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Influence of Kinetics of Nicotine Administration on Tolerance Development and Receptor Levels

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MARKS, M J , J A STITZEL AND A C COLLINS *Influence of kinetics of nicotine administration on tolerance development and receptor levels* PHARMACOL BIOCHEM BEHAV 27(3) 505–512, 1987 —Nicotine was administered intravenously to DBA mice through cannulae implanted in the jugular veins Five groups of animals were treated a control group which received saline and four nicotine treatment groups All of the nicotine treatment groups received a dose of 4 0 mg/kg/hr The first group received continuous infusion, the second group received 1 mg/kg pulses four times an hour, the third group received 2 mg/kg pulses twice an hour, and the fourth group received 4 mg/kg pulses once an hour After a 10-day treatment period, the animals were tested for tolerance to an acute intraperitoneal administration of nicotine Tolerance was measured using a test battery composed of the following tests respiratory rate, acoustic startle response, Y-maze crosses and rears, heart rate, and body temperature Mice from each of the four nicotine treatment groups were tolerant to the acute effect of nicotine, but the extent of tolerance varied among the groups as follows continuous infusion < 1 mg/kg pulses four times/hr < 2 mg/kg pulses twice/hr < 4 mg/kg pulse once/hr Chronic nicotine infusion resulted in significant increases in the binding of L-[^{3}H]nicotine in all six brain regions assayed and in significant increases in the binding of α-[^{125}I]bungarotoxin binding in cerebral cortex and hippocampus All increases in binding resulted from increases in B_{max} for these ligands In contrast to the effects observed for tolerance development, the increases in [^{3}H]nicotine binding were not significantly affected by the kinetics of nicotine infusion However, the binding of α-[^{125}I]BTX tended to increase along with the peak plasma concentration of nicotine and paralleled the differences in tolerance The binding of the muscarinic antagonist, quinuclidinyl benzilate, was unaffected by nicotine treatment While the average blood level of nicotine did not differ significantly among the four groups, peak levels were higher after infusion of both the 2 mg/kg and 4 mg/kg pulses The relationship between the tolerance development and the changes in receptor levels was not dramatic which suggests that additional biochemical and physiological mechanisms must be influencing tolerance development

Tolerance development Receptor levels Nicotine infusion Nicotinic receptors

CHRONIC treatment of rodents with nicotine results in the development of tolerance to the acute behavioral and physiological effects of the drug [3–5, 7–9, 11, 13, 15, 16, 21, 24, 25] and to the effects of nicotine on corticosterone release [2] The tolerance observed for mice appears to be functional rather than dispositional in that chronic treatment by either injection [5] or infusion [11] has little effect on the metabolism of nicotine Several biochemical changes, which may be related to the functional tolerance, have been noted after chronic treatment Nicotinic receptors increase after chronic injection of nicotine in the rat [9, 17, 22, 23] or after chronic nicotine infusion in the mouse [11, 13, 15, 16] The increases in nicotinic binding sites noted for mice increase with nicotine dose [11, 13, 16] and time of nicotine treatment [15] The time- and dose-dependent tolerance to the effects of nicotine in several behavioral or physiological responses in the treated animals parallels the increase in the number of nicotinic receptors [15,16] Although a correlation exists between tolerance and receptor increase, a cause-effect relationship between these variables has not been definitely established

The route of nicotine administration as well as the dosage schedule also influence the development of tolerance Tolerance has been obtained after daily injections, constant infusion, or intake in drinking water [24,25] The tolerance observed after injection of a total dose of 1 0 mg/kg administered in three equal doses exceeded that observed when a total dose of 1 2 mg/kg was administered daily through an implanted reservoir Increasing the dosage administered through the reservoir to about 5 mg/kg/day resulted in tolerance comparable to that observed with the three daily injections These results suggest that the dynamics of drug administration are important for the development of tolerance

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The present study was undertaken to determine whether the timing of nicotine administration influences tolerance development and/or brain nicotinic binding sites Nicotine was administered either by constant infusion or by pulse administration (one, two or four times per hour) The results obtained indicate that the dynamics of drug administration influence the development of tolerance, but at this dose has little influence on the extent of receptor change

METHOD

Mice

Female DBA/2Ibg mice were used in this study This strain has been maintained in the breeding colony at the Institute for Behavioral Genetics for at least 20 generations

Before surgery, mice were housed with littermates and permitted free access to food and water Animals were maintained on a 12 hr light/12 hr dark cycle (lights on 7 a m to 7 p m) The mice were 60–90 days old at the time of surgery

Surgery

A cannula made of silastic tubing was implanted in the right jugular vein of each mouse using the method of Barr *et al* [1] Animals were anesthetized with pentobarbital (45 mg/kg) and chloral hydrate (63 mg/kg) during surgery

The day after the surgery, the mice were transferred to individual cages (15×15×25 cm, l×w×h) and each cannula was attached to tubing that was connected to a 1 ml syringe mounted on a Harvard Infusion Pump The animals were subsequently infused with sterile saline The next day the saline infusion was continued for control animals or drug treatment was begun

