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(54) Title: METHOD AND COMPOSITION FOR TREATING NEUROLOGICAL DISORDERS

(57) Abstract

Compositions useful in the treatment of neurological degenerative disorders which affect cholinergic neurons, as well as methods of use therefor. The compositions include a combination of a neural voltage-dependent potassium blocker and choline or a source of choline.

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METHOD AND COMPOSITION FOR TREATING NEUROLOGICAL DISORDERS

Background

Exogenous choline is known to be required for acetylcholine (ACh) synthesis and to be supplied to cholinergic neurons, as choline or one of its precursors, from a variety of sources (e.g., circulation, breakdown of released ACh, efflux of free choline from the intracellular space of brain cells, hydrolysis of choline-containing membrane phospholipids). There are many diseases in which acetylcholine production and/or release appear to be affected. For example, Alzheimer's disease is accompanied by a cholinergic defect in specific areas of the brain; and a specific defect in choline acetyltransferase, the enzyme which catalyzes acetylcholine production from choline and acetyl-coenzyme A, has been identified in autopsy material from patients with Alzheimer's. Summers, W.K. et al., the New England Journal of Medicine, 315:1241-1245 (1986) Davies, P. and Maloney, A.J.F., Lancet, 2:1403 (1976). Loss of cholinergic function is believed to contribute to the intellectual impairment, memory deficits and dementia which characterize Alzheimer's disease. A. Enz, "Accumulation and Turnover of Ach After ACHE-I in Rat Brain", In: Advances in Alzheimer Therapy: Cholinesterase Inhibitors, An International Symposium, March 1988. Cholinergic deficiency states are also believed to be the basis for other neurological disorders. For example, it is thought that cholinergic deficiency states are present in such

neurological disorders as Tourette's disease, Freidreich's ataxia, Huntington's Chorea amyoletrophic lateral scerosis, familiar dysautonomia, post-stroke, post-traumatic, or post-toxic syndromes affecting memory of cognition and tardive dyskinesia. S. Bajada, Alzheimer's Disease: A Report of Progress. In: Aging, S. Corkin, et al., (ed.) 9:427, Raven Press, New York, NY (1982).

At least in part because of the evidence that a cholinergic defect (apparently low acetylcholine synthetic enzyme concentrations) is present in patients with Alzheimer's disease and that cholinergic mechanisms have a role in learning and memory, treatments have been devised in which choline or one of its precursors is administered in an attempt to counteract the acetyl choline deficiency. S. Bajada, Alzheimer's Disease: A Report of Progress In: Aging, S. Corkin, et al., (ed.) 9:427 Raven Press, New York, NY (1982). Drugs which inhibit the action of cholinesterase, such as physostigmine and tetrahydroaminoacridine (THA) have also been used in treating Alzheimer's patients. Kaye et al., Alzheimer's Disease: A Report of Progress, In: Aging, S. Corkin, et al., (ed.) 9:433 Raven Press, New York, NY (1982); Summers, et al., New England Journal of Med., 315(20): 1241-1245 (1986). However, pharmacological therapy with cholinergic agents has had only limited success in patients with degenerative neurological disorders such as is evident in Alzheimer's Disease.

Summary of the Invention

This invention relates to a composition and a process for the treatment of neurological degenerative

disorders by increasing acetylcholine levels in the brain. It is based on the discovery that administration of a combination of choline or a choline source or precursor and a drug which blocks neural voltage-dependent potassium channels (i.e., potassium channel blockers) dramatically increases the synthesis and release of acetylcholine in neurons. The potentiation of the combined effect of choline or a choline source and a potassium channel blocker is greater than the simple additive effects of choline or a choline precursor and a drug which is a potassium channel blocker.

This combination provides distinct advantages in the treatment of diseases associated with decreased acetylcholine production and/or release. In particular, it enables surviving neurons to liberate large amounts of acetylcholine and thus, in effect, "substitutes" for the damaged neurons.

The combination of the invention can be administered to an individual in an amount effective to substantially increase acetylcholine release by the neurons, to reduce the symptoms of the neurological disorder. In one embodiment of the present invention, a combination of choline, which is a precursor of acetylcholine, and 4-aminopyridine, which blocks voltage dependent potassium channels in excitable membranes, is administered to an individual in a quantity sufficient to substantially increase the release of acetylcholine by the neurons. In one embodiment, choline (or a source thereof) sufficient to double blood choline levels in an individual is administered with at least one drug which is a potassium channel blocker.

