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Nicotine Alters Catecholamines and Electrocortical Activity in Perfused Mouse Brain

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ERWIN, V. G., K. CORNELL AND J. F. TOWELL. *Nicotine alters catecholamines and electrocortical activity in perfused mouse brain.* PHARMACOL BIOCHEM BEHAV **24**(1) 99–105, 1986.—Nicotine differentially altered electrocortical (ECoG) activity and brain catecholamine metabolism in mice (C3H and C57BL) known to differ in behavioral response to nicotine. Nicotine appeared to produce a concentration dependent desynchronization to ECoG activity in isolated perfused mouse brain (IPMB) from C3H mice. Homovanillic acid (HVA) production was unchanged in C3H perfused brains while an apparent reduction in 3-methoxy-4-hydroxyphenethyleneglycol (MPHG) was observed. Brain content of norepinephrine and dopamine remained relatively constant in the various regions tested. In IPMB from C57BL mice, nicotine elicited an enhancement of ECoG amplitude which was accompanied by decreased HVA production rates. A downward trend in MHPG production was also observed. These effects were associated with increased levels of norepinephrine and dopamine in various brain regions.

Electrocortical activity Nicotine Catecholamines

NICOTINE elicits a variety of behavioral responses when administered to animals. Recently, Collins and co-workers [14] observed a dose-dependent increase in locomotor activity in C3H mice and a decrease in activity in C57BL/6J mice following nicotine injections, IP. In assessing possible neurochemical correlates of the CNS effects of nicotine, several investigations have shown reduced norepinephrine levels in the mouse hypothalamus [2,7], following repeated doses of nicotine, *in vivo*. Reduction of catecholamine stores by nicotine, *in vivo*, was reported to be greatest in the median eminence. In rat hypothalamic and striatal slices nicotine, at mM concentrations, increases norepinephrine and dopamine outflow [25], and it has been observed [1,5] that nicotinic receptor agonists increase the firing of dopaminergic neurons as well as increase dopamine turnover in striatal neurons. Others have shown that nicotine and oxotremorine, a muscarinic agonist, elicit up to twofold dose-dependent increases in the activity of tyrosine hydroxylase in the rat nucleus locus coeruleus [11]. These results are consistent with an enhanced activity of noradrenergic and dopaminergic neurons.

Since many behaviors, including locomotor activity, are affected by alteration in catecholaminergic pathways [18,22], it is plausible that the observed *in vivo* and *in vitro* effects of nicotine on catecholamine systems might be related to the actions of this substance on locomotor activity. In the present study, this hypothesis has been examined by determining the effects of nicotine at a concentration of 6.2 μM (which corresponds to a 1 mg/kg dose) on norepinephrine and dopamine turnover in brains of C3H and C57BL/6J mice. The isolated perfused mouse brain (IPMB) was selected for this study because it provided a novel way to determine catecholamine turnover rates in an *intact* mouse brain. By measuring the catecholamine metabolite levels in the brain perfusate, nicotine effects on neuronal activity were studied without destroying the organization of the brain pathways. In previous studies the IPMB was characterized as a model for studying norepinephrine turnover [19,20] by measuring the rate of production of 3-methoxy-4-hydroxyphenethyleneglycol (MHPG), the major norepinephrine metabolite in mouse brain [13]. In this study the IPMB was used to determine the effects of nicotine on production of both MHPG and homovanillic acid (HVA), the major dopamine metabolite in mouse brain. Also, associated electrocortical activities and catecholamine levels in various brain regions were determined.

METHOD

Reagents and Animals

Homovanillic acid (No. 38588, Calbiochem-Behring Corp., Los Angeles, CA); DL-4-hydroxy-3-methoxyphenylglycol, piperazine salt (No. 454205, Calbiochem-Behring Corp.); spectral grade ethyl acetate, redistilled (J. T. Baker, Phillipsburg, NJ); ethyl ether, anhydrous (Fisher Scientific Co., Fair Lawn, NJ); acid washed alumina (No. CF-8010, Bioanalytical Systems, West Lafayette, IN); 3,4-dihydroxybenzylamine HBr (No. D-7012, Sigma Chemical Co., St. Louis, MO); 3-hydroxytyramine HCl (No. H6,025-5, Aldrich Chemical Co., Inc., Milwaukee, WI);

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DL-arterenol HCl (No. A7256, Sigma Chemical Co.); artificial blood, washed bovine erythrocytes in an artificial serum [28]; redistilled nicotine, free base; male mice (C3H/Ibg and C57BL/Ibg) from 60 to 80 days of age.

