The Smoking Cessation Medication Varenicline Attenuates Alcohol and Nicotine Interactions in the Rat Mesolimbic Dopamine System

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ABSTRACT

Varenicline was recently approved as an aid for smoking cessation. Patients treated with varenicline have reported a concomitant reduction in their alcohol consumption. This compound has also been demonstrated to reduce alcohol seeking and consumption in alcohol high-prefering rats. Based on the extensive coabuse of nicotine and alcohol, the aim of the present study was to explore whether interactions among varenicline, nicotine, and ethanol in the brain reward system could indicate the use of varenicline also for alcohol dependence. Using the in vivo microdialysis method, we investigated the effects of systemic injections of varenicline on the extracellular accumbal dopamine levels in response to a systemic challenge of ethanol, nicotine, or the combination of nicotine and ethanol in the experimental rat. Acute systemic coadministration of varenicline and ethanol counteracted each others’ respective enhancing effect on dopamine levels in the nucleus accumbens. However, after 5 days of varenicline pretreatment, acute combined varenicline and ethanol administration raised dopamine levels to the same extent as either drug alone. Furthermore, after varenicline pretreatment an acute injection of varenicline antagonized the dopamine stimulatory effect of acute nicotine as well as that of systemic coadministration of ethanol and nicotine. In contrast, a pronounced additive dopamine increase was observed when nicotine and ethanol were coadministered in vehicle-pretreated rats. The antismoking agent varenicline exhibits properties with respect to its interaction with ethanol and nicotine in the brain reward system that may be beneficial for treating patients with alcohol dependence with (and possibly also without) concomitant nicotine dependence.

Alcoholism is a worldwide problem causing considerable suffering to the individual and enormous costs to society. Three pharmacological compounds are available for the treatment of alcohol dependence (acamprosate, naltrexone, and disulfiram). However, in many patients, these drugs have low or no efficacy, and there is a worldwide increasing need for better pharmacotherapy for this indication.

There is a strong correlation between nicotine- and alcohol addiction, in which, for example, high alcohol consumption is associated with a high consumption of nicotine, and vice versa (Zacny, 1990; Daeppen et al., 2000). Extensive research demonstrates that the association between smoking and drinking cannot exclusively be explained by environmental or psychosocial factors. Nicotinic acetylcholine receptors (nAChRs) constitute a common point of action for nicotine and alcohol in their ability to stimulate the mesolimbic dopamine system, an important part of the brain reward system (Blomqvist et al., 1993; for review, see Söderpalm et al., 2000). In the experimental rat, systemic nicotine or alcohol administration elevates extracellular dopamine levels in the nucleus accumbens (nAc), an effect that requires stimulation of nAChRs in the ventral tegmental area (VTA), in which the mesolimbic dopaminergic cell bodies are located (Blomqvist et al., 1997; Tizabi et al., 2002; Ericson et al., 2003). Moreover, in vitro studies demonstrate that, at behaviorally relevant concentrations, ethanol can act as an allosteric modulator stimulating recombinant human (Cardoso et al., 1999) and rat (Covernton and Connolly, 1997) α4β2* nAChR sub-
types. As a consequence, nAChRs have been proposed to be potential targets for novel selective pharmacological interventions aimed at reducing craving and relapse in alcoholism (Blomqvist et al., 1993; Söderpalm et al., 2000; Balogh et al., 2002; Ericson et al., 2003; Larsson et al., 2004; Jerlhag et al., 2006; Löf et al., 2007). In line with this suggestion, the nonselective nAChR antagonist mecamylamine reduces voluntary alcohol-intake in the experimental rat (Blomqvist et al., 1996; Ericson et al., 1998; Lé et al., 2000). Moreover, clinical studies demonstrate that mecamylamine reduces the euphoric and stimulant subjective effects of acute alcohol intoxication in social drinkers and decreases the subjects’ desire to drink more (Blomqvist et al., 2002; Chi and de Wit, 2003; Young et al., 2005). This nAChR antagonist is, however, associated with substantial side effects, emphasizing the need for new, more selective nAChR modulators for the treatment of alcoholism.

