Pharmacology Biochemistry & Behavior, Vol. 30, pp. 255–267. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

The Comparative Binding Characteristics of Nicotinic Ligands and Their Pharmacology[1]

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SLOAN, J. W., W. R. MARTIN, M. BOSTWICK, R. HOOK AND E. WALA. *The comparative binding characteristics of nicotinic ligands and their pharmacology.* PHARMACOL BIOCHEM BEHAV **30**(1) 255–267, 1988.—Five drugs [(−)- and (+)-nicotine, (−)-lobeline, (−)-anabasine and (−)-cytisine] were infused IV into the urethane-pentobarbital anesthetized rat. Changes in heart rate, blood pressure, respiratory rate, minute and tidal volume, which appeared to be largely centrally mediated, were studied. Each of these compounds produced different pharmacologic profiles. The nature of these dissimilarities is not readily explained on the basis of pharmacokinetic considerations suggesting that the drugs have different mechanisms of action. Binding data obtained with these compounds using the rat brain P_2 preparation also show differences. (−)-Lobeline and (−)-anabasine, like the nicotinic antagonists mecamylamine and hexamethonium, bind predominantly to low affinity sites with K_Ds in the micromolar range whereas (−)-cytisine binds only to a single high affinity site with a K_D in the nanomolar range. Further, the binding patterns of these drugs are different from (−)- and (+)-nicotine which bind to both high and low affinity sites but differ from each other in binding characteristics. Thus the binding data are consistent with the pharmacologic data in suggesting that the drugs have different modes of action and support the concept that the low affinity site has an important role in the central nervous system action of these compounds.

(−)-Nicotine (+)-Nicotine (−)-Lobeline (−)-Anabasine (−)-Cytisine Heart rate Blood pressure Respiratory rate Minute volume Tidal volume Binding vs. pharmacologic studies Centrifugation vs. filtration binding techniques Vagotomy Rats

RECENT studies of the binding characteristics of nicotinic ligands indicate that they interact with multiple sites. In this regard a variety of nicotinic ligands have been studied which differ in their binding characteristics [29–33]. In an effort to determine if these binding sites have pharmacologic relevance, prototypic drugs have been studied by intravenous infusion in the urethane-pentobarbital anesthetized rat. This paper will be concerned with the pharmacologic analysis and binding characteristics of the following nicotinic ligands: (−)-nicotine, (+)-nicotine, (−)-lobeline, (−)-cytisine and (−)-anabasine.

METHOD

Binding Studies Characterizing Nicotinic Ligands

Two techniques were used to study the binding characteristics of these ligands, the filtration [29–33] and centrifugation techniques. A crude synaptosomal (P_2) fraction was prepared at 4°C from the whole brains of female Sprague-Dawley rats (200–300 g) sacrificed between 0800 and 0900 hr for both the filtration and centrifugation methods. Brains were homogenized in 10 volumes of 0.32 M sucrose using a glass vessel and serrated teflon pestle and then centrifuged at 2000 × g for 10 minutes. The supernatent was decanted and centrifuged at 50,000 × g for 20 minutes. The resulting pellet was homogenized with a Brinkmann polytron in 50 volumes of 50 mM Tris-HCl buffer, pH 7.4 and recentrifuged at 50,000 × g for 10 minutes. This final pellet was diluted to a concentration of approximately 4 mg of protein per ml in 50 mM Hepes buffer, pH 7.4 and rehomogenized with the polytron.

For the competition studies, 0.5 ml of the P_2 suspension (~2 mg protein) was transferred to 1.5 ml (centrifugation technique) or 10×75 mm (filtration technique) polypropylene tubes. Either 0.25 ml of 50 mM, pH 7.4 Hepes (total binding) or 0.25 ml of the appropriate concentration of the competing drug adjusted to pH 7.4 in 50 mM Hepes was then added. A wide range of ligand concentrations, 10^{-12} to 10^{-2} M were employed. (−)-[^{3}H]Nicotine (final concentration of 1.2×10^{-8} M) prepared in 50 mM Hepes, pH 7.4, was added last. Nonspecific binding was determined in the presence of 10^{-2} M (−)-nicotine and was used for the construction of inhibition curves. All determinations were in triplicate and each exper-

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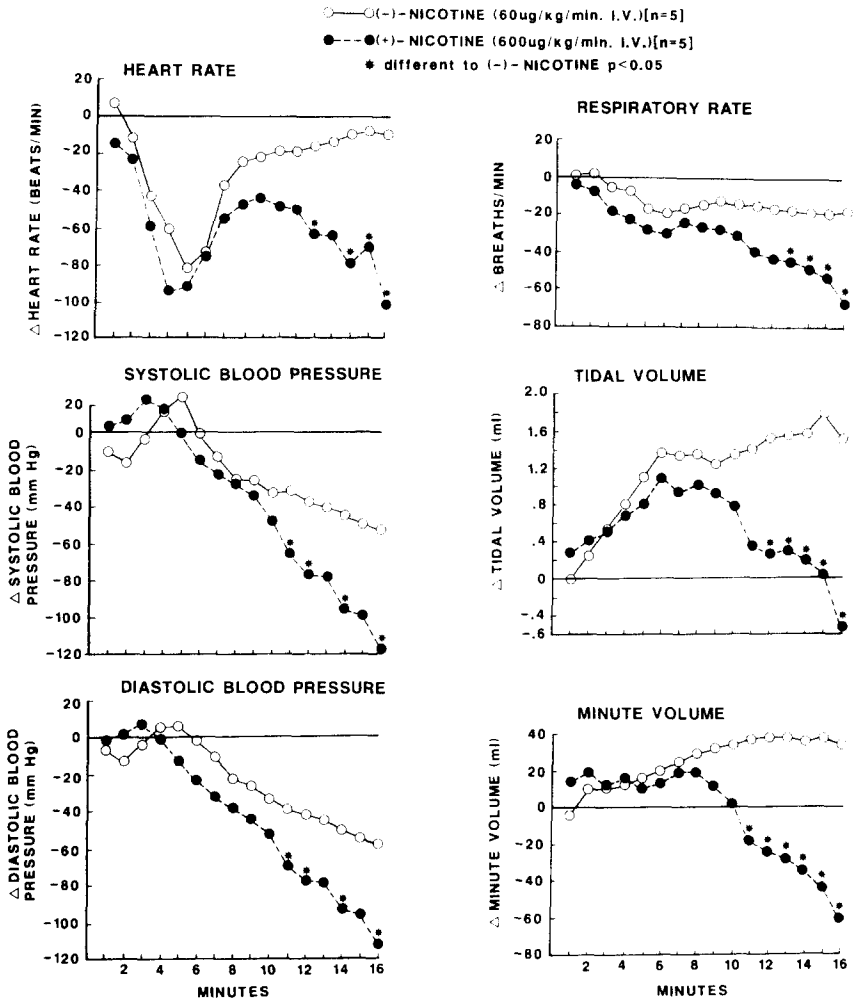


FIG. 1. Comparison of time-response curves for the continuous IV infusion of (−)- and (+)-nicotine on changes in cardiovascular and respiratory parameters. Each point represents the mean change from the predrug control value for each minute for 5 experiments.

iment was repeated 4 to 6 times using different homogenate preparations. Each tube was incubated for exactly 60 minutes (filtration technique) or 55 minutes (centrifugation technique) at 4°C in a shaking bath and vortexed at 10 minute intervals.

