Use of r(+)-n-propargyl-1-aminoindan to treat or prevent hearing loss

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(54) Title: USE OF R(+)-N-PROPARGYL-1-AMINOINDAN TO TREAT OR PREVENT HEARING LOSS

(57) Abstract: A method of treating or inhibiting hearing loss in a mammalian subject, comprising administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof effective to treat or inhibit the hearing loss in the subject.

USE OF R(+)-N-PROPARGYL-1-AMINOINDAN TO TREAT OR PREVENT HEARING LOSS

Throughout this application various publications, published patent applications, and patents are referenced. The disclosures of these documents in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

Background of the Invention

Hearing loss is a serious handicap which affects millions of people. Hearing impairments can be attributed to a wide variety of causes, including infections, mechanical injury, loud sounds, aging, and chemical-induced ototoxicity that damages neurons and/or hair cells of the peripheral auditory system.

The peripheral auditory system consists of auditory receptors, hair cells in the organ of Corti, and primary auditory neurons, the spiral ganglion neurons in the cochlea. The activity of the synapse between the inner hair cells (IHCs) and the type II afferent dendrites is modulated by the lateral olivocochlear (LOC) efferent fibers (Eybalin M, (1993) Neurotransmitters and neuromodulators of the mammalian cochlea. Physiol Rev 73: 309-373; Eybalin M, Pujol R, (1989) Cochlear neuroactive substances. Arch Otorhinolaryngol 246: 228-234; Puel J-L, (1995) Chemical synaptic transmission in the cochlea. Prog Neurobiol 47: 449-476).

Ototoxicity is caused by drugs or chemicals that damage the inner ear or the vestibulocochlear nerve, which sends

balance and hearing information to the brain from the inner ear. Ototoxicity may result in temporary or permanent losses of hearing, balance, or both. Substances that may cause ototoxicity include antibiotics, chemotherapy drugs, environmental chemicals, loop diuretics, aspirin and quinine products.

Rasagiline, R(+)-N-propargyl-1-aminoindan, is a potent second generation monoamine oxidase (MAO) B inhibitor (Finberg JP, Youdim MB, (2002) Pharmacological properties of the anti-Parkinson drug rasagiline; modification of endogenous brain amines, reserpine reversal, serotonergic and dopaminergic behaviours. Neuropharmacology 43(7):1110-8). Rasagiline mesylate in a 1 mg tablet is commercially available as monotherapy or as an adjunct for the treatment of idiopathic Parkinson's disease as Azilect® from Teva Pharmaceuticals Industries, Ltd. (Petach Tikva, Israel) and H. Lundbeck A/S (Copenhagen, Denmark). See, e.g. AZILECT®, Physician's Desk Reference (2006), 60th Edition, Thomson Healthcare. Recent studies have demonstrated that, in addition to its MAO-B inhibitor activity, rasagiline possesses potent neuroprotective activity demonstrated by *in vitro* and *in vivo* experiments. Neuroprotection by rasagiline was achieved in animal models of closed head trauma (Huang W, Chen Y, Shohami E, Weinstock M. (1999) Neuroprotective effect of rasagiline, a selective monoamine oxidase-B inhibitor, against closed head injury in the mouse. Eur J Pharmacol. 366(2-3):127-35), global focal ischemia (Speiser Z, Mayk A, Eliash S, Cohen S. (1999) Studies with rasagiline, a MAO-B inhibitor, in experimental focal ischemia in the rat. 106 (7-8) 593-606) and MPTP(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced neurotoxicity (Sage et al. 2001, 2003) as well as transgenic model of amyotrophic lateral sclerosis (Waibel S. et al. (2004)

Rasagiline alone and in combination with riluzole prolongs survival in an ALS mouse model. 251 (9) 1080-1084) and 6-OHDA(6-hydroxydopamine) model of Parkinson's disease (Blandini, F. et al. (2004) Neuroprotective effect of rasagiline in a rodent model of Parkinson's disease. Exp Neurol. 2004 Jun;187(2):455-9). Cell culture experiments have shown that rasagiline potently suppresses apoptotic cell death initiated by mitochondria (Youdim MBH, et al., (2001) Rasagiline (N-propargyl-1R(+)-aminoindan), a selective and potent inhibitor of mitochondrial monoamine oxidase B. Br. J. Pharmacol., 132:500-6; Akao Y. et al. (2002) Mitochondrial permeability transition mediates apoptosis induced by *N*-methyl(*R*)salsolinol, an endogenous neurotoxin, and is inhibited by Bcl-2 and rasagiline, *N*-propargyl-1(*R*)-aminoindan. 82 (4) 913-923) by preventing preapoptotic swelling of mitochondria, caspase 3 activation, activation of nuclear PARP(poly ADP ribose polymerase)-1, translocation of GADPH(glyceraldehydes-3-phosphate dehydroxenase), and nucleasomal DNA fragmentation (Youdim MBH and Weistock M. (2001) Molecular Basis of Neuroprotective Activities of Rasagiline and the Anti-Alzheimer Drug TV3326 [N-Propargyl-(3R) Aminoindan-5-YL)-Ethyl Methyl Carbamate]. Cell. Mol. Neurobio. 21(6) 555-573; Youdim MBH et al. (2003) Neuroprotective Strategies in Parkinson's Disease: An Update on Progress. CNS Drugs. 17(10):729-762; Bar-am et al. (2004) Regulation of protein kinase C by the anti-Parkinson drug, MAO-B inhibitor, rasagiline and its derivatives, *in vivo*. Journal of Neurochemistry 89 (5), 1119-1125; and Weinreb O. et al. (2004) Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases. The Journal of Nutritional Biochemistry, Volume 15, Issue 9, Pages 506-516). Further, rasagiline induces increase of the anti-apoptotic Bcl-2 and Bcl-xL expression parallel to

downregulation of proapoptotic Bad and Bax (Youdim MBH et al. (2003) The essentiality of Bcl-2, PKC and proteasome-ubiquitin complex activations in the neuroprotective-antiapoptotic action of the anti-Parkinson drug, rasagiline. Biochem Pharmacol. 66(8):1635-41; Yogev-Falach et al. (2003) Amyloid Processing and Signal Transduction Properties of Antiparkinson-Antialzheimer Neuroprotective Drugs Rasagiline and TV3326. *Annals of the New York Academy of Sciences* 993:378-386). Recent evidence from a delayed-start design study in Parkinson's Disease has suggested potential disease-modifying efficacy of rasagiline also in a clinical setting (Parkinson Study, G, A controlled, randomized, delayed-start study of rasagiline in early Parkinson's disease, Arch. Neurol. (2004) 61 (4) : 561-6).

Whether rasagiline has positive effects on the peripheral auditory system has not been heretofore investigated.

