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Locomotor Activity as a Predictor of Times and Dosages for Studies of Nicotine's Neurochemical Actions

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FREEMAN, G. B., K. A. SHERMAN AND G. E. GIBSON. *Locomotor activity as a predictor of times and dosages for studies of nicotine's neurochemical actions.* PHARMACOL BIOCHEM BEHAV 26(2) 305–312, 1987.—Nicotine's action on the central nervous system is complex and likely involves an interaction of neurotransmitters. To determine the time after administration of nicotine and dosage for neurochemical studies, locomotor activity of CD-1 mice was determined at 5 min intervals between 0–60 min. A low nicotine dosage (0.05 mg/kg) did not alter activity 5–15 min after drug injection, but increased activity 28% at 15–25 min post-injection. A high dosage (0.8 mg/kg) reduced total distance 62% and rearing 87% at 5–15 min; at 15–25 minutes total distance declined 56% and rearing 69%; all measures returned to control values after 30 minutes; rearing then increased at 40 min after nicotine. Pretreatment (15 min before nicotine) with mecamylamine (1.0 mg/kg), but not hexamethonium (1.0 mg/kg), prevented the depressant effect of nicotine. Dopamine (DA) and its metabolites as well as acetylcholine (ACh) synthesis were measured at the point of nicotine's maximal depressant action. Striatal levels of dihydroxyphenylacetic acid (DOPAC) were increased and ACh utilization was reduced in striatum (−25%) and cortex (−24%) 10 min after nicotine (0.8 mg/kg). Mecamylamine, while preventing the depressant effect of nicotine on locomotor activity, did not alter its effects on DA metabolism. These results demonstrate that the behavioral outcome of acute nicotine treatment is time and dose-dependent. Nicotine's depressant action appears not to be due to altered DA but may be related to changes in carbohydrate and acetylcholine metabolism.

Nicotine Locomotor activity Acetylcholine Dopamine Mecamylamine Hexamethonium

THE behavioral responses to nicotine are diverse and depend on dosage [12, 14, 32, 47], route of administration [9], time course of drug action [12, 14, 31, 43, 47], prior experience with the drug [14,15], strain [44] and time of day [13]. The exact mechanisms mediating nicotine's behavioral actions are not fully understood, but increasing evidence suggests that these effects are related to changes in neurotransmitter systems [10,45].

Pharmacological and behavioral studies of nicotine have demonstrated cholinergic involvement. Dose-dependent increases or decreases in acetylcholine (ACh) release with nicotine have been associated with similar changes in cortical activation as monitored by changes in the electroencephalogram [6]. Physostigmine potentiates the behaviorally depressant effects of high doses of nicotine while inhibiting the stimulated rate of responding in rats given low doses of nicotine. This suggests that the depressant effects of nicotine are mediated by ACh release whereas the stimulant properties are antagonized by ACh [36]. Increases [34], decreases [17,41] and no change [30] in endogenous ACh levels have been reported with nicotine treatment. Recently, no change in ACh synthesis from labelled choline was observed in various mouse brain regions following a relatively high dose of nicotine (4.0 mg/kg, IP) [40].

In addition to its effects on cholinergic function, nicotine alters DA metabolism. Regional studies using brain slices preloaded with [^{3}H] norepinephrine or dopamine (DA) have demonstrated a stimulatory effect of nicotine on catecholamine release from hypothalamus [26,48], hippocampus [7,8] and striatum [7, 25, 26]. Single injections of nicotine (1.0 mg/kg) increase DA turnover as measured by determination of the decrease in DA concentration after administration of a tyrosine hydroxylase inhibitor [1–4, 33].

In the present experiments, locomotor activity after acute nicotine was used to determine the appropriate time and dosage for investigating nicotine's biochemical effects *in vivo* and to increase the relevance of the neurochemical data. ACh formation from radioactive glucose and DOPAC/DA ratios were used to estimate the turnovers of ACh and DA, respectively.

