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Desensitization of Central Nicotinic Cardiovascular Effects by Nicotine Isomers and a Quaternary Analogue[1]

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DONG, L., A. A. HOUDI AND G. R. VAN LOON. *Desensitization of central nicotinic cardiovascular effects by nicotine isomers and a quaternary analogue.* PHARMACOL BIOCHEM BEHAV **38**(4) 843–852, 1991.—Nicotine produces potent cardiovascular and sympathoadrenal effects. Furthermore, repeated administration of nicotine is associated with development of tolerance for many responses. We sought to compare the effects of initial intracerebral administration of nicotine isomers and a quaternary analogue on cardiovascular and sympathoadrenal responses and to compare the desensitizing properties of these nicotinic compounds on subsequent responses to nicotine. Thus we examined the effects of (−)-nicotine, (+)-nicotine, N′-methylnicotinium iodide (N′MN), a quaternary analogue of (−)-nicotine, and saline vehicle, administered into a lateral cerebral ventricle, on heart rate (HR), systolic, diastolic and mean arterial blood pressure (BP), and plasma concentrations of epinephrine and norepinephrine in conscious, freely moving, adult, male rats. (−)-Nicotine (120 nmol, ICV) produced decrease in HR and increases in all other parameters. (+)-Nicotine at this dose produced only small effects on HR, BP and plasma catecholamines. An equimolar dose of N′MN produced similar effects on these parameters, quantitatively intermediate between those of the two nicotine isomers. Thirty min after administration of these nicotinic agonists, all parameters had returned to baseline. At this time, the effects of subsequent ICV administration of (−)-nicotine 120 nmol was studied in all animals. Prior administration of either (−)-nicotine or (+)-nicotine markedly attenuated the bradycardic response to (−)-nicotine, and N′MN was less effective in this regard. In contrast, neither (−)-nicotine nor N′MN inhibited the pressor responses to subsequent (−)-nicotine, whereas (+)-nicotine did produce some attenuation of this pressor response. Similarly, only (+)-nicotine, was found to inhibit the plasma norepinephrine response to subsequent (−)-nicotine when this drug dose and timing of administration were used. The epinephrine response to subsequent (−)-nicotine was not affected by this dose (120 nmol) and timing of treatment with any of these ligands. These data support the concept that desensitization of the intracerebral effects of nicotine on cardiovascular and sympathoadrenal function may be mediated at binding sites other than those producing nicotinic responses.

(−)-Nicotine (+)-Nicotine N′-Methylnicotinium iodide Heart rate Blood pressure Cardiovascular function Catecholamines, plasma Tolerance

THE effects of nicotine on the cardiovascular control of the central nervous system (CNS) and on the regulation of sympathoadrenal secretion have long been recognized (24,29). Nicotine is a classic sympathoadrenal stimulant, inducing catecholamine release from sympathetic nerve endings and adrenal medulla. These effects of nicotine are generally attributed to nicotinic receptor stimulation at sympathetic ganglia, adrenal chromaffin cells and directly at sympathetic nerve endings. Direct intracerebral administration of nicotine in different animal species also produced variable effects on cardiovascular and sympathoadrenal parameters (13, 16, 27, 28).

As a result of the action of nicotine on receptors in the neuromuscular junction and autonomic ganglia, it seems reasonable to assume that central effects of nicotine are also receptor mediated. Considerable in vitro binding data have been generated over the past few years which support the notion of specific nicotinic receptors in the CNS (2,21). The implication of receptor involvement in the central effects of nicotine has been supported by antagonism studies with ganglionic blockers as well as by determination of structure-activity relationships and stereoselectivity (6, 8, 12, 17). There has been considerable interest in the effects of (−)-nicotine and (+)-nicotine on the CNS since stereoselectivity is an essential factor in establishing the existence of specific binding sites for nicotine. In general, (+)-nicotine exhibits similar pharmacological profiles to (−)-nicotine, but is 3–30 times less potent in most tests (17). It is possible that various nicotinic

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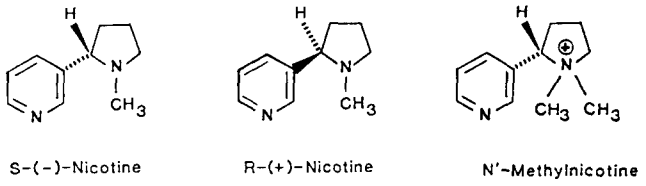

FIG. 1. Structures of nicotine isomers and N'-methylnicotinium ion.

agonists, by binding to nicotinic sites, may inhibit the effects of (−)-nicotine. Thus we hypothesized that (+)-nicotine acts as a partial agonist to inhibit the cardiovascular and sympathoadrenal effects of (−)-nicotine. This desensitization may occur at agonist or other receptor sites (12). The net effect of nicotine on sympathoadrenal secretion and cardiovascular function depends upon a number of factors. These include species differences, state of consciousness, drug concentration and site of administration and accessibility of drug to target sites. In order to assess further both the agonist and desensitizing actions of nicotinic compounds in CNS on cardiovascular and sympathoadrenal function, we studied the intracerebral effects of a quaternary analogue of (−)-nicotine, N'-methylnicotinium iodide (N'MN) (Fig. 1). N'MN exhibits potent nicotine-like properties (3, 6, 8, 10) but does not cross the blood-brain barrier (3).

