NERVE GROWTH INDUCTION, STIMULATION AND MAINTENANCE AND ENZYME POTENTIATION

Inventors: John Paul DaVanzo, Greenville; Joseph West Paul, Jr., Ayden, both of N.C.

Assignee: East Carolina University, Greenville, N.C.

Appl. No.: 545,843
Filed: Oct. 12, 1995

Related U.S. Application Data


Field of Search

References Cited

U.S. PATENT DOCUMENTS

2,719,861 10/1955 Carboni
3,311,539 3/1967 Eberle 514/526
4,287,184 9/1981 Young 514/2
5,023,238 6/1991 DaVanzo et al. 514/21
5,194,426 3/1993 DaVanzo et al. 514/21

OTHER PUBLICATIONS


S. Varon, et al., Biochemistry, 6, 2202 (1967) published in the U.S. and entitled "The Isolation of the Mouse Nerve Growth Factor Protein in a High Molecular Weight Form".


H. Gaahn, et al., Developmental Brain Research, 9, 45 (1983) published in the U.S. and entitled "NGF—Mediated Increase of Choline Acetyltransferase (ChAT) in the Neonatal Rat Forebrain: Evidence for a Physiological Role of NGF in the Brain?".


B.K. Schirler, et al., The Pharmacologist, 30, 128.10 (1988) received Sep. 27, 1988, published in the U.S. and entitled "Triap is Trophic for Cultured Peripheral Neurons".


Primary Examiner—Cecilia J. Tsang
Assistant Examiner—P. Lynn Touzeau
Attorney, Agent, or Firm—Raymond R. Wittekind

ABSTRACT

A composition comprising nerve growth factor and 2-amino-1,13-tricyano—1—propene useful for the induction, stimulation, and maintenance of nerve growth, and methods of potentiating choline O-acetyltransferase and tyrosine hydroxylase by 2-amino-1,13-tricyano—1—propene are disclosed.

40 Claims, No Drawings
NERVE GROWTH INDUCTION, STIMULATION AND MAINTENANCE AND ENZYME POTENTIATION

This is a continuation of application Ser. No. 08/339,886 filed Nov. 14, 1994, now abandoned, which is a division of application Ser. No. 07/998,070 filed Dec. 14, 1992, now abandoned, which is a division of prior application Ser. No. 07/681,310 filed Apr. 8, 1991, now U.S. Pat. No. 5,194,426, which is a division of prior application Ser. No. 07/253,167, filed Oct. 4, 1988, now U.S. Pat. No. 5,023,238.

INTRODUCTION

The present invention relates to nerve growth induction, stimulation, and maintenance, and enzyme potentiation. More particularly, the present invention relates to a composition comprising nerve growth factor and 2-amino-1,1,3-tricyano-1-propene and method of inducing, stimulating, and maintaining nerve growth therewith, and methods of potentiating choline-O-acetyltransferase and tyrosine hydroxylase by means of 2-amino-1,1,3-tricyano-1-propene.

BACKGROUND OF THE INVENTION

Nerve growth plays a major role in the development of host nervous systems, as well as the survival and regeneration of component nerve cells subject to damage or destruction by injury or disease, such as cognitive disorders associated with dementia.

Nerve growth factor, a polypeptide, induces nerve growth in host (for reviews on nerve growth factor, see L. A. Green and E. M. Shooter, Ann. Rev. Neurosci., 3, 353 (1980) and B. A. Yankcr and E. M. Shooter, Ann. Rev. Biochem., 51, 845 (1982)). 2-Amino-1,1,3-tricyano-1-propene, a dimer of malonitrile, also promotes nerve growth in host systems (see, for example, R. T. Houltihan and J. P. DaVanzo, Experimental Neurology, 10, 183 (1964)). It has now been found that nerve growth factor in combination with 2-amino-1,1,3-tricyano-1-propene synergistically induces, stimulates, and maintains nerve growth, thereby rendering the combination more effective than either component in restoring nerve function diminished by injuries or degenerative conditions, e.g., Alzheimer's disease (see F. Hefl and W. J. Weiner, Annals of Neurology, 20, 275 (1986)).

Cholinergic and adrenergic defects are also implicated in nerve degenerative disorders (see, for example, K. L. Davis and R. C. Mohs, The New England Journal of Medicine, 315, 1286 (1986)). It has now also been found that 2-amino-1,1,3-tricyano-1-propene potentiates choline-O-acetyltransferase and tyrosine hydroxylase, thereby augmenting its nerve growth restorative properties and usefulness in nerve degenerative conditions including, e.g., Parkinson's disease, S. H. Appel, Ann. Neurol., 10, 499 (1981).