Recently, varenicline was registered as a new aid for smoking cessation. It acts as a partial agonist at the α4β2* nAChR subtype, slightly increasing the activity of the mesolimbic dopamine system to alleviate the nicotine withdrawal symptoms, while blocking the reinforcing dopamine-stimulatory properties of nicotine (Coe et al., 2005; Rollema et al., 2007). The clinical efficacy of varenicline (Cahill et al., 2007) is attributed to this dual action of the partial agonist. Several anecdotal reports suggest that patients treated with varenicline to reduce their smoking have reported a concomitant reduction in their alcohol consumption. Moreover, varenicline was recently demonstrated to reduce alcohol seeking and consumption in alcohol high-prefering rats (Steensland et al., 2007).

Using the in vivo microdialysis method, the present study investigated the effects of systemic acute or subchronic injections of varenicline on the accumbal dopamine response to a systemic challenge of alcohol, nicotine, or the combination of nicotine and alcohol in the experimental rat.

**Materials and Methods**

**Animals.** Male Wistar rats (Beekay, Sollentuna, Sweden or Tacconic, Ry, Denmark) weighing 270 to 350 g were housed four per cage (55 × 35 × 20 cm) at constant room temperature (22°C) and humidity (65%). The animals were kept under regular light/dark conditions (lights on at 7:00 AM and off at 7:00 PM) and had free access to “rat standard feed” (Harlan UK Limited, Bicester, Oxon, UK) and tap water. In all experiments, drug-naive animals were used. Separate groups of rats were used for each drug/concentration. Animals were allowed to adapt for 1 week to the novel environment before any experiment was performed. This study was performed in agreement with the Declaration of Helsinki and was approved by the Ethics Committee for Animal Experiments, Göteborg, Sweden (337/06).

**Drugs.** Ethanol (Kemetyl AB, Hanninge, Sweden), varenicline (6,7,8,9-tetrahydro-6,10-methano-6H pyrazino[2,3-h][3]benzazepine tartrate; kindly provided by Pfizer Global Research and Development, Groton, CT), and nicotine [nicotine hydrogen tartrate; (S)-3-(1-methyl-2-pyrrrolidinyl)pyridine; Sigma-Aldrich, St. Louis, MO] were all dissolved in 0.9% NaCl and administered systemically. Nicotine doses are expressed as free base.

**Microdialysis Technique.** Brain microdialysis experiments were performed in awake and freely moving animals. Rats were anesthetized by isoflurane, mounted into a stereotaxic instrument (David Kopf Instruments, Tujunga, CA), and put on a heating pad to prevent hypothermia during the surgery. Holes were drilled for the placement of two anchoring screws and an I-shaped dialysis probe custom made in the laboratory. The dialysis probe was lowered into the nAc (AP, +1.85; ML, −1.4 mm relative to bregma; DV, −7.8 mm relative to dura) (Paxinos and Watson, 2005). The dialysis probes were placed in the core-shell borderline region (suggesting that sampling was done in both the core and the shell of the nAc), and the probes and the anchoring screws were fixed to the skull with Harvard cement (Dibo Dental AB, Göteborg, Sweden). After surgery, the rats were allowed to recover for 2 days before the dialysis experiments were initiated. On the experimental day, the sealed inlet and outlet of the probes were cut open and connected to a micro perfusion pump (U-864 syringe pump; AgnTho’s, Lidingo, Sweden) via a swivel allowing the animal to move around freely. The probe was perfused with Ringer’s solution at a rate of 2 μl/min, and dialysate samples (40 μl) were collected every 20 min. The probes were perfused with Ringer’s solution for 1 h to obtain a balanced fluid exchange before baseline sampling began. Animals were sacrificed directly after the experiment, brains were removed, and probe placements were verified using a vibroslicer (Campden Instruments Ltd., Leicester, UK) (Fig. 1).