METHOD

Adult male Sprague-Dawley rats (275–350 g, Harlan Industries, Indianapolis, IN) were used in experiments. The animals were housed in groups of 3–4 prior to surgery and subsequently were housed singly in stainless steel cages with free access to food and water. All the animals were under constant environmental conditions of temperature (24°C), humidity and 12-hour light/dark cycle (lights on 0700–1900 h). After surgery under deep equithesin anesthesia (3.0 ml/kg, IP) prepared as described below, animals were allowed 3 days recovery before an experiment. A polyethylene (PE50) catheter filled with heparinized saline (50 U/ml) was implanted into the left common carotid artery of all rats, and was passed out of the home cage through a stainless spring attached to the animal's back (26). This arrangement provided access to the cannula for blood sampling and cardiovascular monitoring in freely moving rats.

A stainless steel guide cannula (Plastic Products, Roanoke, VA) was implanted over the left lateral cerebral ventricle. Stereotaxic coordinates were 0.8 mm posterior to the bregma, 1.4 mm lateral to the midline and 3.4 mm below the skull. This guide cannula was secured to the skull with dental acrylic and screws.

Thirty minutes before drug administration, animals were handled briefly to lower a drug-filled injector (Plastic Products, Roanoke, VA) through the guide cannula into the lateral ventricle (the tip of the injector is 1 mm below the tip of the guide cannula). The rats were then returned to the home cages where they were allowed to recover from the stress of handling. The injector consisted of a segment of stainless steel tubing connected to a 10 μl Hamilton syringe by PE10 tubing. Microinjection of the drug solution (each treatment consisted of 120 nmoles of drug in 4 μl saline vehicle) was made over one minute using a programmed syringe pump (Tracor Atlas, Houston, TX). A dose of 120 nmol of (−)-nicotine used in this study was selected from our previously constructed dose-response curve for (−)-nicotine administered ICV in rat (13). This dose of (−)-nicotine was found to induce changes in plasma catecholamine secretion and cardiovascular responses. All animals received 2 treatments; in order to avoid handling the rats between treatments, the two drug solutions were drawn up into the tubing and separated by an air bubble, and the second drug solution was separated from saline solution by another air bubble before insertion of the injector.

Heart rate (HR) and arterial blood pressure (systolic pressure, SP; diastolic pressure, DP; mean pressure, MP) were recorded continuously throughout the experimental period. These analog signals were digitized by an A/D converter (Buxco Electronic, Sharon, CT), averaged over six-second epochs and stored using a Buxco data logger and IBM PC/AT.

Blood samples (0.25 ml) for determination of plasma catecholamines were collected from the carotid artery before and 2 min after each treatment. Each blood sample was replaced by an equal volume of saline solution. Plasma concentrations of norepinephrine and epinephrine were determined using a single isotope radioenzymatic assay (22).

Successful ICV injection was confirmed by monitoring the movement of a small air bubble over a calibrated distance in the PE10 tubing during the drug administration. Also at the conclusion of the experiment, each ICV treatment was verified by examining the cerebral ventricles 1 minute after a 4 μl fast green dye injection into the anesthetized rat.

In calculating the baseline values for heart rate and arterial

TABLE 1

BASELINE VALUES FOR CARDIOVASCULAR PARAMETERS AND PLASMA CATECHOLAMINES

Parameter Measured	Experimental Group			
	Saline (n = 8)	(−)-Nicotine (n = 11)	(+)-Nicotine (n = 12)	N'-Methylnicotine (n = 9)
Heart rate (beats/min)	384 ± 12	373 ± 10	378 ± 10	379 ± 11
Systolic blood pressure (mmHg)	137 ± 6	136 ± 5	133 ± 6	134 ± 3
Diastolic blood pressure (mmHg)	107 ± 7	101 ± 4	96 ± 5	94 ± 3
Mean blood pressure (mmHg)	117 ± 7	112 ± 4	108 ± 5	107 ± 3
Plasma norepinephrine (nM)	1.62 ± 0.19	1.33 ± 0.16	1.54 ± 0.12	1.45 ± 0.12
Plasma epinephrine (nM)	2.05 ± 0.50	1.61 ± 0.21	1.98 ± 0.23	2.27 ± 0.53

Data represent mean ± SEM. The value for each cardiovascular parameter used from each rat represents the mean for 10 data points taken during the 1 minute prior to treatment.

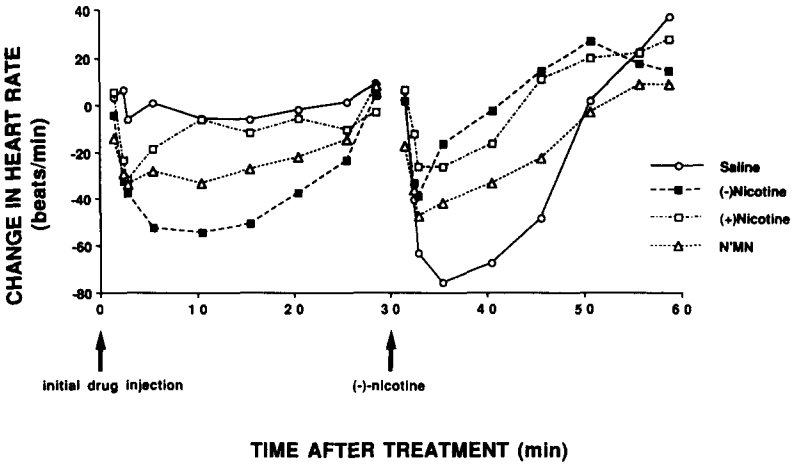

FIG. 2. Heart rate responses to initial treatment with various nicotinic drugs [(−)-nicotine (n = 11), (+)-nicotine (n = 12), N′-methylnicotinium iodide (n = 9), and saline vehicle (n = 8)] and to subsequent treatment of the same rats with (−)-nicotine.

pressure, we used the mean of 10 data points taken during the 1 min prior to drug administration. Responses (change from baseline) to drug treatment within experimental groups were analyzed statistically using Student's t-test; in order to protect against Type 1 error in this test, we required $p<0.02$ for a significant response. Comparisons of responses (change from initial or subsequent baseline) across groups were analyzed statistically using one-way analysis of variance followed by Duncan's multiple range test.