SUMMARY OF THE INVENTION

This subject invention provides a method of treating or inhibiting hearing loss in a mammalian subject, comprising administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof effective to treat or inhibit the hearing loss in the subject.

This subject invention also provides a method of alleviating a symptom of hearing loss in a mammalian subject, comprising administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof effective to alleviate the symptom of hearing loss in the subject.

This subject invention also provides a pharmaceutical composition for the use in the treatment, prevention, or alleviation of symptoms of hearing loss in a subject which comprises a therapeutically effective amount of R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof and pharmaceutically acceptable carrier.

This subject invention also provides a use of R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof for the manufacture of medicament for the treatment, prevention or alleviation of a symptom of hearing loss.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. shows a schematic drawing of the experimental design.

Figure 2. shows the effect of the Rasagiline on dopamine release from the guinea pig cochlea preparation was concentration dependent.

Rasagiline was added to the perfusion fluid from the 8^{th} fraction and was maintained until the end of the experiment. Ratio values of electrically evoked dopamine release (FRS_2/FRS_1) in the absence and the presence of different concentrations of Rasagiline are shown for comparison. Data presented are means ± SEM.

Figure 3A, B, C and D. shows the changes of duration of drug application did not influence the action of Rasagiline. **(A)** Time lapse changes in the release of dopamine from the cochlea *in vitro*. Rasagiline was added to the perfusion 3-21 minutes prior to the second electrical stimulation (in brackets). Dotted line indicates the timing of Rasagiline in our previous study (8^{th} fraction, 15 min). **(B)** The shortest and the longest duration of Rasagiline perfusion are shown separately. **(C)** Ratio values of electrically evoked dopamine. **(D)** Ratio values of resting DA release. Data presented in C and D are means ± SEM.

Figure 4. shows an example for deafening the mouse ear with intratympanic application of neomycine recorded by auditory brainstem response (ABR) measurements in vivo. In the control measurement click stimuli evoked brainstem responses at normal hearing thresholds. After 3 weeks of

intratympanic treatment with neomycine (pretreated) on the right and with saline on the left ear, no brainstem responses could be evoked in the ototoxic drug treated ear with the preservation of normal threshold on the other ear (vehicle control).

DETAILED DESCRIPTION OF THE INVENTION

The subject invention provides a method of treating or inhibiting hearing loss in a mammalian subject, comprising administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof effective to treat or inhibit the hearing loss in the subject.

The subject invention also provides a method of alleviating a symptom of hearing loss in a mammalian subject, comprising administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof effective to alleviate the symptom of hearing loss in the subject.

In an embodiment of the method the symptoms of hearing loss are selected from the group consisting of: muffled hearing, ringing, roaring, hissing, buzzing in the ear, ear pain, loss of hearing in one ear, plugged ear, otitis media and vertigo.

In another embodiment the hearing loss is induced by exposure to an ototoxic agent. The said ototoxic agent may be selected from the group consisting of antibiotics, chemotherapy, sound, environmental chemicals, loop diuretics, aspirin or quinine.

In another embodiment the mammalian subject is a human.

In the methods the amount of R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof administered may be from 0.1mg to 50.0mg based on the weight of the R(+)-N-propargyl-1-aminoindan free base. The administration

may be of the pharmaceutically acceptable salt of R(+)-N-propargyl-1-aminoindan. The pharmaceutically acceptable salt may be esylate, mesylate, sulfate, tannate or tartrate salt of R(+)-N-propargyl-1-aminoindan.

In the methods the administration is otic, oral, intraperitoneal, topical, parenteral or nasal administration. In an embodiment the administration may be topical otic application to the middle ear.

In another embodiment R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof is crystalline.

In another embodiment the R (+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof is in the form of a pharmaceutical composition. The pharmaceutical composition may be in tablet form.

In another embodiment of the method the pharmaceutical composition is in a form suitable for transdermal administration.

In another embodiment of the method the pharmaceutical composition is in a form suitable for sublingual administration.

The subject invention also provides a pharmaceutical composition for the use in the treatment, prevention, or alleviation of symptoms of hearing loss in a subject which comprises a therapeutically effective amount of R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof and pharmaceutically acceptable carrier.

The subject invention also provides use of R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof for the manufacture of medicament for the treatment, prevention or alleviation of a symptom of hearing loss.

ABBREVIATIONS

ABR - auditory brainstem response

AMPA - alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid

DA - dopamine

GADPH - glyceraldehyde-3-phosphate dehydrogenase

HEPES - 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

IHC - inner hair cells

HSD - highly significant differences

LOC - lateral olivocochlear

6-OHDA - 6-hydroxydopamine

MPTP - 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

NMDA - N-methyl-D-aspartic acid

PARP - poly ADP ribose polymerase

R(+)PAI mesylate is commercially available as Azilect® from Teva Pharmaceutical Industries Ltd. and Lundbeck A/S. R(+)PAI may be obtained by optical resolution of racemic mixtures of R- and S-enantiomers of PAI. Such a resolution can be accomplished by any conventional resolution method well known to a person skilled in the art. For example, the resolution may be carried out by preparative column chromatography on a chiral column. Another example of a suitable resolution method is the formation of diastereomeric salts with a chiral acid such as tartaric, malic, mandelic acid or N-acetyl derivatives of amino acids, such as N-acetyl leucine, followed by recrystallization to isolate the diastereomeric salt of the

desired R enantiomer. A complete description of the preparation of R(+)PAI and its salts is described in U.S. Patent Nos. 5,532,415, 5,387,612, 5,453,446, 5,457,133, 5,599,991, 5,744,500, 5,891,923, 5,668,181, 5,576,353, 5,519,061, 5,786,390, 6,316,504, 6,630,514. The R(+)PAI salts include mesylate, maleate, fumarate, tartrate, hydrochloride, hydrobromide, esylate, p-toluenesulfonate, benzoate, acetate, phosphate, tannate and sulfate. For example, rasagiline tannate may be prepared by a process comprising combining a solution of tannic acid with rasagiline base.

As used herein, the term "effective amount" refers to the quantity of a component that is sufficient to yield a desired therapeutic response without undue adverse side effects (such as toxicity, irritation, or allergic response) commensurate with a reasonable benefit/risk ratio when used in the manner of this invention. For example, an amount effective to inhibit, attenuate or reverse hearing loss symptoms. The specific effective amount may vary with such factors as the particular condition being treated, the physical condition of the patient, the type of mammal being treated, the duration of the treatment, the nature of concurrent therapy (if any), and the specific formulations employed and the structure of the compounds or its derivatives.