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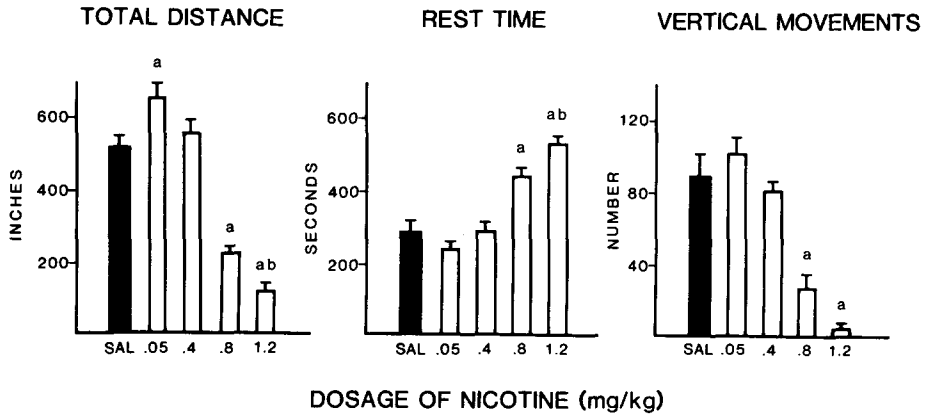

FIG. 1. Effects of nicotine on open field behavior. Mice were injected with nicotine at 0 min, placed in the activity monitor at 15 min and removed at 25 min. Values are means±SEM of 6 animals per 10 min. Significant dose-related effects were evident for total distance, $F(4,19)=39.42$, $p<0.001$; rest time, $F(4,23)=28.93$, $p<0.001$; and vertical movements, $F(4,22)=29.22$, $p<0.001$. Letters denote value differs significantly ($p<0.05$) from [a]saline or [b]0.8 mg/kg of nicotine.

METHOD

Materials

Male CD-1 mice (30–35 days old; 23–25 g) were from Charles River Breeding Laboratories (Stone Ridge, NY). Nicotine, hexamethonium bromide (Sigma Chemical Company, St. Louis, MO) and mecamylamine hydrochloride (Merck, Sharpe and Dohme Research Labs, West Point, PA) were prepared fresh each day. The electrochemical detector (LC-4B) and flow cell (LC-17) were from Bioanalytical Systems, Inc. (West Lafayette, IN). The chromatographic column (μBondapak, C-18, reverse phase), sample delivery system (model 710B WISP and 6000A pump) and data reduction system (model 730 data module and model 721 programmable system controller) were from Millipore, Waters Chromatography Division (Milford, MA). The digiscan (optical digital sensor) animal activity monitor (model RXYZCM-16) was from Omnitech Electronics, Inc. (Columbus, OH). Data was printed automatically on an Epson dot matrix printer (model MX-80IIIF/T). [U-^{14}C]glucose (250 mCi/mmol), [^{3}H-acetyl]choline iodide (80–100 mCi/mmol) and Aquasol 2 liquid scintillation counting fluid were from New England Nuclear (Boston, MA). All other reagents and supplies for acetylcholine and glucose measurements were obtained as described previously [24,42].

Experimental Procedures

Dose response effects of acute nicotine treatment on open field behavior. Animals were housed 6 per cage and maintained on Ralston Purina Company Rodent laboratory Chow No. 5001. The animal quarter was illuminated from 6:00 a.m. to 6:00 p.m. Mice that had been acclimated to our laboratory for one week were weighed and fasted the night before the experiment with free access to water. Animals were matched into four treatment groups according to weight. Nicotine was dissolved in 0.9% saline and neutralized to pH 7.4 with 5 *N*-HCl. On the day of the experiment, nicotine (0, 0.05, 0.4, 0.8 mg/kg) was injected intraperitoneally (0.0075 ml/g). Five minutes later, animals were placed in a digiscan animal activity monitor for 10 min. In a separate experiment, animals were injected with nicotine (0, 0.05, 0.4, 0.8, 1.2 mg/kg). Activity was monitored between 15 and 25 min after drug injection.

The behavioral instrument monitored various components of horizontal and vertical activity of two animals simultaneously (animals in the same group were tested together). Horizontal movement sensors directed eight beams from front to back (x-axis) and eight beams from side to side (y-axis). The vertical movement sensor consisted of eight beams which sensed vertical activity. Interruption of these beams generated data which was accumulated and printed automatically at the end of the sample period on an Epson printer. The dimensions (length × width × height) for each animal compartment were 20×20×30 cm. Animals were tested between the third and seventh hours of their light cycle. The parameters of interest include:

Total distance. The distance in inches traveled by the animal in the given sample period.

