

A Pharmacologic Strategy for the Treatment of Nicotine Addiction

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ABSTRACT Like many psychostimulant drugs, nicotine elevates extracellular and synaptic dopamine (DA) concentrations in the nucleus accumbens (NAc). This elevation has been linked to its reinforcing properties. Dopaminergic transmission within the NAc is modulated by gamma-aminobutyric acid (GABA). Therefore, we examined the utility of gamma vinyl-GABA (GVG, Vigabatrin) for inhibiting nicotine's biochemical effects on NAc DA as well as its effects on behaviors associated with these biochemical changes. Given 2.5 hours prior to nicotine, GVG (75 mg/kg) had no effect on nicotine-induced increases in extracellular NAc DA. However, at 90 mg/kg, GVG significantly inhibited nicotine-induced increases by approximately 50% while at 100 or 150 mg/kg, GVG completely abolished nicotine-induced increases in both naïve and chronically nicotine-treated animals. When given 12 or 24 hours prior to nicotine administration at a dose of 100 mg/kg, GVG-induced inhibition was diminished or abolished, respectively. In addition, at a dose of 18.75 mg/kg GVG abolished the expression of nicotine-induced conditioned place preference (CPP) while a dose of 75 mg/kg abolished the acquisition phase of CPP. Finally, using positron emission tomography (PET) and ¹¹C-raclopride in primates, GVG (100 mg/kg) abolished nicotine-induced increases in synaptic DA while having no effect on the rate of metabolism of the radiotracer or its regional distribution. Together, these data suggest that GVG may be useful for the treatment of nicotine addiction and further support the strategy of targeting the GABAergic system with a suicide inhibitor of GABA-transaminase for the treatment of drug addiction. **Synapse 31:76–86, 1999.** © 1999 Wiley-Liss, Inc.

INTRODUCTION

The health consequences of cigarette smoking are well established. This behavior is directly or indirectly responsible for 300,000–400,000 deaths in the United States annually (Pollin, 1984; Ravenholt 1980; U.S. Dept. of Health and Human Services, 1983, 1987). Each year an estimated 35 million smokers make a serious attempt to quit. Unfortunately, less than 7% achieve more than 1 year of abstinence and most relapse within a few days (Fiore, 1992). Several pharmacologic strategies have been developed for the treatment of nicotine addiction including nicotine gum, transdermal nicotine patches, nasal sprays, nicotine inhalers, and bupropion, the first nonnicotinic treatment for smoking cessation (Henningfield, 1987, 1995; Hurt et al., 1997). Together with these new pharmacologic treatments and

an increased awareness of the potential devastating consequences associated with tobacco use, smoking cessation has increased by approximately 20% each year (Henningfield, 1995).

Considerable evidence indicates that, despite the numerous other compounds present in cigarette smoke, it appears that the addictive potential of cigarettes is strongly (though not completely) related to the pres-

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ence of the alkaloid (-)-nicotine. For example, nicotine is readily self-administered by humans (Henningfield et al., 1983, Henningfield and Goldberg, 1983; Pomerleau and Pomerleau, 1992) and other mammalian species (Deneau and Inoki 1967; Donny et al., 1995; Goldberg et al., 1981; Valentine et al., 1997). In addition, smokers consume cigarettes in a manner that maintains constant nicotine levels (Russell et al., 1995). People report a lower degree of euphoric sensations after consuming ultra-low nicotine cigarettes compared to medium- and high-nicotine cigarettes (Pomerleau and Pomerleau, 1992) and the amount of cigarettes smoked is inversely related to the total amount of nicotine delivered per cigarette (Gritz, 1980).

To date, an extensive literature indicates that the rewarding/reinforcing actions of nicotine may be in part related to its effects on dopamine (DA) neurons in the reward pathways of the brain (Gardner, 1997; Nisell et al., 1995; Pontieri et al., 1996). For example, nicotine shares with other drugs of abuse the property of producing an increase in extracellular DA levels in the nucleus accumbens (NAc), an important component of the reward system (Damsma et al., 1989; Di Chiara and Imperato, 1988; Imperato et al., 1986; Nisell et al., 1994a, 1995; Pontieri et al., 1997). Similarly, the infusion of nicotine into the ventral tegmental area (VTA) of the rodent produces a significant increase in DA levels in the NAc (Nisell et al., 1994b). In contrast, the administration of the nicotinic receptor antagonist mecamylamine into the VTA significantly attenuates the increase in NAc DA levels produced by nicotine (Nisell et al., 1994a). Nicotine stimulates the release of DA from slices and from synaptosomal preparations of the striatum and accumbens (Chesselet, 1984; Rowell et al., 1987) while exposure to cigarette smoke or systemic administration of nicotine increases DA utilization or release in the NAc (Fuxe et al., 1988; Imperato et al., 1986). Following acute nicotine treatment, DA turnover is greatest in the NAc as compared to other brain regions (Lapin et al., 1989) and both DA D₁ and D₂ antagonists, as well as haloperidol, altered nicotine self-administration. Further, i.v. administration of nicotine to rats increases the firing rate and bursting activity of VTA DA neurons, which send terminal projections predominantly to limbic structures including the NAc (Grenhoff et al., 1986; Grenhoff and Svensson, 1988; Mereu et al., 1987; Nisell et al., 1995). The lesioning of DA nerve terminals in the NAc by 6-OHDA significantly reduces nicotine self-administration (Corrigall et al., 1992; Singer et al., 1982) and attenuates nicotine-induced increases in locomotor activity (Clarke et al., 1988). Furthermore, systemic administration of the DA receptor antagonist haloperidol significantly decreases nicotine self-administration (Corrigall and Coen, 1991) in a manner similar to the microinfusion of the nicotinic receptor antagonist dihydro- β -erythrodine into the VTA (Corrigall et al., 1994).