The dosage of the compounds administered in treatment will vary depending upon factors such as the pharmacodynamic characteristics of a specific chemotherapeutic agent and its mode and route of administration; the age, sex, metabolic rate, absorptive efficiency, health and weight of the recipient; the nature and extent of the symptoms; the kind of concurrent treatment being administered; the

frequency of treatment with; and the desired therapeutic effect.

A dosage unit of the compounds may comprise a single compound or mixtures thereof with hearing loss compounds or with other compounds also used to treat neurite damage. The compounds can be administered in oral dosage forms as tablets, capsules, pills, powders, granules, elixirs, tinctures, suspensions, syrups, and emulsions. The compounds may also be administered in intravenous (bolus or infusion), intraperitoneal, subcutaneous, or intramuscular form, or introduced directly, e.g. by injection or other methods, into the cancer, all using dosage forms well known to those of ordinary skill in the pharmaceutical arts.

The compounds can be administered in admixture with suitable pharmaceutical diluents, extenders, excipients, or carriers (collectively referred to herein as a pharmaceutically acceptable carrier) suitably selected with respect to the intended form of administration and as consistent with conventional pharmaceutical practices. The unit will be in a form suitable for oral, rectal, topical, intravenous or direct injection or parenteral administration. The compounds can be administered alone but are generally mixed with a pharmaceutically acceptable carrier. This carrier can be a solid or liquid, and the type of carrier is generally chosen based on the type of administration being used. The active agent can be co-administered in the form of a tablet or capsule, liposome, as an agglomerated powder or in a liquid form. Examples of suitable solid carriers include lactose, sucrose, gelatin and agar. Capsule or tablets can be easily formulated and can be made easy to swallow or chew; other solid forms include granules, and bulk powders. Tablets may contain

suitable binders, lubricants, diluents, disintegrating agents, coloring agents, flavoring agents, flow-inducing agents, and melting agents. Examples of suitable liquid dosage forms include solutions or suspensions in water, pharmaceutically acceptable fats and oils, alcohols or other organic solvents, including esters, emulsions, syrups or elixirs, suspensions, solutions and/or suspensions reconstituted from non-effervescent granules and effervescent preparations reconstituted from effervescent granules. Such liquid dosage forms may contain, for example, suitable solvents, preservatives, emulsifying agents, suspending agents, diluents, sweeteners, thickeners, and melting agents. Oral dosage forms optionally contain flavorants and coloring agents. Parenteral and intravenous forms may also include minerals and other materials to make them compatible with the type of injection or delivery system chosen.

Specific examples of pharmaceutically acceptable carriers and excipients that may be used to formulate oral dosage forms of the present invention are described in U. S. Pat. No. 3,903,297 to Robert, issued Sept. 2, 1975. Techniques and compositions for making dosage forms useful in the present invention are described-in the following references: Modern Pharmaceutics, Chapters 9 and 10 (Banker & Rhodes, Editors, 1979); Pharmaceutical Dosage Forms: Tablets (Lieberman et al., 1981); Ansel, Introduction to Pharmaceutical Dosage Forms 2nd Edition (1976); Remington's Pharmaceutical Sciences, 17th ed. (Mack Publishing Company, Easton, Pa., 1985); Advances in Pharmaceutical Sciences (David Ganderton, Trevor Jones, Eds., 1992); Advances in Pharmaceutical Sciences Vol 7. (David Ganderton, Trevor Jones, James McGinity, Eds., 1995); Aqueous Polymeric Coatings for Pharmaceutical Dosage

Forms (Drugs and the Pharmaceutical Sciences, Series 36 (James McGinity, Ed., 1989); Pharmaceutical Particulate Carriers: Therapeutic Applications: Drugs and the Pharmaceutical Sciences, Vol 61 (Alain Rolland, Ed., 1993); Drug Delivery to the Gastrointestinal Tract (Ellis Horwood Books in the Biological Sciences. Series in Pharmaceutical Technology; J. G. Hardy, S. S. Davis, Clive G. Wilson, Eds.); Modern Pharmaceutics Drugs and the Pharmaceutical Sciences, Vol 40 (Gilbert S. Banker, Christopher T. Rhodes, Eds.).

Tablets may contain suitable binders, lubricants, disintegrating agents, coloring agents, flavoring agents, flow-inducing agents, and melting agents. For instance, for oral administration in the dosage unit form of a tablet or capsule, the active drug component can be combined with an oral, non-toxic, pharmaceutically acceptable, inert carrier such as lactose, gelatin, agar, starch, sucrose, glucose, methyl cellulose, dicalcium phosphate, calcium sulfate, mannitol, sorbitol, microcrystalline cellulose and the like. Suitable binders include starch, gelatin, natural sugars such as glucose or beta-lactose, corn starch, natural and synthetic gums such as acacia, tragacanth, or sodium alginate, povidone, carboxymethylcellulose, polyethylene glycol, waxes, and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, sodium benzoate, sodium acetate, sodium chloride, stearic acid, sodium stearyl fumarate, talc and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum, croscarmellose sodium, sodium starch glycolate and the like.

In development of pharmaceutical compositions, crystallinity is a desirable property in an active pharmaceutical

ingredient. Crystal substances allow for ease in processing and formulating into most types of pharmaceutical dosage forms.

By any range disclosed herein, it is meant that all hundredth, tenth and integer unit amounts within the range are specifically disclosed as part of the invention. Thus, for example, 0.01-50.0 mg means that 0.02, 0.03 ... 0.09; 0.1, 0.2 ... 0.9; and 1, 2 ... 49 mg unit amounts are included as embodiments of this invention.

As used herein, a subject "afflicted" with hearing loss means the subject has been diagnosed with hearing loss or a condition wherein hearing loss can occur.

In this application, a subject diagnosed with hearing loss refers to a subject diagnosed with hearing impairments attributed to a wide variety of causes, including infections, mechanical injury, loud sounds, aging, and chemically induced ototoxicity that damages neurons and/or hair cells of the peripheral auditory system. Symptoms include muffled hearing, ringing, roaring, hissing, buzzing in the ear, ear pain, loss of hearing in one ear, plugged ear otitis media and vertigo. Diagnosis may be by hearing by air conduction, hearing by bone conduction, Rinne's test, audiometry, speech audiometry, speech discrimination, tympanometry, or acoustic reflex testing. Rasagiline may be used as a potential therapeutic prevent or inhibit hearing loss.

This invention will be better understood from the experimental details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the

invention as described more fully in the claims which follow thereafter.

