration was reduced by 61% compared to controls ($p < 0.001$). Compared to animals receiving nicotine 30 min after Nic-IgG, the increase in serum nicotine concentration was smaller (12.3 vs. 18.4-fold increase) but the decrease in brain concentration was similar (61 vs. 65%).

Thirty minutes after administering Nic-IgG to rats, the serum nicotine-specific IgG concentration was 0.21 ± 0.03 g/L. Twenty-four hours after administering Nic-IgG, the serum nicotine-specific IgG concentration was lower, 0.09 ± 0.01 g/L. Assuming a total serum IgG concentration of 10 g/L (13), nicotine-specific IgG represented 2.1% of the total IgG in serum at 30 min and 0.9% of the total IgG in serum at 24 h.

Experiment 2: Effects of Nic–IgG (Passive Immunization) on the Distribution of Repeated Nicotine Doses

This experiment studied whether the ability of Nic-IgG to reduce nicotine distribution to the brain, demonstrated above, is maintained with the administration of five repeated doses of nicotine (equivalent to the nicotine dose absorbed by a human smoking 10 cigarettes over 80 min).

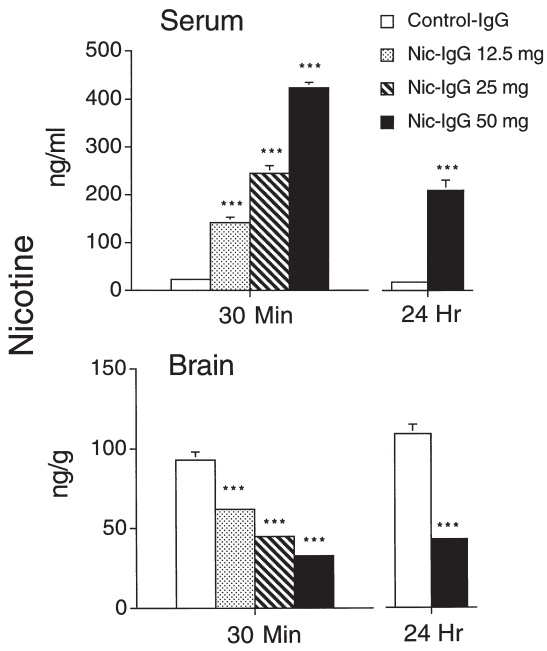

FIG. 1. Serum and brain nicotine concentrations (ng/g wet weight) in rats 3 min after a single dose of nicotine 0.03 mg/kg IV (mean ± SE). Four groups (left panel) were pretreated with control-IgG or Nic-IgG 30 min prior to receiving nicotine. Nic-IgG pretreatment increased the serum nicotine concentration and decreased the brain nicotine concentration in a dose-related manner. Two additional groups (right panel) were pretreated with control-IgG or Nic-IgG, but received nicotine 24 h later. Compared to animals receiving nicotine 30 min after IgG, the increase in serum nicotine concentration was less but the decrease in brain nicotine concentration was similar (65% after 30 min, 61% after 24 h). ***$p < 0.001$.

Methods. Groups of eight rats were anesthetized, a femoral vein catheter placed, and 50 mg Nic-IgG or control-IgG administered. The catheter was removed and animals were allowed to recover. Twenty-four hours later rats were again anesthetized, femoral and jugular vein catheters placed, and nicotine (0.03 mg/kg) was administered IV via the jugular catheter at 0, 20, 40, 60, and 80 min for a total of five doses. Blood (1 ml) was removed via the femoral vein catheter 1 min after the first nicotine dose. Removal of this volume of blood does not alter blood pressure or heart rate (unpublished data). The fifth and final nicotine dose included 3 μCi of ^{3}H-nicotine so that the distribution of that dose could be followed independent of the five cumulative doses. Rats were sacrificed 1 min after the final nicotine dose, and trunk blood and the brain were removed for analysis. Nicotine concentrations were compared using *t*-tests.

