

Varenicline Is a Potent Agonist of the Human 5-Hydroxytryptamine₃ Receptor^S

S. C. R. Lummis, A. J. Thompson, M. Bencherif, and H. A. Lester

Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom (S.C.R.L., A.J.T.); Targacept Inc., Winston-Salem, North Carolina (M.B.); and Division of Biology, California Institute of Technology, Pasadena, California (H.A.L.)

Received June 21, 2011; accepted July 11, 2011

ABSTRACT

Varenicline, a widely used and successful smoking cessation agent, acts as a partial agonist at nicotinic acetylcholine receptors. Here, we explore the effects of varenicline at human and mouse 5-Hydroxytryptamine₃ (5-HT₃) receptors. Application of varenicline to human 5-HT₃ receptors expressed in *Xenopus laevis* oocytes reveal it is almost a full agonist ($R_{max} = 80\%$) with an EC_{50} (5.9 μ M) 3-fold higher than 5-HT. At mouse 5-HT₃ receptors varenicline is a partial agonist ($R_{max} = 35\%$) with an EC_{50} (18 μ M) 20-fold higher than 5-HT. Displacement of the competitive 5-HT₃ receptor antagonist [³H]granisetron reveals similar IC_{50} values for varenicline at mouse and human receptors expressed in human embryonic kidney 293 cells, although studies in these cells using a membrane potential-sensitive dye

show that again varenicline is a 4- or 35-fold less potent agonist than 5-HT in human and mouse receptors, respectively. Thus the data suggest that the efficacy, but not the affinity, of varenicline is greater at human 5-HT₃ receptors compared with mouse. Docking studies provide a possible explanation for this difference, because they suggest distinct orientations of the ligand in the mouse versus human 5-HT₃ agonist binding sites. Additional binding selectivity studies in a broad panel of recombinant receptors and enzymes confirmed an interaction with 5-HT₃ receptors but revealed no additional interactions of varenicline. Therefore, activation of human 5-HT₃ receptors may be responsible for some of the side effects that preclude use of higher doses during varenicline treatment.

Introduction

The 5-HT₃ receptor is a member of the Cys-loop ligand-gated ion channel family, of which the nACh receptor is the prototypic member. These receptors, which play roles in synaptic transmission in both the central and peripheral nervous systems, are pentameric assemblies of one, or, more usually, several subunits, which surround a central ion-conducting pore. Five 5-HT₃ receptor subunits (A–E) have been identified to date, and whereas the A subunit can form functional homomeric receptors, subunits B to E function only as heteromeric receptors in combination with the A subunit (Davies et al., 1999; Niesler et al., 2003). In the human nervous system, 5-HT₃A subunits have been found throughout the adult brain, but are also widely distributed in extra-

neuronal cells (Miyake et al., 1995; Fiebich et al., 2004). 5-HT₃B receptor subunits are not as widespread, but are still detectable in a range of tissues (Davies et al., 1999; Dubin et al., 1999; Niesler et al., 2003; Tzvetkov et al., 2007). The other subunits have been less well studied, and their functional significance is not yet clear (Thompson et al., 2010a).

5-HT₃ receptor antagonists are in use clinically, primarily for controlling chemotherapy- and radiotherapy-induced nausea and vomiting, and in postoperative nausea and vomiting. Alosetron is an effective symptomatic treatment for irritable bowel syndrome. In addition, studies have revealed a diversity of potential disease targets that might be amenable to alleviation by 5-HT₃ receptor-selective compounds; these include addiction, pruritis, emesis, fibromyalgia, migraine, rheumatic diseases, and neurological phenomena such as anxiety, psychosis, nociception, and cognitive function (Thompson and Lummis, 2007).

Structural details of the 5-HT₃ receptor at the molecular level are unresolved, but a wealth of evidence shows that the structure of these receptors is closely related to the structure of the nACh receptor (see Thompson et al., 2010a and Reeves and Lummis, 2002 for reviews). Consequently, the 5-HT₃ receptor is thought to be well represented by cryo-electron

This work was supported by the Wellcome Trust [Grant 81925] (to A.J.T. and S.C.R.L.); the National Institutes of Health National Institute of Neurological Disorders and Stroke [Grant NS11756] (to H.A.L.); and the National Institutes of Health National Institute of General Medical Sciences [Grant GM19375] (to H.A.L.). S.C.R.L. is a Wellcome Trust Senior Research Fellow in Basic Biomedical Science.

Article, publication date, and citation information can be found at <http://jpet.aspetjournals.org>.

doi:10.1124/jpet.111.185306.

^S The online version of this article (available at <http://jpet.aspetjournals.org>) contains supplemental material.

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; nACh, nicotinic acetylcholine; AChBP, acetylcholine binding protein; HEK, human embryonic kidney; ND96, 96 mM NaCl, 2 mM KCl, 1 mM MgCl₂, and 5 mM HEPES, pH 7.5; VAR, varenicline.

microscope images of the nACh receptor and crystal structures of the acetylcholine binding protein (AChBP), a protein homologous with the extracellular domain of the nACh receptor (Brejc et al., 2001). Perhaps unsurprisingly, because the 5-HT₃ and nACh receptors have similar functions (agonist binding results in the activation of a cation-selective ion channel), there are some overlaps in their pharmacological profiles; D-tubocurarine for example, which classically is considered to be a nACh receptor antagonist, is also a highly potent competitive, 5-HT₃ receptor antagonist.

