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Relations Between Nicotine-Induced Convulsive Behavior and Blood and Brain Levels of Nicotine as a Function of Sex and Age in Two Inbred Strains of Mice

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TEPPER, J. M., J. R. WILSON AND K. SCHLESINGER. *Relations between nicotine-induced convulsive behavior and blood and brain levels of nicotine as a function of sex and age in two inbred strains of mice.* PHARMAC. BIOCHEM. BEHAV. **10**(3) 349–353, 1979.—Nicotine levels in blood and whole brain were measured as a function of sex and age in C57BL/6J and DBA/2J mice and compared to the behavioral responses following an intraperitoneal injection of nicotine. The results indicate that blood levels of nicotine alone do not accurately predict either brain levels of nicotine or the behavioral responses to a single injection of nicotine. In general, brain levels of nicotine proved to be a fairly accurate predictor of the behavioral responses to nicotine. The data indicate that the sexes differ in their sensitivity to nicotine. Forty-two-day-old male mice of both strains given comparable doses of nicotine were found to concentrate the drug in the brain more than females. However, there was no corresponding increase in sensitivity to this increased brain concentration as measured by LD50, ED50, latency to tremor or latency to death.

Nicotine Convulsions Blood level LD50 ED50 Brain level

THE IDENTIFICATION of the sources of individual differences in the response of animals to nicotine has been approached by several investigators in many different ways. Age-dependent changes in nicotine-induced convulsions and death have been previously reported [7,8]. Hatchell *et al.* have investigated the development of behavioral and pharmacological tolerance to multiple doses of nicotine and found strain and sex differences [1], and Masner has reported a correlation between nicotine-induced locomotor activity, analgesia, and whole brain levels of nicotine in male mice of the NMRI strain [5].

The purpose of this study was to investigate the relation between levels of nicotine found in the blood and in whole brain following acute intraperitoneal administration with certain behavioral effects of the drug. Specifically: (1) LD50 and ED50 values were derived for each genotype, sex and age of mice used in these experiments, and these were compared to nicotine levels found in blood and brain for the purpose of determining whether the behavioral effects of nicotine were related to levels of nicotine found in these tissues. (2) Blood levels of nicotine were compared to levels of the drug in brain to determine if blood levels predicted brain levels with sufficient accuracy to allow monitoring of brain nicotine levels by taking small blood samples for analysis, thereby leaving the animal alive and intact for long-term studies of the behavioral and pharmacological effects of nicotine.

Animals

C57BL/6J (C57) and DBA/2J (DBA) mice, bred in our laboratories from stock obtained from The Jackson Memorial Laboratories, Bar Harbor, Maine, were used in these experiments. The origin and degree of inbreeding of these mice has been described previously [3]. All mice were weaned at 21 days of age and housed together with littermates of the same sex (2–6 animals per cage) until used in an experiment. Animals were maintained with ad lib access to Purina Mouse Breeder Chow and tap water, under standard laboratory conditions of temperature (74 ± 3°F) and controlled lighting (12 hr light cycle, 7:00 a.m.–7:00 p.m.). All mice were tested at either 21 or 42 days of age, between 11:00 a.m. and 3:00 p.m. Approximately equal numbers of males and females were used, and no mice were used more than once in any experiment.

Materials

N-methyl-^{14}C nicotine-d-bitartrate (47 mCi/mmol) was obtained from the Amersham-Searle Corporation. Nico-

tine(+)hydrogen tartrate was obtained from the Gallard-Schlesinger Chemical Manufacturing Corporation. All other chemicals used were of reagent grade.

Drug Preparation

For the determination of LD50 and ED50 values, nicotine(+)hydrogen tartrate was dissolved in 0.9% saline in 2.0% phosphate buffer. The pH was adjusted by the dropwise addition of 1.0 N NaOH. All injections were administered intraperitoneally (IP) and given in a volume corresponding to 0.01 ml per gram body weight. For the blood and brain nicotine determinations, N-methyl-^{14}C nicotine-d-bitartrate was diluted with nicotine(+)hydrogen tartrate. The specific activity of all labelled injections was 1.0 μCi/ml. All doses are expressed as the free base equivalent of nicotine.

