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(54) **SODIUM-INDEPENDENT SMALL NEUTRAL AMINO ACID TRANSPORTER TRANSPORTING L- AND D- AMINO ACIDS**

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C07K 14/705	(2006.01)

(52) **U.S. Cl.** **424/185.1**; 435/6; 435/69.1; 435/320.1; 435/325; 530/350; 536/23.5; 424/144.1; 530/388.22

(57) **ABSTRACT**

Novel sodium-independent small neutral amino acid transporters which transport L- and D-amino acids. A protein comprising the amino acid sequence represented by SEQ ID NO:1 or 4 or an amino acid derived therefrom by deletion, substitution or addition of one or more amino acids and being capable of sodium-independently transporting L- and D-small neutral amino acids and analogs thereof; a gene encoding the above protein; a method of screening substances inhibiting or promoting the function of the above protein; an antibody against the above protein; and a method of regulating cell function by using the above antibody, function inhibitors, function promoters, etc.

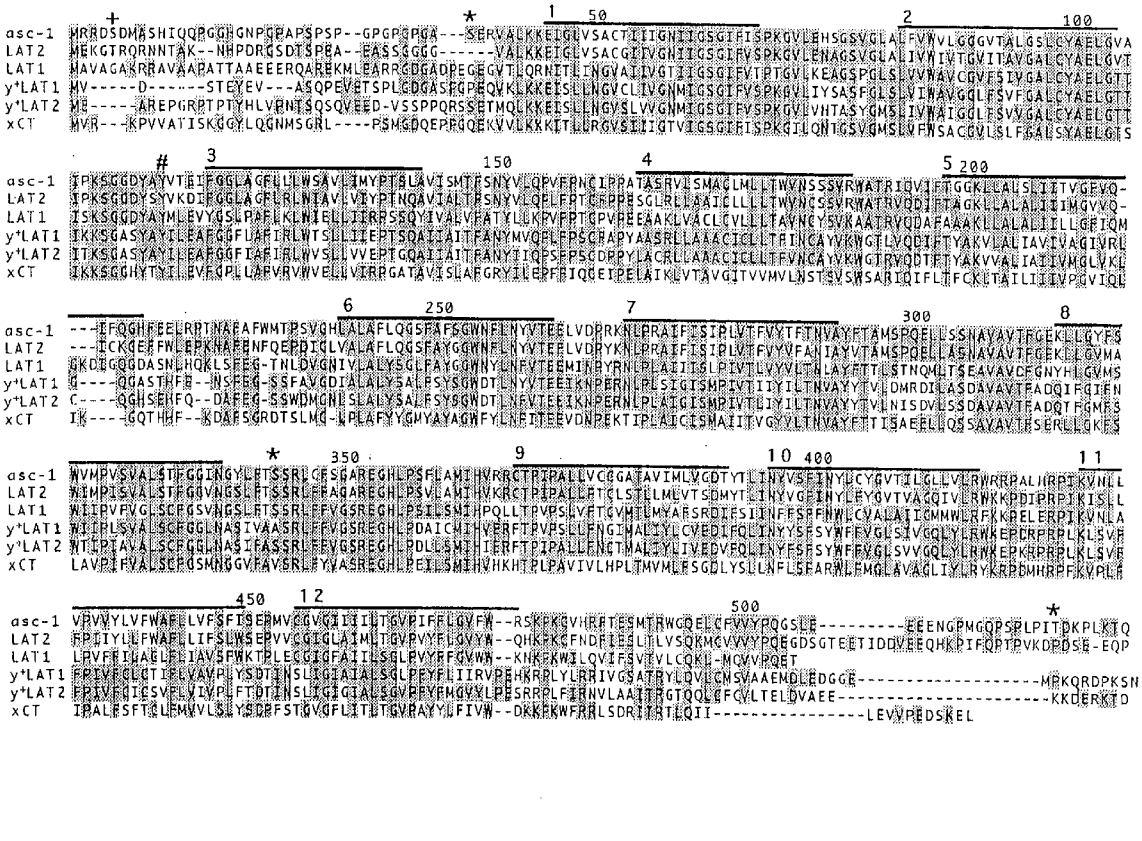


Figure 1

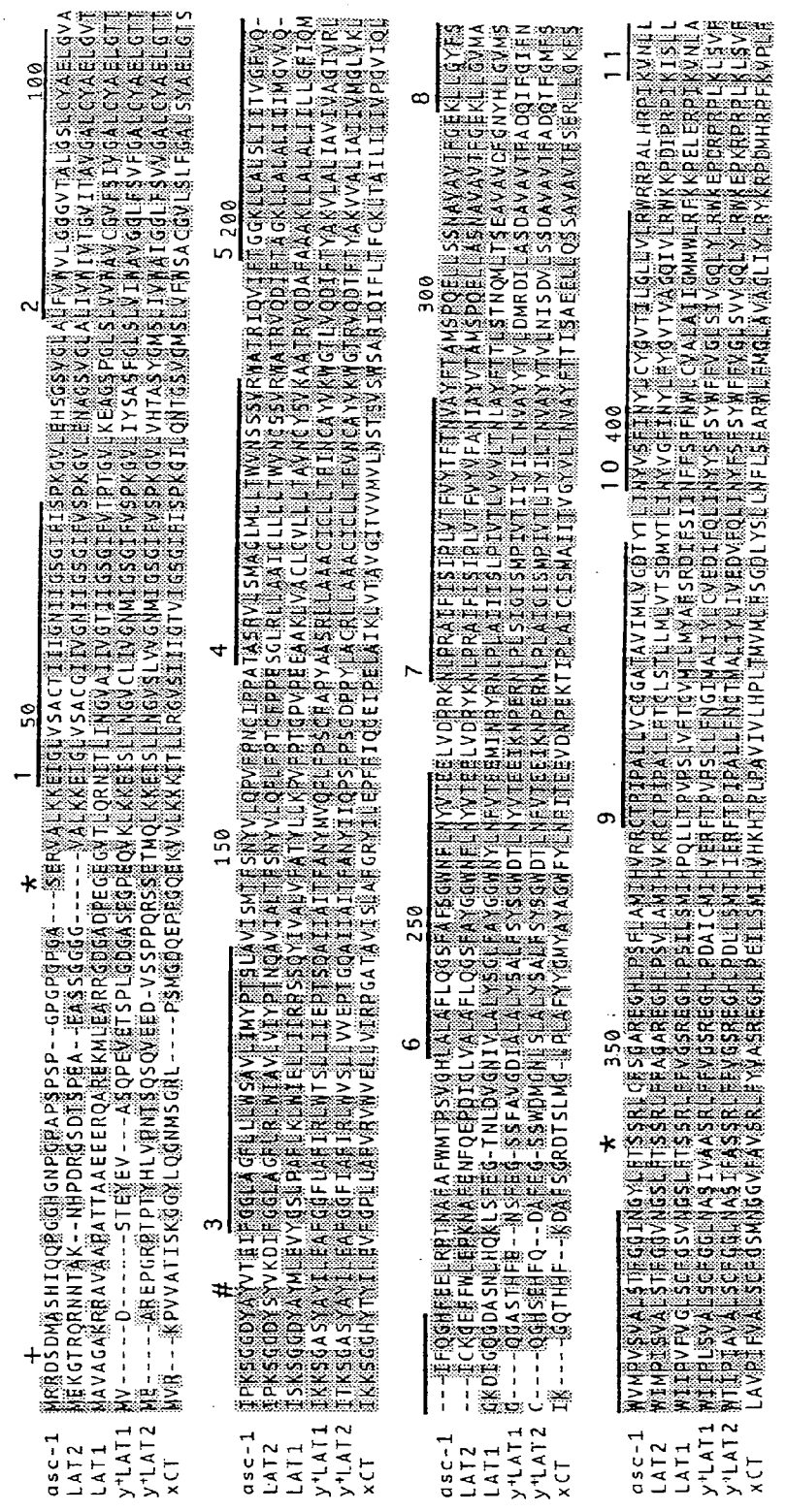


Figure 2

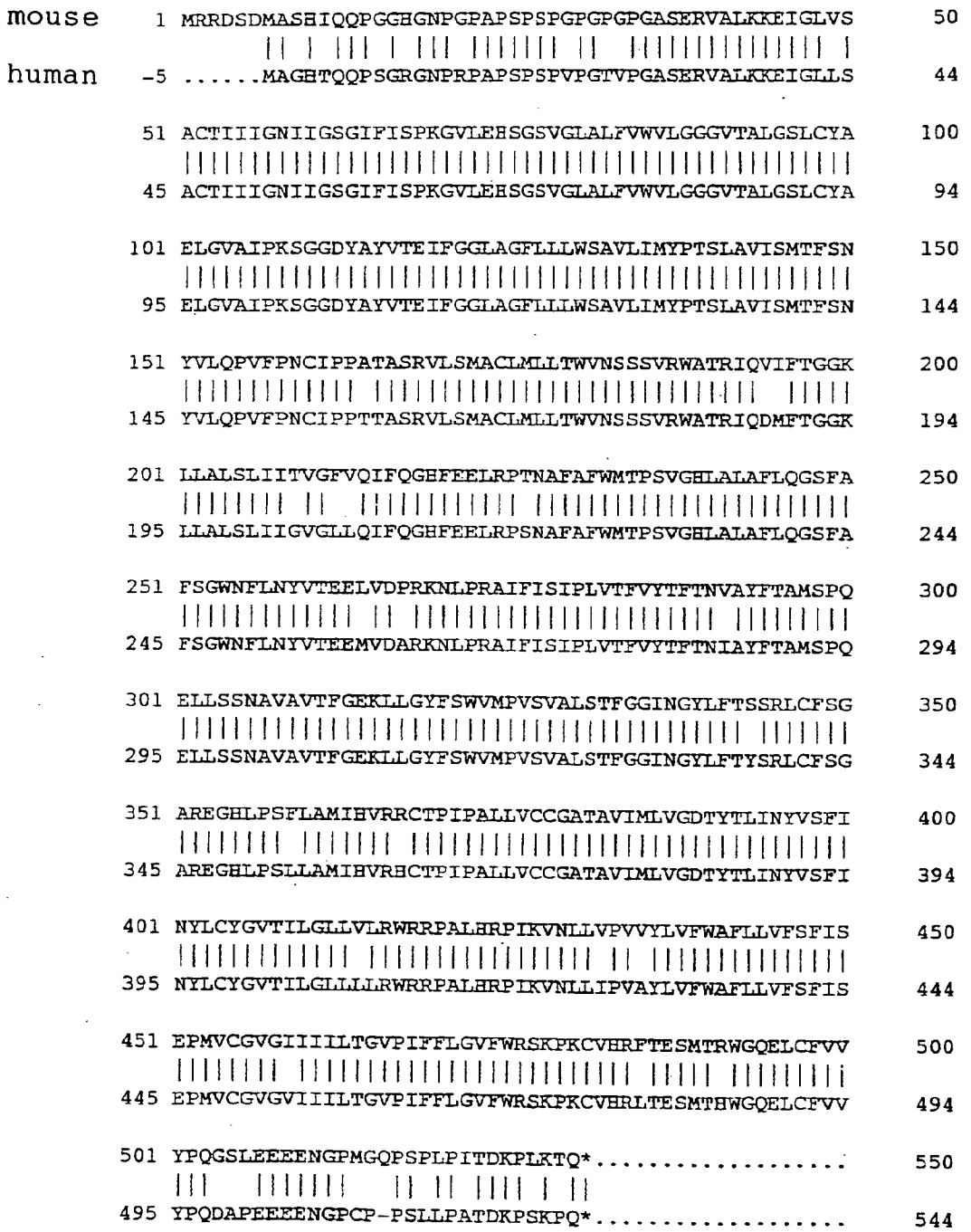

mouse 1 MRRDSDMASHIQQPGGHGNPGPAPSPSPGPGPGPGASERVALKKEIGLVS 50
human -5 .....MAGHTQQPSGRGNPRPAPSPSPVPGTVPGASERVALKKEIGLLS 44
51 ACTIIIGNIIGSGIFISPKGVLEHSGSVGLALFVWVLGGGVTALGSLCYA 100
45 ACTIIIGNIIGSGIFISPKGVLEHSGSVGLALFVWVLGGGVTALGSLCYA 94
101 ELGVAIPKSGGDYAYVTEIFGGLAGFLLLWSAVLIMYPTSLAVISMTFSN 150
95 ELGVAIPKSGGDYAYVTEIFGGLAGFLLLWSAVLIMYPTSLAVISMTFSN 144
151 YVLQPVFPNCIPPATASRVLSMACLMLLTWVNSSSVRWATRIQVIFTGGK 200
145 YVLQPVFPNCIPPTTASRVLSMACLMLLTWVNSSSVRWATRIQDMFTGGK 194
201 LLALSLIITVGFVQIFQGHFEELRPTNAFAFWMTPSVGHLALAFLQGSFA 250
195 LLALSLIIGVGLLQIFQGHFEELRPSNAFAFWMTPSVGHLALAFLQGSFA 244
251 FSGWNFLNYVTEELVDPRKNLPRAIFISIPLVTFVYTFTNVAYFTAMSPQ 300
245 FSGWNFLNYVTEEMVDARKNLPRAIFISIPLVTFVYTFTNIAYFTAMSPQ 294
301 ELLSSNAVAVTFGEKLLGYFSWVMPVSVALSTFGGINGYLFTSSRLCFSG 350
295 ELLSSNAVAVTFGEKLLGYFSWVMPVSVALSTFGGINGYLFTYSRLCFSG 344
351 AREGHLPSFLAMIHVRRCTPIPALLVCCGATAVIMLVGDTYTLINYVSFI 400
345 AREGHLPSLLAMIHVRHCTPIPALLVCCGATAVIMLVGDTYTLINYVSFI 394
401 NYLCYGVTILGLLVLRWRRPALHRPIKVNLLVPVVYLVFWAFLLVFSFIS 450
395 NYLCYGVTILGLLLLRWRRPALHRPIKVNLLIPVAYLVFWAFLLVFSFIS 444
451 EPMVCGVGIIIILTGVPIFFLGVFWRSKPKCVHRFTESMTRWGQELCFVV 500
445 EPMVCGVGVIIILTGVPIFFLGVFWRSKPKCVHRLTESMTHWGQELCFVV 494
501 YPQGSLEEEENGPMGQPSPLPITDKPLKTQ*..... 550
495 YPQDAPEEEENGPCP-PSLLPATDKPSKPQ*..... 544

Figure 3

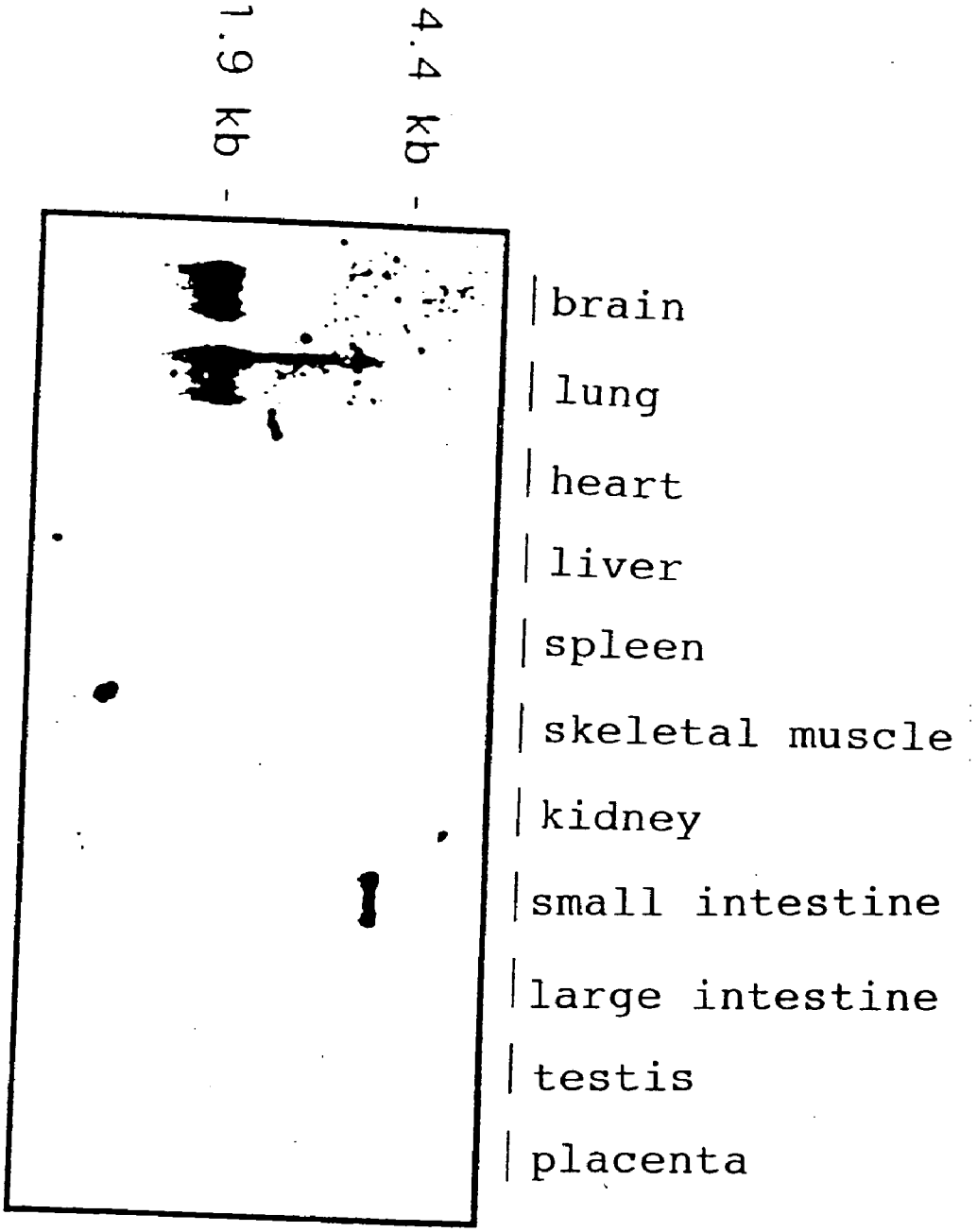

1.9 kb -
4.4 kb -
brain
lung
heart
liver
spleen
skeletal muscle
kidney
small intestine
large intestine
testis
placenta