**Biochemical Assay.** To analyze the dopamine content of the dialysate samples, a high-pressure liquid chromatography system with electrochemical detection was used for the separation and detection of dopamine as described previously (Ericson et al., 2003). To identify the dopamine peak, an external standard was used containing 2.64 fmol/μl dopamine. When at least three consecutive stable values of dopamine were obtained (±5%), the first drug was introduced.

**Experimental Design.** In the first study, we explored the effects of acute application of varenicline on extracellular dopamine levels in the nAc. The animals received an injection of saline or varenicline (0.75, 1.5, 3, or 6 mg/kg s.c.), and dialysate samples were collected for an additional 180 min. The varenicline doses were chosen based on dopamine turnover in the nAc in one of the original articles describing the effects of varenicline (Rollema et al., 2007). In the second set...
of animals, we explored the possible interaction between varenicline and ethanol. These animals received either varenicline 1.5 mg/kg s.c. + 0.9% NaCl i.p., 0.9% NaCl s.c. + ethanol 2.5 g/kg i.p., varenicline 1.5 mg/kg s.c. + ethanol 2.5 g/kg i.p., or 0.9% NaCl s.c. + 0.9% NaCl i.p., and the dopamine levels were monitored for 3 h.

In the third experiment, the animals were redosed with either 0.9% NaCl s.c. or varenicline 1.5 mg/kg s.c. in the afternoon for 5 consecutive days. This treatment paradigm was used to study the effects of varenicline after repeated administration, a subchronic paradigm resembling the one used for aiding human smoking cessation. On the day of the dialysis experiment, the animals received a systemic injection of either saline or varenicline (1.5 mg/kg s.c.), 60 min before nicotine 0.35 mg/kg s.c. + saline 0.1 ml/kg i.p., saline 0.1 ml/kg s.c. + ethanol 2.5 g/kg i.p., or nicotine 0.35 mg/kg s.c. + ethanol 2.5 g/kg i.p., after which dopamine levels were monitored for 3 h.

Statistics. Data were statistically evaluated using a two-way ANOVA with repeated measures (treatment group × time) followed by Fisher’s protected least significant difference (PLSD) test or using a paired t test. Multiple t test comparisons were corrected for with Holm’s sequential rejection procedure, a weighted improvement of the Bonferroni correction (Holm, 1979). A probability value (p) less than 0.057 was considered statistically significant. All values are expressed as means ± S.E.M.

Results

Dose-Dependent Effect of Varenicline. The lowest and the highest doses of varenicline (0.75 and 6 mg/kg) did not influence the dopamine output significantly compared with saline administration. However, the intermediate doses (1.5 and 3 mg/kg s.c.) elevated and tended to elevate extracellular dopamine levels in the nAc to 55% (p < 0.05) and 30% (N.S., p = 0.057) of basal levels, respectively (Fig. 2).

Effect of 1.5 mg/kg Varenicline on Ethanol-Induced Dopamine Release. We investigated the possible influence of varenicline on ethanol-induced elevation of dopamine levels in the nAc with 1.5 mg/kg varenicline, a dose that produced a robust significant dopamine response. A repeated measures ANOVA demonstrated a drug effect (F[3,26] = 4.858, p < 0.05), a time effect (F[6,156] = 4.379, p < 0.001), and a time × drug interaction (F[6,156] = 2.966, p < 0.001) (Fig. 3). Separate administration of varenicline or ethanol produced significant elevations of nAc dopamine compared with saline injections (Fisher’s PLSD test: saline versus varenicline 1.5 mg/kg, p < 0.05; saline versus ethanol 2.5 g/kg, p < 0.05) (Fig. 3). However, when the two drugs were administered concomitantly, they counteracted each other’s dopamine enhancing properties (Fisher’s PLSD test: saline versus varenicline + ethanol, p = 0.4922; varenicline + ethanol versus varenicline, p < 0.05; varenicline + ethanol versus ethanol, p < 0.05) (Fig. 3).