Filtration Technique

After incubation each sample was diluted with 3.5 ml of chilled (~4°C) Hepes and filtered at a reduced pressure (460–510 mmHg) using a filter apparatus (Hoeffer Scientific Instruments, San Francisco, CA) and Whatman GF/C glass fiber filters previously soaked in poly-l-lysine (0.1%). The filters were washed 4 times with 3.5 ml of chilled Hepes and after 20 seconds of suction were transferred to scintillation vials.

Centrifugation Technique

After incubation, the tubes were centrifuged in a Microfuge (West Coast Scientific) at 14,600 × g for 5 minutes and the pellets were washed twice by filling the tubes with 50 mM Hepes buffer, pH 7.4, and carefully aspirating the supernatant fluid. The bottom of the tubes were cut off, transferred to a scintillation vial [1] and the radioactivity counted by liquid scintillation.

Protein Analysis

Protein was estimated by previously described techniques [16,31].

Data Analysis: Binding Studies

The estimation of up-regulation of (−)-[^{3}H]nicotine binding has been described previously [29–32]. Nonspecific binding, K_Ds and site densities were estimated from the least squares best fit of the data obtained using the LIGAND Program [21].

Pharmacologic Studies

Studies were conducted in female Sprague-Dawley rats (200–300 g) while under urethane (1 g/kg, IP)-pentobarbital (20 mg/kg, IP) anesthesia. Blood pressure was measured via a cannula inserted into the left common carotid artery using a pressure transducer (Gould Strain Gauge, P23Db). The cannula was kept free of blood clots by infusing heparin, 2 units/ml, at a rate of ~0.015 ml/min. The trachea was cannulated and inspiratory flow rate was determined using a Rudolph valve and Gould pneumograph flow transducer. Minute volume was obtained using a Grass 7P10E polygraph integrator. Heart rate, blood pressure, respiratory rate and

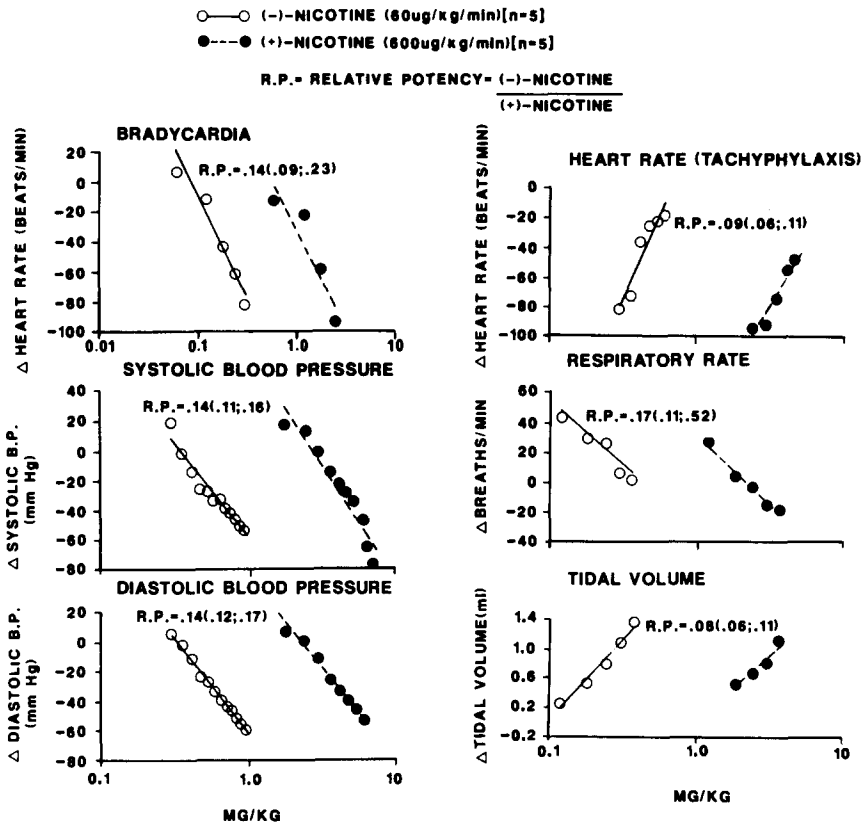


FIG. 2. Dose-response curves constructed from the data shown in Fig. 1 represent the cumulative amount of drug infused minute by minute as described in the Method section. Relative potencies with their upper and lower fiducial limits are shown for each parameter and were estimated using (−)-nicotine as the standard drug.

minute volume were recorded continuously on a polygraph (Grass, Model 7D). After surgery a heat lamp (actuated by a YSI Model 63RC temperature controller connected to the rat by a rectal thermister probe) maintained body temperature at 37±1°C throughout the experiment. Tidal volume was calculated from the minute volume and respiratory rate. Heart rate, respiration and blood pressure were also recorded on tape and subsequently digitized (Apple IIe Microcomputer equipped with an ISAAC Data Acquisition and Lab Control Module) for computer analysis. Drugs were dissolved in normal saline and administered as the free base via a cannula inserted into the right external jugular vein at a rate of 0.025 ml/minute for 16 minutes following a 10 minute predrug or saline control period. At the end of the drug infusion, residual drug was flushed into the vein and measurements of the responses were recorded for a 10 minute recovery period.

Data Analysis: Pharmacologic Studies

The data are presented as the change in response from the appropriate control. The mean change in response for each drug treatment for the different parameters was estimated for each minute and time-response curves were constructed. Dose-response curves were also constructed where the cumulative amount of drug infused was taken to be the dose at each minute. This dose estimate does not take into account distribution, metabolism and elimination of the drug. In the rat, nicotine has been shown to have two half-lives (14.64 minutes and 57.60 minutes) [2]. The data reported herein were collected during the short half-life of (−)-nicotine (14.64 minutes) and the doses have not been corrected for distribution since the half-lives of the other drugs studied are unknown. When the doses of (−)-nicotine are corrected for its short half-life, the equivalent dose for 0.06 mg/kg (minute 1) is 0.0586 mg/kg (98% of the infused dose) and the equivalent dose for 0.96 mg/kg (minute 16) is 0.6733 mg/kg (70% of the administered dose). The slopes of the dose-response curves would therefore have been shifted slightly to the right and would have been less steep had they been corrected. The data were further analyzed by a two-way analysis of variance (ANOVA), by appropriate unpaired comparisons and by a parallel line bioassay for unsymmetric designs [8]. Relative potency estimates ± the 95% fiducial limits were calculated.

Drugs and Chemicals

(−)-[^{3}H]Nicotine, 60–71.9 Ci/mmole, 99% pure, was obtained from New England Nuclear. Its purity was checked and it was stored as previously described [29]. (+)-Nicotine was resolved by Amy Howell and Dr. W. T. Smith of the University of Kentucky, Department of Chemistry [33]; sodium heparin, 1000 units per ml, was obtained from Upjohn (Kalamazoo, MI); urethane, Aldrich Chemical Company, Inc. (Milwaukee, WI); sodium pentobarbital, the Butler Co. (Columbus, OH) and dihydro-beta-erythroidine hydrobromide, Dr. Clement Stone, Merck Sharp and Dohme (West Point, PA). Sources of other drugs and chemicals have been identified previously [33].