Figure 4

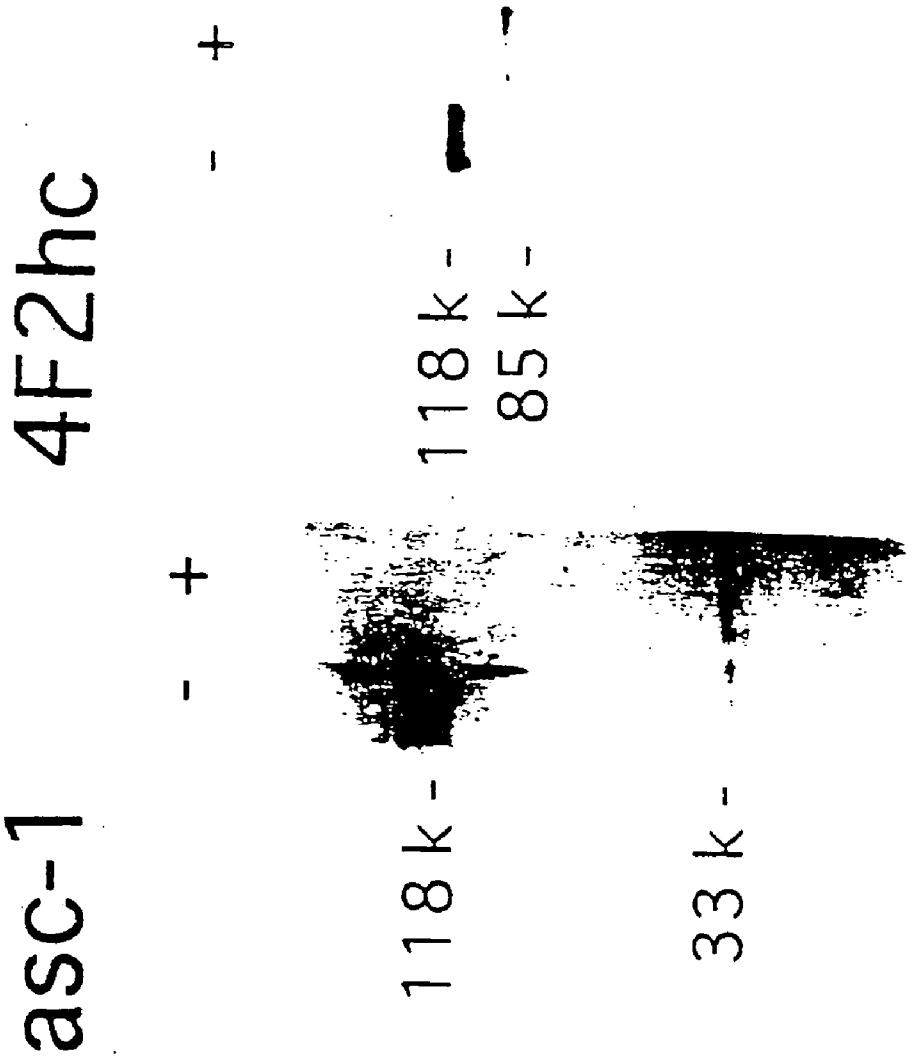


Figure 5

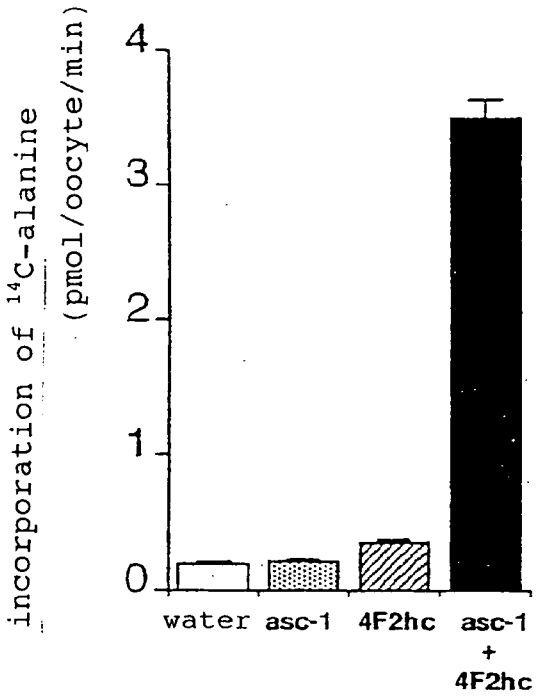


Figure 6

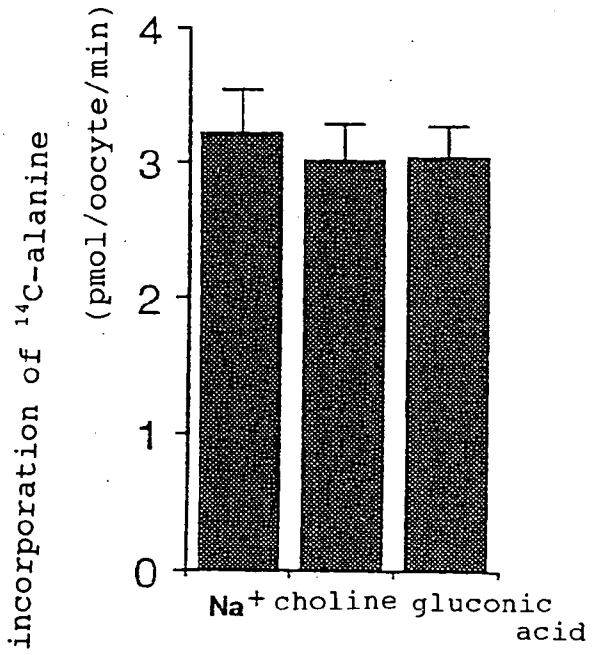


Figure 7

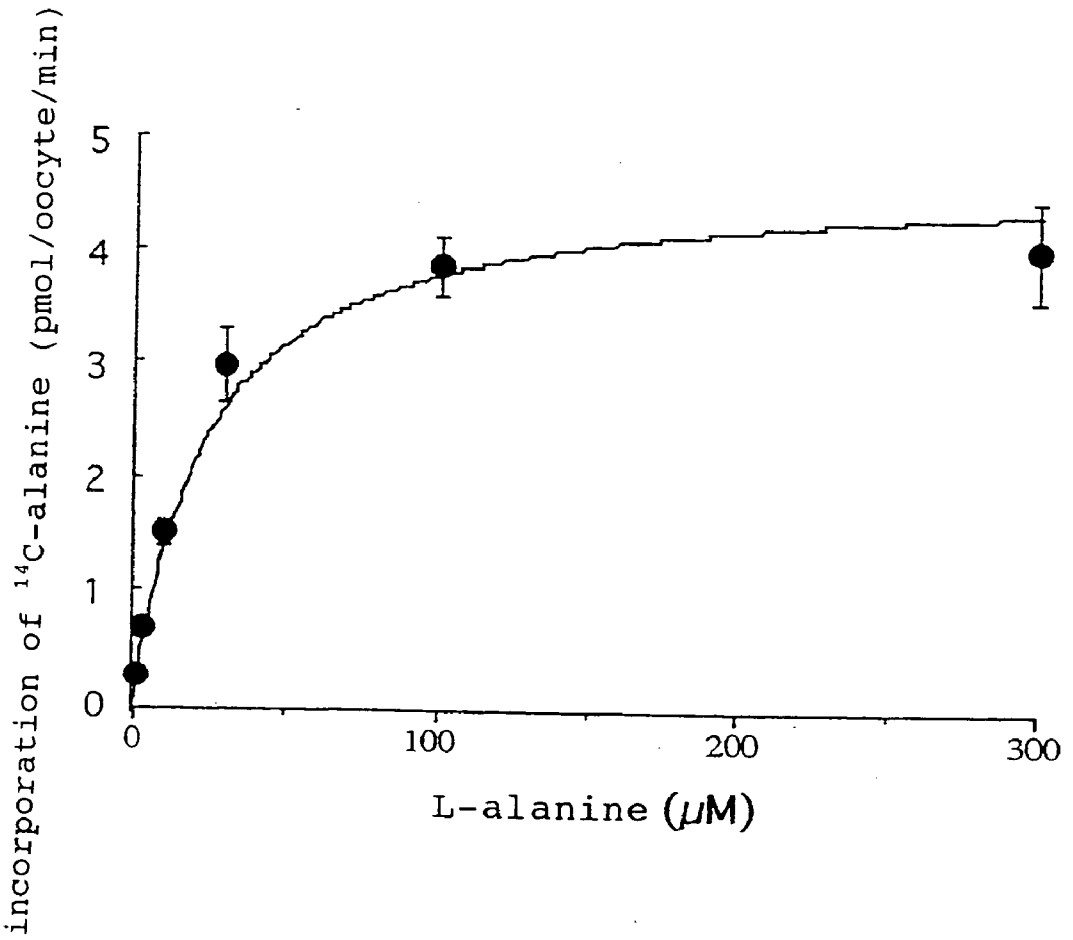

incorporation of ^{14}C-alanine (pmol/oocyte/min)
0
1
2
3
4
5
0
100
200
300
L-alanine (μM)

Figure 8

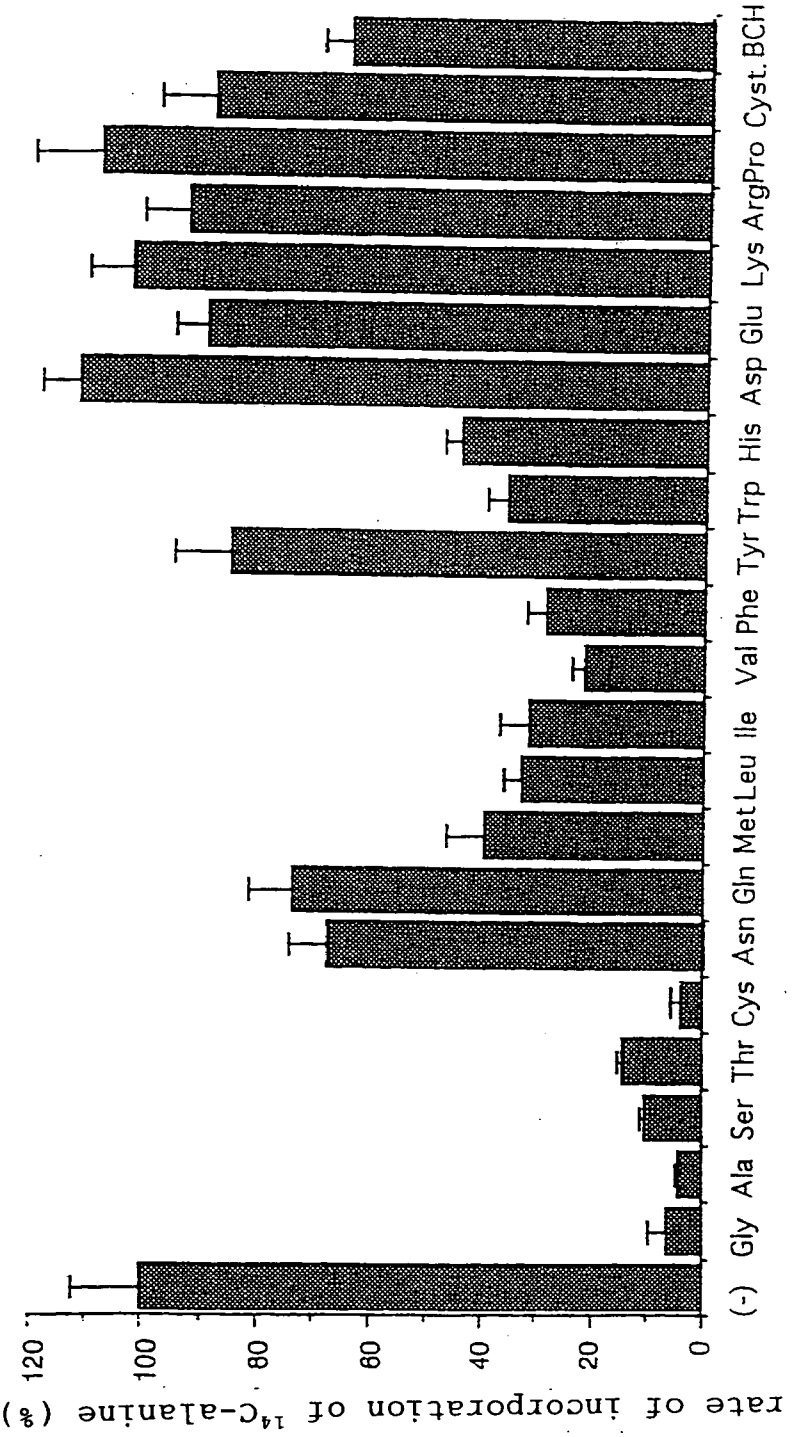

rate of incorporation of ^{14}C-alanine (%)
120
100
80
60
40
20
0
(-) Gly Ala Ser Thr Cys Asn Gln Met Leu Ile Val Phe Tyr Trp His Asp Glu Lys Arg Pro Cyst. BCH

Figure 9

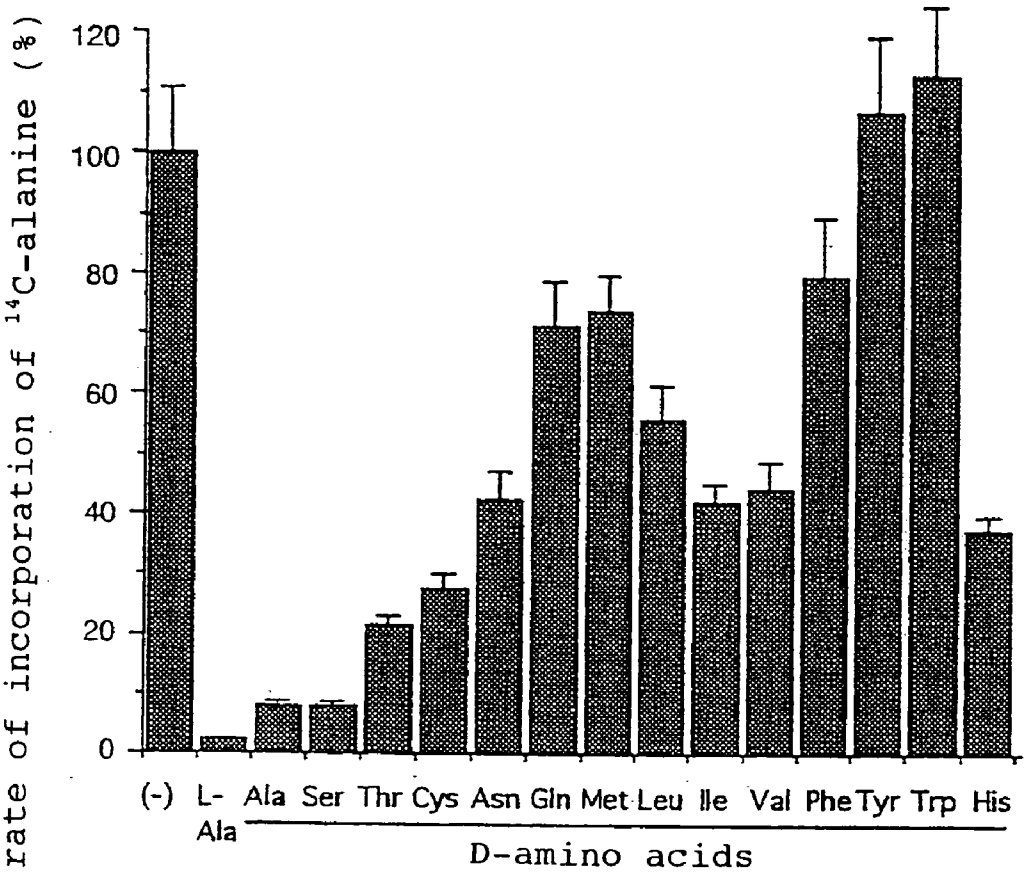

rate of incorporation of ^{14}C-alanine (%)
120
100
80
60
40
20
0
(-) L-Ala
Ala
Ser
Thr
Cys
Asn
Gln
Met
Leu
Ile
Val
Phe
Tyr
Trp
His
D-amino acids

Figure 10

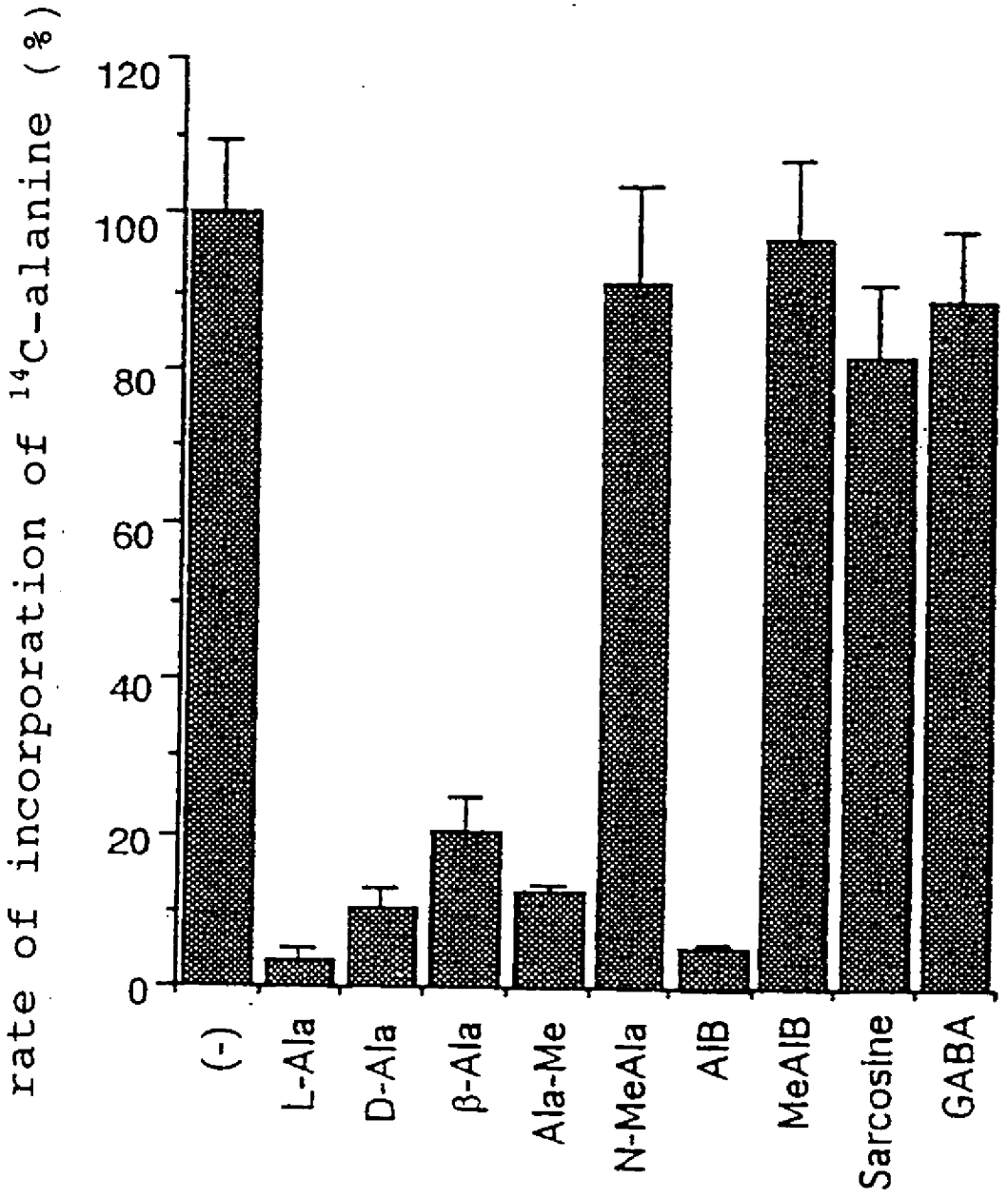

rate of incorporation of ^{14}C-alanine (%)
120
100
80
60
40
20
0
(-)
L-Ala
D-Ala
β-Ala
Ala-Me
N-MeAla
AIB
MeAIB
Sarcosine
GABA

Figure 11

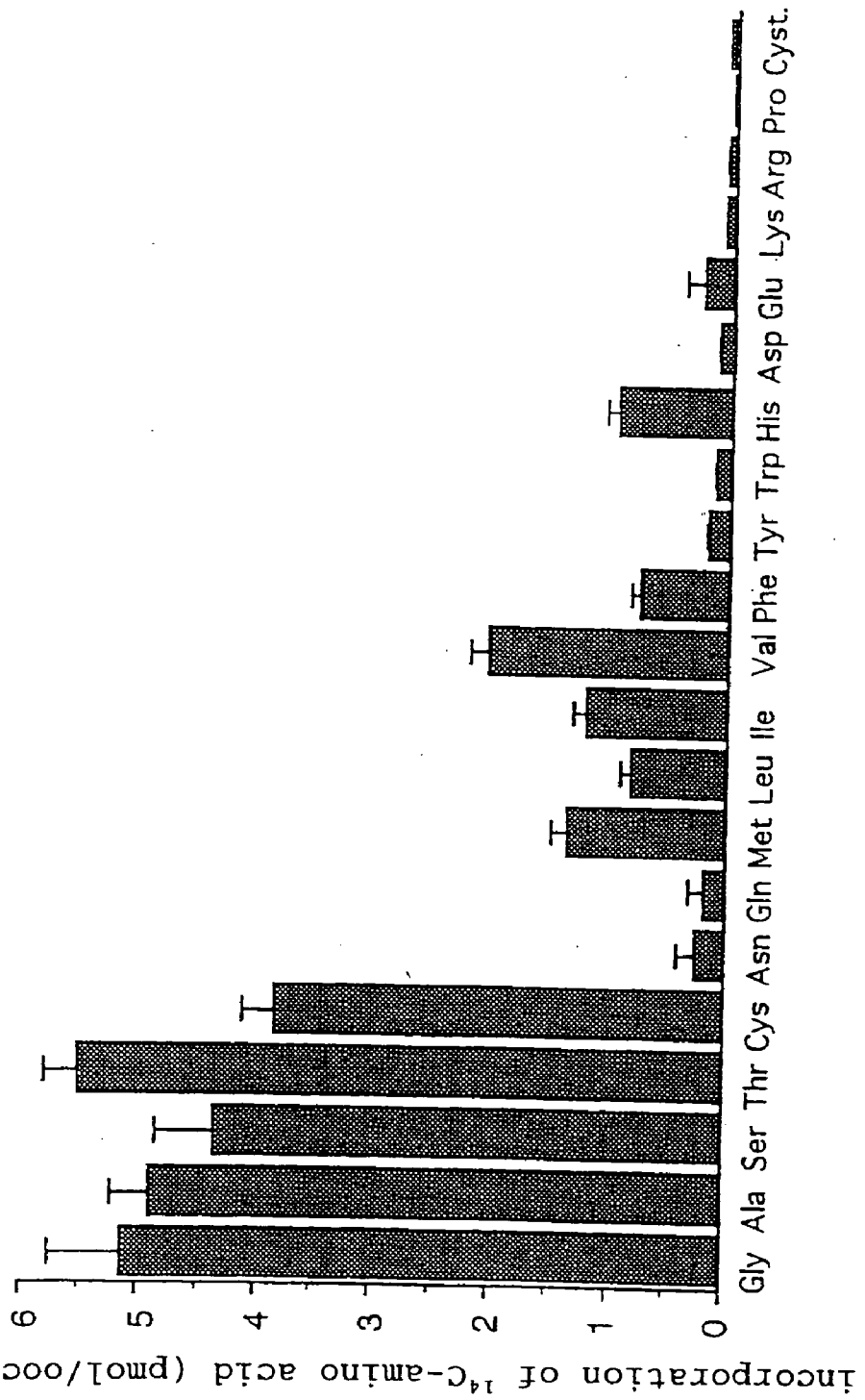

Incorporation of ^{14}C-amino acid (pmol/oocyte/min)
0
1
2
3
4
5
6
Gly Ala Ser Thr Cys Asn Gln Met Leu Ile Val Phe Tyr Trp His Asp Glu Lys Arg Pro Cyst.

