Description

Title of Invention: USE OF CYCLIC DIPEPTIDES IN PRODUCTION OF AGENT FOR INHIBITING DECLINE IN COGNITIVE FUNCTION OR AMELIORATING COGNITIVE DYSFUNCTION

Technical Field

[0001] The present invention relates to use of cyclic dipeptides in the production of an agent for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction. The present invention also relates to a composition for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction, and a method of inhibiting a decline in cognitive function or ameliorating cognitive dysfunction.

Background Art

[0002] Improvement in cognitive function such as memory function is desired by many people regardless of generation. Recently, an aging-related decline in cognitive function has been a problem in countries where the elderly ratio in the population is high, and search has been made for a component capable of inhibiting a decline in cognitive function or ameliorating cognitive dysfunction through daily intake of the component as a food, beverage, or the like.

[0003] For example, Patent Literature 1 discloses an agent containing Cyclo(Gly-Pro) for inhibiting a decline in cognitive function and/or for improving cognitive function. According to the disclosure, oral intake of the agent for inhibiting a decline in cognitive function and/or improving cognitive function by a human can inhibit a decline in attention/concentration, language ability, and visuospatial/constructional ability, and/or can improve these abilities.

Citation List

Patent Literature

[0004] PTL 1: Patent Literature 1: JP 6598412 B

Summary of Invention

Technical Problem

[0005] The present invention aims to provide an agent effective in inhibiting a decline in cognitive function such as memory function or ameliorating cognitive dysfunction. The present invention also aims to provide a composition for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction. The present invention also aims to provide a method of inhibiting a decline in cognitive function or ameliorating cognitive dysfunction.

Solution to Problem

[0006] As a result of extensive studies on the above problems, the present inventors found that combination use of specific cyclic dipeptides significantly increases the effect of inhibiting a decline in cognitive function or ameliorating cognitive dysfunction, and thus completed the present invention.

[0007] Specifically, the present invention relates to, but is not limited to, use, a composition for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction, and a method of inhibiting a decline in cognitive function or ameliorating cognitive dysfunction, for example, which are described as follows.

(1) Use of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) in the production of an agent for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction.

(2) The use according to (1) above, wherein the cognitive dysfunction is aging-related cognitive dysfunction.

(3) The use according to (1) or (2) above, wherein the cognitive function includes at least one selected from the group consisting of memory function, executive function, attention, and concentration.

(4) The use according to (3) above, wherein the memory function includes at least one selected from the group consisting of verbal memory function, nonverbal memory function, visuospatial memory function, gist memory function, and working memory function.

(5) The use according to any one of (1) to (4) above,

wherein the Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) inhibit a decline in cognitive function or ameliorate cognitive dysfunction by inhibition of inflammation in the brain.

(6) The use according to (5) above,

wherein the Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) inhibit the inflammation in the brain by inhibition of the production of nitric oxide.

(7) The use according to any one of (1) to (5) above, wherein the Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) inhibit a decline in cognitive function or ameliorate cognitive dysfunction by inhibition of the production of tumor necrosis factor-α (TNF-α).

(8) The use according to any one of (1) to (7) above, wherein the agent is for oral administration.

(9) The use according to any one of (1) to (8) above, wherein the agent is a food or beverage, or pharmaceutical product.

(10) The use according to any one of (1) to (9) above, wherein the agent is labeled

with one or more function claims selected from the group consisting of "enhancing cognitive function", "inhibiting a decline in cognitive function", "maintaining good cognitive function", "improving memory", "inhibiting a decline in memory", "maintaining good memory", "improving accuracy of memory", "preventing memory disorders", "ameliorating memory disorders", "maintaining memory that is one domain of cognitive function", "function suitable for those who are concerned about a decline in memory", "improving accuracy of memory and/or correctness of judgement that are part of cognitive function", "improving memory retention or consolidation", "sustaining cognitive enhancement", "improving executive function", "promoting attention and concentration", "improving learning ability", "delaying aging-related cognitive decline", "enhancing short-term and long-term memory" and "promoting verbal and visuospatial memory".

(11) Use of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction.

(12) A composition for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction, containing: Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) as active ingredients.

(13) A method of inhibiting a decline in cognitive function or ameliorating cognitive dysfunction, including: administering or feeding Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro).

Advantageous Effects of Invention

[0008] The present invention provides an agent effective in inhibiting a decline in cognitive function such as memory function or ameliorating cognitive dysfunction. The present invention also provides a composition for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction and the like. The agent and the composition of the present invention can be used as foods or beverages, pharmaceutical products, or the like for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction. Cyclic dipeptides are ingestible as foods, beverages, pharmaceutical products, or the like by humans, and are advantageously highly safe. The present invention also provides a method of inhibiting a decline in cognitive function or ameliorating cognitive dysfunction.

Description of Embodiments

[0009] The present invention relates to use of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) in the production of an agent for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction. A combination use of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) shows excellent effects of inhibiting a decline in cognitive function or ameliorating cognitive

dysfunction.

Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) can be used as active ingredients of the agent for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction.

The agent for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction usually contains Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) as active ingredients.

[0010] All of the Cyclo(Phe-Phe) (cyclo-phenylalanyl-phenylalanine), Cyclo(Gly-Pro) (cyclo-glycyl-proline), Cyclo(His-Pro) (cyclo-histidyl-proline), and Cyclo(Val-Pro) (cyclo-valyl-proline) are cyclic dipeptides. Herein, the term "cyclic dipeptide" refers to a compound whose structural units are amino acids and which has a diketopiperazine structure produced by dehydration condensation of an amino group of an amino acid at the N-terminal side and a carboxy group of an amino acid at the C-terminal side. Herein, the description order of amino acids in a cyclic dipeptide is not limited as long as the amino acid structure is unchanged. For example, Cyclo(Gly-Pro) and Cyclo(Pro-Gly) (cyclo-prolyl-glycine) represent an identical cyclic dipeptide.

[0011] Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro) and Cyclo(Val-Pro) can be used in the form of a salt with an inorganic acid or an organic acid or a salt with an inorganic base or an organic base in the present invention. Such an acid or a base can be selected based on the application of the salt. In view of application to foods, beverages, and pharmaceutical products, the following dietary or pharmaceutically acceptable salts are preferred. Examples of the inorganic acid salt include hydrochloride, nitrate, sulfate, methanesulfonate, and p-toluenesulfonate. Examples of the organic acid salt include salts with dicarboxylic acids such as oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, and salts with monocarboxylic acids such as acetic acid, propionic acid, and butyric acid. Examples of the inorganic base include hydroxides, carbonates, and bicarbonates of sodium, lithium, calcium, magnesium, and aluminium and ammonia. Examples of the salt with the organic base include mono-, di-, or trialkylamine salts such as salts of methylamine, dimethylamine, and triethylamine, mono-, di-, or tri-hydroxyalkylamine salts, guanidine salt, and N-methylglucosamine salt.

[0012] In the present invention, Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) may be derived from extracts of natural products such as an extract obtained by heat treatment of natural product or hydrolyzed protein, or may be artificially synthesized products. When these cyclic dipeptides are derived from the extracts of natural products, they can be obtained, for example, by heating protein or protein hydrolysates such as plant protein hydrolysates, poultry protein hydrolysates or collagen hydrolysates in water.

Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) may be used in the form of protein hydrolysates containing these cyclic dipeptides, or may be used in the form of concentrates, dry powder, or purified products thereof. Cyclic dipeptides are ingestible as foods or beverages and are highly safe.

In the present invention, the above protein hydrolysates or heat-treated product thereof can be used to add Cyclo (Phe-Phe), Cyclo (Gly-Pro), Cyclo (His-Pro) and Cyclo (Val-Pro) to the agent or composition.

[0013] In the present invention, the cognitive function refers to the brain function such as memory function, learning ability, memory consolidation, executive function, attention (attention function), and concentration. The cognitive dysfunction means partial or entire failure of the cognitive function.

In the present invention, the phrase "inhibiting a decline in cognitive function" encompasses maintaining cognitive function, slowing down the speed of decline in cognitive function, stopping decline in cognitive function, and the like. The phrase "ameliorating cognitive dysfunction" encompasses alleviating (moderating) the degree of cognitive dysfunction, recovering cognitive function, improving cognitive function, and the like. The phrase "inhibiting a decline in cognitive function or ameliorating cognitive dysfunction" encompasses inhibiting a decline in part of cognitive function or ameliorating part of cognitive dysfunction. The term "recovering" encompasses at least partial recovering.

Examples of causes of the decline in cognitive function or cognitive dysfunction include aging; neurodegenerative diseases such as Alzheimer's disease, Pick's disease, and diffuse Lewy body disease; brain diseases such as cerebral infarction, cerebral hemorrhage, chronic subdural hematoma, brain tumor, encephalitis, and normal pressure hydrocephalus; dementia such as vascular dementia and frontotemporal dementia; and Creutzfeldt-Jakob disease.

In one embodiment, Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro), or an agent containing these cyclic dipeptides can be suitably used to ameliorate aging-related cognitive dysfunction. The aging-related cognitive dysfunction may be aging-related cognitive dysfunction among middle-aged and older people. The middle-aged and older people include the elderly.

[0014] In one embodiment, Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro), or an agent containing these cyclic dipeptides can be suitably used to inhibit a decline in cognitive function or ameliorate cognitive dysfunction, the cognitive function being at least one selected from the group consisting of memory function, executive function, attention, and concentration.

[0015] The memory function is a function for retaining information from past experiences and retrieving the information for use at a later time. The memory function is classified

into, for example, verbal memory function, nonverbal memory function, visuospatial memory function, gist memory function, and working memory function.

[0016] The executive function is a function for making decision, formulating and carrying out plans to achieve goals, and is an essential function for people to behave socially, independently, and creatively. The executive function compares various kinds of information such as visual, auditory, and olfactory information to information from past experiences and coordinate the information in the frontal lobe.

