Effect of co-administration of varenicline and antidepressants on extracellular monoamine concentrations in rat prefrontal cortex

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Abstract

Since a substantial proportion of smokers have comorbid mood disorders, the smoking cessation aid varenicline might occasionally be prescribed to patients who are simultaneously treated with antidepressants. Given that varenicline is a selective nicotinic acetylcholine receptor partial agonist and not a substrate or inhibitor of drug metabolizing enzymes, pharmacokinetic interactions with various classes of antidepressants are highly unlikely. It is, however, conceivable that varenicline may have a pharmacodynamic effect on antidepressant-evoked increases in central monoamine release. Interactions resulting in excessive transmitter release could cause adverse events such as serotonin syndrome, while attenuation of monoamine release could impact the clinical efficacy of antidepressants. To investigate this we examined whether varenicline administration modulates the effects of the selective serotonin reuptake inhibitor sertraline and the monoamine oxidase inhibitor clorgyline, given alone and combined, on extracellular concentrations of the monoamines serotonin, dopamine, and norepinephrine in rat brain by microdialysis. Given the important role attributed to cortical monoamine release in serotonin syndrome as well as antidepressant activity, the effects on extracellular monoamine concentrations were measured in the medial prefrontal cortex. Responses to maximally effective doses of sertraline or clorgyline and of sertraline plus clorgyline were the same in the absence as in the presence of a relatively high dose of varenicline, which by itself had no significant effect on cortical monoamine release. This is consistent with the binding profile of varenicline that has insufficient affinity for receptors, enzymes, or transporters to inhibit or potentiate the pharmacologic effects of antidepressants. Since varenicline neither diminished nor potentiated sertraline- or clorgyline-induced increases in neurotransmitter levels, combining varenicline with serotonergic antidepressants is unlikely to cause excessive serotonin release or to attenuate antidepressant efficacy via effects on cortical serotonin, dopamine or norepinephrine release.

1. Introduction

Nicotine dependence and depression are illnesses that have serious health consequences and affect a large proportion of the population. The World Health Organization predicts that by 2030 more than 8 million deaths per year will be caused by smoking-related illnesses, while depression is one of the leading causes of disability affecting more than 120 million people worldwide (Üstün et al., 2004; World Health Organization, 2008). There is substantial epidemiologic evidence that smokers are more likely to have mood or personality disorders, depressive symptoms, or clinical depression and that the smoking prevalence is approximately 50% among psychiatric outpatients with depression (Kalman et al., 2005; Papera et al., 2004; Ziedonis et al., 2008). Data from the first wave of the US National Epidemiologic Survey on Alcohol and Related Conditions indicate that, among nicotine-dependent individuals, 21.1% had a mood disorder (Grant et al., 2004). The US National Comorbidity Study demonstrated that smoking prevalence in those with a psychiatric diagnosis (41.0%) is about two times the rate seen in those without a...
psychiatric diagnosis (22.5%) (Lasser et al., 2000). An association between smoking and occurrence of depressive symptoms has also been reported in other population-based studies of adults in the United States (Breslau et al., 1998; Kinnunen et al., 2006), Finland (Korhonen et al., 2007), Norway (Klungsøyr et al., 2006), and The Netherlands (Cuijpers et al., 2007).

Treating nicotine dependence and depression are effective ways to improve health, and a substantial number of smokers are thus likely to receive antidepressant and smoking cessation medication simultaneously. Given the possibility of pharmacokinetic or pharmacodynamic drug–drug interactions, it is important to investigate whether and to what extent treatments for smoking cessation could interact with co-administered antidepressants.

