NEW USE

TECHNICAL FIELD

The present invention relates to pharmaceutical compositions comprising a positive modulator of a nicotinic receptor agonist, said positive modulator having the capability to increase the efficacy of the said nicotinic receptor agonist.

BACKGROUND ART

Cholinergic receptors normally bind the endogenous neurotransmitter acetylcholine (ACh), thereby triggering the opening of ion channels. ACh receptors in the mammalian central nervous system can be divided into muscarinic (mAChR) and nicotinic (nAChR) subtypes based on the agonist activities of muscarine and nicotine, respectively. The nicotinic acetylcholine receptors are ligand-gated ion-channels containing five subunits (for reviews, see Colquhon et al. (1997) Advances in Pharmacology 39, 191-220; Williams et al. (1994) Drug News & Perspectives 7, 205-223; Doherty et al. (1995) Annual reports in Medicinal Chemistry 30, 41-50). Members of the nAChR gene family have been divided into two groups based on their sequences; members of one group are considered β subunits, while a second group are classified as α subunits (for reviews, see Karlin & Akabas (1995) Neuron 15, 1231-1244; Sargent (1993) Annu. Rev. Neurosci. 16, 403-443). Three of the α subunits, α7, α8 and α9, form functional receptors when expressed alone and thus presumably form homooligomeric receptors.

An allosteric transition state model of the nAChR involves at least a resting state, an activated state and a "desensitized" closed channel state (Williams et al., *supra*; Karlin & Akabas, *supra*). Different nAChR ligands can thus differentially stabilize the conformational state to which they preferentially bind. For example, the agonists ACh and (–)-nicotine stabilize the active and desensitized states.

Changes of the activity of nicotinic receptors has been implicated in a number of diseases. Some of these, e.g. myasthenia gravis and ADNFLE (autosomal dominant nocturnal front lobe epilepsy) (Kuryatov et al. (1997) J. Neurosci. 17(23):9035-47), are associated with reductions in the activity of nicotinic transmission either through a decrease in receptor number or increased desensitization, a process by which receptors become insensitive to the agonist. Reductions in nicotinic receptors have also been hypothesized to mediate cognitive deficits seen in diseases such as Alzheimer's disease and schizophrenia (Williams et al., *supra*). The effects of nicotine from tobacco are also mediated by nicotinic receptors. Increased activity of nicotinic receptors may reduce the desire to smoke.

The use of compounds which bind nicotinic acetylcholine receptors in the treatment of a range of disorders involving reduced cholinergic function such as Alzheimer's disease, cognitive or attention disorders, attention deficit hyperactivity disorders, anxiety, depression, smoking cessation, neuroprotection, schizophrenia, analgesia, Tourette's syndrome, and Parkinson's disease has been discussed in McDonald et al. (1995) "Nicotinic Acetylcholine Receptors: Molecular Biology, Chemistry and Pharmacology", Chapter 5 in Annual Reports in Medicinal Chemistry, vol. 30, pp. 41-50, Academic Press Inc., San Diego, CA; and in Williams et al. (1994) "Neuronal Nicotinic Acetylcholine Receptors," Drug News & Perspectives, vol. 7, pp. 205-223.

However, treatment with nicotinic receptor agonists which act at the same site as ACh is problematic because ACh not only activates, but also blocks receptor activity through processes which include desensitization (for a review, see Ochoa et al. (1989) Cellular and Molecular Neurobiology 9, 141-178) and uncompetitive blockade (open-channel block) (Forman & Miller (1988) Biophysical Journal 54(1):149-58). Furthermore, prolonged activation appears to induce a long-lasting inactivation. Therefore agonists of ACh can be expected to reduce activity as well as enhance it. At nicotinic receptors in general, and, of particular note, at the α7-nicotinic receptor, desensitization limits the duration of current during agonist application.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1

Model of current traces elicited by agonist, representing determination of increase in agonist efficacy by determination of current amplitude. Bars denote duration of application of compounds.

Fig. 2

Model of current traces elicited by agonist, representing determination of increase in agonist efficacy by determination of "area under the curve". Arrow indicates overlay of ACh current and ACh+modulator current. Bars denote duration of application of compounds.