[0017] The attention is a function for selecting information from a large amount of information. It is an information selective processing function for selectively processing necessary information by not assigning processing resources to unnecessary information in order to effectively use limited processing resources in the brain.

The concentration is a function for investing mental effort or focusing effectively on the task at hand while ignoring distractions in any given situation.

In one embodiment, Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro), or an agent containing these cyclic dipeptides can be suitably used to inhibit a decline in memory function or improve memory function, and can be more suitably used to inhibit a decline in verbal memory function, nonverbal memory function, visuospatial memory function, gist memory function, and working memory function, or improve these functions.

[0018] As described in the later-described examples, Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) when used in combination exhibit an excellent action to inhibit the production of nitric oxide (NO). The effect of inhibiting the production of NO, which is obtainable by the combination of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro), is a significant effect that cannot be predicted from the effect of inhibiting the production of NO which is obtainable when each of these cyclic dipeptides is used alone.

[0019] For example, in an aging-related inflammatory condition, NO is attached to superoxide to form peroxynitrite ($ONOO^-$). Peroxynitrite in the brain is known to cause, for example, inflammation in the brain such as oxidative stress in the brain and neuroinflammation. Inflammation in the brain is considered to be one of the causes of a decline in cognitive function in Alzheimer's disease and the like. Thus, inhibition of the production of peroxynitrite by inhibition of the production of NO is considered to inhibit a decline in cognitive function.

Intake or administration of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) can inhibit the production of NO and in turn can inhibit inflammation in the brain. Inhibition of inflammation in the brain can inhibit a decline in cognitive function or ameliorate cognitive dysfunction.

The action of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro)

to inhibit the production of NO is observed in, for example, microglial cells and the like. Microglial cells are a type of glial cells in the central nervous system, and are considered to be the major central immunocompetent cells. An increase in the production of NO in microglial cells is known to relate to a decline in cognitive function. Inhibition of the production of NO in microglial cells inhibits inflammation in the brain, which in turn can inhibit a decline in cognitive function or ameliorate cognitive dysfunction.

In one embodiment, Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) , or an agent containing these cyclic dipeptides can be used to inhibit inflammation in the brain so to as inhibit a decline in cognitive function or ameliorate cognitive dysfunction. In one embodiment, Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro), or an agent containing these cyclic dipeptides can be used, for example, to inhibit a decline in cognitive function associated with inflammation in the brain or ameliorate cognitive dysfunction associated with inflammation in the brain. Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro), or an agent containing these cyclic dipeptides can be used to inhibit inflammation in the brain by inhibition of the production of NO so as to inhibit a decline in cognitive function or ameliorate cognitive dysfunction.

[0020] As described in the later-described examples, Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) when used in combination have an excellent action to inhibit the production of inflammatory markers. Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) can be suitably used to inhibit the production of particularly tumor necrosis factor-α (TNF-α) among inflammatory markers.

The effect of inhibiting the production of TNF-α, which is obtainable by the combination of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro), is a significant effect that cannot be predicted from the effect of inhibiting the production of inflammatory markers which is obtainable when each of these cyclic dipeptides is used alone.

The action of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) to inhibit the production of inflammatory markers is observed in, for example, microglial cells. An increase in the production of TNF-α is known to relate to a decline in cognitive function. Preferably, inflammation in the brain is inhibited by inhibition of the production of TNF-α in microglial cells, which in turn can inhibit a decline in cognitive function or ameliorate cognitive dysfunction. Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro), or an agent containing these cyclic dipeptides can inhibit a decline in cognitive function or ameliorate cognitive dysfunction by inhibition of the production of TNF-α, and can be suitably used for

such purposes.

[0021] Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro), or an agent containing these cyclic dipeptides can be used to prevent or ameliorate a condition or disease that can be prevented or ameliorated by inhibition of a decline in cognitive function or amelioration of cognitive dysfunction. Such a condition or disease may be a condition or disease that shows a decline in cognitive function, or a condition associated with a decline in cognitive function. Examples thereof include a decline in memory, memory disorders (forgetfulness), aphasia (inability to formulate words), apraxia, agnosia (e.g., getting lost in a familiar place), a decline in verbal and non-verbal learning, a decline in auditory and visual processing, a decline in problem solving, a decline in executive function, executive dysfunction (inability to formulate and carry out plans), a decline in concentration, a decline in attention, a decline in judgement, a decline in spatial awareness, a decline in cognitive flexibility, and a decline in information processing speed. In particular, Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro), or an agent containing these cyclic dipeptides can be suitably used to prevent or ameliorate a decline in memory, memory disorders, aphasia, apraxia, executive dysfunction, a decline in executive function, a decline in concentration, a decline in attention, and the like. In one embodiment, Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro), or an agent containing these cyclic dipeptides can be suitably used to prevent or ameliorate an aging-related decline in memory, memory disorders, aphasia, apraxia, verbal learning, a decline in executive function, a decline in concentration, a decline in attention, and the like. In one embodiment, Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro), or an agent containing these cyclic dipeptides can be used to prevent or ameliorate the above conditions or diseases by inhibition of the production of NO. In one embodiment, Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro), or an agent containing these cyclic dipeptides can be used to prevent or ameliorate the above conditions or diseases by inhibition of the production of TNF-α.