Discussion

Dopamine (DA) was identified as a possible modulator of the IHC-afferent synapse (Safieddine S, Prior AM, Eybalin M, (1997) Choline acetyltransferase, glutamate decarboxylase thyrosine hydroxilase, calcitonin gene-related peptide and opioid peptides coexist in lateral efferent neurons of rat and guinea-pig. Eur J Neurosci 9: 356-367). It is well known that the cochlea is vulnerable to different noxae that can lead to sensorineural hearing loss (Pujol R, Puel J-L, (1999) Excitotoxicity, synaptic repair, and functional recovery in the mammalian cochlea: a review of recent findings. Ann NY Acad Sci 884: 249-54). In addition to the dysfunction of the supplier arteries other pathological noxae (like endolymphatic hydrops or noise trauma) can cause ischemia in the organ of Corti (Vass Z, Brechtelsbauer PB, Nuttall AL, Miller JM, (1995) Effect of endolympahtic hydrops on capsaicin evoked increase in cochlear blood flow. Acta Otolaryngol 115: 754-758). Excitotoxicity is also playing a role in the pathomechanisms of presbyacusis (Seidman MD, Quirk WS, Shirwany NA, (1999) Mechanisms of alterations in the microcirculation of the cochlea. Ann NY Acad Sci 884: 226-232), aminoglycoside-induced ototoxicity (Duan M, Agerman K, Ernfors P, Canlon B, (2000) Complementary roles of neurotrophin 3 and a N-methyl-D-aspartate antagonist in the protection of noise and aminoglycoside-induced ototoxicity. Proc Natl Acad Sci USA 97: 7597-7602) and tinnitus (Sahley TL, Nodar RH, (2001) A biochemical model of peripheral tinnitus. Hear Res 152: 43-54).

Harmful stimuli also activate the cochlear nuclei, which can release protective transmitters in the cochlea: the dopamine-containing LOC efferent fibers were shown to establish a short-loop feedback mechanism between the brainstem and the cochlea (Pujol R, (1994) Lateral and medial efferents: a double neurochemical mechanism to protect and regulate inner and outer hair cell function in the cochlea. Br J Audiol 28: 185-191). In line with the theory of the cochleo-protective role of the LOC substance dopamine, *(i)* D_1 and D_2 receptor agonists inhibited the NMDA(N-methyl-D-aspartic acid)- and AMPA(alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)-induced firing of the primary afferent nerve (Puel J-L, Bobbin RP, Fallon M, (1988) An ipsilateral cochlear efferent loop protects the cochlea during intense sound exposure. Hear Res 37: 65-70), *(ii)* stimulation of the LOC fibers decreased the amplitude of the cochlear compound action potential evoked by intensive sound stimulation (Oestreicher E, Arnold W, Ehrenberger K, Felix D, (1997) Dopamine regulates the glutamatergic inner hair cell activity in guinea pigs. Hear Res 107: 46-52), and finally *(iii)* intracochlear application of the D_2/D_3 dopamine receptor agonist piribedil reduced the characteristic electrophysiological and structural changes evoked by ischemia (d' Aldin C, Eybalin M, Puel J-L, Characon G, Ladrech S, Renard N, Pujol R, (1995a) Synaptic connections and putative functions of the dopaminergic innrevation of the guinea pig cochlea. Eur Arch Otorhinolar 252: 270-274. d' Aldin C, Puel J-L, Leducq R, Crambes O, Eybalin M, Pujol R, (1995b) Effect of a dopaminerg agonist in the guinea pig cochlea. Hear Res 90: 202-211; Gil-Loyzaga P, (1995) Neurotransmitters of the olivocochlear lateral efferent system: with an emphasis on dopamine. Acta Otolaryngol 115: 222-226; Pujol R, Puel J-L, d'Aldin CG, Eybalin M, (1993) Pathophysiology of the

glutamergic synapses in the cochlea. Acta Otolaryngol. 113: 330-334). In addition, it has been shown that experimental ischemia and activation of the known neuroprotective metabotropic glutamate receptors can induce *in vitro* dopamine release from the acutely isolated cochlea (Halmos G, Doleviczenyi Z, , Vizi ES, Lendvai B. Zelles T. (2005) Oxygen-glucose deprivation evokes dopamine release in isolated cochleae. Neuroscience 132: 801-809, Doleviczényi Z, Halmos G, Répássy G, Vizi ES, Zelles T, Lendvai B., (2005) Cochlear dopamine release is modulated by group II metabotropic glutamate receptors via GABAergic neurotransmission. Neurosci. Lett. 385: 93-98). Taken together, these data sufficiently established the potential neuroprotective effect of dopamine during ischemia.

The present invention describes the modulation of dopamine release by rasagiline to evoke this protective factor in ischemia. Rasagiline, at the applied concentrations and timing, can cause an enhancement of the field stimulation-evoked dopamine release as revealed by the FRS_2/FRS_1 values in the cochlea preparation. The increase was significant at the higher concentrations, 100 and 300 µM of Rasagiline.

EXAMPLES

EXAMPLE 1

Animals and tissue preparation

The bulla tympani of a male guinea pig (weighing 150-350 g) was opened. The bony capsule of the cochlea was removed under stereomicroscopic guidance and the stria vascularis was stripped and the cochlea was fractured at the basis of the modiolus. The preparation contained the ganglion spirale, the afferent auditory fibers, the axons and axon terminals of the efferent bundles and both the inner and

outer hair cells. All experiments were carried out in a perilimph-like solution, which contained 150 mM NaCl, 3.5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 2.75 mM HEPES and 2.25 mM Tris-OH at the temperature of 37°C and pH 7.4. The osmolality was set by D-glucose and continuously saturated by 100% O_2.

Microvolume superfusion

The cochleae were incubated with 0.2 µM [^{3}H]dopamine (Amersham, UK, spec. act.: 31.0 Ci/mmol, 6 µCi in 1 ml) for 35 min. Each cochlea was then placed in a microvolume plexi chamber and superfused at 3 ml/min with perilymph-like solution. After 1 hour pre-perfusion the outflow was collected into 3 min-fractions. The released activity was determined by assaying 500 ml-aliquots of each sample with liquid scintillation spectrometry (Packard Tri-Carb 1900TR). After collecting samples for 57 minutes (19 fractions) each cochlea was transferred from the microchambers to 500 µl of 10% trichloroacetic acid for one day and 100 µl was assayed for tissue radioactivity. Earlier HPLC measurements showed that 91-95% of the stimulation-evoked radioactivity could be attributable to [^{3}H]dopamine and its metabolites (Gáborján A, Lendvai B, Vizi ES, (1999a) Neurochemical evidence of dopamine release by lateral olivocochlear efferents and its presynaptic modulation in guinea-pig cochlea. Neuroscience 90: 131-138). Electrical field stimulation was applied for one fraction period (3 min, 360 pulses) at 60 V, 2 Hz and 0.5 ms duration at the 3rd and 13th fractions. The pulses were delivered by a Grass S88 stimulator (West Warwick, USA) through platinum electrodes at the top and bottom of the tissue chamber.