The following drugs and chemicals were used in this experiment: Equithesin composed of chloral hydrate (2.13 g, Sigma Chemical Co., St. Louis, MO), magnesium sulfate (1.07 g, Fisher Scientific Co., Fairlawn, NJ), propylene glycol (14.1 ml, Fisher Scientific Co.), ethanol (3.8 ml), water (22.4 ml) and nembutal (9.8 ml, Abbott Laboratories, N. Chicago, IL); S-(−)-nicotine (Eastman Kodak Co., Rochester, NY); R-(+)-nicotine was prepared by the method of Bowman et al. (7) by resolution of (+)-

TABLE 2

HEART RATE RESPONSES TO INITIAL TREATMENT WITH DIFFERENT NICOTINIC DRUGS AND TO SUBSEQUENT TREATMENT OF THE SAME RAT WITH (−)-NICOTINE

	Treatment Group							
	Saline		(−)-Nicotine		(+)-Nicotine		N′-Methylnicotine	
Time (min)	t	p	t	p	t	p	t	p
0	Treatment With Nicotinic Agonist							
1	0.47	0.66	−0.90	0.39	0.38	0.71	-2.58	0.033
2	0.66	0.53	−3.60	0.0049*	−3.21	0.0083*	−3.03	0.016*
2.5	−0.67	0.53	−4.47	0.0012*	−3.72	0.0034*	−3.26	0.012*
5	−0.08	0.94	−4.94	0.0006*	−1.88	0.087	−2.98	0.018*
10	−1.02	0.34	−4.98	0.0006*	−1.38	0.19	−2.87	0.020*
15	−1.09	0.31	−5.76	0.0002*	−1.97	0.074	−2.59	0.032
20	−0.49	0.64	−4.51	0.0011*	−1.22	0.25	−2.88	0.020*
25	−0.05	0.96	−2.37	0.039	−2.11	0.058	−3.69	0.0061*
28	0.45	0.66	0.18	0.86	−0.75	0.47	1.30	0.23
30	Treatment With (−)-Nicotine							
31	0.98	0.36	0.00	1.0000	0.56	0.58	−1.95	0.088
32	−2.40	0.047	−2.38	0.038	−2.13	0.056	−5.52	0.0006*
32.5	−3.88	0.0061*	−2.15	0.057	−3.08	0.011*	−9.02	0.0001*
35	−3.81	0.0066*	−0.95	0.36	−2.79	0.018*	−6.87	0.0001*
40	−5.29	0.0011*	−0.41	0.69	−2.12	0.058	−3.06	0.0160*
45	−4.37	0.0033*	1.40	0.19	1.08	0.30	−2.27	0.053
50	−0.01	0.99	2.27	0.047	1.64	0.13	−0.62	0.55
55	1.49	0.18	1.47	0.17	3.00	0.012	0.94	0.38
58	2.57	0.037	1.11	0.29	2.51	0.029	0.71	0.50

Response to initial treatment represents change from baseline prior to drug treatment and response to subsequent treatment with nicotine represents change from new baseline 30 min after initial drug treatment. In order to protect against Type 1 error, we required $p<0.02$ for a significant response (*).

TABLE 3

COMPARISONS OF THE HEART RATE RESPONSES AT SPECIFIC TIME POINTS AFTER INITIAL DRUG INJECTION AND SUBSEQUENT INJECTION OF (−)-NICOTINE