Apparatus

The apparatus for HPLC separation and detection of catecholamines and metabolites included: solvent metering pumps, Altex 110A (Altex Scientific); sample injection and window valves, Rheodyne 7125 and 7001 (Rheodyne, Berkeley, CA); RP-2 MPLC cartridge and holder and chromosorb LC-7, C_{18} reverse phase analytical column, 10 μm, 25 cm×4.6 mm i.d. (Brownlee Labs, Santa Clara, CA); μBondapak C_{18} analytical column, 30 cm×3.9 mm i.d., 10 μm (Waters Associates, Milford, MA); electrochemical detector (LC-4), CP-S carbon paste, TL-4 thin-layer transducer cell, RE-1 Ag|AgCl reference electrode (Bioanalytical Systems, West Lafayette, IN); recorder (Houston Instruments Omniscribe); sample filters (No. GSWP 01300, Millipore Corp., Bedford, MA); filter holders (Liqui-Holder, 13 mm, No. 342-0001, Bio-Rad Labs, Richmond, CA).

Procedure

Brain perfusion procedures. Surgical preparation of the IPMB was adapted from the procedure described in detail by Andjus *et al.* [3] for the rat brain. Prior to cannulation, animals were anesthetized and bipolar platinum electrodes were placed on the parietal and frontal (just anterior to the coronal suture) regions of the calvarium and fixed in place with epon dental cement. After 24 hr, the fully recovered animals were anesthetized with urethane (1.5 g/kg, IP), the carotid arteries were ligated bilaterally, the internal carotid arteries were cannulated, and the brains were perfused with a modified Krebs-Henseleit buffer (28°C), pH 7.35, containing washed bovine erythrocytes and 8% bovine serum albumin. The preparation has been characterized [19] according to the electrophysiological, morphological, and biochemical criteria suggested by Woods *et al.* [26]. In addition, the IPMB has been shown to be a useful model for determining endogenous MHPG formation [19]. Evidence that various regions of the IPMB are adequately perfused has been provided by recent glucose utilization studies performed with ^{14}C-2-deoxy-D-glucose in the perfusion medium [21].

Bipolar cortical EEG recordings were made with a Grass Model 795B AC and a Model 7DAF driver amplifier with a 0.1-sec time constant and a 75-Hz half amplitude cutoff. A Grass Model 7P10 summating integrator was used to provide a running record of "total accumulated area under the curve" of input voltage plotted against time. Amplitude of the electrical spikes was readily observed, and total electrical input (frequency × amplitude) was obtained for each perfusion. Control electrocortical activity, in absence of nicotine, was compared with activity in the presence of nicotine at various times and doses. Perfusion fluid was collected for catecholamine metabolite (MHPG and HVA) extraction and determination.

Extraction of catecholamines from brain tissue. At the termination of the perfusion (60 min), brains were rapidly cooled in ice-cold 0.9% saline for 3 min. They were then dissected on ice according to Glowinski and Iversen [6] and regions were frozen on dry ice, weighed, and stored at −80°C until extractions could be performed.

The extractions were adapted from the method of Felice *et al.* [6]. One ml 0.05 M $HClO_4$ and 0.1 ml of the internal standard solution containing 500 pmoles of DBA were added to the brain part. These amounts were the same for all of the brain regions except the cerebral cortex, in which case all amounts were doubled. The resulting mixture was homogenized and then centrifuged at 12,000 g for 10 min. The supernatant was transferred to a screw-cap tube containing 50 mg acid washed alumina and 0.5 ml 3 M Tris buffer, pH 8.6. The tube was capped and shaken for 15 min on a reciprocal shaker. The supernatant was then aspirated and the alumina was washed once with Tris buffer, pH 8.6 (6 mM), and twice with distilled water. After the third wash, care was taken to aspirate all water from the tube, and 0.25 ml of 0.05 M $HClO_4$ was added to the alumina. After thorough mixing for several minutes, the mixture was filtered and injected onto the HPLC system. Percent recovery of catecholamines from brain tissue was typically 70%.