Figure 12

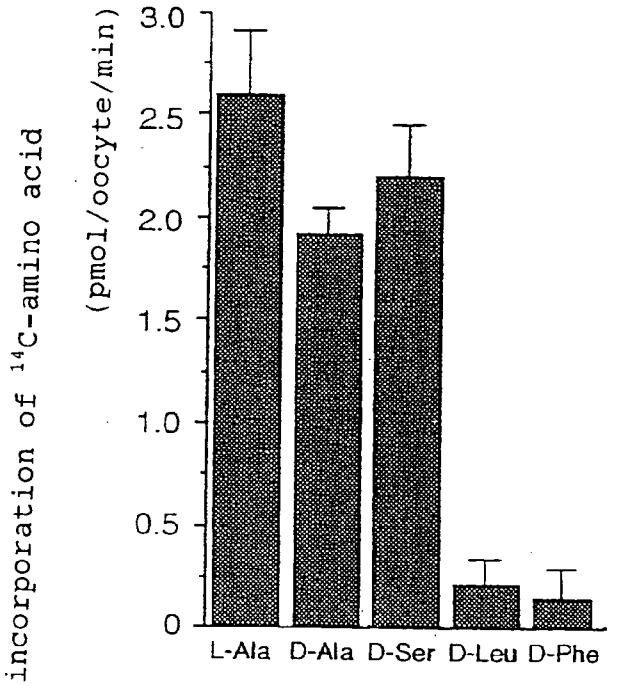


Figure 13

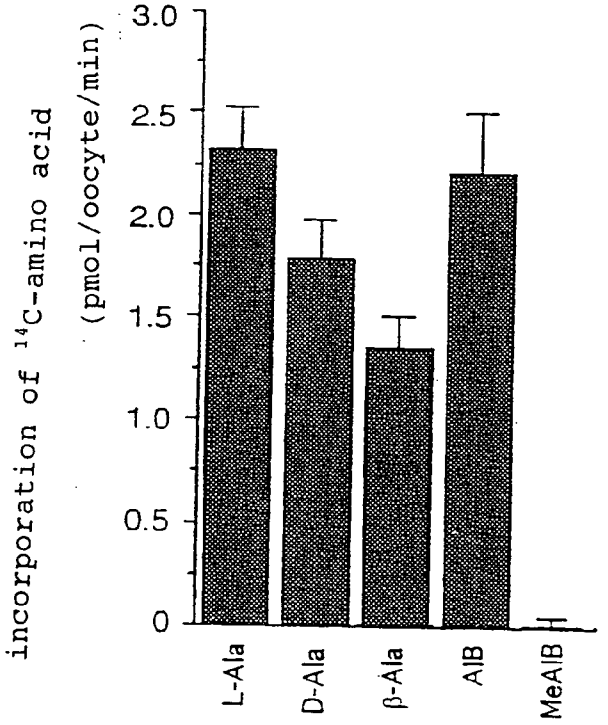


Figure 14

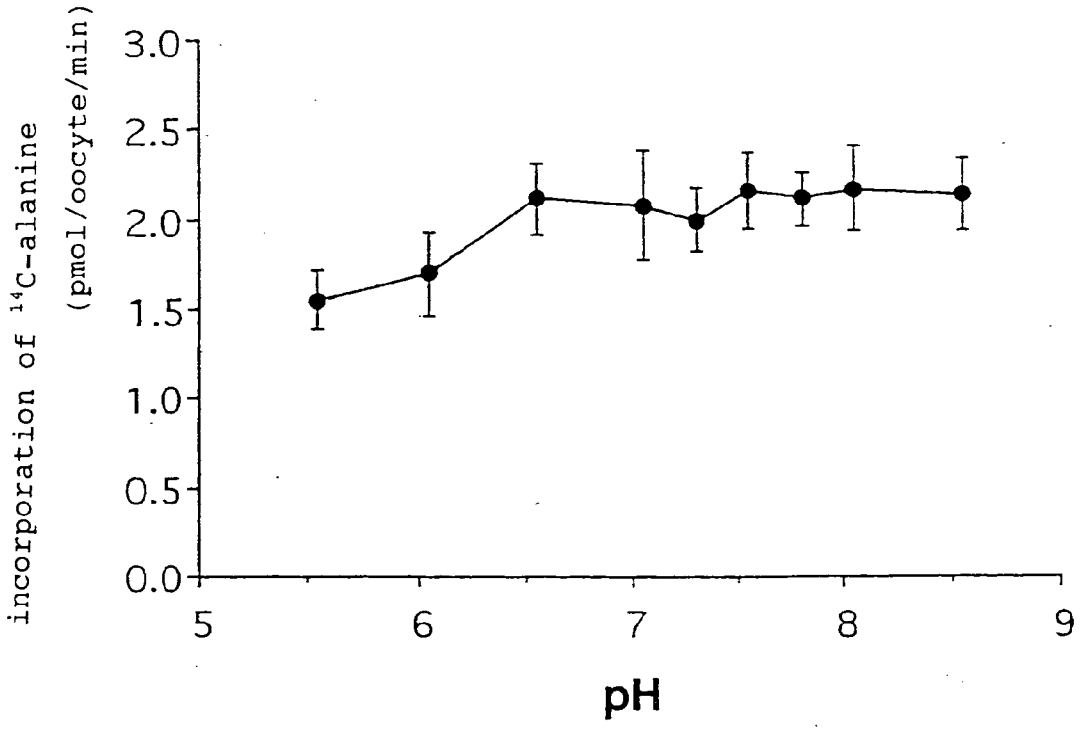


Figure 15

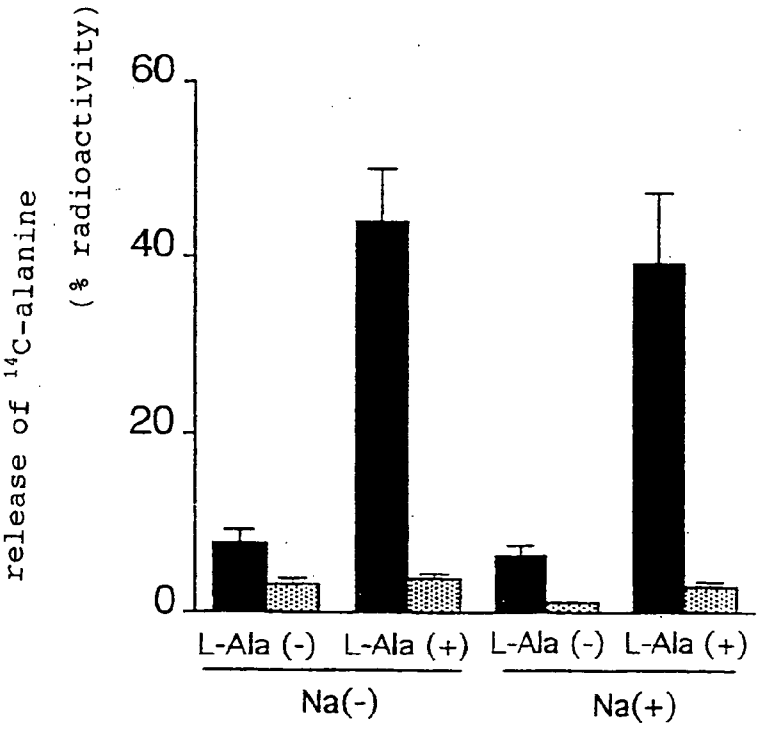


Figure 16

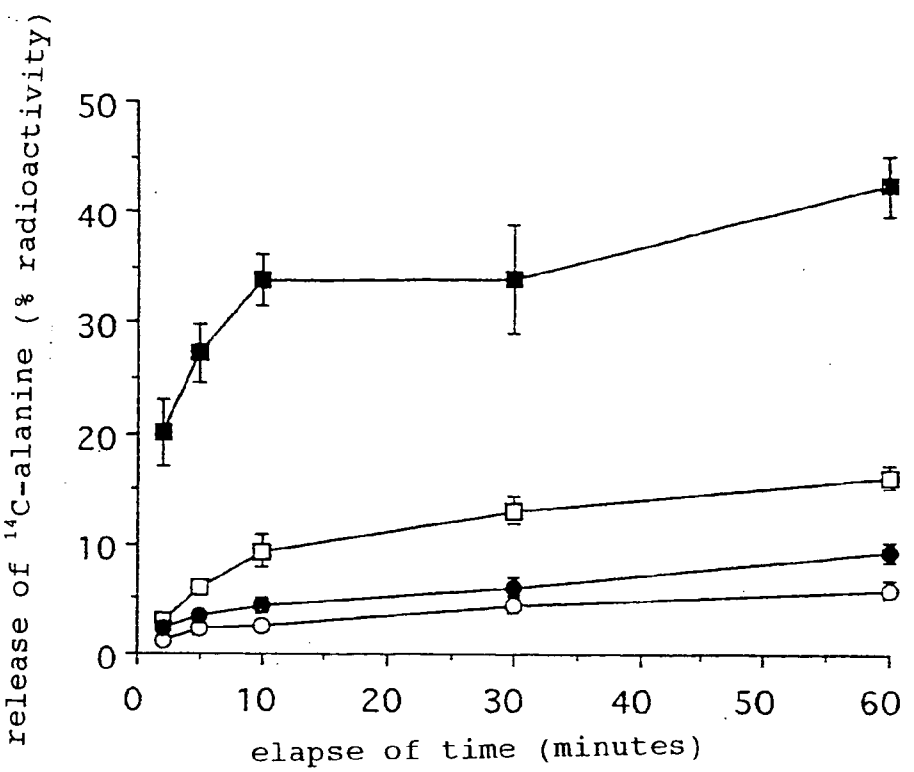


Figure 17

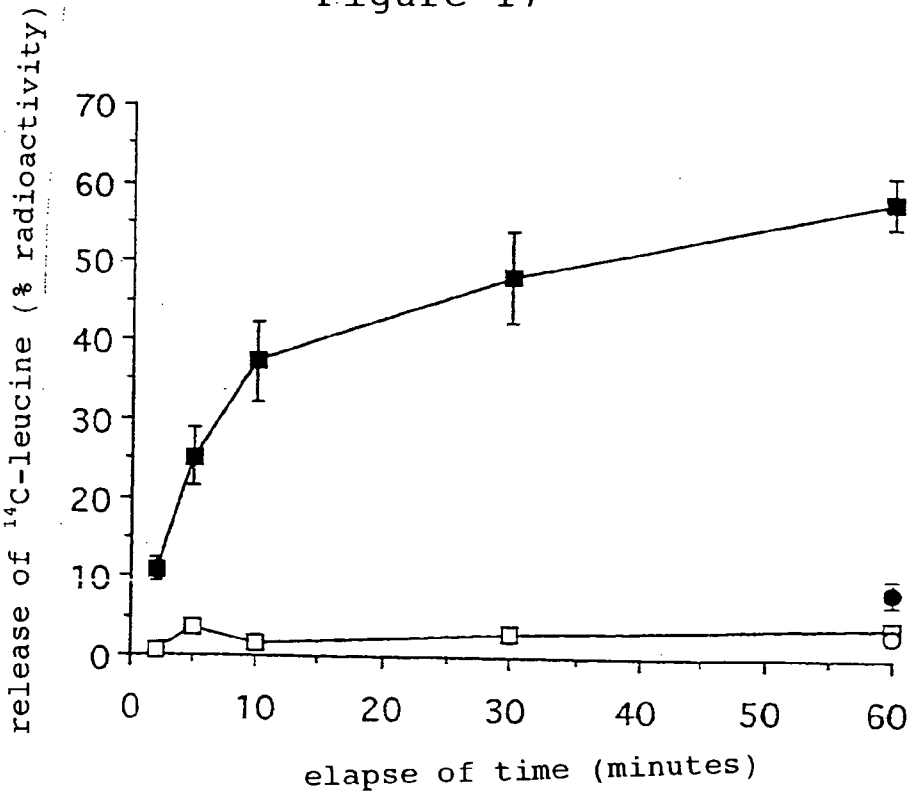


Figure 18

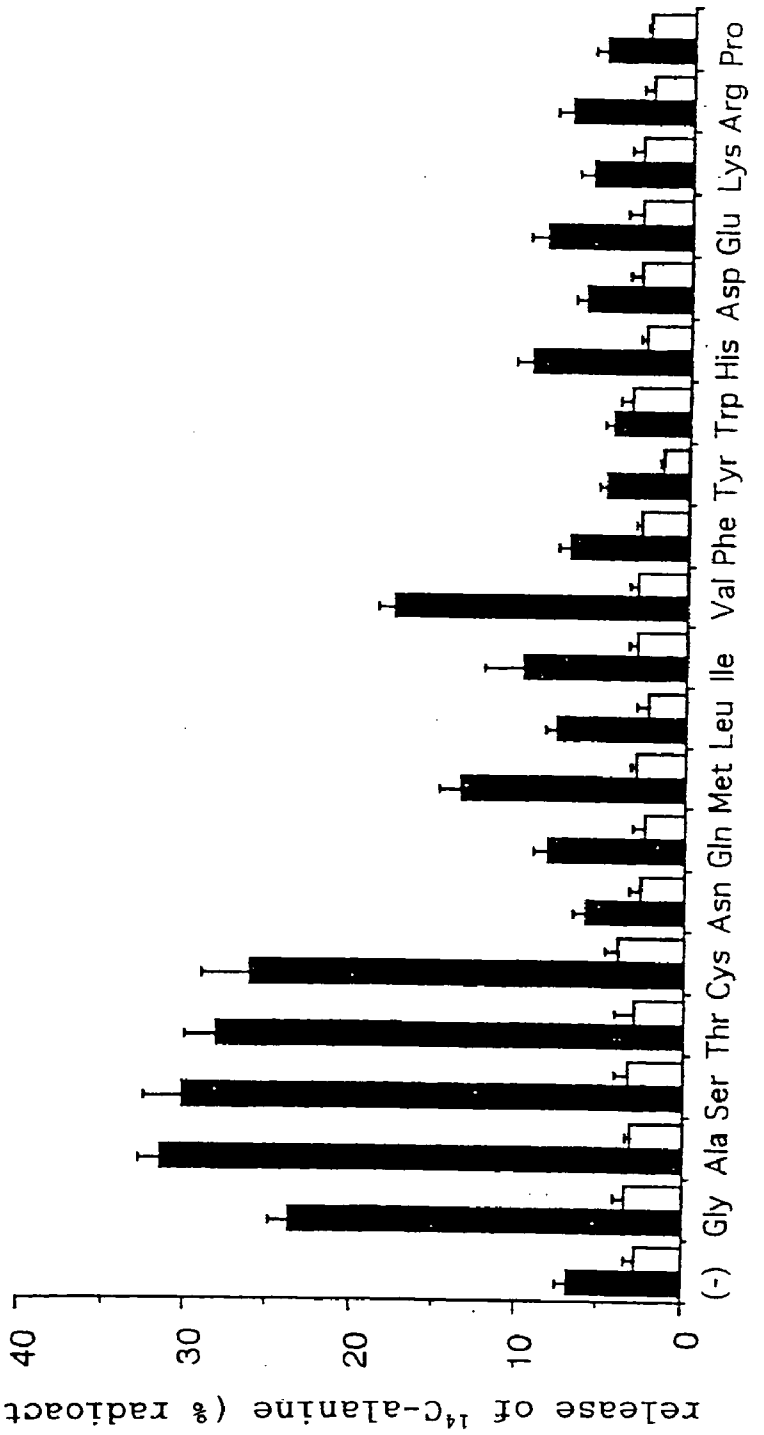

release of ^{14}C-alanine (% radioactivity)
0
10
20
30
40
(-) Gly Ala Ser Thr Cys Asn Gln Met Leu Ile Val Phe Tyr Trp His Asp Glu Lys Arg Pro

