Synergistic Interactions Between Nicotine and Cocaine or Methylphenidate Depend on the Dose of Dopamine Transporter Inhibitor

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ABSTRACT There is a greater prevalence of cigarette smoking among cocaine-dependent individuals and hyperactive children treated with stimulants (e.g., methylphenidate, MP). However, little is known about the neurochemical basis of the interaction between nicotine and cocaine or MP. It is thought that the reinforcing effects of cocaine and MP are due partly to increases in synaptic DA in the nucleus accumbens (NAc). These measurable increases are secondary to the blockade of the DA transporter. In contrast, nicotine stimulates acetylcholine receptors located presynaptically on dopaminergic projections from the ventral tegmental area (VTA) to the NAc and increases DA transmission. Here we investigate the effects of nicotine on NAc DA in animals simultaneously injected with cocaine or MP. Coadministration of nicotine (0.4 mg/kg s.c.) and cocaine (10 mg/kg i.p.) or MP (5 mg/kg i.p.) increased the extracellular NAc DA levels in an additive manner, while coadministration of nicotine (0.4 mg/kg s.c.) and a higher dose of cocaine (20 mg/kg) or MP (10 mg/kg) clearly produced a synergistic elevation in NAc DA. These findings suggest that the degree of DA transporter (DAT) occupancy contributes to the synergistic interaction between nicotine and cocaine or MP.


INTRODUCTION

Nicotine is one of the most commonly abused reinforcing agents. In addition to the reward system, it affects several other systems that could alter behavior, including cognition, learning, and memory (Levin, 1992; Levin et al., 1996). Cigarette smoking is a serious health problem in the US, but it is causing serious concern in cocaine abusers (Schloring et al., 1994) and in patients with attention deficit hyperactivity disorder (ADHD) (Milberger et al., 1997a, b). Epidemiological studies show the prevalence of cigarette smoking is significantly greater among cocaine-dependent individuals compared to nonabusers and in children with ADHD relative to healthy controls (Budney et al., 1993; Lambert and Hartsough, 1998). The latter has generated concerns regarding the potential contribution of methylphenidate (MP), the drug currently being used to treat ADHD, to nicotine dependence in medicated ADHD patients. However, little is known about the neurochemical interaction between nicotine and cocaine or MP.

An extensive literature indicates that increases in dopamine (DA) transmission in the nucleus accumbens (NAc) underlie the rewarding/reinforcing effects of addictive drugs (Di Chiara, 1999; Di Chiara and Imperato, 1988; Gardner, 1997; Koob and Bloom, 1988). However, involvement of other monoamine transporters is also currently debated. Nicotine, cocaine, and MP have in common the ability to stimulate DA transmission in the main reward pathway of the brain, although the exact mechanism by which they achieve this effect is different. Nicotine is postulated to activate acetylcholine receptors located presynaptically on dopaminergic projections from the ventral tegmental area.
(VTA) to the NAc (Stolerman and Shoabi, 1991), while cocaine and MP both act through inhibition of a dopamine transporter responsible for the reuptake of DA from the synaptic cleft (Madras et al., 1989; Ritz et al., 1987). We have recently shown that there is a high correlation between the increase in synaptic DA as assessed by measuring the D₂ receptor occupancy with [11C]raclopride before and after i.v. MP and the intensity of the self-reported “high” (Volkow et al., 1999c). Due to the limited spatial resolution of positron emission tomography (PET), the measurements were made in the dorsal striatum and not in the NAc.

The reported high rate of “coabuse” of nicotine and cocaine or MP implies a neurochemical interaction, presumably at the levels of limbic brain regions. Zernig et al. (1997) demonstrated that coadministration of cocaine (3 mg/kg s.c.) and nicotine (0.1 mg/kg s.c.) produced an additive increase in NAc DA. Similarly, Sztiraki et al. (1999) administered cocaine (250 μg/kg) and nicotine (50 μg/kg) i.v. and observed the additive effect of the combination on DA overflow in NAc. However, another cocaine combination of high abuse potential, i.e., heroin/cocaine (speedball) clearly produces synergistic effect on the increases in NAc DA in both self-administration (Hemby et al., 1999) and forced (Gerassimov and Dewey, 1999) injection paradigms.