Herein, the expression "prevention of a condition or disease" encompasses preventing the onset of a condition or disease, delaying the onset of a condition or disease, reducing the incidence, reducing the onset risk, and the like. The expression "amelioration of a condition or disease" encompasses helping a subject recover from a condition or disease, alleviating a symptom of a condition or disease, changing a symptom of a condition or disease for the better, delaying or preventing the progress of a condition or disease, and the like.

[0022] In the present invention, the agent containing Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) is applicable for either therapeutic use (medical

use) or non-therapeutic use (non-medical use). The "non-therapeutic" is a concept that does not include medical activities, i.e., a concept that does not include surgery, therapy, or diagnosis of humans.

The agent can be provided directly as a composition, or can be provided as a composition containing the agent.

[0023] In the present invention, the agent may be either for oral administration or parenteral administration. Preferably, the agent is for oral administration. The agent can be provided, for example, as a food or beverage, pharmaceutical product, quasi-pharmaceutical product, or feed. A food or beverage, or pharmaceutical product is preferred. The agent can be used by being added to a food or beverage, pharmaceutical product, quasi-pharmaceutical product, feed, or the like.

For example, when the agent is provided as a food or beverage, components usable in a food or beverage (e.g., food or beverage materials and optional food or beverage additives) can be added to Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) to provide various foods or beverages. Non-limiting examples of the food or beverage include general foods or beverages, health foods, health beverages, foods with function claims, foods for specified health uses, health supplements, and foods or beverages for the sick. Examples of the health foods, health beverages, the foods with function claims, foods for specified health use, and health supplements can be used in various forms of preparations such as fine granules, tablets, granules, powders, capsules, chewable tablets, dry syrups, syrups, liquids, beverages, soft gel, jelly and liquid foods.

[0024] An example of a preferred form of the agent is a beverage.

Non-limiting examples of the beverage include tea, coffee, alcoholic beverages, non-alcoholic beverages such as non-alcoholic beer-taste beverages, carbonated beverages, functional beverages, fruit and/or vegetable beverages, dairy beverages, soy milk, flavored water, energy drink, fermented drink such as kombucha, poultry meat extract, poultry meat essence, and protein drink.

[0025] When the agent is provided as a pharmaceutical product or a quasi-pharmaceutical product, various dosage forms of pharmaceutical or quasi-pharmaceutical products can be provided by, for example, adding a pharmacologically acceptable carrier, an optional additive, or the like to Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro). Such a carrier, an additive, or the like may be of any pharmacologically acceptable type that can be used in pharmaceutical or quasi-pharmaceutical products. Examples thereof include excipients, binders, disintegrants, lubricants, antioxidants, and colorants. One or more of these can be used. Examples of the form of administration (intake) of the pharmaceutical product or quasi-pharmaceutical product include oral administration and parenteral administration (e.g., transdermal,

transmucosal, enteral administration, and injection). When the agent is provided as a pharmaceutical product or quasi-pharmaceutical product, a pharmaceutical product for oral administration or a quasi-pharmaceutical product for oral administration is preferred. Examples of dosage forms for oral administration include liquids, tablets, powders, fine granules, granules, sugar-coated tablets, capsules, suspensions, emulsions, and chewable tablets. Examples of dosage forms for parenteral administration include injections, intravenous agents, ointments, lotions, adhesive skin patches, suppositories, nasal agents, and transpulmonary agents (inhalants). The pharmaceutical product may be for non-human animals.

[0026] The agent contains Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro), and may further contain various types of additives such as diluents, acidulants, antioxidants, stabilizers, preservatives, flavoring or masking agents, emulsifiers, pigments, seasonings, pH adjusters, and nutrient enhancers which are accepted as additives to foods, pharmaceutical products, or the like.

[0027] When the agent is provided as feed, Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) are added to feed. The feed also encompasses feed additives. Examples of the feed include livestock feed for animals such as cows, pigs, chickens, sheep, and horses; feed for small animals such as rabbits, rats, and mice; and pet food for animals such as dogs, cats, and birds.

[0028] The form of a food or beverage, pharmaceutical product, quasi- pharmaceutical product, or feed is not particularly limited, and any commonly used container can be used. The beverage may be, for example, a packaged beverage. Such a container includes paper containers such as paper packs; plastic containers such as PET bottles and press through pack sheets (PTP sheets); metal containers such as aluminium cans and steel cans; glass containers such as glass bottles; and wooden containers such as barrels.

A food or beverage, pharmaceutical product, quasi- pharmaceutical product, or feed may be in bulk in a single container or a single dose in one or more pouches.