Chronic Drug Treatment

For those mice to be treated with nicotine (L-nicotine base, Sigma Chemical Co , St Louis, MO), drug treatment was begun on the second day of infusion The infusion dose for all drug treatment groups was 4 0 mg/kg/hr and the hourly infusion volume was 35 μl The animals were divided equally among five treatment groups (one saline infused and the other four nicotine infused) The drug treatment differed among the four groups as follows (1) Constant nicotine infusion, (2) Nicotine infusion in pulses of 1 mg/kg Pulses 2 min in duration were spaced at 15 min intervals, (3) Nicotine infusion in pulses of 2 mg/kg Pulses were 4 min in duration and were spaced at 30 min intervals, and (4) Nicotine infusion in a pulse of 4 mg/kg Pulses were 8 min in duration and were administered hourly The dosage per minute in the three pulse infusion groups was 0 5 mg/kg/min The mice were infused for 10 days before testing

Tolerance Tests

Tolerance was measured after 10 days of treatment using a battery consisting of the following tests respiratory rate, acoustic startle response, Y-maze activity (both line crossings and rears), heart rate, and body temperature All tests were conducted on each individual 2 hours after cessation of treatment Details of the test method have been published previously [14] Each animal was tested with only one nicotine challenge dose but three different doses were employed for each treatment group to allow the construction of dose-response curves Isoeffective doses (ED values) were calculated for each of these dose-response curves The values calculated were the dose that elevated respiratory rate

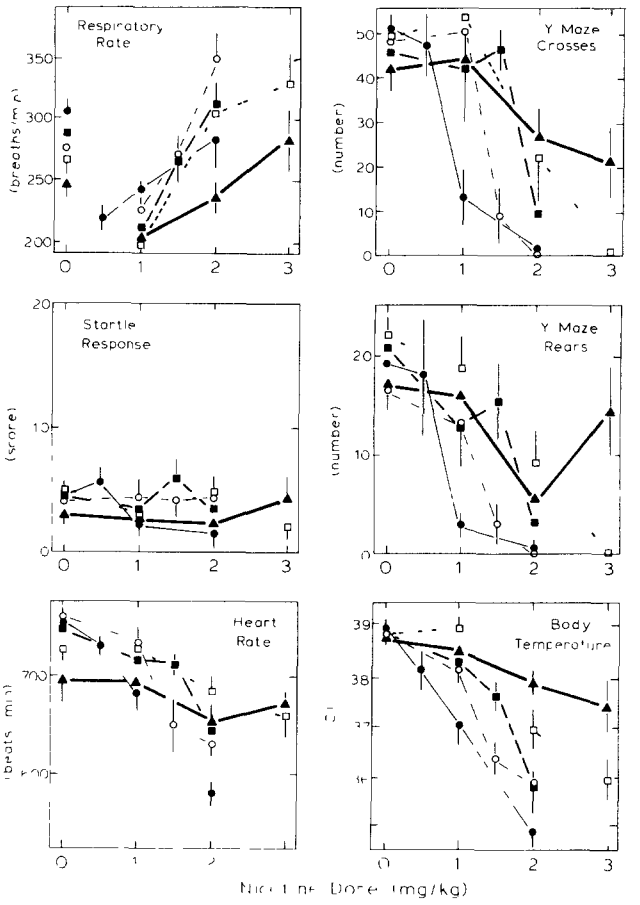

FIG 1 Nicotine effects after chronic treatment Mice were chronically treated by intravenous infusion of saline (●) or 4 0 mg/kg/hr nicotine Nicotine was administered continuously (○), in four 1 mg/kg pulses/hr (■), in two 2 mg/kg pulses/hour (□) or in one 4 mg/kg pulse/hr (▲) Responses of mice of each treatment group were measured with a test battery after intraperitoneal administration of the indicated doses of nicotine Points represent mean±SEM for 15–16 mice after saline injection and for 5–6 mice at each nicotine dose

to 260 breaths/min (ED_{260}), the dose that decreased heart rate by 100 beats/min (ED_{-100}), the dose that decreased Y-maze crosses and rears by 50% (ED_{50}), and the dose that decreased body temperature 2° (ED_{-2}) In addition, an overall responsiveness index was calculated as described previously [16] This value is a weighted average of the individual drug effects After completion of the tolerance test each mouse was returned to its infusion chamber and drug treatment was resumed The following day each animal was removed from its infusion chamber and after 2 hr was tested for its baseline responses (saline injection) The continued treatment for the day between the tolerance test and the baseline test was included to minimize possible changes in response and biochemical parameters that may have occurred had treatment been stopped for 24 hr

Tissue Preparation

After completion of the tolerance test, the mouse was sacrificed by cervical dislocation and its brain was removed and the blood rinsed off The brain was dissected into seven

regions cortex, cerebellum, hindbrain (pons-medulla), hypothalamus, hippocampus, striatum, and midbrain (midbrain areas remaining after removal of the hypothalamus, hippocampus, and striatum) The cerebellum was discarded owing to its low level of cholinergic activity The tissue pieces were placed in 10 vol of HEPES-buffered Ringer's solution (NaCl, 118 mM, KCl, 4 8 mM, $CaCl_2$, 2 5 mM, $MgSO_4$, 1 2 mM, HEPES, 20 mM, pH adjusted to 7 5 with NaOH) and quickly frozen (−70°) On the day of assay, the samples were thawed and homogenized with a glass-teflon homogenizer The particulate fraction was prepared using the method of Romano and Goldstein [19] Prior to each of the three centrifugation steps, the homogenates were incubated for 5 min at 37° to promote the dissociation of any nicotine that may have been in the tissue [11]