DESCRIPTION OF THE INVENTION

The present invention relates to a composition comprising nerve growth factor and 2-amino-1,1,3-tricyano-1-propene useful for the induction, stimulation, and maintenance of nerve growth in hosts. The present invention also relates to the potentiation of choline-O-acetyltransferase and tyrosine hydroxylase by 2-amino-1,1,3-tricyano-1-propene.

As used throughout the specification and appended claims, the phrase "inducing nerve growth" refers to the production of nerve cells from non-neuritic cells; the phrase "stimulating nerve growth" refers to the enhanced production of nerve cells from neuritic cells; the phrase "maintain-
Nerve growth induction, stimulation, and maintenance are achieved when the compositions are administered to a subject requiring such treatment as an effective oral, parenteral, intracerebral, or intravenous dose of from about 15 to about 45 μg/kg of body weight per day. A particularly preferred effective amount is about 20 μg/kg of body weight per day. It is to be understood, however, that for any particular subject, specific dosage regimens should be adjusted to the individual need and the professional judgment of the person administering or supervising the administration of the aforesaid compositions. It is to be further understood that the dosages set forth herein are exemplary only and they do not, to any extent, limit the scope or practice of the invention.

Administration of 2-amino-1,1,3-tricyano-1-propene to a host, a mammal, for example, a mouse, or a rabbit, potentiates the effects of choline O-acetyltransferase and tyrosine hydroxylase. The potentiation of the effects of choline-O-acetyltransferase is demonstrated as follows:

Rat adrenal/pheochromocytoma (PC-12) cells (1×10^5 per well) are plated on collagen treated 24 well plates (Costar) in dilutions of nerve growth factor, 2-amino-1,1,3-tricyano-1-propene, 2-amino-1,1,3-tricyano-1-propene and nerve growth factor, and media without additives for 4 days, and choline-O-acetyl transferase activity is measured by the method of B. K. Schrier and L. Shutter, J. Neurochem., 14, 977 (1967). Briefly, after incubation media is removed from the plated cells and the wells are washed three times with phosphate buffer. Cells are lysed with a solution of triton X-100 and luci of 14C-acetyl coenzyme is added to each well. The plates are then incubated at 37°C for 1 hour and stopped with the addition to each well of 1 ml of cold water. The fluid in each well is poured over an anion exchange column, the effluent is counted by addition of Scinti-Verse E, and the activity is determined by measuring radioactivity on a scintillation counter.

### RESULTS

<table>
<thead>
<tr>
<th>Compound or Composition</th>
<th>Peak Conc</th>
<th>Count (of cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-amino-1,1,3-tricyano-1-propene nerve growth factor</td>
<td>0.1 μg/ml @ 48 hr</td>
<td>3.0</td>
</tr>
<tr>
<td>2-amino-1,1,3-tricyano-1-propene nerve growth factor and</td>
<td>0.1 μg/ml</td>
<td>54</td>
</tr>
<tr>
<td>2-amino-1,1,3-tricyano-1-propene nerve growth factor and</td>
<td>132 μg/ml @ 48 hr</td>
<td>50</td>
</tr>
<tr>
<td>2-amino-1,1,3-tricyano-1-propene nerve growth factor and</td>
<td>20 μg/ml</td>
<td>3.0</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Choline O-acetyltransferase and tyrosine hydroxylase potentiation is achieved when the compound is administered to a subject requiring such treatment as an effective oral, parenteral, intracerebral, or intravenous dose of from about 15 to about 45 μg/kg of body weight per day. A particularly preferred effective amount is about 20 μg/kg of body weight per day. It is to be understood, however, that for any particular subject, specific dosage regimens should be adjusted to the individual need and the professional judgment of the person administering or supervising the administration of the aforesaid compositions. It is to be further understood that the dosages set forth herein are exemplary only and they do not, to any extent, limit the scope or practice of the invention.

Effective amounts of the compound and compositions may be administered to a subject by any one of various methods, for example, orally as in capsules or tablets, or intracerebrally, intravenously, or parenterally in the form of sterile solutions. The compound or compositions, while effective themselves, may be formulated and administered in the form of their pharmaceutically acceptable addition salts for purposes of stability, convenience of crystallization, increased solubility and the like.

The compound and compositions may be administered orally, for example, with an inert diluent or with an edible carcer. They may be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the aforesaid compound and compositions may be incorporated with excipients and used in the form of tablets, troches, capsules, elixirs, suspensions, syrups, wafers, chewing gums, and the like. These preparations should contain at least 0.5% of active compound, but may be varied depending upon the particular form and may conveniently be between 4.0% to about 70% of the weight of the unit. The amount of compound and compositions in such composition is such that a suitable dosage will be obtained. Preferred compositions and preparations according to the present invention are prepared so that an oral dosage unit form contains between 1.0–300 mgs of active compound.