Fig. 3

Effect of 5-hydroxyindole on ACh activity on the α7-nicotinic receptor. The current value of 100% is the extrapolated maximum from the ACh curve.

(●) ACh

(O) ACh + 0.5 mM 5-hydroxyindole

Fig. 4

Effect of 5-hydroxyindole on ACh activity on the α7-nicotinic receptor (human, rat and chick) expressed in *Xenopus* oocytes.

Fig. 5

Effect of 5-hydroxyindole on ACh (open staples) and (-)-Spiro[1-Azabicyclo[2.2.2.]Octane-3,5*-Oxazolidine]-2*-One (filled staples) activity on the α7-nicotinic receptor expressed in *Xenopus* oocytes.

Fig. 6

Effect of nAChR α7 modulator on agonist activity as measured by Ca^{2+} flux through nAChR α7 expressed in HEK-293 cells. The agonist is represented by (-)-Spiro[1-Azabicyclo[2.2.2.]Octane-3,5*-Oxazolidine]-2*-One.

DISCLOSURE OF THE INVENTION

It has surprisingly been found that certain compounds, e.g. 5-hydroxyindole (5-OHi), can enhance the efficacy of agonists at nicotinic receptors. This increase in efficacy can be greater than 2-fold. It is believed that compounds having this type of action (hereinafter referred to as "positive modulators") will be particularly useful for treatment of conditions associated with reductions in nicotinic transmission. In a therapeutic setting such compounds could restore normal interneuronal communication without affecting the temporal profile of activation. In addition, they would not produce long-term inactivation as prolonged application of agonist may.

The presence of this efficacy enhancing activity could not be predicted by the prior art. Albuquerque et al. have reported on another allosteric site on nicotinic receptors, which they call a "noncompetitive agonist" site. Compounds acting at this site are also called "allosterically potentiating ligands" (APL's). Compounds which appear to act at this site include several cholinesterase inhibitors, codeine, and 5-HT. It has been stated that activity via this noncompetitive agonist site "does not affect the level of maximum response to ACh; it shifts the dose-response curve to the left" (Maelicke & Albuquerque (1996) DDT, vol. 1, 53-59). In specific distinction, compounds acting at the discovered site increase the maximum response to ACh (its efficacy).

Another distinction between APL's and the present invention is the effect the modulators have on total current (as measured by area under the curve) in the presence of a saturating

concentration of agonist. APL's have little to no effect on area under the curve on nAChR α7 expressed in oocytes; 8-10% increases in area under the curve for a 1 second agonist application have been observed. In contrast, 5-OHi causes a robust increase in area under the curve (~400% increase) under the same conditions (see Fig. 4, top trace).

Specificity of the effect within the nicotinic receptor family is yet another distinguishing characteristic between APL's and the invention. APL's exert their positive modulatory effect on all nicotinic receptors tested, including muscle type (α1βδe).

At some non-nicotinic receptors, compounds have been found which can decrease receptor desensitization. At AMPA-type excitatory amino acid receptors, compounds such as cyclothiazide, some lectins like wheat-germ agglutinin, piracetam-like nootropics, and AMPAkines have been shown to decrease desensitization (Partin et al. (1993) Neuron 11, 1069-1082). Glycine has been reported to reduce desensitization of NMDA-type excitatory amino acid receptors (Mayer et al. (1989) Nature 338, 425-427). However, compounds which decrease desensitization on one receptor group have been found, in general, not to have the same affect on other receptor groups. For instance cyclothiazide has little or no effect on the NMDA and KA subtypes of glutamate receptors (Partin et al. (1993) Neuron 11, 1069-1082); moreover cyclothiazide is found to block 5-HT_3 receptors (D.A. Gurley, unpublished results). Glycine has no effect on 5-HT_3 receptors (Gurley and Lanthorn, (in press) Neurosci. Lett.).