Varenicline was recently introduced as a novel efficacious smoking cessation aid that acts as an α4β2 nicotinic acetylcholine receptor (nAChR) partial agonist (Coe et al., 2005; Rollema et al., 2007). Since varenicline is virtually not metabolized, has low plasma protein binding, is excreted unchanged renally, and does not inhibit or induce the activity of the major cytochrome P450 enzymes (Burstein et al., 2007; Faessel et al., 2010; Obach et al., 2006), pharmacokinetic interactions between varenicline and antidepressant drugs are highly unlikely. It was for instance shown that concomitant administration of varenicline with the dopamine (DA) and norepinephrine (NE) reuptake inhibitor, bupropion, in a randomized, placebo-controlled study in smokers did not result in clinically relevant effect on bupropion's pharmacokinetics (Faessel et al., 2010).

However, both varenicline and antidepressant agents affect monoaminergic neurotransmission and it is conceivable that a pharmacodynamic interaction could cause either a reduction or an increase in the antidepressant-induced release of the monoamine neurotransmitters DA, NE, and serotonin (5-HT). Pharmacodynamic interactions with the nAChR partial agonist varenicline could occur at nAChRs, since most antidepressant monoamine reuptake inhibitors have weak nAChR antagonist properties (Fryer and Lukas, 1999; Hennings et al., 1999; Szaszi et al., 2007), but can also be mediated via other, unknown mechanisms. Interactions that would result in large increases in monoamine release might be associated with adverse events, such as serotonin syndrome via accumulation of extracellular 5-HT levels and excessive activation of central nervous system and peripheral serotonergic receptors (Boyer and Shannon, 2005; Gillman, 2006; Shioda et al., 2004; Sundelstein et al., 2008). On the other hand, a reduction of antidepressant-induced central monoamine release could impact the clinical efficacy of monoaminergic antidepressants. To investigate this possibility in a preclinical in vivo model, we measured the effects of varenicline treatment on basal and antidepressant-induced elevated extracellular concentrations of 5-HT, DA, and NE in rat brain. Since the primary purpose of the study was to examine the potential impact on serotonin syndrome, which is mostly associated with combinations of serotonergic antidepressants, we used the selective serotonin reuptake inhibitor (SSRI) sertraline and the monoamine oxidase inhibitor (MAO-I) clorgyline. Both compounds were given at doses that are known to maximally inhibit 5-HT transport and MAO, respectively (Artigas and Adell, 2007; Segal et al., 1992), and were combined with a varenicline dose known to result in levels above the human therapeutic exposure (Faessel et al., 2010; Rollema et al., 2009b) and to have maximal effect on dopamine release in nucleus accumbens (Coe et al., 2005; Rollema et al., 2007). Monoamine release was measured in the medial prefrontal cortex (mPFC), a brain area that plays an important role in serotonin syndrome (Gillman, 2006) as well as in the effects of antidepressants (Artigas and Adell, 2007). In addition, we compiled in vitro data of varenicline to examine whether its binding affinities or inhibitory potencies for relevant receptors, enzymes, or transporters would be sufficient to inhibit or potentiate the pharmacologic effects of antidepressants at therapeutic doses.

2. Materials and methods

2.1. Materials

Varenicline tartrate and sertraline HCl were synthesized at Pfizer (Gorton, CT, USA); clorgyline HCl and all other chemicals were purchased from Sigma (St. Louis, MO, USA) unless indicated otherwise.