Figure 19

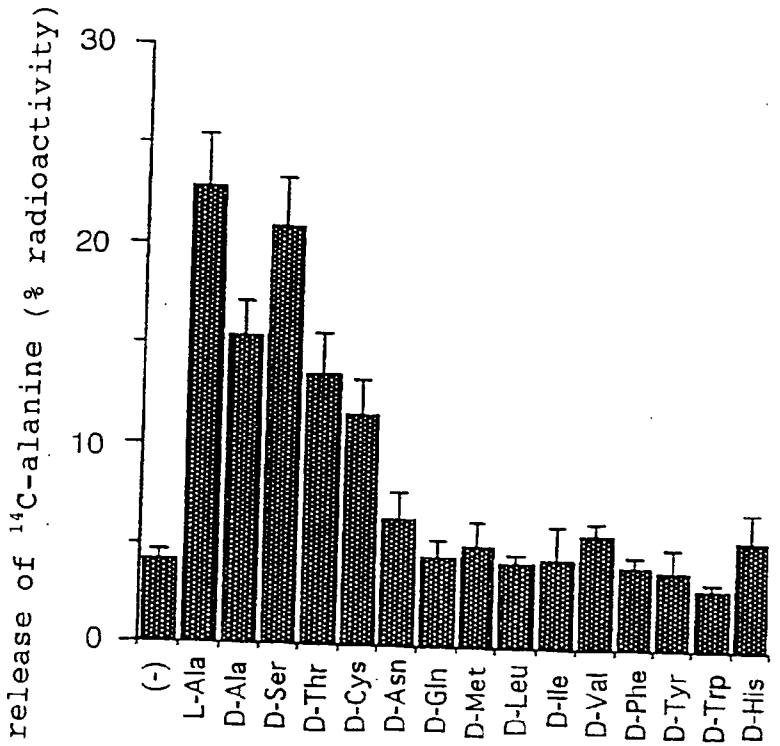


Figure 20

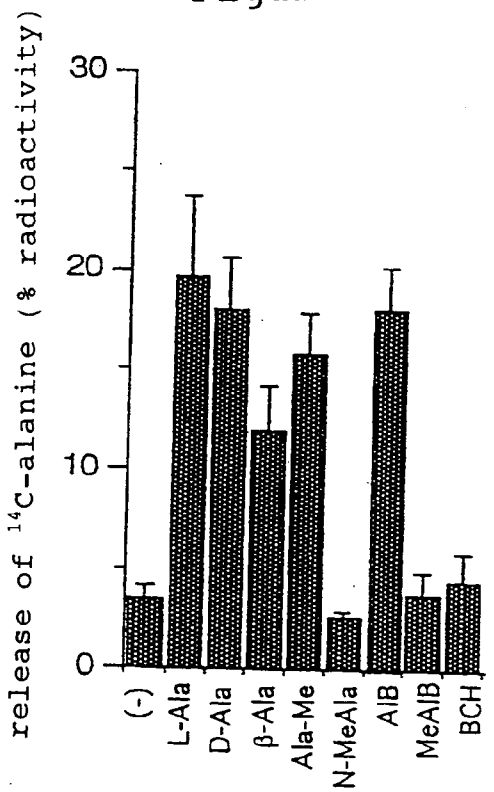
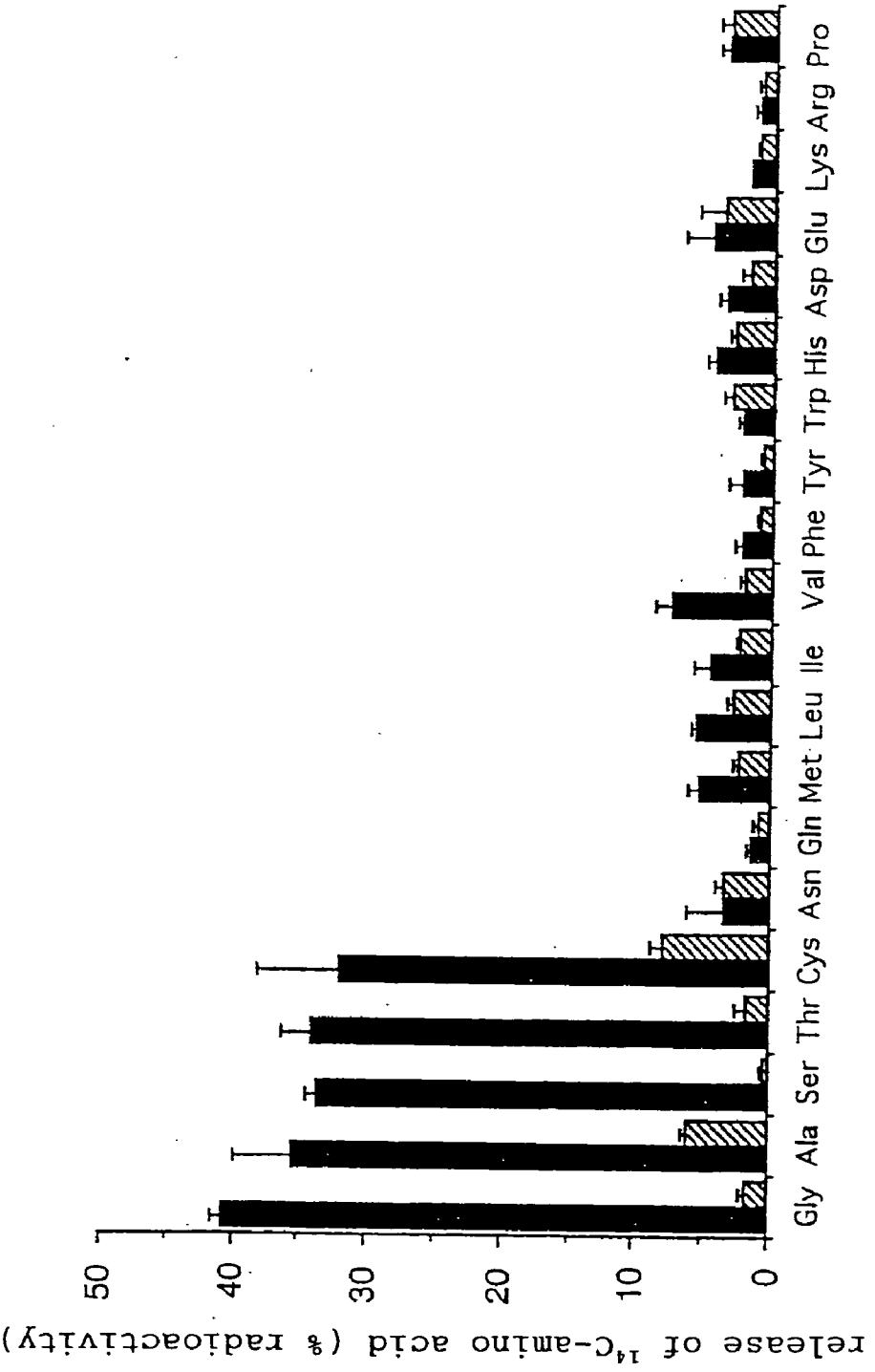


Figure 21


release of ^{14}C-amino acid (% radioactivity)
0
10
20
30
40
50
Gly Ala Ser Thr Cys Asn Gln Met Leu Ile Val Phe Tyr Trp His Asp Glu Lys Arg Pro

SODIUM-INDEPENDENT SMALL NEUTRAL AMINO ACID TRANSPORTER TRANSPORTING L- AND D- AMINO ACIDS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of co-pending U.S. application Ser. No. 10/214,867, filed Aug. 7, 2002, now allowed, which is a continuation of PCT Application No. PCT/JP01/00031, filed Jan. 9, 2001, which claims the benefit of Japanese Application No. 2000-028822 filed Feb. 7, 2000. The contents of all of these applications are hereby incorporated by reference in their entirety.

TECHNICAL FIELD

[0002] The present invention relates to a gene concerning a sodium-independent transport of a small neutral amino acid and analogs thereof, to protein encoding the genes and to an antibody against the protein. The present invention further relates to a method for screening the substance to be tested using the said protein.

BACKGROUND ART

[0003] Cells are required that amino acids are to be always incorporated thereinto as nutrition and such a function is carried by an amino acid transporter which is a membrane protein existing in cell membrane. The amino acid transporter is aligned in a specific site in each tissue in multicellular living things and plays an important role in expression of specific functions in each tissue.

[0004] A transport system asc is an amino acid transport system which transports small neutral amino acids mostly alanine, serine and cysteine and, originally, it was found in erythrocyte membrane and mentioned in many literatures. After that, its existence was confirmed in cultured cells as well (Christensen, *Physiol. Rev.*, volume 70, page 43, 1990). The transport system asc is a transporter which is sodium-independent or, in other words, it does not need sodium ion for its function. Its transport substrate selectivity and transport characteristic have been known to have some differences depending upon cells and animal species.

[0005] The transport system asc shows a high affinity to a transport substrate such as alanine, serine or cysteine and, as a transport system similar thereto, there is a transport system C where small neutral amino acid such as alanine, serine or cysteine is a transport substrate as well but affinity to a transport substrate is low (Young et al., *Biochem. J.*, volume 154, page 43, 1976; Young et al., *Biochem. J.*, volume 162, page 33, 1977). The transport system C is believed to be a subsystem for the transport system asc. Sheep where the transport system C is genetically deficient was found, reduction in glutathione content in its erythrocytes was shown and the importance of incorporation of cysteine mediated by cell membrane in the production of glutathione was proved (Young, et al., *Nature*, volume 254, page 156, 1975).

[0006] However, in the conventional methods, it is difficult to analyze the details of transport of amino acid and analogs thereof mediated by the amino acid transport system asc and the functional role in vivo and there has been a demand to isolate gene of neutral amino acid transporter carrying a function of the amino acid transport system asc so as to make the detailed function analysis possible.

[0007] As to a small neutral amino acid transporter, there were cloned ASCT1 and ASCT2 (Kanai, *Curr. Opin. Cell Biol.*, volume 9, page 565, 1997). However, they are sodium-dependent transporters and are entirely different from a sodium-independent amino acid transport system asc. In the meanwhile, glycine transporter and proline transporter were cloned (Amara and Kuhar, *Annu. Rev. Neurosci.*, volume 16, page 73, 1993) but both of them transport only glycine and proline in a sodium-dependent manner and are different from the transport system asc.

[0008] cDNA of rBAT and 4F2hc which are type II membrane glycoproteins having only one transmembrane structure which is considered to be an activating factor of an amino acid transporter were cloned although they are not transporters per se and it was known that, when they were expressed in oocytes of Xenopus, incorporation of basic amino acid together with neutral amino acid was activated (Palacin, *J. Exp. Biol.*, volume 196, page 123, 1994).

[0009] With regard to a transporter which selectively transports neutral amino acid, there were cloned neutral amino acid transporter LAT1 (Kanai et al., *J. Biol. Chem.*, volume 273, pages 23629-23632, 1998) and LAT 2 (Segawa et al., *J. Biol. Chem.*, volume 274, pages 19745-19751, 1999) corresponding to a transport system L. It was further shown that LAT1 and LAT2 functioned only when they coexist together with a cofactor 4F2hc. Both are not dependent on Na^+ but LAT1 shows an exchange transport activity for transporting the large neutral amino acids such as leucine, isoleucine, valine, phenylalanine, tyrosine, tryptophane, methionine and histidine and LAT2 has a wide substrate selectivity transporting the small neutral amino acids such as glycine, alanine, serine, cysteine and threonine in addition to large neutral amino acids. However, even they are different from the amino acid transport system asc in terms of substrate selectivity.

[0010] With regard to proteins analogous to the neutral amino acid transporters LAT1 and LAT2, there were cloned the above-mentioned y^+LAT1 and y^+LAT2 having a function of a transport system y^+L transporting the neutral amino acids and basic amino acids (Torrents et al., *J. Biol. Chem.*, volume 273, pages 32437-32445, 1998). It was also shown that both y^+LAT1 and y^+LAT2 functioned only when coexisted together with the cofactor 4F2hc. y^+LAT1 and y^+LAT2 mainly transport glutamine, leucine and isoleucine as neutral amino acids and are different from the amino acid transport system asc in terms of the substrate selectivity.

[0011] With regard to a transporter demanding the cofactor 4F2hc for the expression of the function, there was cloned xCT which is a protein analogous to the neutral amino acid transporters LAT1 and LAT2 (Sato et al., *J. Biol. Chem.*, 274: 11455-11458, 1999). The xCT transports cystine and glutamic acid and is different from the amino acid transport system asc in terms of the substrate selectivity.

[0012] With regard to a transporter demanding other cofactor rBAT having a structure analogous to 4F2hc for expressing the function, there was cloned BAT1 which is a protein analogous to the neutral amino acid transporters LAT1 and LAT2 (Chairoungdua et al., *J. Biol. Chem.*, 274: 28845-28848, 1999). BAT1 transports cystine, neutral amino acids and basic amino acids and is different from the amino acid transport system asc in terms of substrate selectivity.

[0013] As such, molecular substances of the transporters which function upon linking to 4F2hc and rBAT were made clear and it was clarified that there was a group of transporters which achieve a transporting function when a molecular complex is formed with the type II glycoprotein.

DISCLOSURE OF THE INVENTION

[0014] An object of the present invention is to provide a gene of a transporter which transports small neutral amino acid in a sodium-independent manner and shows a function of transport system asc and to provide a sodium-independent small neutral amino acid transporter which is a polypeptide encoded by the gene. Other objects will be apparent from the following description.

[0015] The present inventors have checked the EST (expressed sequence tag) database using the base sequence of translated region of cDNA of LAT1 and identified a base sequence analogous to LAT1. They have prepared a probe corresponding to that, screened a cDNA library and cloned a gene coding for a novel protein. They have further expressed this genetic product in oocytes of Xenopus and made clear that 4F2hc is essential when the genetic product achieves the function and that, although the expressed function corresponds to the neutral amino acid transport system asc, it makes not only L-amino acids but also D-amino acids into high-affinity transport substrates unlike the conventionally mentioned property of the transport system asc whereupon the present invention has been achieved.

[0016] Thus, the present invention relates to a protein comprising an amino acid sequence represented by SEQ ID NO: 1 or 4 or an amino acid sequence where one or more amino acid(s) therein is/are deleted, substituted or added and being capable of transporting the small neutral amino acid and analogs thereof in a Na^+-independent manner. The protein of the present invention is a protein having an ability of transporting the small neutral amino acids and analogs thereof in a sodium-independent manner when co-existed together with a protein having an amino acid sequence represented by SEQ ID NO: [[3]] 7 or [[6]] 8 or a protein comprising an amino acid sequence where one or more amino acid(s) is/are deleted, substituted or added.

[0017] The present invention further relates to a gene coding for the above-mentioned protein of the present invention. To be more specific, the present invention relates to a gene coding for a protein having an ability of transporting the small neutral amino acid and analogs thereof in a sodium-independent manner comprising a base sequence represented by SEQ ID NO: 2 or 5 or a base sequence being able to hybridize with DNA comprising the said base sequence under a stringent condition.

[0018] The novel protein of the present invention being capable of transporting small neutral amino acids and analogs thereof in a sodium-independent manner or an asc-type amino acid transporter 1 has an ability of transporting (incorporating) the small neutral amino acid such as glycine, L-alanine, L-serine, L-cysteine and L-threonine in a highly affinitive manner when co-existed together with an amino acid transport activating factor 4F2hc. It further transports L-valine, L-methionine, L-isoleucine, L-leucine, L-histidine and L-phenylalanine in a lowly affinitive manner. The asc-1 further transports D-alanine, D-serine, D-cysteine and D-threonine and particularly D-serine in a highly affinitive manner. In addition, the asc-1 transports the substances analogous to amino acid such as a-aminoisobutyric acid, β-alanine and alanine methyl ester.

[0019] Further, the sodium-independent small neutral amino acid transporter asc-1 of the present invention which transports L- and D-amino acids is mostly expressed in brain, lung, small intestine and placenta in vivo. Particularly, asc-1 transports D-serine which is believed to be an endogenous function-modifying substance for an NMDA-type glutamic acid receptor and, therefore, there is a possibility that it participates in the kinetics of D-serine in brain and affects the functioning state of the NMDA receptor. Furthermore, since asc-1 transports cysteine, it is believed to be a factor regulating the production amount of glutathione produced from cysteine as a material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] FIG. **1** is a drawing which shows a comparison of amino acid sequence of mouse asc-1 (SEQ ID NO: 1) with those of rat LAT2 (SEQ ID NO: 9), rat LAT1 (SEQ ID NO: 10), human y^+LAT1(SEQ ID NO: 11), human y^+LAT2 (SEQ ID NO: 12) and mouse xCT (SEQ ID NO: 13). The presumed transmembrane sites are shown by the lines added.

[0021] FIG. **2** is a drawing which shows a comparison of amino acid sequences of mouse asc-1 (SEQ ID NO: 1) and human asc-1 (SEQ ID NO: 4).

[0022] FIG. **3** is a picture which is a substitute for a drawing which shows the result of analysis of expression of asc-1 gene mRNA in various organ tissues of mouse by a northern blotting.

[0023] FIG. **4** is a picture which is a substitute for a drawing which shows the result of by a western blotting analysis using mouse brain membrane sample conducted under a non-reductive condition (−) and a reductive condition (+) using anti-asc-1 antibody (left) and anti-4F2hc antibody (right).

[0024] FIG. **5** is a drawing which shows the result of an experiment for incorporation of alanine by oocytes into which cRNA of mouse asc-1 gene and/or mouse 4F2hc gene are/is injected.

[0025] FIG. **6** is a drawing which shows the result of checking the influence of salt added in an experiment of incorporation of alanine by oocytes into which cRNA of asc-1 gene of mouse and cRNA of 4F2hc gene of mouse are injected.

[0026] FIG. **7** is a drawing which shows the result of checking the influence of concentration of alanine substrate in an experiment of incorporation of alanine by oocytes into which cRNA of asc-1 gene of mouse and cRNA of 4F2hc gene of mouse are injected.

[0027] FIG. **8** is a drawing which shows the result of checking the influence of addition of various L-amino acids or analogous compounds thereof to the system in an experiment of incorporation of alanine by oocytes into which cRNA of asc-1 gene of mouse and cRNA of 4F2hc gene of mouse are injected.

[0028] FIG. **9** is a drawing which shows the result of checking the influence of addition of various D-amino acids

to the system in an experiment of incorporation of alanine by oocytes into which cRNA of asc-1 gene of mouse and cRNA of 4F2hc gene of mouse are injected.

[0029] FIG. **10** is a drawing which shows the result of checking the influence of addition of alanine or analogous compounds thereof to the system in an experiment of incorporation of alanine by oocytes into which cRNA of asc-1 gene of mouse and cRNA of 4F2hc gene of mouse are injected.

[0030] FIG. **11** is a drawing which shows the result of checking the incorporation of radiolabeled L-amino acid by oocytes into which cRNA of asc-1 gene of mouse and cRNA of 4F2hc gene of mouse are injected.

[0031] FIG. **12** is a drawing which shows the result of checking the incorporation of radiolabeled D-amino acid by oocytes into which cRNA of asc-1 gene of mouse and cRNA of 4F2hc gene of mouse are injected.

[0032] FIG. **13** is a drawing which shows the result of checking the incorporation of radiolabeled L-alanine or analogous compounds thereof by oocytes into which cRNA of asc-1 gene of mouse and cRNA of 4F2hc gene of mouse are injected.

[0033] FIG. **14** is a drawing which shows the result of checking the influence of pH in an experiment of incorporation of alanine by oocytes into which cRNA of asc-1 gene of mouse and cRNA of 4F2hc gene of mouse are injected.

[0034] FIG. **15** is a drawing which shows the result of checking the release of ^{14}C-alanine by oocytes into which cRNA of asc-1 gene of mouse and cRNA of 4F2hc gene of mouse are injected. The ordinate shows the rate (%) of the released radioactivity to the radioactivity injected into the oocytes.

[0035] FIG. **16** is a drawing which shows the result of checking the progress of release of ^{14}C-alanine with a lapse of time by oocytes into which cRNA of asc-1 gene of mouse and cRNA of 4F2hc gene of mouse are injected. In the drawing, ○ is the case where, in the release of ^{14}C-alanine in the oocytes into which water is injected as a control instead of cRNA of asc-1 gene of mouse and cRNA of 4F2hc gene of mouse, a Na^+-free uptake solution to which no alanine is added is used; ● is the case where, in the release of ^{14}C-alanine in the oocytes into which water is injected as a control instead of cRNA of asc-1 gene of mouse and cRNA of 4F2hc gene of mouse, a Na^+-free uptake solution to which alanine is added is used; □ is the case where, in the release of ^{14}C-alanine in the oocytes into which cRNA of asc-1 gene of mouse and cRNA of 4F2hc gene of mouse are injected, a Na^+-free uptake solution to which no alanine is added is used; and □ is the case where, in the release of ^{14}C-alanine in the oocytes into which cRNA of asc-1 gene of mouse and cRNA of 4F2hc gene of mouse are injected, a Na^+-free uptake solution to which alanine is added is used. The ordinate shows the rate (%) of the released radioactivity to the radioactivity injected into the oocytes.

[0036] FIG. **17** is a drawing which shows the result of checking the progress of release of ^{14}C-leucine with a lapse of time by oocytes into which cRNA of LAT1 gene of rat and cRNA of 4F2hc gene of rat are injected. In the drawing, 0 is the case where, in the release of ^{14}C-leucine in the oocytes into which water is injected as a control instead of cRNA of LAT1 gene of rat and cRNA of 4F2hc gene of rat, a Na^+-free uptake solution to which no leucine is added is used; ● is the case where, in the release of ^{14}C-leucine in the oocytes into which water is injected as a control instead of cRNA of LAT1 gene of rat and cRNA of 4F2hc gene of rat, a Na^+-free uptake solution to which leucine is added is used; □ is the case where, in the release of ^{14}C-leucine in the oocytes into which cRNA of LAT1 gene of rat and cRNA of 4F2hc gene of rat are injected, a Na^+-free uptake solution to which no leucine is added is used; and ■ is the case where, in the release of ^{14}C-leucine in the oocytes into which cRNA of LAT1 gene of rat and cRNA of 4F2hc gene of rat are injected, a Na^+-free uptake solution to which leucine is added is used. The ordinate shows the rate (%) of the released radioactivity to the radioactivity injected into the oocytes.