Chromatography. A model LC-4 electrochemical detector (Bioanalytical Systems) with a carbon paste (CP-S) thin-layer transducer cell (TL-4) was equipped with a 30 cm×3.9 mm i.d. reverse phase C_{18} column (Waters Associates, Milford, MA). Samples were injected into a 20-μl loop on a 7125 Rheodyne valve. The mobile phase was composed of three parts 0.1 M citric acid, two parts 0.1 M Na_2HPO_4, and made 0.3 mM in sodium octyl sulfate. The detector potential was +0.72 V vs. an Ag|AgCl reference electrode and the flow rate was 1 ml/min.

Extraction of MHPG and HVA from perfusion fluid. Following removal of erythrocytes by centrifugation, MHPG was extracted from the fluid by shaking 10-ml samples for 10-min intervals with three 10-ml additions of ethyl acetate. Ethyl acetate extracts were maintained at 5°C until they were rotoevaporated to dryness [20]. The residues were then dissolved in methanol, dried, dissolved in mobile phase, and filtered.

For HVA extraction ethyl ether (10 ml) was added to the remaining serum "perfusion fluid" from the MHPG extraction. The mixture was shaken for 10 min, the ether decanted, and nitrogen bubbled through the serum to evaporate the remaining ether. The pH of the fluid was then adjusted to 1.5 to 2.0 with 4 N HCl. HVA was extracted by shaking with ethyl ether (20 ml) for 15 min. The ethyl ether extracts were evaporated to dryness with nitrogen and the residue was dissolved in 1 ml distilled water and filtered. Appropriate serum blanks and standards (serum + MHPG and HVA) were analyzed with each set of samples.

Chromatography. A model (LC-4) electrochemical detector (Bioanalytical Systems) with a carbon paste (CP-S) thin-layer transducer cell (TL-4) was used. The MHPG and HVA samples were injected into a 100-μl loop on a 7125 Rheodyne injection valve. The mobile phase was 0.01 M KH_2PO_4 (pH 6.0) and the flow rate was 1 ml/min. The detector potential was +0.8 V vs. an Ag|AgCl reference electrode.

Because of extraneous components that eluted from the analytical column 60 to 75 min after the MHPG and HVA injection, a column switching technique was used to eliminate these late eluting components. An HPLC RP-2 cartridge was precalibrated for the retention times of MHPG and HVA. During the interval in which these compounds eluted, a "window valve" [20] was opened allowing them to flow onto the chromosorb LC-7 analytical column. The "window valve" was then closed and the extraneous components were eluted from the HPLC column into the waste container. The retention times for HVA and MHPG were 11 and 19 min and the extraction yielded recoveries of 45% and 40%, respectively. Calibration curves, analyzed by least-squares

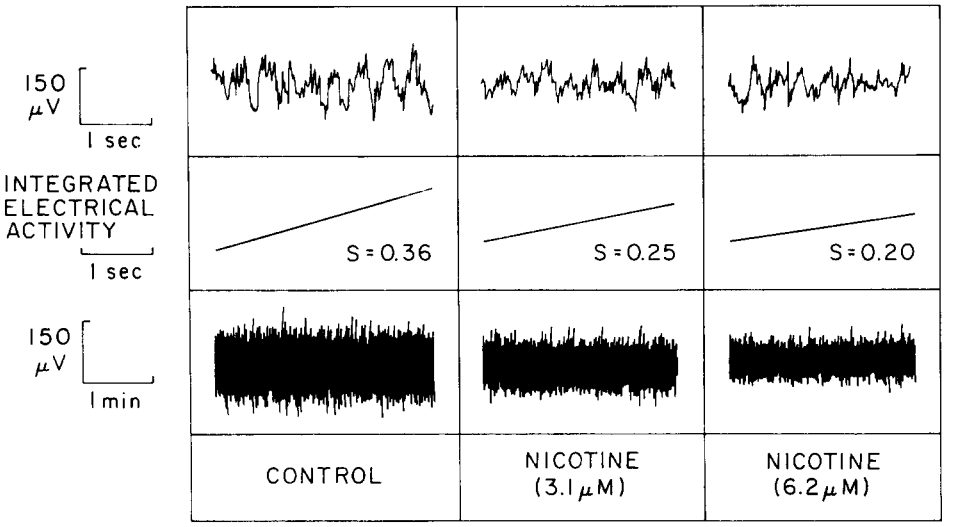

FIG. 1. Effects of nicotine on electrocortical activity in C3H mice. Electrocortical (ECoG) activities of IPMBs from C3H mice were measured as described in the text. The ECoG tracings represent typical patterns obtained at two chart speeds and show the effects of nicotine at 3.1 and 6.2 μM concentrations in the perfusion fluid. The integrated ECoG (frequency $\times$ amplitude) as a function of time is represented as a slope with values (S) as shown.