METHOD

Behavioral Testing

In order to determine the ED50, i.e., that dose inducing tremor, 159 21-day-old DBA and C57 mice received IP injections of either 0.75, 1.0, 1.25, 1.5, 1.75 or 2.0 mg/kg nicotine. In order to determine the ED50 for 42-day-old mice, 140 DBA and C57 mice received IP injections of either 2.0, 3.0, 3.25, 3.5, 4.0 or 4.5 mg/kg. For determination of the LD50, 140 21-day-old DBA and C57 mice received an IP injection of either 1.0, 1.5, 1.75, 2.0, 3.0, 4.0 or 4.5 mg/kg nicotine, whereas 210 42-day-old C57 and DBA mice received an IP injection of either 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0 or 13.0 mg/kg. Mice were observed for five minutes post-injection, and records kept as to the incidence of tremor, wild running, clonic, tonic, and lethal seizures. In addition, post-injection latencies to the onset of tremor and the onset of death were recorded.

Blood and Brain Nicotine Determination

One hundred sixteen 21-day-old C57 and DBA mice of both sexes received an acute IP injection of ^{14}C-labelled nicotine of either 0.5, 1.0, 1.5, 1.75, or 2.0 mg/kg. One hundred eighty-three 42-day-old mice of both strains and sexes received 2.0, 3.0, 4.0, 5.0, or 6.0 mg/kg. Mice were sacrificed by decapitation 5 min post-injection and nicotine levels in blood and whole brain were determined by a modification of the procedure described by Hucker *et al.* [2]. Blood was collected in a 25 ml beaker containing 2.0 ml cold saline and 0.1 ml heparin (250 units/ml) and weighed. Brains were removed, washed free of any blood and blotted dry, weighed, and homogenized in 5 volumes cold saline. Samples were then centrifuged at 10,800 × g for 10 minutes, and the supernatant decanted into 25 ml culture tubes containing 7.5 ml washed heptane (washed in equal volumes of 1.0 N HCl, 1.0 N NaOH, and distilled, deionized water three times) containing 1.5% (v/v) isoamyl alcohol, and 1.0 ml 0.9 N NaOH. Samples were shaken at 2°C for 20 min and centrifuged at 120 × g for 5 min. A 5.0 ml aliquot of the organic phase was removed and transferred to another culture tube containing 2.5 ml 0.1 N HCl. The extraction was repeated twice more, and the three pooled heptane aliquots were shaken in the 2.5 ml HCl for 5 min at 2°C and centrifuged at 120 × g for 5 min. The organic phase was removed and the remaining contents of the tube were transferred to a scintillation vial containing 15 ml Triton-X-100 cocktail (5.3 g PPO, 450 ml Triton-X-100 and 500 ml toluene) and counted for 20 min in a Beckman LS-250 liquid scintillation counter. Internal standards were run every three days and these were used to calculate μg nicotine/g tissue from raw DPM/g tissue. The recovery from blood and brain samples to which known amounts of nicotine had been added was between 80% and 85% and quite replicable. All recovered values of nicotine are expressed in terms of the free base.

Data Analysis

Separate analyses of variance for blood and brain levels of nicotine, the brain/blood ratio, and the latency measures were computed by strain, sex and age, and a multiple regression of brain nicotine levels by blood nicotine levels, strain, sex, age, and dose was performed. LD50 and ED50 values were computed and compared, along with 95% confidence limits by the method of Litchfield and Wilcoxon [4].

RESULTS

Behavioral Measures

Genotype effects. The effects of genotype, sex and age on nicotine-induced convulsions and death are summarized in Tables 1 and 2. At 21 days of age there were no significant differences between C57 and DBA mice in LD50, ED50, latency to tremor and latency to death. At 42 days of age, however, C57 mice showed a significantly shorter latency to tremor than DBA mice, $F(1/140)=9.344$, $p<0.033$. C57 mice also showed a significantly shorter latency to death at this age, $F(1/95)=32.535$, $p<0.001$. In addition, C57 mice showed a significantly lower LD50 ($p<0.05$) (Table 1) and a significantly smaller ED50 ($p<0.05$) (Table 2) than DBA mice at this age.

Sex effects. Twenty-one-day-old DBA females showed a significantly shorter latency to death than did males of this strain, $F(1/41)=6.713$, $p<0.015$. No other significant behavioral sex differences were found in 21-day-old C57's nor in either strain at 42 days of age.