Results (Fig. 2). Administration of Nic-IgG increased nicotine retention in serum and reduced nicotine distribution to brain even after five repeated doses of nicotine. This was true of both the cumulative nicotine dose (as measured by unlabeled nicotine concentrations) and the final nicotine dose alone (as measured by ^{3}H-nicotine concentrations).

The mean serum unlabeled nicotine concentration measured after the first nicotine dose was 11.7 times higher in the group receiving Nic-IgG than in controls ($p < 0.001$), and after the final (fifth) dose of nicotine the mean serum nicotine

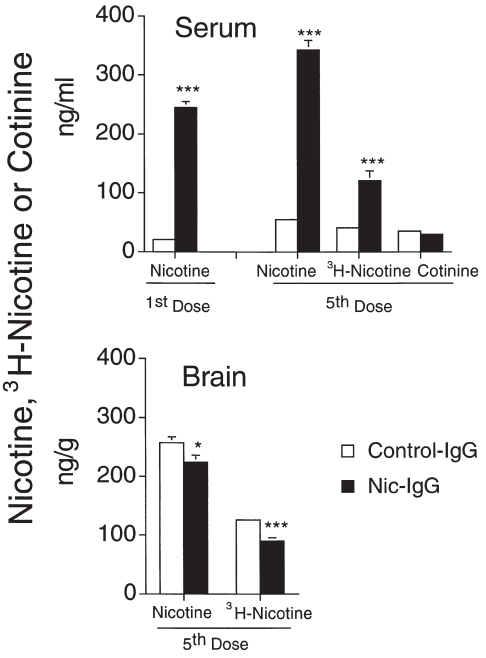

FIG. 2. Serum and brain nicotine concentration in rats receiving five repeated doses of nicotine after pretreatment with 50 mg control-IgG or Nic-IgG (mean ± SE). ^{3}H-nicotine was added to the fifth nicotine dose to allow measurement of effects on this dose independent of the cumulative five doses. After the first dose (top left), the serum nicotine concentration was increased in rats pretreated with Nic-IgG. After the fifth dose (top right) both the serum unlabeled nicotine concentration (representing cumulative nicotine dosing) and the ^{3}H-nicotine concentration (representing the fifth nicotine dose) were increased compared to controls, while that of the nicotine metabolite cotinine was not. Brain concentrations (lower panel) of both unlabeled nicotine and ^{3}H-nicotine were decreased in animals pretreated with Nic-IgG compared to controls. $*p < 0.05$. $***p < 0.001$.

concentration was 6.2 times higher ($p < 0.001$). The serum ^{3}H-nicotine concentration after the final nicotine dose, in the group receiving Nic-IgG, was 3.0 times higher than in controls ($p < 0.001$). Serum cotinine concentrations after the fifth nicotine dose were slightly lower in rats receiving Nic-IgG than in those receiving control-IgG (30 ± 1 vs. 35 ± 2 ng/ml, mean ± SE, $p = 0.52$).

The mean brain unlabeled nicotine concentration (representing cumulative nicotine dosing) in rats treated with Nic-IgG was reduced by 13% compared to controls ($p < 0.05$). However, the mean brain concentration of ^{3}H-nicotine (representing the fifth dose of nicotine alone) in rats treated with Nic-IgG was reduced by 29% compared to controls ($p < 0.001$).

Twenty-four hours after administering Nic-IgG to rats, the serum nicotine-specific IgG concentration was 0.1 ± 0.004 g/L (mean ± SE). Assuming a total serum IgG concentration of 10 g/L (13), nicotine-specific IgG represented 1% of total IgG in serum.

Experiment 3: Effects of Active Immunization on the Distribution of a Single Dose of Nicotine

The previous two experiments examined passive immunization with Nic-IgG. The current experiment examined active immunization, to allow comparison of the serum antibody concentrations and effects on nicotine distribution between these two methods of immunization.