L-[^3H]Nicotine Binding

The binding of L-[^{3}H]nicotine (N-methyl ^{3}H, specific activity=75 7 Ci/mmol, New England Nuclear, Boston, MA) was measured at 37°C using a modification of the method of Romano and Goldstein [19] as described previously [12] Nicotine binding was determined in the six brain regions Assays were conducted using a single nicotine concentration of 5 1 nM In addition, at least three full saturation curves were constructed for each brain region to determine the effect of drug treatment on the binding parameters

α-[^{125}I]Bungarotoxin (BTX) Binding

The binding of α-[^{125}I]BTX (tyr-^{125}I, initial specific activity=138 Ci/mmol, New England Nuclear, Boston, MA) was measured as described previously [12] Assays were conducted for each brain region using a single concentration of ligand (1 6 nM) In addition, at least three full saturation curves were constructed for each brain region to determine the effect of drug treatment on the binding parameters

L-[^3H]Quinuclidinyl Benzilate (QNB) Binding

The binding of L-[^{3}H]QNB was determined using a modification of the method of Yamamura and Snyder [27] as described previously [12] Binding in each brain region was measured using 87 4 pM L-[^{3}H]QNB

Scintillation Counting

After the samples were washed, the glass fiber filters were placed in polypropylene scintillation vials (7 ml) and 2 5 ml of scintillation fluid (Budget-Solve, Research Products International, Mt Prospect, IL) were added The samples were mechanically shaken for 30 min and radioactivity was determined on a Beckman LS 1800 liquid scintillation spectrometer Tritium was counted at 40% efficiency and ^{125}I was counted at 44% efficiency

Protein Assay

Protein was measured using the method of Lowry *et al* [10] with bovine serum albumin (Sigma Chemical Co , St Louis, MO) as the standard

Nicotine Blood Levels

The concentration of nicotine in the blood of mice of the various treatment groups was determined by methods described previously [5, 6, 11] Mice were treated with nicotine solutions containing 10 μCi of L-[^{3}H]nicotine per ml

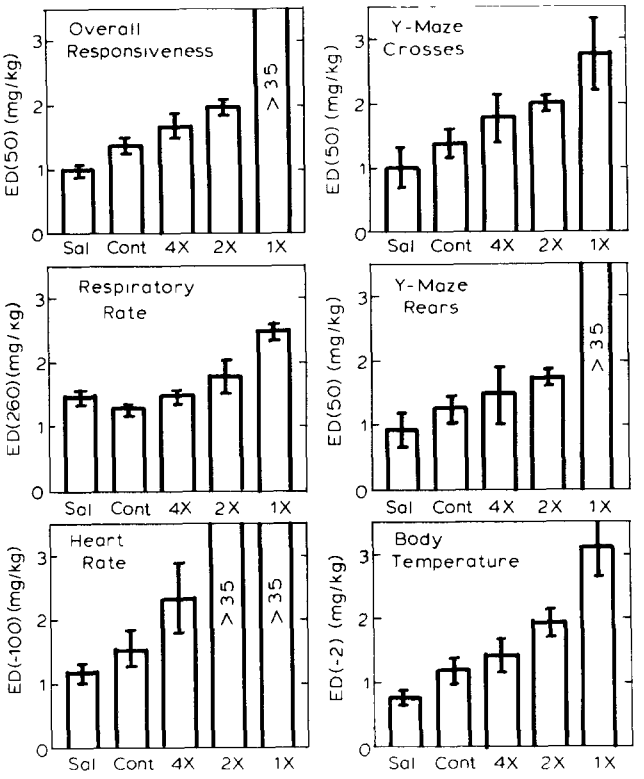

FIG 2 Effect of nicotine treatment on ED values Isoeffective doses (mean±SEM) of nicotine for five tests in the battery and for overall responsiveness were calculated by regression analysis of curves shown in Fig 1 Values differing significantly from controls ($p<0$ 05) are indicated by asterisks (*) The abbreviations for the five treatment groups are Sal-Saline infused, Cont-continuous nicotine infusion of 4 0 mg/kg/hr, 4X-Nicotine infusion as pulses of 1 mg/kg four times an hour, 2X-Nicotine infusion as pulses of 2 mg/kg twice an hour, and 1X-Nicotine infusion as pulses of 4 mg/kg once an hour

Animals were treated with the tracer-containing solutions for at least 3 hr prior to sampling of blood levels The timing of nicotine administration corresponded to that of the chronic treatments Five min prior to the beginning of the sampling period each mouse was anesthesized by administration of 400 mg/kg chloral hydrate At the completion of the administration, the mouse was removed from the infusion chamber and 40 μl of blood was removed from the retro-orbital sinus using a heparin-coated capillary tube Blood was sampled at various times thereafter continuing to the time at which the next pulse was to be administered Mice in the continuous infusion group remained attached to the infusion pumps throughout the sampling period