Administration of choline or a choline source and at least one potassium channel blocker according to the method of the invention is beneficial to individuals suffering from neurological disorders, because it results in replacement or replenishment of acetylcholine lacking or not made/released by affected neurons.

Brief Description of the Figure

The Figure is a graphic representation of the relationship between release of acetylcholine from superfused slices of rat striatum and addition to the medium of choline alone, 4-aminopyridine alone or choline and 4-aminopyridine in combination.

Detailed Description of the Invention

The invention relates to a composition to be administered to enhance the synthesis and release of acetylcholine from neurons, as well as to a method of administering choline (or a choline source) and at least one potassium channel blocker to individuals for treatment of neurological disorders which selectively involve cholinergic neurons.

The composition of the invention comprises choline or a choline source and at least one potassium channel blocker. Choline itself can be used in the composition. Alternatively, a choline source, such as phosphatidylcholine, glycerophosphocholine or commercial lecithin, can be used. The potassium channel blocker enhances the release of acetylcholine. Drugs which are potassium channel blockers include tetraethylammonium, guanethidine, cesium ions (Cs^{++}), tetrahydroaminoacridine

(THA), aminopyridine compounds, apamin, quinine, quinidine, charybdotoxin, calcium channel blockers which block transient potassium currents and neurotransmitter agonists which regulate potassium channels (e.g., alpha-1 agonists which act on dorsal raphe serotoninergic neurons and cholinergic neurons).

The agents or drugs can be administered orally, by subcutaneous or other injection, intravenously, parenterally, transdermally, rectally or via an implanted reservoir containing choline or a choline source and the potassium channel blocker(s). The form in which the drugs will be administered (e.g. powder, tablet, capsule, solution, emulsion) will depend on the route by which it is administered. The quantity of the drugs to be administered will be determined on an individual basis, and will be based at least in part on consideration of the individual's size, the severity of the symptoms to be treated and the result sought. In general, quantities of choline or a choline source sufficient to double blood choline levels will be administered (Blood choline levels generally range from 7-9 nanomoles/ml.). For example, approximately 9 gm. of pure phosphaphotidylcholine a day (given in one dose or a number of smaller doses) will be adequate in most individuals to produce the desired doubling. In general, 3-100 gm. of phosphatidylcholine will be given in conjunction with the potassium channel blocker(s). Normally, lecithin is not available in pure form and is available as a mixture of lecithin and other phospholipids; typically 20-30 weight percent of such mixtures is lecithin. Mixtures such as these in which lecithin is one component are referred to as commercial lecithin.

The composition of the present invention can optionally include, in addition to choline or a choline source and potassium channel blocker(s), other components. The components included in a particular composition are determined primarily by the manner in which the composition is to be administered. For example, a composition to be administered orally in tablet form can include, in addition to the drugs, a filler (e.g. lactose), a binder (e.g.carboxymethyl cellulose, gum arabic, gelatin), an adjuvant, a flavoring agent, a coloring agent and a coating material (e.g. wax or a plasticizer). A composition to be administered in liquid form can include the combination of drugs of the present invention, and, optionally, an emulsifying agent, a flavoring agent and/or a coloring agent.

In general, the composition of the present invention is administered to an individual periodically as necessary to improve symptoms of the disease being treated. The length of time during which the drugs are administered and the dosage will depend on the disease being treated, the type and severity of the symptoms, and the physical condition of the individual being treated.

The composition of the present invention can be used to treat neurological disorders which are characterized by degeneration of cholinergic neurons or other neurological disorders which cause deficiencies in acetylcholine release. Such diseases include Alzheimer's disease, post-polio syndrome, myasthenia gravis, Huntington's disease, age-related memory disorders, post-traumatic, post-stroke or post-toxic syndromes affecting memory or cognition, dysautonomia or any other disorder affecting memory or cognition.

The potassium channel blocker enhances the release of acetylcholine, and the choline source provides a source of free choline. The availability of extracellular choline can influence the synthesis and release of acetylcholine, the synthesis of phophatidylcholine and levels of phosphatidylcholine in membranes. When extracellular choline is inadequate, choline in membrane phosphatidylcholine can be mobilized to serve as a precursor for acetylcholine synthesis. This can be problematic, however, because neuron membrane phospholipids can be depleted. This depletion can be reduced by supplying choline to the neurons. Ulus and Wurtman, The New England Journal of Medicine, 318(3):191 (1988).