Rest time. The difference in seconds between total sample time (600 seconds) and time spent moving.

Number of vertical or rearing movements. A movement was recorded each time the animal reared up. The mouse had to go below the level of the vertical sensor for at least one second before the next vertical movement could be registered.

Time-response of nicotine's effects on open field activity. Mice were starved the night before the experiment. On the day of the experiment, nicotine (0.8 mg/kg) was injected at 0 min and animals were placed into the activity monitor for 5 min intervals up to 60 min post-injection. Mice not immediately placed in the activity chamber were put back into their home cage until testing. Mice that received saline injections were also placed into the monitor at the different times represented in the time course range. The various parameters of open field activity were measured as described previously.

Effects of pretreatment with mecamylamine or hexamethonium on nicotine-induced alterations of open field behavior. Animals that had been starved the night before, were injected with mecamylamine (1.0 mg/kg), hexamethonium (1.0 mg/kg) or saline at 0 min. Fifteen min

TABLE 1

DOSE-RESPONSE OF NICOTINE ON OPEN FIELD BEHAVIOR

Dose of Nicotine	Total Distance	Rest Time	Vertical Movements
Control	545 ± 54	301 ± 24	80 ± 9
0.05	519 ± 70	307 ± 25	78 ± 11
0.4	559 ± 72	330 ± 29	65 ± 10
0.8	208 ± 18*	476 ± 15*	10 ± 3*

Mice were injected with nicotine (mg/kg) at 0 min. At 5 min animals were placed in the activity monitor and were removed at 15 min. Values are means ± SEM of 11–12 animals. Significant dose response effects of nicotine were evident for total distance, $F(3,43)=8.72$, $p<0.001$; rest time, $F(3,43)=12.23$, $p<0.001$; and vertical movements, $F(3,43)=12.79$, $p<0.001$.

*Value differs from saline control, $p<0.001$.

later mice were treated with nicotine (0.05 or 0.8 mg/kg) or saline. Animals were placed into the activity monitor at 20 min and removed at 30 min. Open field behavior was measured as previously described.

Effect of nicotine on striatal dopamine and its interaction with mecamylamine or hexamethonium. Animals were fasted the night before the experiment with free access to water. On the morning of the experiment, mice were injected intraperitoneally with saline, mecamylamine (1.0 mg/kg) or hexamethonium (1.0 mg/kg) at 0 min. At 15 min, mice received an injection of saline or nicotine (0.8 mg/kg). Ten minutes after the second injection, animals were placed into fenestrated Plexiglas holders and sacrificed by focussed microwave irradiation (2.2 kW, 0.35 sec; Gerling Moore). Brains were quickly removed and the striatum was free hand dissected and weighed. The striata (about 30 mg) were homogenized with a Polytron homogenizer (Brinkman Instruments Co., Westbury, NY) in 0.7 ml of ice cold 1 *N* formic acid/acetone (vol/vol: 15/85) and the homogenate was centrifuged (15,000 × g at 4°C). An aliquot of the supernatant (300 μl) was transferred to 5 ml conical tubes. Three volumes (900 μl) of cold heptane/chloroform (vol/vol: 8/1) were mixed with the supernatant and then the tubes were centrifuged. The organic layer was aspirated and the aqueous layer was dried with nitrogen and stored at −20°C until assay. The dried extract was reconstituted in 110 μl of mobile phase and 100 μl was injected onto the high pressure liquid chromatograph (HPLC).

Dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were separated by HPLC. The mobile phase contained 0.66 *M*-chloroacetic acid, 0.046 *M*-dibasic sodium phosphate, 0.06 mM-Na_2EDTA, 1.66 mM-octanesulfonic acid sodium salt monohydrate, 7.5–12.5% methanol at pH 3.2. The mobile phase was filtered through a 0.22 μm filter and degassed by sonication. The flow rate was 1.0–1.2 ml/min. The stationary phase was a 10 micron μBondapak 3.9 mm internal diameter ×30 cm C18 reverse phase column which was maintained at a temperature of 27°C. The detector consisted of an LC-4B electronic controller and LC-17 oxidative flow cell with a TL-5 glassy carbon working electrode. The applied potential was +0.875 V versus a Ag/AgCl reference electrode. Sensitivity was set at 20 nanoamps per volt. Samples were injected onto the column with a model 710B WISP autosample injection module and a 6000A solvent delivery system. Data reduction was