Varenicline, a successful smoking cessation agent, is a derivative of cytosine, a nACh receptor agonist (Coe et al., 2005; Lowe et al., 2010). Varenicline is also a nACh receptor agonist, and, like cytosine, it has different potencies and efficacies at different subtypes of nACh receptors. At the α 4 β 2 receptor, varenicline is a potent partial agonist, whereas at the α 3 β 4 receptor it is a weak partial agonist. However, at the α 7 nACh receptor it is a full agonist, albeit with a comparatively high EC₅₀ (Mihalak et al., 2006). Here, we explore the effect of varenicline at the 5-HT₃ receptor, a protein with striking homology to the nAChR. The data show that varenicline is a potent agonist at the human but not the mouse 5-HT₃ receptor.

Materials and Methods

Materials. All cell culture reagents were obtained from Invitrogen (Paisley, UK), except fetal calf serum, which was from Labtech International (Ringmer, U.K.). [³H]granisetron (63.5 Ci/mmol) was from PerkinElmer Life and Analytical Sciences (Waltham, MA). All other reagents were of the highest obtainable grade.

Cell Culture. Human embryonic kidney (HEK) 293 cells were maintained on 90-mm tissue culture plates at 37°C and 7% CO₂ in a humidified atmosphere. They were cultured in Dulbecco's modified Eagle's medium/nutrient mix F12 (1:1) with GlutaMAX I media containing 10% fetal calf serum. For radioligand binding studies cells were transfected by using electroporation and plated in 90-mm dishes. For FlexStation studies, cells were transfected and then plated in 96-well plates. Cells were incubated 1 to 2 days before assay.

Oocytes. Harvested stage V to VI *Xenopus laevis* oocytes were washed in four changes of ND96 (96 mM NaCl, 2 mM KCl, 1 mM MgCl₂, and 5 mM HEPES, pH 7.5), defolliculated in 1.5 mg · ml⁻¹ collagenase type 1A for approximately 2 h, washed again in four changes of ND96, and stored in ND96 containing 2.5 mM sodium pyruvate, 50 mM gentamycin, and 0.7 mM theophylline. Human 5-HT_{3A} (GenBank accession number P46098) and mouse 5-HT_{3A} (GenBank accession number Q6J1J7) receptor subunit cDNAs were cloned into pGEMHE for oocyte expression (Liman et al., 1992). cRNA was transcribed in vitro from linearized plasmid cDNA template using the mMessage mMachine T7 transcription kit (Ambion, Austin, TX). Stage V and VI oocytes were injected with 20 ng of cRNA, and currents were recorded 2 to 3 days postinjection.

Electrophysiology. Using a two-electrode voltage clamp, *X. laevis* oocytes were clamped at -60 mV with an OpusXpress (Molecular Devices, Wokingham, UK) as described previously (Lummis et al., 2005). Analysis and curve fitting was performed using Prism version 3.0 (GraphPad Software Inc., San Diego, CA; www.graphpad.com). Concentration-response data for each oocyte were normalized to the maximum current for that oocyte. The mean and S.E.M. for a series of oocytes were plotted against agonist concentration and iteratively fitted to the four-parameter logistic equation using Prism. Values are presented as mean ± S.E.M.

Radioligand Binding. This was undertaken as described previously (Price and Lummis, 2004) with minor modifications. In brief,

transfected HEK293 cell membranes were incubated in 0.5 ml of HEPES buffer containing the 5-HT₃ receptor antagonist [³H]granisetron (0.1 nM). Nonspecific binding was determined using 1 μM quipazine. Data were analyzed by iterative curve fitting using Prism. Values are presented as mean ± S.E.M.

FlexStation Analysis. This technique uses fluorescent voltage-sensitive dyes to detect changes in the membrane potential and has been used to examine various ion channels including 5-HT₃ receptors (Price and Lummis, 2005; Thompson et al., 2010b). The methods were as described previously (Price and Lummis, 2005). In brief, fluorescent membrane potential dye (Molecular Devices) was diluted in Flex buffer (10 mM HEPES, 115 mM NaCl, 1 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, and 10 mM glucose, pH 7.4) and added to transfected cells grown on a 96-well plate. The cells were incubated at room temperature for 45 min and then fluorescence was measured in a FlexStation (Molecular Devices) at 2-s intervals for 200 s. Control (Flex buffer), 5-HT, or varenicline (0.001 μM–1.0 mM) was added to each well after 20 s. The percentage change in fluorescence was calculated as F (peak fluorescence) minus F_{\min} (baseline fluorescence at 20 s) divided by F_{\max} (peak fluorescence at 30 μM 5-HT). Concentration-response data were fitted to the four-parameter logistic equation using Prism software.

Modeling and Docking. Amino acid sequences of the human and mouse 5-HT_{3A} receptor subunits were aligned with that of AChBP using Fugue (<http://www-cryst.bioc.cam.ac.uk/fugue>). Three-dimensional homology models of human and mouse 5-HT_{3A} receptors were generated using MODELLER (University of California, San Francisco) based on the crystal structure of AChBP with carbamoylcholine docked (Protein Data Bank ID1UV6) as described previously (Thompson et al., 2008). The protonated form of varenicline was constructed in Chem3DUltra (CambridgeSoft Corporation, Cambridge, MA), energy-minimized using the MM2 force field, and docked into the models using GOLD (The Cambridge Crystallographic Data Centre, Cambridge, UK). The binding site was defined as being within 10 Å of W183, a critical ligand binding residue (e.g., Reeves et al., 2002). Ten genetic algorithm runs were performed, and the structures were categorized using their final ligand orientations.