A preferred choline source is choline. Other useful compounds, which serve as choline sources, are, for example, phosphatidylcholine, glycerophosphocholine and commercial lecithin. A preferred potassium blocker is 4-aminopyidrine (4-AP). The combination of 4-AP with choline results in a potentiation of the release of acetylcholine which is much greater than the sum of each compound acting alone. The synergistic effect of the combination will be useful in treating Alzheimer's disease and/or other neurological disorders involving cholinergic neurons.

The invention is illustrated by the following exemplification, which is not to be seen as limiting in any way.

EXEMPLIFICATION

Potentiation of Acetylcholine from Rat Brain

Methods

Preparation of the slices. Male Sprague-Dawley rats

(180-250 g) were decapitated, and their striata were rapidly dissected and kept in ice-cold physiological solution. Slices 0.3 mm thick were prepared with a Mellwain tissue chopper (The Mickle Laboratory Engineering Co., Gomstall, Surrey) and collected in cold medium. The physiological solution had the following composition (mM): NaCl, 120; CKl, 3.5; $Cacl_2$, 1.3; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 10 (equilibrated with 95% O_2 and 5% CO_2, pH 7.3). The tissue sections were washed several times with ice cold medium to remove most of the membrane debris.

Superfusion of the slices. The slices were transferred to a superfusion chamber (volume 0.7 ml). The chamber was maintained at 37°C in a water-bath. A peristaltic pump (Sage Instruments, type 375A, Cambridge, MA) delivered the pre-heated physiological solution (constantly bubbled with a mixture of 95% O_2 and 5% CO_2) containing 20 uM eserine salicylate. Drugs were added to the superfusion medium, as indicated in the text. The slices (approximately 90 mg wet weight) were equilibrated for 30 min in superfusion medium, flowing at a rate of 0.5 $ml.min^1$. At the end of this equilibration period, the perfusate was collected at 10 minute intervals in glass tubes kept on ice.

At the end of the 1 hour collection period, the slices were removed from the superfusion chamber and homogenized for determination of DNA contents.

Extraction of acetylcholine and choline from media. ACH and choline were separated, as described by Gilberstadt and Kussel, and then measured by the enzymatic method of Goldberg and McCaman. Gilberstadt and Kussel,

Analytical Biochemistry, 138:78-85 (1984); Goldberg and McCaman, Journal of Neural Chemistry, 20:1-8 (1973).

In brief, the endogenous choline was phosphorylated with [^{32}P]- ATP into labelled phosphocholine which was then separated from excess ATP and quantitated. For the determination of ACh, the endogenous choline was first converted to unlabelled phosphocholine; the ACh was then hydrolized by acetylcholinesterase, and the choline resulting from this hydrolysis was assayed as indicated above for endogenous choline. In samples where choline concentrations were much greater than those of ACh, the reliability of the ACh assay depended on the complete phosphorylation of the free choline during a first incubation with non-labelled ATP. Since this phosphorylation might not have been complete, we generated appropriate blanks for the proportion of free choline not phosphorylated by incubating aliquots in the presence of acetylcholinesterase (2.5 U) during the initial incubation with non-labelled ATP; these blank values were then subtracted from those for the ACh assay. This correction allowed us to assay for ACh samples containing as much as 50 times more choline than ACh. For this reason, the maximum concentration of exogenous choline in the superfusion solution was 20 uM or below in all experiments where ACh was measured; this concentration is within the physiologic range (Tucek, 1978).

If the release of acetylcholine (ACh) from superfused slices of rat striatum (a brain region rich in ACh-releasing neurons) is measured, the addition to the medium of choline alone (20 micromolar) or of 4-AP alone (50 micromolar) significantly enhances ACh's spontaneous

release. But when both substances are added to the medium (physiological solution containing, NaCl, 120 mM; KCl, 3.5 mM; $CaCl_2$, 1.3 mM; Mg_2O_4, 1.2 mM; NaH_2PO_4, 1.2 mM; $NaHCO_3$, 25 mM; glucose, 10 mM; and esterine salicylate, 0.02 mM) to allow the measurement of released ACh, there is marked potentiation of their effects: While choline or 4-AP alone raised ACh release from about 2.8 to 4.1 or 4.2 picomols/microgram DNA/ten minutes, respectively, the addition of both substances simultaneously raised it to 8.2 picomoles/microgram DNA/ten minutes. The results are shown in the Figure.