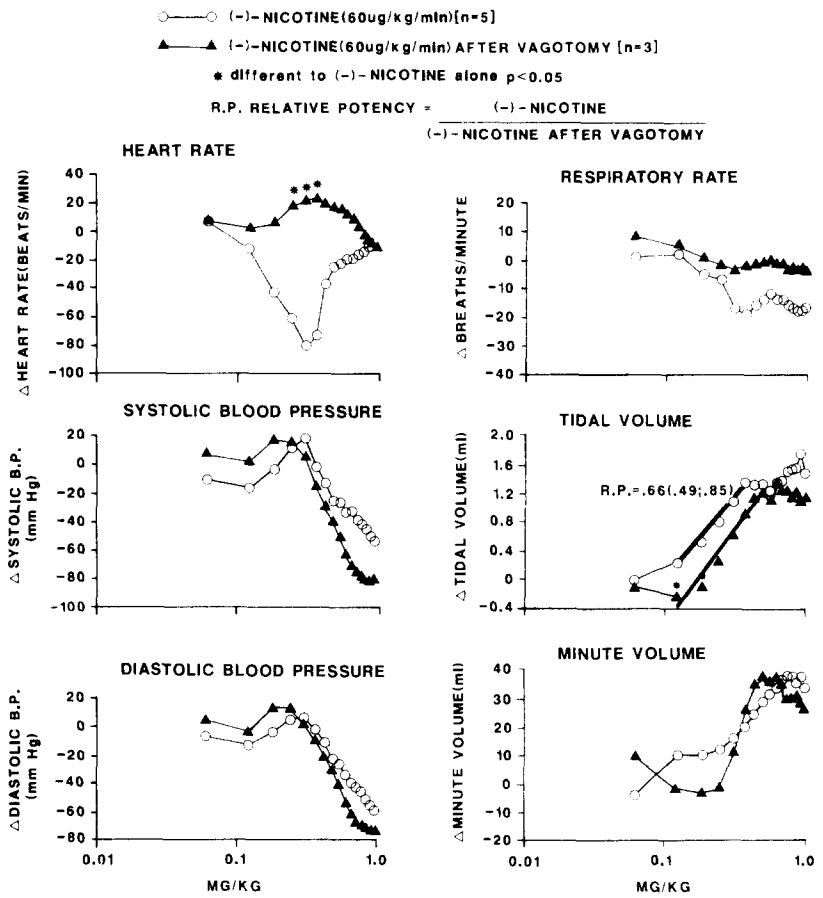


FIG. 3. Dose-response curves constructed from the continuous infusion of (−)-nicotine (60 μg/kg/minute) in the intact and bilaterally vagotomized rat. The relative potency for tidal volume was estimated as described for Fig. 2 using the points comprising the heavy lines.

RESULTS

Pharmacologic Studies

Figures 1 and 2 show the effects of an infusion of (−)-nicotine (60 μg/kg/min) in the urethane-pentobarbital anesthetized rat. As can be seen, nicotine produced bradycardia, both an increase and decrease in systolic and diastolic blood pressure, an increase in tidal volume and a dose-related increase in minute volume. Respiratory rate was depressed. During the course of infusion, tachyphylaxis was seen for some of the effects of nicotine. This is most dramatic for bradycardia which showed a high degree of tachyphylaxis midway through the infusion. At about the same concentrations, the effect of nicotine on increasing tidal volume and minute volume reached a plateau. On the other hand, blood pressure showed a progressive decrease throughout the infusion. A series of experiments were conducted to provide insight into the site of action of (−)-nicotine. Following vagotomy, (−)-nicotine did not produce bradycardia, and as a matter of fact the higher concentrations produced a small degree of tachycardia to which tachyphylaxis developed (Fig. 3). Vagotomy did not markedly alter (−)-nicotine's effects on blood pressure or respiration. Somewhat higher concentrations of (−)-nicotine were required to increase tidal volume. However, this may have been a consequence of the abolition of the Hering-Breuer reflex. Higher concentrations of (−)-nicotine produced a profound depression of respiratory rate which has led to death [28]. The respiratory depressant effects of nicotine were antagonized by naltrexone, whereas adrenalectomy did not alter this effect [28]. These two pieces of data taken together provide evidence supporting the idea that nicotine produces bradycardia and respiratory depression through a central action, effects that have been studied in the rat [6, 12–14, 25]. Some of the later effects of nicotine on blood pressure are not only a consequence of its ability to depress central vasomotor centers but are also due to respiratory depression since artificial respiration can reverse the hypotension produced by nicotine (Fig. 4A).

Figures 5 and 6 illustrate the effects of (−)-lobeline infusion. (−)-Lobeline's potency ranged from $^1/_{10}$ to $^1/_{50}$ of that of (−)-nicotine in producing bradycardia, decreasing systolic blood pressure, decreasing respiratory rate and enhancing tidal volume. As can be seen from Fig. 5, (−)-lobeline produced a greater bradycardia, a greater decrease in blood pressure and a greater stimulation of respiratory rate and minute volume than did (−)-nicotine. However, its ability to enhance tidal volume was less. (−)-Lobeline's ability to induce tachyphylaxis to the bradycardic effects was less than that of (−)-nicotine.

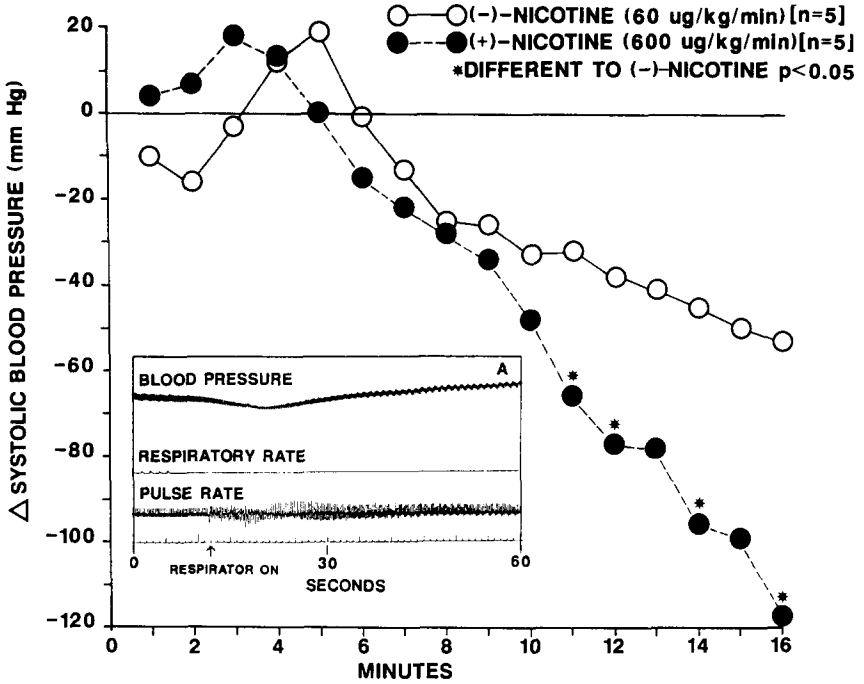


FIG. 4. Changes in systolic blood pressure induced by the continuous infusion of (−)- and (+)-nicotine. Insert "A" shows the effect of artificial respiration on (+)-nicotine induced cardiovascular responses.

Figures 7 and 8 show the effects of (−)-anabasine on the same parameters. As can be seen, the potencies of (−)-anabasine on the various physiologic parameters vary greatly. It is about 1/3 as potent as (−)-nicotine in increasing minute volume but only 1/25 as potent as (−)-nicotine in producing bradycardia. Although all of these potency estimates are based on a valid bioassay, none of them are statistically significantly different. Hence, (−)-anabasine appears to be approximately 1/10 as potent as (−)-nicotine which is consistent with the rate of infusion [13.3 times as much (−)-anabasine was infused as (−)-nicotine]. However, inspection of Fig. 7 shows that although (−)-anabasine shares common pharmacologic properties with (−)-nicotine, it differs in several ways. (−)-Anabasine produces a greater respiratory depression than (−)-nicotine and a greater fall in blood pressure. Further, tachyphylaxis to its ability to depress heart rate was not seen. The doses and rates of infusion of (−)- and (+)-nicotine as well as (−)-lobeline, (−)-anabasine and (−)-cytisine were selected to produce approximately equal degress of bradycardia.