[0037] FIG. **18** is a drawing which shows the result of checking the release of ^{14}C-alanine when various L-amino acids are added to a Na^+-free uptake solution containing no sodium ion by oocytes into which cRNA of asc-1 gene of mouse and cRNA of 4F2hc gene of mouse are injected (black bars) or by oocytes into which water is injected instead of cRNA as a control (white bars). (−) shows the release of ^{14}C-alanine mediated by asc-1 of mouse when no amino acid is added to a Na^+-free uptake solution. The ordinate shows the rate (%) of the released radioactivity to the radioactivity injected into the oocytes.

[0038] FIG. **19** is a drawing which shows the result of checking the release of ^{14}C-alanine mediated by mouse asc-1 when various kinds of D-amino acids are added to a Na^+-free uptake solution. (−) shows the release of ^{14}C-alanine mediated by asc-1 of mouse when no amino acid is added to a Na^+-free uptake solution. The ordinate shows the rate (%) of the released radioactivity to the radioactivity injected into the oocytes.

[0039] FIG. **20** is a drawing which shows the result of checking the release of ^{14}C-alanine mediated by asc-1 of mouse when various kinds of alanine-analogous compounds are added to a Na^+-free uptake solution. (−) shows the release of ^{14}C-alanine mediated by asc-1 of mouse when no amino acid is added to a Na^+-free uptake solution. The ordinate shows the rate (%) of the released radioactivity to the radioactivity injected into the oocytes.

[0040] FIG. **21** is a drawing which shows the result of checking the release of the injected ^{14}C-amino acid from oocytes into which cRNA of asc-1 gene of mouse and cRNA of 4F2hc gene of mouse are injected. Black bars show the case where alanine is added to a Na^+-free uptake solution while bars with oblique lines show the case where alanine is not added to a Na^+-free uptake solution.

BEST MODE FOR CARRYING OUT THE INVENTION

[0041] SEQ ID NO: 2 and NO: 1 in the Sequence Listing which will be mentioned later stand for a full-length cDNA base sequence (about 1.6 kbp) of gene of sodium-independent small neutral amino acid transporter (asc-1 of mouse) transporting L- and D-amino acid derived from brain of mouse and an amino acid sequence (530 amino acids) of protein encoded in its translated region.

[0042] SEQ ID NO: 5 and NO: 4 in the Sequence Listing which will be mentioned later stand for a full-length cDNA

base sequence (about 1.9 kbp) of gene of sodium-independent small neutral amino acid transporter (human asc-1) transporting L- and D-amino acid derived from human brain and an amino acid sequence (523 amino acids) of protein encoded in its translated region.

[0043] When the base sequence or the amino acid sequence represented by the above SEQ ID NO: 1 or 2 or SEQ ID NO: 4 or 5 was subjected to a homology check for all sequences contained in the known DNA databases (GenBank™ and EMBL) and protein databases (NBRF and SWISS-PROT), there was nothing which was identical whereby all those sequences are believed to be novel.

[0044] With regard to the protein of the present invention, that which has an amino acid sequence represented by SEQ ID NO: 1 or 4 and, in addition, protein having an amino acid sequence where one or more amino acid(s) is/are deficient, substituted or added in the amino acid sequence represented by SEQ ID NO: 1 or 4 for example may be listed. Defect, substitution or addition of amino acid may be within such an extent that the neutral amino acid transport activity is not lost and that is usually from 1 to about 106 or, preferably, from 1 to about 53. Like the amino acid sequence represented by SEQ ID NO: 1 or 4, such a protein has usually 80% or, preferably, 90% homology of amino acid.

[0045] With regard to the gene of the present invention, that which contains DNA being hybridizable under a stringent condition with DNA comprising a base sequence represented by SEQ ID NO: 2 or 5 may be exemplified in addition to that having a base sequence represented by SEQ ID NO: 2 or 5. The DNA which is hybridizable as such may be in such an extent that the protein encoded by the DNA is capable of transporting the neutral amino acid. Such a DNA has usually not less than 70% or, preferably, not less than 80% of homology of a base sequence to the base sequence represented by SEQ ID NO: 2 or 5. Such a DNA includes mutant gene found in nature, artificially modified mutant and homologous gene derived from living things of difference species.

[0046] Hybridization under a stringent condition according to the present invention is usually carried out in such a manner that hybridization is carried out in a hybridization solution of 5×SSC or the equivalent salt concentration at the temperature condition of 37-42° C. for about 12 hours, a preliminary washing is carried out upon necessity by a solution of 5×SSC or the equivalent salt concentration and a washing is carried out in a solution of 1×SSC or the equivalent salt concentration.

[0047] The sodium-independent small neutral amino acid transporter gene of the present invention which transports L- and D-amino acids can be isolated and obtained by carrying out a screening using appropriate mammalian tissues or cells as a gene source. With regard to mammals, human being may be listed in addition to non-human animals such as dog, cow, horse, goat, sheep, monkey, pig, rabbit, rat and mouse.

[0048] Screening and isolation of gene may be appropriately carried out by a homology cloning, etc.

[0049] For example, brain of mouse or human being is used as a gene source and mRNA (poly(A)$^+$ RNA) is prepared therefrom. A cDNA library is constructed therefrom and a screening is carried out for the cDNA library using a probe corresponding to LAT1-homologous sequence (such as GenBank™/EBI/DDBJ accession No. N32639) obtained by checking an EST (expressed sequence tag) database whereupon clone containing cDNA of asc-1 gene is obtained.

[0050] With regard to the obtained cDNA, its base sequence is determined by a conventional method, the translated region is analyzed and an amino acid sequence of the protein encoded thereby, i.e. asc-1, can be determined.

[0051] The fact that the resulting cDNA is a cDNA of a sodium-independent small neutral amino acid transporter gene which transports L- and D-amino acids or, in other words, it is a genetic product encoded by cDNA is a sodium-independent small neutral amino acid transporter which transports L- and D-amino acids is, for example, able to be tested as follows. Thus, RNA (cRNA) (a capped one) complementary thereto prepared from the resulting cDNA of asc-1 gene is expressed by introducing into oocytes together with cRNA having a base sequence of 4F2hc represented by SEQ ID NO: 3 or 6 and an ability of transporting (incorporating) a neutral amino acid into cells can be confirmed by measuring the incorporation of a substrate into the cells by means of a conventional incorporation test (Kanai and Hediger, *Nature*, volume 360, pages 467-471, 1992) where an appropriate neutral amino acid is a substrate.

[0052] An asc-1 protein is synthesized by means of an in vitro translation method (Hediger, et al., *Biochim. Biophys. Acta*, volume 1064, page 360, 1991) using RNA (cRNA) which is complementary thereto prepared from the resulting cDNA of asc-1 whereupon it is possible to check the size of protein, addition or non-addition of sugar, etc. by means of electrophoresis.

[0053] Since the cDNA of gene of 4F2hc was reported already (Broer, et al., *Biochem. J., volume* 312, page 863, 1995), it is possible to prepare a gene of 4F2hc easily by means of PCR or the like. cRNA (a capped one) can be synthesized from the resulting cDNA of 4F2hc.

[0054] The same incorporation experiment is applied to expression cells whereupon it is possible to check the characteristics of the asc-1 such as a characteristic that asc-1 conducts transport of an exchange type of amino acid as well as substrate selectivity of asc-1 and pH-dependency.

[0055] When an appropriate genomic DNA library or cDNA library prepared from different gene source is screened using the resulting cDNA of asc-1 gene, it is possible to isolate homologous gene or chromosomal gene derived from different living things.

[0056] When a synthetic primer designed on the basis of information of the disclosed base sequence of gene of the present invention (a base sequence represented by SEQ ID NO: 2 or 5 or a part thereof) is used and a conventional PCR (polymerase chain reaction) is carried out, it is possible to isolate a gene from a cDNA library or a genomic DNA library.

[0057] DNA libraries such as a cDNA library and a genomic DNA library can be prepared by a method mentioned in, for example, "Molecular Cloning" (by Sambrook, J., Fritsh, E. F and Manitis, T.; published by Cold Spring Harbor Press). Alternatively, when a commercially available library is available, that may be used.

[0058] The sodium-independent small neutral amino acid transporter (asc-1) transporting D- and L-amino acids according to the present invention may be produced by, for example, a gene recombination technique using the cDNA coding therefor. For example, DNA (such as cDNA) coding for asc-1 is incorporated into an appropriate expression vector and the resulting recombinant DNA can be introduced into an appropriate host cell. Examples of the expression system (host-vector system) for the production of polypeptide are expression systems of bacteria, yeasts, insect cells and mammalian cells. Among them, the use of insect cells and mammalian cells is preferred for the preparation of functional protein.

[0059] For example, in the case of expression of polypeptide in mammalian cells, DNA coding for a sodium-independent small neutral amino acid transporter asc-1 which transports the L- and D-amino acids is inserted into the downstream of an appropriate promoter (such as cytomegalovirus promoter, SV 40 promoter, LTR promoter or elongation **1***a* promoter) in an appropriate expression vector (such as vector of adenovirus type, vector of retrovirus type, papilloma virus vector, vaccinia virus vector or vector of SV 40 type) whereupon an expression vector is constructed. After that, an appropriate animal cell is transformed by the resulting expression vector and the transformant is incubated in an appropriate medium whereupon an aimed polypeptide is produced. Examples of the mammalian cell used as a host are cell strains such as simian COS-7 cell, CHO cell of Chinese hamster and human HeLa cell.

[0060] With regard to the DNA coding for a sodium-independent small amino acid transporter asc-1 which transports the L- and D-amino acids, the cDNA having a base sequence represented by SEQ ID NO: 2 or 5 may be used for example and, in addition, it is not limited to the above-mentioned DNA sequence but DNA corresponding to amino acid may be designed and used as a DNA coding for the polypeptide. In that case, as to the codon for coding for one amino acid, from 1 to 6 kinds of codon(s) is/are known for each and, although the codon used may be selected freely, it is possible to design a sequence having higher expression efficiency by taking the frequency of use of codon of the host utilized for the expression into consideration. DNA having a designed base sequence can be prepared by chemical synthesis of DNA, by fragmentation of the above-mentioned cDNA and combination thereof, by a partial modification of a base sequence, etc. Artificial modification of a base sequence and introduction of variation can be carried out by means of a site-specific mutagenesis (Mars, D. F., et al., *Proceedings of National Academy of Sciences*, volume 81, page 5662, 1984), etc. utilizing a primer comprising a synthetic oligonucleotide coding for the desired modification.

[0061] The present invention further relates to nucleotide containing a partial sequence of continuous 14 or more bases, preferably 20 or more bases or, more preferably, 30 or more bases in the base sequence represented by SEQ ID NO: 2 or 5 of the Sequence Listing or a complementary sequence thereof. The nucleotide of the present invention may be used as a probe for the detection of gene coding for a protein being capable of transporting a sodium-independent small neutral amino acid and analogs thereof.

[0062] When the sodium-independent small neutral amino acid transporter of the present invention transporting the L- and D-amino acids or a polypeptide having the immunological homology thereto is used, an antibody against that can be prepared. The antibody can be utilized for the detection or the purification of the sodium-independent small neutral amino acid transporter which transports the L- and D-amino acids. The antibody can be manufactured using the sodium-independent small neutral amino acid transporter of the present invention transporting the L- and D-amino acids, a fragment thereof, a synthetic peptide having a partial sequence thereof or the like as an antigen. A polyclonal antibody can be manufactured by a conventional method where antigen is inoculated to a host animal (such as rat or rabbit) and the immunized serum is recovered therefrom while a monoclonal antibody can be manufactured by a conventional technique such as a hybridoma method.

[0063] The sodium-independent small neutral amino acid transporter asc-1 of the present invention transporting the L- and D-amino acids, gene thereof and expression cell thereof can be used in an in vitro test for permeation efficiency at the cell membrane where asc-1 is present or at the site where asc-1 is presumed to be present.

[0064] Further, the sodium-independent small neutral amino acid transporter asc-1 of the present invention transporting the L- and D-amino acids, gene thereof and expression cell thereof can be used in the development of compounds which efficiently permeate the cell membrane where asc-1 is present or at the site where asc-1 is presumed to be present. Furthermore, the sodium-independent small neutral amino acid transporter asc-1 of the present invention transporting the L- and D-amino acids, gene thereof and expression cell thereof can be used in an in vitro test for a drug interaction at the cell membrane where asc-1 is present or at the site where asc-1 is presumed to be present.

[0065] When the sodium-independent small neutral amino acid transporter asc-1 of the present invention which transports the L- and D-amino acids is inhibited, it is possible to limit the permeation of specific compounds at the cell membrane where asc-1 is expressed or at the site where asc-1 is presumed to be present. Further, the sodium-independent small neutral amino acid transporter asc-1 of the present invention which transports the L- and D-amino acids, gene thereof and expression cell thereof can be used in the development of drugs (such as specific inhibitor for asc-1) which limit the passing of the cell membrane or the permeation at the site where asc-1 is presumed to be present of a compound transported by asc-1.

[0066] Accordingly, the present invention provides a method for detection, identification or quantification of action as a substrate of a substance to be tested to the ability of the present invention for transporting a small neutral amino acid and analogs thereof in a sodium-independent manner using the protein of the present protein. In accordance with the method of the present invention, it is possible to screen a substance which promotes the function of the protein of the present invention or a substance which inhibits

that. When an uptake solution containing an amino acid which is labeled by radioactivity or by fluorescence such as ^{14}C-alanine is used and the amount of the said incorporated or released amino acid is measured in the presence of the substance to be tested, it is now possible to test the action of the said substance to be tested to the protein of the present invention.

[0067] The present invention further provides a method for controlling the resistance of cells to oxidative stress where the protein of the present invention, a specific antibody thereof, a substance for promoting the function thereof or a substance for inhibiting the function thereof is used to modulate an ability of transporting a small neutral amino acid of the said protein or analogs thereof.

[0068] The present invention furthermore provides a method for controlling the activity of a glutamic acid receptor of an NMDA type in a nervous system where the protein of the present invention, a specific antibody thereof, a substance for promoting the function thereof or a substance for inhibiting the function thereof is used to modulate an ability of transporting a small neutral amino acid of the said protein or analogs thereof; a method for controlling the plasticity of synaptic transmission in which a glutamic acid receptor of an NMDA type is participated by means of the above-mentioned method; and a method for controlling the neuronal death in which a glutamic acid receptor of an NMDA type is participated by means of the above-mentioned method.

[0069] The present invention provides a method for controlling such as inhibition or promotion of growth of cells where the protein of the present invention, a specific antibody thereof, a substance for promoting the function thereof or a substance for inhibiting the function thereof is used to modulate an ability of transporting a small neutral amino acid of the said protein or analogs thereof.

[0070] The present invention provides a method for changing the fate of a drug transported by the protein in vivo where the protein of the present invention, a specific antibody thereof, a substance for promoting the function thereof or a substance for inhibiting the function thereof is used to modulate an ability of transporting a neutral amino acid of the said protein or analogs thereof.

[0071] The present invention provides a method for changing the fate of a toxin or an exogenous substance transported by the protein in vivo where the protein of the present invention, a specific antibody thereof, a substance for promoting the function thereof or a substance for inhibiting the function thereof is used to modulate an ability of transporting a neutral amino acid of the said protein or analogs thereof.

[0072] The present invention will now be further illustrated by way of the Examples although the present invention is not limited thereto.

[0073] In the following Examples, each of the operation was carried out, unless otherwise clearly mentioned, by a method descried in "Molecular Cloning" (by Sambrook, J., Fritsh, E. F. and Manitis, T.; published by Cold Spring Harbor Press in 1989) or, when a commercially available reagent or kit is used, it was carried out according to the direction for use of the said commercially available product.

EXAMPLES

Example 1

Mouse and Human cDNA Cloning of Sodium-Independent Small Neutral Amino Acid Transporter which Transports L- and D-Amino Acids

[0074] (1) Isolation of cDNA of 4F2hc of Mouse and Human Being and Preparation of cRNA

[0075] A cDNA library was prepared from poly(A)$^+$ RNA purified from brain of mouse or from poly(A)$^+$ RNA derived from human placenta (purchased from Clontec) using a kit for the synthesis of cDNA (trade name: Superscript Choice System; manufactured by Gibco) and was integrated into a site cleaved by a restriction enzyme EcoRI of phage vector λZipLox (manufactured by Gibco). A segment which corresponds to the bases of from 135th to 580th bases of 4F2hc gene of rat (Broer, et al., *Biochem. J.*, volume 312, page 863, 1995) was amplified and labeled with ^{32}P-dCTP and the resulting one was used as a probe whereby a cDNA library of brain of mouse and a cDNA library of human placenta were screened. Hybridization was carried out for one night in a solution for hybridization and a filter membrane was washed at 37° C. with 0.1×SSC/0.1% SDS. As to the solution for hybridization, there was used a buffer of pH 6.5 containing 5×SSC, 3× Denhard's solution, 0.2% SDS, 10% dextran sulfate, 50% formamide, 0.01% Abtiform B (trade name; Sigma) (antifoaming agent), 0.2 mg/ml salmon sperm modified DNA, 2.5 mM sodium pyrophosphate and 25 mM-MES. A cDNA moiety of λZipLox phage into which cDNA was integrated was integrated into a plasmid pZL1. In cDNA of human 4F2hc, a cDNA moiety of λZipLox phage into which cDNA was integrated was recombined to a plasmid pZL1.

[0076] The resulting clone or the clone containing cDNA of 4F2hc of mouse and human being was subjected to a base sequence determination of cDNA by a diterminator cycle sequencing method (Applied Biosystems) using a synthetic primer for determination of base sequence. As a result, the cloned cDNA was confirmed to be that of gene of 4F2hc of mouse or human being. The base sequences of the resulting 4F2hc are represented by SEQ ID NO: 3 and 6 of the Sequence Listing which will be mentioned later.

[0077] From the plasmid containing cDNA of 4F2hc of mouse and human being prepared as above, cRNA (RNA complementary to cDNA) was prepared using a T7RNA polymerase.

[0078] (2) Isolation of cDNA of Mouse of Sodium-Independent Small Neutral Amino Acid Transporter Asc-1 which Transports L- and D-Amino Acids and Preparation of cRNA

[0079] A sense primer (5'-CTCTTCACATGCATCTCCAC-3') (SEQ ID NO: 14) corresponding to 35-54 bp of LAT1-analogous sequence GenBank™/EBI/DDBI accession No. N32639 obtained by retrieval of EST (expressed sequence tag) database using a base sequence of translated region of LAT1, an antisense primer (5'-GGTACACGACCACACACATC-3') (SEQ ID NO: 15) corresponding to 397-416 bp thereof and an IMAGE (Integrated and Molecular Analysis of Genomes and their Expression) cDNA clone No. 267666 were used as templates whereby DNA fragment

was amplified by PCR. The resulting DNA fragment was labeled with ^{32}P-dCTP and was used as a probe to screen a cDNA library of brain of mouse.

[0080] The cDNA library was prepared from poly(A)$^+$ RNA derived from brain of mouse using a kit for the synthesis of cDNA (trade name: Superscript Choice System; manufactured by Gibco) and was integrated into a site of phage vector λZipLox (Gibco) cleaved by a restriction enzyme EcoRI. Hybridization by a probe labeled with ^{32}P_dCTP was carried out for one night in a solution of hybridization at 37° C. and a filter membrane was washed at 37° C. with 0.1×SSC/0.1% SDS. As to the solution for hybridization, there was used a buffer of pH 6.5 containing 5×SSC, 3× Denhard's solution, 0.2% SDS, 10% dextran sulfate, 50% formamide, 0.01% Abtiform B (trade name; Sigma) (antifoaming agent), 0.2 mg/ml salmon sperm modified DNA, 2.5 mM sodium pyrophosphate and 25 mM-MES. A cDNA moiety of % ZipLox phage into which cDNA was integrated was integrated into a plasmid pZL1 and was further subcloned to a plasmid pBluescript II SK$^-$ (manufactured by Stratagene).