Binding to Other Targets. A survey was conducted at NovaScreen/Caliper Life Sciences, Inc. (Hanover, MD). Assays included the nicotinic drug [³H]epibatidine binding to α -bungarotoxin-insensitive nACh receptors, the 5-HT₃ inhibitor [*N*-methyl-³H]GR65630 to mouse receptors in N1E-115 cells, and human receptors expressed in a clonal cell line.

Results

This study sought to determine whether varenicline is an agonist at human 5-HT₃ receptors. Because the homomultimeric human 5-HT_{3A} receptor is ~20-fold more sensitive to agonists than the heteromultimeric 5-HT_{3AB} receptor (Thompson and Lummis, 2008), we experimented on the former.

Oocyte Expression Studies. Application of 5-HT to *X. laevis* oocytes expressing human or mouse 5-HT_{3A} receptors produced concentration-dependent, rapidly activating inward currents. Plotting current amplitude against a series of 5-HT concentrations allowed curves to be fitted (Fig. 1). The pEC₅₀ values and Hill slopes determined from these curves are in Table 1.

The effect of varenicline on human 5-HT_{3A} receptors expressed in oocytes revealed that it was a relatively potent agonist with application of 10 μM routinely eliciting peak current responses of 2 to 10 μA. Challenging the receptor with a range of varenicline concentrations revealed an EC₅₀ of 5.9 μM, compared with an EC₅₀ of 2.0 μM for 5-HT (Table 1). Examination of the maximum responses to the two compounds revealed that varenicline had an I_{\max} value 79 ± 3%

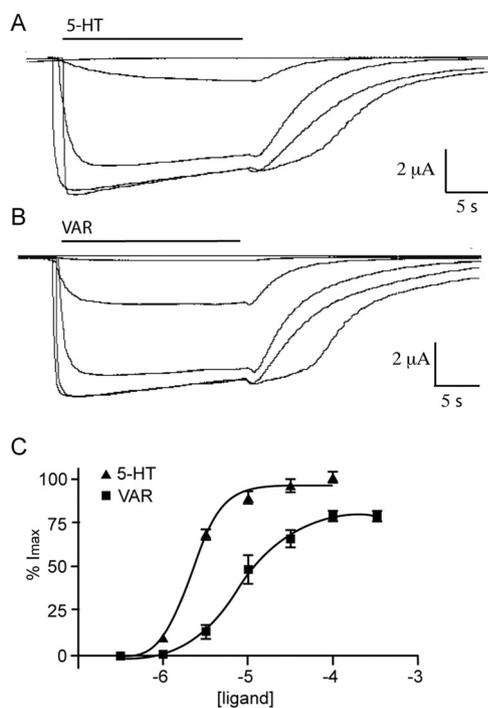


Fig. 1. A and B, typical responses of a single oocyte expressing human 5-HT₃A receptors to application of various 5-HT (A; 0.3–100 μM) or varenicline (VAR) (B; 0.3–100 μM) concentrations. C, concentration response curves are shown (mean ± S.E.M.; *n* = 7–10).

TABLE 1

Parameters for 5-HT₃ receptors expressed in *X. laevis* oocytes assayed using two-electrode voltage clamp
Data are mean ± S.E.M.

| Agonist | pEC ₅₀ | EC ₅₀ | nH | <i>n</i> |
|---------------------------|-------------------|------------------|-----------|----------|
| | μM | | | |
| Human 5-HT ₃ A | | | | |
| 5-HT | 5.70 ± 0.03 | 2.0 | 2.2 ± 0.2 | 10 |
| Varenicline | 5.23 ± 0.06 | 5.9 | 1.8 ± 0.3 | 10 |
| Mouse 5-HT ₃ A | | | | |
| 5-HT | 5.99 ± 0.03 | 1.0 | 1.9 ± 0.2 | 7 |
| Varenicline | 4.74 ± 0.08 | 18.3 | 1.2 ± 0.2 | 7 |

of that of 5-HT, indicating it is a near full agonist (Fig. 1). There were no significant differences in the Hill slopes.

Application of varenicline to mouse 5-HT₃A receptors expressed in oocytes revealed this compound was also an agonist, but it had a considerably lower potency than 5-HT, with an EC₅₀ close to 20 μM. The Hill slope was also significantly reduced. Examination of the maximum responses to the two compounds revealed that the maximum response to varenicline was 22 ± 2% of that of 5-HT, indicating it is a partial agonist (Fig. 2).

