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BRIEF COMMUNICATION

Chronic Nicotine-Induced Changes in Dopaminergic System: Effect on Behavioral Response to Dopamine Agonist

HENRY SERSHEN, AUDREY HASHIM, LASZLO HARSING AND ABEL LAJTHA

Center for Neurochemistry, N. S. Kline Institute, Orangeburg, NY 10962

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SERSHEN, H., A. HASHIM, L. HARSING AND A. LAJTHA. *Chronic nicotine-induced changes in dopaminergic system: Effect on behavioral response to dopamine agonist.* PHARMACOL BIOCHEM BEHAV **39**(2) 545–547, 1991.—The effect of chronic nicotine on dopamine-agonist-mediated locomotor activity response was measured in BALB/cBy and C57BL/6 mice. Mice were injected twice daily for 10 days with 1.2 mg/kg SC (−)-nicotine di-(+)tartrate. Subsequent locomotor activity response to apomorphine (1 mg/kg SC) was measured. Apomorphine induced hypomotility in both strains of mice, with the BALB/cBy mice showing a greater hypomotility compared to the C57BL/6 mice. The response to apomorphine was attenuated in both strains of mice that were treated previously with repeated injections of nicotine. The results suggest that chronic nicotine may induce changes in the dopaminergic system, which is reflected in altered behavioral response to a dopamine agonist.

Nicotine Dopaminergic system D2 autoreceptor Apomorphine Locomotor activity

NICOTINE administration to rodents produces behavioral and biological effects mediated in part by stimulation of nicotinic receptors located presynaptically on dopamine terminals in the CNS. Genetic variability in the sensitivity to nicotine or alteration in response has been suggested to be related in part to genetic differences in the number of nicotinic receptors and their subsequent up-regulation seen after repeated administration (12–15). Genetic factors also regulate tolerance development, which may be related to strain differences in the number and regional distribution of these receptors (5). Secondarily, the location of nicotine binding sites presynaptic on dopamine terminals suggests that behavioral and physiological effects of nicotine are mediated by a presynaptic modulation of amine transmitter release. In particular, the effects of nicotine on the release of dopamine have been well documented (2, 16–18, 23).

Nicotine has a complicated pharmacology, with acute or chronic tolerance developing to many of its effects. Responses that fail to show either acute or chronic tolerance include its locomotor stimulant and reinforcing actions (3, 10, 11, 20). A lack of tolerance to nicotine-induced dopamine release was observed in the nucleus accumbens in chronically treated rats (6), which could be related to the reinforcing properties of this drug. The results also suggest that modulation of dopaminergic function may be an important component of nicotine action. Harsing et al. (7) recently reported that [^{3}H]dopamine release evoked by K^+ or electrical stimulation was increased in striatal tissue from mice treated chronically with nicotine. The study also showed that D2 agonists- or antagonists-induced release of [^{3}H]dopamine evoked by electrical stimulation was attenuated in striatal tissue from mice pretreated with repeated injections of nicotine, suggesting that chronic nicotine may decrease the sensitivity of the D2 autoreceptor. To further test this hypothesis, the following experiments examined whether chronic nicotine would alter the behavioral response to a dopamine agonist in two strains of mice with differences in dopaminergic properties (19,21).

METHOD

BALB/cBy and C57BL/6 adult mice (4–6 months old) were used. The mice were given daily injections of nicotine [1.2 mg (−)-nicotine di-(+)-tartrate/salt/kg SC, twice daily for ten days] or saline (0.1 ml SC).

One day after the last injection, mice were treated with the dopamine agonist apomorphine (1.0 mg/kg SC) and locomotor activity measured. Each mouse was housed in individual cages (27 × 17 × 12) one day prior to locomotor activity measurements (after the last nicotine injection). Behavioral testing was started, after injection of apomorphine, by placing the animal back in its own home cage in an Opto-Varimex-Minor activity monitor (Columbus Instruments) and replacing the lid with a flat top without food and water, as described by Sershen et al. (20). By placing the animals in their home cage the day before activity measurements, the possibility of procedure habituation is reduced. Control groups included chronic saline- and nicotine-treated mice challenged with either saline or nicotine. After basal

activity was measured for 10 min in 5-min segments, the animal was injected with apomorphine and locomotor activity measured for an additional 20 min (in 5-min segments). The number of activity counts produced by interrupting consecutive infrared beams in the postinjection period was divided by the average counts produced during a 5-min segment in the preinjection period. This ratio was defined as the locomotor stimulation ratio. Saline- and nicotine-pretreated animals were tested concurrently.