Initial Drug Injection							(−)-Nicotine Injection						
Time Interval* (min)	F	p	Experimental Group	n	Mean	Group Differences‡	Time Interval*† (min)	F	p	Experimental Group	n	Mean	Group Differences‡
1	1.42	0.253	+Nic	12	3.50		31(1)	1.26	0.304	+Nic	12	4.67	
			Sal	8	1.25					Sal	8	4.00	
			−Nic	11	−6.18					−Nic	11	0.00	
			NMN	9	−16.2					NMN	9	−19.22	
2	3.37	0.029	Sal	8	3.75	A	32(2)	1.22	0.317	+Nic	12	−14.00	
			+Nic	12	−25.5	B				−Nic	11	−35.27	
			NMN	9	−30.8	B				NMN	9	−38.22	
			−Nic	11	−34.2	B				Sal	8	−42.00	
2.5	1.74	0.176	Sal	8	−8.13		32.5(2.5)	1.20	0.324	+Nic	12	−28.08	
			+Nic	12	−33.7					−Nic	11	−41.00	
			NMN	9	−35.6					NMN	9	−49.00	
			−Nic	11	−39.6					Sal	8	−65.00	
5	3.82	0.018	Sal	8	−0.88	A	35(5)	2.70	0.059	−Nic	11	−18.36	A
			+Nic	12	−20.9	A B				+Nic	12	−28.25	A
			NMN	9	−30.2	A B				NMN	9	−43.56	A B
			−Nic	11	−54.3	B				Sal	8	−77.63	B
10	6.30	0.002	Sal	8	−7.75	A	40(10)	6.10	0.002	−Nic	11	−4.45	A
			+Nic	12	−8.17	A				+Nic	12	−18.25	A
			NMN	9	−35.0	A B				NMN	9	−35.22	A
			−Nic	11	−56.0	B				Sal	8	−69.25	B
15	5.33	0.004	Sal	8	−8.13	A	45(15)	8.61	0.0002	−Nic	11	12.55	A
			+Nic	12	−13.5	A				+Nic	12	9.33	A
			NMN	9	−29.3	A B				NMN	9	−24.44	B
			−Nic	11	−52.6	B				Sal	8	−50.00	B
20	4.28	0.011	Sal	8	−4.13	A	50(20)	1.38	0.264	−Nic	11	25.18	
			+Nic	12	−7.67	A				+Nic	12	17.92	
			NMN	9	−24.22	A B				Sal	8	−0.13	
			−Nic	11	−39.27	B				NMN	9	−5.00	
25	1.37	0.266	Sal	8	−0.63		55(25)	0.40	0.753	Sal	8	20.75	
			+Nic	12	−12.25					+Nic	12	20.17	
			NMN	9	−16.67					−Nic	11	15.73	
			−Nic	11	−25.45					NMN	9	7.00	
28	0.28	0.842	Sal	8	7.375		58(28)	1.21	0.320	Sal	8	35.38	
			NMN	9	6.556					+Nic	12	25.75	
			−Nic	11	2.364					−Nic	11	12.36	
			+Nic	12	−4.667					NMN	9	6.78	
							61(31)	0.66	0.584	+Nic	12	60.17	
										−Nic	11	54.91	
										NMN	9	51.44	
										Sal	8	38.25	
							63(33)	0.64	0.596	−Nic	11	59.00	
										+Nic	12	54.00	
										Sal	8	40.88	
										NMN	9	37.78	

*Minutes after first drug injection.
†Minutes after subsequent injection of (−)-nicotine are presented in parentheses.
‡Groups which share letter A or B are not significantly different.
Comparisons were made using one-way ANOVA followed by Duncan's multiple range test.

nicotine with di-(p-toluoyl)-D-tartaric acid (Aldrich, Milwaukee, WI) (specific rotation, measured by polarimetry at 589 nm) afforded $@^{25} = +135.6°$ (25 mg/ml 100% methanol) indicating >99.5% optically pure R-(+)-nicotine since S-(−)-nicotine under similar conditions yielded $@^{25} = -136.0°$; N′-methylnicotinium iodide (this quaternary nicotine analogue was obtained from Tobacco and Health Research Institute, University of Kentucky). All other reagents and solvents were analytical grade and obtained from either Fisher Scientific Co. or Sigma Chemical Co. (−)-Nicotine, (+)-nicotine and N′-methylnicotinium iodide were

TABLE 4

STATISTICAL COMPARISON OF THE AREAS UNDER THE HEART RATE RESPONSE CURVES

	N'MN(1)		−Nic(1)		+Nic(1)		Sal(1)		N'MN(2)		−Nic(2)		+Nic(2)		Sal(2)	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p	F	p
N'MN(1)			1.04	NS	0.96	NS	3.81	0.055	0.01	NS	3.75	0.057	4.95	0.029	0.11	NS
−Nic(1)					4.56	0.036	9.17	0.003	1.29	NS	10.1	0.002	11.9	0.001	0.42	NS
+Nic(1)							1.28	NS	0.75	NS	1.10	NS	1.88	NS	1.68	NS
Sal (1)									3.41	NS	0.03	NS	0.00	NS	5.12	0.027
N'MN(2)											3.32	NS	4.44	0.039	0.19	NS
−Nic(2)													0.07	NS	4.91	0.030
+Nic(2)															6.23	0.015
Sal (2)																

p is the p value for the F-test for the significance of contrast of equal areas under 2 response curves.

NS refers to nonsignificant.

Responses to initial treatment with N'methylnicotinium iodide [N'MN(1)], (−)-nicotine [−Nic(1)], (+)-nicotine [+Nic(1)] or saline [Sal(1)] are presented. Responses to subsequent treatment with (−)-nicotine of rats treated initially with N'-methylnicotinium iodine, (−)-nicotine, (+)-nicotine or saline are presented as N'MN(2), −Nic(2), +Nic(2) and Sal(2), respectively.

Comparisons were made using repeated measures ANOVA with between subjects factor corresponding to the four experimental groups and within subjects factor corresponding to the two treatment periods, namely initial drug treatment and subsequent treatment with (−)-nicotine.

dissolved in saline and solution were adjusted to pH 7.5 with 1.5 N HCl.

RESULTS

Baseline Values (Table 1)

No significant differences were noted among experimental groups for any parameters.

Heart Rate Response to Nicotinic Agonists

ICV administration of (−)-nicotine, 120 nmol, produced a marked bradycardia which was sustained from 2–20 min (Fig. 2 and Tables 2, 3, 4). Tables 2, 3 and 4 present the statistical analyses of the data presented in Fig. 2. In contrast, ICV administration of (+)-nicotine (120 nmol) produced only a mild and transient bradycardia which was significant at 2–2.5 min after drug administration. The quaternary analogue, N'MN, administered ICV in an equimolar dose also produced a mild bradycardia which appeared to be sustained similar to that seen with (−)-nicotine. ICV administration of saline vehicle, 4 μl, produced no effects on heart rate.