[0081] The resulting clone or the clone containing cDNA of asc-1 of mouse was subjected to a base sequence determination of cDNA by a diterminator cycle sequencing method (Applied Biosystems) using a synthetic primer for determination of base sequence.

[0082] As a result, a base sequence of asc-1 gene of mouse was obtained. Further, a base sequence of cDNA was analyzed by a conventional method to determine a translated region of cDNA and an amino acid sequence of asc-1 encoded there.

[0083] Those sequences are represented by SEQ ID NO: 1 (amino acid sequence) and 2 (base sequence) in the Sequence Listing which will be mentioned later.

[0084] The asc-1 had a homology of 45% in terms of amino acid sequence to a rat transporter LAT1 corresponding to a neutral amino acid transport system $^-$L and the homology of 65% to LAT2. Further, the asc-1 had a homology of 45% to a human transporter y$^+$LAT1 corresponding to a neutral and basic amino acid transport system y$^-$L and the homology of 45% to y+LAT2. Furthermore, the asc-1 had a homology of 45% to a transporter xCT of mouse corresponding to cystine and an acidic amino acid transport system x$^-$c and a homology of 44% in terms of amino acid sequence to a transporter BAT1 of rat corresponding to cystine and a neutral and basic amino acid transport system b$^{0,+}$.

[0085] Comparison of asc-1 with LAT2 of rat, LAT1 of rat, human y$^+$LAT1, human y$^+$LAT2 and xCT of mouse in terms of amino acid sequence is shown in FIG. **1**.

[0086] As a result of analysis of an amino acid sequence of asc-1 by an SOSUI algorithm (Hirokawa, T. et al., *Bioinformatics*, volume 14, page 378 (1998)), 12 membrane-spanning domains were expected as shown by the lines in FIG. **1**. There were also the sites which were believed to be tyrosine phosphorylated site in the second hydrophilic loop, protein kinase C-dependent phosphorylated site in N-terminal intracellular region, the eighth hydrophilic loop and C-terminal intracellular region and cAMP-dependent phosphorylated site in N-terminal intracellular region.

[0087] (3) Isolation of Human cDNA of Sodium-Independent Small Neutral Amino Acid Transporter Asc-1 which Transports L- and D-Amino Acids and Preparation of cRNA.

[0088] Fragment cleaved by NcoI of asc-1 cDNA of mouse (corresponding to 523-1366 bp of asc-1 cDNA of mouse) was labeled with ^{32}P-dCTP and this was used as a probe for screening a human brain cDNA library.

[0089] The cDNA library was prepared from poly(A)$^+$ RNA (purchased from Clontech) derived from human brain using a kit for the synthesis of cDNA (trade name: Superscript Choice System; manufactured by Gibco) and integrated into a site of phage vector λZipLox (manufactured by Gibco) cleaved by a restriction enzyme EcoRI. Hybridization by a probe labeled with ^{32}P-dCTP was carried out at 37° C. in a solution for hybridization for one night and a filter membrane was washed at 37° C. with 0.1×SSC/0.1% SDS. As to the solution for hybridization, there was used a buffer of pH 6.5 containing 5×SSC, 3× Denhard's solution, 0.2% SDS, 10% dextran sulfate, 50% formamide, 0.01% Abtiform B (trade name; Sigma) (antifoaming agent), 0.2 mg/ml salmon sperm modified DNA, 2.5 mM sodium pyrophosphate and 25 mM-MES. A cDNA moiety of λZipLox phage into which cDNA was integrated was integrated into a plasmid pZL1.

[0090] The resulting clone or the clone containing cDNA of human asc-1 was subjected to a base sequence determination of cDNA by a diterminator cycle sequencing method (Applied Biosystems) using a synthetic primer for determination of base sequence.

[0091] As a result, a base sequence of human asc-1 gene was obtained. Further, a base sequence of cDNA was analyzed by a conventional method to determine a translated region of cDNA and an amino acid sequence of asc-1 encoded there.

[0092] Those sequences are represented by SEQ ID NO: 4 (amino acid sequence) and 5 (base sequence) in the Sequence Listing which will be mentioned later.

[0093] Comparison of the expected amino acid sequence of human asc-1 and asc-1 of rat is shown in FIG. **2**.

[0094] (4) Expression of Asc-1 Gene in Various Tissues of Mouse (Analysis by a Northern Blotting)

[0095] cDNA fragment corresponding to 1-512 bases of asc-1 gene was excised by restriction enzymes EcoRI and XhoI and labeled with ^{32}P-dCTP and the resulting one is used as a probe for conducting a northern blotting as follows to RNA extracted from various tissues of mouse. Thus, 3 μg of poly(A)$^+$ RNA were subjected to electrophoresis with 1% agarose/formaldehyde gel and transferred to a nitrocellulose filter. This filter was subjected to hybridization for one night using a hybridization solution containing asc-1 cDNA fragment labeled with ^{32}P_dCTP. The filter was washed at 65° C. with 0.1×SSC containing 0.1% SDS.

[0096] Result of the northern blotting is shown in FIG. **3** by a picture which is a substitute for a drawing. As a result, a band was detected near 1.9 kb in brain, lung and placenta. In addition, a band was detected at about 4.4 kb in small intestine.

[0097] (5) Expression of asc-1 and 4F2hc Protein in Brain of Mouse

[0098] Specific antibody to a synthetic oligopeptide [PSPLPITDKPLKTQC] (SEQ ID NO: 16) corresponding to 517-530 amino acid residues of asc-1 of mouse and to a synthetic oligopeptide [CEGLLLQFPFVA] (SEQ ID NO: 17) (cysteine residue of C-terminal or N-terminal was introduced for a conjugation with KLH (keyhole limpet hemocyanine)) corresponding to 516-526 amino acid residue of 4F2hc of mouse was prepared according to a method of Altman, et al. (Altman, et al., *Proc. Natl. Acad. Sci. USA*, volume 81, pages 2176-2180, 1984).

[0099] Fraction of cerebral membrane of mouse was prepared according to a method of Thorens, et al. (Thorens, et al., *Cell*, volume 55, pages 281-290, 1988). A protein sample was treated at 100° C. for 5 minutes in the presence (under reducing condition) or absence (under non-reducing condition) of 5% 2-mercaptoethanol, subjected to electrophoresis by SDS-polyacrylamide gel, subjected to blotting to a Hybond-P PVDV transfer membrane and treated with an anti-asc-1 antiserum (1:10,000) or an anti-4F2hc antiserum (1:10,000).

[0100] The result is shown in a picture of FIG. **4** as a substitute for a drawing. Left side of FIG. **4** is for the anti-asc-1 antibody while right side thereof is for the anti-4F2hc antibody. They were carried out under non-reducing condition (−) and reducing condition (+), respectively.

[0101] As shown in FIG. **4**, in the anti-asc-1 antiserum, a band of 118 kDa observed under a non-reducing condition disappeared under a reducing condition and transferred to a band of 33 kDa. In the anti-4F2hc antiserum, a band of 118 kDa observed under a non-reducing condition disappeared under a reducing condition and a band of 85 kDa appeared. Those results suggest that asc-1 and 4F2hc were connected by a disulfide bond to form a heterodimer.

Example 2

Characterization of Sodium-Independent Small Neutral Amino Acid Transporter Asc-1 which Transports L- and D-Amino Acids

[0102] (1) Role of 4F2hc in Transport Activity of asc-1

[0103] Incorporation of alanine when asc-1 gene cRNA of mouse was solely expressed in oocytes of Xenopus and when asc-1 gene cRNA of mouse and 4F2hc gene cRNA of mouse were expressed in oocytes of Xenopus together was compared.

[0104] Into oocytes were injected 12 ng of asc-1 gene cRNA of mouse, 13 ng of 4F2hc gene cRNA of mouse or 12 ng asc-1 gene cRNA of mouse/13 ng of 4F2hc gene cRNA of mouse to express followed by incubating for 3 days. With regard to the oocytes in which asc-1 gene cRNA, 4F2hc gene cRNA or asc-1 gene cRNA/4F2hc gene cRNA was injected, there was carried out an experiment for incorporation of the substrate according to a method by Kanai, et al. (Kanai and Hediger, *Nature, volume* 360, pages 467-471, 1992) using alanine as a substrate as follows. Thus, the oocytes were allowed to stand for 30 minutes in a Na^+-free uptake solution [100 mM choline chloride, 2 mM potassium chloride, 1.8 mM calcium chloride, 1 mM magnesium chloride, 5 mM HEPES; pH 7.4] containing ^{14}C-alanine (100 μM) as a substrate and the rate of the substrate incorporated into the cells was measured by way of counting the radioactivity incorporated thereinto.

[0105] The result is shown in FIG. **5**. In the oocytes where only asc-1 was expressed, incorporation of alanine was in the same level as in the case of the oocytes into which water was injected as a control while, in the oocytes where both asc-1 and 4F2hc were expressed together, a big incorporation of alanine was shown whereby it is believed that 4F2hc is necessary for asc-1 to achieve its function.

[0106] (2) Dependency of Transport Activity Asc-1 on Salt

[0107] In an experiment of incorporation of alanine by oocytes into which both asc-1 gene cRNA and 4F2hc gene cRNA of mouse were injected together, influence of the salt added to the medium was investigated.

[0108] The experiment of incorporation of alanine was carried out in accordance with the method mentioned in the above Example 2(1) using oocytes into which both asc-1 gene cRNA and 4F2hc gene cRNA of mouse were injected together. When the influence of sodium ion was checked however, a standard uptake solution (where 100 mM choline chloride was substituted with 100 mM sodium chloride) was used in place of the Na^+-free uptake solution as the uptake solution. When the influence of chlorine ion was checked, a gluconic acid uptake solution (where 100 mM sodium chloride was substituted with 100 mM sodium gluconate) was used in place of the standard uptake solution.

[0109] The result is shown in FIG. **6**. Even when choline outside the cells was changed to sodium or even when chlorine ion outside the cells was changed to gluconic acid ion, that does no affect the incorporation of alanine at all. From those, it is shown that asc-1 is a transporter which acts independently of sodium ion and chlorine ion.

[0110] (3) A Michaelis-Menten Kinetic Test for Asc-1

[0111] A Michaelis-Menten kinetic test was carried out for a sodium-independent small neutral amino acid transporter asc-1 which transports L- and D-amino acids. By checking the changes in the rate of incorporation of alanine due to the difference in concentration of the alanine substrate, the Michaelis-Menten kinetic test of asc-1 was carried out.

[0112] An experiment for the incorporation of alanine was carried out according to the method mentioned in the above Example 2(1) using the oocytes into which both asc-1 gene cRNA of mouse and 4F2hc gene cRNA of mouse were injected. The result is shown in FIG. **7**. The result was that the Km value was 23.0±5.1 μM (mean value±standard error; n=4).

[0113] With regard to amino acids other than alanine which were used as substrates, a Michaelis-Menten kinetic was carried out as well in the same manner whereupon Km values and Vmax values were calculated. The result is shown in the following Table 1. Each Vmax value in Table 1 was shown in terms of the ratio when the Vmax value of alanine was defined as 1.00.

TABLE 1

Km Values and Vmax values of Amino Acids Used as Substrates

Amino Acid	Km μM	Vmax[a]
L-Alanine	23.0	(1.00)
Glycine	7.8	0.89
L-Serine	11.3	1.02
L-Threonine	19.3	0.86
L-Cysteine	23.7	0.82
L-Valine	112	1.17
L-Methionine	139	1.15
L-Isoleucine	160	1.33
L-Leucine	245	0.58
L-Histidine	368	0.79
L-Phenylalanine	464	1.09
AIB	22.7	0.81
D-Alanine	100	0.86
D-Serine	52.0	1.22
β-Alanine	281	0.92

[a]The Vmax value for each amino acid was shown in terms of the ratio to the Vmax value for alanine

[0114] (4) Substrate Selectivity of Asc-1 (Experiment for Inhibition by Addition of Amino Acid and Analogs Thereof)

[0115] In an experiment of incorporation of alanine by oocytes into which both asc-1 gene cRNA of mouse and 4F2hc gene cRNA of mouse were injected, influence of addition of various amino acid and analogs thereof was investigated.

[0116] In an experiment of incorporation of alanine, a method according to that mentioned in the above Example 2(1) was carried out using oocytes into which both asc-1 gene cRNA of mouse and 4F2hc gene cRNA of mouse were injected. Here, a Na^+-free uptake solution was used and incorporation of ^{14}C-alanine (50 μM) was measured in the presence and absence of 5 mM of various compounds (non-labeled).

[0117] The result in the presence and absence (−) of various L-amino acids or analogous compounds thereof is shown in FIG. **8**. The result in the presence and absence (−) of various D-amino acids is shown in FIG. **9**. The result in the presence and absence (−) of alanine or analogous compounds thereof is shown in FIG. **10**.

[0118] In various neutral L-amino acids, a cis-inhibiting effect was observed. Glycine, alanine, serine, threonine and cysteine particularly strongly inhibited the incorporation of ^{14}C-alanine mediated by asc-1 (refer to FIG. **8**).

[0119] Among the D-amino acids, D-alanine and D-serine strongly inhibited the incorporation of ^{14}C-alanine mediated by asc-1. D-Threonine and D-cysteine mediumly inhibited the incorporation of ^{14}C-alanine mediated by asc-1 (refer to FIG. **9**).

[0120] Even in the case of the substances other than standard amino acids, β-alanine, alanine methyl ester and α-aminoisobutyric acid (α-methylalanine) also inhibited the incorporation of ^{14}C-alanine mediated by asc-1 (refer to FIG. **10**). Acidic amino acids, basic amino acids, transport system L-specific inhibitor 2-amino-2-norbornane-carboxylic acid (BCH), γ-aminoisobutyric acid and N-methylamino acids (N-methylalanine, α-aminomethylisobutyric acid and sarcosine) did not affect the incorporation of ^{14}C-alanine mediated by asc-1 (refer to FIG. **8** and FIG. **10**).

[0121] (5) Substrate Selectivity of Asc-1 (Test for Incorporation Using Various Amino Acids and Analogs as Substrate)

[0122] Incorporation by asc-1 was investigated using various amino acids and analogs thereof as substrates. Experiment for incorporation of various amino acids and analogs thereof was carried out according to the method mentioned in the above Example 2(1) using oocytes into which asc-1 gene cRNA of mouse and 4F2hc gene cRNA of mouse were injected together. With regard to the substrates however, various compounds labeled with radioactivity were used in place of ^{14}C-alanine.

[0123] Result of incorporation of the radiolabeled L-amino acids is shown in FIG. **11**. Result of incorporation of the radiolabeled D-amino acids is shown in FIG. **12**. Result of incorporation of the radiolabeled L-alanine or analogous compound thereof is shown in FIG. **13**.

[0124] As a result, a big incorporation into the oocytes was noted when glycine (a ^{14}C compound), L-alanine (a ^{14}C compound), L-serine (a ^{14}C compound), L-threonine (a ^{14}C compound), L-cysteine (a ^{14}C compound) (for those, refer to FIG. **11**), D-alanine (a ^{14}C compound), D-serine (a ^{14}C compound) (for those, refer to FIG. **12**), β-alanine (a ^{14}C compound) and a-aminoisobutyric acid (a ^{14}C compound) (for those, refer to FIG. **13**) were used as substrates.

[0125] (6) pH-Dependency of Transport Activity of Asc-1

[0126] Influence of pH was checked in an experiment for incorporation of alanine by oocytes into which both asc-1 gene cRNA and 4F2hc gene cRNA of mouse were injected together. With regard to an experiment for incorporation of alanine, the method according to that mentioned in the above Example 2 (1) was carried out using oocytes into which both asc-1 gene cRNA of mouse and 4F2hc gene cRNA of mouse were injected together.

[0127] Result where the influence of pH in the experiment for incorporation of alanine was checked is shown in FIG. **14**. As a result thereof, there was no significant dependency on pH in the incorporation of alanine (refer to FIG. **14**).

[0128] (7) Test of Release of Amino Acid Mediated by Asc-1

[0129] Release of a preloaded ^{14}C-alanine mediated by asc-1 was checked in the oocytes into which both asc-1 gene cRNA and 4F2hc gene cRNA of mouse were injected together. ^{14}C-Alanine (−3nCi) of 100 μM in an amount of 100 nl was injected into the oocytes into which both asc-1 gene cRNA and 4F2hc gene cRNA of mouse were injected together, washed with an ice-cooled Na^+-free uptake solution containing no alanine and transferred to a Na^+-free uptake solution to which alanine (100 μM) was added or not added at room temperature (18° C.-22° C.) and the amount of ^{14}C-alanine released outside the cells was measured.

[0130] Further, ^{14}C-leucine was similarly injected into oocytes into which LAT1 gene cRNA and 4F2hc gene cRNA of rat were injected together (Kanai et al., *J. Biol. Chem.*, volume 273, page 23629, 1988), washed with an ice-cooled Na^+-free uptake solution containing no leucine and transferred to a Na^+-free uptake solution to which leucine (100

μM) was added or not added at room temperature (18° C.-22° C.) whereupon the amount of ^{14}C-leucine released outside the cells was measured.

[0131] Those results are shown in FIG. **15**, FIG. **16** and FIG. **17**.

[0132] FIG. **15** shows the result of checking the release of ^{14}C-alanine from the oocytes into which cRNA of asc-1 gene of mouse and cRNA of 4F2hc gene of mouse were injected and the ordinate in the drawing shows a rate (%) of the released radioactivity to the radioactivity injected into the oocytes. Left side of FIG. **15** shows the case in the absence of Na (−) while right side thereof shows that in the presence of Na (+) and, in each graph, L-Ala(−) shows the case where L-alanine was not added while L-Ala(+) shows the case where L-alanine was added.

[0133] FIG. **16** shows the result of checking the progress of release of ^{14}C-alanine with a lapse of time by oocytes into which cRNA of mouse asc-1 gene and cRNA of mouse 4F2hc gene are injected. In the drawing, ○ is the case where, in the release of ^{14}C-alanine in the oocytes into which water is injected as a control instead of cRNA of mouse asc-1 gene and cRNA of mouse 4F2hc gene, a Na^+-free uptake solution to which no alanine is added is used; ● is the case where, in the release of ^{14}C-alanine in the oocytes into which water is injected as a control instead of cRNA of mouse asc-1 gene and cRNA of mouse 4F2hc gene, a Na^+-free uptake solution to which alanine is added is used; □ is the case where, in the release of ^{14}C-alanine in the oocytes into which cRNA of mouse asc-1 gene and cRNA of mouse 4F2hc gene are injected, a Na^+-free uptake solution to which no alanine is added is used; and ■ is the case where, in the release of ^{14}C-alanine in the oocytes into which cRNA of mouse asc-1 gene and cRNA of mouse 4F2hc gene are injected, a Na^+-free uptake solution to which alanine is added is used. The ordinate in the drawing shows the rate (%) of the released radioactivity to the radioactivity injected into the oocytes.

[0134] FIG. **17** shows the result of checking the progress of release of ^{14}C-leucine with a lapse of time by oocytes into which cRNA of rat LAT1 gene and cRNA of rat 4F2hc gene are injected. In the drawing, 0 is the case where, in the release of ^{14}C-leucine in the oocytes into which water is injected as a control instead of cRNA of rat LAT1 gene and cRNA of rat 4F2hc gene, a Na^+-free uptake solution to which no leucine is added is used; ● is the case where, in the release of ^{14}C-leucine in the oocytes into which water is injected as a control instead of cRNA of rat LAT1 gene and cRNA of rat 4F2hc gene, a Na^+-free uptake solution to which leucine is added is used; ● is the case where, in the release of ^{14}C-leucine in the oocytes into which cRNA of rat LAT1 gene and cRNA of rat 4F2hc gene are injected, a Na^+-free uptake solution to which no leucine is added is used; and ■ is the case where, in the release of ^{14}C-leucine in the oocytes into which cRNA of rat LAT1 gene and cRNA of rat 4F2hc gene are injected, a Na^+-free uptake solution to which leucine is added is used. The ordinate in the drawing shows the rate (%) of the released radioactivity to the radioactivity injected into the oocytes.