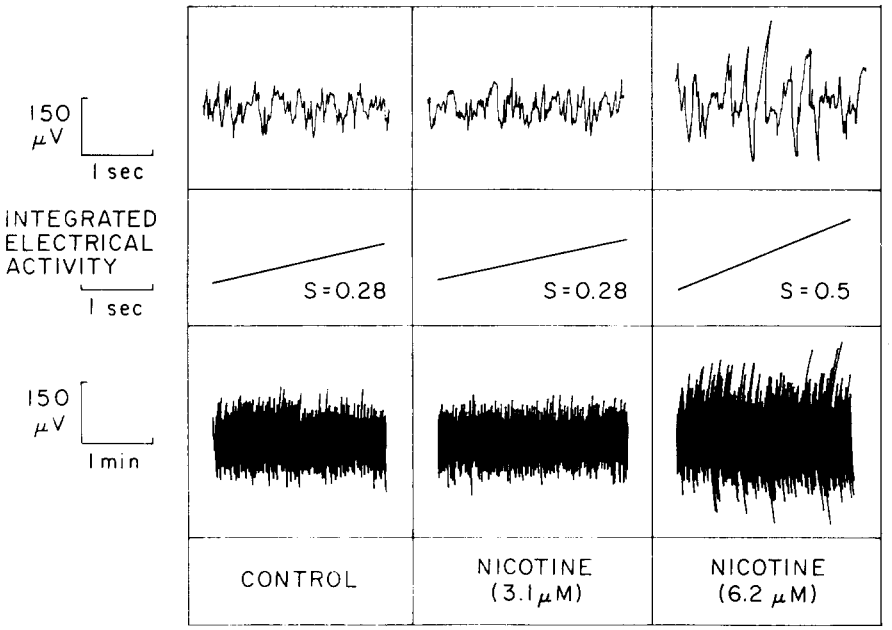

FIG. 2. Effects of nicotine on electrocortical activity in C57BL mice. Electrocortical (ECoG) activities of IPMBs from C57BL mice were measured as described in the text. The ECoG tracings represent typical patterns obtained at two chart speeds and show the effects of nicotine at 3.1 and 6.2 μM concentrations in the perfusion fluid. The integrated ECoG (frequency $\times$ amplitude) as a function of time is represented as a slope with values (S) as shown.

linear regression, for HVA and MHPG were run with 20 samples ranging from 10.0 to 850 pmoles; the correlation coefficients for these assays were 0.94 and 0.80, respectively.

Statistical analysis. In experiments comparing the effects of nicotine on catecholamine levels in various brain regions, a two-factor ANOVA followed by a Duncan posthoc test was employed for statistical significance. For analysis of some results, significance was determined by use of the non-parametric Mann-Whitney U test. This test was used because of large differences in estimated population variances and a small number of subjects (n). Values are expressed as mean$\pm$S.E.M. Values of $p<0.05$ were considered statistically significant.

RESULTS

The isolated perfused mouse brain (IPMB) was used to

TABLE 1

MHPG AND HVA PRODUCTION RATES FROM BRAINS OF C3H AND C57BL MICE

Strain	Metabolite (MHPG or HVA)	Sample Period (minutes)				Mean† Rate	Difference %	p‡ Values
		0–15	15–30	30–45	45–60			
C3H	MHPG	6.17 ± 0.5*	5.53 ± 0.4	4.98 ± 0.7	5.58 ± 0.6	5.52 ± 0.3	—	
		n=3	n=4	n=3	n=4	n=15		
C57BL	MHPG	10.25 ± 3.2	12.26 ± 4.8	8.05 ± 1.3	14.17 ± 4.6	11.70 ± 1.97	+112	$p<0.05$
		n=2	n=5	n=2	n=3	n=12		U(12,15)=0
C3H	HVA	16.70 ± 4.2	26.33 ± 9.8	33.48 ± 12.0	13.30 ± 3.8	22.45 ± 3.9	—	
		n=4	n=4	n=4	n=4	n=16		
C57BL	HVA	28.03 ± 10.3	24.66 ± 3.5	45.15 ± 28.1	61.77 ± 14.5	37.15 ± 6.0	+65	$p<0.05$
		n=3	n=5	n=2	n=3	n=13		U(13,16)=53

Brains from C3H and C57BL mice were perfused as described in the text with no nicotine in the perfusion fluid. At the 15-min time intervals shown, samples of perfusion medium were extracted and assayed for 3-methoxy-4-hydroxyphenethyleneglycol (MHPG) and 3-methoxy-4-hydroxyphenylacetic acid (Homovanillic acid, HVA) as described in the text.