For more than a decade, we have been using positron emission tomography (PET) and in vivo microdialysis techniques to study neurotransmitter interactions in the human, non-human primate, and rodent brain (Schloesser et al., 1996). In the course of developing a novel approach for the treatment of schizophrenia, we focused on GABA's ability to modulate NAc and striatal DA (Dewey et al., 1992). In an extension of these findings to another question of clinical relevance, we recently assessed the utility of gamma vinyl-GABA (GVG, Vigabatrin[®]), a selective and irreversible (suicide) inhibitor of GABA-transaminase (GABA-T) for dose-dependently attenuating cocaine-induced increases in extracellular and synaptic dopamine (DA) (Dewey et al., 1998). In addition to these biochemical studies, we examined the effects of GVG on cocaine-induced conditioned place preference (CPP), cocaine self-administration (using both a fixed ratio 5 and a progressive ratio schedule, Kushner et al., 1997a), cocaine-induced lowering of brain stimulation reward thresholds (Kushner et al., 1997b), catalepsy, locomotor behavior and drug metabolism, and systemic delivery. GVG dose-dependently blocked the biochemical effects of cocaine on brain DA concentrations in naïve and chronically cocaine-treated rodents while it also blocked these effects in primates as well. In addition, GVG abolished both cocaine-induced CPP and cocaine self-administration (drug seeking and drug taking behavior, respectively), and abolished cocaine-induced sensitization (unpublished), and attenuated cocaine-induced lowering of brain stimulation reward thresholds. However, GVG did not alter CPP for a food reward, feeding behavior, or gross locomotor activity. Furthermore, in order to establish the safety of these two drugs in combination, we determined the effects of acute GVG pretreatment on a cocaine challenge in conscious, freely moving animals. These data demonstrated that acute co-administration of GVG and cocaine did not result in immediate cardiotoxicity or hepatic toxicity of enough significance to preclude further clinical trials (unpublished). Finally, we demonstrated that the selective GABA_B antagonist, SCH 50911, completely abolished GVG's attenuation of cocaine-induced increases in NAc DA suggesting that the mechanism underlying this attenuation is mediated in part, if not completely, through the GABA_B receptor (Ashby et al., 1999).

For the treatment of brain disorders such as drug addiction, Parkinson's disease, and mental illness, the use of specific suicide inhibitors of an enzyme, like GVG, offers advantages unique to their biochemical mode of action. First, suicide inhibitors are typically long acting, as the reversal of their effects is dependent upon the de novo synthesis of enzyme. Second, suicide inhibitors do not produce tolerance or symptoms of withdrawal that are more commonly associated with specific neurotransmitter receptor-acting drugs. Finally, these drugs are themselves not addictive, which

is especially important when considering their use for the treatment of substance abuse. Consistent with the demonstrated ability of GVG to lower synaptic DA, is the notion that this strategy of targeting the GABAergic system with a suicide inhibitor could be applicable to the treatment of addiction to other drugs whose reinforcing properties are linked to elevations in NAc DA. Specifically, drugs including nicotine, amphetamine and methamphetamine, alcohol, and heroin have been reported to alter DA in the NAc in a manner similar to that of cocaine. In this report, we focused on the effects of GVG on nicotine-induced changes in biochemistry and behavior. Ongoing studies are currently investigating the utility of this approach for treating addictions to amphetamine and methamphetamine, alcohol, heroin, and morphine.

In the present study, we examined the effects of GVG on nicotine-induced increases in NAc DA as well as on behaviors associated with these biochemical effects. Specifically, this was accomplished by: (1) using *in vivo* microdialysis in freely moving naïve and chronically-nicotine treated animals to measure the effects of GVG and nicotine on extracellular NAc DA; (2) using positron emission tomography (PET) to measure the effect of GVG on nicotine-induced decreases in ¹¹C-raclopride binding in the striatum of anesthetized, female baboons; and (3) examining the effect of GVG on CPP to nicotine. The CPP paradigm is widely used to evaluate the incentive motivational effects of drugs in laboratory animals (Altman et al., 1996; Carr et al., 1989; Hoffman, 1989; Van Der Kooy, 1987).

MATERIALS AND METHODS

In vivo microdialysis studies

Male Sprague-Dawley animals were used in all studies (200–225 g, 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 hour light/dark cycle. All animals were used under an IACUC-approved protocol and with strict adherence to NIH guidelines.

In vivo microdialysis studies were conducted as detailed previously (Dewey et al., 1998; Morgan and Dewey, 1998). In the first series of studies performed in naïve animals (Group 1), nicotine (0.4 mg/kg, sc) was administered 2.5 hours after GVG (75, 90, 100, or 150 mg/kg, i.p.). In a separate series of experiments (Group 2) animals were treated for 21 days with nicotine (0.4 mg/kg, s.c., twice daily). On the day of the study, GVG (100 mg/kg) was administered either 2.5, 12, or 24 hours prior to nicotine (0.4 mg/kg, s.c.) challenge. In all studies, animals were placed in the microdialysis test chambers the night before the experiment and artificial cerebrospinal fluid (ACSF) was perfused through the microdialysis probes at a flow rate of 2.0 µl/min. At the end of each study, animals were sacrificed and their brains removed and sectioned for probe placement verification.