Data Analysis

Dose-response curves obtained from the tolerance tests and Scatchard plots of the binding data were subjected to linear regression analysis Lines were subsequently compared using *t*-tests Individual binding data were analyzed using one-way analysis of variance Those groups showing significant overall effects of treatment were compared using Duncan's test

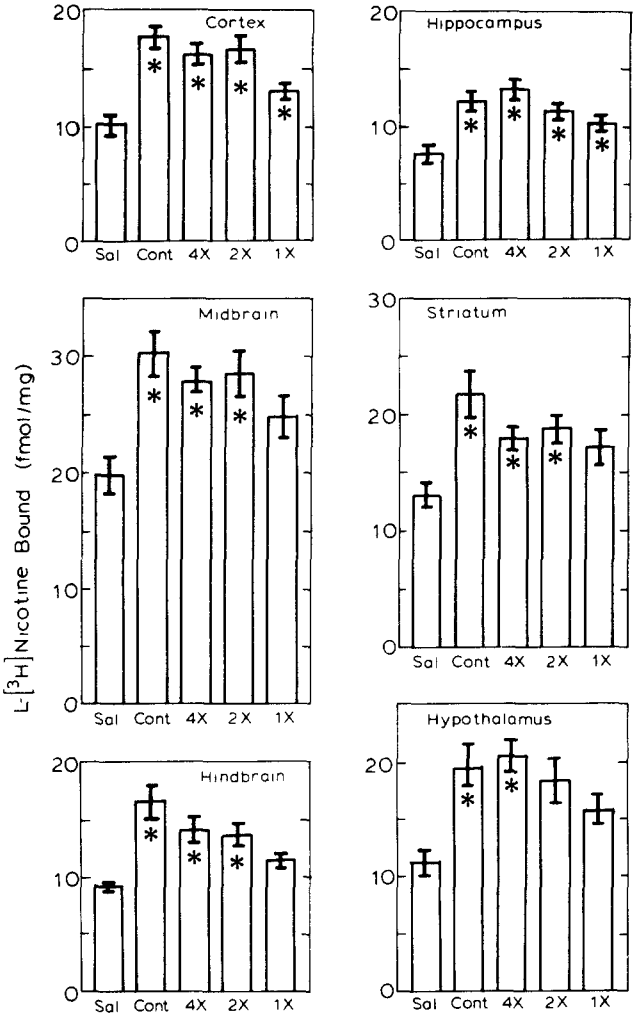

FIG 3 L-[^{3}H]Nicotine binding The binding of L-[^{3}H]nicotine was measured at an average ligand concentration of 5 nM Values represent mean±SEM of 11 individual assays Values differing from saline-infused controls are marked with asterisks (*) Group abbreviations are given in the legend to Fig 2

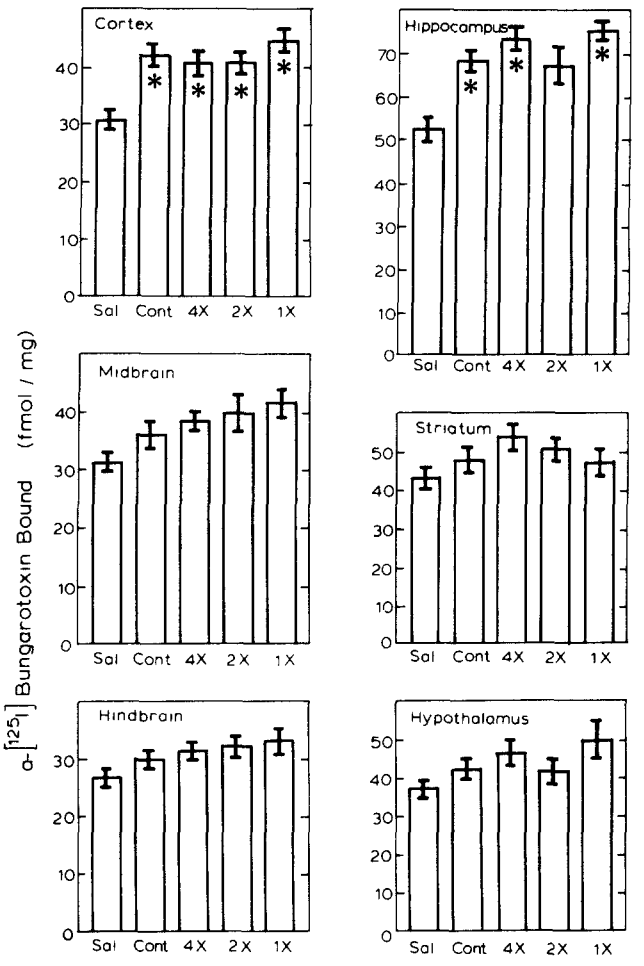

FIG 4 α-[^{125}I]BTX binding The binding of α-[^{125}I]BTX was measured at an average ligand concentration of 1 6 nM Values represent mean±SEM of 12 individual assays Values differing from saline-infused controls are marked with asterisks (*) Group abbreviations are given in the legend to Fig 2