Heart Rate Response to Subsequent (−)-Nicotine

In rats pretreated with saline ICV, subsequent ICV administration of (−)-nicotine, 120 nmol, produced a marked, sustained bradycardia similar to that seen in response to the initial treatment of the (−)-nicotine treatment group (Fig. 2 and Tables 2 and 4). In rats pretreated with nicotinic agonists, heart rate had returned to baseline 30 min after drug administration; thus heart rate was basal in all experimental groups at the time of subsequent administration of (−)-nicotine. Rats pretreated with (−)-nicotine, showed marked attenuation of the bradycardic response to subsequent (−)-nicotine (Fig. 2 and Tables 2, 3 and 4). Also, pretreatment with (+)-nicotine inhibited the bradycardic response to subsequent (−)-nicotine (Fig. 2 and Tables 2, 3 and 4). (−)-Nicotine and (+)-nicotine appeared equally effective in desensitizing the heart rate response to subsequent (−)-nicotine (Fig. 2 and Table 4). Although pretreatment with N'MN attenuated the bradycardic response to subsequent (−)-nicotine, this attenuation was less than that produced by (−)- or (+)-nicotine, and in the N'MN-pretreated group, subsequent (−)-nicotine did produce significant, sustained bradycardia (Fig. 2 and Tables 2, 3 and 4).

Arterial Blood Pressure Responses to Nicotinic Agonists

ICV administration of (−)-nicotine, 120 nmol, produced rapid and marked increases in systolic, diastolic and mean arterial pressure (Fig. 3 and Tables 5 and 6). Systolic pressure had increased significantly within 1 min after (−)-nicotine administration, that is, the pressor response was even more rapid in onset than the heart rate response. However, the increases in systolic, diastolic and mean arterial pressure were not sustained as long as the bradycardic response. In contrast, (+)-nicotine (120 nmol, ICV) produced only a very small and transient increase in systolic arterial pressure. The quaternary analogue, N'MN, administered ICV, in a dose equimolar to (−)-nicotine, produced a small increase in arterial pressure which was maintained for the same duration as that produced by (−)-nicotine (Fig. 3 and Tables 5 and 6). Saline vehicle ICV did not alter arterial pressure.

Arterial Blood Pressure Responses to Subsequent (−)-Nicotine

In all experimental groups, systolic, diastolic and mean blood pressure had returned to basal level 30 min after initial drug treatment. In rats pretreated with saline ICV, subsequent ICV administration of (−)-nicotine 120 nmol produced marked increases in systolic, diastolic and mean arterial pressure similar to those seen in response to the initial treatment of the (−)-nicotine treatment group (Fig. 3 and Tables 5 and 6). Also, in rats pretreated with (−)-nicotine, subsequent ICV nicotine produced increases in arterial pressure similar to those seen either in response to the initial treatment with (−)-nicotine in this group or in response to (−)-nicotine in the saline pretreatment group (Fig. 3 and Tables 5 and 6). That is, no significant attenuation of the pressor responses to (−)-nicotine was produced by administration of (−)-nicotine 120 nmol given 30 min earlier. Also, no significant attenuation of the systolic pressor response to (−)-nicotine was produced by prior administration of N'MN. On the other hand,