In the present study we used in vivo brain microdialysis in freely moving rats to examine the manner in which nicotine affects increases in extracellular NAc DA levels induced by cocaine or MP.

**MATERIALS AND METHODS**

**In vivo microdialysis studies**

Male Sprague-Dawley rats were used in all experiments (200–300g, Taconic Farms, Germantown, NY) and were given food and water ad libitum. Temperature and humidity were kept relatively constant. Each animal was housed individually on a 12/12 h light/dark cycle. All animals were used under an IACUC-approved protocol and with strict adherence to NIH guidelines.

Animals were anesthetized and siliconized guide cannulae were stereotactically implanted into the right NAc (2.0 mm anterior and 1.0 mm lateral to bregma, and 7.0 mm ventral to the cortical surface) at least 4 days prior to study. Microdialysis probes (2.0 mm, Bioanalytical Systems, BAS, West Lafayette, IN) were positioned within the guide cannulae and artificial cerebrospinal fluid (ACSF, 155 mM Na⁺, 1.1 mM Ca²⁺, 2.9 mM K⁺, 132.76 mM Cl⁻, and 0.83 mM Mg²⁺) was administered through the probe using a CMA/100 microinfusion pump (BAS) at a flow rate of 2.0 μl/min. Animals were placed in bowls and probes were inserted and flushed with ACSF overnight. On the day of study, a minimum of three samples were injected to determine baseline stability. Samples were collected for 20 min and injected on-line (CMA/160, BAS). The average dopamine concentration of these three stable samples was defined as control (100%), and all subsequent treatment values were transformed to a percentage of that control. The high-pressure liquid chromatography (HPLC) system consists of a BAS reverse-phase column (3.0 μ C-18), a BAS LC-4C electrochemical transducer with a dual glassy carbon electrode set at 650 mV, a computer that analyzes data on-line using a commercial software package (Chromgraph, BAS), and a dual pen chart recorder. The mobile phase (flow rate 1.0 ml/min) consisted of 7.0% methanol, 50 mM sodium phosphate monobasic, 1.0 mM sodium octyl sulfate, and 0.1 mM EDTA, pH 4.0.

**Pharmacologic challenge regimens**

(--)Nicotine (Aldrich Chemical Company, Milwaukee, WI) was dissolved in saline and injected subcutaneously (0.4 mg/kg).

(--)Cocaine hydrochloride (NIDA) (10 or 20 mg/kg) and methylphenidate (5 or 10 mg/kg) (a racemic mixture of d-threo and 1-threo- methylphenidate; Research Biochemicals, Natick, MA) were dissolved in saline and injected intraperitonealy.

Drug combination group animals received one drug (nicotine) subcutaneously and the other (cocaine or MP) intraperitonealy. A separate group of animals was used for every challenge regimen (see Table I).

Univariate analysis of peak effects (percent change from basal DA levels) were used to determine significant differences between treatment groups.

**RESULTS**

Nicotine alone (0.4 mg/kg) increased extracellular DA concentrations in the NAc by approximately 100% 40 min following administration. Administration of cocaine alone (10 and 20 mg/kg) resulted in significant increase in NAc DA levels, by 160% and 400%, respectively. Coadministration of nicotine (0.4 mg/kg) and the lower dose of cocaine (10 mg/kg) resulted in an additive increase of 240% above baseline. The observed increase in DA levels for the combination group was not statistically different from the calculated combined effects of each drug alone (P > 0.1, ANOVA and Newman-Keuls test) (Fig. 1A,B). DA levels returned to baseline values 140 min following the administration of either drug alone or their combination.

When nicotine was combined with the higher dose of cocaine (20 mg/kg), the resulting effect on the NAc DA concentrations was greater than the additive effect for
individual doses of nicotine and cocaine combined. That is, it peaked 880% above baseline values 40 min after the injection of the drug combination (Fig. 3A). In all experiments with a demonstrated synergistic effect, the time period necessary for dopamine levels to return to baseline was considerably longer (240 min) than in studies demonstrating an additive effect (140 min).