RESULTS

The dose-response curves constructed for mice of each of the five treatment groups are displayed in Fig 1 Nicotine had no measurable effect on the startle response displayed by these mice and chronic nicotine infusion did not alter this Chronic nicotine infusion had significant effects for each of the other five tests in the battery For respiratory rate, only those mice infused with nicotine in a single 4 mg/kg pulse hourly displayed tolerance to the respiratory stimulation induced by nicotine For Y-maze crosses, Y-maze rears, heart rate and body temperature, nicotine-infused mice of each treatment group differed from controls In general, for each of these four tests the shifts to the right of the dose-response curves proceeded (from left to right) as follows continuous infusion at a rate of 4 mg/kg/hr < infusion of a 1 mg/kg dose four times per hour < infusion of a 2 mg/kg dose twice per hour < infusion of a 4 mg/kg dose once an hour Thus, the greater the amount of nicotine in each pulse the greater the tolerance development

The results presented in Fig 2 provide the ED values calculated for the lines in Fig 1 A measure of overall responsiveness representing a weighed average response for five of the tests of the battery (excluding startle) has been included to provide an indication of total sensitivity to the effects of nicotine The trends in the ED values reinforce the results generated from the dose-response curves shown in Fig 1 The higher the amount of nicotine contained in an individual pulse, the greater the degree of tolerance Tolerance to the effects of nicotine on Y-maze rears and overall responsiveness in animals treated once an hour with a 4 mg/kg pulse was so great that the ED value exceeded the highest challenge dose administered The tolerance to the effects on heart rate was substantial for animals receiving nicotine pulses either once or twice an hour

To determine if the kinetics of nicotine administration affected binding sites for putative nicotinic receptors, brains of animals from each of the treatment groups were assayed for the binding of L-[^{3}H]nicotine and α-[^{125}I]BTX In addition, the effects of nicotine treatment on the binding of

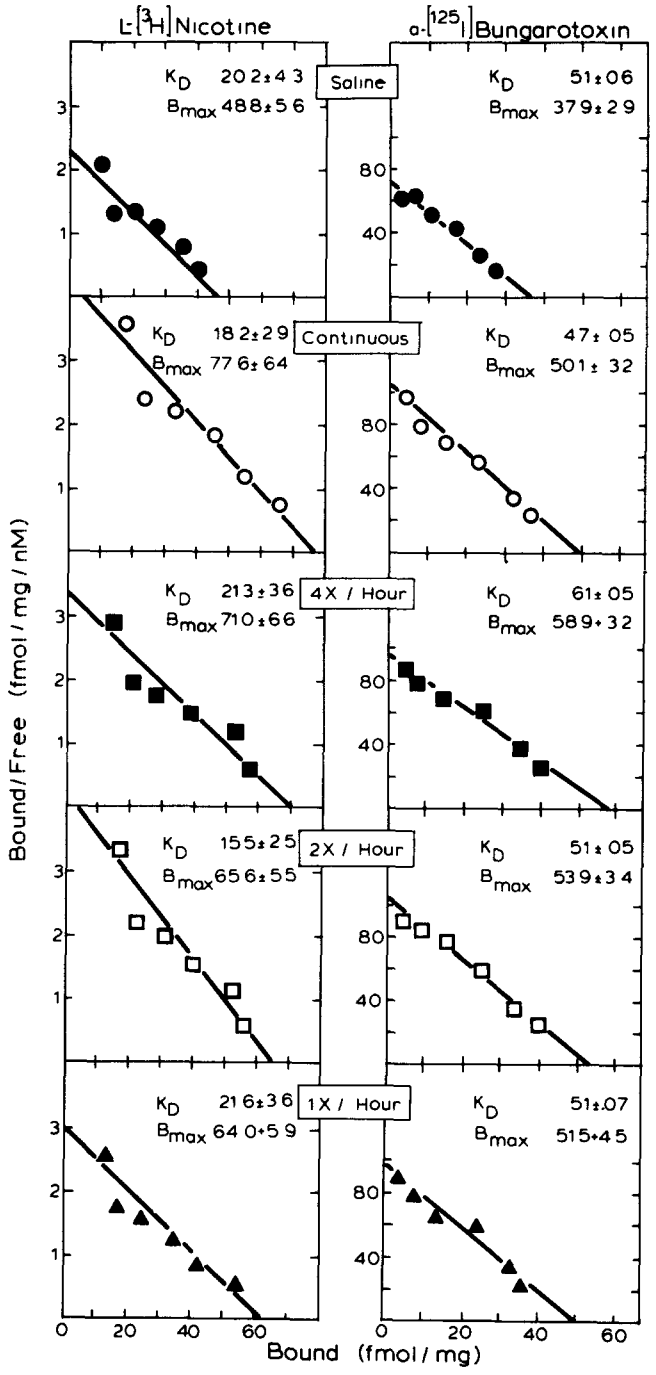

FIG 5 Scatchard plots for L-[^{3}H]nicotine and α-[^{125}I]BTX binding in cerebral cortex Scatchard plots for the two ligands were constructed in cortices of mice of each of the 5 treatment groups Values of K_D and B_{max} were calculated by linear regression Each point represents the mean of 4–5 individual experiments