The site was discovered using a compound (5-OHi) which is known to decrease desensitization at the 5-HT_3 receptor (Kooyman. A.R. et al. (1993) British Journal of Pharmacology 108, 287-289). However, only one other compound which produces or increases activity at the 5-HT_3 receptor, 5-HT itself, has been reported to increase activity at nicotinic receptors (Schrattenholz et al. (1996) Molecular Pharmacology 49, 1-6) although this activity has never been reported in *Xenopus* oocytes. Most agonists at the 5-HT_3 receptor have no activity or are antagonists at nicotinic receptors (unpublished results). In addition, the present inventors have been unable to reproduce the finding that 5-

HT increases activity at a nicotinic receptor. Therefore the enhancing effect of 5-OHi at nicotinic receptors could not have been predicted.

Consequently, the present invention provides in a first aspect a pharmaceutical composition comprising a positive modulator of a nicotinic receptor agonist together with a pharmaceutically acceptable carrier said positive modulator having the capability to increase the efficacy of the said receptor agonist. For the purposes of the present invention, the term "positive modulator" or "positive modulator of a nicotinic receptor agonist" shall be understood as a compound having the capability to increase the maximum efficacy of a nicotinic receptor agonist.

It will be understood that the invention includes compositions comprising either a positive modulator as the only active substance, thus modulating the activity of endogenous nicotinic receptor agonists, or a positive modulator in combination with a nicotinic receptor agonist. Thus, the said pharmaceutical compositions containing a positive modulator of a nicotinic receptor agonist may, in addition comprise a nicotinic receptor agonist.

In a preferred form of the invention, the said positive modulator is 5-hydroxyindole.

In another preferred form of the invention, the said nicotinic receptor agonist is an α7-nicotinic receptor agonist. Example of an α7-nicotinic receptor agonist is (-)-Spiro[1-Azabicyclo[2.2.2.]Octane-3,5*-Oxazolidine]-2*-One. Several α7-nicotinic receptor agonists are known in the art, e.g. from WO 96/06098, WO 97/30998 and WO 99/03859.

In a further aspect, the invention provides a method for the treatment of a condition associated with reduced nicotine transmission, by administering to a patient in need of such treatment, a medically effective amount of a positive modulator of a nicotinic receptor agonist, said positive modulator having the capability to increase the efficacy of the said nicotinic receptor agonist.

It will be understood that the methods of treatment of this invention includes either a positive modulator as the only active substance, thus modulating the activity of endogenous nicotinic receptor agonists, or a positive modulator administered together with a nicotinic receptor agonist.

In a preferred form of the invention, the said method of treatment includes the positive modulator is 5-hydroxyindole.

In another preferred form of the invention, the said method of treatment includes a nicotinic receptor agonist which is an α7-nicotinic receptor agonist. Example of an α7-nicotinic receptor agonist is (-)-Spiro[1-Azabicyclo[2.2.2.]Octane-3,5*-Oxazolidine]-2*-One. Several α7-nicotinic receptor agonists are known in the art, e.g. from WO 96/06098, WO 97/30998 and WO 99/03859.

A further aspect of the invention is the use of a pharmaceutical composition according to the invention in the manufacture of a medicament for the treatment or prophylaxis of a condition associated with reduced nicotinic receptor transmission or a condition associated with reduced nicotinic density which could be one of the below mentioned diseases or conditions which comprises administering a therapeutically effective amount of compounds according to the invention to a patient.

It will be understood that the use includes compositions comprising either a positive modulator as the only active substance, thus modulating the activity of endogenous nicotinic receptor agonists, or a positive modulator in combination with a nicotinic receptor agonist. Thus, the said use of pharmaceutical compositions containing a positive modulator of a nicotinic receptor agonist may, in addition comprise a nicotinic receptor agonist.

In a preferred form of the invention, the use comprises the positive modulator is 5-hydroxyindole.

In another preferred form of the invention, the use of the said nicotinic receptor agonist is represented by an α7-nicotinic receptor agonist. Example of an α7-nicotinic receptor agonist is (-)-Spiro[1-Azabicyclo[2.2.2.]Octane-3,5*-Oxazolidine]-2*-One. Several α7-nicotinic receptor agonists are known in the art, e.g. from WO 96/06098, WO 97/30998 and WO 99/03859.

Examples of diseases or conditions include schizophrenia, mania and manic depression, anxiety, Alzheimer's disease, learning deficit, cognition deficit, attention deficit, memory loss, and Attention Deficit Hyperactivity Disorder, Parkinson's disease, Huntington's disease, Tourette's syndrome, jetlag, and nicotine addiction (including that resulting from exposure to products containing nicotine).