*Values represent mean ± standard error of the mean (S.E.M.) and are expressed as pmoles/min/g of brain; n represents number of values in each computed mean. No significant time effects were found as determined by a mixed design two-factor ANOVA.

†Mean rate represents the computed mean production rate of all values obtained from 0–60 min.

‡Significance for differences in mean rates was determined by use of the nonparametric Mann-Whitney U test. A nonparametric test was used because of the large differences in estimated population variances and the small n. The small n is unavoidable due to the expense and time required to perform experiments with the isolated perfused brain.

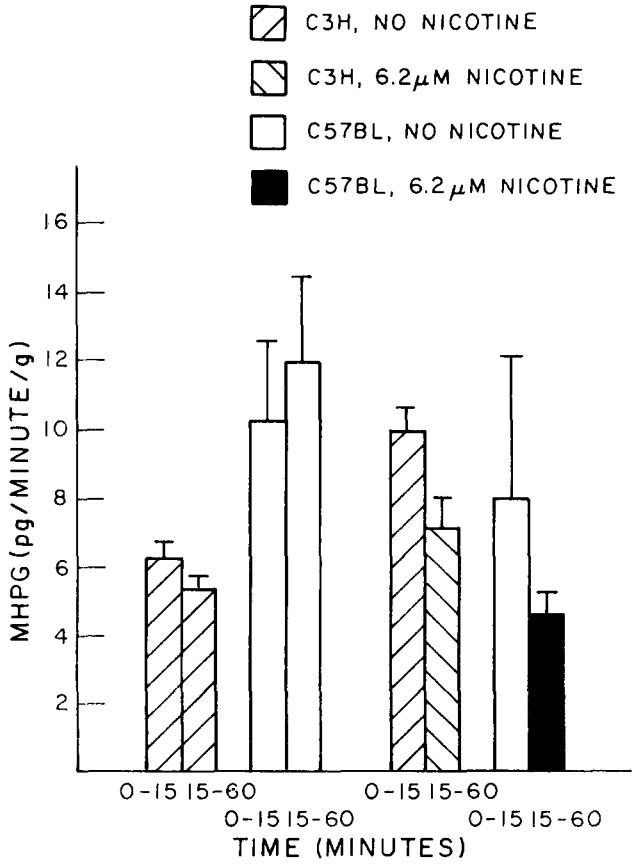

FIG. 3. Effect of 6.2 μM nicotine on MHPG production in C3H and C57BL perfused brains. Conditions and procedures are as described in Table 1 and in the text. Values represent mean rates (0–15 min or 15–60 min) ±S.E.M. as indicated. Nicotine did not significantly ($p<0.05$) alter MHPG production.

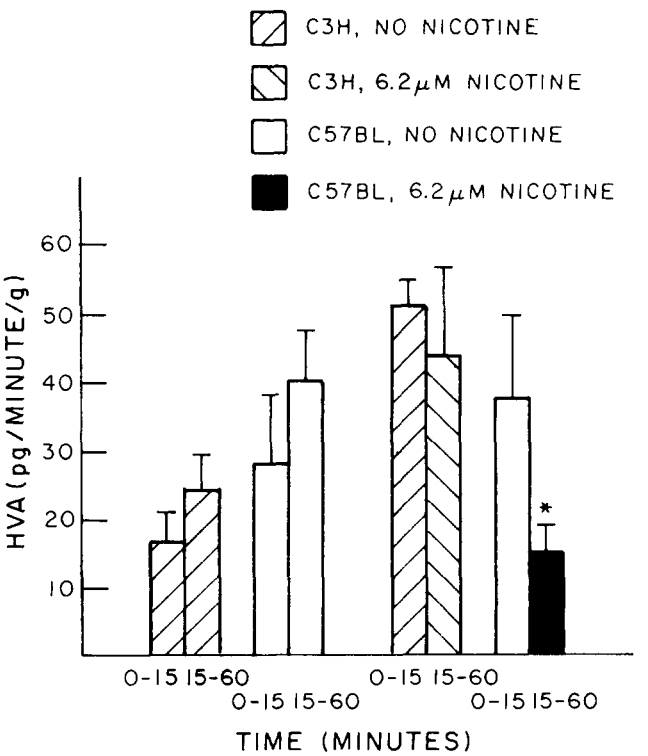

FIG. 4. Effect of 6.2 μM nicotine on HVA production in C3H and C57BL perfused brains. Perfusions were performed as described in Fig. 3 and Table 1. *Mean value is significantly different from control value, $p<0.05$, U(3,10)=3, Mann-Whitney U test.