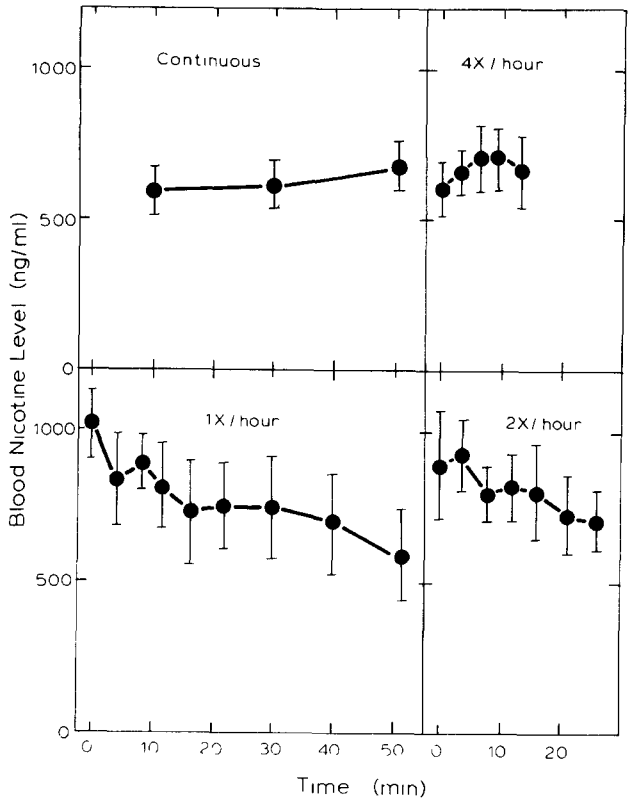

FIG 6 Blood levels of nicotine L-Nicotine containing L-[^{3}H]nicotine tracer was administered to mice of the four treatment groups indicated Blood samples (40 μl) were taken and the nicotine extracted as indicated in the Method section Each point represents mean±SEM of 6–7 individual measurements

L-[^{3}H]QNB were measured to determine whether changes observed were selective for nicotinic binding sites

The results displayed in Fig 3 are histograms of L-[^{3}H]nicotine binding measured in six brain regions at a single ligand concentration Nicotine treatment resulted in a significant increase in the binding of L-[^{3}H]nicotine in all six regions assayed However, in contrast to the results obtained for the tolerance tests, no increase in binding was observed as the amount of nicotine contained in a single pulse increased The binding observed for the continuously infused mice tended to be greater than that for all other treatment groups in all regions

The results displayed in Fig 4 are histograms of α-[^{125}I]BTX binding measured in six brain regions at a single ligand concentration Nicotine treatment resulted in a significant increase in α-[^{125}I]BTX binding in two regions cerebral cortex and hippocampus The binding observed for all nicotine treatment groups was similar for both of these brain regions

Nicotine infusion had no effect on L-[^{3}H]QNB binding in any brain region (results not shown)

The changes observed in both L-[^{3}H]nicotine and α-[^{125}I]BTX binding after nicotine treatment could have arisen from changes in K_D and/or B_{max} for either ligand Previous studies in which nicotine was chronically infused have demonstrated that the changes in binding arise from B_{max} changes alone, but inasmuch as the dynamics of drug administration employed in the present study differed from the continuous infusion used in the past, Scatchard plots were constructed using tissue obtained from all six regions of all five treatment groups The results shown in Fig 5 are Scatchard plots obtained for cerebral cortex Drug treatment had no effect on the K_D for either L-[^{3}H]nicotine or α-[^{125}I]BTX, the changes in binding observed were changes in B_{max} The values calculated for the binding parameters for