It will be understood that the said positive modulator can be administered either with the purpose of acting on endogenous nicotine receptor agonists, or in combination with an exogenous nicotinic receptor agonist.

A further aspect of the invention relates to a pharmaceutical composition for treating or preventing a condition or disorder as exemplified above arising from dysfunction of nicotinic acetylcholine receptor neurotransmission in a mammal, preferably a human, compositions comprising either a positive modulator as the only active substance, thus modulating the activity of endogenous nicotinic receptor agonists, or a positive modulator in combination with a nicotinic receptor agonist. Thus, the said use of pharmaceutical compositions containing a positive modulator of a nicotinic receptor agonist may, in addition comprise a nicotinic receptor agonist, effective in treating or preventing such disorder or condition and an inert pharmaceutically acceptable carrier.

For the above-mentioned uses the dosage administered will, of course, vary with the composition employed, the mode of administration and the treatment desired. However, in general, satisfactory results will be obtained when the active components are administered at a daily dosage of from about 0.1 mg to about 20 mg per kg of mammlian body weight, preferably given in divided doses 1 to 4 times a day or in sustained release form. For man,

the total daily dose is in the range of from 5 mg to 1,400 mg, more preferably from 10 mg to 100 mg, and unit dosage forms suitable for oral administration comprise from 2 mg to 1,400 mg of the active components admixed with a solid or liquid pharmaceutical carrier or diluent.

The compositions mentioned above, may be used on their own or in the form of appropriate medicinal preparations for enteral, parenteral, oral, rectal or nasal administration.

Examples of suitable diluents and carriers are:
– for tablets and dragees: lactose, starch, talc, stearic acid; for capsules: tartaric acid or lactose;
– for injectable solutions: water, alcohols, glycerin, vegetable oils; for suppositories: natural or hardened oils or waxes.

There is also provided a process for the preparation of such a pharmaceutical composition which comprises mixing the ingredients simultaneously or sequentially.

In a further aspect, the invention provides a method for identifying a positive modulator of a nicotinic receptor agonist. Compounds are considered "positive modulators" if, in the presence of saturating concentrations of the nAChR α7 agonist ACh, current is elicited that exceeds 200% of control current (100% potentiation) when measured baseline to peak (see Experimental Methods). Control current is defined as the current elicited by agonist in the absence of modulator. A saturating concentration of ACh is defined as 10-times the EC_{50} for the specific nAChR α7 type used. EC_{50} is defined as the concentration which elicits a half-maximal response. EC_{50} values for nAChR α7 subtypes typically range between 100–300 μM (Bertrand et al. (1992) Neuroscience Letters 146, 87-90; Peng et al. (1994) Molecular Pharmacology 45, 546-554). Further, compounds are considered "positive modulators" if, in the presence of saturating concentrations of agonist, total current through the receptor (flux) exceeds 200% of control current. One measure of total current is area under the curve (current trace) during an agonist application.

Consequently, the method according to the invention for identifying a positive modulator of a nicotinic receptor agonist, can comprise the steps (a) expressing a nicotinic receptor on the surface of a cell; (b) contacting the said nicotinic receptor with a compound known to be a nicotinic receptor agonist and a compound to be tested for positive modulating activity; (c) determining whether the compound to be tested exhibits a positive modulation on the effect of the said nicotinic receptor agonist, resulting in current amplitude (measured baseline to peak) or total current (measured as area under the curve for the current trace) greater than 200% of control (100% potentiation). The cell could be a *Xenopus* oocyte, HEK-293 cell or a cultured neuron. The nicotinic receptor could be either a human, rat, chick, mouse or bovine nicotinic receptor.

In a further aspect of the present invention related to the method for identifying a positive modulator of a nicotinic receptor agonist, the nicotinic receptor is an α7-nicotinic receptor.