This potentiation of 4-AP's effect on ACh release by choline is much greater than simple additivity. It was previously known that either compound, provided alone, could enhance ACh release; choline by providing more precursor for ACh synthesis and 4-AP by blocking certain potassium channels. However, there was no reason to anticipate the synergistic effect resulting from the interaction of these compounds.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

Claims

1. A composition for treatment of neurological disorders, comprising: a combination of choline or a choline source and a potassium channel blocker.

2. A composition of Claim 1 wherein the choline source is selected from the group consisting of: phosphatidylcholine, glycerophosphocholine, and lecithin.

3. A composition of Claim 1 wherein the potassium channel blocker is a drug selected from the group consisting of: tetraethylammonium, guanethidine, tetrahydroaminoacridine, apamin, quinine, quinidine, charybdotoxin and aminopyridine compounds.

4. A composition of Claim 1 comprising choline and 4-aminopyridine.

5. A composition for treating neurological disorders which selectively involve cholinergic neurons, comprising: choline or a choline source and a drug selected from the group consisting of 4-aminopyridine and derivatives thereof and 3,4-aminopyridine and derivatives thereof.

6. A composition for administration to persons having neurological disorders in which cholinergic neurons are affected, comprising at least two acetylcholinergic drugs.

7. A method of treating neurological disorders in which cholinergic neurons are affected, comprising administering choline or a choline source and an effective quantity of a potassium channel blocker.

8. A method of Claim 7 wherein the choline source is from the group consisting of phosphatidylcholine, glycerochosphocholine and lecithin and the potassium channel blocker is 4-aminopyridine, or a derivative thereof, or 3,4-diaminopyridine, or a derivative thereof.

9. In a composition for treating neurological disorders in which cholinergic neurons are affected, the improvement comprising choline and 4-aminopyridine.

10. In a method of treating neurological disorders in which cholinergic neurons are affected, the improvement comprising administering to an affected person a therapeutically effective amount of choline and 4-aminopyridine.

11. A composition for treatment of neurological disorders, comprising: a combination of choline or a choline source in an amount sufficient to double blood choline levels and a potassium channel blocker.

12. A composition of Claim 11 wherein the choline source is selected from the group consisting of:

phosphatidyl choline, glycerophosphocholine and lecithin.

13. A composition of Claim 11 wherein the potassium channel blocker is a drug selected from the group consisting of: tetraethylammonium, guanethidine, tetrahydroaminoacridine, apamin, quinine, quinidine, charybdotoxin and aminopyridine compounds.

14. A composition of Claim 11 comprising choline and 4-aminopyridine.

15. A composition for treating neurological disorders which selectively involve cholinergic neurons, comprising: choline or a choline source in an amount sufficient to double blood choline levels and a drug selected from the group consisting of: 4-aminopyridine, 3,4-aminopyridine and derivatives thereof.