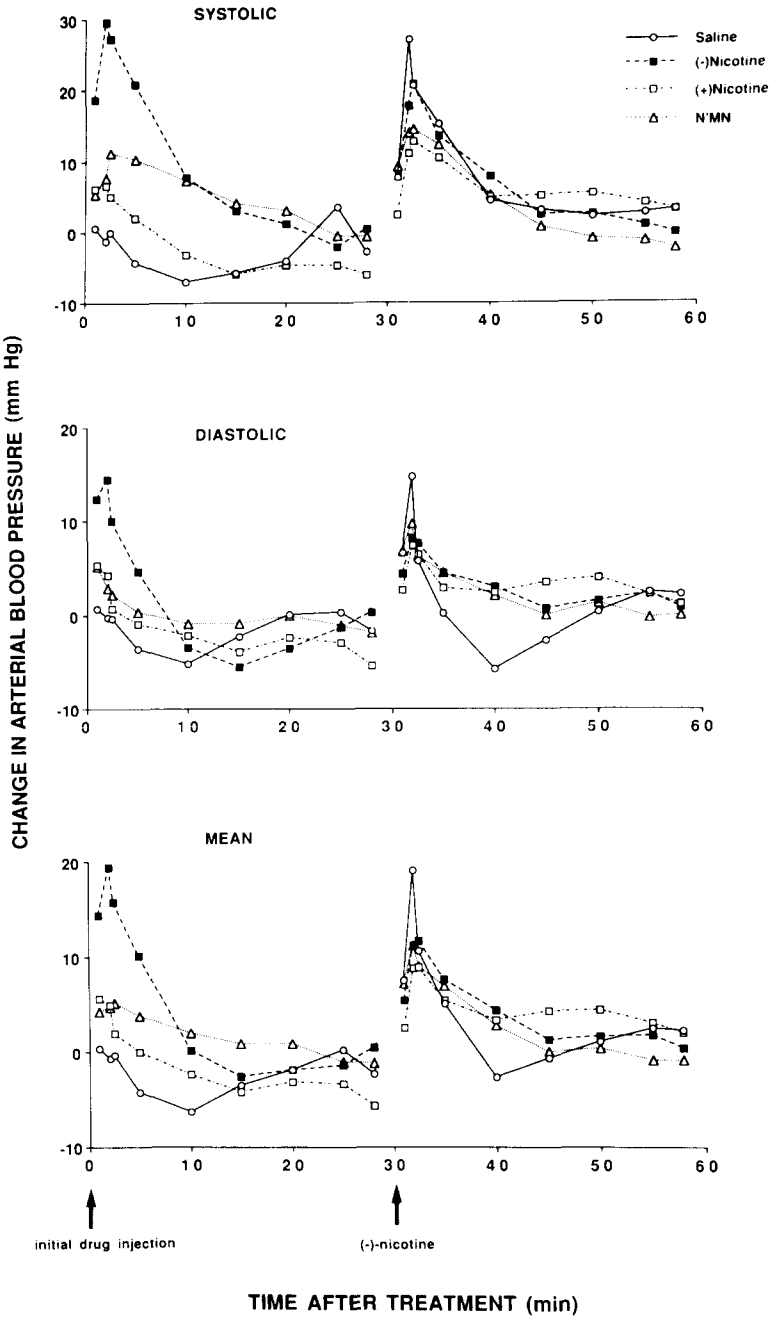

FIG. 3. Systolic, diastolic and mean arterial blood pressure responses to initial treatment with various nicotinic drugs [(−)-nicotine (n = 11), (+)-nicotine (n = 12), N′-methylnicotinium iodide (n = 9), and saline vehicle (n = 8)] and to subsequent treatment of the same rats with (−)-nicotine.

(+)-nicotine did attenuate the systolic pressor response to subsequent (−)-nicotine (Table 5).

Plasma Catecholamine Responses to Nicotinic Agonists

ICV administration of (−)-nicotine produced a marked increase in plasma epinephrine and a small increase in norepinephrine, 1 min after drug administration (Fig. 4). (+)-Nicotine produced smaller increases in plasma concentrations of both epinephrine and norepinephrine. N′MN produced increases in plasma epinephrine and norepinephrine similar to those seen with (−)- nicotine.

Plasma Catecholamine Responses to Subsequent (−)-Nicotine

In rats pretreated with saline ICV, subsequent ICV administration of (−)-nicotine increased plasma epinephrine and norepinephrine concentrations similar to the increases seen in response

TABLE 5

SYSTOLIC BLOOD PRESSURE RESPONSES TO INITIAL TREATMENT WITH DIFFERENT NICOTINIC DRUGS AND TO SUBSEQUENT TREATMENT OF THE SAME RAT WITH (−)-NICOTINE

	Treatment Group							
	Saline		(−)-Nicotine		(+)-Nicotine		N′-Methylnicotine	
Time (min)	*t*	*p*	*t*	*p*	*t*	*p*	*t*	*p*
0				Treatment With Nicotinic Agonist				
1	0.28	0.79	5.37	0.0003*	2.84	0.016*	2.10	0.069
2	−0.72	0.50	5.45	0.0003*	2.36	0.038	2.57	0.0333
2.5	−0.04	0.97	4.80	0.0007*	1.83	0.095	4.24	0.0028*
5	−2.97	0.021	4.80	0.0007*	0.97	0.35	3.62	0.0068*
10	−3.96	0.0054*	3.00	0.0132*	−1.46	0.17	1.90	0.094
15	−2.49	0.041	1.31	0.22	−2.46	0.032	1.19	0.27
20	−1.68	0.14	0.47	0.65	−1.93	0.08	0.71	0.50
25	0.74	0.48	−0.82	0.43	−2.11	0.058	−0.24	0.81
28	−1.15	0.29	0.09	0.93	−2.00	0.071	−0.40	0.70
30				Treatment With (−)-Nicotine				
31	2.05	0.08	7.42	0.0001*	0.86	0.41	2.46	0.04
32	3.33	0.0126*	6.32	0.0001*	2.35	0.039	3.99	0.0040*
32.5	3.20	0.0151*	5.88	0.0002*	2.68	0.021	4.99	0.0011*
35	3.04	0.0188*	6.05	0.0001*	2.17	0.053	4.71	0.0015*
40	1.80	0.12	4.60	0.0010*	1.22	0.25	2.75	0.025
45	0.98	0.36	2.81	0.0186*	1.36	0.20	0.29	0.78
50	0.70	0.50	1.96	0.078	1.65	0.13	−0.79	0.45
55	0.89	0.40	0.37	0.72	1.05	0.32	−0.70	0.51
58	1.02	0.34	−0.16	0.88	1.06	0.31	−0.70	0.50

Response to initial treatment represents change from baseline prior to drug treatment and response to subsequent treatment with nicotine represents change from new baseline 30 min after initial drug treatment. In order to protect against Type 1 error, we required $p<0.02$ for a significant response (*).

to the initial treatment of the (−)-nicotine treatment group (Fig. 4). Also, (−)-nicotine produced similar increases in plasma epinephrine and norepinephrine in rats pretreated with either (−)-nicotine or N′MN, that is pretreatment with either (−)-nicotine or N′MN did not attenuate the plasma epinephrine or norepinephrine responses to subsequent (−)-nicotine. On the other hand, pretreatment with (+)-nicotine did attenuate the norepinephrine, but not the epinephrine response to subsequent (−)-nicotine.