RESULTS

Figure 1A shows the effect in BALB/cBy mice of a challenge injection of saline or nicotine in chronic saline- or nicotine-injected controls. There was no significant difference in the locomotor stimulation ratio between chronic saline-injected (Sal-Sal) or chronic nicotine-injected (Nic-Sal) mice challenged with saline. An acute challenge with nicotine in chronic saline-injected mice (Sal-Nic) resulted in a lowering of the locomotor stimulation ratio at 10–20 min after injection (n = 10 per group, mean ± SEM, $^{**}p<0.01$ compared to Sal-Sal, Nic-Sal, or Nic-Nic; ANOVA followed by protected t-test). In the chronic nicotine-injected mice, a nicotine challenge (Nic-Nic) stimulated locomotor activity at 0–5 min after injection (n = 10 per group, mean ± SEM, $^{*}p<0.05$ compared to Sal-Sal, Nic-Sal, or Sal-Nic; ANOVA followed by protected t-test).

Figure 1B and C shows the effect of the mixed D1/D2 agonist apomorphine (1 mg/kg) on locomotor activity in BALB/cBy and C57BL/6 mice. In saline-pretreated C57BL/6 mice, apomorphine slightly reduced the post/prelocomotor activity ratio, whereas, in BALB/cBy mice, a marked hypoactivity was seen. In both C57BL/6 and BALB/cBy mice, the hypoactivity induced by apomorphine was attenuated in mice pretreated with nicotine. In fact, apomorphine induced hyperactivity in C57BL/6 mice pretreated with nicotine.

The averages of the total motor activity counts measured over two 5-min periods before apomorphine were BALB/cBy = 385 ± 61 saline group and 263 ± 33 nicotine group; C57BL/6 = 310 ± 45 saline group and 253 ± 30 nicotine group. The average of the total activity counts of four 5-min periods after apomorphine were BALB/cBy = 14 ± 3* saline group and 29 ± 1*·** nicotine group; C57BL/6 = 202 ± 10* saline group and 423 ± 48*·** nicotine group (n = 6 per group, mean ± SEM, $^{*}p<0.01$ compared to preactivity counts; $^{**}p<0.01$ compared to saline postinjection activity, Student's t-test).

DISCUSSION

Drugs that increase the release of dopamine can be expected under chronic conditions to influence either or both the pre- and postsynaptic receptors. The D2 postsynaptic receptor has been shown to undergo up-regulation after lesioning of the presynaptic terminals, or chronic drug administration, for example, with D2 antagonists. The presynaptic process is also subjected to regulatory feedback control, for example, the D2 autoreceptor, which regulates neurotransmitter synthesis and release. Our biochemical studies suggested that, in chronic nicotine-treated mice, the D2 postsynaptic receptor is not affected (25); however, the release of dopamine is increased, showing no tolerance to this effect, and that D2 autoreceptor-mediated release of [^{3}H]dopamine may be diminished (7). Recently, the dopamine-releasing effect of chronic cocaine was shown to induce subsensitization of the D2 autoreceptor (26). In the case of chronic cocaine, the effect of the dopamine agonist N-0437 to inhibit release of dopamine was abolished (26). Also, chronic cocaine caused significant subsensitivity to the inhibitory effects of apomorphine on extracellular single-unit recording of A10 dopamine neurons (8). With chronic nicotine, the dopamine D2 antagonist