In yet a further aspect, the invention provides a method for identifying a compound which is a nicotinic receptor agonist, said method comprising the steps (a) expressing a nicotinic receptor on the surface of a cell; (b) contacting the said nicotinic receptor with a compound to be tested for nicotinic receptor agonist activity, in the presence of a positive modulator of a nicotinic receptor agonist; and (c) determining whether the compound to be tested exhibits nicotinic receptor agonist activity. The cell could be a *Xenopus* oocyte, HEK-293 cell or a cultured neuron. The nicotinic receptor could be either a human, rat, ovine, murine or bovine nicotinic receptor. It will be understood by the skilled person that "nicotinic receptor agonist activity" can be determined by methods known in the art, such as those methods described in the section "Experimental Methods" below.

In a further aspect of the present invention, related to the method for identifying a compound which is a nicotinic receptor agonist, the nicotinic receptor is an α7-nicotinic receptor.

EXPERIMENTAL METHODS

(a) *Xenopus* oocyte current recording

The *Xenopus* oocyte has provided a powerful means of assessing the function of proteins thought to be subunits of ligand-gated ion-channels. Injection of RNA transcribed from cDNA clones encoding the appropriate receptor subunits, or injection of cDNA in which the coding sequence is placed downstream of a promoter, results in the appearance of functional ligand-gated ion-channels on the surface of the oocyte (see e.g. Boulter et al. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 7763-7767).

Consequently, one convenient technique to assess the enhancement of nicotinic efficacy is two-electrode voltage-clamp recording from *Xenopus* oocytes expressing α7-nicotinic receptors from cRNA.

Xenopus laevis frogs (Xenopus I, Kalamazoo, MI) were anesthetized using 0.15% tricaine. Oocytes were removed to OR2 solution (82 mM NaCl, 2.5 mM KCl, 5 mM HEPES, 1.5 mM NaH_2PO_4, 1 mM $MgCl_2$, 0.1 mM EDTA; pH 7.4). The oocytes were defolliculated by incubation in 25 ml OR2 containing 0.2% collagenase 1A (Sigma) two times for 60 min on a platform vibrating at 1 Hz and stored in Leibovitz's L-15 medium (50 µg/ml gentomycin, 10 Units/ml penicillin, and 10 µg/ml streptomycin). Approximately 50 ng of cRNA was injected in each oocyte the following day. cRNA was synthesised from cDNA using Message Machine (purchased from Abion).

The external recording solution consisted of 90 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, 1 mM $BaCl_2$, 5 mM HEPES; pH 7.4. Two-electrode voltage-clamp recording was carried out using an Oocyte Clamp amplifier (OC 725C; Warner Instrument, Hamden, CT). Oocytes were impaled with two electrodes of 1-2 MΩ tip resistance when filled with 3M KCl. Recordings were begun when membrane potential became stable at potentials negative to –20mV (resting membrane potentials are less negative when Ba^{++} replaces Ca^{++} in bathing solutions). Membrane potential was clamped at –80 mV. ACh was purchased from Sigma.

Oocytes were continuously perfused (5 ml/min) with recording solution with or without ACh.

Current amplitude was measured from baseline to peak. EC_{50} values, maximal effect, and Hill slopes were estimated by fitting the data to the logistic equation using GraphPad Prism (GraphPad Software, Inc., San Diego, CA).

Increases in agonist efficacy elicited by a positive modulator can be calculated in two ways:

(1) As percent potentiation of current amplitude which is defined as $100(I_m-I_c)/I_c$ where I_m is current amplitude in the presence of modulator and I_c is current in the absence of modulator (Fig. 1).

(2) As percent potentiation of "area under curve" of an agonist trace. Area under the curve is a common representation of the total ion flux through the channel (Fig. 2). In the example shown in Fig. 2, although current amplitude is not increased, area under the curve is potentiated roughly 100% over control for the duration of the agonist application

(b) Ca^{2+} flux imaging

Imaging of Ca^{2+} flux through nAChR α7 receptors transiently expressed in a cell line is another means of assaying modulator activity.