Nicotine-induced CPP

CPP apparatus

The CPP apparatus was made entirely of plexiglass, except for the floor in one of the pairing chambers, which was made of a stainless steel plate with holes (0.5 mm in diameter) spaced 0.5 mm from edge to edge. The two pairing chambers differed in visual and tactile cues. One chamber was entirely light blue with the stainless steel floor and the second chamber was light blue with horizontal black stripes (2.5 cm wide) spaced 3.8 cm apart with a smooth plexiglass floor. The two pairing chambers were separated by a third, neutral connecting tunnel (10 x 14 x 36 cm) with clear plexiglass walls and a plexiglass floor. The visual and tactile cues were balanced such that no significant side preference was exhibited by animals prior to conditioning.

Test procedure for effect of GVG on expression of CPP to nicotine

The CPP conditioning procedure consisted of 20 sessions carried out consecutively over 20 days. The first three sessions were habituation sessions, during which the animals were handled for 5 minutes per day and exposed to the sights and sounds of the test room. This was followed by 16 sessions of 8 pairings with (1) vehicle/vehicle (1 ml/kg i.p. 0.9% saline) or (2) saline/nicotine (0.4 mg/kg s.c.) with 10 animals in each group. Half the animals exposed to the second conditioning procedure (saline/nicotine) received nicotine before exposure to the blue chamber and the other half received saline before exposure to the blue and black striped chamber. The animals that received saline or nicotine were injected and confined to the appropriate compartment for 30 minutes by using guillotine plexiglass doors to block access to the rest of the chamber. The final session (day 20) was a test session, in which animals received one of the following treatments 30 minutes before the test: (1) saline or (2) GVG (18.75, 37.5, 75, or 150 mg/kg i.p.). The entrances to both pairing chambers were opened, and the animals were allowed to freely move between the 3 chambers for 15 minutes. The amount of time spent in each chamber was recorded using an automated infrared beam electronically coupled to a timer.

Test procedure for the effect of GVG on acquisition of CPP to nicotine.

PET primate studies

Papio anubis, 13–18 kg) were used for all imaging studies and carbon-11 labeled raclopride (¹¹C-raclopride), previously shown to be sensitive to changes in synaptic DA (Dewey et al., 1993), was synthesized as previously described (Ehrin et al., 1987). Animals were placed into 5 groups as detailed in Table I. Control animals (Group 1) received two injections of ¹¹C-raclopride without any drug intervention in order to deter-

TABLE I. Groups for primate PET studies

Group	Condition
1	Test/Retest (no challenge)
2	GVG (300 mg/kg)
3	Nicotine (0.3 mg)
4	GVG (100 mg/kg), Nicotine (0.3 mg)
5	GVG (300 mg/kg), Nicotine (0.3 mg)

TABLE II. Effects of drug challenge on the mean distribution volume (DV) ratio

Group	% Change in mean DV ratio
1	7.16 ± 1.2
2	18.8 ± 3.2
3	-12.3 ± 2.6
4	9.45 ± 2.1
5	15.1 ± 2.8

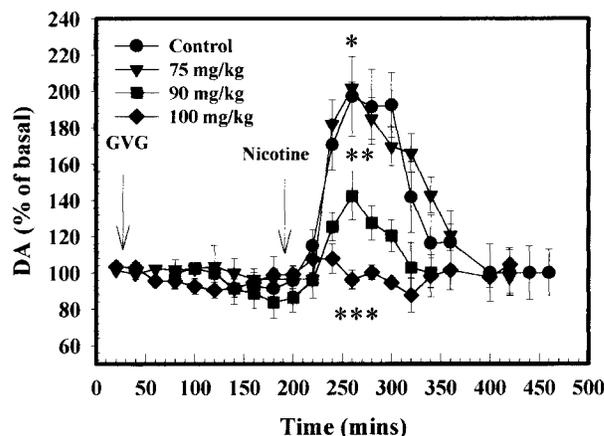
mine the test/retest variability of the measurement. These data have been reported previously (Dewey et al., 1998). Group 2 animals received GVG alone (300 mg/kg) 2.5 hours prior to the second injection of ^{11}C -raclopride. Like Group 1 animals, these data have been reported previously (Dewey et al., 1992). Group 3 animals received nicotine alone (0.3 mg total, approximately 0.02 mg/kg) 30 minutes prior to the second injection of ^{11}C -raclopride. In the combined GVG/nicotine studies, GVG was administered intravenously (i.v.) at doses of 100 (Group 4) or 300 mg/kg (Group 5) 2.5 hours prior to nicotine administration. Nicotine (0.3 mg total, i.v.) was administered 30 minutes prior to the second injection of ^{11}C -raclopride. Arterial blood samples were obtained throughout the study and selected plasma samples were analyzed for the presence of unchanged ^{11}C -raclopride. Animals were not removed from the gantry between isotope injections. Data analysis was performed using the Logan method as detailed previously (Logan et al., 1990).