DISCUSSION

Nicotine administered ICV produces bradycardia and increases in systolic, diastolic and mean arterial blood pressure and plasma

TABLE 6

STATISTICAL COMPARISON OF THE AREAS UNDER THE SYSTOLIC BLOOD PRESSURE RESPONSE CURVES

	N′MN(1)		−Nic(1)		+Nic(1)		Sal(1)		N′MN(2)		−Nic(2)		+Nic(2)		Sal(2)	
	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
N′MN(1)			3.72	0.058	3.06	NS	4.36	0.040	0.01	NS	0.64	NS	0.11	NS	1.24	NS
−Nic(1)					15.4	0.0002	16.4	0.0001	3.41	NS	1.70	NS	2.99	NS	0.49	NS
+Nic(1)							0.28	NS	3.35	NS	7.35	0.008	6.10	0.016	8.25	0.005
Sal (1)									4.67	0.034	8.76	0.004	6.45	0.013	11.7	0.001
N′MN(2)											0.52	NS	0.06	NS	1.08	NS
−Nic(2)													0.27	NS	0.15	NS
+Nic(2)															0.75	NS
Sal (2)																

p is the *p* value for the F-test for the significance of contrast of equal areas under 2 response curves.

NS refers to nonsignificant.

Responses to initial treatment with N′-methylnicotinium iodide [N′MN(1)], (−)-nicotine [−Nic(1)], (+)-nicotine [+Nic(1)] or saline [Sal(1)] are presented. Responses to subsequent treatment with (−)-nicotine of rats treated initially with N′-methylnicotinium iodide, (−)-nicotine, (+)-nicotine or saline are presented as N′MN(2), −Nic(2), +Nic(2) and Sal(2), respectively.

Comparisons were made using repeated measures ANOVA with between subjects factor corresponding to the four experimental groups and within subjects factor corresponding to the two treatment periods, namely initial drug treatment and subsequent treatment with (−)-nicotine.

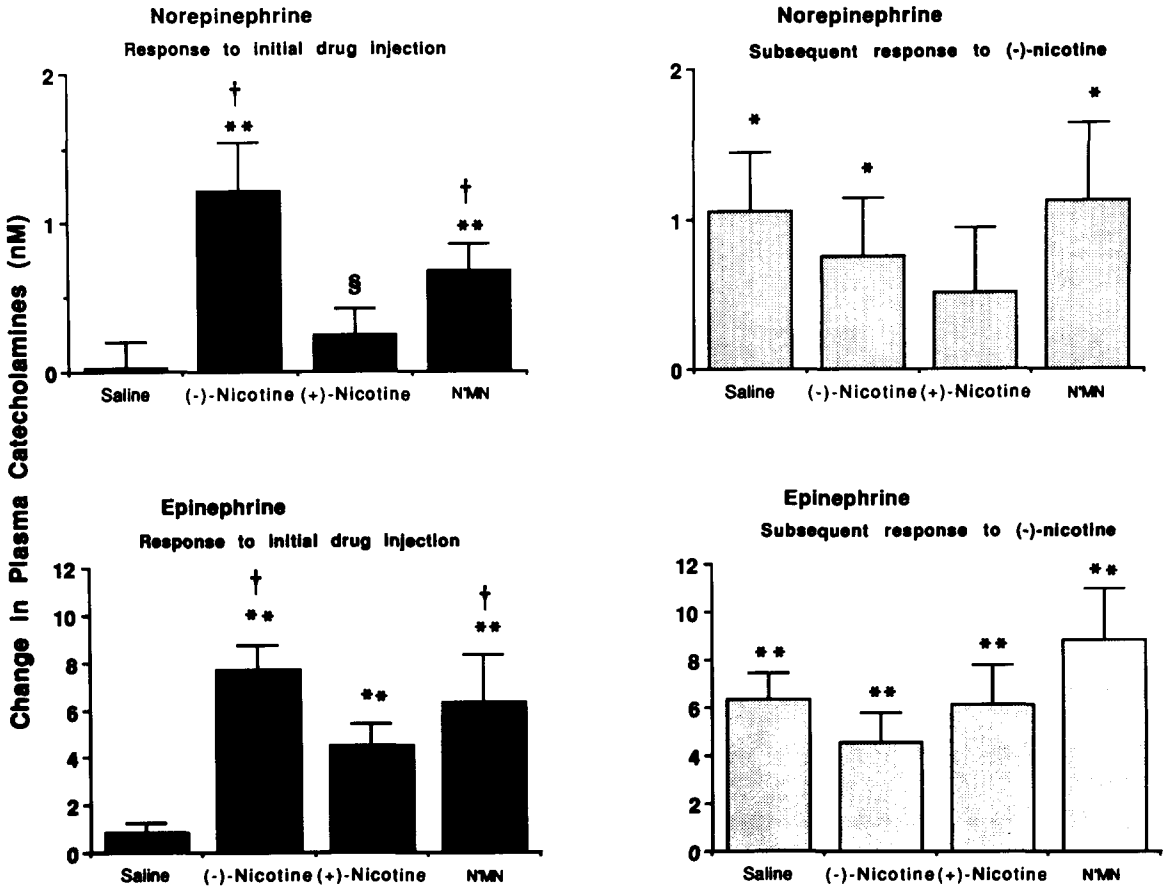

FIG. 4. Comparison of plasma catecholamine responses to initial treatment with various nicotinic drugs [(−)-nicotine (n=11), (+)-nicotine (n=12), N′-methylnicotinium iodide (n=9), and saline vehicle (n=8)] and to subsequent treatment of the same rats with (−)-nicotine. Significant difference from baseline (paired Student's *t*-test) is represented by * at $p<0.05$, ** at $p<0.01$. Significant difference from corresponding saline control group (one-way ANOVA plus Duncan's test) is represented by †at $p<0.05$. Significant difference from initial (−)-nicotine (one-way ANOVA plus Duncan's test) is represented by §at $p<0.05$.

