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CHRONIC NICOTINE USE BLOCKS HALOPERIDOL-INDUCED INCREASE IN STRIATAL D_2-DOPAMINE RECEPTOR DENSITY

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Epidemiologic studies have suggested a positive association in man between nicotine use and the incidence of tardive dyskinesia, a disease characterized by dopaminergic supersensitivity after chronic neuroleptic therapy . In rats, repeated administration of neuroleptics results into dopaminergic supersensitivity and increased density of striatal D_2- dopamine receptors. We investigated the effects of 6-week continuous nicotine intake on the neuroleptic (haloperidol)-induced increase in murine striatal D_2-dopamine receptor density. Contrary to expectations, our data show that nicotine blocked the increase in D_2- dopamine receptor density after neuroleptic administration.

Tardive dyskinesia (TD) is a disorder characterized by abnormal involuntary movements caused by prolonged exposure to neuroleptics (1). Several factors that increase the risk of TD in man include sex, age, and duration of use and dosage of neuroleptics (2-5). Two recent independent epidemiologic studies, however, have shown a prevalence of TD in chronic psychiatric outpatients that is higher in smokers than in nonsmokers (6-7). In rodents, dopaminergic supersensitivity consequent to repeated neuroleptic administration is reflected in increased striatal D_2- dopamine receptor density (8). Our experiments were designed to determine the effect of nicotine use on the development of dopaminergic supersensitivity in rodents. The results of this study show, apparently for the first time, that chronic nicotine intake blocks haloperidol (neuroleptic)-induced increase in the Bmax of striatal D_2-dopamine receptors.

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MATERIALS AND METHODS

[Benzene ring-^{3}H]- Domperidone (45 Ci/mmol) was purchased from New England Nuclear, Boston, MA. Haloperidol and nicotine [L-1-methyl-2-(3-pyridyl) pyrrolidine] were products of Sigma Chemical Co., St. Louis, MO.

Male Sprague-Dawley rats (200-250 g) were purchased from Holtzmann Co. and housed in a temperature- and light-controlled (22^{o}C, 12-h light) room with free access to Purina Chow and water. Fifty rats were randomly assigned to 2 groups. The first group (n=25), serving as a control, received water; the second group (n=25) had free access to water containing 1.0 mg% nicotine. The mean daily nicotine intake during the 6-week treatment period was 0.7 ± 0.01 mg/kg body weight. At the end of week 6, both groups were injected subcutaneously (once in the morning) with haloperidol (1 mg/kg body weight). Haloperidol solution (3 mg/ml) was prepared daily in 75% ethanol in saline. Two days after the last injection, the rats were killed by decapitation, striata dissected (9) from the brain, frozen over dry ice, and stored at -70^{o}C until assayed for D_2-dopamine receptor.

Rat striata were homogenized in 20 volumes of ice-cold buffer A (50 mM Tri-HCl, pH 7.4) using Polytron (setting 6, 1 X 20 sec) and homogenate filtered through three layers of moist cheese-cloth. The filtrate was centrifuged at 48,000 X g for 10 min at 4^{o}C, the pellet resuspended in the original volume of buffer A, and recentrifuged. The final pellet was resuspended in about one-half of the original volume (3-4 mg protein/ml) of buffer B (50 mM Tris-HCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4) and used for receptor binding. All other reagents used in the receptor assay were prepared in buffer B. The reaction mixture containing 0.12 to 2.5 nM [^{3}H]-domperidone and 100-125 ug membrane protein in a total volume of 1.0 ml was incubated at 37^{o}C for 30 min. Binding reactions were terminated by adding 4.0 ml of cold buffer A, and the tubes were kept on ice until filtration (less than 3 hours). The reaction mixture was rapidly filtered under vacuum through Whatman GF/B glass fiber filters, washed twice with 4.0 ml ice-cold

buffer A, placed in a mini-vial containing 1 ml of ethyleneglycol monoethylether, vortexed, and their radioactivity counted in 2.5 ml of scintillation fluid. The nonspecific binding was defined as that displaced by 5 uM haloperidol. The specific binding (total binding minus nonspecific binding) data were analyzed by Scatchard analysis (10) to calculate the K_D and Bmax of the receptor. Protein concentration of membrane preparation was analyzed by the method of Lowry et al. (11). The data were analyzed statistically by one-way ANOVA followed by Student's t-test for the group comparison.