[0029] When the agent is provided as, for example, a food or beverage, pharmaceutical product, quasi-pharmaceutical product, or feed, the production method is not limited. Any of these products can be produced by a common method using the four cyclic dipeptides, i.e., Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro).

[0030] In the present invention, the amount of each of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) in the agent is not limited, and can be determined depending on the form or the like of the agent.

In one embodiment, the total amount of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) in the agent is, for example, preferably 0.0000001 wt% or more, more preferably 0.000001 wt% or more and is preferably 99 wt% or less,

more preferably 90 wt% or less. In one embodiment, the total amount of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) in the agent is, for example, preferably 0.0000001 to 99 wt%, more preferably 0.000001 to 90 wt%. The amount of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) can be quantitated by a known method such as high performance liquid chromatography (HPLC)and liquid chromatography mass spectroscopy (LCMS).

[0031] In the present invention, the percentage of each cyclic dipeptide in the agent is not limited. The percentage may be the same or different for each cyclic dipeptide.

In the present invention, the ratio of Cyclo(Phe-Phe) to Cyclo(Gly-Pro), i.e., Cyclo(Gly-Pro)/Cyclo(Phe-Phe) is preferably 0.02 to 450, more preferably 0.5 to 10, still more preferably 0.5 to 6. The ratio of Cyclo(Phe-Phe) to Cyclo(His-Pro), i.e., Cyclo(His-Pro)/Cyclo(Phe-Phe) is preferably 0.01 to 200, more preferably 0.1 to 10, still more preferably 0.5 to 3. The ratio of Cyclo(Phe-Phe) to Cyclo(Val-Pro), i.e., Cyclo(Val-Pro)/Cyclo(Phe-Phe) is preferably 0.01 to 250, more preferably 0.1 to 10, still more preferably 0.5 to 4.

[0032] In the present invention, preferably, the agent is orally fed (orally administered). The dose (intake) of the agent is not limited. The dose of the agent may be any amount that provides the effect of inhibiting a decline in cognitive function or the effect of ameliorating cognitive dysfunction. The dose may be suitably set according to the dosage form, administration method, body weight of a subject, and the like.

[0033] In one embodiment, when the agent is orally fed or administered to a human (adult), the dose in terms of the total dose of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) is preferably 0.001 mg or more, more preferably 0.01 mg or more, still more preferably 0.1 mg or more, and is preferably 50000 mg or less, more preferably 10000 mg or less, still more preferably 1000 mg or less, per 60 kg body weight per day. In one embodiment, the oral dose of the agent in terms of the total dose of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) for a human (adult) is preferably 0.001 to 50000 mg, more preferably 0.01 to 10000 mg, still more preferably 0.1 to 1000 mg, per 60 kg body weight per day. Intake or administration of the above amount in one or more portions per day, for example, one to several times (e.g., two to three times) per day, is preferred. In one embodiment, preferably, the above amount of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) is orally fed or administered to a human. In one embodiment, the agent can be used to feed or administer the above amount of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) to a human per 60 kg body weight per day.

[0034] In the present invention, preferably, the agent is fed or administered continuously. The above effects are expected to increase by continuous feeding or administration of

Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro). In one embodiment, the agent for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction is continuously fed or administered preferably for at least one week, more preferably for at least four weeks, still more preferably for at least eight weeks.

[0035] In the present invention, a subject to be fed or administered (administration subject) with the agent is not limited. The subject is preferably a human or non-human mammal, more preferably a human.

In one embodiment, an administration subject of the agent may be, for example, one who needs or wants to inhibit a decline in cognitive function or ameliorate cognitive dysfunction. Examples of such a subject include those who need or want to inhibit a decline in cognitive function or ameliorate cognitive dysfunction, and those who need or want to prevent or ameliorate an inflammatory condition or disease. In one embodiment, examples of the administration subject in the present invention include middle-aged and older people. The middle-aged and older people may be those who are 40 years old or older, for example. In one embodiment, the elderly are preferred subjects among the middle-aged and older people. The elderly may be those who are 60 years old or older or 65 years old or older. The agent may be used on healthy people. For example, the agent can be used on healthy people for a purpose such as inhibiting a decline in cognitive function or preventing cognitive dysfunction. The agent can be used for subjects with a decline in cognitive function or cognitive dysfunction, or for subjects with conditions or diseases associated with a decline in cognitive function or cognitive dysfunction.

[0036] The agent may be labeled with one or more of function claims. Such labels are also referred to as function claims, and the contents of the labels are not limited. Examples of such labels include "enhancing cognitive function", "inhibiting a decline in cognitive function", "maintaining good cognitive function", "improving memory", "inhibiting a decline in memory", "maintaining good memory", "improving accuracy of memory", "preventing memory disorders", "ameliorating memory disorders", "maintaining memory that is one domain of cognitive function", "function suitable for those who are concerned about a decline in memory", "improving accuracy of memory and/or correctness of judgement that are part of cognitive function", "improving memory retention or consolidation", "sustaining cognitive enhancement", "improving executive function", "promoting attention and concentration", "improving learning ability", "delaying aging-related cognitive decline", "enhancing short-term and long-term memory", "promoting verbal and visuospatial memory", and labels or function claims equated to these labels.