2.2. In vivo microdialysis

Male Wistar rats (280–350 g; Harlan, Horst, The Netherlands) were individually housed in plastic cages (30 cm × 30 cm × 40 cm) under a 12:12 h light/dark cycle (starting at 7:00 a.m.) and had access to food and water ad libitum. Experiments were conducted in accordance with the declarations of Helsinki and were approved by the Institutional Animal Care and Use Committee of the University of Groningen, The Netherlands. Rats were anesthetized using isoflurane (2%, 800 ml/min O2). Bupivacain/epinephrine was used for local anesthesia and fentanyl (0.1%, 1 ml/kg) for pre/per operative analgesia. The animals were placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA), and I-shaped probes (Hospal membrane, 4 mm exposed surface; Brainlink, Groningen, The Netherlands) were inserted into the mPFC. Coordinates for the tips of the probes were posterior (AP) +3.4 mm to bregma, lateral +0.8 mm to midline, and ventral –5.0 mm to dura; the toothbar was set at –3.3 mm (Paxinos and Watson, 1982). Experiments were performed 24–48 h after probe implantation. On the day of the experiment, the probes were connected with flexible PEEK tubing to a microperfusion pump (Syringe pump UV 8301S01, TSE, Karlsruhe, Germany) and perfused with artificial cerebrospinal fluid (aCSF) containing 147 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl2, and 1.2 mM MgCl2 at a flow rate of 1.5 μl/min. Microdialysates samples were collected for 15-min periods into mini-vials containing 7.5 μl of 0.02 M formic acid, using an automated fraction collector (CMA 142, CMA Microdialysis, Solna, Sweden), and stored at −80 °C pending analysis. At least four pre-drug basal samples were collected before compounds were administered (varenicline, 1 mg/ml free-base in saline; clorgyline, 4 mg/ml free-base in saline; sertraline 17.8 mg/ml free-base in 20% solutol; all compounds and vehicles were administered s.c. in a volume of 1 ml/kg s.c.). Effects of each compound alone on monoamine dialysate levels were determined by administering varenicline, sertraline, clorgyline or corresponding vehicles and collecting 15-min samples for 4.5 h. For the combination experiments varenicline and sertraline, which have similar half lives in the rat of approximately 4–5 h (Obach et al., 2006; Tremaine et al., 1989) were administered together and clorgyline was administered 2.5 h later. For each of the five treatment groups the appropriate vehicles were given at the same time points (see Table 1) and six animals were used per group. After the experiment, rats were euthanized and brains were removed and stored for 3 days in a 4% solution of paraformaldehyde. The position of each probe was historically verified by making coronal sections of the brain (Paxinos and Watson, 1982).

2.3. Neurotransmitter assays

Microdialysate concentrations of 5-HT, NE, and DA were determined by HPLC combined with tandem mass spectrometry (MS/MS) detection. Dialysate samples (22.5 μl) were collected in 0.3 ml polypropylene vials containing 7.5 μl of 20 mM formic acid. After addition of 4 μl of internal standards (deuterated 5-HT, NE, and DA) to each vial, 40 μl of the derivatization reagent SymDaQ (Klein et al., 2009) was added with an autosampler (SIL-10AD vp, Shimadzu, Kyoto, Japan). Two minutes later, 50 μl of the mixture was injected onto the HPLC system and analytes were separated over a reversed phase Phenomenex 100 × 3.0 mm (2.5 μm) column at 30 °C, using a linear gradient of acetonitrile/0.1% formic acid at a flow rate of 0.3 ml/ min. The starting acetonitrile concentration (5%) was increased to 30% after 4 min,

<table>
<thead>
<tr>
<th>Varenicline (VAR) and/or sertraline and/or appropriate vehicles (saline or 20% solutol) were administered at t = 0 min and either clorgyline or vehicle (saline) was administered at t = 150 min.</th>
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<tr>
<td><strong>Dosing:</strong></td>
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<td><strong>t = 0 min</strong></td>
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</table>
to 70% after 6.5 min, and decreased to 5% after 7 min. A post-column make-up flow of mobile phase at 0.15 ml/min was added to the column effluent to enhance ionization efficiency. The flow was diverted to waste for 2.5 min to avoid source contamination and then switched to the MS, consisting of an API 4000 MS/MS detector and a Turbo Ion Spray interface (Applied Biosystems, Ijssel, The Netherlands). The acquisitions were performed in positive ionization mode with the ion spray voltage set at 3 kV and a probe temperature of 200 °C, using multiple-reaction-monitoring (MRM). The collision gas (nitrogen) pressure was held at 2 psig. Data were calibrated and quantified using the Analyst™ data system (Applied Biosystems, version 1.4.2). Mass transitions were: 5-HT-d4 Q1 426.2 and Q3 482.4; NE Q1 415.3 and Q3 371.7; NE-d6 Q1 421.3 and Q3 377.7; DA Q1 399.2 and Q3 355.4; DA-d4 Q1 403.2 and Q3 359.4.