FlexStation Expression Studies. Application of varenicline to HEK293 cells transfected with human 5-HT₃A subunit DNA and analyzed using membrane potential fluorescent dye in the FlexStation revealed an EC₅₀ ~4-fold higher than that of 5-HT, i.e., comparable with the data obtained in oocytes (Table 2; Fig. 3). Cells expressing mouse 5-HT₃A receptors had an EC₅₀ for varenicline ~35-fold higher than 5-HT and again there was a decrease in the Hill slope. Relative EC₅₀ values determined using the FlexStation are therefore similar, but absolute EC₅₀ values are lower than those determined using voltage clamp, as we (and others)

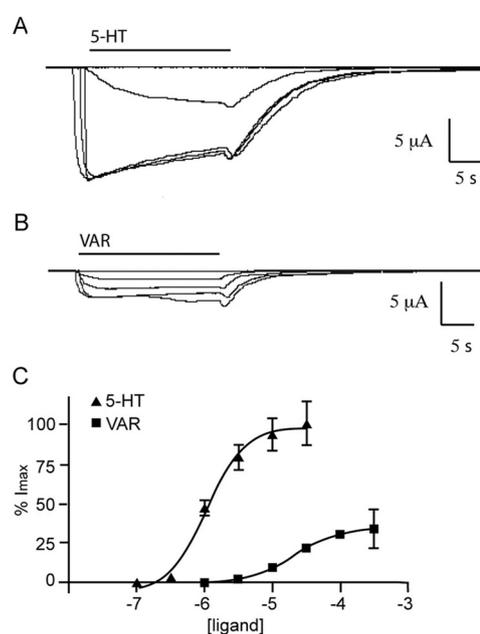


Fig. 2. A and B, typical responses of a single oocyte expressing mouse 5-HT₃A receptors to application of various 5-HT concentrations (A; 0.1–30 μM) or varenicline (B; 0.3–300 μM). C, concentration response curves are shown (mean ± S.E.M.; *n* = 7–10).

TABLE 2

Parameters derived from 5-HT₃ receptors expressed in HEK293 cells assayed using membrane potential-sensitive dye

Data are mean ± S.E.M.

| Agonist | pEC ₅₀ | EC ₅₀ | nH | <i>n</i> |
|---------------------------|-------------------|------------------|-----------|----------|
| | μM | | | |
| Human 5-HT ₃ A | | | | |
| 5-HT | 7.13 ± 0.08 | 0.07 | 2.7 ± 0.6 | 6 |
| Varenicline | 6.57 ± 0.06 | 0.27 | 2.9 ± 0.9 | 6 |
| Mouse 5-HT ₃ A | | | | |
| 5-HT | 6.85 ± 0.03 | 0.14 | 3.0 ± 0.4 | 4 |
| Varenicline | 5.31 ± 0.08 | 4.93 | 1.8 ± 0.2 | 4 |

have observed previously (e.g. Price and Lummis 2005). This is expected because different phenomena are being measured. For electrophysiological studies, EC₅₀ values represent the agonist concentration required to open 50% of channels, whereas in fluorescent studies they represent the agonist concentration required to depolarize the membrane potential to 50% of its original value.

Radioligand Binding Studies. Human and mouse 5-HT₃A receptors were expressed in HEK293 cells, and competition of varenicline with the competitive antagonist [³H]granisetron was explored. IC₅₀ values for varenicline and 5-HT were similar at both receptor types (Table 3; Fig. 4). Thus, given the large differences in EC₅₀ values, these data suggest that varenicline has different efficiencies of transducing the binding information to channel opening in the two species.

Modeling and Docking Studies. Many critical residues in the ligand binding site of human and mouse 5-HT₃A receptors are identical, but homology models of their binding sites reveal several subtle differences (Fig. 5). These differences arise from differing side chains, especially in loop C, which in turn lead to differing orientations of identical residues. Several types of data suggest that loop C closes over the bound agonist; this may be a critical step in channel gating.

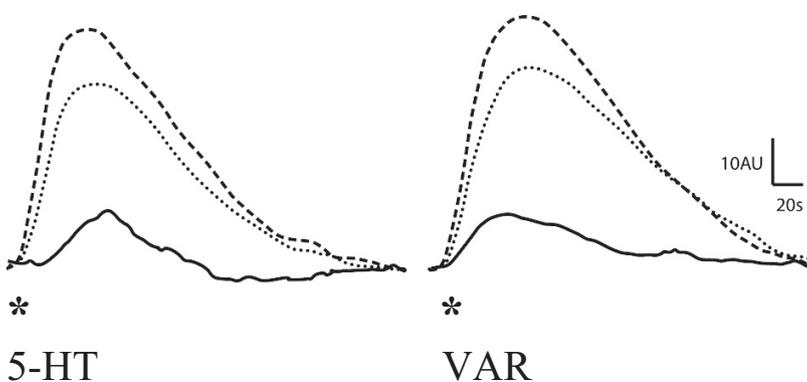


Fig. 3. Typical FlexStation responses of HEK293 cells transfected with human 5-HT_{3A} receptors. Dose-dependent increases in fluorescence in cells loaded with membrane potential fluorescent dye were observed with both 5-HT (0.03, 0.1, or 0.3 μ M) or varenicline (0.1, 0.3, or 1 μ M), which were added at *. AU, arbitrary fluorescent units.

TABLE 3
IC₅₀ values derived from radiolabeled antagonist-inhibition curves
Data are mean \pm S.E.M.

| | 5-HT IC ₅₀ | <i>n</i> | Varenicline IC ₅₀ | <i>n</i> |
|--------------------------|-----------------------|----------|------------------------------|----------|
| | μ M | | μ M | |
| Human 5-HT _{3A} | 0.09 \pm 0.01 | 5 | 0.58 \pm 0.10 | 4 |
| Mouse 5-HT _{3A} | 0.11 \pm 0.03 | 4 | 0.53 \pm 0.14 | 4 |

Docking of varenicline reveals different orientations in human and mouse 5-HT_{3A} receptor binding sites (Fig. 6). Relative to the loops that form the binding site, the angle of the relatively planar varenicline molecule differs by nearly 90°. Differing orientations of an agonist in the binding site could markedly affect C-loop closure and hence subsequent activation. Thus, these docking studies data provide a possible explanation for the different efficacies of varenicline at human and mouse receptors.