[0135] As a result, even when alanine was not added to the outside of the cells, a significant release of ^{14}C-alanine was observed in the case of asc-1 and such a release significantly increased by addition of alanine to the outside of the cells (refer to FIG. **15** and FIG. **16**). On the other hand, in the case of LAT1 which is a complete exchange transport mediating the forced exchange, release of leucine was observed only when leucine was added to the outside of the cells (refer to FIG. **17**). Accordingly, although asc-1 is mostly in an exchange transport mode, it was found to be a transporter where a transport mode of a promotion diffusion type is mixed as well.

[0136] (8) Investigation of Substrate Selectivity of Asc-1 Utilizing the Release Test of Amino Acids

[0137] In oocytes into which cRNA of asc-1 gene and cRNA of 4F2hc gene of mouse were injected together, release of preloaded ^{14}C-alanine mediated by asc-1 was investigated whereupon it was checked whether the compound which inhibited the incorporation of ^{14}C-alanine mediated by asc-1 was a substrate of asc-1.

[0138] ^{14}C-Alanine (−3nCi) of 100 μM in an amount of 100 nl was injected into the oocytes into which both asc-1 gene cRNA and 4F2hc gene cRNA of mouse were injected together, washed with an ice-cooled Na^+-free uptake solution containing no alanine and transferred to a Na^+-free uptake solution to which amino acid or amino acid analog (100 μM) was added or not added at room temperature (18° C.-22° C.) and the amount of ^{14}C-alanine released outside the cells was measured.

[0139] The result is shown in FIG. **18**. Black bars in FIG. **18** are the case where there were used oocytes into which cRNA of mouse asc-1 gene and cRNA of mouse 4F2hc gene were injected while white bars are the case where there were used oocytes into which water was injected instead of cRNA as a control. (−) shows the case where no amino acid was added to a Na^+-free uptake solution. The ordinate in FIG. **18** shows the rate (%) of the released radioactivity to the radioactivity injected into the oocytes.

[0140] As a result, a high increase in the release of ^{14}C-alanine was observed in glycine, alanine, serine and threonine and a medium increase therein was observed in methionine and valine (refer to FIG. **18**). Such a result coincides with the result of the test for incorporation of amino acids (refer to FIG. **11**) and the test for release of amino acids was shown to be able to be used for determination of substrate selectivity of asc-1.

[0141] The result where further investigation was conducted for D-amino acids and amino acid analogs using the said method is shown in FIG. **19** and FIG. **20**. With regard to D-amino acids, D-alanine, D-serine, D-threonine and D-cysteine resulted in a significant increase in the release of ^{14}C-alanine (refer to FIG. **19**). With regard to amino acid analogs, β-alanine, alanine methyl ester and α-aminoisobutyric acid (AIB) resulted in a significant increase in the release of ^{14}C-alanine (refer to FIG. **20**). It was therefore found that D-threonine, D-cysteine and alanine methyl ester which have been unable to be subjected to an incorporation experiment using radiolabeled ones because of unavailability of radiolabeled compounds are now able to be substrates for asc-1. As such, when an amino acid releasing test is used, it is now possible to screen whether a compound can be a substrate for asc-1 or, in other words, whether it can be transported by asc-1 even in the case of the compound where no radiolabeled one is available for the investigation.

[0142] (9) Investigation of Substrate Selectivity of Intracellular Substrate-Binding Site of Asc-1 Utilizing the Release Test of Amino Acids

[0143] In oocytes into which cRNA of asc-1 gene and cRNA of 4F2hc gene of mouse were injected together, release of preloaded ^{14}C-amino acid mediated by asc-1 was checked whereby the substrate selectivity of intracellular substrate-binding site of asc-1 was checked.

[0144] ^{14}C-Amino acid (~3nCi) of 100 μM in an amount of 100 nl was injected into the oocytes into which both asc-1 gene cRNA and 4F2hc gene cRNA of mouse were injected together, washed with an ice-cooled Na^+-free uptake solution containing no alanine and transferred to a Na^+-free uptake solution to which alanine (100 μM) was added or not added at room temperature (18° C.-22° C.) and the amount of ^{14}C-amino acid released outside the cells was measured.

[0145] The result is shown in FIG. **21**. Black bars in FIG. **21** show the case where alanine was added to a Na^+-free uptake solution while bars with oblique lines show the case where alanine was not added to a Na^+-free uptake solution. The ordinate of FIG. **21** shows the rate (%) of the release radioactivity to the radioactivity injected into oocytes.

[0146] As a result, an increase in release of ^{14}C-labeled glycine, alanine, serine, threonine and cysteine injected into the cells by extracellular alanine was observed. Therefore, it was shown that the intracellular substrate-binding site shows a substrate selectivity for receiving small neutral amino acid such as glycine, alanine, serine, threonine and cysteine as same as in the extracellular case.

[0147] (10) Confirmation of Human Asc-1

[0148] From a plasmid containing cDNA of human asc-1 obtained in Example 1(3), cRNA (RNA complementary to cDNA) was prepared using a T7 RNA polymerase. Comparison was made between incorporation of ^{14}C-alanine in the case where human asc-1 gene cRNA was solely expressed in oocytes with that in the case where both human asc-1 gene cRNA and human 4F2hc gene cRNA were expressed in oocytes.

[0149] Expression was carried out by injection of 12.5 ng of human asc-1 gene cRNA, 12.5 ng of human 4F2hc gene cRNA or 12.5 ng of human asc-1 gene cRNA/12.5 ng of human 4F2hc gene cRNA into oocytes and incubation was conducted for three days. With regard to the oocytes into which human asc-1 gene cRNA, 4F2hc gene cRNA or human asc-1 gene cRNA/4F2hc gene cRNA was injected, an experiment for incorporation of substrate was carried out according to Example 2(1) using alanine as a substrate.

[0150] The result is as follows. Like in the case of asc-1 of mouse, the oocytes where only asc-1 was expressed showed incorporation of alanine in the same level as in the case of oocytes into which water was injected as a control while, in the oocytes where both asc-1 and 4F2hc were expressed together, a big incorporation of alanine was observed. Accordingly, like asc-1 of mouse, human asc-1 was also shown to achieve the function only when it was present together with 4F2hc. It is also noted that the human asc-1 shows the same property as the above-mentioned asc-1 of mouse.

INDUSTRIAL APPLICABILITY

[0151] The sodium-independent small neutral amino acid transporter according to the present invention which transports L- and D-amino acids and gene thereof makes it possible to conduct an in vitro investigation of transport of small neutral amino acids of L- and D-forms and amino acid analogous compounds including exogenous matters at the expressed part of the said transporter and also to conduct an in vitro presumption of fate of those compounds in vivo on the basis of the above. Further, that is useful for the development of medicaments which efficiently permeate the expressed part of the said transporter and the present invention provides novel amino acid transporters. Furthermore, as a result of modulation of an ability of the said transporter for transporting the small neutral L- and D-amino acids and analogs thereof, it is useful as a method for controlling the resistance of cells to oxidative stress, a method for controlling the activity of glutamic acid receptor of an NMDA type in nervous system, a method for controlling the cell growth and a method for screening the medicament having such activities.

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         55                  60                  65

cct ggc tgg gtg cgc acc cgc tgg gcg ctg ctg ctg ctc ttc tgg ctc      357
Pro Gly Trp Val Arg Thr Arg Trp Ala Leu Leu Leu Leu Phe Trp Leu
     70                  75                  80

ggt tgg ctg ggc atg ctg gcg ggc gcc gtg gtt atc atc gtt cgg gcg      405
Gly Trp Leu Gly Met Leu Ala Gly Ala Val Val Ile Ile Val Arg Ala
 85                  90                  95                 100

ccg cgc tgc cgt gag ctg cct gta cag agg tgg tgg cac aag ggc gcc      453
Pro Arg Cys Arg Glu Leu Pro Val Gln Arg Trp Trp His Lys Gly Ala
                105                 110                 115

ctc tac cgc atc ggc gac ctt cag gcc ttt gta ggc cgg gat gcg gga      501
Leu Tyr Arg Ile Gly Asp Leu Gln Ala Phe Val Gly Arg Asp Ala Gly
            120                 125                 130

ggc ata gct ggt ctg aag agc cat ctg gag tac ttg agc acc ctg aag      549
Gly Ile Ala Gly Leu Lys Ser His Leu Glu Tyr Leu Ser Thr Leu Lys
        135                 140                 145

gtg aag ggc ctg gtg tta ggc cca att cac aag aac cag aag gat gaa      597
Val Lys Gly Leu Val Leu Gly Pro Ile His Lys Asn Gln Lys Asp Glu
    150                 155                 160

atc aat gaa acc gac ctg aaa cag att aat ccc act ttg ggc tcc cag      645
Ile Asn Glu Thr Asp Leu Lys Gln Ile Asn Pro Thr Leu Gly Ser Gln
165                 170                 175                 180

gaa gat ttt aaa gac ctt cta caa agt gcc aag aaa aag agc att cac      693
Glu Asp Phe Lys Asp Leu Leu Gln Ser Ala Lys Lys Lys Ser Ile His
                185                 190                 195

atc att ttg gac ctc act ccc aac tac cag ggc cag aat gcg tgg ttc      741
Ile Ile Leu Asp Leu Thr Pro Asn Tyr Gln Gly Gln Asn Ala Trp Phe
            200                 205                 210
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ctc cct gct cag gct gac att gta gcc acc aaa atg aag gaa gct ctg      789
Leu Pro Ala Gln Ala Asp Ile Val Ala Thr Lys Met Lys Glu Ala Leu
        215                 220                 225

agt tct tgg ttg cag gac ggt gtg gat ggt ttc caa ttc cgg gat gtg      837
Ser Ser Trp Leu Gln Asp Gly Val Asp Gly Phe Gln Phe Arg Asp Val
    230                 235                 240

gga aag ctg atg aat gca ccc ttg tac ttg gct gag tgg cag aat atc      885
Gly Lys Leu Met Asn Ala Pro Leu Tyr Leu Ala Glu Trp Gln Asn Ile
245                 250                 255                 260

acc aag aac tta agt gag gac agg ctt ttg att gca ggg act gag tcc      933
Thr Lys Asn Leu Ser Glu Asp Arg Leu Leu Ile Ala Gly Thr Glu Ser
                265                 270                 275

tct gac ctg cag caa att gtc aac ata ctt gaa tcc acc agc gac ctg      981
Ser Asp Leu Gln Gln Ile Val Asn Ile Leu Glu Ser Thr Ser Asp Leu
            280                 285                 290

ctg ttg acc agc tcc tac ctg tca aat tcc act ttc act ggg gag cgt     1029
Leu Leu Thr Ser Ser Tyr Leu Ser Asn Ser Thr Phe Thr Gly Glu Arg
        295                 300                 305

act gaa tcc cta gtc act agg ttt ttg aat gcc act ggc agc caa tgg     1077
Thr Glu Ser Leu Val Thr Arg Phe Leu Asn Ala Thr Gly Ser Gln Trp
    310                 315                 320

tgc agc tgg agt gtg tcg caa gca gga ctc ctc gca gac ttt ata ccg     1125
Cys Ser Trp Ser Val Ser Gln Ala Gly Leu Leu Ala Asp Phe Ile Pro
325                 330                 335                 340

gac cat ctt ctc cga ctc tac cag ctg ctg ctc ttc act ctg cca ggg     1173
Asp His Leu Leu Arg Leu Tyr Gln Leu Leu Leu Phe Thr Leu Pro Gly
                345                 350                 355

act cct gtt ttt agc tac ggg gat gag ctt ggc ctt cag ggt gcc ctt     1221
Thr Pro Val Phe Ser Tyr Gly Asp Glu Leu Gly Leu Gln Gly Ala Leu
            360                 365                 370

cct gga cag cct gcg aag gcc cca ctc atg ccg tgg aat gag tcc agc     1269
Pro Gly Gln Pro Ala Lys Ala Pro Leu Met Pro Trp Asn Glu Ser Ser
        375                 380                 385

atc ttt cac atc cca aga cct gta agc ctc aac atg aca gtg aag ggc     1317
Ile Phe His Ile Pro Arg Pro Val Ser Leu Asn Met Thr Val Lys Gly
    390                 395                 400

cag aat gaa gac cct ggc tcc ctt ctt acc cag ttc cgg cgg ctg agt     1365
Gln Asn Glu Asp Pro Gly Ser Leu Leu Thr Gln Phe Arg Arg Leu Ser
405                 410                 415                 420

gac ctt cgg ggt aag gag cgc tct ctg ttg cac ggt gac ttc cat gca     1413
Asp Leu Arg Gly Lys Glu Arg Ser Leu Leu His Gly Asp Phe His Ala
                425                 430                 435

ctg tct tcc tca cct gac ctc ttc tcc tac ata cga cac tgg gac cag     1461
Leu Ser Ser Ser Pro Asp Leu Phe Ser Tyr Ile Arg His Trp Asp Gln
            440                 445                 450

aat gag cgt tac ctg gtg gtg ctc aac ttc cga gat tcg ggc cgg tca     1509
Asn Glu Arg Tyr Leu Val Val Leu Asn Phe Arg Asp Ser Gly Arg Ser
        455                 460                 465

gcc agg cta ggg gcc tcc aac ctc cct gct ggc ata agc ctg cca gcc     1557
Ala Arg Leu Gly Ala Ser Asn Leu Pro Ala Gly Ile Ser Leu Pro Ala
    470                 475                 480

agc gct aaa ctt ttg ctt agt acc gac agt gcc cgg caa agc cgt gag     1605
Ser Ala Lys Leu Leu Leu Ser Thr Asp Ser Ala Arg Gln Ser Arg Glu
485                 490                 495                 500

gag gac acc tcc ctg aag ctg gaa aac ctg agc ctg aat cct tat gag     1653
Glu Asp Thr Ser Leu Lys Leu Glu Asn Leu Ser Leu Asn Pro Tyr Glu
                505                 510                 515
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ggc ttg ctg tta cag ttc ccc ttt gtg gcc tgatccttcc tatgcagaac       1703
Gly Leu Leu Leu Gln Phe Pro Phe Val Ala
            520                 525

ctaccaccct cctttgttct ccccaggcct tttggattct agtcttcctc tccttgtttt   1763

taaacttttg cagattacat acgaattctt atactgggtg tttttgtctt caaataaaaa   1823

catcacccct gcctcaaaaa aaaaaaaaa                                     1852


<210> SEQ ID NO 4
<211> LENGTH: 523
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Met Ala Gly His Thr Gln Gln Pro Ser Gly Arg Gly Asn Pro Arg Pro
  1               5                  10                  15

Ala Pro Ser Pro Ser Pro Val Pro Gly Thr Val Pro Gly Ala Ser Glu
             20                  25                  30

Arg Val Ala Leu Lys Lys Glu Ile Gly Leu Leu Ser Ala Cys Thr Ile
         35                  40                  45

Ile Ile Gly Asn Ile Ile Gly Ser Gly Ile Phe Ile Ser Pro Lys Gly
     50                  55                  60

Val Leu Glu His Ser Gly Ser Val Gly Leu Ala Leu Phe Val Trp Val
 65                  70                  75                  80

Leu Gly Gly Gly Val Thr Ala Leu Gly Ser Leu Cys Tyr Ala Glu Leu
                 85                  90                  95

Gly Val Ala Ile Pro Lys Ser Gly Gly Asp Tyr Ala Tyr Val Thr Glu
            100                 105                 110

Ile Phe Gly Gly Leu Ala Gly Phe Leu Leu Leu Trp Ser Ala Val Leu
        115                 120                 125

Ile Met Tyr Pro Thr Ser Leu Ala Val Ile Ser Met Thr Phe Ser Asn
    130                 135                 140

Tyr Val Leu Gln Pro Val Phe Pro Asn Cys Ile Pro Pro Thr Thr Ala
145                 150                 155                 160

Ser Arg Val Leu Ser Met Ala Cys Leu Met Leu Leu Thr Trp Val Asn
                165                 170                 175

Ser Ser Ser Val Arg Trp Ala Thr Arg Ile Gln Asp Met Phe Thr Gly
            180                 185                 190

Gly Lys Leu Leu Ala Leu Ser Leu Ile Ile Gly Val Gly Leu Leu Gln
        195                 200                 205

Ile Phe Gln Gly His Phe Glu Glu Leu Arg Pro Ser Asn Ala Phe Ala
    210                 215                 220

Phe Trp Met Thr Pro Ser Val Gly His Leu Ala Leu Ala Phe Leu Gln
225                 230                 235                 240

Gly Ser Phe Ala Phe Ser Gly Trp Asn Phe Leu Asn Tyr Val Thr Glu
                245                 250                 255

Glu Met Val Asp Ala Arg Lys Asn Leu Pro Arg Ala Ile Phe Ile Ser
            260                 265                 270

Ile Pro Leu Val Thr Phe Val Tyr Thr Phe Thr Asn Ile Ala Tyr Phe
        275                 280                 285

Thr Ala Met Ser Pro Gln Glu Leu Leu Ser Ser Asn Ala Val Ala Val
    290                 295                 300

Thr Phe Gly Glu Lys Leu Leu Gly Tyr Phe Ser Trp Val Met Pro Val
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305                 310                 315                 320

Ser Val Ala Leu Ser Thr Phe Gly Gly Ile Asn Gly Tyr Leu Phe Thr
                325                 330                 335

Tyr Ser Arg Leu Cys Phe Ser Gly Ala Arg Glu Gly His Leu Pro Ser
            340                 345                 350

Leu Leu Ala Met Ile His Val Arg His Cys Thr Pro Ile Pro Ala Leu
        355                 360                 365

Leu Val Cys Cys Gly Ala Thr Ala Val Ile Met Leu Val Gly Asp Thr
    370                 375                 380

Tyr Thr Leu Ile Asn Tyr Val Ser Phe Ile Asn Tyr Leu Cys Tyr Gly
385                 390                 395                 400

Val Thr Ile Leu Gly Leu Leu Leu Leu Arg Trp Arg Arg Pro Ala Leu
                405                 410                 415

His Arg Pro Ile Lys Val Asn Leu Leu Ile Pro Val Ala Tyr Leu Val
            420                 425                 430

Phe Trp Ala Phe Leu Leu Val Phe Ser Phe Ile Ser Glu Pro Met Val
        435                 440                 445

Cys Gly Val Gly Val Ile Ile Ile Leu Thr Gly Val Pro Ile Phe Phe
    450                 455                 460

Leu Gly Val Phe Trp Arg Ser Lys Pro Lys Cys Val His Arg Leu Thr
465                 470                 475                 480

Glu Ser Met Thr His Trp Gly Gln Glu Leu Cys Phe Val Val Tyr Pro
                485                 490                 495

Gln Asp Ala Pro Glu Glu Glu Glu Asn Gly Pro Cys Pro Pro Ser Leu
            500                 505                 510

Leu Pro Ala Thr Asp Lys Pro Ser Lys Pro Gln
        515                 520
```

<210> SEQ ID NO 5
<211> LENGTH: 1918
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