Concentration dependence

Rasagiline was added to the perfusion fluid, 15 minutes prior to the second field stimulation (8^{th} fraction) at the concentration of 1, 10, 100 and 300 μM and maintained until the end of the experiments. At the low concentration, Rasagiline did not change cochlear dopamine release while higher doses elevate cochlear DA release (Fig. 2).

Effect of timing of Rasagiline application on the evoked cochlear dopamine release

The examination of the timing effect of Rasagiline perfusion came to address the possible involvement of slow intracellular processes in the action of Rasagiline in the cochlea, such as interaction with phosphorylation of intracellular enzymes or receptors or interference with protein synthesis. The assumption was that the longer the time before the second electrical stimulation, the larger the effect on the amplitude of the evoked dopamine release. In these experiments Rasagiline (100 μM) was added to the perfusion 21, 18, 12, 9, 6, and 3 minutes prior to the second field stimulation.

The different timing of Rasagiline perfusion did not cause different enhancement of the evoked dopamine release (Fig. 3A-C). Furthermore, Rasagiline given even at the longest duration (21 minutes prior to stimulation) failed to induce significant differences in the resting release of dopamine (Fig. 3B, D).

All groups with the various timing of Rasagiline perfusion caused a significant enhancement of evoked dopamine release (Table 1). In contrast, the p-values of post hoc test revealed no further differences between timing groups. Numbers as presented in the table are probability (p)

values of Tukey HSD post hoc comparisons (a statistical method used for testing the statistical significance of unplanned pair wise comparisons, Winer, Michels & Brown, 1991) between treatment groups of 3, 6, 9, 12, 18, 21 minutes perfusion with Rasagiline and a control. Significant p-values are indicated by underline.

Table 1. p-values of Rasagiline timing experiments showing significant enhancement of dopamine release.

	3-min	6-min	9-min	12-min	18-min	21-min
6-min	0.999763					
9-min	0.867797	0.975706				
12-min	0.998655	0.970966	0.535902			
18-min	1.000000	0.999780	0.887884	0.999156		
21-min	0.999926	0.992801	0.699156	0.999996	0.999957	
control	0.006352	0.002175	0.000205	0.016949	0.011157	0.014986

Data analysis

To best describe the release of dopamine during one collecting period, the fractional release (FR) of the tritium-outflow was determined as the percentage of total radioactivity present in the tissue at the time of sample collection. The fractional release evoked by field stimulations (S_1 and S_2) was calculated by the area-under-the-curve, i.e., by subtracting the mean of the basal release from the total fractional release during the electrical stimulation (Halmos G, Gáborján A, Lendvai B, Répássy G, Z Szabó L, Vizi ES, (2000) Veratridine-evoked release of dopamine from guinea pig isolated cochlea. Hear Res 144: 89-96, Halmos G, Lendvai B, Gáborján A, Baranyi M,

Z Szabó L, Csokonai Vitéz L, (2002) Simultaneous measurement of glutamate and dopamine release from isolated guinea pig cochlea. Neurochem Int 40: 243-248). The effects of drugs on the field stimulation-evoked [^{3}H]dopamine release were expressed by the calculated ratio of FRS_2 over FRS_1, in the presence and in the absence of the drug, respectively. The effect of drugs on the resting outflow of tritium was determined as the ratio of the sum of the two highest consecutive resting FR values in the presence of the drug and before the drug reached the cochleae (FRR_2/FRR_1). Data are expressed as the means ±S.E.M. ANOVA was used for statistical analysis. Tukey post-hoc test was applied to determine the significance of pair wise comparisons.

EXAMPLE 2

In vivo auditory brainstem response (ABR) measurements in mice

Summary

Mice were treated with intratympanic neomycin (200 mg/ml) on the right ear on two consecutive days. Left ears were left for vehicle control (NaCl injection). In order to develop significant hearing loss, the measurement of brainstem evoked potentials were made 3 weeks after the pretreatment with neomycin (under general anaesthesia). Click stimulations to the ear including various frequencies were used to evoke the auditory response in the brainstem that was measured by multiple electrodes placed on the head of the animal.

Discussion:

Control experiments showed the hearing threshold of each ear (Fig. 4). Pretreatment with the known ototoxic neomycin

produced evident hearing loss in the treated side (Fig. 4). Hearing impairment was not due to the intervention itself because the vehicle-treated side showed no hearing deficit (Fig. 4).

Next, the potential protective role of Rasagiline on neomycin-induced hearing loss *in vivo* was evaluated. The examination of the anticipated protective effect of Rasagiline took place in two sets of experiments. In the first experiment, Rasagiline was applied topically in the middle ear, by injecting 0.5 mg Rasagiline intratympanically together with neomycine one time (0.5 mg/day) (n=4). Rasagiline completely inhibited the ototoxic effect of neomycine in this series of experiments; the hearing threshold of the intratympanically treated animals remained unchanged compared to the threshold prior to the neomycine treatment. In the second experiment the effect of systemic application of Rasagiline was studied. Rasagiline (100 µL) was applied intraperitoneally on the day of intratympanic neomycine treatment and the next three days (50 mg/kg Rasagiline daily) (n=4). In contrast to the topical application, the systemic use of Rasagiline could not prevent the ototoxic effect of the aminoglicoside drug. All neomycine treated ears became deaf.

Intratympanic application of Rasagiline have neuroprotective effect against aminoglicoside ototoxicity indicating the potential therapeutic use of Rasagiline to prevent hearing loss.

EXAMPLE 3

In vivo auditory brainstem response (ABR) measurements in Guinea pigs

Animals

Guinea pigs weighing 250-300 g were used in all experiments. All interventions(including ABR measurements and intratympanic treatments) are performed under general anesthesia using i.p. injection of a cocktail of Ketamine and Xylazine.

ABR measurement

A loudspeaker is placed in each ear and the ABR responses were detected by four surface electrodes. These electrodes are placed two in the mastoid region and one in vertex as positive and fourth electrode was placed on forehead as the ground electrode. Calibrated acoustic signals are obtained to evoke brainstem response. The visual detection threshold is determined by decrement sound pressure in 5 dB steps. The evoked responses are then filtered and averaged with 500 sweeps using a signal processor. All ABR tests are performed bilaterally and baseline Peak Equivalent Sound Pressure Levels (PESPLs) are obtained in terms of decibel (dB). All animals undergo baseline hearing test (ABR) before any kind of treatment in order to rule out previous hearing loss. Control ABR threshold measurements are compared to pretreatment values. Auditory threshold shift is subjected to statistical analysis, statistical significance is determined.