Methods. Five rats actively immunized with nicotine immunogen were studied 7–14 days after their second booster dose. Four sham-immunized rats served as controls. Rats were anesthetized, cannulated, and serum was obtained to measure prenicotine antibody titers. Animals then received nicotine 0.03 mg/kg IV and were sacrificed 3 min later, as described above. Brain and serum nicotine concentrations were compared using *t*-tests.

Results (Fig. 3). The mean serum nicotine concentration measured 3 min after the nicotine dose was 8.5 times higher in immunized rats than that in controls ($p < 0.001$), and the brain nicotine concentration was reduced by 64% compared to controls ($p < 0.001$).

The serum IgG affinity for nicotine in rats actively immunized with nicotine immunogen was $2.6 \pm 0.6 \times 10^7$ M^{-1} and binding capacity for nicotine was 0.2 ± 0.1 g/L. Assuming a total serum IgG concentration of 10 g/L, nicotine-specific IgG represented 2% of total IgG.

Experiment 4: Effects of Nic–IgG (Passive Immunization) on Nicotine-Induced Stimulation of Blood Pressure

The effects of a single nicotine dose on systolic blood pressure were established, and the ability of pretreatment with Nic-IgG to prevent this effect was studied.

Methods. Sixteen male Sprague–Dawley rats, weighing 350–450 g, were habituated to a cylindrical restrainer and to tail pressure from an inflatable cuff. Each rat was habituated four times for durations increasing from 20 to 60 min. A day after the final habituation, each rat was injected IP with either 50, 100, or 150 mg of Nic-IgG or with phosphate-buffered saline (PBS) alone. Nic-IgG was administered IP 1 day prior to nicotine, rather than IV immediately prior to nicotine (as in the pharmacokinetic experiments) to minimize manipulations that might influence blood pressure. Twenty-five hours later, each rat was placed in the restrainer at an ambient temperature of 29°C, and systolic blood pressure was determined by tail-vein occlusion using an automated cuff (Harvard Apparatus, Natick, MA). The rat was then challenged by injection of nicotine 0.035 mg/kg SC. This dose of nicotine was selected on the basis of preliminary dose–response experiments to produce the desired increase in systolic blood pressure. (These preliminary experiments established that saline injection per se had virtually no effect on blood pressure). Systolic

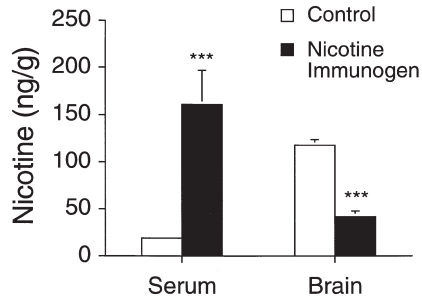

FIG. 3. Serum and brain nicotine concentrations in actively immunized rats (mean ± SE). Three minutes after a single nicotine dose of 0.03 mg/kg, the serum nicotine concentration was higher in immunized rats compared to controls, and brain nicotine concentration was decreased by 64%. $***p < 0.001$.

blood pressure was measured at 2.5, 5.0, and 7.5 min after injection. The dependent variable was the average of the three post-injection readings minus the pre-injection reading. Differences in systolic blood pressure were compared using one-way ANOVA and Dunnett's test for individual post hoc comparisons, and linear trend analysis.

To determine the effects of control-IgG on nicotine-induced stimulation of blood pressure, an additional five rats were pretreated with control-IgG 150 mg IP, and four rats were pretreated with PBS. Effects of nicotine 0.035 mg/kg SC on blood pressure were then studied as above.