TABLE 2

BRAIN CATECHOLAMINE LEVELS IN REGIONS OF PERFUSED C3H AND C57BL BRAINS

Mouse Strain	Amine*	Nicotine (6.2 μM)	Brain Region† Cereb	Ctx	Hypo	Med Pons	Midbr	Striat	Whole Brain
C3H	NE	0	1.77	1.23	5.41	3.81	2.56	0.08	1.77
C3H	NE	+	1.12	1.50	5.14	2.84	2.26	0.83	1.81
C3H	DA	0	0.07	3.89	4.66	0.81	1.41	77.59	7.65
C3H	DA	+	0.84	4.35	5.25	0.86	1.67	53.72	6.88
C57	NE	0	1.07 (+80%)‡ $p<0.05$	1.79	3.88	2.10	1.91	0.81	1.65
C57	NE	+	1.93 n=3	2.08 n=3	4.70 n=3	4.81 n=3	3.53	1.56	2.73
C57	DA	0	1.17	3.03	3.40	0.59	1.21	31.79	4.27
C57	DA	+	0.65 n=3	4.79 n=3	3.56 n=3	0.71 n=3	1.55	53.30	7.37

Brains from perfusions described in the text were rapidly chilled in ice-cold saline at the termination of the 60-min perfusion. Subsequently, brains were dissected and norepinephrine and dopamine were extracted and assayed as described in the text.

*Amines are designated norepinephrine (NE) and dopamine (DA).

†Brain regions are as indicated: cerebellum (cereb); cerebrum (ctx); hypothalamus (hypo); medulla pons (med pons); midbrain (midbr); striatum including globus pallidus (striat), as per Glowinski and Iversen [8].

‡Value in parentheses indicates significant difference (two-factor ANOVA followed by Duncan posthoc test) in mean levels of amine expressed as nmoles/g brain tissue. n=4 unless otherwise indicated.

determine the effects of nicotine on electrocortical (ECoG) activity in C3H and C57BL mice. At concentrations of 3.1 μM and 6.2 μM in the perfusion fluid, nicotine depressed the amplitude but not the frequency of the ECoG recordings from bipolar electrodes placed on the surface of the cortex in C3H mice (Fig. 1). The total integrated electrical activity was decreased. Nicotine at 3.1 μM concentration did not alter ECoG activity from similar electrodes placed on the cortex of C57BL mouse brains (Fig. 2). At a higher concentration of nicotine (6.2 μM) the ECoG amplitude from the isolated perfused C57BL mouse brain was enhanced. Typical seizure patterns were observed at a concentration of 12.4 μM (amplitude of spikes increased from ca. 100 μvolts to greater than 300 μvolts). In IPMBs from C57BL mice nicotine (6.2 μM) often produced an initial transitory decrease in the ECoG followed by the increased activity.

The data presented in Table 1 show the MHPG and HVA production rates in C3H and C57BL isolated perfused brains during the course of a 60-min perfusion with no nicotine present. These control perfusions were performed to determine if there were any significant increases or decreases in MHPG and HVA production over time and to evaluate inbred strain differences. Although there was a great deal of variability in values between sample periods, there were no statistically significant time effects, as determined by a mixed design two-factor ANOVA. The mixed design was used because of the "within-subjects" and "between-subjects" factors within the experimental design [10]. Comparison of the mean production rates of the two strains shows significant differences in basal levels with the C57BL IPMBs having 112% greater MHPG production rates, $U(12,15)=0$, $p<0.01$, than C3H IPMBs and 65% greater HVA production rates, $U(13,16)=53$, $p<0.05$. Significance was determined by the nonparametric Mann-Whitney U test due to large differences in estimated population variances. It is important to note that these rates of MHPG and HVA production in C57BL perfused brains are virtually identical to *in vivo* values reported for norepinephrine and dopamine turnover in the C57BL mouse strain [9].