RESULTS

Microdialysis studies

In Group 1 animals, nicotine elevated extracellular DA concentrations in the NAc by approximately 100% 80 minutes following administration (Fig. 1A). That is, DA levels were elevated to approximately 200% of basal levels. DA returned to basal levels approximately 160 minutes following administration. GVG dose-dependently inhibited this increase as seen in Figure 1A. At 75 mg/kg, GVG had no effect on nicotine-induced increases in DA while at 90 mg/kg, GVG inhibited this response by approximately 50% and at 100 mg/kg it completely abolished this effect. The highest dose of 150 mg/kg completely abolished the effects as well (data not shown). Of particular note is the finding that at the three higher doses (90, 100, or 150 mg/kg) GVG lowered basal DA levels prior to nicotine administration. The lowest dose (75 mg/kg) had no effect on basal DA levels

A.



B.

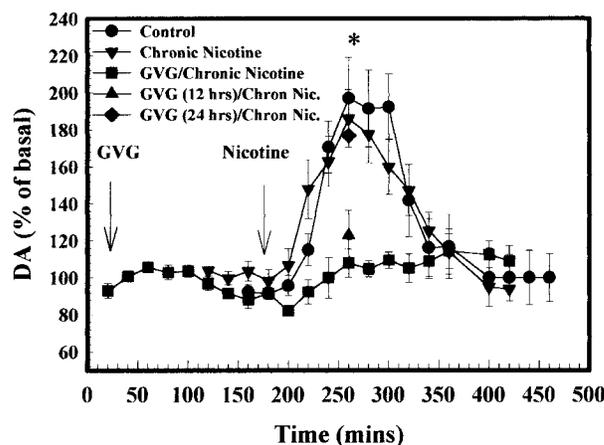


Fig. 1. **A:** Effect of (-)-nicotine on extracellular NAc DA in naive freely moving rats. *Values obtained following GVG (75 mg/kg) are not statistically different than normal controls (ANOVA and Student Neuman-Keuls test, $P > .1$). ** Values are statistically different ($P < 0.01$) from control values (ANOVA and Student Neuman-Keuls test). *** Values are statistically different ($P < 0.01$) from control values (ANOVA and Student Neuman-Keuls test). **B:** Effect of (-)-nicotine on extracellular NAc DA in chronically treated freely moving rats. *Values obtained following nicotine challenge in nicotine-treated animals and animals that received GVG (100 mg/kg) 24 hours prior to a nicotine challenge are not statistically different than normal control values (ANOVA and Student Neuman-Keuls test).

and subsequently no effect on nicotine's ability to elevate extracellular NAc DA.

In Group 2 animals, nicotine increased extracellular NAc DA levels within the same time period and to the same extent measured in Group 1 animals (approximately 100% above baseline, Fig. 1B). Similar to our findings in Group 1, when administered 2.5 hours prior to nicotine administration GVG (100 mg/kg) completely abolished nicotine-induced increases in extracellular DA. However, when administered 12 hours prior to challenge, nicotine increased extracellular DA levels approximately 25% above baseline values (Fig. 1B). In

TABLE III. Effect of saline and GVG on expression of conditioned place preference response to 0.4 mg/kg s.c. of (-)-nicotine

Treatment pairings	Drug given on test day	Time spent in chambers (min) ¹	
		Paired	Unpaired
saline/saline	Saline ²	7.4 ± 0.3	7.6 ± 0.3
saline/nicotine	Saline	9.6 ± 0.6	5.4 ± 0.6
saline/nicotine	GVG, 18.75 mg/kg	7.5 ± 0.7*	7.5 ± 0.7
saline/nicotine	GVG, 37.5 mg/kg	6.8 ± 1.0**	8.2 ± 1.0
saline/nicotine	GVG, 75 mg/kg	6.4 ± 0.3**	8.6 ± 0.3
saline/nicotine	GVG, 150 mg/kg	5.0 ± 0.9**	10.0 ± 0.9

¹Each value represents the mean number of minutes spent in each chamber ± S.E.M. A total of 8–10 rats were examined for each treatment pairing. All animals received 8 pairings with nicotine and saline prior to the test day. On the test day, animals received either saline or GVG 2.5 hours before being placed into the CPP apparatus.

²Saline was 1 ml/kg s.c. of 0.9% saline.

*Significantly less than Saline/Nicotine pairing with saline on test day, $P < 0.05$, ANOVA and Student-Newman-Keuls test.

**Significantly less than Saline/Nicotine pairing with saline on test day, $P < 0.01$, ANOVA and Student-Newman-Keuls test.

Group 2 animals that received GVG 24 hours prior to nicotine challenge, extracellular DA levels increased to values similar to those measured in control animals (Fig. 1B). Consistent with our previous findings (Dewey et al., 1997), GVG did not alter gross locomotor activity during the 2.5-hour pretreatment interval. However, nicotine increased gross locomotor activity in all animals regardless of the dose of GVG they received.

Nicotine-induced CPP studies

As previously reported, the administration of saline did not produce a chamber preference (Tables III and IV; Dewey et al., 1998). However, nicotine (0.4 mg/kg s.c.) produced a significant and reliable CPP response where animals spent 9.6 ± 0.6 minutes on the paired (nicotine) side compared with 5.4 ± 0.6 minutes on the unpaired (saline) side (Tables III and IV). Statistical analysis of the expression data indicated a treatment effect ($F(5, 50) = 21.6$, $P < 0.001$). Post hoc analysis revealed that GVG at doses of 18.75, 37.5, 75.0, or 150 mg/kg but not saline, abolished the expression phase of nicotine-induced CPP (Table III).