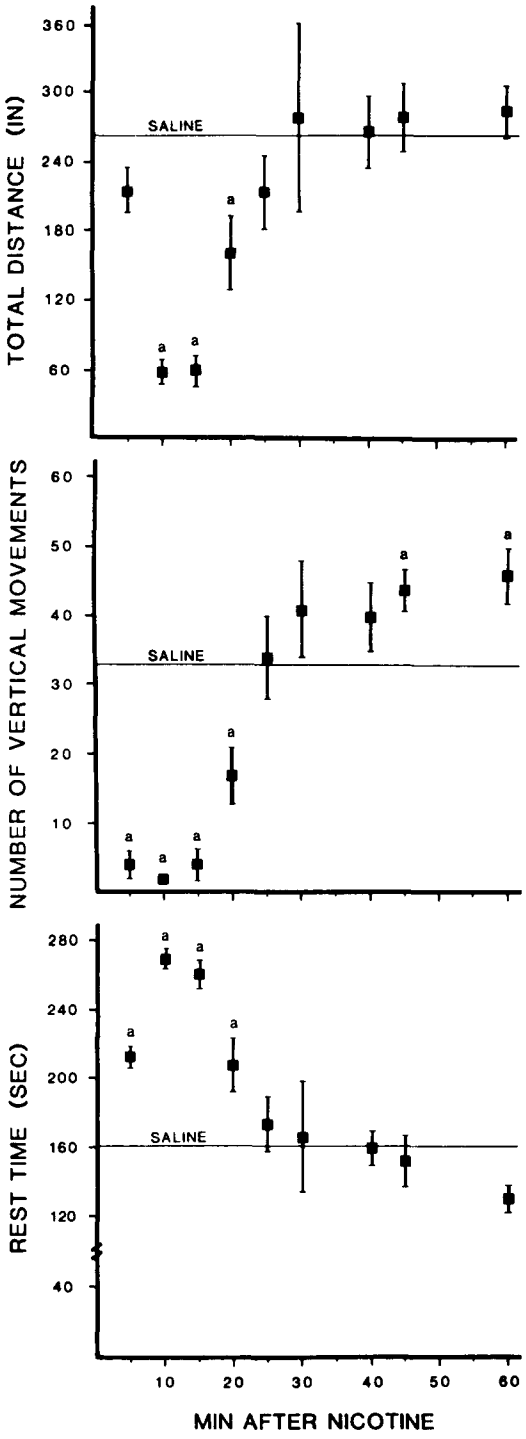

FIG. 2. Time response effects of nicotine on open field behavior. Mice were injected with 0.8 mg/kg of nicotine at 0 minutes and were placed into the activity monitor for 5 min intervals up to 60 min. The first interval is 0–5 min and the last interval is 55–60 min. Values are means±SEM of 4–18 animals per 5 min interval. The saline value represents an average of all the time intervals. Significant effects were indicated for total distance, $F(10,127)=9.30$, $p<0.001$; vertical movements, $F(10,128)=20.70$, $p<0.001$; and rest time, $F(10,126)=14.13$, $p<0.001$. [a]Value differs significantly from saline, $p<0.05$.

TABLE 2

EFFECTS OF PRETREATMENT WITH MECAMYLAMINE OR HEXAMETHONIUM ON NICOTINE-INDUCED ALTERATIONS OF OPEN FIELD BEHAVIOR

Treatment	Total Distance	Rest Time	Vertical Movements
Saline + Saline	626 ± 27	274 ± 13	95 ± 5
Saline + Nicotine (0.8)	352 ± 65*	436 ± 27*	33 ± 8*
Saline + Nicotine (0.05)	762 ± 86	257 ± 23	101 ± 8
Mecamylamine + Saline	646 ± 31	275 ± 11	106 ± 4
Hexamethonium + Saline	622 ± 36	281 ± 14	96 ± 7
Mecamylamine + Nicotine (0.8)	619 ± 45†	304 ± 18†	89 ± 7†
Mecamylamine + Nicotine (0.05)	728 ± 61	262 ± 21	108 ± 6
Hexamethonium + Nicotine (0.8)	324 ± 65‡	433 ± 32‡	48 ± 13‡