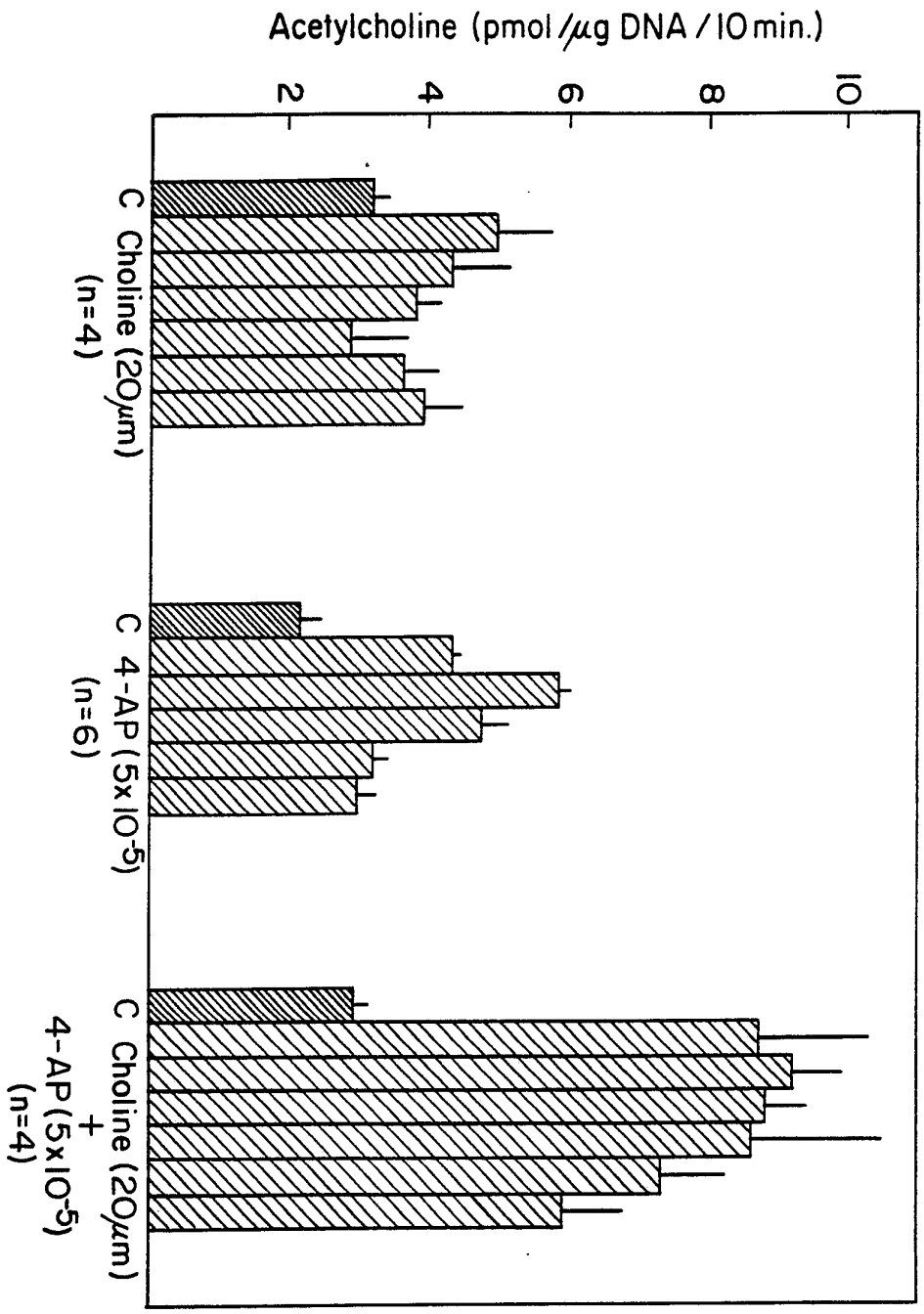
16. A composition for administration to a human having a neurological disorder in which cholinergic neurons are affected, said composition comprising at least two drugs which enhance the synthesis and release of acetylcholine from neurons.

17. A method of treating a neurological disorder in which cholinergic neurons are affected in a human, comprising administering to said human choline or a choline source in an amount sufficient to the double

blood choline level in said human, and an effective quantity of a potassium channel blocker.

18. A method of Claim 17 wherein the choline source is selected from the group consisting of: phosphatidyl choline, glyercophosphocholine and lecithin, and the potassium channel blocker is selected from the group consisting of: tetraethylammonium, guanethidine, tetrahydroaminoacridine, apamin, quinine, quinidine, charybdotoxin and aminopyridine compounds.

19. A method of Claim 18 wherein the choline source comprises choline and the potassium channel blocker comprises 4-aminopyridine.


Acetylcholine (pmol/μg DNA / 10 min.)
2
4
6
8
10
C Choline (20μm)
(n=4)
C 4-AP (5x10-5)
(n=6)
C Choline (20μm)
+
4-AP (5x10-5)
(n=4)

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 89/01402

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: A 61 K 31/685, A 61 K 31/44,//(A 61 K 31/685, 31:44, 31:14)

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	A 61 K, C 07 D

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4386095 (G.E. GIBSON) 31 May 1983, see column 1, line 38 - column 2, line 38 --	1-6,9,11-16
Y	US, A, 4385053 (B. REISBERG) 24 May 1983, see column 3, lines 25-45; column 6, line 64 - column 7, line 13 --	1-6,9,11-16
A	US, A, 4346084 (J.H. GROWDON) 24 August 1982 ----	1-6,9,11-16

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document 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

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"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

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
2nd August 1989	28. 08. 89
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. VAN MOL

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

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

1. [X] Claim numbers ..XX... because they relate to subject matter not required to be searched by this Authority, namely:

xx claims 7, 8, 10, 17 - 19

pls. see Rule 39.1 (iv) - PCT:

Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers ....., 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. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

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 of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] 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 claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 8901402 SA 28155

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 17/08/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 4386095	31-05-83	None	
US-A- 4385053	24-05-83	None	
US-A- 4346084	24-08-82	US-A- 4351831	28-09-82
		US-A- 4346085	24-08-82
		US-A- 4355027	19-10-82
		US-A- 4430330	07-02-84
		US-A- 4569929	11-02-86