RESULTS AND DISCUSSION

The data presented in Figure 1 (bottom panel) show that once daily subcutaneous administration of haloperidol (1 mg/kg) to control rats led to a progressive increase in maximal binding capacity or receptor density (Bmax) of striatal D_2-dopamine receptor (ANOVA: F-ratio=3.32, p=0.0438). The maximal increase in Bmax was observed on the seventh day of haloperidol treatment (Bmax, day 0 = 848.5 ± 119.8 and day 7 = 2161.0 ± 524.2; p=0.04), an increase of more than 150%, and it remained unchanged on further treatment for at least 12 days. In contrast, such haloperidol-mediated increases in

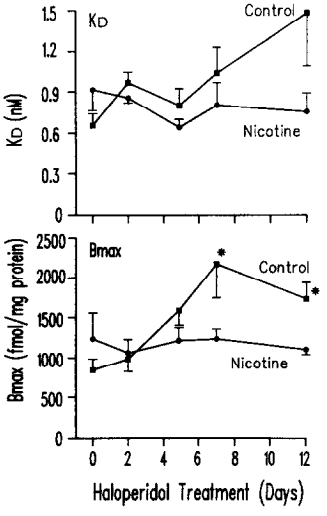

Fig. 1: Effect of nicotine on haloperidol-induced changes in the properties of rat striatal D_2-dopamine receptor. *P=0.04 compared to control (non-paired t-test).

Bmax were completely blocked in rats receiving nicotine (0.7 mg/kg/day) in their drinking water (ANOVA: F-ratio=0.17, p=0.9483). The affinity constant (K_D) of the receptor also seemed to increase with haloperidol treatment in control, but not nicotine-treated rats (Figure 1, bottom panel). The observed changes were statistically insignificant (ANOVA; control: F-ratio=1.65, p=0.2199; nicotine-treated: F-ratio=0.68, p=0.6177), however, because the data varied so greatly. In conclusion, the data presented here show for the first time that nicotine use blocks the increase in striatal D_2- dopamine receptor density after chronic neuroleptic (haloperidol) administration.

Use of nicotine in large doses is known to decrease appetite and body weight gain (12). In addition, chronic reduction in body weight by dietary restrictions is known to attenuate age-associated decreases in striatal D_2-receptor density (13,14). However, in a recent study we have observed that on chronic intake of 1 mg% nicotine-water for 2 months, food and water intakes and body weights of rats remained within 95-98% ($p > 0.05$) of the control group (unpublished observations). Furthermore, after one-year of continuous consumption of 1 mg% nicotine-water the increase in the body weight of control group (66.9±1.5%, n=28) was not significantly ($p > 0.05$) different than the nicotine group (63.8±1.3%, n=25). Therefore, the observed blockade of haloperidol-induced increase in striatal D_2-DA receptor density is not due to the effect of nicotine on food and water intake, or changes in the body weight of rats.

Physical symptoms of TD in man suggest the existense of dopaminergic supersensitivity, a condition easily produced in rats after repeated neuroleptic administration (8). Does this mean that nicotine can abate the incidence of TD in man? The answer will depend on our understanding of the role played by various factors such as age, sex, dopaminergic as well as nondopaminergic neurons on the development of behavioral supersensitivity of D_2-dopamine receptor agonists after chronic nicotine use. For example, not only reduced brain serotonin levels (produced by low tryptophan diet), but also agents affecting cholinergic or noradrenergic neurons clearly modulate

stereotypic behavior elicited by dopaminergic agonists (15-17). In summary, these data show that association between nicotine use and TD observed in epidemiologic studies (6-7) should be interpreted with caution when deciphering the biologic mechanism of TD.

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