TABLE 1

BINDING CONSTANTS FOR NICOTINIC RECEPTORS AFTER CHRONIC TREATMENT

Group	Brain Region					
	Cortex	Midbrain	Hindbrain	Hippocampus	Striatum	Hypothalamus
	L-[^{3}H]Nicotine Binding					
B_{max} Values (fmol/mg protein)						
Saline	48 9 ±5 6	78 2 ±6 4	34 7 ±4 7	23 4 ± 5 1	37 9 ± 5 1	48 2 ± 7 0
Continuous	77 6 ±6 4*	103 8 ±6 6*	60 9 ±3 9*	47 6 ± 6 9*	81 5 ±12 0*	74 6 ± 8 0*
4 Pulses/Hour	71 0 ±6 9*	103 1 ±7 4*	47 7 ±4 3*	47 4 ± 6 3*	89 6 ± 9 3*	65 9 ± 8 5*
2 Pulses/Hour	65 6 ±5 2*	96 0 ±8 0*	51 2 ±5 0*	39 6 ±10 4	81 5 ± 7 1*	74 5 ± 8 9*
1 Pulse/Hour	64 0 ±5 9*	90 1 ±6 4*	51 8 ±6 1*	44 8 ± 6 9*	75 6 ±15 2*	68 3 ± 7 4*
K_D Values (nM)						
Saline	20 2 ±4 3	17 0 ±2 7	16 0 ±4 4	10 1 ± 5 6	8 0 ± 3 0	15 0 ± 4 6
Continuous	18 2 ±2 9	14 0 ±1 9	13 0 ±1 8	16 0 ± 1 8	12 6 ± 4 1	13 1 ± 3 1
4 Pulses/Hour	21 3 ±3 6	15 3 ±2 2	10 8 ±2 2	13 2 ± 3 9	19 4 ± 3 8	8 8 ± 2 8
2 Pulses/Hour	15 5 ±2 5	13 5 ±2 5	15 5 ±3 1	10 5 ± 6 8	15 0 ± 2 6	13 6 ± 3 6
1 Pulse/Hour	21 6 ±3 6	11 9 ±1 9	18 2 ±4 2	14 8 ± 4 8	16 1 ± 6 6	13 5 ± 3 2
	α-[^{125}I]Burgarotoxin Binding					
B_{max} Values (fmol/mg protein)						
Saline	37 9 ±2 9	42 8 ±1 9	32 4 ±4 8	51 8 ± 3 1	58 0 ± 3 1	40 6 ± 5 8
Continuous	50 1 ±3 2*	40 3 ±4 1	38 2 ±5 1	72 8 ± 6 0*	54 3 ± 3 5	48 1 ± 7 5
4 Pulses/Hour	58 9 ±3 3*	42 7 ±3 3	35 6 ±5 0	83 0 ± 6 3*	53 4 ± 8 9	60 0 ±11 3
2 Pulses/Hour	53 9 ±3 4*	48 6 ±3 1	36 6 ±5 5	65 8 ± 2 6*	44 9 ± 6 8	40 0 ± 2 6
1 Pulse/Hour	51 5 ±4 4*	44 2 ±4 6	41 3 ±2 9*	85 3 ± 8 3*	53 9 ± 5 8	48 6 ± 4 4
KD Values (nM)						
Saline	0 51±0 06	0 48±0 03	0 47±0 11	0 42± 0 04	0 56± 0 08	0 29± 0 08
Continuous	0 47±0 05	0 42±0 07	0 43±0 09	0 48± 0 06	0 38± 0 04	0 42± 0 11
4 Pulses/Hour	0 61±0 05	0 48±0 06	0 44±0 10	0 43± 0 05	0 44± 0 12	0 54± 0 16
2 Pulses/Hour	0 51±0 05	0 48±0 05	0 47±0 11	0 39± 0 03	0 35± 0 09	0 28± 0 03
1 Pulse/Hour	0 51±0 07	0 35±0 06	0 48±0 05	0 52± 0 08	0 39± 0 07	0 41± 0 06

K_D and B_{max} values were calculated from at least 3 separate Scatchard plots for each ligand in each of the 6 brain regions Values represent mean ± SEM Those values differing from saline-treated controls are marked with asterisks (*)

both ligands in all six brain regions are summarized in Table 1 The pattern observed in every region is the same as that observed for cortex K_D values were unaffected, any significant changes arose from changes in B_{max} The pattern observed from the single point assays are confirmed by the patterns observed for B_{max} values For nicotine treatment the greatest B_{max} values for L-[^{3}H]nicotine binding were found after continuous infusion and the B_{max} values tended to decrease as the nicotine content per pulse increased, but the B_{max} values were greater for all treatment groups than they were for the saline-treated mice The B_{max} values for α-[^{125}I]BTX binding were affected in cortex and hippocampus and were similarly elevated in all nicotine treatment groups

To more fully describe the dynamics of nicotine levels, nicotine content in the blood of mice of the various treatment groups was measured The results of these measurements are shown in Fig 6 The concentration of nicotine in the blood of mice continuously infused with nicotine was relatively constant and averaged 670 ng/ml The levels of nicotine in the blood of mice administered 1 mg/kg of nicotine four times an hour were also relatively constant, and the average level was approximately 660 ng/ml The blood levels of nicotine in mice administered the drug in 2 mg/kg and 4 mg/kg pulses varied with time Peak levels were observed soon after completion of the pulsing and were higher than the levels observed for either the continuously infused mice or those pulsed with 1 mg/kg (peak level for the 2 mg/kg pulse was 880 ng/ml and for the 4 mg/kg pulse was 1000 ng/ml) The drug levels declined steadily from peak values to a low point immediately before the next pulse was to be administered The average pre-pulse levels were 700 ng/ml for the 2 mg/kg pulse group and 580 ng/ml for the 4 mg/kg pulse group

The areas under the concentration/time curves (the average nicotine concentration in the blood) were calculated for each of the four treatment groups The average areas under the curves were 670±109 ng/ml for continuous infusion,

660±112 ng/ml for infusion four times per hour, 773±139 ng/ml for infusion twice an hour, and 707±153 ng/ml for infusion once per hour The standard errors indicated for these values were estimated from the errors observed for the individual points, not from differences in drug level as a function of time Average blood levels did not differ significantly among the four treatment groups

DISCUSSION

In agreement with the results of Stolerman *et al* [24], animals administered nicotine in discrete pulses developed more tolerance to the effects of the drug than did animals administered the same dose of the drug continuously Peak blood levels were slightly higher in mice administered pulses of nicotine once or twice an hour but the average nicotine levels in these animals did not differ significantly from those of mice pulsed four times per hour or from those infused continuously Assuming that blood nicotine level is related to the drug concentration at the site of action, it would appear that peak drug concentration is related to the differences in tolerance development This is consistent with our earlier observation that tolerance increases with increasing treatment dose administered by constant infusion [11, 13, 16]