Figure 3 is a representation of the effect of nicotine on the mean production rate of MHPG in C3H and C57BL IPMBs. Due to the variability of production rates from perfusion to perfusion, it was determined, after 10 min of perfusion, to allow the effects of the urethane anesthesia to wash out, that a 0- to 15-min control production rate for each brain should be compared with a mean production rate from the subsequent 15- to 60-min period during which the isolated perfused brain either remained untreated or received 6.2 μM nicotine in the perfusion fluid. Unfortunately, the variability of both MHPG and HVA production rates is quite large, undoubtedly reflecting the variability of the extraction and assay procedure coupled with that of the IPMB procedure where subtle differences in perfusion pressure and flow rates exist. However, no obvious association was found between MHPG or HVA production rates and either perfusion pressure or flow rate. Each animal was used as its own control in an attempt to remedy this situation. Comparison of the mean production rates of the 0- to 15-min control periods to those of the subsequent 15- to 60-min periods in the untreated C3H and C57BL mice reveals that there are no significant changes in the production of MHPG in these two strains of mice.

When 6.2 μM nicotine was added to the perfusion fluid at 15 min, there was an apparent 28% decrease in MHPG production in C3H brains; however, the decrease was not significant. A larger apparent decrease (41%) in C57BL MHPG was also not significant, $U(3,11)=9$, using a Mann-Whitney U test. The latter test was used due to large differences in the estimated population variance.

The effect of a 6.2 μM concentration of nicotine on HVA production in C3H and C57BL IPMBs was examined in the

same manner. Figure 4 shows that HVA production in the untreated IPMBs did not change significantly over time. However, when 6.2 μM nicotine was added to the perfusion reservoir at 15 min, it had no significant effect on HVA production in C3H brains while the HVA production rate in C57BL brains decreased 60%, U(3,10)=3, $p<0.05$, Mann-Whitney U test.

Since MHPG and HVA production rates are probably reflections of noradrenergic and dopaminergic neuronal activity, we determined the effects of nicotine on norepinephrine and dopamine levels in various regions of brains from C3H and C57BL mice.

Results presented in Table 2 show that perfusion of C3H brains with medium containing 6.2 μM nicotine did not alter regional endogenous norepinephrine levels. Likewise, endogenous dopamine levels were not significantly altered. However, levels of norepinephrine in brains of C57BL mice were increased by perfusion with medium containing nicotine. The data show that nicotine increased norepinephrine levels in cerebellum by 80%. In perfused C57BL brains dopamine levels were higher in whole brain when perfused with medium containing 6.2 μM nicotine.

DISCUSSION

Brains from C3H and C57BL/6J mice showed differential responses to the effect of nicotine; ECoG activity was desynchronized or displayed seizures in brains from C3H or C57/BL mice, respectively. These differences in ECoG response in perfused mouse brains are of interest because it has been shown that these mice differ in their behavioral response (locomotor activity and hypothermia) due to administration of comparable doses of nicotine *in vivo* [14]. For example, at doses of 0.75 to 1.5 mg/kg, C3H mice show a marked elevation in locomotor activity and display excitatory behavior, whereas these doses of nicotine elicit a depressed locomotor activity and behavior in C57BL mice. The effects of nicotine on ECoG activity in IPMB from C3H mice are comparable to those observed by Ryan *et al.* [17] who demonstrated that nicotine, IP, depressed the amplitude of the EEG in LS/Ibg but not in SS/Ibg mice. Although nicotine depressed the amplitude, the frequency of the ECoG in C3H perfused brains did not change. This was probably due to the induction of hippocampal theta activity by nicotine. This effect of nicotine has been previously observed by Yamamoto *et al.* [27]. Posterior placement of electrodes on the cortex adequately records hippocampal rhythmic activity in mice due to volume conduction through the layer of neocortex that overlies the hippocampus [24]. It is of interest to note that nicotine (6.2 μM) in IPMB from C57BL mice produced effects similar to those reported by Armitage *et al.* [4] for cat brains, i.e., the ECoG exhibited high voltage spiking, suggesting pre-seizure activity. Longo *et al.* [12] reported that small doses of nicotine (0.02 to 0.05 mg/kg) resulted in desynchronization of the EEG in pre-pontine brain-stem transected rabbit, and larger doses (1 to 2 mg/kg) provoked seizure followed by electrical silence.