Cells expressing α7 receptors (for example HEK-293 cells or cell cultured neurons) are grown to confluence in 96 well plates and loaded with fluo-3, a fluorescent calcium indicator. To screen for α7 modulatory activity, the 96 well plate is placed in a fluorescence imaging plate reader (FLIPR) and test compounds along with an α7 agonist are applied simultaneously to all wells. Receptor activation is measured by calcium influx into cells which is quantified by the increase in fluorescence intensity of each well, recorded simultaneously by the FLIPR. A modulatory effect is determined by the increase

in fluorescence over that of agonist alone. Similarly, to test for nAChR α7 agonist activity, test compounds along with an α7 modulator are applied simultaneously to all wells. Receptor activation is measured by calcium influx into cells which is quantified by the increase in fluorescence intensity of each well, recorded simultaneously by the FLIPR. An agonist effect is determined by the increase in fluorescence over that of modulator alone.

Cell-cultured neurons are prepared according to the following method: Eighteen day old Sprague-Dawley rat fetuses (E-18) were asceptically removed from the pregnant male, sacrificed, the frontal cortices of the brains removed, the menniges stripped, and the cleaned cortex placed into cold HBSS. If hippocampus was desired, the hippocampus was dissected away from the cortex and then placed into cold HBSS. The tissues were mechanically dispersed, washed once in HBSS (200 g for 30 minutes in 4C) resuspended in a modification of Sato's medium supplemented with glutamine, antibiotics, potassium chloride, insulin, transferrin, selenium, and 5% heat-inactivated fetal bovine serum (FBS; endotoxin free) and plated into each of a 24-well plate (coated with poly-L-lysine). The wells could contain glass coverslips which were also coated with PLL. The plates were incubated at 37C in a CO_2 incubator. After 24 hours the medium was removed, fresh medium added, and the cells allowed to grow for at least another 11 days, feeding when necessary.

EXAMPLE 1

Changes in efficacy of nicotinic agonists was assessed by measuring the combined effects of a nicotinic agonist with test compounds. In general, the protocol consisted of pretreatment with test compound plus coapplication of agonist and test compound. 5-hydroxyindole was tested at 500 μM against a range of concentrations of ACh. ACh was first tested by itself so that an EC_{50} and maximal response could be determined. Then the same concentrations of ACh were applied along with 5-OH-indole. The results (Fig. 3)

were that the maximal response to ACh was increased (maximum amplitude increased 2-fold).

The effect of 5-OHi (0.5 mM) on "area under curve" for saturating concentration of agonist (3 mM ACh) was determined. 5-OHi caused a robust increase in area under the curve (~400% increase) (Fig. 4).

Applied by itself, 5-hydroxyindole did not induce current in oocytes injected with cRNA for α7 nicotinic receptors.

EXAMPLE 2

The effect of 5-hydroxyindole on various nicotinic agonists was tested. The increase in efficacy afforded by 5-hydroxyindole was seen with all nicotinic agonists tested, e.g. (-)-Spiro[1-Azabicyclo[2.2.2.]Octane-3,5*-Oxazolidine]-2*-One (Fig. 5). Open boxes current elicited by ACh (3 mM) with (+) and without (–) modulator. Solid boxes current elicited by a nicotinic agonist designated AR-R 17779 (100 μM) with (+) and without (–) modulator. Modulator in this instance was 1 mM 5-OHi.

Compounds tested with similar results include (–)-nicotine and choline (data not shown). Therefore the effect appears to be general for any cholinergic agonist.

EXAMPLE 3

The increase in efficacy afforded by 5-hydroxyindole was not seen on any other nicotinic receptors, e.g. mouse muscle-type nicotinic receptors.

EXAMPLE 4

A series of related compounds were tested for positive modulation on ACh activity. Only a few compounds retained efficacy enhancing activity. In particular, serotonin (5-HT) did not increase efficacy. This preliminary analysis of close analogues indicates a fairly tight structure-activity relationship, suggesting a selective site of action.

EXAMPLE 5

Effect of nAChR α7 modulator on agonist activity was measured by Ca^{2+} flux through nAChR α7 expressed in HEK-293 cells. The nicotinic agonist (-)-Spiro[1-Azabicyclo[2.2.2.]Octane-3,5*-Oxazolidine]-2*-One, was used. The results are shown in Fig. 6. No discernible signal was obtained in the presence of agonist alone (no modulator). In the presence of agonist together with modulator, a significant increase in agonist activity was seen.

CLAIMS

1. A pharmaceutical composition comprising a positive modulator of a nicotinic receptor agonist together with a pharmaceutically acceptable carrier, said positive modulator having the capability to increase the efficacy of the said nicotinic receptor agonist.