Study groups

All treatment group contain the intratympanic application of neomycin and performed under stereomicroscopic guidance. A fine needle is used for myringotomy and drugs are delivered through the same needle. The whole middle ear is filled until the fluid can be seen in the external auditory

canal. Animals are laid on the opposite side for 5 min after intratympanic injection in order to avoid the leakage of drugs from the middle ear. Typical injections are approximately 100 ml of solution into the middle ear to avoid spillover at larger volumes. Concentration is calculated using this volume. For example, a 50 mg/kg Rasagiline dose for a 300 g guinea pig is achieved by giving 15 mg Rasagiline dissolved in 100 ml solution. After the baseline threshold measurement animals are divided into different treatment groups as follows:

Control group: Right ear: neomycin 1 mg/kg, left ear: vehicle, n=6 animals;

Rasagiline IP study group: N=6-8 animals, three days before intratympanic neomycin treatment: Rasagiline 1 mg/kg day; Intratympanic neomycine (1 mg/kg) in both ears.

After the intratympanic neomycine application: Rasagiline 1 mg/kg day intraperitoneally or per os for 3 weeks (during the whole duration of ABR measurement). Rasagiline intratympanic effect study group. N=6 animals right ear: Neomycine 1 mg/kg and Rasagiline, 50 mg/kg, left ear: Neomycin only 1 mg/kg. Intratympanic application only to assess the effect of local Rasagiline administration (same design as in the preliminary experiment).

To estimate the threshold of hearing the sound stimulus is applied at different frequencies. In control animals at a particular frequency the sound induces a response in the ear. In deaf animals response is usually not detected at any frequency.

Discussion:

Control experiments show the hearing threshold of each ear. Pretreatment with the known ototoxic neomycin produces evident hearing loss in the treated side. Hearing impairment is not due to the intervention itself because the vehicle-treated side shows no hearing deficit.

Next, the potential protective role of Rasagiline on neomycin-induced hearing loss *in vivo* is evaluated. The examination of the anticipated protective effect of Rasagiline takes place in two sets of experiments. In the first experiment, Rasagiline is applied topically in the middle ear, by injecting 0.5 mg Rasagiline intratympanically together with neomycine one time. Rasagiline completely inhibits the ototoxic effect of neomycine in this series of experiments; the hearing threshold of the intratympanically treated animals remains unchanged compared to the threshold prior to the neomycine treatment. In the second experiment the effect of systemic application of Rasagiline is studied. Rasagiline is applied intraperitoneally on the day of intratympanic neomycine treatment and the next three days. In contrast to the topical application, the systemic use of Rasagiline can not prevent the ototoxic effect of the aminoglicoside drug. All neomycine treated ears become deaf.

Intratympanic application of Rasagiline has a neuroprotective effect against aminoglicoside ototoxicity indicating the potential therapeutic use of Rasagiline to prevent hearing loss.

Claims

1. A method of treating or inhibiting hearing loss in a mammalian subject, comprising administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof effective to treat or inhibit the hearing loss in the subject.

2. A method of alleviating a symptom of hearing loss in a mammalian subject, comprising administering to the subject an amount of R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof effective to alleviate the symptom of hearing loss in the subject.

3. The method of claim 2, wherein the symptoms of hearing loss are selected from the group consisting of: muffled hearing, ringing, roaring, hissing, buzzing in the ear, ear pain, loss of hearing in one ear, plugged ear, otitis media and vertigo.

4. The method any of claims 1-3, wherein the hearing loss is induced by exposure to an ototoxic agent.

5. The method of claim 4, wherein said ototoxic agent is selected from the group consisting of antibiotics, chemotherapy, sound, environmental chemicals, loop diuretics, aspirin or quinine.

6. The method of any of claims 1-5, wherein the mammalian subject is a human.

7. The method of any of claims 1-6, wherein the amount of R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof administered is from 0.1 mg to

50.0 mg based on the weight of the R(+)-N-propargyl-1-aminoindan free base.

8. The method of claim 7, wherein the amount of R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof administered is from 0.1 mg to 10.0mg based on the weight of R(+)-N-propargyl-1-aminoindan free base.

9. The method of any of claim 1-8, wherein the administration is of the pharmaceutically acceptable salt of R(+)-N-propargyl-1-aminoindan.

10. The method of claim 9, wherein the pharmaceutically acceptable salt is esylate, mesylate, sulfate, tannate or tartrate salt of R(+)-N-propargyl-1-aminoindan.

11. The method of claim 9, wherein the pharmaceutically acceptable salt is the mesylate salt of R(+)-N-propargyl-1-aminoindan.

12. The method of any of claims 1-11, wherein the administration is otic, oral, intraperitoneal, topical, parenteral or nasal administration.

13. The method of claim 12, wherein the administration is topical otic application to the middle ear.

14. The method of any one of claims 1-13 wherein R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt is crystalline.

15. The method of any of claim 1-14, wherein the R (+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof is in the form of a pharmaceutical composition.

16. The method of claim 15, wherein the pharmaceutical composition is in tablet form.

17. The method of claim 15 wherein the pharmaceutical composition is in a form suitable for transdermal administration.

18. The method of claim 15 wherein the pharmaceutical composition is in a form suitable for sublingual administration.

19. A pharmaceutical composition for the use in the treatment, prevention, or alleviation of symptoms of hearing loss in a subject which comprises a therapeutically effective amount of R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof and pharmaceutically acceptable carrier.

20. Use of R(+)-N-propargyl-1-aminoindan or pharmaceutically acceptable salt thereof for the manufacture of medicament for the treatment, prevention or alleviation of a symptom of hearing loss.