epinephrine and norepinephrine concentrations in conscious freely moving rats. These studies confirm our findings in conscious rats (13,27) and earlier studies in anesthetized animals (9,15). It is unclear whether the nicotine-induced bradycardia is mediated by activation of the baroreflex, especially since the decrease in heart rate in this study followed the increase in blood pressure, or by acting directly on the vagal nuclear complex. The bradycardic response to ICV nicotine is antagonized by systemic methylatropine (15), and the pressor response to intracisternally administered nicotine was antagonized by phenoxybenzamine indicating that nicotine activates both sympathetic and parasympathetic systems. The epinephrine response and bradycardia are antagonized by pretreatment with ICV hexamethonium (13). Also the effects of both systemic and ICV nicotine were blocked by mecamyl amine and hexamethonium (27). These data suggested that the nicotinic receptor mediating the action of nicotine in the brain is similar to the peripheral ganglionic nicotinic receptor. The marked increase in plasma epinephrine and very small increase in plasma norepinephrine seen in response to the dose (120 nmol) of nicotine given ICV in our study suggests that ICV nicotine may be more accessible to pathways mediating adrenomedullary responses than to pathways regulating peripheral sympathetic nerves.

In general, (+)-nicotine has been found to produce similar but less potent pharmacologic effects than (−)-nicotine, when administered systemically (4, 12, 18). In the present study, we also found similar but less potent effects of (+)-nicotine administered ICV on heart rate, blood pressure and catecholamine secretion. Of interest, it has been found that (+)-nicotine occupies only one-tenth as many binding sites as (−)-nicotine (21).

ICV administration of the quaternary analogue of (−)-nicotine, N′MN, appeared equipotent with (−)-nicotine in increasing plasma concentrations of epinephrine and norepinephrine, but less potent in increasing arterial pressure and decreasing heart rate.

N′MN has been shown to be more potent than (−)-nicotine in eliciting tail-flick antinociception when given ICV in mice (3). Since N′MN does not cross the blood-brain barrier, our data and those of Aceto et al. (3) demonstrating nicotine-like effects of this compound when given ICV but lack of effect when given systemically by others (3, 6, 8, 10) strongly supports the intracerebral mediation of these pharmacologic effects of N′MN. This further supports central mediation of many of the pharmacologic effects of nicotine.

Repeated administration of nicotine produces tolerance or desensitization to the effects of subsequent nicotine. The onset and duration of this desensitization depend upon dose and frequency of drug and parameter studied (1, 5, 11, 13, 14, 19, 20, 25, 27). Previously, we found tolerance of the epinephrine-stimulating effect of (−)-nicotine present 30 min but not 24 h after prior systemic administration of nicotine 100 μg/kg (13). Aceto et al. (5) found tolerance to the pressor effect 1 min after a prior systemic

injection of 10 μg/kg, but not 30 min after this smaller dose. In contrast, nicotine 120 nmol when given ICV (comparable to 90 μg/kg in our rats) produced much longer lasting (24 h) tolerance (13). In the present study, we found that nicotine 120 nmol ICV produced tolerance to the bradycardic but not the pressor or catecholamine-stimulating effects of a subsequent injection of nicotine within 30 min. Thus bradycardia is the cardiovascular/sympathoadrenal parameter most readily desensitized by prior intracerebral exposure to nicotine, and ICV nicotine appears to produce desensitization of the bradycardic response more readily and with longer duration than does systemic nicotine (5,27).

In our study, (+)-nicotine appeared equal to (−)-nicotine in desensitizing the bradycardic response to subsequent (−)-nicotine, and even more effective than (−)-nicotine in desensitizing the pressor and norepinephrine responses. Perhaps relevant in this regard, (+)-nicotine has been found to be more potent and more effective than (−)-nicotine in up-regulating the binding of (−)-[H^3]-nicotine (21). Advantage may be taken of the partial agonist nature of (+)-nicotine to use this isomer in selective dosage with minimal agonist activity as a nicotinic antagonist for certain functions.

Ikushima et al. (12) also found the desensitizing effects of (+)-nicotine to be at least equipotent with (−)-nicotine on (−)-nicotine stimulation of rabbit pulmonary artery and neuromuscular junction of rat diaphragm in vitro. These authors suggested that desensitization of adrenergic nerve terminals by (+)-nicotine was presynaptic and noncompetitive and was produced without mediation by nicotinic receptors. Previous studies showing that subthreshold agonist concentrations of nicotine inhibit nicotine-induced vasoconstriction of rabbit ear artery (23) also suggested that desensitization occurs at sites other than those producing nicotinic stimulation. It seems indeed reasonable to suggest, then, that desensitization results from binding of the ligand to a site other than the nicotinic agonist sites. Abood et al. (2) have shown that some of the central actions of nicotine are not readily explained by assuming that it interacts only with nicotinic cholinergic receptors. It remains to be determined whether one of the nicotinic binding sites of Martin and Sloan (21) represents such a nicotinic antagonist site.

We found the quaternary derivative, N′MN, to be less effective than (−)- or (+)-nicotine in desensitizing the bradycardic response to (−)-nicotine. It is possible that the quaternary analog is less accessible to or has less affinity for the desensitization site. In any case, this desensitization of (−)-nicotine-induced bradycardia by N′MN provides strong support for an intracerebral site for the desensitization of nicotinic responses by nicotine. Desensitization or tolerance to the effects of nicotine may be related to the development and persistence of smoking behavior. However, the mechanisms of action of nicotine in brain to affect behavior, cardiovascular function and many other parameters in both health and disease are complex and remain poorly defined.

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