In studies of (−)-cytisine, the onset of some of its effects (e.g., bradycardia) were delayed (Fig. 9). (−)-Cytisine is 1.4 times as potent as (−)-nicotine in decreasing blood pressure but it is 1/4 to 1/8 as potent as (−)-nicotine in decreasing heart rate, slowing respiratory rate and enhancing minute volume (Fig. 10). (−)-Cytisine produced little enhancement of tidal volume (Fig. 9).

Figures 1 and 2 compare the effects of the infusion of (−)-and (+)-nicotine. As can be seen, the infusion concentration of (+)-nicotine was 10 times that of (−)-nicotine and overall (+)-nicotine on all physiologic parameters was approximately 1/10 as potent as (−)-nicotine. However, there are differences between (+)- and (−)-nicotine at later infusion times (Fig. 1). For example, (−)-nicotine produces a greater tachyphylaxis to the slowing of heart rate, does not produce as great a depression of respiratory rate and does not produce as much depression in minute volume as (+)-nicotine. Thus, (+)-nicotine appears to be a drug that has a greater potential for producing, on a relative potency basis, respiratory depression and bradycardia than (−)-nicotine. (+)-Nicotine's greater propensity to produce a fall in blood pressure is in all probability related to its ability to depress respiration (Figs. 1 and 4A).

The cardiovascular and respiratory changes produced by the drugs in this study are quite different as can be seen from a summary of their relative potencies (Table 1).

Binding Studies

With regard to the nicotine binding sites, the issue has arisen as to the multiplicity of these sites, their pharmacology and their physiologic significance. In an effort to determine if the filtration procedure provided misleading information concerning estimates of the K_Ds and B_{max}s of the various ligands as a consequence of elution of the radioligand, two types of experiments were conducted. Many of the experiments were repeated using a centrifugation procedure where elution of the radioligand by washing from the binding site would be minimized. As can be seen from Table 2, there is quite good agreement between the K_Ds and site densities for sites II, IV and V between data acquired using the filtration and centrifugation techniques for (−)-nicotine, (−)-lobeline and (−)-anabasine. It is important to note that site V was only seen with (−)-nicotine when the centrifugation technique was used but was seen with (−)-anabasine when either the filtration or centrifugation techniques was used.

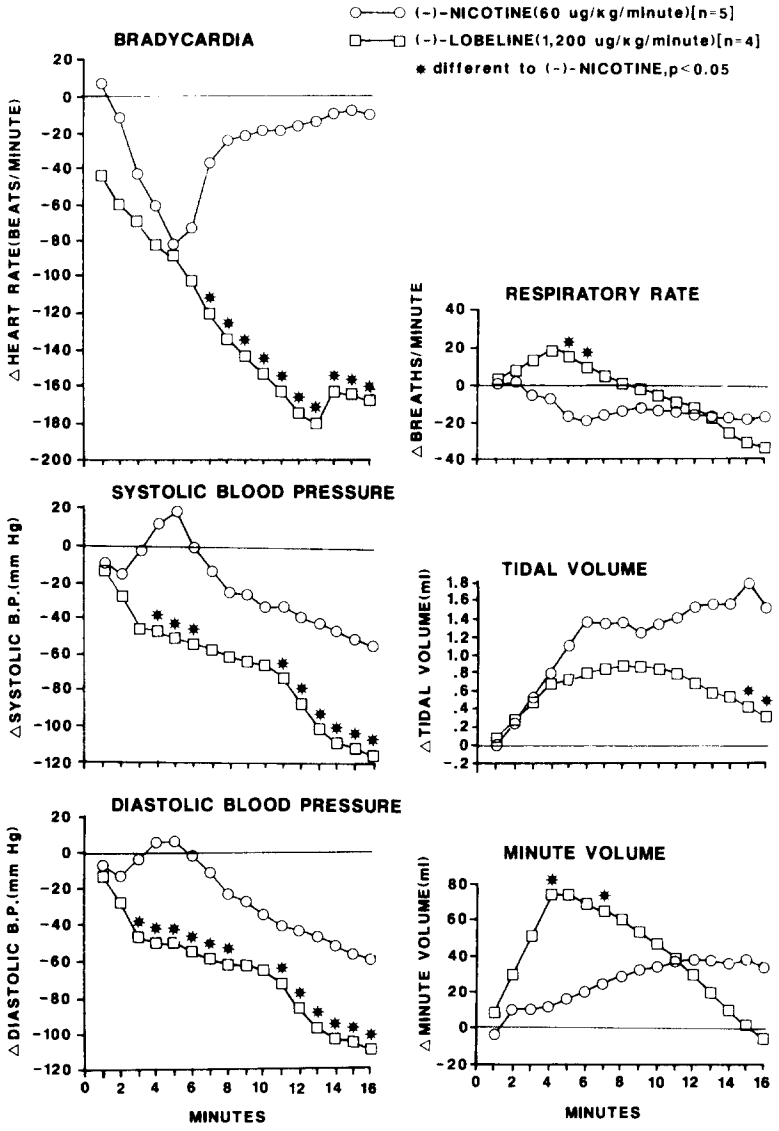


FIG. 5. Comparison of the time-response curves for (−)-nicotine and (−)-lobeline on changes in cardiovascular and respiratory parameters. Each point represents the mean change from the predrug control value for the number of experiments indicated in brackets.

DISCUSSION

The thrust of these studies has been to identify ligands of different specificities and compare their pharmacologies with the end of understanding the pharmacologic and physiologic significance of the different binding sites. It is clear that the ligands studied produce different pharmacologic changes in the urethane-pentobarbital anesthetized rat. The urethane-pentobarbital anesthetized rat shows little evidence of the ganglionic stimulant action of these ligands in the dose levels employed while depressant effects dominate. Indirect evidence suggest that most of the nicotinic depressant effects seen are a direct or indirect consequence of the ligands' actions on the central nervous system. A review of the literature, however, reveals a wealth of other experimental data indicating pharmacologic differences between these ligands.

(−)-Nicotine produced a dose-related bradycardia to which tachyphylaxis developed rapidly. Vagotomy abolished this effect indicating that its site of action was central to the cardiac parasympathetic ganglia. (−)-Nicotine also slowed respiratory rate and increased tidal volume. The respiratory rate depressant effect will dominate with higher doses however and lead to hypoxia and death [28]. The depressant effects of (−)-nicotine on blood pressure are in part due to a medullary site of action [12–14, 25] and in higher concentrations to respiratory depression.