2.4. Data analysis

Four consecutive pre-treatment microdialysis samples with less than 50% variation were taken as baseline and the mean concentration was set at 100%. Drug effects were expressed as percentages of baseline level (mean ± SE) within the same subject. Statistical analysis was performed using SigmaStat for Windows (SPSS Corp, Chicago, IL, USA).

Treatment effects were compared with baseline and between treatments, using two-way analysis of variance (ANOVA) for repeated measurements followed by Student Newman Keuls post hoc test. Treatment and time were the main effects. The level of statistical significance was set at p < 0.05.

2.5. In vitro profile

In vitro data on the interaction of varenicline with selected receptors, transporters, and enzymes were taken from the literature or generated by standard methods (indicated in Table 4).

3. Results

3.1. Microdialysis of monoamines

3.1.1. Basal and drug-evoked extracellular monoamine concentrations

Basal concentrations of 5-HT, NE, and DA in microdialysates from rat mPFC did not differ between treatment groups, with mean values (not corrected for recovery) of 5.30 ± 0.48 (n = 30), 16.55 ± 1.31 (n = 30), and 7.65 ± 0.91 fmol/30 μl sample (n = 30), respectively. Effects of vehicle and drug treatments on extracellular monoamine concentrations are expressed as percentages of baseline levels ± SEM and shown as time courses in Figs. 1–4. Results of statistical analyses are summarized in Tables 2 and 3.

3.1.2. Effects of administration of test compounds alone on extracellular monoamine levels

Varenicline, given at 1 mg/kg, caused no significant increases in the extracellular levels of 5-HT, NE, and DA in rat mPFC compared with vehicle treatment (Fig. 1; statistical evaluation in Table 2). Sertraline, when given alone at a dose of 17.8 mg/kg, increased cortical 5-HT levels approximately 2.5-fold within 1 h after dosing, while at this dose sertraline caused a small, significant increase in NE levels to about 1.5-fold of baseline levels 2 h after administration and had no significant effect on extracellular DA levels (Fig. 1 and Table 2). Clorgyline administration (4 mg/kg) alone effectively inhibited MAO and caused the expected increases in extracellular levels of all three monoamines, 5-HT to 1.5-fold, NE to 2.5-fold, and DA to 2.5-fold of basal levels (Fig. 1 and Table 2).

3.1.3. Effect of varenicline on sertraline- and clorgyline-induced extracellular 5-HT levels

Varenicline (1 mg/kg s.c.) had no significant effect on the increases in extracellular 5-HT levels in rat mPFC produced either by sertraline or by clorgyline alone. Administration of 4 mg/kg clorgyline to rats pretreated with 17.8 mg/kg sertraline increased extracellular 5-HT concentrations to more than 10-fold of baseline levels. This massive 5-HT increase was not affected by pretreatment with varenicline and time courses for the effects of sertraline, clorgyline, or sertraline with clorgyline on 5-HT in the absence and presence of varenicline were taken as baseline and the mean concentration was set at 100%.
**3.1.5. Effect of varenicline on sertraline- and clorgyline-induced extracellular DA levels**

Varenicline and sertraline, which when given separately do not affect extracellular DA concentrations, had also no significant effect when administered together. The small clorgyline-induced DA increases appeared to be affected by varenicline pretreatment. We have no reasonable explanation for this observation, although it could be due to the MAO-A inhibitory activity of clorgyline that has an indirect and variable effect on DA release via 5-HT and/or NE increases. Importantly, varenicline administration showed a trend to reducing the 2.5-fold DA increase produced by co-administration of sertraline and clorgyline, but this effect was not significant (Fig. 4, $F_{1,72} = 0.762; \ p = 0.403$).