Mice were injected with mecamylamine (1.0 mg/kg), hexamethonium (1.0 mg/kg) or saline at 0 min. At 15 min mice were treated with nicotine (0.05 or 0.8 mg/kg) or saline. Animals were placed into the activity monitor at 20 min and were removed at 30 min. Values are means ± SEM of 12–24 animals. Univariate tests indicated significant effects of drug treatment on total distance, $F(7,133)=8.59$, $p<0.001$; rest time, $F(7,134)=12.99$, $p<0.001$; and vertical movements, $F(7,133)=16.02$, $p<0.001$. Letters denote value differs significantly ($p<0.01$) from *saline + saline, †saline + 0.8 mg/kg nicotine and ‡hexamethonium + saline.

TABLE 3

EFFECT OF NICOTINE ON STRIATAL DOPAMINE AND ITS INTERACTION WITH MECAMYLAMINE OR HEXAMETHONIUM

Treatment	DA	DOPAC	DOPAC/DA	HVA
Saline + Saline	60.0 ±4.7	2.03 ±0.31	0.031 ±0.001	4.77 ±0.63
Saline + Nicotine	70.7 ±3.6	3.31* ±0.03	0.046† ±0.004	4.57 ±0.19
Mecamylamine + Nicotine	70.5 ±4.2	2.80 ±0.18	0.040 ±0.003	4.84 ±0.39
Hexamethonium + Nicotine	65.4 ±3.7	2.57 ±0.28	0.039 ±0.003	4.64 ±0.04
Mecamylamine + Saline	64.2 ±5.8	2.67 ±0.03	0.038 ±0.006	4.75 ±0.41
Hexamethonium + Saline	56.9 ±2.8	2.20 ±0.01	0.045 ±0.005	4.03 ±0.26

Mice were injected IP with saline, mecamylamine (1.0 mg/kg) or hexamethonium (1.0 mg/kg) at 0 min. At 15 min animals were injected with saline or nicotine (0.8 mg/kg). Mice were sacrificed at 25 min by focussed microwave irradiation. Values (pmol/mg of tissue) are means ± SEM of 4 animals. An analysis of variance indicated a significant effect of drug treatment on DOPAC, $F(5,14)=3.83$, $p<0.025$.

*Value differs from saline + saline, $p<0.025$.

†Overall F not significant, but value differs from saline + saline by least significant difference test, $p<0.05$.

performed with a model 730 data module and a model 721 programmable system controller. Quantitation was by integration of peak area and comparison with a standard mixture of monoamines and metabolites [20].

Acetylcholine and carbohydrate metabolism during nicotine treatment. Male mice were fasted the night before the experiment, but allowed free access to water. On the morning of the experiment, saline or nicotine (0.05 or 0.8 mg/kg) was injected intraperitoneally (0.0075 mg/g). All mice were injected (9 min) intravenously with [U-^{14}C]glucose (8 μCi/g; 160 μCi/0.1 ml) 1 min prior to sacrifice (10 min) by head focussed microwave irradiation (2.2 kW; 0.35 sec). The brains were quickly removed and the hippocampus, striatum and cortex were dissected and added to 0.83 ml of 0.2 *N*-perchloric acid that contained 2.5 nanomoles of trimethylacetylcholine and 5000 dpm of ^{3}H-ACh as internal standards. The samples were homogenized and the probe was rinsed twice with 0.83 ml of 0.2 *N*-perchloric acid. The rinses and homogenates were combined and centrifuged for 10 min at 15,000 × g. Two ml of the perchloric acid extract were used for the determination of ACh and ^{14}C-ACh [20, 25, 43]. The remainder was utilized for triplicate measurements of glucose, glucose specific activity and of total radioactivity in the brain [23]. All values are expressed per mg of protein [27]. Glucose utillzation was calculated by the method of Gaitonde [22].

$$\text{Glucose utilized} = \frac{\text{Total non-glucose acid soluble dpm accumulated in 1 min/mg protein}}{\text{dpm in glucose/nmol of glucose}}$$

The rate ACh synthesis (nmol/mg protein per min) was estimated by the following equation [23].

$$\text{ACh rate of synthesis} = \frac{[\text{ACh (dpm/mg protein per min)}]\ (3)}{\text{dpm in glucose/nmol glucose}}$$

A factor of 3 is used because there are three times as many carbon atoms in glucose as there are in ACh.