Results (Fig. 4). The baseline systolic blood pressure did not differ among groups (means of 105, 118, 110, and 115 mmHg for PBS, Nic-IgG 50, Nic-IgG 100, and Nic-IgG 150 mg groups, $p > 0.05$). Systolic blood pressure increased equally in rats pretreated with either PBS or control-IgG ($p = 0.6$). Nic-IgG pretreatment attenuated nicotine-induced stimulation of systolic blood pressure in a dose-related manner according to linear trend analysis ($p < 0.01$). All Nic-IgG groups differed significantly from PBS controls.

Expeirment 5: Effect of Nic–IgG (Passive Immunization) on Nicotine-Induced Stimulation of Locomotor Activity

This experiment determined the effect of nicotine 0.28 mg/kg SC on locomotor activity and the ability of Nic-IgG to prevent this nicotine effect.

Methods. The subjects were 28 male Sprague–Dawley rats weighing 353–543 g. The behavioral apparatus was a Stoelting activity monitor connected to an IBM Thinkpad computer. The system tallied horizontal activity by cumulating the crossing of photoelectric beams placed 2 cm apart. The subjects were habituated to this apparatus three times for 40 min each time. The habituation routine included each rat being removed from the apparatus after 20 min, and held for a sham injection before being returned to the apparatus for another 20 min. On the experiment day, each rat was placed in the apparatus and habituated for 15 min. Its activity counts during the subsequent 5 min were recorded. The rat was then injected SC with either 0.28 mg/kg nicotine in PBS or with PBS alone. This dose was chosen on the basis of pilot experiments showing that 0.28 mg/kg was the highest dose of nicotine that increased activity without inducing any observable locomotor abnormalities such as stiff gait or ataxia. Immediately following injection, the rat was returned to the activity monitor and its activity counts were cumulated over the subsequent 5 min. The subject's activity change score was the number of postinjection counts minus the number of preinjection counts.

To determine the effect of Nic-IgG on nicotine-induced locomotor stimulation, an additional 14 male Sprague–Dawley rats weighing 380–505 g were studied. The methods were identical to those above with the following exception: 25 h prior to the experiment, six rats were injected IP with 50 mg control-IgG and eight rats received Nic-IgG. All subjects were tested as above for locomotor activity during 5 min intervals before and after receiving nicotine 0.28 mg/kg SC. The dependent variable was the change in activity counts from pre- to post-nicotine injection. Locomotor activity scores were compared using *t*-tests.

To determine the serum nicotine concentrations achieved in this experiment, a separate group of six rats received nicotine 0.28 mg/kg SC, and were decapitated 5 min later. Trunk blood was collected for measurement of serum nicotine levels.

Results (Fig. 5). The baseline locomotor activity scores for animals receiving control-IgG or Nic-IgG did not differ (132 ± 19 vs. 129 ± 31 activity counts/5 min, $p > 0.5$). Nicotine alone resulted in an increase of 70.7 ± 33.9 activity counts/5 min (mean ± SE). Saline resulted in a decrease of 1.6 ± 25.6 activity counts/5 min. This difference was significant ($p < 0.05$).

Rats pretreated with control-IgG increased their activity by 71.0 ± 32.7 counts/ 5 min after receiving nicotine. Rats pretreated with Nic-IgG showed no increased activity in response to nicotine injection; their activity decreased 15.0 ± 24.1 counts/5 min. This difference was significant ($p < 0.05$).

Serum nicotine concentration 5 min after administration of nicotine 0.28 mg SC was 168 ± 16 ng/ml (mean ± SE).