Age effects. The only doses common to 21- and 42-day-old animals were of 3.0, 4.0, and 5.0 mg/kg nicotine. At these doses 21-day-old mice were found to have significantly shorter latencies to tremor, $F(1/82)=34.440$, $p<0.001$, and death, $F(1/55)=24.172$, $p<0.001$, than the 42-day-old mice. In addition, the LD50 and ED50 were significantly lower for the 21-day-old mice at the $p<0.05$ level.

Biochemical Measures

Genotype effects. The effects of genotype, sex and age on nicotine levels in blood and brain are summarized in Tables 3 and 4. In 21-day-old mice, there was no significant strain difference in brain levels of nicotine. There was, however, a significant difference in the mean blood levels of nicotine with the DBA mice having a greater concentration than the C57 mice, $F(1/115)=16.872$, $p<0.001$. In addition, there was a significant difference in the brain/blood ratio at this age with the higher ratio found in the C57 mice, $F(1/115)=36.638$, $p<0.001$. At 42 days of age there was no significant strain difference in mean blood levels of nicotine but there was a significant difference in mean brain levels of nicotine where the greater concentration was found in the C57 mice, $F(1/127)=28.083$, $p<0.001$. The brain/blood ratios also differed significantly at this age between strains with the C57 mice having the greater ratio, $F(1/127)=17.237$, $p<0.001$.

Sex effects. At 21 days of age there were no significant

TABLE 1

LD50

Strain	Age	Sex	LD50 (mg/kg)*†	Latency to Death in Seconds‡
DBA/2J	21	F	2.30 (1.71–3.09)	72.17
DBA/2J	21	M	1.70 (1.39–2.08)	96.13
DBA/2J	42	F	10.00 (6.93–14.44)	140.21
DBA/2J	42	M	8.00 (6.10–10.40)	137.21
C57BL/6J	21	F	1.70 (1.30–2.27)	79.48
C57BL/6J	21	M	2.33 (1.80–3.00)	83.19
C57BL/6J	42	F	6.20 (5.10–7.54)	82.50
C57BL/6J	42	M	7.20 (6.27–8.26)	85.86

*Numbers in parentheses indicate 95% confidence limits.
†LD50 values are expressed as mg/kg of the free base equivalent.
‡Latencies are reported as mean latencies across the range of doses.

TABLE 2

ED50

Strain	Age	Sex	ED 50 (mg/kg)*†	Latency to Tremor in Seconds‡
DBA/2J	21	F	1.35 (1.14–1.59)	44.55
DBA/2J	21	M	1.35 (1.21–1.51)	44.50
DBA/2J	42	F	3.90 (3.51–4.34)	48.34
DBA/2J	42	M	3.95 (3.47–4.50)	48.28
C57BL/6J	21	F	1.35 (1.13–1.62)	58.53
C57BL/6J	21	M	1.20 (1.03–1.40)	60.13
C57BL/6J	42	F	3.15 (2.86–3.46)	39.87
C57BL/6J	42	M	3.40 (3.04–3.80)	41.72

*Numbers in parentheses indicate 95% confidence limits.
†ED50 values are expressed as mg/kg of the free base equivalent.
‡Latencies are reported as mean latencies across the range of doses.