Analysis of the acquisition data indicated a treatment effect ($F(3,32) = 11.8$, $P < 0.05$). Post hoc analyses indicated that GVG (37.5 mg/kg) did not significantly block the acquisition of the nicotine-induced CPP (Table IV) but that at 75 mg/kg, GVG significantly blocked the acquisition phase of nicotine-induced CPP (Table IV).

Primate PET studies

Each primate ($n = 16$) received two ¹¹C-raclopride injections. The first served as a baseline for the second that followed GVG, nicotine, or both. Test/retest primates ($n = 7$, Group 1, Table I) received placebo (0.9% saline, 1 ml/kg) 30 minutes prior to the second radiotracer injection in order to determine the test/retest variability of the method. All remaining primates ($n = 9$) received a systemic injection of GVG, nicotine, or both prior to the second [¹¹C]-raclopride injection.

TABLE IV. Effect of saline and GVG on acquisition of conditioned place preference response to 0.4 mg/kg s.c. of (-)-nicotine

Treatment pairings	Time spent in chambers (min) ¹	
	Paired	Unpaired
saline/saline ²	7.3 ± 0.3	7.7 ± 0.3
saline/nicotine	9.6 ± 0.6	5.4 ± 0.6
nicotine/GVG, 37.5 mg/kg i.p.	8.8 ± 0.5	6.2 ± 0.5
nicotine/GVG, 75 mg/kg i.p.	6.9 ± 0.9*	8.1 ± 0.9

¹Each value represents the mean number of minutes spent in each chamber ± S.E.M. A total of 8–10 rats were examined for each treatment pairing. Animals were pretreated with either saline, 37.5 or 75 mg/kg i.p. of GVG and 2.5 hours later, each animal received 0.4 mg/kg s.c. of nicotine, except for one group, which received saline followed by saline treatment (saline/saline pairing). Eight pairings were performed with each animal.

²The saline was 1 ml/kg s.c. of 0.9% saline.

*Significantly less than Saline/Nicotine pairing with saline on test day, $P < 0.05$, ANOVA and Student-Newman-Keuls test.

As reported previously (Dewey et al., 1998), the test/retest mean distribution volume (DV) ratio variability of labeled raclopride in the primate striatum was slightly greater than 7% (Table II). The DV is a measurement of receptor availability. GVG administration (300 mg/kg, Group 2) significantly increased the mean DV ratio by 18% (Table II). These data are consistent with microdialysis studies demonstrating that GVG dose-dependently decreases extracellular DA in freely moving animals. Nicotine administration (Group 3), however, produced the opposite effect of GVG and significantly reduced the mean DV ratio by 12% (Table II). This is again consistent with our microdialysis data demonstrating that nicotine increases extracellular DA in freely moving animals. When administered sequentially, GVG (100 mg/kg, Group 4) abolished the decrease in the mean DV ratio produced by nicotine alone (Group 3). At this dose of GVG, the mean DV ratio was similar to the test/retest value obtained in Group 1 animals (9%, Table II). However, when administered at a dose of 300 mg/kg (Group 5), the mean DV ratio for labeled raclopride was significantly higher (15%) than the test/retest values and was in fact, similar to the values obtained in Group 2 animals that received GVG alone (Table II). GVG, nicotine, or both did not alter the rate of systemic metabolism of labeled raclopride nor the regional distribution of the radiotracer. Recovery from each study was unremarkable.

DISCUSSION

In the present study, nicotine (0.4 mg/kg s.c.) increased NAc DA by approximately 100% (or 200% above baseline) in freely moving animals approximately 80 minutes following administration. Previous microdialysis studies in male Sprague-Dawley animals reported that higher doses of nicotine (0.6 or 0.8 mg/kg, s.c.) produced a 220 and 179% increase in extracellular DA levels in the NAc, respectively (Brazell et al., 1990; Di Chiara and Imperato, 1988; Imperato et al., 1986). Although not directly comparable, our results appear to agree with previous data obtained with s.c. nicotine.

Furthermore, in our animals exposed chronically to nicotine, a nicotine challenge produced a 90% increase in extracellular NAc DA levels. This finding is consistent with previous data indicating that chronic nicotine administration does not produce tolerance or sensitization to an acute challenge with nicotine (Damsma et al., 1989).

With respect to our findings using GVG, we demonstrated that it dose-dependently inhibited nicotine-induced increases in NAc DA in both naïve and chronically nicotine treated animals. This is the first study to report such an action of GVG. At a dose of 75 mg/kg, GVG had no effect as nicotine increased extracellular DA by nearly 200% while a dose of 90 mg/kg produced an inhibition of nearly 50%. At the two highest doses examined (100 and 150 mg/kg), GVG completely abolished nicotine-induced increases in extracellular NAc DA levels. Previously, we demonstrated that an acute injection of GVG (300 mg/kg i.p.) produced a 25% decrease in cocaine-induced increases in NAc DA (Dewey et al., 1998). However, chronic treatment with GVG, at a similar dose, produced a greater inhibition (Morgan and Dewey, 1998). Together these data suggest that the dose of GVG needed to attenuate drug-induced increases in NAc DA levels is dependent not only on the challenge drug used (e.g., cocaine, nicotine), but also on the dose at which the challenge drug is administered. Further support for this statement is evidenced by our recent work with methamphetamine and alcohol. GVG administration, 300 or 600 mg/kg, reduced by approximately 35 and 58%, respectively, increases in extracellular NAc DA levels following methamphetamine challenge (2.5 mg/kg i.p.) while GVG (300 mg/kg) reduced by 55% increases in NAc DA following alcohol administration (unpublished data). Thus, the rank order of nicotine, cocaine, alcohol, and methamphetamine to increase NAc DA levels is methamphetamine (2,500%) > cocaine (450%) > alcohol (280%) > nicotine (90%), which parallels the rank order of the size of an acute dose of GVG needed to decrease drug-induced increases in NAc DA.