**3.2. In vitro binding profile of varenicline**

The in vitro binding profile (Table 4) shows that varenicline is highly selective for $\alpha_4\beta 2$ containing nAChRs ($K_i = 0.4 \text{ nM}$) and binds with at least 200-fold lower affinity to other nAChR subtypes ($K_i \geq 85 \text{ nM}$). Varenicline does not have significant affinity for other ion channels or for neurotransmitter receptors, uptake sites and enzymes, including targets of common antidepressant drugs, such as the monoamine transporters, the metabolizing enzyme MAO, and the various 5-HT, NE, and DA receptor subtypes ($K_i$ values $\geq 1 \mu \text{M}$), with the exception of its affinity for the 5-HT$_{3A}$ receptor ($K_i = 0.35 \mu \text{M}$).

**4. Discussion**

Since a large proportion of smokers have comorbid depression and smoking prevalence is approximately 50% among psychiatric outpatients with depression (Grant et al., 2004; Kalman et al., 2005; Paperwalla et al., 2004; Ziedonis et al., 2008), nicotine dependence and depression will sometimes be treated simultaneously with a smoking cessation aid and an antidepressant, which theoretically at least could lead to either pharmacokinetic or pharmacodynamic drug–drug interactions. This has not yet been investigated in preclinical models for the recently introduced smoking cessation aid varenicline, a partial agonist at the $\alpha_4\beta 2$ nAChR (Cahill et al., 2007; Coe et al., 2005; Rollema et al., 2007). Since its pharmacokinetic profile (Burstein et al., 2007; Faessel et al., 2010; Obach et al., 2006) makes it highly unlikely that varenicline will affect the disposition of concomitantly administered antidepressants, this study investigated potential pharmacodynamic interactions in an animal model with two antidepressants that act via different mechanisms. The results show that varenicline neither reduces nor further increases elevated monoamine neurotransmitter levels produced by administration of an SSRI and an MOA-I, or by combined SSRI and MOA-I administration, consistent with its in vitro profile.

Currently, antidepressant pharmacotherapy is aimed at improving dysfunctional monoaminergic neurotransmission by chronically elevating extracellular monoamine levels through multiple mechanisms, such as inhibition of monoamine re-uptake by tricyclic antidepressants (TCAs), selective inhibition of serotonin or noradrenaline and norepinephrine reuptake by SSRIs or SNRIs, and inhibition of monoamine metabolism by MAO-Is (Slattery et al., 2004). The neurochemical effects of these antidepressants have been well characterized in numerous in vivo intracerebral microdialysis studies by assessment of the effects on extracellular transmitter levels in rodent brain areas, such as hippocampus and prefrontal cortex (Artigas and Adell, 2007). Varenicline is known to increase DA release in the nucleus accumbens and striatum, which is key to its clinical efficacy as a smoking cessation aid (Coe et al., 2005; Rollema et al., 2007), but does not significantly modulate DA or NE release in the prefrontal cortex (Coe et al., 2005; Rollema et al., 2007).
Enzymes

IC<sub>Ki</sub> (efficacy ( Lipper et al., 1979 ). The effect of co-administration of an
sertraline is an efficacious SSRI ( Cipriani et al., 2009 ) that has, like
in the action of antidepressants. We used sertraline and clorgyline:
monoamines in rat prefrontal cortex, an area that is believed to be
have a different mechanism of action, on extracellular levels of
regulation of monoamine release.