Statistical comparisons between groups were made by analysis of variance with the least significant difference test [46].

RESULTS

Dose Response Effects of Nicotine Treatment on Open Field Behavior

Nicotine's behavioral effects were dose-dependent. At 5–15 min after drug administration, low and moderate doses of nicotine did not signigicantly alter open field behavior, whereas the highest dosage (0.8 mg/kg) reduced activity (Table 1). When the dose-response to nicotine was monitored 15–25 min after injection, in a separate experiment, effects were apparent at lower dosages (Fig. 1). The low dose of nicotine (0.05 mg/kg) increased activity 28% whereas 0.8 and 1.2 mg/kg reduced total distance, 56% and 77%, respectively. Moreover, the decrease in total distance with 1.2 mg/kg was significantly different from the 0.8 mg/kg dosage ($p<0.05$).

TABLE 4

CARBOHYDRATE METABOLISM DURING NICOTINE TREATMENT

Treatment	Brain Region: Cortex	Hippocampus	Striatum
	Glucose (nmol/mg protein)		
Saline	20.6 ± 2.1	29.4 ± 3.1	20.0 ± 1.7
0.05 mg/kg	19.8 ± 1.5	31.4 ± 3.7	20.1 ± 1.8
0.8	24.0 ± 1.0	32.3 ± 1.9	22.5 ± 0.7
	Glucose Specific Activity (dpm/nmol)		
Saline	5015 ± 356	3313 ± 335	4309 ± 391
0.05	4805 ± 518	2706 ± 383	4031 ± 465
0.8	3323 ± 292*	1958 ± 197*	2811 ± 226*
	Total Acid Soluble dpm not in Glucose × 10^{-3} mg Protein		
Saline	44.0 ± 2.9	32.5 ± 3.0	42.0 ± 4.0
0.05	41.2 ± 4.6	31.5 ± 4.4	39.1 ± 5.0
0.8	23.0 ± 2.3*	22.2 ± 2.8	24.7 ± 2.7*
	Glucose Utilization (nmol/mg protein per min)		
Saline	8.5 ± 0.5	9.5 ± 0.5	9.4 ± 0.4
0.05	8.1 ± 0.4	10.4 ± 0.5	9.1 ± 0.7
0.8	6.7 ± 0.2*	10.5 ± 0.8	7.8 ± 0.3

Mice that had been starved overnight received an intraperitoneal injection of saline or nicotine (0.05 or 0.8 mg/kg; 0.0075 ml/g). All mice were injected with [U-^{14}C]glucose (8 μCi/g; 250–300 mCi/mmol; 0.005 ml/g) at 9 min and sacrificed at 10 min by focussed microwave irradiation. Glucose, glucose specific activities, and glucose utilization rates were determined as described in the Method section. Values are means ± SEM for 8–10 animals per treatment group from two separate experiments.

*Value differs from saline, $p<0.05$.

TABLE 5

ACETYLCHOLINE METABOLISM DURING NICOTINE TREATMENT

Treatment	Brain Region: Cortex	Hippocampus	Striatum
	ACh (nmol/mg protein)		
Saline	0.145 ± 0.009	0.174 ± 0.012	0.485 ± 0.024
0.05 mg/kg	0.136 ± 0.007	0.148 ± 0.012	0.496 ± 0.019
0.8	0.151 ± 0.005	0.166 ± 0.014	0.543 ± 0.022
	Choline (nmol/mg protein)		
Saline	0.193 ± 0.022	0.157 ± 0.009	0.189 ± 0.010
0.05	0.178 ± 0.015	0.141 ± 0.006	0.218 ± 0.005
0.8	0.293 ± 0.042*	0.206 ± 0.011*	0.239 ± 0.011*
	ACh Specific Activity (dpm/nmol per min)		
Saline	378 ± 50	168 ± 22	643 ± 99
0.05	373 ± 47	165 ± 22	555 ± 78
0.8	178 ± 24*	88 ± 13*	257 ± 27*
	ACh (dpm/mg protein)		
Saline	58.9 ± 7.7	30.6 ± 4.6	327.0 ± 53.3
0.05	53.8 ± 7.5	29.3 ± 4.8	275.5 ± 44.0
0.8	29.7 ± 3.4*	13.0 ± 1.7*	154.4 ± 14.3*
	ACh Utilization (nmol/mg protein per min)		
Saline	0.033 ± 0.003	0.027 ± 0.003	0.209 ± 0.019
0.05	0.031 ± 0.002	0.028 ± 0.002	0.191 ± 0.019
0.8	0.025 ± 0.002*	0.022 ± 0.003	0.156 ± 0.006*