Injections of MP alone (5 and 10 mg/kg) produced elevations in NAc DA of 198% and 345% above baseline, respectively, that peaked 40 min after administration. DA concentrations returned to baseline after 140 min. Simultaneous administration of nicotine and the lower dose of MP (5 mg/kg) further increased the levels of extracellular DA in NAc in an additive manner; that is, it peaked 350% above baseline. The observed increase in DA levels for the combination group was not statistically different from the calculated combined effects of each drug alone \( (P > 0.1) \) (Fig. 2A,B). There was no difference between the groups in the time it took for DA levels to reach the peak values.

In contrast, coadministration of nicotine and the higher (10 mg/kg) dose of MP produced an increase of 1325% above baseline that was clearly synergistic (Fig. 3B). Again, it took considerably longer for DA levels to return to baseline as compared to the single drug challenge study (220 min vs. 140 min).
DISCUSSION

In the present study we demonstrate that the combined effects of nicotine and cocaine as well as the combined effects of nicotine and MP on extracellular NAc DA levels may be either additive or synergistic, depending on the dose of DAT blocker.

Inasmuch as the reinforcing/rewarding properties of these psychostimulants are associated with increases in brain DA, our results correspond well with epidemiological data indicating a higher incidence of cigarette smoking among cocaine abusers and ADHD patients treated with MP.

Numerous reports indicate pharmacologic similarities between cocaine and MP. Both drugs have similar brain distribution, equivalent levels of uptake, and similar rates of uptake (Volkow et al., 1995). The differences in the potencies in blocking the DAT in the human brain between cocaine and MP (ED50 values are 0.13 and 0.075 mg/kg i.v., respectively) (Volkow et al., 1999b) correspond to differences in their in vitro affinities for DAT (Ki for inhibition of DA uptake is 640 and 390 nM, respectively) (Ritz et al., 1987). Interestingly, despite similar dose-occupancy relationships for the two drugs, the locomotor activation by MP is greater in magnitude and duration than that of cocaine (Gatley et al., 1999). Accordingly, in agreement with our previous primate PET studies where twice the dose of cocaine compared to MP was needed to produce the same decrease in 11C raclopride binding (Volkow et al., 1999a), the present study demonstrates similar increases in extracellular NAc DA levels can be induced by doses of nicotine twice those of MP. However, when interpreting these data one should keep in mind that MP is administered as a 50/50 mixture of two enantiomers, while cocaine is administered in enantiomerically pure form. We have demonstrated that binding of the inactive enantiomer of MP in the human brain is mostly non-specific. Moreover, systemic administration of pharmacologically inactive enantiomer does not affect extracellular striatal DA levels in rats (Ding et al., 1997).

Stimulation of NAc DA release by nicotine in rodents does not show a strong dose dependence. On the contrary, based on numerous literature reports as well as our own findings, it appears that the elevation in extracellular DA levels elicited by increasing doses of nicotine reaches a plateau. That is, the doses ranging from 0.1 to 0.8 mg/kg s.c. produced increases in NAc DA similar in magnitude (165–220% above baseline) (Dewey et al., 1999; Di Chiara and Imperato, 1988; Imperato et al., 1986; Brazell et al., 1990; Benwell and Balfour, 1992). It has been demonstrated that direct inhibition of the DAT is not involved in the effects of nicotine (Damaj et al., 1999). Since DAT is one of the most important regulators of dopaminergic function (Jones et al., 1998), it is conceivable that in the presence of fully functional DAT transient increase in synaptic DA induced by nicotine is quickly counteracted and that the temporal and spatial resolutions of the in vivo microdialysis technique does not allow for the detection of “real” increase. In contrast, when DAT is blocked by cocaine or MP, DA released upon stimulation of nicotinic receptors freely escapes into extracellular space, where it can be detected.

Interestingly, Izenwasser and colleagues have reported that nicotine inhibits 50% of [3H]dopamine uptake in striatum by a mechanism which appears to be
different from that of cocaine (Izenwasser and Cox, 1992; Izenwasser et al., 1991a, b). Furthermore, it seems that the effects of nicotine on DA uptake and release are mediated via separate mechanisms, since the IC$_{50}$ value for inhibition of [3H]dopamine uptake was much lower than the concentrations necessary for activating nicotinic acetylcholine receptors and for stimulating DA release. Thus, it is possible that modulation of transporter function induced indirectly by nicotine may augment the inhibitory effect of cocaine or MP, leading to a more complete DAT blockade and, subsequently, enhanced DA concentrations.