We applied the microdialysis technique to examine the effects
of varenicline alone and combined with two antidepressants that
have a different mechanism of action, on extracellular levels of
monoamines in rat prefrontal cortex, an area that is believed to be
involved in the underlying mechanism of serotonin syndrome and
in the action of antidepressants. We used sertraline and clorgyline:
sertaline is an efficacious SSRI ( Cipriani et al., 2009 ) that has, like
other SSRIs, weak nAChR antagonist activity ( Fryer and Lukas, 1999 ),
and clorgyline is a potent MAO-I with antidepressant
efficacy ( Lipper et al., 1979 ). The effect of co-administration of an
SSRI and an MAO-I was studied because that combination is most
frequently associated with serotonin syndrome ( Gillman, 2006 ).
For this study we selected a dose of each compound known to be
maximally effective in previous microdialysis studies in rodents
(Artigas and Adell, 2007; Coe et al., 2005; Rollema et al., 2009b;
Segal et al., 1992; Sprouse et al., 1996; Waldmeier et al., 1976). We
used a relatively high dose of 1 mg/kg varenicline that is associated
with peak rat plasma concentrations of about 150 ng/ml at 30 min
(Rollema et al., 2009b), which, with a half life in rats of 4.5 h ( Obach
et al., 2006 ), decline to about 40 ng/ml after 3 h. The 1 mg/kg dose
results in higher rat plasma concentrations throughout the
duration of the microdialysis experiment than steady state
therapeutic plasma levels in patients on 1 mg BID, which are
about 10 ng/ml ( Faessell et al., 2010 ).

Varenicline by itself caused no significant changes in extracellu-
lar levels of 5-HT, NE, or DA in rat prefrontal cortex, in agreement
with previous reports, which showed that varenicline only
produces robust increases in cortical DA and NE release after a
high dose of 10 mg/kg ( Rollema et al., 2009b ). This dose is
associated with very high brain concentrations of > 1 μM, which
can interact with several other nAChR subtypes than α6β2
nAChRs. The lack of effects of varenicline on cortical monoamine
release is also consistent with its in vitro properties, since
varenicline has very low affinity for the 5-HT transporter or 5-
HT receptors and can thus neither increase 5-HT by blocking 5-HT
reuptake or by interacting with central 5-HT receptors. Although
varenicline has modest affinity for 5-HT<sub>3A</sub> receptors ( K<sub>a</sub> = 0.35 μM)
at which it is a full agonist ( EC<sub><sub>50</sub></sub> = 1 μM ), the therapeutic unbound
varenicline brain concentrations are predicted to be approximately
50 nM and thus insufficient for activating central 5-HT<sub>3A</sub> receptors.
Furthermore, varenicline does not bind to DA receptors
( K<sub>a</sub> > 1 μM ) that can modulate 5-HT release, and does not inhibit
the enzyme that metabolizes 5-HT, MAO-A ( IC<sub><sub>50</sub></sub> > 1 μM ).

Varenicline produced the well-documented robust and long-
lasting increase in extracellular 5-HT levels in rat brain ( Artigas
and Adell, 2007; Sprouse et al., 1996 ). Adding clorgyline to
sertraline-treated rats significantly further elevated 5-HT levels
and produced the expected increases in extracellular DA and NE as
a consequence of MAO inhibition ( Segal et al., 1992; Waldmeier
et al., 1976 ), confirming numerous previous reports on the effects
of antidepressants on extracellular neurotransmitter levels.
Administration of 1 mg/kg varenicline did not significantly change
the elevated extracellular monoamine levels induced by sertraline
alone, by clorgyline alone, or by sertraline followed by clorgyline
treatment. The lack of an effect of varenicline on antidepressant-
induced transmitter increases is again consistent with its in vitro
profile of a highly selective α6β2 nAChR partial agonist that has
poor affinity for the receptors, transporters, or enzymes that can
affect central monoamine levels ( Table 4 ).

