Short Communications

The effect of nicotine on blood pressure in the genetically hypertensive mouse[1]

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Summary. Mice genetically selected for high and low blood pressure were exposed to nicotine via a single injected dose or addition to drinking water for 52 weeks. In the acute study, the response of mice with high blood pressure was a statistically significant increase in blood pressure. In the chronic study the pattern of response to nicotine ingestion was similar for mice with high blood pressure and those with low. Both lines responded with an increase in blood pressure after 6 weeks followed by a decrease to below baseline blood pressure at 12 weeks.
Key words. Mice, genetically hypertensive; hypertension, mouse; blood pressure; nicotine.

Nicotine has been implicated as one of the substances in cigarette smoke related to cardiovascular disease and is known to elevate blood pressure and heart rate in man[2]. In rats, acute exposure to nicotine produces a transient increase in blood pressure immediately after exposure[3], probably brought about by the stimulation of the sympathetic ganglia resulting in a rise in catecholamine levels. After long term exposure, individuals exhibit resting blood pressure that is lower than those unexposed[4]. The mechanism for the chronic response is unknown. Studies by Wenzel et al.[5] and Roth et al.[6] suggested that the response to nicotine may be different for individuals who are hypertensive compared to normotensives.

A study of animal models of hypertension may provide information about the differential effects of nicotine on hypertensive, hypotensive, and normotensive individuals. One such model, the genetically hypertensive mouse (GMH[7]), was used in this study. Its response to nicotine was compared to that of normotensive and hypotensive mice.

Materials and methods. Mice. The mice used in this study were from the lines developed for high (HBP) or low blood pressure (LBP) through selective matings over a number of generations, and from a randomly bred control normotensive line (RBP)[8]. Many of the mice in the HBP strain have systolic blood pressure in excess of 140 mm Hg and are designated genetically hypertensive[9]. Males for the acute study were from generations 23 and 24 of the ongoing selection program. Males for the chronic study were from generation 24. The animals were all between 100 and 200 days old at the start of the experiments. The mean, standard error, and sample size for blood pressure of the males measured in generation 23 were: 133 ± 1.2 mm Hg (172) for the high line, 83 ± 1.3 (60) for the low line, and 108 ± 1.7 (39) for the random bred control line. Within each line, pairs of male siblings were used for the experiments. One animal of each pair was assigned to the treatment group, and its sibling was assigned to the control group. The assignments were made randomly.

Blood pressure. Systolic blood pressure was measured indirectly by occluding the flow of blood in the caudal artery and detecting the return of a pulse upon deflation of the occluding cuff (Narco BioSystems physiograph). Details are given in Schlager and Weibust[10].

Statistical analysis. The difference between the blood pressures of the treatment and control individuals of a sibling pair was statistically equal to zero ($p > 0.10$) for each line when the experiments were begun. Statistical analyses were then done on data recorded as treatment minus control blood pressures in mm Hg for each sibling pair. In the acute study a t-test for paired comparisons was used to determine the effects of nicotine treatment on blood pressure in each line. Model I 2-way analysis of variance for treatment differences[11] was used to determine if significant interactions existed between mouse line and nicotine treatment.

In the chronic study a model I 2-way analysis of variance was performed on treatment minus control values for each sibling pair classified by line and time. Comparisons of individual mean values were done by Tukey's w-procedure (Steel and Torrie[11], page 185) using the standard error from the analysis of variance; the standard error is shown on the figure.

Acute study. For the acute study, 11 sibling treatment and control pairs from the hypertensive line and 12 pairs from the hypotensive line were measured. The treated animal was injected with the two-pack-a-day equivalent (2.28 mg/kg)[12] dose of nicotine dissolved in physiological saline. The sibling control animal of each pair was injected with physiological saline only. Blood pressure was remeasured after injection.

Compared to sibling controls 20 min after the injection, the blood pressure of the nicotine treated mice increased by 17 ± 7.3 mm Hg ($n = 11$) in the HBP and by 5 ± 3.2 ($n = 12$) in the LBP. A t-test for paired comparisons showed that the increase in the HBP was statistically significant ($p < 0.05$) while that in the LBP was not ($p > 0.10$). Analysis of variance of differences between treatment and control litter mates also demonstrated a significant treatment effect overall, but there was no interaction between mouse line and treatment. Although there was a difference in the magnitude of the increase, statistically the 2 mouse lines reacted in a similar manner to the injection of nicotine. These results are similar to findings in humans where it was shown that smoking a cigarette causes an immediate increase in blood pressure[2].

Chronic study. The chronic study used 10 treatment and control littermate pairs from each of the 3 blood pressure lines. Blood pressure was measured, and the animals were housed individually with food and water or a solution of nicotine dissolved in water provided ad libitum. The nicotine solution provided a daily dose of 2.28 mg/kg based on an average weight of 27 g per mouse. Blood pressure was remeasured after 6, 12, 24, and 52 weeks of nicotine ingestion, and again 4 weeks after the cessation of nicotine ingestion.