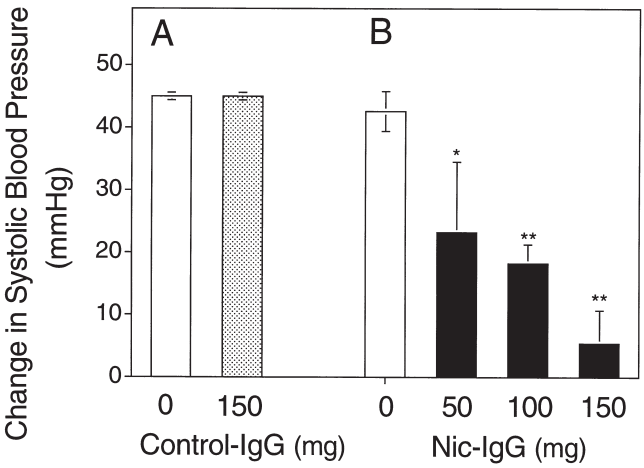

FIG. 4. Changes in systolic blood pressure following a single nicotine dose of 0.035 mg/kg SC (mean ± SE). (A) Systolic blood pressure increased equally in rats pretreated with either PBS or control-IgG. (B) The nicotine-induced increase in blood pressure was attenuated by pretreatment with Nic-IgG in a dose-related manner, and was nearly completely suppressed at the highest Nic-IgG dose. $*p < 0.05$. $**p < 0.01$.

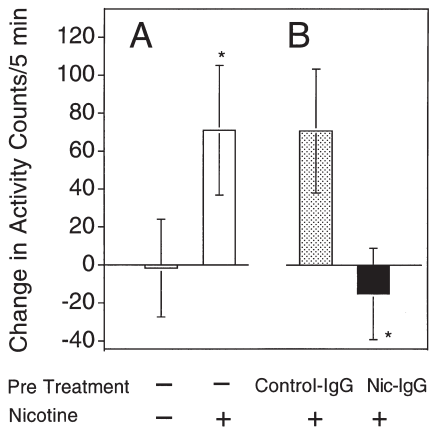

FIG. 5. Changes in activity counts in rats after treatment with nicotine 0.28 mg SC. (A) In rats receiving no pretreatment, nicotine significantly increased locomotor activity scores. (B) Nic-IgG completely prevented the nicotine-induced increase in locomotor activity observed in rats receiving control IgG. $*p < 0.05$.

Experiment 6: Effects of Nic–IgG (Passive Immunization) on Cocaine-Induced Locomotor Stimulation

This experiment determined whether the effects of Nic-IgG were specific to nicotine-induced locomotor stimulation as opposed to drug-induced stimulation in general. The effects of Nic-IgG on locomotor stimulation by an unrelated drug, cocaine, were studied to evaluate Nic-IgG specificity.

Methods. The methods were identical with those used to study nicotine-induced locomotor stimulation with the following exceptions. Six male Sprague–Dawley rats weighing 368 –476 g were pretreated with 50 mg Nic-IgG IP, while six received the same amount of control-IgG. Twenty-five hours later, each rat was pretested for locomotor activity as above, injected with cocaine HCl 0.18 mg/kg SC (as the base) and retested. The dependent variable was the change in activity counts from the last 5 min of pre-test to 5 min of post-test. The dose of cocaine was chosen based on pilot dose–response experiments suggesting that it produced a comparable activity increase to that seen after 0.28 mg/kg nicotine in the experiment described above. Locomotor activity scores were compared using *t*-tests.

Results. Nic-IgG did not attenuate the locomotor stimulant effect of cocaine. The group receiving Nic-IgG responded to cocaine with an increase of 107.6 ± 46.5 counts/5 min (mean ± SE) counts, while the group receiving control-IgG responded with and increase of 60.2 ± 44.8 counts/5 min (data not shown). This difference was not significant ($p > 0.48$).

DISCUSSION

Most existing or candidate treatments for drug abuse target brain receptors or neurotransmitter pathways. Because these targets also mediate normal brain function, side effects and lack of specificity are common. Drug-specific antibodies can bind drug outside of the central nervous system, reducing drug acces to receptor sites (12,31). This peripheral site of action, as well as the high specificity of antibody-drug binding, makes the use of drug-specific antibodies potentially attractive as an alternative therapeutic approach. In the current study, immunization reduced nicotine distribution to the brain, and attenuated nicotine-induced increases in systolic blood pressure and locomotor activity. These data support the further investigation of immunization as a means of altering the reinforcing effects of nicotine.