(+)-Nicotine differed from (−)-nicotine in that it had a greater (on a relative potency basis) respiratory depressant action. All other differences between (+)- and (−)-nicotine (on the parameters studied in these experiments) probably are a consequence of this effect. These results are in keeping

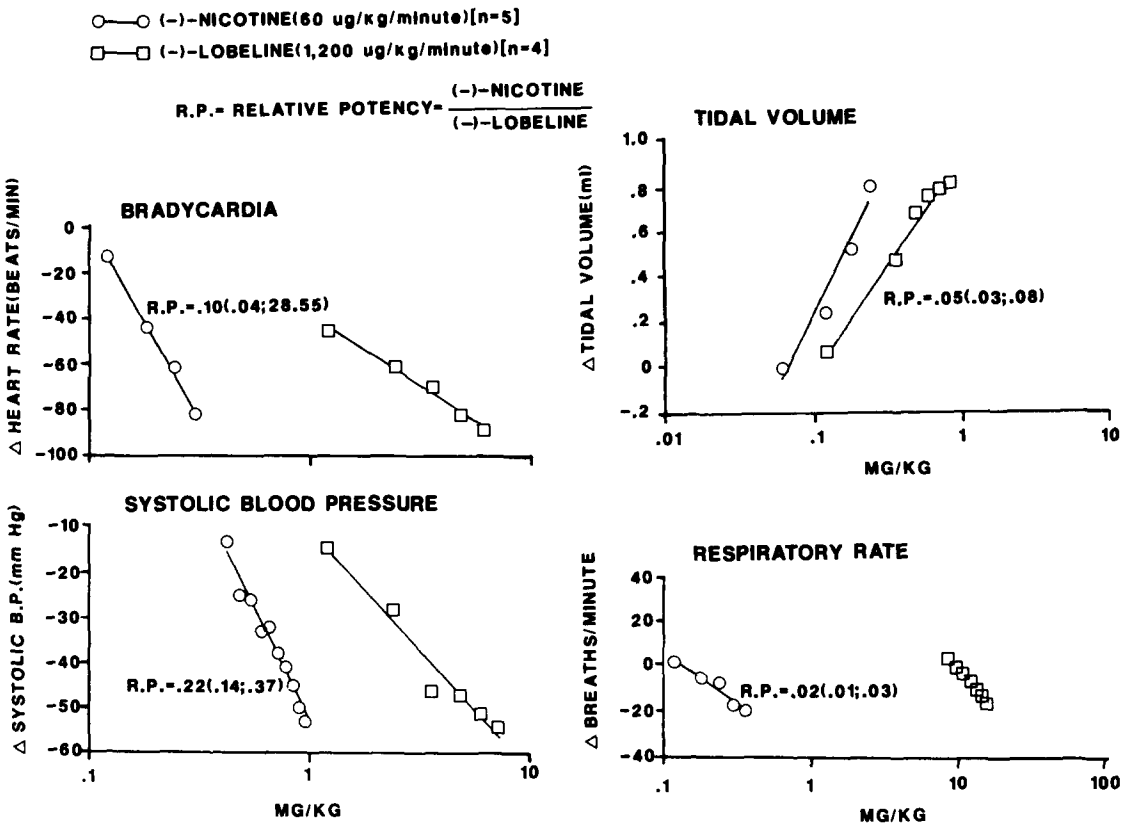


FIG. 6. Dose-response curves constructed as described in the legend of Fig. 2 for the data shown in Fig. 5 for (−)-nicotine and (−)-lobeline.

TABLE 1

COMPARISON OF RELATIVE POTENCIES* (± THEIR 95% FIDUCIAL LIMITS) OF NICOTINIC DRUGS IN INDUCING CARDIOVASCULAR AND RESPIRATORY CHANGES IN THE ANESTHETIZED RAT

	Relative Potency			
Physiologic Measures	(+)-Nicotine	(−)-Lobeline	(−)-Anabasine	(−)-Cystisine
Bradycardia	.14(.09; .23)	.10(.04; 28.55)	.04(.02; .05)	.12(.09; .15)
Systolic B.P.↓	.14(.11; .16)	.22(.14; .37)	.06(.05; .08)	.71(.59; .86)
Diastolic B.P.↓	.14(.12; .17)	Invalid	.07(.05; .08)	.71(.60; .83)
Bradypnea	.17(.11; .52)	.02(.01; .03)	.06(.04; .13)	.13(.08; .20)
Minute vol ↑	Invalid	Invalid	$.28(4\times10^{-5}; 3.08)$	.26(.03; .65)
Tidal vol ↑	.08(.06; .11)	.05(.03; .08)	.11(.04; .25)	Invalid

*Relative potencies estimated using (−)-nicotine as the standard drug and represent the mg/kg of (−)-nicotine required to produce the same effect as 1 mg/kg of the test drug.

with the observations that (−)- and (+)-nicotine are equipotent in producing death [9,17]. (+)-Nicotine is $^1/_{14}$ as potent as (−)-nicotine as a discriminative stimulus [34]. (−)-Nicotine, on the other hand, is about 30 times more potent in producing analgesia than (+)-nicotine in rats [19]. The pharmacologic profiles of (+)- and (−)-nicotine have been reviewed [18, 20, 32, 33]. It was found that (+)-nicotine exhibited specificity in producing EEG synchronization, while (−)-nicotine exibited specificity in producing EEG desynchronization, analgesia and bradycardia when administered into the 4th ventricle of the dog [20].

Clearly the pharmacology of (−)-lobeline in the anesthetized rat is different from that of (−)-nicotine in several respects. Although it is $^1/_{10}$ to $^1/_{50}$ as potent as (−)-nicotine, it is capable of producing a greater depression of heart rate and blood pressure, a lesser enhancement of tidal volume and greater stimulation of respiratory rate than (−)-nicotine. Further, it produces a lesser degree of

TABLE 2

BINDING CHARACTERISTICS* OF PROTOTYPIC AGONISTS

Presumed Site		Filtration	Centrifugation
		(−)-Nicotine	
I			
II	K_D	2.5×10^{-8}	7.6×10^{-9}
	B_{max}	2.8×10^{-15}	1.6×10^{-15}
IV	K_D	1.5×10^{-5}	2.1×10^{-6}
	B_{max}	1.3×10^{-13}	1.6×10^{-13}
V	K_D		2.5×10^{-3}
	B_{max}		4.0×10^{-10}
		(−)-Lobeline	
IV	K_D	4.0×10^{-7}	5.8×10^{-7}
	B_{max}	4.0×10^{-13}	1.1×10^{-13}
		(−)-Anabasine	
IV	K_D	1.3×10^{-6}	4.0×10^{-7}
	B_{max}	1.2×10^{-13}	6.8×10^{-14}
V	K_D	2.4×10^{-4}	8.8×10^{-5}
	B_{max}	1.2×10^{-10}	2.1×10^{-11}
		Carbachol	
IV	K_D	3.7×10^{-5}	1.0×10^{-6}
	B_{max}	8.4×10^{-13}	1.75×10^{-13}
V	K_D		1.9×10^{-2}
	B_{max}		5.0×10^{-9}
		(−)-Cytisine	
II	K_D	3.7×10^{-9}	
	B_{max}	8.5×10^{-16}	

*K_D expressed in Molar Units: B_{max}=Moles per mg of tissue.

TABLE 3

BINDING CHARACTERISTICS* OF PROTOTYPIC ANTAGONISTS

Presumed Site		Filtration	Centrifugation
		Dihydro-Beta-Erythroidine	
II	K_D		5.5×10^{-13}
	B_{max}		9.8×10^{-16}
IV	K_D	1.1×10^{-6}	8.5×10^{-6}
	B_{max}	4.2×10^{-14}	9.3×10^{-13}
V	K_D	3.5×10^{-2}	1.1×10^{-3}
	B_{max}	7.4×10^{-11}	3.2×10^{-10}
		Mecamylamine	
IV	K_D	4.8×10^{-5}	8.2×10^{-5}
	B_{max}	9.7×10^{-13}	1.6×10^{-11}
V	K_D		6.7×10^{-3}
	B_{max}		2.2×10^{-9}
		Hexamethonium	
V	K_D	2.0×10^{-3}	1.4×10^{-2}
	B_{max}	2.5×10^{-10}	5.1×10^{-9}

*K_D expressed in Molar Units; B_{max} = Moles per mg of tissue.