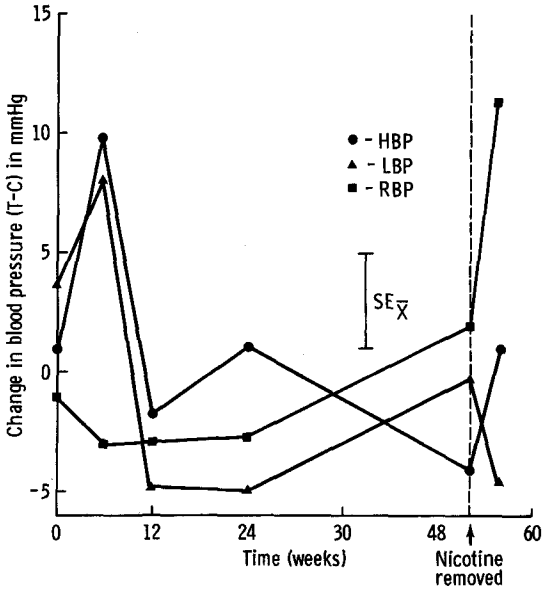
The figure presents the average differences in blood pressure between treatment and control sibling pairs over the 1-year course of exposure to nicotine. A year represents approximately 65% of the average lifespan in HBP and 40% in the LBP[9], therefore the mice in these lines were exposed to nicotine for a significant portion of their lives. Blood pressures in the nicotine-treated mice of both the HBP and LBP lines were higher than those of control litter mates at 6 weeks. At 12 and 24 weeks the LBP nicotine-treated mice had lower than control blood pressures, while the HBP differences were near zero. Statistical analysis showed that none of these comparisons was significant at $p = 0.05$. The HBP and LBP reacted more or less the same to nicotine treatment, but both may have a different reaction earlier on than the normotensive random-mated mice.

Discussion. It has been reported that chronic heavy smokers generally tend to have lower resting blood pressure than non-

smokers[13]. The difference was as much as 5 mm Hg for systolic blood pressure and up to 4 mm Hg for diastolic. These differences were statistically significant when standardized by age for a large number of people. Rats have shown similar trends when exposed to cigarette smoke daily for up to 28 months[3]. Systolic blood pressure in the treated rats was as much as 10 mm Hg lower than the controls; these differences were not statistically significant. In the same study, Haag et al.[3] demonstrated a statistically significant increase in blood pressure if the measurements were done immediately before and immediately after smoke exposure. In another short term study, Westfall[14] found a statistically significant increase of 14 mm Hg in systolic blood pressures of rats injected with nicotine for 8 weeks. Our findings in the mouse show trends similar to these earlier studies. The response to nicotine exposure was an increase in blood pressure as seen in both the acute study and the first part of the chronic study. In the chronic study, blood pressure dropped dramatically between 6 and 12 weeks, and remained close to the baseline level for the duration of nicotine exposure. When nicotine exposure was discontinued the HBP and RBP responded by showing more elevated blood pressures, while there was a small decrease in the LBP.

During the course of the experiment the nicotine concentration in the water bottles remained constant, based on a 27-g mouse. The LBP generally are somewhat smaller (10–25%) than the HBP and, consequently, received a larger dose/kg. The RBP are the largest animals being about 5% heavier than the HBP. The differences in weight remained constant during the chronic study and the small differences in dose/kg may be partially responsible for the differences in response seen in the figure. The HBP and LBP have been found to have large differences in norepinephrine content of the brain[7,15]. Since some of the cardiovascular changes brought about by nicotine are thought to occur through the stimulation of the sympathetic nervous system, we anticipated that the exposure to nicotine in the HBP and LBP would bring about different responses. This was not the case. In neither the acute nor the chronic study was there a significant difference in the way the 2 strains responded. Both strains showed an increase in blood pressure 20 min after an injection of nicotine. This increase was, however, 3-fold larger in the HBP than the LBP. The pattern of response over time in the chronic study was the same for the LBP and HBP lines and agrees with previously published reports using rats[5,12]. There was an initial pressor response followed by a depressor effect that lowered blood pressure to a level equal to or below baseline levels. A pressor rebound was previously demonstrated in normal and renal hypertensive rats when nicotine was withdrawn during the depressor phase of chronic exposure to high doses (3.42 or 4.56 mg/kg/day)[12]. This pressor effect was also observed in the HBP and RBP lines of this study. However, blood pressure actually decreased in the LBP line when nicotine was withdrawn. A Tukey w-procedure[11] comparing the last blood pressure measured during nicotine ingestion and the blood pressure measured four weeks after nicotine withdrawal demonstrated a statistically significant difference between the LBP and the other 2 lines ($p < 0.01$).

This unexpected difference in reaction to the withdrawal of nicotine treatment may be related to physiological differences between the HBP and LBP mouse lines. For example, although it has been shown that the differences in whole brain norepinephrine content is not related to blood pressure in these strains, differences in norepinephrine content in specific areas of the brain[16] may be important in the physiological response to nicotine. It is possible that nicotine acts on different components of the central nervous system to produce a similar net decrease in blood pressure during chronic exposure while producing opposite effects in the two lines after withdrawal. A study involving a shorter nicotine treatment time and longer post-withdrawal monitoring would be necessary to determine if these tends are really manifestations of a physiological difference between these mouse lines selected for high or low blood pressure.



Mean change in blood pressure during 52 weeks of nicotine ingestion via drinking water and 4 weeks without nicotine treatment. Each point represents the average difference between treatment and control male siblings. The $SE_{\bar{x}}$ is based on the $\sqrt{\frac{\text{Error ms}}{n}}$ in the analysis of variance and was used to make comparisons between means with the Tukey w-procedure.

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