Both active and passive immunization significantly reduced the distribution of a single dose of nicotine to the brain. This reduction was dose dependent, with a substantial 65% reduction at the highest Nic-IgG dose. The reduction in nicotine distribution to the brain in actively immunized animals (64%) was greater than that observed previously (38%) in comparable experiments using a different immunogen (15). The greater efficacy of the current immunogen was probably due to the fourfold higher affinity for nicotine of the elicited antibodies (1×10^8 vs. 2.4×10^7 M^{-1}), as the serum antibody concentrations elicited by the two immunogens were comparable.

Nicotine distribution to the brain was significantly reduced even with the administration of five repeated doses of nicotine. A lesser effect was observed with repeated nicotine doses than with a single dose. However, distribution to brain of the fifth (radiolabeled) nicotine dose alone was still reduced by 29%, showing that Nic-IgG had not been saturated. The nicotine doses administered in this experiment were substantial (equivalent to the nicotine absorbed by a human from 10 cigarettes over 80 min), and each individual dose was infused rapidly (the nicotine equivalent of two cigarettes infused over 10 s) (4). These factors may have impacted on the ability of antibody to prevent nicotine distribution to brain. A

TABLE 1

MOLAR RATIOS OF IgG BINDING SITES TO NICOTINE

Experiment	Nic-IgG Dose* (mol of Binding Sites)	Nicotine Dose† (mol)	Molar Ratio IgG Binding Sites: Nicotine
Pharmacokinetics (Passive IgG, single-dose nicotine)	1×10^{-8}	6.1×10^{-8}	0.16
	2×10^{-8}	6.1×10^{-8}	0.33
	4×10^{-8}	6.1×10^{-8}	0.66
Pharmacokinetics (Passive IgG, repeated-dose nicotine)	4×10^{-8}	3.1×10^{-7}	0.13
Blood pressure	4×10^{-8}	7.2×10^{-8}	0.60
	8×10^{-8}	7.2×10^{-8}	1.20
	1.2×10^{-7}	7.2×10^{-8}	1.70
Locomotor activity	4×10^{-8}	5.7×10^{-7}	0.07

Ratios represent the maximum fraction of the administered nicotine dose that could have been bound by antibody. Values were calculated using molecular weights of 162 Da for nicotine, 150 kDa for IgG, and assuming two binding sites per IgG. In the pharmacokinetic experiment, efficacy in reducing nicotine distribution to brain increased as the IgG:nicotine ratio approached 1. Nic-IgG was most effective in preventing blood pressure increases at ratios greater than 1, while nicotine-induced locomotor activation was prevented by a much lower IgG:nicotine ratio, 0.07.

*Molar doses were calculated using molecular weights of 75 kDa per binding site for IgG and 162 Da for nicotine. Ratios are corrected for Nic-IgG having a nicotine-specific IgG content of 6.2%.

†Because nicotine was dosed on a mg/kg basis, the values represented here are for a 333-g rat.

higher Nic-IgG dose, as was used in the blood pressure experiment, would likely have been more effective.

The Nic-IgG doses used in this study were intended to simulate antibody concentrations produced by active immunization. The 50-mg dose of Nic-IgG produced a serum nicotine-specific IgG concentration of 0.1 g/L or approximately 1% of total IgG, while active immunization resulted in a concentration of 0.2 g/L or 2% of total IgG. Thus the Nic-IgG doses used to reduce nicotine's cardiovascular and behavioral effects were within the range that is achievable with active immunization. Similar antibody concentrations have been achieved in humans as well with selected immunogens (18,33).