2. The pharmaceutical composition according to claim 1, in addition comprising a nicotinic receptor agonist.

3. The pharmaceutical composition according to claim 1 or 2 wherein the said positive modulator is 5-hydroxyindole.

4. The pharmaceutical composition according to any one of claims 1 to 3 wherein the said nicotinic receptor agonist is an α7-nicotinic receptor agonist.

5. A method for the treatment of a condition associated with reduced nicotine transmission, by administering to a patient in need of such treatment, a medically effective amount of a positive modulator of a nicotinic receptor agonist, said positive modulator having the capability to increase the efficacy of the said nicotinic receptor agonist.

6. The method according to claim 5 wherein the said positive modulator is administered together with a nicotinic receptor agonist.

7. The method according to claim 5 or 6 wherein the said positive modulator is 5-hydroxyindole.

8. The method according to any one of claims 5 to 7 wherein the said nicotinic receptor agonist is an α7-nicotinic receptor agonist.

This Sheet Has Been Replaced

9. The method according to any one of claims 5 to 8 for the treatment of Alzheimer's disease, Attention Deficit Hyperactivity Disorder, schizophrenia, anxiety or nicotine addiction.

10. The method according to any one of claims 5 to 8 for the treatment of Alzheimer's disease.

11. The method according to any one of claims 5 to 8 for the treatment of Attention Deficit Hyperactivity Disorder.

12. The method according to any one of claims 5 to 8 for the treatment of schizophrenia.

13. The method according to any one of claims 5 to 8 for the treatment of nicotine addiction.

14. A method for identifying a positive modulator of a nicotinic receptor agonist, said method comprising the steps (a) expressing a nicotinic receptor on the surface of a cell; (b) contacting the said nicotinic receptor with a compound known to be a nicotinic receptor agonist and a compound to be tested for positive modulating activity; (c) determining whether the compound to be tested exhibits a positive modulation on the effect of the said nicotinic receptor agonist.

15. A method for identifying a compound which is a nicotinic receptor agonist, said method comprising the steps (a) expressing a nicotinic receptor on the surface of a cell; (b) contacting the said nicotinic receptor with a compound to be tested for nicotinic receptor agonist activity, in the presence of a positive modulator of a nicotinic receptor agonist; and (c) determining whether the compound to be tested exhibits nicotinic receptor agonist activity.

16. A method according to claim 14 or 15 wherein the cell is a *Xenopus* oocyte, a HEK-293 cell or a cell-cultured neuron.

This Sheet Has Been Replaced

17. A method according to claim 14 or 15 wherein the nicotinic receptor is an α7-nicotinic receptor.

18. A method according to claim 14 or 15 wherein the nicotinic receptor is either a human, rat, chick, mouse or bovine nicotinic receptor.

19. A compound identifiable by a method according to any one of claims 10 to 18.

20. Use of a positive modulator of a nicotinic receptor agonist in the manufacture of a medicament for treatment of or prophylaxis of a condition associated with reduced nicotine transmission.

21. Use of a positive modulator of a nicotinic receptor agonist together with a nicotinic receptor agonist in the manufacture of a medicament for treatment of a condition associated with reduced nicotine transmission.

22. The use according to claim 20 or 21 wherein the modulator is 5-hydroxyindole.

23. The use according to claim 20 or 21 wherein the nicotinic receptor agonist is an α7-nicotinic receptor agonist.

24. The use according to any one of claims 20 to 23 in the manufacture of a medicament for the treatment of Alzheimer's disease, attention deficit hyperactivity disorder, schizophrenia, anxiety or nicotine addiction.

25. The use according to any one of claims 20 to 23 in the manufacture of a medicament for the treatment of Alzheimer's disease.

26. The use according to any one of claims 20 to 23 in the manufacture of a medicament for the treatment of attention deficit hyperactivity disorder.

This Sheet Has Been Replaced

27. The use according to any one of claims 20 to 23 in the manufacture of a medicament for the treatment of schizophrenia.

28. The use according to any one of claims 20 to 23 in the manufacture of a medicament for the treatment of nicotine addiction.

This Sheet Has Been Replaced