Results from many studies suggest that noradrenergic and dopaminergic pathways are involved in numerous animal behaviors including locomotor activity and thermoregulation. These behaviors are altered by agents which affect the metabolism of norepinephrine or dopamine [18,22]. Therefore, we determined associated effects of nicotine at 6.2 μM concentrations on norepinephrine and dopamine turnover in brains from C3H and C57BL mice. Previous studies [19] have demonstrated that the MHPG production rates in the IPMB were direct measures of the turnover rates of norepinephrine. Likewise, results reported in the present study suggest that HVA production rates in the IPMB are indications of dopamine turnover. As shown in Table 1, C3H and C57BL mice brains differed significantly in basal rates of MHPG and HVA production which parallel the differences in baseline locomotor activity reported by Marks *et al.* [14]. C57BL mice have a significantly higher baseline locomotor activity, 194±27, than do C3H mice, 131±34. Another study by Morrison *et al.* [16] using inbred rat strains noted that strains with high control activity (Lister and Sprague-Dawley) tended to exhibit reduced activity after nicotine, whereas low-activity (Wistar) rats showed increased activity after nicotine administration. This was the case with C57BL and C3H mice when tested for open-field activity following nicotine.

The ratio of HVA to MHPG production in C57BL perfused brains remained virtually unchanged following 6.2 μM nicotine, 3.3:1 vs. 3.2:1. However, there was a significant 60% reduction of HVA production and an apparent 41% reduction of MHPG production which could indicate reduced dopaminergic and noradrenergic neuronal activity. It is known that drugs which cause depletion of norepinephrine suppress motor activity [23]. The importance of the effects of dopamine depletion on motor activity is also well documented. This overall reduction of metabolite production of both norepinephrine and dopamine, which indicates reduced noradrenergic and dopaminergic neuronal activity, might account for the marked reduction of motor activity following nicotine in C57BL mice.

The reduction of MHPG production in C57BL perfused brains was accompanied by an 80% increase in cerebellar norepinephrine, indicating a major nicotine effect in this brain region. Decreased HVA production in C57BL perfused brains was accompanied by a 73% increase in whole brain dopamine, with the largest apparent increases occurring in the cortex and striatum. There were no significant changes in brain content of norepinephrine or dopamine in the C3H perfused brains. The apparent decrease in MHPG production (−28%) was somewhat less than that observed in C57BL perfused brains (−41%). These observations utilizing the IPMB are of interest in that previous investigations [2,7] have demonstrated effects of repeated doses of nicotine on catecholamine turnover *in vivo*. For example, Fuxe and coworkers found that using repeated injections of nicotine (1 to 2 mg/kg) produces a reduction in catecholamine stores only in the median eminence of the hypothalamus. In the present studies nicotine-induced changes in a discrete area of the brain would not be detectable. Also, recent studies in this laboratory [15] have demonstrated tachyphylaxis to nicotine-induced changes in the ECoG and nicotine-induced secretion of αMSH-ir and β-endorphin-ir in perfused C3H mouse brains. It is possible that C3H perfused brains exhibit a rapid tachyphylaxis to the activating effects of nicotine when exposed to steady-state nicotine, which would be difficult to detect with the experimental design utilized in the present studies. Conversely, C57BL brains, when perfused with steady-state nicotine, showed a marked reduction in HVA production during the 45 min of nicotine perfusion, which would indicate that tachyphylaxis to the depressant effects of nicotine was not present.

In summary, nicotine produced what appeared to be a concentration-dependent decrease in the ECoG input voltage of C3H mice which was associated with an apparent

decrease in MHPG production but with no change in HVA production. There were no changes in the norepinephrine and dopamine content of various brain regions. These ECoG and neurochemical changes may be associated with the known enhanced behavioral effect of nicotine in C3H mice *in vivo*.

In C57BL mice perfusion with nicotine produced a dose-dependent enhancement of the ECoG amplitude culminating in high-frequency, high-amplitude seizure activity, and these electrocortical effects were accompanied by a nicotine-induced decrease in HVA production rates and an apparent decrease in MHPG production. These changes were likewise associated with alterations in levels of catecholamines in various brain regions. For example, perfused C57BL brain norepinephrine and dopamine levels were increased. These increases in amine levels are consistent with an inhibition of turnover of norepinephrine and dopamine as reflected in the decreased MPHG and HVA production rates. The ECoG activities and effects on catecholamines may be associated with the known decreased behavioral effects (locomotor activity) of nicotine in C57BL mice, *in vivo*. While these experiments show nicotine-induced changes in catecholamine metabolism and in ECoG input voltage, any causal relationship between these effects remains to be elucidated.

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