The fact that cocaine's ability to elevate extracellular DA hinges on the level of ongoing cell activity offers another possible explanation of the observed results. Nicotine is known to produce a dose-related increase in the firing rate of ventral tegmental area (VTA) DA cells via activation of cholinergic receptors (Mereu et al., 1987). Cocaine, however, causes reduction in spontaneous DA cell firing, partly as a result of elevating DA at somatodendritic autoreceptors (Lacey et al., 1990; Kalivas and Duffy, 1991; Einhorn et al., 1988). It is plausible then, that nicotine would counteract the inhibitory effect of cocaine on DA cell firing within the VTA. On the other hand, we have previously proposed a model for estimating DAT occupancy and related increase in synaptic DA (Gatley et al., 1997). According to this model, synaptic DA rises exponentially with increasing DAT occupancy. One might argue, based on the model, that the qualitative difference between the responses to the combination of nicotine and DAT inhibitor (additive vs. synergistic) is due to the change in the degree of DAT blockade, however small it might be, coupled with activation of DA cell firing. Relevant to this point, we have previously demonstrated that increasing the dose of intravenous cocaine or MP four times (from 0.25 to 1 mg/kg) results in increases in DAT occupancy from 50–80% (Gatley et al., 1999). Simultaneous microPET scanning and brain microdialysis sampling would provide further opportunities to clarify this issue.

We have previously demonstrated in humans that doses of cocaine commonly taken by addicts achieve almost total blockade of DAT (Volkow et al., 1996). Nevertheless, even larger doses are often abused (Verheye and Gold, 1988). The doses of cocaine and MP used in the present study are above minimal doses known to be behaviorally active in rodents. For example, both drugs elicit robust conditioned place preference at 5 mg/kg i.p. (Patkina and Zvartau, 1998; Sora et al., 1998). We have commented on the possibility that even though the higher doses are unlikely to further increase peak DAT occupancy, they may increase instead the rate of attaining the “ceiling” occupancy (Volkow et al., 1996). Thus, the higher doses are more potent reinforcers, since the rate of the change in the concentration of DA (rate of change from baseline) secondary to the inhibition of DAT affects reinforcing properties of the drug (Balster and Schuster, 1973). This hypothesis is relevant to the present study, where doubling the dose of cocaine or MP used in combination with nicotine produced a dramatic change in the measured increases in extracellular DA levels (344% vs. 1,325% for MP and 400% vs. 870% for cocaine). Interestingly, since it took the same time for the peak values to be reached in all the present studies, it appears that the rate of increase in DA levels was much higher in the studies with synergistic outcome. Hence, the combination might be predicted to be more reinforcing.

Although the direct evidence that nicotine augments the reinforcing effect of cocaine or MP is lacking, one available study showed that prior exposure to daily nicotine injections subsequently increased the rate of acquisition of cocaine self-administration in rats (Horger et al., 1992). The relevance of that study to our results is limited, however, since the effect of a chronic nicotine exposure cannot be directly compared with the effect of an acute challenge. Also, as was mentioned above, epidemiological studies are consistent with pharmacologic interactions between these drugs.

Our data may be relevant to the debate over the role of other monoamine transporters in the reinforcement. Unlike cocaine, MP appears to have very weak potency both in binding to the serotonin transporter and as an in vitro inhibitor of serotonin uptake (Gatley et al., 1996; Wall et al., 1995). Additionally, Kuczenski and Segal (1997) have shown that MP has no effect on extracellular caudate putamen serotonin levels in vivo. Similar trends in the interaction between nicotine and cocaine or MP observed in the present study suggest that the ability of cocaine to block serotonin uptake may not play an appreciable role in the mutually enhancing interaction with nicotine.

The implications of this potential enhancement by nicotine of stimulant-induced effects are significant. Primarily, this interaction suggests that smoking might influence the onset and pattern of cocaine abuse. Second, if treatment with MP enhances the pleasure associated with cigarette smoking, it could lead to a quicker development of nicotine dependence.

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