Mice that had been starved overnight received an intraperitoneal injection of saline or nicotine (0.05 or 0.8 mg/kg; 0.0075 ml/g). All mice were injected with [U-^{14}C]glucose (8 μCi/g; 250–300 mCi/mmol; 0.005 ml/g) at 9 min and sacrificed at 10 min by focussed microwave irradiation. ACh, ACh specific activity and ACh utilization rates were determined as described in the Method section. Values are means ± SEM for 8–10 animals per treatment group from two separate experiments.

*Value differs from saline, $p<0.05$, by the least significant difference test.

Time-Response of Nicotine's Effects on Open Field Behavior

The effects of nicotine (0.8 mg/kg) varied with time. The onset of the depressant effect of nicotine was evident early (0–5 min post-injection) and was maximal at 5–10 min after drug administration. For most measures, control levels were regained by 25 min. After an initial decrease in vertical movements, nicotine enhanced rearing by 40 min after treatment ($p<0.05$) (Fig. 2).

Effects of Pretreatment With Mecamylamine or Hexamethonium on Nicotine-Induced Alterations of Open Field Behavior

Nicotine's depressant effects were sensitive to mecamylamine but not to hexamethonium. In saline mice, pretreatment with mecamylamine or hexamethonium did not alter any aspect of open field behavior (Table 2). Total distance traveled increased nonsignificantly (+22%), whereas rest time and vertical movements were unchanged with 0.05 mg/kg of nicotine. Pretreatment of low dose nicotine animals (0.05 mg/kg) with mecamylamine did not alter locomotor behavior. The depressant action of nicotine (0.8 mg/kg) was reflected in all measures of open field behavior. Total distance declined 44%, rest time increased 59% and the number of vertical movements decreased 65%. In the presence of mecamylamine, deficits in locomotor behavior were ameliorated, whereas hexamethonium failed to block nicotine's depressant effects.

Effect of Nicotine on Striatal Dopamine and its Interaction With Mecamylamine or Hexamethonium

Striatal concentrations of DA and its metabolites were minimally affected by nicotine and the various drug combinations. Nicotine (0.8 mg/kg) significantly increased DOPAC levels (+63%) compared to control (Table 3). Although an overall analysis of variance did not indicate significant changes in DOPAC/DA ratios, the 48% increase with 0.8 mg/kg nicotine was statistically different from saline by the least significant difference test. Mecamylamine's antagonism of nicotine's depressant effects on behavior was probably not due to an action on dopamine metabolism since DA, DOPAC and DOPAC/DA levels of mecamylamine-nicotine animals were not different from saline-nicotine animals.

Carbohydrate and Acetylcholine Metabolism During Nicotine Treatment

Glucose concentrations in the striatum, hippocampus and cortex were not altered by nicotine (Table 4). The glucose specific activity (dpm/nmol per min) was relatively unaffected by 0.05 mg/kg nicotine but was significantly reduced by 0.8 mg/kg in striatum (−35%), hippocampus (−41%) and cortex (−34%). The estimated rate of glucose utilization decreased nonsignificantly in striatum (−17%), was unchanged in hippocampus and significantly reduced in cortex (−21%) with 0.8 mg/kg nicotine.

Regional concentrations of choline were increased by nicotine (0.8 mg/kg) in cortex (+52%), hippocampus (+31%) and striatum (+26%) while concentrations of ACh were unchanged by either 0.05 or 0.8 mg/kg of nicotine (Table 5). ACh formation (dmp/nmol per min) in hippocampus or cortex was not altered by 0.05 mg/kg nicotine whereas in striatum ACh specific activity declined nonsignificantly (−14%). With 0.8 mg/kg, ACh specific activity was reduced in all regions (striatum, −60%; hippocampus −48%; cortex, −53%). Estimates of ACh rate of formation from [U-^{14}C]glucose were unaltered by the 0.05 mg/kg dosage of nicotine. However, significant reductions in ACh utilization in striatum (−25%) and cortex (−24%) and a nonsignificant decrease in hippocampus (−18%) occurred with 0.8 mg/kg.