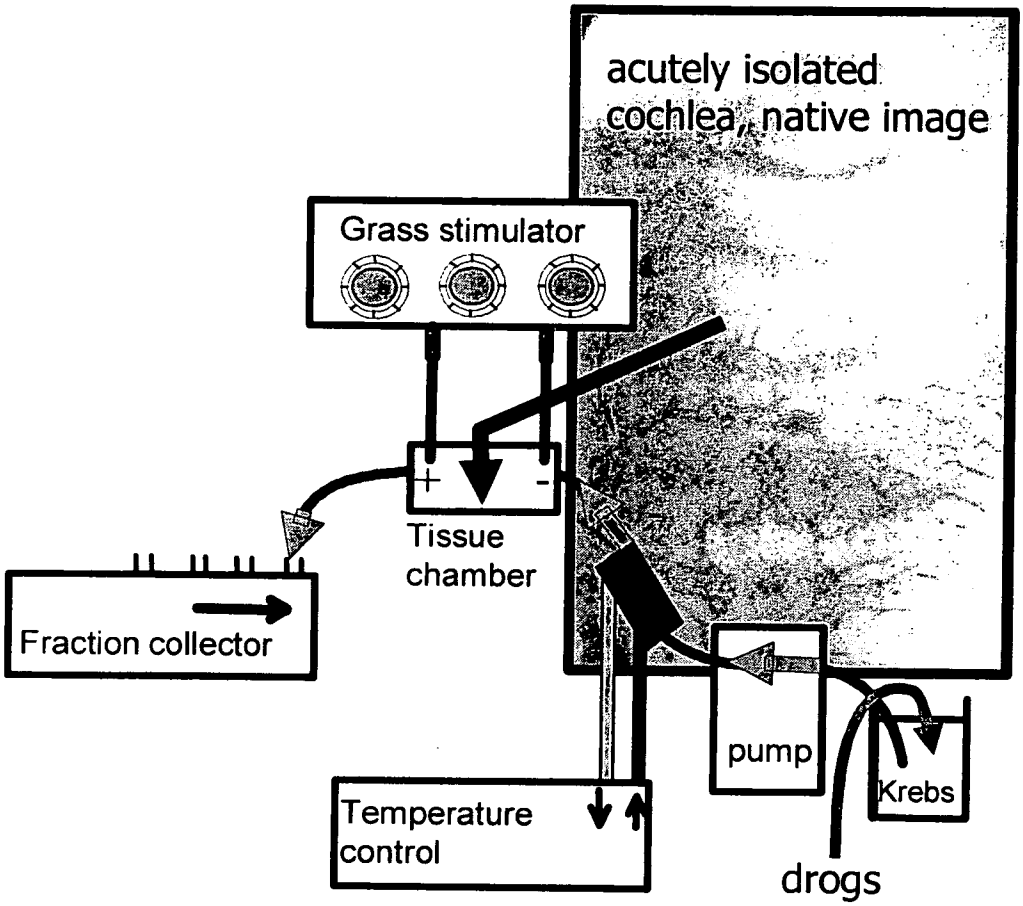

acutely isolated
cochlea, native image
Grass stimulator
+
-
Tissue
chamber
Fraction collector
pump
Krebs
Temperature
control
drogs