These results have both safety and therapeutic implications. Most
importantly, the data do not suggest safety concerns related to
exaggerated neurotransmitter release when combining varenici-
line with serotonergic antidepressants. A well-known potential
side effect of excessive 5-HT release is serotonin syndrome, a
potentially life-threatening adverse reaction to therapeutic drug
use or inadvertent interactions between drugs, resulting in 5-HT
accumulation and excess activation of serotonergic receptors
(Gillman, 2006; Shioda et al., 2004; Sun-Edelstein et al., 2008 ). A
wide variety of drugs and drug combinations have been associated
with this condition, including MAOIs, TCAs, SSRIs, SNRIs, opiate
analgesics, antibiotics, anti-emetics, and drugs that inhibit
cytosol and GABA<sub>0</sub> isoforms. However, serotonin syndrome has
been most frequently observed after combined administration of
antidepressant drugs that act via increased serotonergic neuro-
transmission, in particular co-administration of SSRIs and MAOIs
(Gillman, 2006; Shioda et al., 2004; Sun-Edelstein et al., 2008).

Previous microdialysis studies on the neurochemistry of serotonin

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Time (min)</th>
<th>Analyte</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varenicline + sertraline vs. vehicle + sertraline</td>
<td>0–150</td>
<td>5-HT</td>
<td>F&lt;sub&gt;1,15&lt;/sub&gt; = 0.0002</td>
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<td></td>
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<td>NE</td>
<td>F&lt;sub&gt;1,15&lt;/sub&gt; = 2.705</td>
<td>p = 0.131 ns</td>
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<td></td>
<td></td>
<td>DA</td>
<td>F&lt;sub&gt;1,15&lt;/sub&gt; = 13.315</td>
<td>p = 0.587 ns</td>
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<tr>
<td>Varenicline + vehicle + clorgyline vs. vehicle + vehicle + clorgyline</td>
<td>150–270</td>
<td>5-HT</td>
<td>F&lt;sub&gt;3,30&lt;/sub&gt; &lt; 0.001</td>
<td>p = 0.996 ns</td>
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<td></td>
<td></td>
<td>NE</td>
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<td></td>
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<td>DA</td>
<td>F&lt;sub&gt;3,30&lt;/sub&gt; = 10.257</td>
<td>p = 0.011 s</td>
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<td>Varenicline + sertraline + clorgyline vs. vehicle + sertraline + clorgyline</td>
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<td>F&lt;sub&gt;3,30&lt;/sub&gt; = 5.01</td>
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<td></td>
<td>NE</td>
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<td>p = 0.852 ns</td>
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<td></td>
<td></td>
<td>DA</td>
<td>F&lt;sub&gt;3,30&lt;/sub&gt; = 0.365</td>
<td>p = 0.559 ns</td>
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</table>

Table 3

Statistical analysis of effects of varenicline vs. vehicle treatment on antidepressant-induced extracellular monoamine concentrations.

Table 4

Binding affinities and inhibitory potencies of varenicline for ion channels, receptors, uptake sites, and enzymes. K<sub>i</sub> or IC<sub><sub>50</sub></sub> values > 1 or 10 μM indicate no significant inhibition of radioligand binding at 1 or 10 μM varenicline. Data from Rollema et al. (2007, 2009b) and on file ( Pfizer Inc. ).

<table>
<thead>
<tr>
<th>Ion channels</th>
<th>K&lt;sub&gt;i&lt;/sub&gt; (nM)</th>
<th>Receptors</th>
<th>K&lt;sub&gt;i&lt;/sub&gt; (μM)</th>
<th>Uptake sites</th>
<th>K&lt;sub&gt;i&lt;/sub&gt; (μM)</th>
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<tr>
<td>α6β2 nAChR</td>
<td>0.4</td>
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<td>Neurokinin NK&lt;sub&gt;1&lt;/sub&gt;</td>
<td>&gt;10</td>
<td>MAO-A</td>
<td>&gt;1</td>
</tr>
<tr>
<td>Histamine H&lt;sub&gt;1&lt;/sub&gt;</td>
<td>&gt;10</td>
<td>Cyt-P450</td>
<td>&gt;10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ns: varenicline treatment did not produce a significant effect compared with vehicle treatment.
s: varenicline treatment produced a significant effect compared with vehicle treatment.
syndrome have shown a close correlation between the magnitude of cortical extracellular 5-HT increases produced by antidepressant drugs and the severity of serotonin syndrome in patients taking these drugs (Shioda et al., 2004). Since the present study shows that varenicline does not increase central 5-HT levels and does not have an additive or synergistic effect on 5-HT increases produced by an SSRI, by an MAO-I, or by an SSRI and MAO-I combination, varenicline is unlikely to cause or exacerbate serotonin syndrome.