TABLE 4

IC_{50}S* AND POTENCY RATIOS OF NICOTINIC COMPOUNDS OBTAINED FROM BINDING STUDIES EMPLOYING RAT WHOLE BRAIN HOMOGENATES AND (−)-[^{3}H]NICOTINE (N), (±)-[^{3}H]NICOTINE, [^{3}H]ACETYLCHOLINE (ACH) AND [^{3}H]DIHYDRO-BETA-ERYTHROIDINE (DBE) AS THE LABELLED LIGANDS

	(−)-[^{3}H]N			(±)-[^{3}H]N	[^{3}H]ACH	[^{3}H]DBE
Compound	1 (F) CS	2 (C) CM	3 (F) CM(L)	4 (F) CM(L)	5 (F) CM	6 (F) CM
(−)-Nicotine	40 (1)	0.3 (1)	25 (1)	62 (1)	12 (1)	67 (1)
(+)-Nicotine	800 (20)	1 (3)	1062 (42)	3900 (63)	264 (22)	432 (6)
(−)-Lobeline	630 (16)		76 (3)	300 (5)		21 (0.3)
(−)-Anabasine	2800 (70)	500 (1667)		4600 (74)		1970 (29)
(−)-Cytisine	25 (0.6)		2 (0.1)	14 (0.2)	2 (0.2)	6 (0.1)
Carbachol	50000 (1250)	800 (2667)	850 (34)	2400 (39)	24 (2)	

*IC_{50}=Concentration necessary to inhibit binding of labelled ligand 50%, outside parenthesis. Values are presented in nanomolar units.

Potency ratio = IC_{50} of compound/IC_{50} (−)-N, inside parenthesis.

F = Filtration procedure, C = centrifugation procedure, CS = crude synaptosomal fraction, unlysed, CM = crude membrane preparation, unlysed, CM(L) = crude membrane preparation, lysed.

1—Sloan *et al.* (present studies).

2—Abood *et al.* [1].

3—Lippiello and Fernandez [15].

4—Romano and Goldstein [23].

5—Schwartz *et al.* [29]; Kellar *et al.* [11].

6—Williams and Robinson [38].

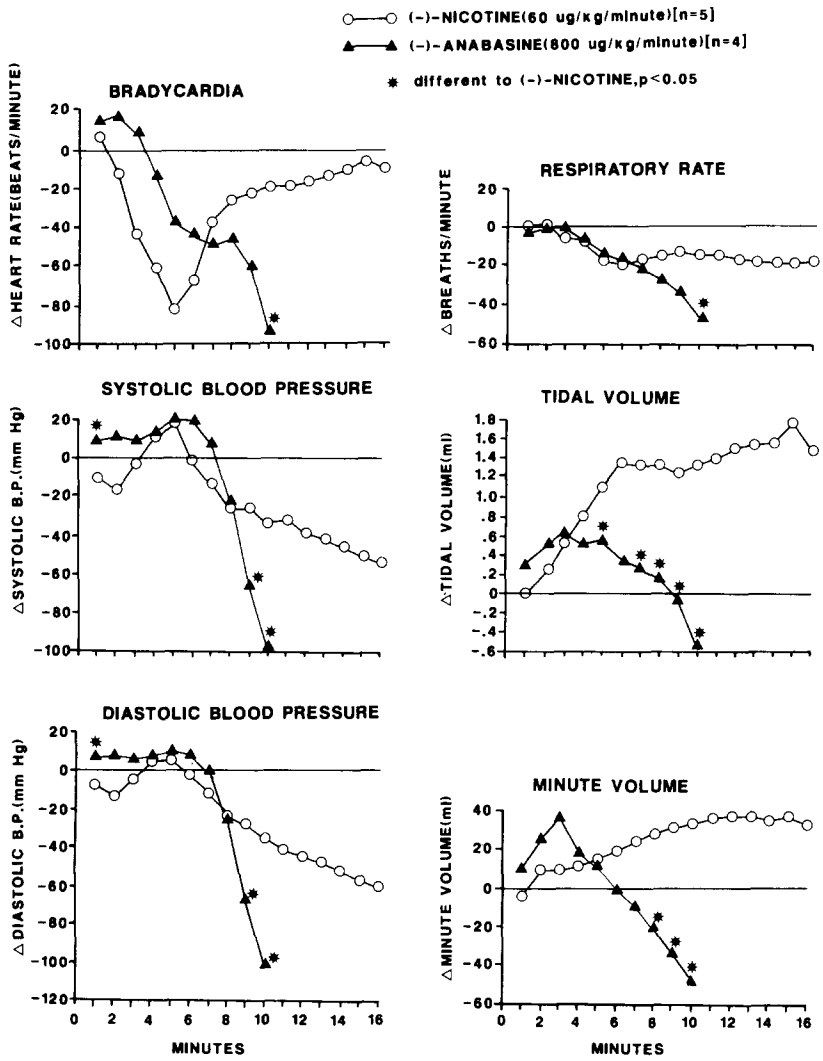


FIG. 7. Comparison of time-response curves for the continuous IV infusion of (−)-nicotine and (−)-anabasine on changes in cardiovascular and respiratory measures. Each point represents the mean change from the predrug control value for each minute for the number of experiments indicated in brackets.

tachyphylaxis than (−)-nicotine to its bradycardic effects. Overall, in low doses it is a more effective respiratory stimulant than (−)-nicotine but in higher doses produces a greater degree of respiratory depression. The older literature on (−)-lobeline has stressed its emetic and respiratory stimulant effects. Analysis of its actions on different cholinergic synapses are complex. Thus at the frog myoneural junction, (−)-lobeline does not cause depolarization but produces a noncompetitative antagonism of (−)-nicotine's excitatory effects [37]. On the other hand, (−)-lobeline will produce depolarization of the cat superior cervical ganglion [10]. (−)-Lobeline's stimulant action on the guinea pig ileum was found to be more sensitive to hexamethonium antagonism than was (−)-nicotine's which the investigators attributed to an atropine-like action of (−)-lobeline [4]. Using drug discrimination techniques other investigators found that (−)-lobeline did not generalize to (−)-nicotine [24,26]. One explanation which has been proposed for this observation is that (−)-lobeline may not penetrate the CNS [35], a hypothesis that has not been tested directly. Clearly, (−)-lobeline's greater ability to produce bradycardia as well as vaso and respiratory depression cannot be a consequence of limited access to the brain. Further, (−)-lobeline is as lipid soluble as (−)-nicotine [24]. In a study of the effect of (−)-lobeline on the rat gut it was concluded that it did not have nicotine-like actions because its ability to relax the oxotremorine-contracted colon could not be antagonized by tetrodotoxin [22].

(−)-Anabasine produced a pharmacologic profile similar to that of (−)-lobeline in that it depressed heart rate, blood pressure and minute volume. It had less respiratory stimulant effects than (−)-lobeline. Unlike (−)-nicotine, tachyphylaxis to the bradycardia was not seen. A relatively modest amount of literature exists on the pharmacology of (−)-anabasine. (−)-Anabasine, a minor alkaloid of tobacco, and the piperidine analog of nicotine, produces a nicotine-like syndrome when injected into the lateral ventricles of the cat [3]. It has been found that rats trained to discriminate (−)-nicotine generalized to anabasine and in this regard (−)-anabasine was $^1/_{57}$ as potent as (−)-nicotine [35]. Cardiovas-