In one embodiment of the present invention, preferably, the agent is a food with one

or more of the above labels. The above labels may be labels indicating use of the agent for obtaining the above functions. These labels may be directly attached to the agent or may be attached to a container or package of the agent.

[0037] In one embodiment, the agent for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction can also be referred to as a cognitive function decline inhibitor or a cognitive dysfunction ameliorator.

In one embodiment, the agent for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction of the present invention can also be used as a composition for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction.

[0038] The present invention also relates to a composition for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction, the composition containing Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) as active ingredients. The composition for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction of the present invention is also simply referred to as the composition of the present invention.

Embodiments of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) used as active ingredients and amounts thereof in the composition are as described above for the agent. The composition of the present invention contains the above active ingredients and may further contain various additives and the like described above.

The form of the composition of the present invention and its preferred embodiments are as described above for the agent. The subject to be fed or administered with the composition of the present invention, dose of the composition of the present invention, and their preferred embodiments are as described above for the agent. The composition for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction of the present invention can inhibit a decline in cognitive function such as memory function or ameliorate cognitive dysfunction. A condition or disease that can be prevented or ameliorated by the composition for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction of the present invention is as described above for the agent.

[0039] The present invention also encompasses the following method.

A method of inhibiting a decline in cognitive function or ameliorating cognitive dysfunction, the method including administering or feeding Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro).

The present invention also encompasses the following use.

Use of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction.

The method may be a therapeutic or non-therapeutic method. The use may be therapeutic or non-therapeutic use. The dose, dosage, subject, and the like of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) are as described above for the agent.

Examples

[0040] Hereinafter, the present invention is described in more detail with reference to examples, but these examples are not intended to limit the scope of the present invention.

[0041] <Evaluation method of anti-inflammatory activity>

In Examples and Comparative Examples, the effect of inhibiting the production of inflammatory markers was evaluated by the following method.

[0042] (Reagents)

Gibco Dulbecco's Modified Eagle's Medium:Nutrient Mixture F-12 Ham 1:1 (DMEM/F12, Cat. #D6421), fetal bovine serum (FBS, Cat. #10500), horse serum (HS, Cat. #26050088) and Penicillin/Streptomycin (P/S, Cat. #15070063) were obtained from ThermoFischer Scientific.

Lipopolysaccharide O111:B4 (LPS, Cat. #LPS25) was purchased from Sigma-Aldrich.

Cyclic dipeptides also known as diketopiperazines (DKPs) including cyclo-phenylalanine-phenylalanine (Cyclo(Phe-Phe), CFF), cyclo-glycine-proline (Cyclo(Gly-Pro), CGP), cyclo-histidine-proline (Cyclo(His-Pro), CHP) and cyclo-valine-proline (Cyclo(Val-Pro), CVP) were bought from Bachem.

Poly-L-lysine (PLL) coated 96-well plates were provided by Corning.

[0043] (Cell culture and Treatments)

SIM-A9 murine microglial cells (ATCC) were maintained in DMEM/F12 supplemented with 10% of FBS, 5% of HS, 1% of P/S and 0.5% of dimethyl sulfoxide (DMSO). Cells (passage number:14 (P14)) were seeded in a PLL-coated 96-well plate at a cell density of 20,000 cells/well and incubated overnight at 37°C in a humidified gas chamber containing 5% of CO_2. Each of 22 μg/mL of Cyclo(Phe-Phe), 71 μg/mL of Cyclo(Gly-Pro), 28 μg/mL of Cyclo(His-Pro), 34 μg/mL of Cyclo(Val-Pro) or a combination of four cyclic dipeptides was added to the chondrocytes separately and the chondrocytes were pre-treated for 24 hours prior to induction with 100 ng/mL of LPS in the presence of the treatment in serum-free medium for another 24 hours.

Cells were centrifuged at 1100 g for 5 min and the supernatant was then used for cytokine analysis.

[0044] (Multiplex cytokine analysis)

The Mouse Cytokine Multiplex Assay (Bio-Rad) was used to analyse the cytokines

in the culture media. The following 23-plex analyses were performed: IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-17A, keratinocytes-derived chemokine (KC/CXCL1), eotaxin (CCL11), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-γ, monocyte chemotactic protein 1 (MCP-1; CCL2), macrophage inflammatory protein-1α (MIP-1α; CCL3), MIP-1β (CCL4), regulated upon activation normal T cell expressed and activated (RANTES) (CCL5) and TNF-α. Multiplex assays were carried out according to the manufacturers' instructions and run on the Luminex xPONENT for MAGPIX platform. Bio-Plex Manager version 6.0 was used for data processing. Cytokine and chemokine concentrations were calculated by reference to the standard curve. The sensitivity of the multiplex kit was <5 pg/mL.