Effect of Semichronic Varenicline on Ethanol-Induced Dopamine Release. In the semichronic study, animals were pretreated with either saline or varenicline (1.5 mg/kg) once a day for five consecutive days. On the day of the dialysis experiment, baseline dopamine levels were recorded before drug administration. There were no differences in basal dopamine levels in animals pretreated with saline (3.18 ± 0.388 fmol/μl) compared with varenicline (2.32 ± 0.298 fmol/μl). Animals that were pretreated with saline received an acute saline injection, and animals that were pretreated with varenicline received an acute varenicline injection. One hour later, all animals were injected with either nicotine (0.35 mg/kg s.c.), ethanol (2.5 g/kg i.p.), or nicotine plus ethanol, and nAc dopamine levels were monitored for another 3 h. A repeated measures ANOVA revealed a significant drug effect (F[5,36] = 2.911, p < 0.05), a time effect (F[12,432] = 20.786, p < 0.0001), and a time × drug interaction (F[60,432] = 1.833, p < 0.001) (Fig. 4).

Varenicline produced a significant increase in nAc dopamine levels in varenicline-pretreated animals acute at time points 20, 40, and 60 min compared with time point 0 min (p < 0.001 in all time points, paired t tests corrected for multiple comparisons) (Fig. 4, A and B). In saline-pretreated

Fig. 2. Effect of saline or varenicline (0.75, 1.5, 3, or 6 mg/kg s.c.) on extracellular dopamine levels in the nAc as measured by in vivo microdialysis in awake, freely moving rats. Drugs were administered as indicated by the arrow. Results are presented as means ± S.E.M., n = 7 to 8. Statistics: ANOVA with repeated measures followed by Fisher’s PLSD test.

Fig. 3. Effects of saline, varenicline (1.5 mg/kg s.c.), ethanol (2.5 g/kg), or the combination of varenicline (1.5 mg/kg s.c.) and ethanol (2.5 g/kg i.p.) on extracellular dopamine levels in the nAc as measured by in vivo microdialysis in awake, freely moving rats. Drugs were administered as indicated by the arrow. Results are presented as means ± S.E.M., n = 7 to 9. Statistics: ANOVA with repeated measures over time points 0 to 60 followed by Fisher’s PLSD test.
animals, nicotine produced a significant increase in nAc dopamine levels ($p < 0.05$, time point 60 min versus baseline) compared with baseline. Significant increases in nAc dopamine levels after a varenicline injection compared with time point 0 min ($p < 0.001$). #, significant increases in nAc dopamine levels compared with time point 60 min ($p < 0.05$) after an acute injection of nicotine or ethanol in vehicle-pretreated animals and after an acute injection of ethanol in varenicline-pretreated animals or the combination of nicotine and ethanol on dopamine levels in the nAc (B). $\dagger$, significant increases in nAc dopamine levels after a varenicline injection compared with baseline. #, significant increases in nAc dopamine levels compared with time point 60 min ($p < 0.05$) after acute coadministration of nicotine and ethanol in saline-pretreated animals. $\ddagger$, significant difference in nAc dopamine response to the coadministration of ethanol and nicotine in animals pretreated with saline compared with varenicline ($p < 0.05$). Drugs were administered as indicated by the arrows. Statistics: ANOVA with repeated measures over time points 20 to 220 min followed by Fisher's PLSD test. Data are presented as means ± S.E.M., n = 5 to 9.

Varenicline was recently approved as an aid for smoking cessation. Based on the extensive coabuse of nicotine and alcohol (Zacny, 1990; Daeppen et al., 2000) and a potential common point of action (nAChRs) for these drugs in the brain reward system (Blomqvist et al., 1993; Söderpalm et al., 2000), the aim of the present study was to preclinically explore the potential benefits of varenicline as a pharmacological treatment of alcohol dependence. Using the in vivo microdialysis method, three sets of experiments measured the effects of subcutaneous injections of varenicline on the accumbal dopamine response to an acute systemic challenge with ethanol or with the combination of nicotine and ethanol in the rat.