Of total body IgG, an estimated 65–75% exists outside of the serum, leaving only 25–35% in the serum (2). Administering nicotine 30 min after Nic-IgG might exaggerate the benefit of immunization, in that most of the IgG would still be in serum and might be more available to bind nicotine than IgG outside of serum. The effects of delaying nicotine administration for 24 h after Nic-IgG, to allow more complete distribution of IgG out of serum, were therefore studied. A 24-h interval (equal to 4 IgG distribution half-lives) was selected to allow sufficient time to approximate the steady-state distribution of IgG. Delaying nicotine dosing for 24 h reduced the amount of nicotine bound in serum, as would be expected with less nicotine-specific IgG remaining in serum, but did not appreciably reduce the effects of Nic-IgG on nicotine distribution to the brain. These data suggest that nicotine-specific IgG outside of serum contributed to nicotine binding. The participation of extravascular IgG in nicotine binding is important because it substantially increases the fraction of a nicotine dose that can be bound, even in the first few minutes after nicotine dosing.

Nicotine-induced increases in blood pressure and heart rate have been studied as immediate and readily quantitated nicotine effects that occur with clinically relevant nicotine doses (1,26). In the current study, the effect of nicotine on blood pressure was reduced by Nic-IgG in a dose-dependent manner, and largely eliminated by the largest (150 mg) dose. This effect was specific, as control-IgG did not alter the nicotine-induced increase in blood pressure. These data demonstrate that immunization can alter some of nicotine's pharmacologic effects, and begin to establish the range of nicotine-specific IgG doses required.

Further evidence of the ability of immunization to alter nicotine's effects is provided by the marked reduction of nicotine-induced locomotor activation by Nic-IgG. Nicotine in various doses can cause both increases (7,19,21,27) and decreases (7,10,27,28) in several measures of locomotor activity. The stimulant effect of nicotine in the current study was generally consistent with the effects of a similar nicotine dose range on numbers of horizontal movements in studies by others (19,21). Pretreatment with control-IgG did not alter the response to nicotine, whereas Nic-IgG completely prevented nicotine-induced locomotor activation. This effect of Nic-IgG appeared to be selective for nicotine, as it did not attenuate locomotor stimulation from cocaine. Serum nicotine concentrations in this experiment (mean 168 ng/ml) were 5–10 times higher than those typical of cigarette smoking (10–40 ng/ml) (3). Thus, Nic-IgG was effective in preventing this centrally mediated nicotine effect despite the presence of serum nicotine concentrations higher than those likely to be encountered clinically.

The Nic-IgG:nicotine ratios required to produce effects in these experiments varied substantially (Table 1). Preventing nicotine-induced stimulation of blood pressure required much higher ratios (up to 1.7) than preventing nicotine-induced stimulation of locomotor activity (ratio of 0.07). The reason for this difference is not clear. In part, administering nicotine SC may have exaggerated the ratio if nicotine was incompletely absorbed from the injection site at the time of study. However, a similar finding was reported for studies of cocaine administered IV in which an IgG:cocaine ratio of just 0.08 was sufficient to abolish cocaine self-administration in rats (12).

Although passive immunization was used in this study primarily as a means of controlling antibody dose and studying dose–effect relationships, the possibility of using passive immunization for therapeutic purposes may also have merit. Most relapse to smoking among patients who quit occurs in the first few weeks to months (29,30). With a terminal half-life of over 3 weeks in humans (32), passively administered IgG could have a sufficiently prolonged effect to provide a therapeutic option. The lack of any permanent effect on the immune system, in contrast to the life-long effect of active immunization, might be advantageous. Allergic reactions or serum sickness in response to exogenous IgG may occur, but can be minimized by using human IgG, or by a variety of strategies to "humanize" heterologous antibodies.

Collectively, the current studies suggest that antibody against nicotine can alter the distribution of nicotine sufficiently to interfere with immediate nicotine effects associated with serum nicotine concentrations equal to or greater than those typical of heavy cigarette smoking. This raises the possibility that immunological interventions might attenuate those actions of nicotine that maintain smoking, such as positive reinforcement (8) and relief of the nicotine abstinence syndrome (23).

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