[0045] (Statistical analyses)

Statistical analysis was conducted using GraphPad Prism version 5.0 (San Diego). All results are expressed as mean ± standard deviation. Statistical analysis was performed by analysis of variance (ANOVA) followed by posthoc Tukey's multiple comparison test. Data was considered to be significant when P-value, $p < 0.05$.

[0046] (Comparative Examples 1-2 and Example 1)

To assess the influence of the excellent effect of the combination of cyclic dipeptides on the inflammatory marker, TNF-α, produced by the SIM-A9 microglial cells, following four different conditions were compared: cells before treatment (vehicle control), LPS-treated cells (Comparative Example 1), LPS + single cyclic dipeptide-treated cells (Comparative Example 2) and LPS + combination of four cyclic dipeptides (4DKPs)-treated cells (Example 1). LPS + single cyclic dipeptide-treated cells were pre-treated with LPS and any one of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro) and Cyclo(Val-Pro). In the pre-treatment, Cyclo(Phe-Phe) concentration was 22 μg/mL, Cyclo(Gly-Pro) concentration was 71 μg/mL, Cyclo(His-Pro) concentration was 28 μg/mL, Cyclo(Val-Pro) concentration was 34 μg/mL. In the LPS + 4DKPs-treated cells, the concentration of each cyclic dipeptide in 4DKPs was 22 μg/mL for Cyclo(Phe-Phe), 71 μg/mL for Cyclo(Gly-Pro), 28 μg/mL for Cyclo(His-Pro) and 34 μg/mL for Cyclo(Val-Pro).

As shown in Table 1, combination of four cyclic dipeptides provided an excellent effect in reducing inflammation compared to treatment with single cyclic dipeptide. Data is represented as means ± SD (n=3). * denotes significant difference with $p<0.05$, ** denotes $p<0.01$, *** denotes $p<0.001$ against vehicle control (untreated) unless indicated. # denotes significant difference with $p<0.05$, ## denotes $p<0.01$, ### denotes $p<0.001$ against LPS-treated cells unless indicated. $ denotes significant difference with $p<0.05$, $$ denotes $p<0.01$, $$$ denotes $p<0.001$ against LPS + single cyclic dipeptide-treated cells unless indicated. The name of cyclic dipeptide in

parentheses after the $, $$ or $$$ mark indicates the cyclic dipeptide to which 4DKP has a significant difference.

[0047] [Table 1]

		Concentration of TNF-α (pg/mL)
	Vehicle control	0 (undetectable) ± 143.35
Comparative Example 1	LPS	3445.68 ± 46.43 ***
Comparative Example 2	CFF	2945.90 ± 79.66 ###
	CGP	2493.91 ± 80.80 ###
	CHP	2832.08 ± 180.37 ###
	CVP	2719.44 ± 218.99 ###
Example 1	4 DKPs	2417.44 ± 154.41 ### $(CVP), $$(CHP), $$$(CFF)

[0048] (Comparative Examples 3-4 and Example 2)

The excellent effect of the combination of cyclic dipeptides on the inflammatory marker was examined in the same manner as in Example 1 and Comparative Examples 1-2, except that cells having the passage number of 16 (P16) were used and cultured in a medium not containing DMSO. In Table 2, "SFM control" means "serum-free medium control".

As shown in Table 2, * denotes significant difference with $p<0.05$, ** denotes $p<0.01$, *** denotes $p<0.001$ against SFM control (untreated) unless indicated.

[0049] [Table 2]

		Concentration of TNF-α (pg/mL)
	SFM control	18.18 ± 0.7842
Comparative Example 3	LPS	613.2 ± 48.62 ***
Comparative Example 4	CFF	362.6 ± 27.18 ###
	CGP	234.0 ± 7.913 ###
	CHP	359.0 ± 24.00 ###
	CVP	325.5 ± 58.07 ###
Example 2	4 DKPs	203.2 ± 4.128 ### $(CVP), $$(CHP), $$$(CFF)

[0050] <Evaluation method of inhibition of the production of NO>

In Examples and Comparative Examples, the effect of inhibiting the production of nitric oxide (NO) was evaluated by the following method.

Same reagents and statistical analyses were used as the evaluation method of anti-inflammatory activity.

[0051] (Cell culture and Treatments)

For Nitrite assay, cells were plated in a PLL-coated 96-well plate, at 20000 cells/well. Each of four cyclic dipeptides or a combination of four cyclic dipeptides was added to the cells separately and the cells were pre-incubated for 24 hours. After the pre-incubation period, LPS was added at 2.5μg/mL for a further 24 hours. Cells were centrifuged at 1,000rpm for 5 min. Supernatant was collected and assayed 48 hours

post-plating.

[0052] (Nitrite Analysis)

To determine the amount of nitric oxide (NO) produced, the culture medium collected was incubated with Griess Reagent (Promega) according to manufacturer's instructions. Nitrite ion (NO_2^-), a stable derivative of NO, was measured and quantified with a plate reader at 540nm (ref 690nm).