Lack of Binding to Other Sites. The binding assays for other targets were performed using 10 μ M varenicline and included 31 neurotransmitter-related targets, two steroid-related targets, six ion channels, nitric-oxide synthase, three prostaglandin receptors, four growth factor receptors, 13 brain/gut peptide receptors, and five enzymes (Supplemental Table 1). Positive results were obtained by competition for nACh receptors. Displacement of specific binding was also obtained in assays for mouse and human 5-HT₃ receptors. No other targets were detected.

Discussion

This study shows that varenicline is a relatively potent agonist of human 5-HT_{3A} receptors and binds to these recep-

tors at clinically relevant concentrations (32–131 nM; Rollema et al., 2010). It is, however, only a weak agonist at murine 5-HT_{3A} receptors. In addition to differences in EC₅₀, we observed large differences in efficacy. Varenicline was a near full agonist at human receptors (R_{max}/R_{max} 5-HT = ~80%) but a partial agonist at mouse receptors (R_{max}/R_{max} 5-HT ~35%), possibly because of different orientations of varenicline in the two different binding sites. Varenicline is widely used as a smoking cessation drug, an effect exerted through its action at nACh receptors (Faessel et al., 2006). However, its high potency and efficacy at human 5-HT_{3A} receptors suggests there may also be 5-HT₃-mediated effects in patients taking varenicline. One such effect may be nausea, which is the most common adverse event reported for varenicline in smoking cessation trials, and occurs at rates approaching 40% of patients (Aubin et al., 2008; Faessel et al., 2009). Nausea rarely causes patients to discontinue treatment but it does cause distress. It is noteworthy that early investigations established that nausea also limits the doses of varenicline tolerated by patients (Faessel et al., 2006). If nausea did not occur at higher doses, varenicline might be a more effective smoking cessation drug. The present data also show that 5-HT₃ agonism begins to increase at precisely the high end of clinically useful doses. Many studies show that 5-HT₃ receptor antagonists are antiemetic; some studies also show that 5-HT₃ receptor agonists directly cause emesis (Miller and Nonaka, 1992).

Previous commentaries suggest that varenicline side effects arise primarily from actions on α 3 β 4 and/or α 7 nACh receptors, where oocyte expression reveals EC₅₀ values of 55 and 18 μ M, respectively (Coe et al., 2005; Mihalak et al.,

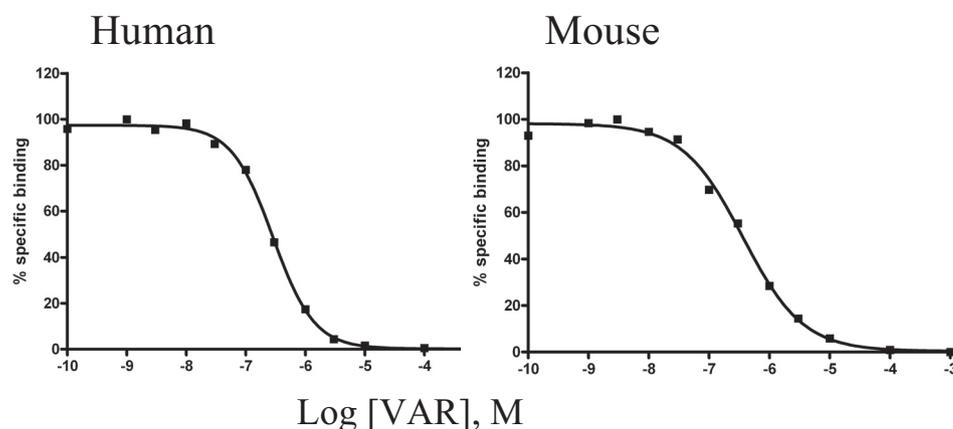


Fig. 4. Typical experiments showing varenicline displacement of [³H]granisetron binding to human and mouse 5-HT_{3A} receptors expressed in HEK293 cells. Parameters obtained from these data are shown in Table 3.