DISCUSSION

Open field behavior in an automated activity monitor was sensitive to pharmacological manipulation by nicotine. Low doses of nicotine (0.05 mg/kg) increased activity and higher doses (0.8 and 1.2 mg/kg) reduced open field activity in agreement with previous behavioral findings [12, 16, 18, 28, 37, 44, 47]. The time course of nicotine's behavioral effects revealed important aspects of nicotine's action that would not have been evident with a single time point observation and has important implications for the neurochemical findings. The increase in activity with a low dosage of nicotine (0.05 mg/kg) depended upon the time after drug injection that the animal was tested. No effect was observed 5–15 min following nicotine administration, whereas 28% and 22% increases occurred 15–25 min and 20–30 minutes post-injection, respectively. The initial depression of activity with 0.8 mg/kg was followed by an increase which is consistent with other studies that demonstrate a biphasic effect of high dose nicotine treatment [14,43]. Open field behavior decreased early following nicotine (0.8 mg/kg) and did not attain control levels until approximately 30 min after injection. The number of vertical movements was particularly sensitive to the depressant effects of 0.8 mg/kg of nicotine. This behavior dropped quickly during the first few minutes and was the only parameter to increase significantly beyond control levels during the stimulation phase. Higher dosages of nicotine produce a longer period of decreased activity [40].

The pharmacological and neurochemical studies suggest that the behavioral effects of nicotine were probably due to its central cholinergic action. Mecamylamine, a nicotinic cholinergic antagonist which is thought to enter the brain readily, has been found to prevent both the stimulant and depressant behavioral actions of nicotine [11, 14, 38, 39]. In the present experiments, mecamylamine, but not hexamethonium (a peripheral nicotine antagonist), blocked the depressant effects of acute nicotine. At the time and dosage of nicotine's maximal depressant effects, ACh turnover was reduced in striatum and cortex. ACh utilization rates were not altered by 0.05 mg/kg nicotine which is consistent with its lack of effect on behavior 10 min after administration.

The effects of nicotine on DA metabolism and its relation to altered behavior are less clear. Although increases in DOPAC levels and DOPAC/DA ratios occurred with 0.8 mg/kg, these changes did not appear to be related to the behavioral changes produced by nicotine since mecamylamine did not affect DOPAC concentrations in nicotine-treated animals, but totally prevented the depressant effect of the drug. The lack of an inhibitory effect on DA metabolism with nicotine's ability to reduce locomotor activity seems contradictory to the general concept that impaired locomotor activity accompanies impaired DA metabolism. For example, hypoxia reduced locomotor activity in parallel with a decline in DA turnover. Morphine, given acutely, antagonized both the behavioral and neurochemical effects of hypoxia. Thus, unlike nicotine, the behavioral effects of morphine, hypoxia and their combination may more closely reflect neurochemical changes in DA [21]. Other studies also suggest that changes in central catecholaminergic systems may not underlie the behavioral effects of nicotine. Nicotine, in a dose-related fashion up to 0.3 mg/kg, increased response rates of rats in a fixed-interval 30 to 120 sec schedule. Mecamylamine (1.0 mg/kg) blocked the changes produced by nicotine while chlorpromazine (1.0 mg/kg), a dopaminergic antagonist, failed to alter the behavioral effects of nicotine and, instead, had an additive effect [49].

Although nicotine (0.8 mg/kg) depressed activity and ACh utilization rates, the effects of stress associated with high doses of nicotine may have complicated interpretations of the present results. Relatively high doses of nicotine produce a marked hypothermic effect [40], increased corticosterone secretion [5, 9, 29] and may lead to increased blood glucose levels [35]. The decreased glucose specific activity may have been due to dilution of the labelled glucose. However, the equation for ACh utilization takes the decrease in glucose specific activity into consideration.

Nicotine's effects on behavior and brain function are complex. The behavioral effects of nicotine are time-dependent, which must be considered in any effort to identify the exact biochemical mechanisms by which nicotine acts. The reduction of striatal and cortical ACh utilization at the time of nicotine's maximal depressant effect may have important implications for its receptor-mediated actions. The multiple effects of nicotine may be mediated through several neurotransmitters. Further analysis of these neurotransmitter interactions will require additional time and dose response studies.

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