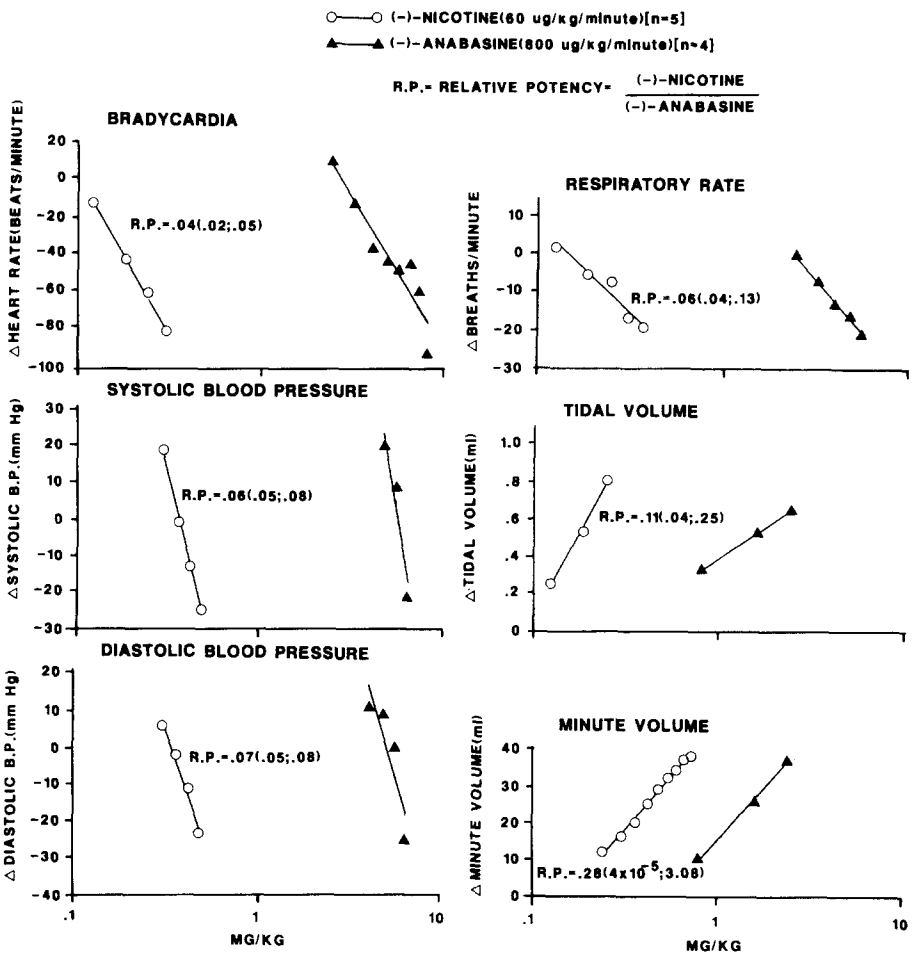


FIG. 8. Dose-response curves constructed as described in the legend of Fig. 2 for the data shown in Fig. 7 for (−)-nicotine and (−)-anabasine.

cular depressant effects of (−)-lobeline and (−)-anabasine were not a consequence of respiratory depression.

(−)-Cytisine does not appear to be a nicotine-like drug. Its ability to produce bradycardia is less than that of (−)-nicotine while its ability to depress blood pressure is greater. The onset of (−)-cytisine's depressant action on blood pressure was the same as that of (−)-nicotine and of approximately the same magnitude. However, it produced a lesser degree of bradycardia and little enhancement of tidal volume. Hexamethonium is about equieffective in antagonizing (−)-cytisine's and (−)-nicotine's stimulant action on the guinea pig ileum [4]. Other investigators [5,7] found that (−)-cytisine was somewhat more potent than (−)-nicotine in elevating blood pressure, a ganglionic stimulant effect. Both drugs produced respiratory depression. (−)-Cytisine is far less potent than (−)-nicotine as a discriminative stimulus [24, 35, 36] (Table 5). (−)-Cytisine's low activity as a discriminative stimulus has been attributed to its low lipid solubility and slow entry into the brain [24].

Tables 2 and 3 summarize binding studies conducted using these prototypic nicotinic agonists as well as several nicotinic antagonists. As can be seen, there is reasonably good agreement between the filtration and centrifugation procedures with regard to the binding characteristics of these

TABLE 5

RELATIVE POTENCY* OF NICOTINIC DRUGS

Drug	I Rat Discrimination	II Rat Colon	III Rat Vasomotor, Cardiac and Respiratory Effects
(−)-Nicotine	1 (1)	1 (1)	1 (1)
(+)-Nicotine	14 (13)	11 (63)	7–12.5 (20)
(−)-Lobeline	Inactive	4 (5)	10–50 (16)
(−)-Anabasine	57 (52)	9 (74)	4–25 (70)
(−)-Cytisine	14 (0.2)	1 (0.2)	1.4–8 (0.6)

*Relative potency = mg of nicotinic drug required to produce same effect as 1 mg (−)-Nicotine. Values in parenthesis are potency ratios (Table 4) based on the IC_{50}s of binding studies.

I—Stolerman [34]; Stolerman *et al.* ([35] and Personal Communication).

II—Romano [22]; Romano and Goldstein [23].

III—Sloan *et al.* (present study).

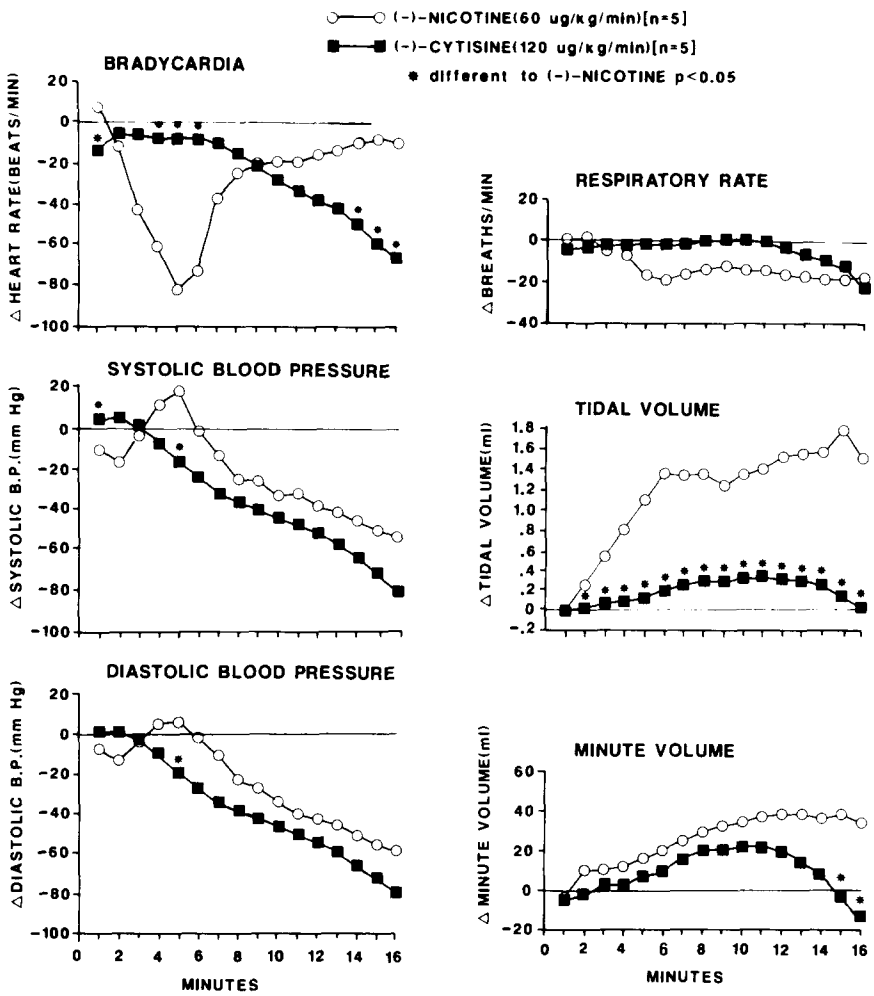


FIG. 9. Comparison of time-response curves for the continuous IV infusion of (−)-nicotine and (−)-cytisine on changes in cardiovascular and respiratory measures. Each point represents the mean change from the predrug control value for each minute for 5 experiments.

compounds. In the present studies a comparison of the centrifugation with the filtration technique revealed that the total and specific binding of (−)-[^{3}H]nicotine was approximately 7 times greater with the centrifugation than with the filtration technique (filter washed 5 times). The unwashed filter, however, had more total and specific binding than the centrifuged pellet. The percent of the total binding which was specific was about the same on the unwashed filter and in the centrifugation pellet but was reduced by over 30% on the washed filter. Although washing elutes the ligand from receptors, probably preferentially from the lower affinity sites, this most likely has only a modest effect on either the estimates of K_D or B_{max} of the lower affinity site since the low affinity site(s) account for over 99% of the specific binding as determined using the LIGAND program. Thus both methods reveal the existence of a low affinity (−)-nicotine site which has a site density of approximately 10^{-13} moles per mg of tissue.