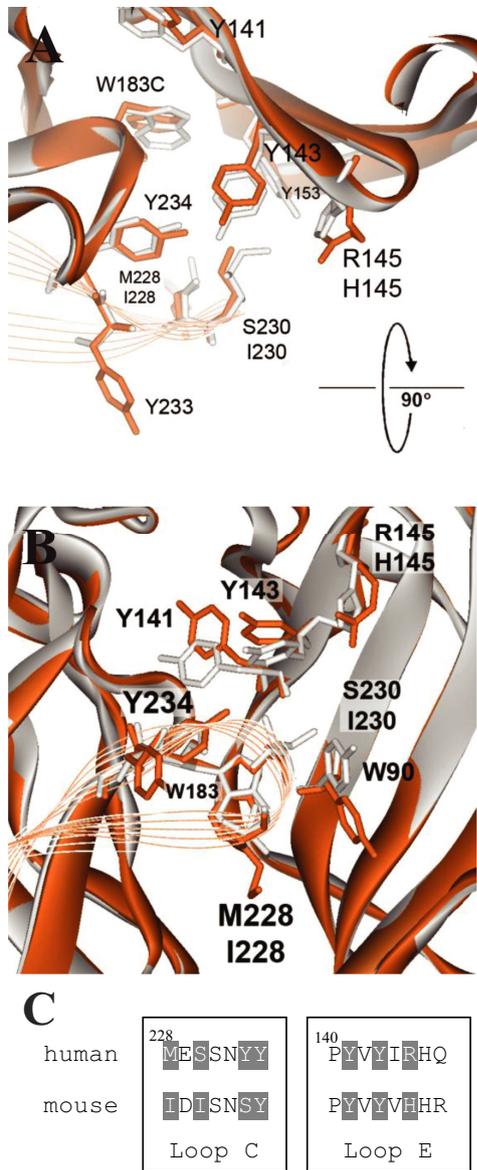


Fig. 5. A and B, an overlay of the human (orange) and mouse (white) 5-HT₃ A+A- binding interface, with differing residues and some critical binding residues highlighted. For clarity, the C-loop is rendered as a wire. The interface is shown from the membrane (A) and the extracellular compartment (B). C, an alignment of the loop C and loop E residues, some of which differ.

2006). We now suggest that the 5-HT₃ receptor is a possible target for some side effects. Our oocyte expression experiments show that varenicline functions as a nearly full (~80%) 5-HT_{3A} receptor agonist at human 5-HT₃ receptors. The EC₅₀ is ~6 μM for the human 5-HT_{3A} receptor, which is in the same range as, or lower than, the values for nACh receptors. Some nerve terminals coexpress both 5-HT₃ and α4β2 nACh receptors (Nayak et al., 2000), leading to the possibility of synergistic activation. In our comprehensive survey of potential targets, no binding was detected to sites other than nACh and 5-HT₃ receptors.

The data suggest that, at the submicromolar varenicline concentrations found in the plasma of patients, α4β2 and 5-HT₃ receptors are activated to approximately the same extent by varenicline (Table 4). We assume that varenicline has a Hill slope (n_H) of 1.8 for the human 5-HT₃ receptor

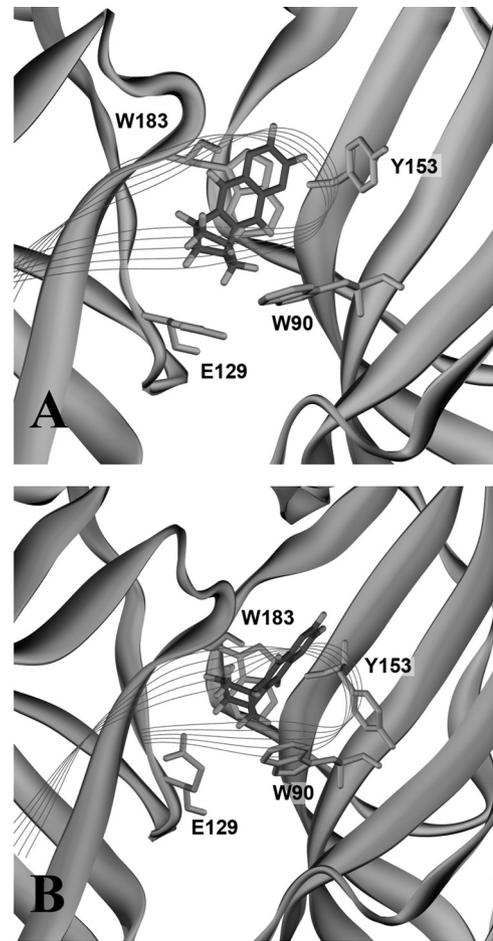


Fig. 6. Varenicline docked into mouse (A) and human (B) 5-HT₃A receptors. The favored docked poses for varenicline lie in the same general regions but have quite distinct orientations.

(Table 1), and 1.4 for the α4β2 receptor [based on H. A. Lester's unpublished results, which show a higher EC₅₀ than measured by Mihalak et al. (2006)]. The clinically used 1- and 2-mg daily doses of varenicline result in steady-state plasma varenicline concentrations of 8.5 and 15 ng/ml (40 and 71 nM, respectively) (Faessel et al., 2006). The calculations of Table 4 suggest that varenicline at these doses produces very low fractional activation of several nACh receptors, including the α4β2 thought to participate in the therapeutic effects of varenicline and the α3β4 or α7 nACh receptors previously considered as targets for the unpleasant side effects of varenicline (Coe et al., 2005; Mihalak et al., 2006). The fractionally small 5-HT₃ receptor activation is of the same order as for nACh receptors if calculated from the 5-HT₃ receptor oocyte data. The α7 nACh receptor seems most strongly activated; however, the Hill coefficients measured by Mihalak et al. (2006) are surprisingly low for agonist activation of α7; and if the usual estimates of >2 are used, then α7 activation resembles that for other receptors in Table 4.