[0053] (Comparative Examples 5-6 and Example 3)

To assess the influence of the excellent effect of the combination of cyclic dipeptides on the production of NO produced by the SIM-A9 microglial cells, following four different conditions were compared: cells before treatment (vehicle control), LPS-treated cells (Comparative Example 5), LPS + single cyclic dipeptide-treated cells (Comparative Example 6) and LPS + 4DKPs-treated cells (Example 3). LPS + single cyclic dipeptide-treated cells were pre-treated with LPS and any one of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro) and Cyclo(Val-Pro). In the pre-treatment, Cyclo(Phe-Phe) concentration was 22 μg/mL, Cyclo(Gly-Pro) concentration was 71 μg/mL, Cyclo(His-Pro) concentration was 28 μg/mL, Cyclo(Val-Pro) concentration was 34 μg/mL. In the LPS + 4DKPs-treated cells, the concentration of each cyclic dipeptide in 4DKPs was 22 μg/mL for Cyclo(Phe-Phe), 71 μg/mL for Cyclo(Gly-Pro), 28 μg/mL for Cyclo(His-Pro) and 34 μg/mL for Cyclo(Val-Pro).

As shown in Table 3, combination of four cyclic dipeptides provided an excellent effect in reducing production of NO compared to treatment with single cyclic dipeptide. Data is represented as means ± SD (n=3). * denotes significant difference with $p<0.05$, ** denotes $p<0.01$, *** denotes $p<0.001$ against vehicle control (untreated) unless indicated. # denotes significant difference with $p<0.05$, ## denotes $p<0.01$, ### denotes $p<0.001$ against LPS-treated cells unless indicated. $ denotes significant difference with $p<0.05$, $$ denotes $p<0.01$, $$$ denotes $p<0.001$ against LPS + single cyclic dipeptide-treated cells unless indicated. The name of cyclic dipeptide in parentheses after the $, $$ or $$$ mark indicates the cyclic dipeptide to which 4DKP has a significant difference.

[0054] [Table 3]

		NO_2^- / μM
	Vehicle control	0 (undetectable) ± 0.0105
Comparative Example 5	LPS	3.005 ± 0.3052 ***
Comparative Example 6	CFF	2.555 ± 0.2007
	CGP	2.413 ± 0.1087 #
	CHP	2.871 ± 0.3403
	CVP	3.076 ± 0.2372
Example 3	4 DKPs	2.091 ± 0.1799 ### $(CFF), $$$(CHP, CVP)

Claims

[Claim 1] Use of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) in the production of an agent for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction.

[Claim 2] The use according to claim 1,
wherein the cognitive dysfunction is aging-related cognitive dysfunction.

[Claim 3] The use according to claim 1 or 2,
wherein the cognitive function includes at least one selected from the group consisting of memory function, executive function, attention, and concentration.

[Claim 4] The use according to claim 3,
wherein the memory function includes at least one selected from the group consisting of verbal memory function, nonverbal memory function, visuospatial memory function, gist memory function, and working memory function.

[Claim 5] The use according to any one of claims 1 to 4,
wherein the Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) inhibit a decline in cognitive function or ameliorate cognitive dysfunction by inhibition of inflammation in the brain.

[Claim 6] The use according to claim 5,
wherein the Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) inhibit the inflammation in the brain by inhibition of the production of nitric oxide.

[Claim 7] The use according to any one of claims 1 to 5,
wherein the Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) inhibit a decline in cognitive function or ameliorate cognitive dysfunction by inhibition of the production of tumor necrosis factor-α (TNF-α).

[Claim 8] The use according to any one of claims 1 to 7,
wherein the agent is for oral administration.

[Claim 9] The use according to any one of claims 1 to 8,
wherein the agent is a food or beverage, or pharmaceutical product.

[Claim 10] The use according to any one of claims 1 to 9,
wherein the agent is labeled with one or more function claims selected from the group consisting of "enhancing cognitive function", "inhibiting a decline in cognitive function", "maintaining good cognitive

function", "improving memory", "inhibiting a decline in memory", "maintaining good memory", "improving accuracy of memory", "preventing memory disorders", "ameliorating memory disorders", "maintaining memory that is one domain of cognitive function", "function suitable for those who are concerned about a decline in memory", "improving accuracy of memory and/or correctness of judgement that are part of cognitive function", "improving memory retention or consolidation", "sustaining cognitive enhancement", "improving executive function", "promoting attention and concentration", "improving learning ability", "delaying aging-related cognitive decline", "enhancing short-term and long-term memory" and "promoting verbal and visuospatial memory".

[Claim 11] Use of Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction.

[Claim 12] A composition for inhibiting a decline in cognitive function or ameliorating cognitive dysfunction, comprising:
Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro) as active ingredients.

[Claim 13] A method of inhibiting a decline in cognitive function or ameliorating cognitive dysfunction, comprising:
administering or feeding Cyclo(Phe-Phe), Cyclo(Gly-Pro), Cyclo(His-Pro), and Cyclo(Val-Pro).