TABLE 3

MEAN BRAIN AND BLOOD LEVELS OF NICOTINE IN μg/g TISSUE IN 21 DAY OLD MICE*

		Nicotine Dose				
Strain	Sex	0.5 mg/kg	1.0 mg/kg	1.5 mg/kg	1.75 mg/kg	2.0 mg/kg
C57	M	0.287 ± 0.063	0.637 ± 0.069	1.09 ± 0.185	1.18 ± 0.326	1.26 ± 0.387
		0.085 ± 0.040	0.260 ± 0.074	0.414 ± 0.069	0.386 ± 0.117	0.518 ± 0.292
C57	F	0.314 ± 0.100	0.762 ± 0.103	0.826 ± 0.244	1.35 ± 0.355	1.28 ± 0.259
		0.107 ± 0.047	0.258 ± 0.041	0.410 ± 0.267	0.489 ± 0.135	0.505 ± 0.244
C57	M+F	0.300 ± 0.081	0.694 ± 0.104	0.968 ± 0.246	1.27 ± 0.336	1.27 ± 0.306
		0.096 ± 0.043	0.259 ± 0.058	0.412 ± 0.179	0.437 ± 0.132	0.511 ± 0.253
DBA	M	0.957 ± 0.985	0.716 ± 0.187	1.46 ± 0.427	1.25 ± 0.381	1.25 ± 0.337
		0.443 ± 0.452	0.338 ± 0.137	0.682 ± 0.222	0.586 ± 0.237	0.601 ± 0.096
DBA	F	0.215 ± 0.045	0.523 ± 0.228	0.861 ± 0.141	1.10 ± 0.243	1.31 ± 0.176
		0.128 ± 0.028	0.274 ± 0.104	0.480 ± 0.093	0.560 ± 0.075	0.736 ± 0.112
DBA	M+F	0.586 ± 0.769	0.620 ± 0.223	1.16 ± 0.437	1.18 ± 0.315	1.28 ± 0.249
		0.207 ± 0.251	0.306 ± 0.121	0.581 ± 0.192	0.573 ± 0.168	0.674 ± 0.122

*Upper numbers denote μg nicotine/g brain (wet weight) ± standard error. Lower numbers denote μg nicotine/g blood ± standard error.

TABLE 4

MEAN BRAIN AND BLOOD LEVELS OF NICOTINE IN μg/g TISSUE IN 42 DAY OLD MICE*

Strain	Sex	Nicotine Dose 2.0 mg/kg	3.0 mg/kg	4.0 mg/kg	5.0 mg/kg	6.0 mg/kg
C57	M	1.41 ± 0.291	2.52 ± 0.464	3.15 ± 0.731	3.72 ± 0.589	5.15 ± 0.972
		0.586 ± 0.171	0.897 ± 0.256	1.08 ± 0.357	1.44 ± 0.376	2.18 ± 0.562
C57	F	0.996 ± 0.243	1.61 ± 0.046	2.99 ± 0.600	3.51 ± 0.911	4.34 ± 0.826
		0.326 ± 0.069	0.568 ± 0.267	1.00 ± 0.222	1.26 ± 0.473	1.60 ± 0.647
C57	M+F	1.18 ± 0.331	2.06 ± 0.647	3.07 ± 0.647	3.62 ± 0.745	4.71 ± 0.947
		0.444 ± 0.180	0.733 ± 0.303	1.04 ± 0.288	1.35 ± 0.422	1.86 ± 0.653
DBA	M	0.860 ± 0.354	2.15 ± 0.370	2.60 ± 0.351	3.47 ± 0.341	4.79 ± 0.916
		0.327 ± 0.167	0.889 ± 0.264	0.910 ± 0.284	1.36 ± 0.429	1.94 ± 0.539
DBA	F	0.605 ± 0.170	1.22 ± 0.429	1.68 ± 0.524	2.71 ± 0.878	3.32 ± 0.898
		0.386 ± 0.124	0.535 ± 0.138	0.772 ± 0.158	1.35 ± 0.389	1.78 ± 0.530
DBA	M+F	0.738 ± 0.296	1.72 ± 0.617	2.10 ± 0.643	3.06 ± 0.768	4.20 ± 1.15
		0.357 ± 0.143	0.726 ± 0.277	0.841 ± 0.229	1.35 ± 0.390	1.88 ± 0.522

*Upper numbers denote μg nicotine/g brain (wet weight) ± standard error. Lower numbers denote μg nicotine/g blood ± standard error.

sex differences within either strain in mean blood levels of nicotine. There was, however, a significantly greater mean concentration of nicotine in the brains of male DBA mice at this age compared to the female DBA mice, $F(1/56)=9.058$, $p<0.004$. Within 21-day-old DBA mice, the brain/blood ratio also differed significantly between the sexes with the males having the greater ratio, $F(1/56)=6.908$, $p<0.011$. The sexes did not differ significantly at this age in the C57 strain with regard to mean blood levels of nicotine, mean brain levels, or the brain/blood ratio.