The tablets, pills, capsules, troches and the like may also contain the following ingredients: a binder such as micro-

### RESULTS

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conc</th>
<th>Rate of Formation of H₂O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-amino-1,1,3-tricyano-1-propene</td>
<td>132 μg/ml</td>
<td>12.78 ± 0.97 pmol/hr/μg</td>
</tr>
<tr>
<td>Control</td>
<td>6.25 ± 0.6 pmol/hr/μg</td>
<td>of total protein</td>
</tr>
<tr>
<td>of total protein</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The potentiation of the effects of tyrosine hydroxylase is demonstrated as follows:

Rat adrenal pheochromocytoma (PC-12) cells (1×10^5 cells) are plated in collagen treated 60 mm petri dishes in dilutions of nerve growth factor, 2-amino-1,1,3-tricyano-1-propene, nerve growth factor plus 2-amino-1,1,3-tricyano-1-propene, and media without additives, and incubated at 37°C for 1 hour. Tyrosine hydroxylase activity is measured by the method of Nagatsu, et al., Analyt. Biochem., 9, 112 (1964) with minor modifications. Briefly, after incubation, the plates are washed three times in phosphate buffer, scraped into 400 μl of tris acetate buffer, and frozen until assayed. For the assay, the cells are thawed, homogenized, and centrifuged. Supernatant (50 μl) is assayed for tyrosine hydroxylase activity by addition of 0.5 μCi of [3H]-tyrosine in 50 μl of buffer, and incubation of the mixture at 37°C for thirty minutes. The reaction is terminated by addition of 200 μl of acetic acid, and activity is determined by scintillation counting of tritiated water contained in the effluent of the sample after treatment on an anion exchange column.
crystalline cellulose, gum tragacanth or gelatin; and excipi-
tent such as starch or lactose, a disintegrating agent such as
alginic acid, Primogel, corn starch and the like; a lubricant
such as magnesium stearate or Sterolets; a glidant such as
colloidal silicon dioxide; and a sweetening agent such as
sucrose or sorbitol. A flavoring agent such as peppermint,
methyl salicylate, or orange flavoring may be added. When
the dosage unit form is a capsule it may contain, in addition
to materials of the above type, a liquid carder such as a fatty
oil. Other dosage unit forms may contain other various
materials which modify the physical form of the dosage unit.
for example, as coatings. Thus tablets or pills may be coated
with sugar, shellac, or other enteric coating agents. A syrup
may contain, in addition to the active compound or
compositions, sucrose as a sweetening agent and certain
preservatives, dyes, colorings, and flavors. Materials used in
preparing these and other compositions should be pharma-
aceutically pure and non-toxic in the amounts used.

For the purpose of parenteral, intravenous, or intracere-
bral therapeutic administration, compound and composi-
tions may be incorporated into a solution or suspension.
These preparations should contain at least 0.1% of the
aforesaid compound or compositions, but may be varied
between 0.5% and about 50% of the weight thereof.
The amount of active compound or composition in such com-
positions is such that a suitable dosage will be obtained.
Preferred compositions and preparations according to the
present invention are prepared so that a parenteral,
intravenous, or intracerebral dosage unit contains between
0.5 to 100 mgs of the active compound or composition.
The solutions or suspensions may also include the fol-
lowing components: a sterile diluent such as water for
injection, saline solution, fixed oils, polyethylene glycols,
glycerine, propylene glycol or other synthetic solvents;
antibacterial agents such as benzyl alcohol or methyl para-
bens; antioxidants such as ascorbic acid or sodium bisulfite;
chelating agents such as ethylenediaminetetraacetic acid;
buffers such as acetates, citrates or phosphates and agents for
the adjustment of tonicity such as sodium chloride or
dextrose. The parenteral, intravenous, or intracerebral prepa-
ration can be enclosed in ampoules, disposable syringes or
multiple dose vials made of glass or plastic.