The present data further demonstrate that the effectiveness of GVG is related to its dose-dependent ability to lower basal DA concentrations prior to drug challenge. For example, the 75 mg/kg dose had no effect on basal DA and on nicotine-induced increases in DA. However, at a dose of either 90 or 100 mg/kg, GVG lowered basal DA levels and reduced by 50% or abolished the effects of nicotine, respectively. Therefore, it appears that the dose-dependent attenuation of either nicotine or cocaine-induced increases in NAc DA is due to a pre-lowering of basal DA concentrations, subsequent to an increase in endogenous GABA produced by GVG. This is consistent with data indicating that augmentation of GABAergic function reduces DA in the NAc.

In an extension of our previous work with GVG and cocaine, we examined the temporal course of GVG's effects on nicotine-induced increases in NAc DA in animals chronically treated with nicotine for 21 days. When administered 2.5 hours prior to nicotine at a dose of 100 mg/kg, GVG completely abolished drug-induced increases in NAc DA. However, when administered at the same dose 12 hours prior to challenge, nicotine increased extracellular DA by approximately 25%. Administered 24 hours prior to nicotine challenge at the same dose, GVG had no effect on nicotine-induced increases in NAc DA. Clearly, our microdialysis and behavioral data suggest that even small changes in GABA-T inhibition produced by increasing doses of GVG have a profound effect on the inhibition of nicotine-induced elevations in NAc DA and CPP, respectively. These data are particularly interesting in light of the synthesis rate of GABA-T, the half-life of GVG in the rodent brain, the duration of the effect on GABA, and the sharp dose response curve detailed here. Previous findings demonstrate that the biologic half-life of GABA-T in the rodent brain is 3.4 days while the half-life of GVG in the brain is approximately 16 hours. In addition, total brain GABA levels do not begin to decrease until 24 hours following acute GVG administration (Jung et al., 1977). The disparity between the sustained brain GABA levels measured 24 hours following a single dose of GVG and the normal response to a nicotine challenge observed at the same time point suggests that GABAergic inhibition of the mesotelencephalic reward pathway may not be a simple reflection of total brain GABA levels. That is, while total brain GABA levels are still significantly elevated 24 hours following an acute dose of GVG, small functional differences in specific pathways may be masked by these global measurements. Alternatively, it is conceivable that GABA receptors have become desensitized to GABA over the 24-hour period, although there is no direct evidence in the GABA system to support such a conjecture. Finally, it is possible that some compensatory changes occurred efferent to the GABAergic neurons.

In the present study, we demonstrated that 8 saline-nicotine pairings produced a reliable CPP response. Our results are in agreement with previous studies indicating that nicotine (0.1–1.2 mg/kg s.c.) produces a dose-dependence CPP response in male Sprague-Dawley animals (Fudala et al., 1985; Fudala and Iwamoto, 1986). We have also shown that Lewis, but not F344 animals, show a CPP response to nicotine after 10 pairings (Horan et al., 1997). However, a previous report has shown that 4 nicotine-vehicle pairings did not elicit a CPP response in male hooded animals (Clarke and Fibiger, 1987). Thus, it appears that nicotine-induced CPP may be strain-specific, although this may be confounded by the fact that the studies quoted utilized different numbers of pairings. The nicotine-induced CPP response reported in the

present study is consistent with the notion that nicotine produces a positive effect on incentive motivational behavior.

This is the first study demonstrating that GVG can block the biochemical and behavioral effects of nicotine using the CPP paradigm. The CPP data clearly indicate that, at a dose as low as 18.75 mg/kg, GVG abolishes the expression of the CPP response produced by nicotine. Our data also indicate that a dose of 75 mg/kg, but not 37.5 mg/kg, blocked the acquisition of the CPP response to nicotine. Based on these dose findings, the dose of GVG needed for the treatment of smoking cessation might be predicted to be a total of 250–500 mg a day (compared with 2–4 g/day for epilepsy), a range considerably lower than that given to epileptics. The effects of GVG on nicotine-induced CPP are unlikely to be related to its producing a rewarding or aversive effects directly as we have previously shown that GVG alone (75–300 mg/kg i.p.) does not produce CPP or aversion (Dewey et al., 1998). Furthermore, it is unlikely that GVG abolishes nicotine's behavioral actions by interfering with memory or locomotor activity of the animals as GVG does not block food reward or locomotor activity at doses as high as 300 mg/kg (Dewey et al., 1998). Finally, it has been shown that GVG is not self-administered by rhesus monkeys and animals withdrawn from chronic GVG treatment do not exhibit withdrawal signs or symptoms (Takada and Yanagita, 1997). Thus, GVG, unlike other drugs used in the pharmacotherapy of certain addictions (e.g., methadone, antabuse), is itself not addicting and does not produce significant aversive effects.