It has been appreciated for some time that smoked concentrations of nicotine produce fractionally small activation of nAChRs; other agonist-receptor interactions, such as desensitization and pharmacological chaperoning, may therefore underlie some of the mechanisms of nicotine and varenicline (Lester et al., 2009; Miwa et al., 2011). In addition, when both varenicline and a full agonist are present, varenicline can act

TABLE 4

Calculated maximal activation at several receptors for two clinically effective daily doses of varenicline

Values were obtained using a simplified Hill equation appropriate for fractional activation of receptors by agonist at concentrations much lower than EC_{50} : fractional activation = peak response/(1 + (EC_{50} /agonist)^{n_H}).

| Receptor | | EC_{50} | n_H | Efficacy | Fractional Activation | |
|-------------------------------------|-------------|-----------|-------|----------|----------------------------|---------------------------|
| | | | | | Plasma Concentration | |
| | | | | | 8.6 ng/ml* (1 mg daily) | 15 ng/ml* (2 mg daily) |
| | | μM | | | | |
| 5-HT ₃ [@] | Oocyte | 2.0 | 1.8 | 0.80 | 1.0E-04 | 2.8E-04 |
| 5-HT ₃ [@] | FlexStation | 0.27 | 2.9 | 0.80 | 3.3E-03 | 1.7E-02 |
| $\alpha 4\beta 2$ ^{#&} | | 2.5 | 1.4 | 0.13 | 4.1E-04 | 8.9E-04 |
| $\alpha 3\beta 4$ [#] | | 55 | 2 | 0.75 | 4.1E-07 | 1.3E-06 |
| $\alpha 7$ [#] | | 1.8 | 1 | 0.93 | 2.1E-02 | 3.7E-02 |

* Data from Faessel et al., 2006.

@ Data from this study.

Data from Mihalak et al., 2006.

& H. A. Lester, unpublished data.

to decrease a full agonist-mediated response to the level of the lower, maximal varenicline response (Rollema et al., 2007). As a partial agonist, varenicline is especially advantageous in smoking cessation, because it decreases activation when nicotine is present (i.e., decreasing the pleasurable aspects of smoking), but activates weakly when nicotine is absent (i.e., during abstinence), presumably countering withdrawal symptoms.

Because varenicline is nearly a full agonist at 5-HT₃ receptors, it is unlikely to produce an analogous decreased response when both varenicline and 5-HT are present. Antagonists of 5-HT₃ receptors are used clinically as antiemetics. Some literature suggests that 5-HT₃ receptor agonists directly cause emesis (Miller and Nonaka, 1992). Our present suggestion, that the clinically relevant emetic side effects of varenicline involve 5-HT₃ receptor activation, favors the explanation that varenicline's emetic side effects arise via activation rather than antagonism of 5-HT₃ receptors. Using the EC_{50} values obtained from oocyte data indicates that receptor activation is low, but if we use the values obtained from FlexStation data it is considerably higher (assuming an efficacy of 0.8 as in the oocyte data; see Table 4). Because the latter is derived from changes in membrane potential rather than ion flux, it may well be more representative of the situation in vivo. Because the concentration of varenicline in the human brain is sufficient to activate nAChR (albeit less effectively than desensitizing them; Rollema et al., 2010), it is at a sufficiently high concentration to activate 5-HT₃ receptors. In addition, as mentioned above, there are potentially alternative mechanisms such as desensitization and/or pharmacological chaperoning. Pharmacological chaperoning by agonists is much stronger than by antagonists at nAChRs (Lester et al., 2009; Miwa et al., 2011). If the same is true of 5-HT₃ agonists at 5-HT₃ receptors, the varenicline-5-HT₃ receptor interaction and/or the downstream sequelae must therefore be considered as a cause of the nausea that limits the effectiveness of varenicline therapy.

Authorship Contributions

Participated in research design: Lummis, Thompson, Bencherif, and Lester.

Conducted experiments: Lummis and Thompson.

Performed data analysis: Lummis, Thompson, and Lester.

Wrote or contributed to the writing of the manuscript: Lummis, Thompson, Bencherif, and Lester.