Figure 1

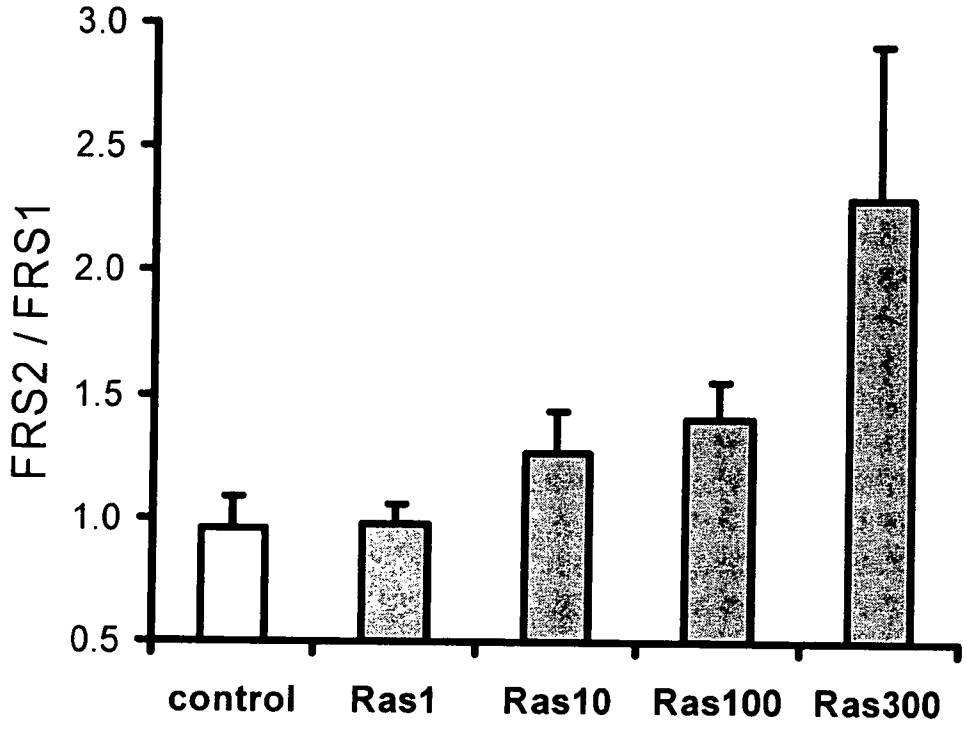


Figure 2

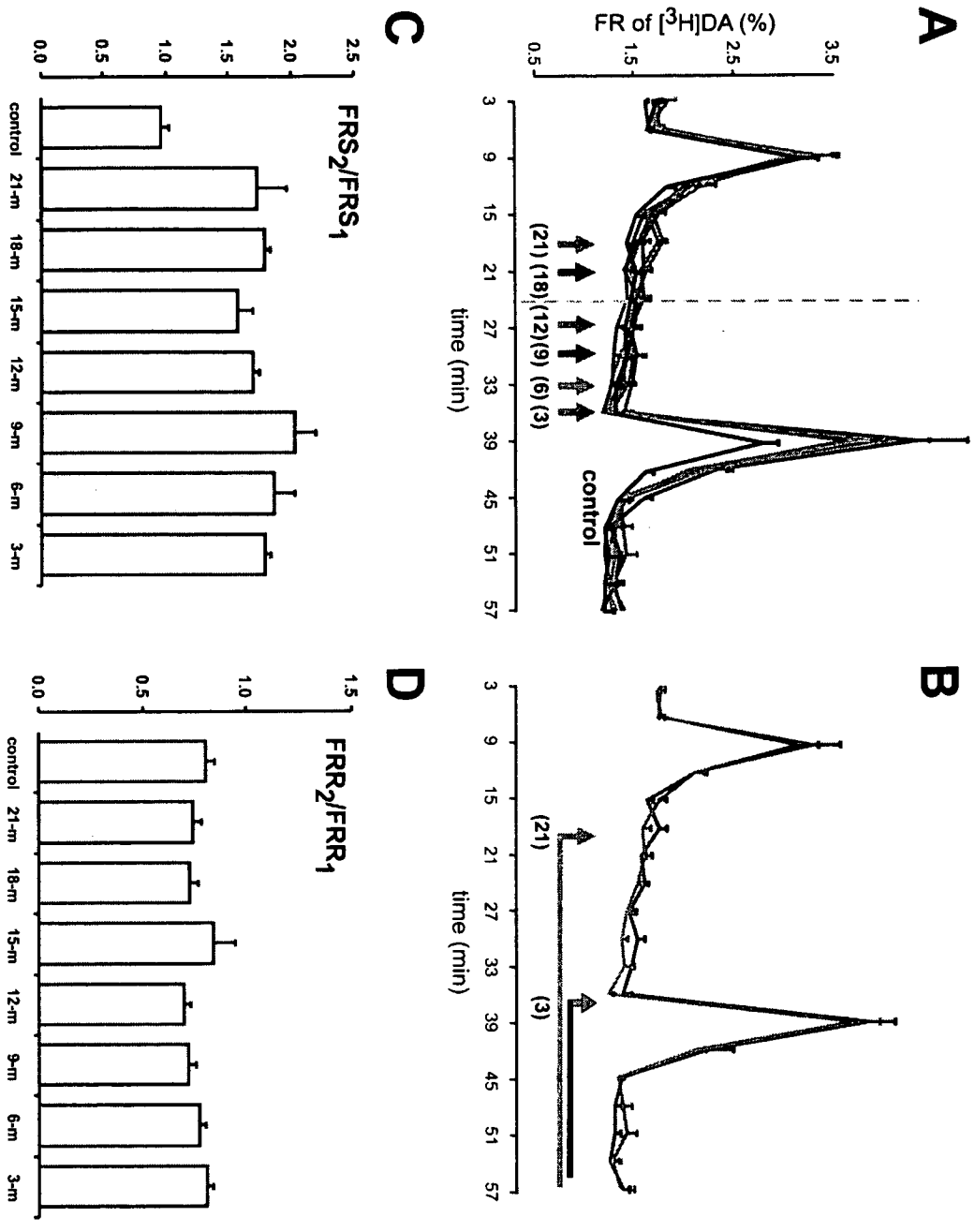
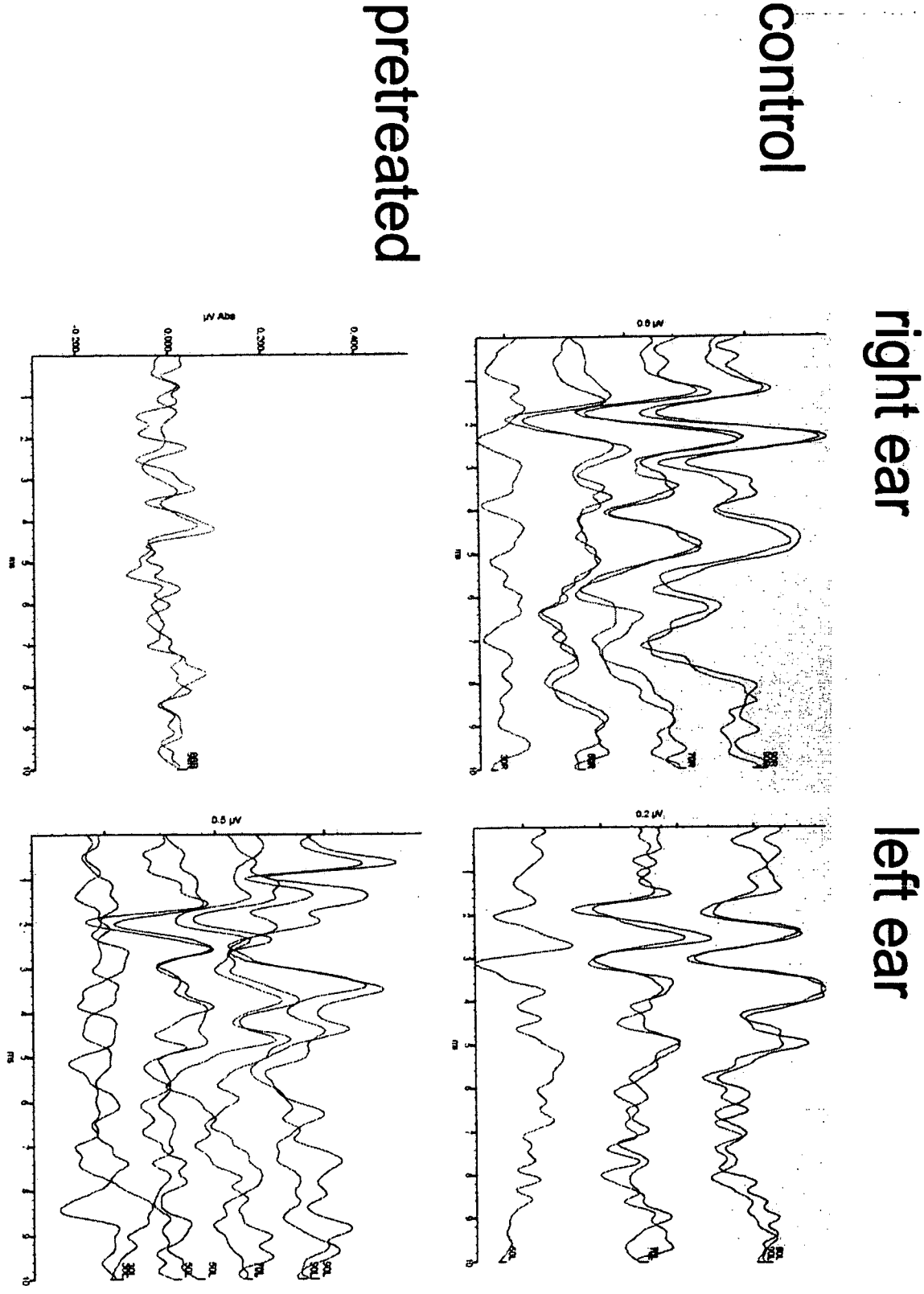

A
FR of [3H]DA (%)
0.5
1.5
2.5
3.5
3
9
15
21
27
33
39
45
51
57
time (min)
(21) (18) (12) (9) (6) (3)
control
B
(21)
(3)
C
FRS2/FRS1
0.0
0.5
1.0
1.5
2.0
2.5
control
21-m
18-m
15-m
12-m
9-m
6-m
3-m
D
FRR2/FRR1
0.0
0.5
1.0
1.5

Figure 3

Figure 4



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 08/10836

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 33/12; A61K 31/135 (2008.04)
USPC - 514/657
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 514/657

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/553-554 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(USPT,PGPB,EPAB,JPAB); GoogleScholar
Search R(+)-N-propargyl-1-aminoindan, hearing loss, hearing impairment, hearing deficiency, otitis, ototoxic agent, human, therapeutically effective amount

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2006/0094783 A1 (YOUDIM et al.) 4 May 2006 (04.05.2006) Abstract; para [0033], [0050], [0131], [0244]	1-5 and 19-20
Y	US 2007/0021352 A1 (ANDERSON et al.) 25 January 2007 (25.01.2007) Abstract; para [0186], [0219]	1-5 and 19-20

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 November 2008 (26.11.2008)	03 DEC 2008
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 08/10836

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-18
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.