While a recent report of a potential association between varenicline and development of serotonin syndrome was based on seven cases of suspected serotonin syndrome in patients taking varenicline that were spontaneously reported to the World Health Organization’s VigiBase database (Johansson and Hill, 2009), in all but one of the reported cases, varenicline was used in combination with antidepressants associated with serotonin syndrome. In addition to varenicline’s lack of effect on central extracellular 5-HT levels, clinical data provide further support that varenicline is unlikely to have played a role in the reported cases of serotonin syndrome, but rather was coincidentally taken by patients already at higher risk of the event due to underlying comorbid illness and antidepressant medication. In 10 randomized, double-blind, placebo-controlled phase II–IV Pfizer-sponsored varenicline trials completed through end of March 2009 (Tonstad et al., 2010), and three open-label varenicline trials (Aubin et al., 2008; Pfizer Inc., 2007; Tonstad et al., 2006), which excluded patients with depression requiring antidepressant treatment, there were no reports of serotonin syndrome amongst over 5000 varenicline patients. In addition, a keyword search in the literature (key-words: “varenicline” and “serotonin syndrome” or “serotonin toxicity”) through January 2010 revealed no other published reports of serotonin syndrome or serotonin toxicity associated with varenicline.

The pharmacotherapeutic significance of this study is related to the potential impact of varenicline on the clinical efficacy of co-administered SSRIs and MAO-I antidepressants. Since varenicline does not attenuate increases in central extracellular 5-HT levels induced by those antidepressants, it is unlikely to reduce the action of serotonergic antidepressant drugs, e.g., via blunting of the 5-HT response. On the contrary, several preclinical studies have shown that α4β2 nAChR partial agonists can augment the effect of classical antidepressants, most likely via reduction of cholinergic signaling (Picciotto et al., 2008; Shytte et al., 2002). The data also indicate that the recent observation that varenicline augments sertraline’s antidepressant-like activity in a preclinical murine antidepressant model (Rollemo et al., 2009a) is unlikely due to a synergistic effect on SSRI-induced 5-HT increase, but rather a consequence of varenicline’s interaction with α4β2 nAChRs. Finally, the lack of effect on evoked increases in cortical DA and NE release suggests that varenicline would not have an effect on the efficacy of other types of antidepressants that act via elevated DA and/or NE levels. However, since the present experiments were only conducted with single doses of a typical SSRI and MAO-I in the prefrontal cortex, further studies would be needed to examine potential interactions with NE or DA reuptake-inhibitors.

5. Conclusions

In view of the high comorbidity of nicotine dependence with mood disorders, this study investigated potential interactions of the smoking cessation agent varenicline with two antidepressant drugs, an SSRI (sertraline) and an MAOI (clorglyline), in a preclinical animal model. Varenicline by itself has no effect on the cortical release of 5-HT, NE, and DA and when combined with one or with both antidepressant drugs, it did not cause significant effects on the increased neurotransmitter levels induced by sertraline and/or clorglyline. These results demonstrate that a pharmacodynamic interaction between varenicline and the SSRIs or MAO-I class of antidepressants is unlikely and that varenicline has little risk of either triggering adverse events by excessive neurotransmitter release or reducing the efficacy of concomitant therapy with serotonergic antidepressants. Taken together with clinical and post-marketing data, the available evidence at this time does not demonstrate that varenicline is likely to have pharmacodynamic or pharmacokinetic interactions with serotonergic antidepressant antidepressants; nor does the data support the view that varenicline plays a role in serotonin syndrome reported in some patients.

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