References

- Aubin HJ, Bobak A, Britton JR, Oncken C, Billing CB Jr, Gong J, Williams KE, and Reeves KR (2008) Varenicline versus transdermal nicotine patch for smoking cessation: results from a randomised open-label trial. *Thorax* **63**:717–724.
- Brejck K, van Dijk WJ, Klaassen RV, Schuurmans M, van Der Oost J, Smit AB, and Sixma TK (2001) Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. *Nature* **411**:269–276.
- Coe JW, Brooks PR, Vetelino MG, Wirtz MC, Arnold EP, Huang J, Sands SB, Davis TI, Lebel LA, Fox CB, et al. (2005) Varenicline: an $\alpha 4\beta 2$ nicotinic receptor partial agonist for smoking cessation. *J Med Chem* **48**:3474–3477.
- Davies PA, Pistis M, Hanna MC, Peters JA, Lambert JJ, Hales TG, and Kirkness EF (1999) The 5-HT_{3B} subunit is a major determinant of serotonin-receptor function. *Nature* **397**:359–363.
- Dubin AE, Huvar R, D'Andrea MR, Pyati J, Zhu JY, Joy KC, Wilson SJ, Galindo JE, Glass CA, Luo L, et al. (1999) The pharmacological and functional characteristics of the serotonin 5-HT_{3A} receptor are specifically modified by a 5-HT_{3B} receptor subunit. *J Biol Chem* **274**:30799–30810.
- Faessel H, Ravva P, and Williams K (2009) Pharmacokinetics, safety, and tolerability of varenicline in healthy adolescent smokers: a multicenter, randomized, double-blind, placebo-controlled, parallel-group study. *Clin Ther* **31**:177–189.
- Faessel HM, Gibbs MA, Clark DJ, Rohrbacher K, Stolar M, and Burstein AH (2006) Multiple-dose pharmacokinetics of the selective nicotinic receptor partial agonist, varenicline, in healthy smokers. *J Clin Pharmacol* **46**:1439–1448.
- Fiebich BL, Akundi RS, Seidel M, Geyer V, Haus U, Müller W, Stratz T, and Candelario-Jalil E (2004) Expression of 5-HT_{3A} receptors in cells of the immune system. *Scand J Rheumatol Suppl* **119**:9–11.
- Lester HA, Xiao C, Srinivasan R, Son CD, Miwa J, Pantoja R, Banghart MR, Dougherty DA, Goate AM, and Wang JC (2009) Nicotine is a selective pharmacological chaperone of acetylcholine receptor number and stoichiometry. Implications for drug discovery. *AAPS J* **11**:167–177.
- Liman ER, Tytgat J, and Hess P (1992) Subunit stoichiometry of a mammalian K⁺ channel determined by construction of multimeric cDNAs. *Neuron* **9**:861–871.
- Lowe JA 3rd, DeNinno SL, Coe JW, Zhang L, Mente S, Hurst RS, Mather RJ, Ward KM, Shrikhande A, Rollema H, et al. (2010) A novel series of [3.2.1] azabicyclic biaryl ethers as $\alpha 3\beta 4$ and $\alpha 6/4\beta 4$ nicotinic receptor agonists. *Bioorg Med Chem Lett* **20**:4749–4752.
- Lummis SC, Beene D, Harrison NJ, Lester HA, and Dougherty DA (2005) A cation- π binding interaction with a tyrosine in the binding site of the GABAC receptor. *Chem Biol* **12**:993–997.
- Mihalak KB, Carroll FI, and Luetje CW (2006) Varenicline is a partial agonist at $\alpha 4\beta 2$ and a full agonist at $\alpha 7$ neuronal nicotinic receptors. *Mol Pharmacol* **70**:801–805.
- Miller AD and Nonaka S (1992) Mechanisms of vomiting induced by serotonin-3 receptor agonists in the cat: effect of vagotomy, splanchnicectomy or area postrema lesion. *J Pharmacol Exp Ther* **260**:509–517.
- Miwa JM, Freedman R, and Lester HA (2011) Neural systems governed by nicotinic acetylcholine receptors: emerging hypotheses. *Neuron* **70**:20–33.
- Miyake A, Mochizuki S, Takemoto Y, and Akuzawa S (1995) Molecular cloning of human 5-hydroxytryptamine₃ receptor: heterogeneity in distribution and function among species. *Mol Pharmacol* **48**:407–416.
- Nayak SV, Rondé P, Spier AD, Lummis SC, and Nichols RA (2000) Nicotinic receptors co-localize with 5-HT₃ serotonin receptors on striatal nerve terminals. *Neuropharmacology* **39**:2681–2690.
- Niesler B, Frank B, Kapeller J, and Rappold GA (2003) Cloning, physical mapping, and expression analysis of the human 5-HT₃ serotonin receptor-like genes HTR3C, HTR3D and HTR3E. *Gene* **310**:101–111.
- Price KL and Lummis SC (2004) The role of tyrosine residues in the extracellular domain of the 5-hydroxytryptamine₃ receptor. *J Biol Chem* **279**:23294–23301.
- Price KL and Lummis SC (2005) FlexStation examination of 5-HT₃ receptor function

- using Ca²⁺- and membrane potential-sensitive dyes: advantages and potential problems. *J Neurosci Methods* **149**:172–177.
- Reeves DC and Lummis SC (2002) The molecular basis of the structure and function of the 5-HT₃ receptor: a model ligand-gated ion channel (review). *Mol Membr Biol* **19**:11–26.
- Rollema H, Chambers LK, Coe JW, Glowa J, Hurst RS, Lebel LA, Lu Y, Mansbach RS, Mather RJ, Rovetti CC, et al. (2007) Pharmacological profile of the α 4 β 2 nicotinic acetylcholine receptor partial agonist varenicline, an effective smoking cessation aid. *Neuropharmacology* **52**:985–994.
- Rollema H, Shrikhande A, Ward KM, Tingley FD 3rd, Coe JW, O'Neill BT, Tseng E, Wang EQ, Mather RJ, Hurst RS, et al. (2010) Pre-clinical properties of the α 4 β 2 nicotinic acetylcholine receptor partial agonists varenicline, cytisine and dianicline translate to clinical efficacy for nicotine dependence. *Br J Pharmacol* **160**:334–345.
- Thompson AJ and Lummis SC (2007) The 5-HT₃ receptor as a therapeutic target. *Expert Opin Ther Targets* **11**:527–540.
- Thompson AJ and Lummis SC (2008) Antimalarial drugs inhibit human 5-HT₃ and GABA_A but not GABA_C receptors. *Br J Pharmacol* **153**:1686–1696.
- Thompson AJ, Lester HA, and Lummis SC (2010a) The structural basis of function in Cys-loop receptors. *Q Rev Biophys* **43**:449–499.
- Thompson AJ, Verheij MH, Leurs R, De Esch LJ, and Lummis SC (2010b) An efficient and information-rich biochemical method design for fragment library screening on ion channels. *Biotechniques* **49**:822–829.
- Tzvetkov MV, Meineke C, Oetjen E, Hirsch-Ernst K, and Brockmüller J (2007) Tissue-specific alternative promoters of the serotonin receptor gene HTR3B in human brain and intestine. *Gene* **386**:52–62.

Address correspondence to: Sarah C. R. Lummis, Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, United Kingdom. E-mail: sl120@cam.ac.uk