The first experiment analyzed the impact of different doses of varenicline on extracellular dopamine levels in the nAc. Varenicline is a partial nAChR agonist that binds to $\alpha 4 \beta 2$ nAChRs with greater affinity, but less efficacy, than nicotine (Coe et al., 2005; Mihalak et al., 2006; Rollema et al., 2007). The subsequent effect of varenicline on the mesolimbic dopamine system is, in addition to a blockade of the nicotine-induced dopamine elevation, a slight intrinsic dopamine enhancement that is suggested to alleviate nicotine-withdrawal symptoms (Coe et al., 2005; Rollema et al., 2007). It is plausible that the ability of varenicline to elevate dopamine can provide relief also from withdrawal symptoms and craving related to other drugs of abuse, including alcohol, at least in a certain dose range. In the present dose-response study, an intermediate dose of 1.5 mg/kg produced the highest dopamine elevation in the nAc and was consequently selected for the remaining experiments. In line with previous studies (Rollema et al., 2007), the present experiment demonstrated an inverted U-shaped dose-response curve. A possible explanation for this feature could be that, at high doses, varenicline may become an antagonist at nAChRs important for dopamine turnover, alternatively it may start to modulate other nAChR subtypes or non-nAChRs.

A second experiment explored whether acute coadministration of varenicline and ethanol interact in the mesolimbic dopamine system with respect to elevation of nAc dopamine overflow. As demonstrated previously, dopamine was increased after acute administration of varenicline alone (Coe et al., 2005; Rollema et al., 2007) and ethanol alone (Blomqvist et al., 1993). It is interesting that dopamine levels were not increased when the same doses of ethanol and varenicline were administered concomitantly; thus, varenicline seems to block the effect of ethanol and ethanol seems to block the effect of varenicline. Whether there are conse-
quences on the efficacy for the drug in aiding smoking cessation when ethanol is present remains to be established, but no reports have indicated that varenicline has lower efficacy after ethanol consumption in humans. The effect of varenicline on ethanol-induced dopamine overflow in the rat nAc resembles its antagonism of dopamine stimulation by nicotine in the same brain area (Rollema et al., 2007), suggesting that varenicline may be an efficient treatment also for alcohol dependence. This conclusion is supported by behavioral data demonstrating that varenicline reduces ethanol seeking and consumption in ethanol high-prefering rats (Steensland et al., 2007). Moreover, the present results suggest that ethanol and varenicline most probably influence the same nAChR subtypes.

Ethanol-induced dopamine elevations in the rat nAc requires stimulation of nAChRs in the VTA (Blomqvist et al., 1997; Tizabi et al., 2002; Ericson et al., 2003). In the rodent VTA, several functional nAChR subtypes have been identified, such as α7+, α4β2+, and α6β2β3+ (Klink et al., 2001; Wooltorton et al., 2003). Varenicline is mainly considered a selective partial α4β2+ nAChR agonist (Coe et al., 2005; Rollema et al., 2007). It is also a full α7+ nAChR agonist (Mihalak et al., 2006) and possesses weak, partial agonistic properties at α3β4+, α6+, and α3β2+ nAChRs (Mihalak et al., 2006; Rollema et al., 2007). In vitro studies suggest an interaction between ethanol and the α4β2+ nAChRs (Aistrup et al., 1999; Cardoso et al., 1999). Moreover, there are several in vivo studies supporting the idea that α4β2+ (Owens et al., 2003; Butt et al., 2004) and α7+ (Wehner et al., 2004) nAChRs are modulated by ethanol, whereas other pharmacological studies in experimental animals seem to exclude the involvement of these nAChR subtypes in important aspects of alcohol reinforcement. In rodents, the α4β2+ nAChR antagonist dihydro-β-erythroidine does not affect alcohol consumption (Lé et al., 2000), ethanol-induced locomotor activity (Larsson et al., 2002; Kamens and Phillips, 2008), dopamine overflow (Larsson et al., 2002; Ericson et al., 2003), or conditioned reinforcement to ethanol (Lof et al., 2007). Moreover, the α7+ nAChRs antagonist methyllycaconitine citrate had no effect on ethanol-induced dopamine elevations or locomotor activity in rodents (Larsson et al., 2002). Instead, the selective α3β2+ and α6+ nAChR antagonist α-conotoxin MII blocks the ethanol-induced effects on these parameters (Larsson et al., 2004; Jerlhag et al., 2006; Lof et al., 2007).