The attenuation of the acquisition of the CPP response to nicotine by GVG may be interpreted as a decrease in the positive incentive value of nicotine. These data suggest that GVG decreases the likelihood that an animal will acquire the association of a positive incentive effect following nicotine administration. Interestingly, our results indicated that the dose of GVG required to block the expression phase of the CPP response produced by nicotine was $\frac{1}{4}$ of the amount needed to block the acquisition of the CPP response. This finding is congruent with our previous data indicating that a higher dose of GVG was required to block the acquisition, as opposed to the expression of CPP to cocaine (Dewey et al., 1998). The explanation for this difference is unknown. Since GVG attenuates the expression of the CPP response to nicotine, one might hypothesize that GVG is decreasing the drug-seeking behavior of the animal as the animal has already acquired the positive incentive value of the drug. Thus, tentatively, our data suggest that GVG may be more effective in blocking the craving for nicotine than it is at blocking the positive incentive value or rewarding action of nicotine. This is consistent with the concept that the "liking" or pleasurable effects of a drug are different from those related to "wanting" or craving

(Robinson and Berridge, 1993). Finally, at the highest dose tested, 150 mg/kg, GVG produced a significant aversive response on the test day (Table IV) where animals spent 5.0 ± 0.9 minutes on the paired (nicotine) side and 10.0 ± 0.9 minutes on the unpaired (saline) side. These data suggest that there might be a ceiling effect at which GVG in high doses becomes aversive in animals treated with nicotine and tested in a drug-free state. These data may have implications in developing the dose limits to be tested in human clinical trials.

The exact implications of our finding that GVG blocks the expression of nicotine-induced CPP are not clear. However, the following argument can be made. In the CPP paradigm, animals are tested in a drug-free state to determine whether they prefer an environment in which they previously received nicotine as compared to an environment in which they previously received saline. If the animal, in a drug-free state, consistently chooses the environment previously associated with nicotine, the inference is drawn that the appetitive value of nicotine was encoded in the brain and is accessible in the drug-free state (Gardner, 1997). Indeed, on the test day, the approach and association of the animals with the drug-paired side may be considered drug-seeking behavior. In essence, environmental stimuli and other cues that were previously neutral or lacked salience have through repeated pairings with nicotine, become salient. Subsequently, when the animals are re-exposed to these cues, a CPP response is produced, i.e., the cues can elicit the drug effect. Thus, drug-related cues produce a Pavlovian conditioned response. This is critical as it is known that non-pharmacologic factors, in addition to pharmacologic ones, play a role in mediating the incentive value of drugs of addiction (Jarvik and Henningfield, 1988; Robinson and Berridge, 1993). In fact, it has been demonstrated clinically that in detoxified addicts, exposure to stimuli that were previously associated with drug use, can elicit relapse (Childress et al., 1986a,b, 1988; Ehrman et al., 1992; O'Brien et al., 1992; Wikler, 1965). Thus, a hypothetical clinical extension of these findings would be that since GVG blocks the expression of the nicotine-induced CPP response, one might hypothesize that GVG would block nicotine craving or seeking. Therefore, we hypothesize that GVG may prove effective in the treatment of individuals who have the desire to stop smoking cigarettes. We believe that GVG may be particularly effective as it abolishes the expression of the CPP response to nicotine and, based on the above discussion, should attenuate craving in the face of environmental cues previously associated with smoking. However, our hypothesis can only be validated by conducting the appropriate clinical trials with GVG to determine its effectiveness in preventing relapse to cigarette smoking. It should be pointed out that other non-pharmacologic factors, such as the taste of the

smoke, play a role in cigarette smoking (Jarvik and Henningfield, 1988) and the effect of GVG on these is currently unknown. Therefore, simply blocking the pharmacologic action of nicotine may be an inadequate approach to stop smoking. Interestingly, at this time, bupropion (Zyban), a drug that is currently being used to aid people in their effort to stop smoking (Goldstein, 1998; Hurt et al., 1997), has not been tested for its efficacy in blocking the expression or acquisition phases of nicotine-induced CPP.

Our primate PET data are consistent with previous findings using multiple pharmacologic challenges that demonstrate ^{11}C -raclopride binding is sensitive to both increases and decreases in synaptic DA (Dewey et al., 1993a; Seeman et al., 1989). As evidenced in Group 3 animals (Table II), the mean DV ratio was consistently decreased relative to baseline values following nicotine administration. This decrease exceeded the test/retest variability of labeled raclopride and is less than the decrease measured with GBR-12909 (Dewey et al., 1993a) or scopolamine (Dewey et al., 1993b). Pretreatment with GVG at a dose of 100 mg/kg 2.5 hours prior to nicotine produced a mean DV ratio similar to Group 1 animals (Table II). However, when the dose of GVG was increased to 300 mg/kg, the mean DV ratio was elevated to values consistent with Group 2 animals. These data suggest that the lower dose of GVG produced a decrease in synaptic DA roughly equivalent to the increase produced by nicotine while the higher dose of GVG produced a decrease that far exceeded nicotine's ability to increase DA. Our microdialysis studies support this hypothesis as higher doses of GVG produce a greater decrease in extracellular DA in freely moving animals.