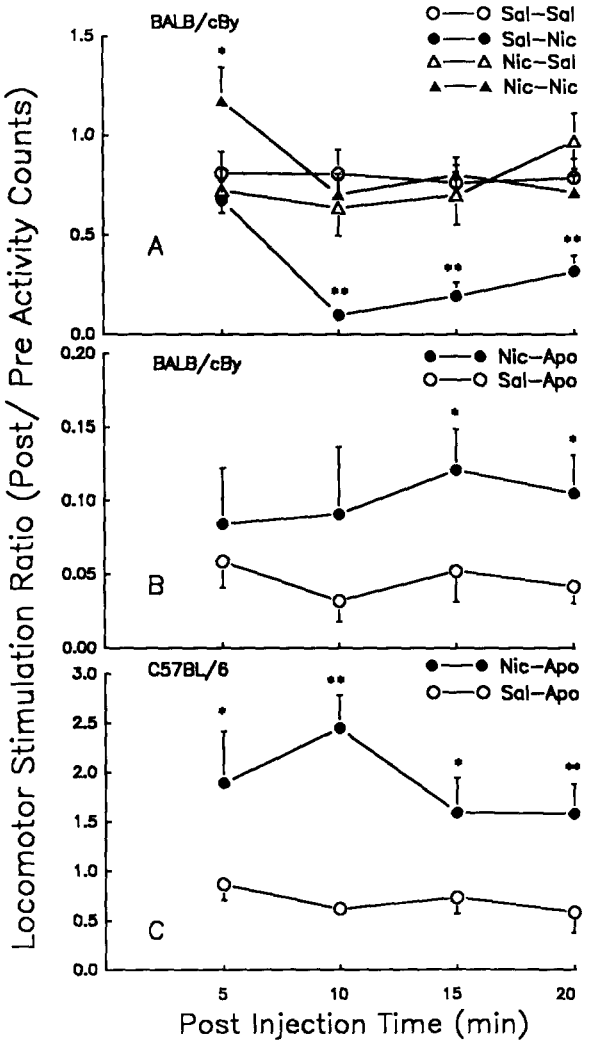

FIG. 1. BALB/cBy and C57BL/6 mice were treated for 10 days with (−)-nicotine di-(+)tartrate (1.2 mg/kg SC, twice daily). On the next day, locomotor activity was monitored before and after an injection with apomorphine (1 mg/kg SC). Data were analyzed by ANOVA and the post hoc protected t-test (GBStat statistical program) $^{*}p<0.05$, $^{**}p<0.01$. Panel A shows the effect of a saline or nicotine challenge to control BALB/cBy mice given repeated saline or nicotine injections (n = 10; mean ± SEM); saline injected with saline challenge (Sal-Sal) or nicotine challenge (Sal-Nic); nicotine injected with saline challenge (Nic-Sal) or nicotine challenge (Nic-Nic). Panel B shows the effect of an injection of apomorphine (1 mg/kg) in BALB/cBy mice [saline injected (Sal-Apo) and nicotine injected (Nic-Apo)] (n = 6, mean ± SEM). Panel C shows the effect of apomorphine (1 mg/kg) in C57BL/6 mice given repeated saline injections (Sal-Apo) or nicotine injections (Nic-Apo) (n = 6, mean ± SEM).

(sulpiride)-stimulated release or the D2 agonist (quinpirole)-inhibited release was abolished in the chronic nicotine-treated striatal tissue (7). Similarly, tolerance appears to develop to the ability of apomorphine to inhibit ventral tegmental neuronal activity, and this effect is mediated by a subsensitivity of the inhibitory dopamine autoreceptor induced by long-term treatment with d-amphetamine (9).

Apomorphine is a mixed D1/D2 agonist, and its behavioral effects depend on its site of action. At high doses, it can act postsynaptically to stimulate locomotor activity, or, at low doses,

it has a higher affinity to the presynaptic autoreceptor, reducing dopamine release and thereby inducing hypomotility. The influence of genotype on the effects of locomotor activity are known, and generally the C57BL/6 strain is more active compared to the BALB/cBy mice in open-field activity and response to stimulating drugs acting on the dopamine receptor (17,19). Severson (21) reported differences in striatal D2 dopamine receptor density in these two strains of mice, with BALB/cBy mice having the higher density. The administration of D2 agonists, and, for example, apomorphine, at least at low doses, is expected to induce hypomotility in mice by its action on the D2 autoreceptor (22). In the BALB/cBy mice, this was clearly seen and, to a lesser degree, in the C57BL/6 mice. In both strains, repeated administration of nicotine attenuated the apomorphine-induced hypomotility effect on locomotor activity. Although the mouse strains may vary in sensitivity to this effect, this cannot be clearly confirmed unless dose-response curves are done. The results suggest that repeated nicotine in some way alters the dopaminergic system. One possibility is a resulting desensitization of the D2 autoreceptor receptor. The resulting decrease in autoreceptor stimulation, which would disinhibit dopamine neuronal activity, will result in enhanced dopamine release in the terminal fields, as has been reported after repeated nicotine (6, 7, 24). A similar lack of tolerance to the locomotor stimulatory effect of nicotine was reported by others (4, 10, 20). This effect could underlie the increase in the locomotor stimulation ratio seen in the Nic-Nic group of mice. However, since apomorphine affects both D1 and D2 receptors, we must caution that, consequently, a behavioral assessment will not clearly distinguish between D1 and D2 pre- and postsynaptic effects. Additional studies would benefit from the use of more specific D1 and D2 agonists to better characterize the changes in the dopaminergic system after chronic nicotine.

The observed response to apomorphine following subchronic nicotine pretreatment suggests that, most likely, both nicotinic and dopaminergic (D1 and D2) receptors systems are involved and undergo changes. Understanding the nature of their interactions and their selective changes with chronic nicotine administration will further elucidate the mechanisms of the action and reinforcing properties of nicotine.

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