At 42 days of age there was no significant sex difference within the DBA strain in regard to mean blood levels of nicotine, but male DBA mice showed a significantly greater concentration of nicotine in the brain than female DBA mice, $F(1/65)=38.842$, $p<0.001$. The brain/blood ratio was also found to be significantly greater in DBA males than DBA females at this age, $F(1/66)=17.471$, $p<0.001$. Within 42-day-old C57 mice, there were significant differences both in mean blood levels of nicotine, $F(1/61)=8.006$, $p<0.006$, and in mean brain levels of nicotine, $F(1/61)=8.117$, $p<0.006$, with the males having a greater concentration in each tissue than the females. There was no significant sex difference in the brain/blood ratio for 42-day-old C57 mice.

Age effects. The only dose common to 21- and 42-day-old mice was 2.0 mg/kg nicotine. Following this dose, the 21-day-old mice showed significantly greater mean concentrations of nicotine both in blood, $F(1/44)=14.488$, $p<0.001$, and in brain, $F(1/44)=14.975$, $p<0.001$, than 42-day-old mice. There was no significant difference in the brain/blood ratios between 21- and 42-day-old mice in either strain.

DISCUSSION

The data presented above indicate that both blood and brain levels of nicotine vary as a function of genotype, sex and age. Predicting brain levels of nicotine solely on the basis of blood levels of the drug accounts for only 15% of the variance ($r=0.387$). However, if the genotype of the animal, its sex, age and injected dose of nicotine are also considered, one can account for 80% of the variance in brain levels of nicotine ($r=0.894$).

Twenty-one-day-old DBA mice show a higher mean level of nicotine in blood than do 21-day-old C57 mice, but there is no corresponding difference in mean brain levels of the drug. Furthermore, there is no difference in the LD50 curves for the two strains at this age, nor in any of the other behavioral measures. By 42 days of age, the significant difference in blood levels between the two strains has vanished, but a significant difference in brain nicotine levels between strains appears. At this age, there is a significant difference in the LD50 curves for the two strains showing the C57 mice to be more sensitive to the effects of nicotine than the DBA mice. In addition to the lower LD50 at this age, the C57 mice have shorter latencies both to tremor and to death than the DBA mice. These data would seem to indicate that (1) blood levels of nicotine alone do not accurately predict either brain levels of nicotine, or any measure of the behavioral response to the drug, and (2) brain levels of nicotine do predict the behavioral response, at least in a general way. In other words, mice that exhibit higher brain concentrations of nicotine after an IP injection also suffer more frequent and more severe convulsions with a shorter latency than mice that exhibit lower concentrations of nicotine in the brain.

However, such an interpretation of the data is complicated by considering the sex differences in brain nicotine levels in 42-day-old mice of both genotypes. In animals of both strains, males were found to exhibit significantly higher mean concentrations of nicotine than females following identical doses. However, there is no corresponding difference in LD50, ED50, latency to tremor or latency to death.

The fact that neither the LD50 nor the ED50 measures differ might simply reflect the fact that these parameters are not very sensitive. However, latency measures have, in the past, been found to be fairly sensitive measures of the strength of a behavioral response. The greater nicotine concentration found in the brains of males of both strains is probably due to a slower metabolism of nicotine by male mice than by female mice [1]. One would expect this significant difference in brain nicotine levels, common to both strains at 42 days of age, to be reflected in behavioral differences. The fact that male mice of both strains have greater nicotine levels in brain without any observable behavioral differences in response to the drug seems to suggest a possible sex difference in the sensitivity to nicotine in mice of the strains studied. This difference in sensitivity might be due to differences in circulating levels of hormones, and we are repeating some of these experiments on mice that have been gonadectomized or treated with the opposite sex hormone.

Twenty-one-day-old mice of both strains showed significantly greater concentrations of nicotine both in blood and in brain than did 42-day-old mice following identical doses of the drug. In addition, these younger mice had significantly shorter latencies to tremor and death than the older mice, and significantly lower LD50 and ED50 values. These results are in agreement with those of Stalhandske [7] who found that the ability of the liver to detoxify nicotine increased from 12 days of age to 35 days of age, with a concurrent increase in LD50 values. All the above data point to the fact that in view of the tremendous differences in behavioral and biochemical responses seen as a function of genotype, sex and age, appropriate dose-response measures must be made for each particular strain, sex and age of mice used in any study of the behavioral or pharmacological effects of nicotine.

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