The microdialysis and PET findings combined with the CPP data provide unique insight into the long held theory that increases in DA in the NAc alone underlie the addictive liability of drugs of abuse. First, these data, combined with our earlier work using cocaine, suggest that *in vivo* microdialysis studies or PET measurements of endogenous DA alone may not necessarily be predicative of the efficacy of drugs used to treat diseases thought to be neurotransmitter-specific in nature. Second, both the microdialysis data and the PET data clearly demonstrate that at a dose of 100 or 150 mg/kg, GVG completely blocked nicotine-induced increases in NAc DA levels, whereas a dose of 75 mg/kg had no effect. In contrast, GVG, at a dose as low as 18.75 mg/kg, completely abolished the expression phase of nicotine-induced CPP while it took a dose of 75 mg/kg to abolish the acquisition phase. Based upon the dose-response curve obtained from the microdialysis data, GVG at a dose of 18.75 mg/kg would not be expected to have any effect on nicotine-induced increases in NAc DA. Furthermore, we observed a similar effect using cocaine where a dose of 300 mg/kg of GVG reduced cocaine-induced increases in NAc DA levels by 25 %

while a dose of 150 mg/kg completely abolished the expression and acquisition phase of cocaine-induced CPP (Dewey et al., 1997, 1998). Together, these data suggest at least two plausible and perhaps combined explanations. First, differential changes in DA following pharmacologic challenge in regions other than the NAc alone may be responsible for the addictive liability of a particular drug. Indeed, it has been reported that various addictive drugs can alter DA levels in brain areas other than the NAc including the amygdala, corpus striatum, and frontal cortex, (Chen et al., 1990; Dewey et al., 1997; Di Chiara and Imperato, 1988; Hurd et al., 1997; Marshall et al., 1997). Second, neurotransmitters other than DA may play a vital role in the addictive liability of drugs of abuse (Mansbach et al., 1998). For example, a CPP response to cocaine is still maintained in mice that lack the DA and 5-HT transporters (Rocha et al., 1998; Sora et al., 1998). Furthermore, it is known that neurotransmitters such as 5-HT, acetylcholine, enkephalins, and glutamate, play a role in mediating the effects of addictive drugs, including nicotine (Bardo, 1998; Gardner, 1997). Taken together, these data suggest that GVG may be inhibiting the effects of cocaine and nicotine through changes in DA in regions other than the NAc. Concomitantly, GVG may be inhibiting other neurotransmitters that either modulate DA directly or are themselves involved in mediating the effects of drugs of addiction. Further studies designed to assess the multiple effects of GVG on other neurotransmitters are ongoing.

Previously, we demonstrated that the ability of GVG to attenuate cocaine-induced increases in NAc DA is completely abolished by pretreating animals with the selective GABA_B receptor antagonist SCH 50911 (Bolser et al., 1995), a drug that does not significantly alter DA levels when given alone (Ashby et al., 1999). Therefore, we can hypothesize that GVG abolishes the action of nicotine via its increase in GABA levels, which subsequently stimulates GABA_B receptors. This is consistent with data indicating that the administration of baclofen, a selective GABA_B agonist (Bowery and Pratt, 1992; Kerr et al., 1990), into the VTA significantly attenuates the CPP response in animals produced by systemic morphine (Tsuji et al., 1995). Furthermore, systemic administration of baclofen attenuates cocaine self-administration in rats on either progressive ratio or discrete trials reinforcement schedules (Roberts et al., 1996; Roberts and Andrews, 1997). One might argue that GVG attenuates the pharmacologic and behavioral actions of nicotine simply by altering the amount that effectively enters the brain either by changing blood brain barrier permeability or by increasing the systemic rate of metabolism of nicotine. This possibility is unlikely for a number of reasons. First, GVG had no effect on the blood brain barrier transport of ^{11}C -cocaine, an alkaloid previously shown to increase NAc DA, in both the rodent or primate brain. Second,

GVG is excreted primarily in the unchanged form by the kidneys (Grant and Heel, 1991; Porter and Meldrum, 1998), whereas nicotine is metabolized by enzymes in the liver. Finally, GVG does not interact with hepatic microsomal enzymes (Grant and Heel, 1991; Porter and Meldrum, 1998) and thus would not induce or inhibit these enzymes.

The size of the NAc is well below the resolution of our tomograph, making its specific analysis outside the capabilities of this technique. Therefore, our analysis included the corpus striatum bilaterally and the cerebellum. Marshall et al. (1995) have demonstrated that nicotine increased DA equally in both the NAc and the corpus striatum, while our own microdialysis data demonstrates that GVG decreases DA concentrations equally in both regions as well (Dewey et al., 1997). These primate data further support the use of this imaging technique to evaluate the functional consequences of pharmacologic challenges in the living brain. Furthermore, this medical imaging technique provides a unique window into the interactions that have been shown to exist between functionally-linked neurotransmitters in both the primate and human brain.

Combined with an exhaustive literature supporting the fundamental principle that neurotransmitters interact in both functionally-specific and regionally specific neuroanatomic foci, it is becoming increasingly clear that new treatment strategies for brain disorders (including addictions to cocaine, nicotine, heroin, and methamphetamine) should be developed with a more global awareness of this fundamental and well-documented principle. While changes in individual neurotransmitter concentrations may indeed underlie the etiology of a specific disorder, it is likely that disease progression and symptom development are linked to compensatory or disease-induced changes in other neurotransmitters functionally linked to the original target. With this knowledge, we have been developing novel treatment strategies specifically designed to alter one or more neurotransmitters by targeting another. Our findings with nicotine, cocaine, methamphetamine, alcohol, and GVG represent the potential utility of such a fundamental approach.

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