As mentioned above, varenicline is also a partial agonist at α3β4+ nAChRs (Mihalak et al., 2006), although with 800-fold lower binding affinity than for α4β2+ nAChRs (Rollema et al., 2007), and blockade of this nAChR subunit composition reduces alcohol (and nicotine) consumption in the rat (Rezvani et al., 1997; Glick et al., 2000; Maissonneuve and Glick, 2003). Taken together, preclinical pharmacological studies indicate that other nAChR subtypes than the α4β2+ may play a role in the alcohol reinforcement processes. Therefore, it cannot be excluded that the ability of varenicline to inhibit the ethanol-induced dopamine elevation in the present study may be mediated via other nAChR subtypes than the α4β2+, despite the low potency of varenicline at these receptors (Mihalak et al., 2006; Rollema et al., 2007).

In the last experiment, rats were subchronically pretreated with varenicline or saline once daily for 5 consecutive days. On day 6, we investigated the effect of varenicline on the accumbal dopamine response to systemic injections of alcohol, nicotine, or the combination of nicotine and alcohol. Varenicline pretreatment prevented the elevation of dopamine induced by a single nicotine injection. This result is in line with published data demonstrating that acute varenicline abolishes nicotine-induced dopamine overflow in the nAc (see above), but this is the first study illustrating the same effect after subchronic varenicline pretreatment. In contrast, the dopamine elevation after a single acute ethanol injection was unaffected by varenicline pretreatment, a result that differs from the acute effects of varenicline on ethanol-induced dopamine overflow in the second experiment. Whether the observed dopamine response to varenicline and ethanol represents a failure of varenicline to block the ethanol effect or varenicline's dopamine-elevating effect by itself is, however difficult to determine, the dopamine elevations produced by ethanol and varenicline are similar in magnitude and the peak effect of varenicline is probably not reached until approximately 100 min after injection (see experiment 1). The discrepancies between the effects of acute versus semi-chronic varenicline administration on drug-induced dopamine elevations may either be explained by alterations induced by the subchronic varenicline regimen or by a different experimental design, i.e., that varenicline was given 60 min before ethanol in the last experiment. Regardless, the subchronic varenicline administration should be more compatible with the clinical situation, where ethanol would be inflicted upon a relatively constant varenicline exposure.

It is noteworthy that, in the last experiment, coadministration of ethanol and nicotine produced an additive effect on the accumbal dopamine levels, whereas coadministration of ethanol and varenicline did not. This result supports the notion that nicotine and ethanol elevate dopamine via different pathways, such as different nAChR subtypes (Larsson et al., 2002, 2004; Ericson et al., 2003). Moreover, the dopamine stimulatory effect of the combination of nicotine and ethanol was completely abolished by varenicline. If translated to the clinical situation, patients who are treated with varenicline as a smoking cessation aid may not experience an additional dopamine stimulatory effect of alcohol on top of nicotine and/or varenicline. Such a phenomenon could explain why some of these patients report a reduction in their alcohol consumption during treatment with varenicline.

The present study demonstrates different effects of acute varenicline on nAc dopamine response to ethanol in naive animals compared with varenicline-pretreated animals. The α4β2+ nAChR subtype desensitizes after stimulation by physiologically relevant levels of nicotine (Benwell et al., 1995) and is hypothesized to become up-regulated after long-term stimulation (Schwartz and Kellar, 1985; Collins et al., 1994), although data are inconsistent and contradictory. The different effects of acute and subchronic varenicline on the dopamine response to ethanol in the present study may thus be due to compensatory alterations of α4β2+ nAChRs provoked by repeated administrations of varenicline. It is also possible that long-term consumption of alcohol and/or nicotine alters the subtype composition of nAChRs toward an increased significance of α4β2+ nAChRs also in alcohol reinforcement, thus in favor of the α4β2+-mediated properties of varenicline.

In summary, this set of in vivo neurochemical experiments suggests that varenicline reduces neurochemical events associated also with alcohol reinforcement, at least in individ-
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References