Several other studies have demonstrated that chronic nicotine treatment results in an increase in the number of brain nicotinic receptors [9, 11, 13, 15–17, 22, 23] This is a surprising finding especially considering chronic treatment results in reduction in drug response (tolerance) We [11] and others [17,23] have speculated that nicotine treatment serves to initially stimulate brain nicotinic receptors and that a longer lasting desensitization of these receptors then occurs Perhaps neural tissue responds to this long-lasting desensitization by increasing the synthesis or decreasing the catabolism of receptor molecules, but it may well be that the absolute number of "activatable" (non-desensitized) receptors has decreased This would result in tolerance to the effects of nicotine

The blood levels of nicotine varied much less than would have been predicted by a simple one-compartment pharmacokinetic model In an earlier analysis of nicotine pharmacokinetics [6] we obtained data that suggested nicotine has a half-life of 5–7 min in several inbred mouse strains This half-life calculation was based on the assumption that nicotine is distributed into a single compartment If nicotine was distributed in this fashion, nearly 100% of the drug should have been eliminated within 6–7 half-lives (30–49 min) Therefore, the blood levels in the group treated with one 4 mg/kg infusion every hour should have been at or near zero immediately before the next scheduled infusion Because the blood levels in the 1X and 2X groups were quite high immediately before the next scheduled infusions, it seems likely that nicotine pharmacokinetics in the mouse cannot be explained by monophasic, one-compartment kinetics Indeed, it has been demonstrated that nicotine elimination in mouse approximates first-order kinetics at short times after a single injection, but at later times the elimination kinetics appear to be multiphasic [6,18] Nicotine elimination in humans is also multi-phasic [20]

The effects of continuous and pulsed infusion of nicotine in the cat have been studied in detail [26] In this study, cats were either pulse infused once every min with 4 μg/kg for 20 min or continuously infused with the same total dose The results obtained were similar to ours in that the pulse infused animals had higher levels of nicotine in the blood at the end of infusion than did continuously infused animals and, as was the case with infused mice, the blood levels of nicotine were virtually identical in pulse- and continuously-infused cats within an hour after infusion stopped The complexities of nicotine metabolism and distribution may well contribute to the buffering of drug levels in the blood after pulse administration

While tolerance increased with dosage contained in an individual pulse, the elevation of L-[^{3}H]nicotine binding was not significantly affected by the kinetics of drug administration There were no statistically significant differences among the various nicotine infusion groups (as assessed by an ANOVA) in L-[^{3}H]nicotine binding, but an examination of Fig 3 will reveal that a trend may exist In virtually every brain region the L-[^{3}H]nicotine binding in the continuously infused group tended to be slightly greater than was the binding in the pulse infused groups and within the pulse infused groups the order was usually 4X>2X>1X (highest to lowest mean binding) Thus, there is a hint of a differential effect of the various treatment methods on L-[^{3}H]nicotine binding but, as noted above, the ANOVA failed to detect any statistically significant differences We have observed that the binding of L-[^{3}H]nicotine increases with dose [11, 13, 16], and that the increase is maximal after treatment with 4 mg/kg/hr [16] Although the peak blood levels of nicotine are higher after repeated pulse administration, it may be that differences in up-regulation were not observed because a dose of 4 mg/kg/hr induces maximal up-regulation of L-[^{3}H]nicotine binding The apparent trend (continuous >4X>2X>1X) in changes in L-[^{3}H]nicotine binding may relate to the proposed mechanism for the up-regulation of the L-[^{3}H]nicotine binding sites If the increase in receptor number is related to the extent of desensitization of the receptor it seems reasonable to expect that resensitization of the receptor would reduce the extent of up-regulation More resensitization would be predicted for a system where nicotine levels fluctuate than in a system where levels are constant Perhaps if we had used a lower total dose, or if we had increased the intervals between pulse infusions, significant differences in receptor levels would have been achieved

We have reported that the binding of α-[^{125}I]BTX continues to increase with constant infusion dose up to at least 8 0 mg/kg/hr [11, 13, 16] An increase in α-[^{125}I]BTX binding was seen in the present study as well Binding was significantly elevated in only two of the brain regions (cortex and hippocampus), and in these regions all of the treatment groups were affected similarly Non-significant increases in mean binding were seen in the other four regions but in three of these regions a potential trend was discernable (1X>2X->4X>continuous) This parallels the peak plasma concentrations We have argued previously that the L-[^{3}H]nicotine binding site is a high affinity nicotine receptor and that the α-[^{125}I]BTX binding site is a lower affinity nicotine receptor The observation that not all brain regions show statistically significant elevations of α-[^{125}I]BTX binding is consistent with the hypothesis that the α-[^{125}I]BTX site is a low affinity nicotine receptor as is the observation that the apparent increases in several brain regions parallel the peak blood-nicotine concentrations Furthermore, it may be that tolerance to lower doses of nicotine is related primarily to changes in the number of L-[^{3}H]nicotine binding sites and that tolerance at higher doses or concentrations involve changes in the number of α-[^{125}I]BTX binding sites

Clearly the differences in the levels of putative nicotinic receptors measured in relatively large brain regions cannot completely explain the differences in tolerance development Additional explanations for these differences must be sought, but the mechanisms involved may well be secondary to receptor activation

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