(−)-Lobeline, (−)-anabasine and carbachol all bind with a K_D in the micromolar range and a binding density of approximately 10^{-13} moles per mg of tissue, parameters which are similar to the parameters of the low affinity binding site of (−)-nicotine. In contrast (−)-cytisine binds to a single high affinity site with a K_D in the nanomolar range and a site density of $\sim 10^{-15}$ moles per mg of tissue. Table 4 compares the IC_{50}s for several nicotinic ligands obtained from different laboratories. There is reasonably good agreement between the binding data herein presented with the observations of some investigators [23]. However, there are marked disparities between laboratories when radioligands other than (−)-nicotine are employed and when there are other procedural differences. A plausable interpretation of the findings is that internal consistency does not exist and that agonist binding data do not allow a simple interpretation.

The failure of binding data obtained with nicotinic antagonist to agree with pharmacologic data has also been disturbing. As can be seen in Table 3, both dihydro-beta-erythroidin and mecamylamine exhibit a low affinity binding site. Dihydro-beta-erythroidin may also bind to a high affinity site with a K_D in the nanomolar range which agrees with data obtained by others [38]. Although LIGAND analysis of the hexamethonium data revealed only a very low affinity

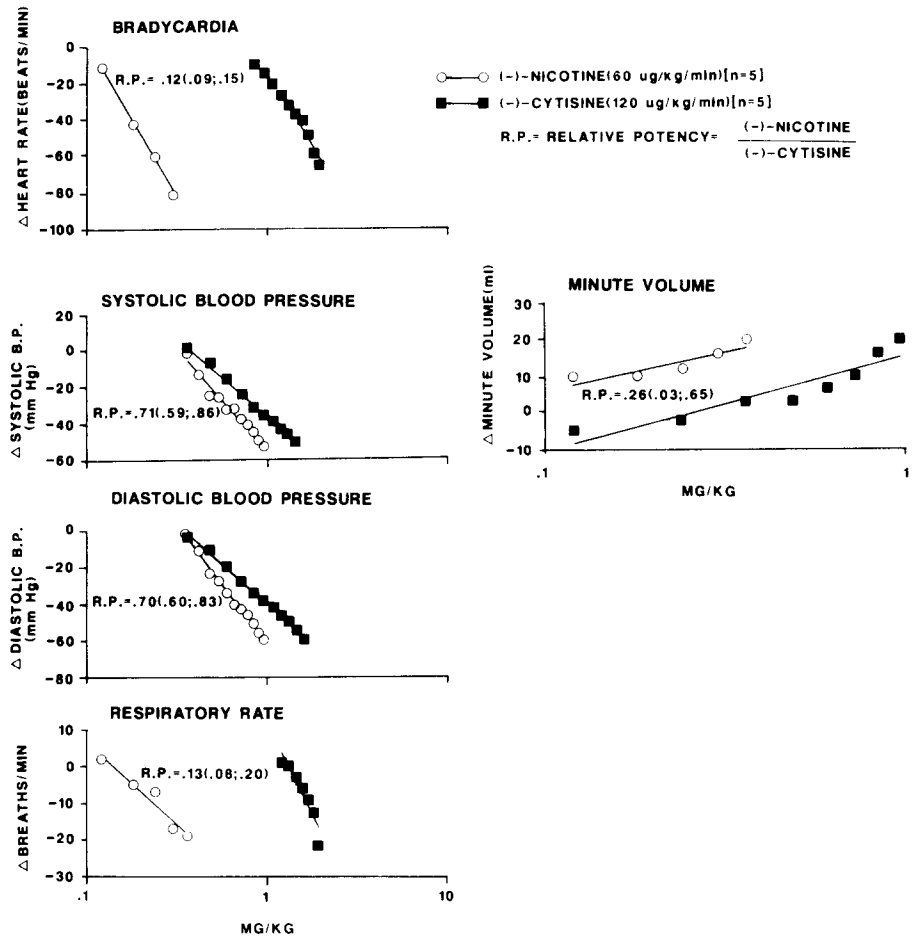
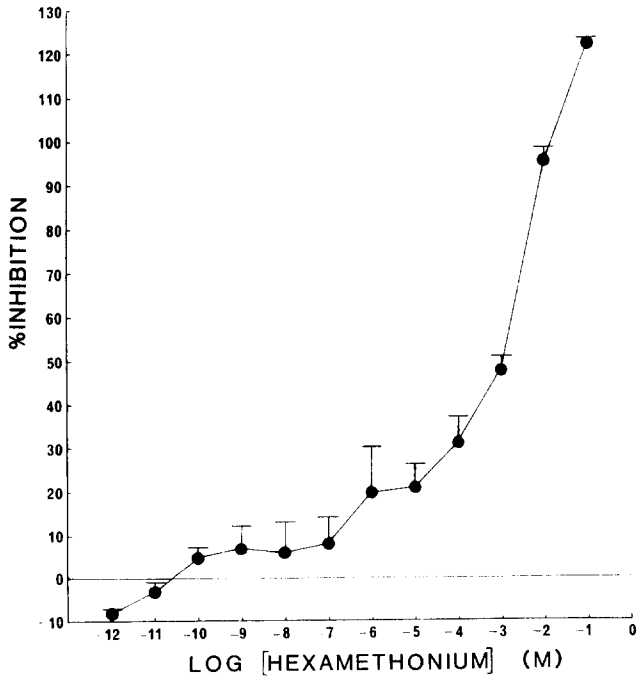


FIG. 10. Dose-response curves constructed as described in the legend of Fig. 2 for the data shown in Fig. 9 for (−)-nicotine and (−)-cytisine.



site, it did inhibit some 20% of (−)-[^{3}H]nicotine binding in concentrations of 10^{-10} to 10^{-5} M (Fig. 11). Hence the possibility that hexamethonium can interact with a subpopulation of low affinity binding sites cannot be excluded.

Table 5 summarizes the relative potencies of the nicotinic ligands studied for several different pharmacologic parameters. The correlation between these potency estimates and any set of brain binding data is poor. There are admittedly many confounding variables in both pharmacologic and binding studies which may partially explain this lack of correlation. Nevertheless, despite this unexplained lack of correlation, we feel that these data provide support for the hypothesis that the low affinity binding sites play an important role in the pharmacology of the nicotinic ligands studied.

FIG. 11. The inhibition of (−)-[^{3}H]nicotine binding by graded concentrations of hexamethonium using the filtration technique. The inhibition produced by hexamethonium is shown as a percentage of the maximum displacement achieved by 10^{-2} M (−)-nicotine. Each point, ± its standard error, is the mean of 3 experiments.

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