We claim:
1. A method of stimulating nerve growth in a host
requiring nerve growth stimulation comprising adminis-
tering a nerve growth stimulating effective amount of a com-
position consisting essentially of nerve growth factor and
2-amino-1,13-tricyano-1-propane.
2. A method of maintaining nerve growth in a host
requiring nerve growth maintaining comprising adminis-
tering a nerve growth maintaining effective amount of a
composition consisting essentially of nerve growth factor
and 2-amino-1,13-tricyano-1-propane.
3. A method according to claim 1 wherein the effective
amount of the composition is between about 15 µg/kg of
body weight and about 20 µg/kg of body weight.
4. The method according to claim 3 wherein the effective
amount of the composition is about 20 µg/kg of body weight.
5. A method according to claim 2 wherein the effective
amount of the composition is between about 15 µg/kg of
body weight and about 20 µg of body weight.
6. The method according to claim 5 wherein the effective
amount of the composition is about 20 µg/mg of body
weight.
7. A method according to claim 1 wherein the nerve is part
of the central nervous system.
8. A method according to claim 1 wherein the nerve is part
of the peripheral nervous system.
9. A method according to claim 1 wherein the nerve is part
of the autonomic nervous system.
10. A method according to claim 1 wherein the nerve is part
of the central nervous system.
11. A method according to claim 1 wherein the nerve is part
of the peripheral nervous system.
12. A method according to claim 1 wherein the nerve is part
of the autonomic nervous system.
13. A method according to claim 2 wherein the nerve is part
of the central nervous system.
14. A method according to claim 2 wherein the nerve is part
of the peripheral nervous system.
15. A method according to claim 2 wherein the nerve is part
of the autonomic nervous system.
16. The method according to claim 10 wherein the nerve
of the central nervous system is selected from the group
consisting of the sciatic, ulnar, radial, and median nerves.
17. The method according to claim 14 wherein the nerve of
the peripheral nervous system is selected from the group
consisting of the sciatic, ulnar, radial, and median nerves.
18. The method according to claim 12 wherein the nerve
of the autonomic nervous system is selected from the group
consisting of the vagus, facial, glosso-pharyngeal, and
splanchnic nerves.
19. The method according to claim 13 wherein the nerve
of the central nervous system is selected from the group
consisting of cholinergic and adrenergic nerves.
20. The method according to claim 14 wherein the nerve of
the peripheral nervous system is selected from the group
consisting of the sciatic, ulnar, radial, and median nerves.
21. The method according to claim 15 wherein the nerve
of the autonomic nervous system is selected from the group
consisting of the vagus, facial, glosso-pharyngeal, and
splanchnic nerve.
22. The method according to claim 1 wherein nerve
growth refers to the bulk of the nerve.
23. The method according to claim 2 wherein nerve
growth refers to the bulk of the nerve.
24. The method according to claim 1 wherein nerve
growth refers to the extension of the nerve.
25. The method according to claim 2 wherein nerve
growth refers to the extension of the nerve.
26. A method according to claim 1 wherein the induction
of nerve growth involves the potentiation of nerve growth
factor by 2-amino-1,13-tricyano-1-propane.
27. A method according to claim 1 wherein the induction
of nerve growth involves the potentiation of nerve growth
factor by 2-amino-1,13-tricyano-1-propane.
28. A method according to claim 2 wherein the induction
of nerve growth involves the potentiation of nerve growth
factor by 2-amino-1,13-tricyano-1-propane.
29. A method according to claim 1 wherein the mainte-
nance of nerve growth involves the potentiation of nerve
growth factor by 1,13-tricyano-2-amino-1-propane.
30. A method according to claim 1 wherein the mainte-
nance of nerve growth involves the potentiation of nerve
growth factor by 1,13-tricyano-2-amino-1-propane.
31. A method according to claim 2 wherein the mainte-
nance of nerve growth involves the potentiation of nerve
growth factor by 1,13-tricyano-2-amino-1-propane.
32. A method according to claim 6 wherein the mainte-
nance of nerve growth involves the potentiation of nerve
growth factor by 1,13-tricyano-2-amino-1-propane.
33. A method according to claim 6 wherein the stimula-
tion of nerve growth involves the potentiation of nerve
growth factor by 1,13-tricyano-2-amino-1-propane.
34. A method according to claim 2 wherein the mainte-
nance of nerve growth involves the potentiation of nerve
growth factor by 1,13-tricyano-2-amino-1-propane.
35. A method of restoring nerve growth by potentiating choline O-acetyltransferase in a host requiring nerve growth restoration comprising administering to a host a nerve growth restorative effective amount of 1,1,3-tricyano-2-amino-1-propene.

36. A method of restoring nerve growth by potentiating tyrosine hydroxylase in a host requiring nerve growth restoration comprising administering to a host a nerve growth restorative effective amount of 1,1,3-tricyano-2-amino-1-propene.

37. A method according to claim 35 wherein the effective amount of 2-amino-1,1,3-tricyano-1-propene is from about 15 µg/kg to about 45 µg/kg of body weight.

38. The method according to claim 37 wherein the effective amount of 2-amino-1,1,3-tricyano-1-propene is about 20 µg/kg of body weight.

39. A method according to claim 36 wherein the effective amount of 2-amino-1,1,3-tricyano-1-propene is from about 15 µg/kg to about 45 µg/kg of body weight.

40. The method according to claim 39 wherein the effective amount of 2-amino-1,1,3-tricyano-1-propene is about 20 µg/kg of body weight.

* * * * *