```
cggctgcgag ggccgtgagc tcacggaccg acggaccgac gggcggccgg ccggacagac     60

ggggcagcgc agggagcggg gacgcggcgg gacagcgaca tggccggcca cacgcagcag    120

ccgagcgggc gcgggaaccc caggcctgcg ccctcgccct ccccagtccc agggaccgtc    180

cccggcgcct cggagcgggt ggcgctcaag aaggagatcg ggctgctgag cgcctgcacc    240

atcatcatcg ggaacatcat cggctcgggc atcttcatct cgcccaaggg ggtcctggag    300

cactcaggct ccgtgggtct ggccctgttc gtctgggtcc tgggtggggg cgtgacggct    360

ctgggctccc tctgctatgc agagctggga gtcgccatcc ccaagtctgg cggggactac    420

gcctacgtca cagagatctt cgggggcctg gctggctttc tgctgctctg gagcgccgtc    480

ctcatcatgt accccaccag ccttgctgtc atctccatga ccttctccaa ctacgtgctg    540

cagcccgtgt tccccaactg catcccccccc accacagcct cccgggtgct gtccatggcc    600

tgcctgatgc tcctgacatg ggtgaacagc tccagtgtgc gctgggccac gcgcatccag    660

gacatgttca caggcgggaa gctgctggcc ttgtccctca tcatcggcgt gggccttctc    720

cagatcttcc aaggacactt cgaggagctg aggcccagca atgcctttgc tttctggatg    780

acgccctccg tgggacacct ggccctggcc ttcctccagg gctccttcgc cttcagtggc    840
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tggaacttcc tcaactatgt caccgaggag atggttgacg cccgaaagaa cctacctcgc    900

gccatcttca tctccatccc actggtgacc ttcgtgtaca cgttcaccaa cattgcctac    960

ttcacggcca tgtcccccca ggagctgctc tcctccaatg cggtggctgt gaccttcggg   1020

gagaagctgc tgggctactt ttcttgggtc atgcctgtct ccgtggctct gtcaaccttc   1080

ggagggatca atggttacct gttcacctac tccaggctgt gcttctctgg agcccgcgag   1140

gggcacctgc ccagcctgct ggccatgatc cacgtcagac actgcacccc catccccgcc   1200

ctcctcgtct gttgcggggc cacagccgtc atcatgctcg tgggcgacac gtacacgctc   1260

atcaactatg tgtccttcat caactacctc tgctacggcg tcaccatcct gggcctgctg   1320

ctgctgcgct ggaggcggcc tgcactccac aggcccatca aggtgaacct tctcatcccc   1380

gtggcgtact tggtcttctg ggccttcctg ctggtcttca gcttcatctc agagcctatg   1440

gtctgtgggg tcggcgtcat catcatcctt acgggggtgc ccattttctt tctgggagtg   1500

ttctggagaa gcaaaccaaa gtgtgtgcac agactcacag agtccatgac acactggggc   1560

caggagctgt gtttcgtggt ctacccccag gacgcccccg aagaggagga gaatggcccc   1620

tgcccaccct ccctgctgcc tgccacagac aagccctcga agccacaatg agatttttgt   1680

agagactgaa gcagttgttt ctgtttacat gttgtttatt gaggaggtgt tttggcaaaa   1740

aagttttgtt ttgttttttt ctggaaaaaa aagaaaaaag atacgactct cagaagcctg   1800

ttttaaggaa gccctaaaat gtggactggg tttcctgtct tagcactgcc ctgctagctc   1860

ttcctgaaaa ggcctataaa taaacagggc tggctgttaa aaaaaaaaaa aaaaaaaa     1918


<210> SEQ ID NO 6
<211> LENGTH: 1897
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (112)..(1698)

<400> SEQUENCE: 6

ctgcgcggag gcacagaggc cggggagagc gttctgggtc cgagggtcca ggtaggggtt     60

gagccaccat ctgaccgcaa gctgcgtcgt gtcgccggtt ctgcaggcac c atg agc     117
                                                          Met Ser
                                                            1

cag gac acc gag gtg gat atg aag gag gtg gag ctg aat gag tta gag      165
Gln Asp Thr Glu Val Asp Met Lys Glu Val Glu Leu Asn Glu Leu Glu
          5                  10                  15

ccc gag aag cag ccg atg aac gcg gcg tct ggg gcg gcc atg tcc ctg      213
Pro Glu Lys Gln Pro Met Asn Ala Ala Ser Gly Ala Ala Met Ser Leu
     20                  25                  30

gcg gga gcc gag aag aat ggt ctg gtg aag atc aag gtg gcg gaa gac      261
Ala Gly Ala Glu Lys Asn Gly Leu Val Lys Ile Lys Val Ala Glu Asp
 35                  40                  45                  50

gag gcg gag gcg gca gcc gcg gct aag ttc acg ggc ctg tcc aag gag      309
Glu Ala Glu Ala Ala Ala Ala Lys Phe Thr Gly Leu Ser Lys Glu
                 55                  60                  65

gag ctg ctg aag gtg gca ggc agc ccc ggc tgg gta cgc acc cgc tgg      357
Glu Leu Leu Lys Val Ala Gly Ser Pro Gly Trp Val Arg Thr Arg Trp
            70                  75                  80

gca ctg ctg ctg ctc ttc tgg ctc ggc tgg ctc ggc atg ctt gct ggt      405
Ala Leu Leu Leu Leu Phe Trp Leu Gly Trp Leu Gly Met Leu Ala Gly
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         85                  90                  95

gcc gtg gtc ata atc gtg cga gcg ccg cgt tgt cgc gag cta ccg gcg      453
Ala Val Val Ile Ile Val Arg Ala Pro Arg Cys Arg Glu Leu Pro Ala
    100                 105                 110

cag aag tgg tgg cac acg ggc gcc ctc tac cgc atc ggc gac ctt cag      501
Gln Lys Trp Trp His Thr Gly Ala Leu Tyr Arg Ile Gly Asp Leu Gln
115                 120                 125                 130

gcc ttc cag ggc cac ggc gcg ggc aac ctg gcg ggt ctg aag ggg cgt      549
Ala Phe Gln Gly His Gly Ala Gly Asn Leu Ala Gly Leu Lys Gly Arg
                135                 140                 145

ctc gat tac ctg agc tct ctg aag gtg aag ggc ctt gtg ctg ggt cca      597
Leu Asp Tyr Leu Ser Ser Leu Lys Val Lys Gly Leu Val Leu Gly Pro
            150                 155                 160

att cac aag aac cag aag gat gat gtc gct cag act gac ttg ctg cag      645
Ile His Lys Asn Gln Lys Asp Asp Val Ala Gln Thr Asp Leu Leu Gln
        165                 170                 175

atc gac ccc aat ttt ggc tcc aag gaa gat ttt gac agt ctc ttg caa      693
Ile Asp Pro Asn Phe Gly Ser Lys Glu Asp Phe Asp Ser Leu Leu Gln
    180                 185                 190

tcg gct aaa aaa aag agc atc cgt gtc att ctg gac ctt act ccc aac      741
Ser Ala Lys Lys Lys Ser Ile Arg Val Ile Leu Asp Leu Thr Pro Asn
195                 200                 205                 210

tac cgg ggt gag aac tcg tgg ttc tcc act cag gtt gac act gtg gcc      789
Tyr Arg Gly Glu Asn Ser Trp Phe Ser Thr Gln Val Asp Thr Val Ala
                215                 220                 225

acc aag gtg aag gat gct ctg gag ttt tgg ctg caa gct ggc gtg gat      837
Thr Lys Val Lys Asp Ala Leu Glu Phe Trp Leu Gln Ala Gly Val Asp
            230                 235                 240

ggg ttc cag gtt cgg gac ata gag aat ctg aag gat gca tcc tca ttc      885
Gly Phe Gln Val Arg Asp Ile Glu Asn Leu Lys Asp Ala Ser Ser Phe
        245                 250                 255

ttg gct gag tgg caa aat atc acc aag ggc ttc agt gaa gac agg ctc      933
Leu Ala Glu Trp Gln Asn Ile Thr Lys Gly Phe Ser Glu Asp Arg Leu
    260                 265                 270

ttg att gcg ggg act aac tcc tcc gac ctt cag cag atc ctg agc cta      981
Leu Ile Ala Gly Thr Asn Ser Ser Asp Leu Gln Gln Ile Leu Ser Leu
275                 280                 285                 290

ctc gaa tcc aac aaa gac ttg ctg ttg act agc tca tac ctg tct gat     1029
Leu Glu Ser Asn Lys Asp Leu Leu Leu Thr Ser Ser Tyr Leu Ser Asp
                295                 300                 305

tct ggt tct act ggg gag cat aca aaa tcc cta gtc aca cag tat ttg     1077
Ser Gly Ser Thr Gly Glu His Thr Lys Ser Leu Val Thr Gln Tyr Leu
            310                 315                 320

aat gcc act ggc aat cgc tgg tgc agc tgg agt ttg tct cag gca agg     1125
Asn Ala Thr Gly Asn Arg Trp Cys Ser Trp Ser Leu Ser Gln Ala Arg
        325                 330                 335

ctc ctg act tcc ttc ttg ccg gct caa ctt ctc cga ctc tac cag ctg     1173
Leu Leu Thr Ser Phe Leu Pro Ala Gln Leu Leu Arg Leu Tyr Gln Leu
    340                 345                 350

atg ctc ttc acc ctg cca ggg acc cct gtt ttc agc tac ggg gat gag     1221
Met Leu Phe Thr Leu Pro Gly Thr Pro Val Phe Ser Tyr Gly Asp Glu
355                 360                 365                 370

att ggc ctg gat gca gct gcc ctt cct gga cag cct atg gag gct cca     1269
Ile Gly Leu Asp Ala Ala Ala Leu Pro Gly Gln Pro Met Glu Ala Pro
                375                 380                 385

gtc atg ctg tgg gat gag tcc agc ttc cct gac atc cca ggg gct gta     1317
Val Met Leu Trp Asp Glu Ser Ser Phe Pro Asp Ile Pro Gly Ala Val
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            390                 395                 400

agt gcc aac atg act gtg aag ggc cag agt gaa gac cct ggc tcc ctc     1365
Ser Ala Asn Met Thr Val Lys Gly Gln Ser Glu Asp Pro Gly Ser Leu
        405                 410                 415

ctt tcc ttg ttc cgg cgg ctg agt gac cag cgg agt aag gag cgc tcc     1413
Leu Ser Leu Phe Arg Arg Leu Ser Asp Gln Arg Ser Lys Glu Arg Ser
    420                 425                 430

cta ctg cat ggg gac ttc cac gcg ttc tcc gct ggg cct gga ctc ttc     1461
Leu Leu His Gly Asp Phe His Ala Phe Ser Ala Gly Pro Gly Leu Phe
435                 440                 445                 450

tcc tat atc cgc cac tgg gac cag aat gag cgt ttt ctg gta gtg ctt     1509
Ser Tyr Ile Arg His Trp Asp Gln Asn Glu Arg Phe Leu Val Val Leu
                455                 460                 465

aac ttt ggg gat gtg ggc ctc tcg gct gga ctg cag gcc tcc gac ctg     1557
Asn Phe Gly Asp Val Gly Leu Ser Ala Gly Leu Gln Ala Ser Asp Leu
            470                 475                 480

cct gcc agc gcc agc ctg cca gcc aag gct gac ctc ctg ctc agc acc     1605
Pro Ala Ser Ala Ser Leu Pro Ala Lys Ala Asp Leu Leu Leu Ser Thr
        485                 490                 495

cag cca ggc cgt gag gag ggc tcc cct ctt gag ctg gaa cgc ctg aaa     1653
Gln Pro Gly Arg Glu Glu Gly Ser Pro Leu Glu Leu Glu Arg Leu Lys
    500                 505                 510

ctg gag cct cac gaa ggg ctg ctg ctc cgc ttc ccc tac gcg gcc         1698
Leu Glu Pro His Glu Gly Leu Leu Leu Arg Phe Pro Tyr Ala Ala
515                 520                 525

tgacttcagc ctgacatgga cccactaccc ttctcctttc cttcccaggc cctttggctt   1758

ctgatttttc tctttttaa aaacaaacaa acaaactgtt gcagattatg agtgaacccc   1818

caaatagggt gttttctgcc ttcaaataaa agtcacccct gcatggtgaa gtcttccctc   1878

taaaaaaaaa aaaaaaaaa                                             1897


<210> SEQ ID NO 7
<211> LENGTH: 526
<212> TYPE: PRT
<213> ORGANISM: Mus sp.

<400> SEQUENCE: 7

Met Ser Gln Asp Thr Glu Val Asp Met Lys Asp Val Glu Leu Asn Glu
  1               5                  10                  15

Leu Glu Pro Glu Lys Gln Pro Met Asn Ala Ala Asp Gly Ala Ala Ala
             20                  25                  30

Gly Glu Lys Asn Gly Leu Val Lys Ile Lys Val Ala Glu Asp Glu Thr
         35                  40                  45

Glu Ala Gly Val Lys Phe Thr Gly Leu Ser Lys Glu Glu Leu Leu Lys
     50                  55                  60

Val Ala Gly Ser Pro Gly Trp Val Arg Thr Arg Trp Ala Leu Leu Leu
 65                  70                  75                  80

Leu Phe Trp Leu Gly Trp Leu Gly Met Leu Ala Gly Ala Val Val Ile
                 85                  90                  95

Ile Val Arg Ala Pro Arg Cys Arg Glu Leu Pro Val Gln Arg Trp Trp
            100                 105                 110

His Lys Gly Ala Leu Tyr Arg Ile Gly Asp Leu Gln Ala Phe Val Gly
        115                 120                 125

Arg Asp Ala Gly Gly Ile Ala Gly Leu Lys Ser His Leu Glu Tyr Leu
    130                 135                 140
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Ser Thr Leu Lys Val Lys Gly Leu Val Leu Gly Pro Ile His Lys Asn
145                 150                 155                 160

Gln Lys Asp Glu Ile Asn Glu Thr Asp Leu Lys Gln Ile Asn Pro Thr
                165                 170                 175

Leu Gly Ser Gln Glu Asp Phe Lys Asp Leu Leu Gln Ser Ala Lys Lys
            180                 185                 190

Lys Ser Ile His Ile Ile Leu Asp Leu Thr Pro Asn Tyr Gln Gly Gln
        195                 200                 205

Asn Ala Trp Phe Leu Pro Ala Gln Ala Asp Ile Val Ala Thr Lys Met
    210                 215                 220

Lys Glu Ala Leu Ser Ser Trp Leu Gln Asp Gly Val Asp Gly Phe Gln
225                 230                 235                 240

Phe Arg Asp Val Gly Lys Leu Met Asn Ala Pro Leu Tyr Leu Ala Glu
                245                 250                 255

Trp Gln Asn Ile Thr Lys Asn Leu Ser Glu Asp Arg Leu Leu Ile Ala
            260                 265                 270

Gly Thr Glu Ser Ser Asp Leu Gln Gln Ile Val Asn Ile Leu Glu Ser
        275                 280                 285

Thr Ser Asp Leu Leu Leu Thr Ser Ser Tyr Leu Ser Asn Ser Thr Phe
    290                 295                 300

Thr Gly Glu Arg Thr Glu Ser Leu Val Thr Arg Phe Leu Asn Ala Thr
305                 310                 315                 320

Gly Ser Gln Trp Cys Ser Trp Ser Val Ser Gln Ala Gly Leu Leu Ala
                325                 330                 335

Asp Phe Ile Pro Asp His Leu Leu Arg Leu Tyr Gln Leu Leu Leu Phe
            340                 345                 350

Thr Leu Pro Gly Thr Pro Val Phe Ser Tyr Gly Asp Glu Leu Gly Leu
        355                 360                 365

Gln Gly Ala Leu Pro Gly Gln Pro Ala Lys Ala Pro Leu Met Pro Trp
    370                 375                 380

Asn Glu Ser Ser Ile Phe His Ile Pro Arg Pro Val Ser Leu Asn Met
385                 390                 395                 400

Thr Val Lys Gly Gln Asn Glu Asp Pro Gly Ser Leu Leu Thr Gln Phe
                405                 410                 415

Arg Arg Leu Ser Asp Leu Arg Gly Lys Glu Arg Ser Leu Leu His Gly
            420                 425                 430

Asp Phe His Ala Leu Ser Ser Ser Pro Asp Leu Phe Ser Tyr Ile Arg
        435                 440                 445

His Trp Asp Gln Asn Glu Arg Tyr Leu Val Val Leu Asn Phe Arg Asp
    450                 455                 460

Ser Gly Arg Ser Ala Arg Leu Gly Ala Ser Asn Leu Pro Ala Gly Ile
465                 470                 475                 480

Ser Leu Pro Ala Ser Ala Lys Leu Leu Leu Ser Thr Asp Ser Ala Arg
                485                 490                 495

Gln Ser Arg Glu Glu Asp Thr Ser Leu Lys Leu Glu Asn Leu Ser Leu
            500                 505                 510

Asn Pro Tyr Glu Gly Leu Leu Leu Gln Phe Pro Phe Val Ala
        515                 520                 525
```

<210> SEQ ID NO 8
<211> LENGTH: 529

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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

```
Met Ser Gln Asp Thr Glu Val Asp Met Lys Glu Val Glu Leu Asn Glu
  1               5                  10                  15

Leu Glu Pro Glu Lys Gln Pro Met Asn Ala Ala Ser Gly Ala Ala Met
             20                  25                  30

Ser Leu Ala Gly Ala Glu Lys Asn Gly Leu Val Lys Ile Lys Val Ala
         35                  40                  45

Glu Asp Glu Ala Glu Ala Ala Ala Ala Ala Lys Phe Thr Gly Leu Ser
     50                  55                  60

Lys Glu Glu Leu Leu Lys Val Ala Gly Ser Pro Gly Trp Val Arg Thr
 65                  70                  75                  80

Arg Trp Ala Leu Leu Leu Leu Phe Trp Leu Gly Trp Leu Gly Met Leu
                 85                  90                  95

Ala Gly Ala Val Val Ile Ile Val Arg Ala Pro Arg Cys Arg Glu Leu
            100                 105                 110

Pro Ala Gln Lys Trp Trp His Thr Gly Ala Leu Tyr Arg Ile Gly Asp
        115                 120                 125

Leu Gln Ala Phe Gln Gly His Gly Ala Gly Asn Leu Ala Gly Leu Lys
    130                 135                 140

Gly Arg Leu Asp Tyr Leu Ser Ser Leu Lys Val Lys Gly Leu Val Leu
145                 150                 155                 160

Gly Pro Ile His Lys Asn Gln Lys Asp Asp Val Ala Gln Thr Asp Leu
                165                 170                 175

Leu Gln Ile Asp Pro Asn Phe Gly Ser Lys Glu Asp Phe Asp Ser Leu
            180                 185                 190

Leu Gln Ser Ala Lys Lys Lys Ser Ile Arg Val Ile Leu Asp Leu Thr
        195                 200                 205

Pro Asn Tyr Arg Gly Glu Asn Ser Trp Phe Ser Thr Gln Val Asp Thr
    210                 215                 220

Val Ala Thr Lys Val Lys Asp Ala Leu Glu Phe Trp Leu Gln Ala Gly
225                 230                 235                 240

Val Asp Gly Phe Gln Val Arg Asp Ile Glu Asn Leu Lys Asp Ala Ser
                245                 250                 255

Ser Phe Leu Ala Glu Trp Gln Asn Ile Thr Lys Gly Phe Ser Glu Asp
            260                 265                 270

Arg Leu Leu Ile Ala Gly Thr Asn Ser Ser Asp Leu Gln Gln Ile Leu
        275                 280                 285

Ser Leu Leu Glu Ser Asn Lys Asp Leu Leu Leu Thr Ser Ser Tyr Leu
    290                 295                 300

Ser Asp Ser Gly Ser Thr Gly Glu His Thr Lys Ser Leu Val Thr Gln
305                 310                 315                 320

Tyr Leu Asn Ala Thr Gly Asn Arg Trp Cys Ser Trp Ser Leu Ser Gln
                325                 330                 335

Ala Arg Leu Leu Thr Ser Phe Leu Pro Ala Gln Leu Leu Arg Leu Tyr
            340                 345                 350

Gln Leu Met Leu Phe Thr Leu Pro Gly Thr Pro Val Phe Ser Tyr Gly
        355                 360                 365

Asp Glu Ile Gly Leu Asp Ala Ala Ala Leu Pro Gly Gln Pro Met Glu
    370                 375                 380
```

-continued

```
Ala Pro Val Met Leu Trp Asp Glu Ser Ser Phe Pro Asp Ile Pro Gly
385                 390                 395                 400

Ala Val Ser Ala Asn Met Thr Val Lys Gly Gln Ser Glu Asp Pro Gly
                405                 410                 415

Ser Leu Leu Ser Leu Phe Arg Arg Leu Ser Asp Gln Arg Ser Lys Glu
            420                 425                 430

Arg Ser Leu Leu His Gly Asp Phe His Ala Phe Ser Ala Gly Pro Gly
        435                 440                 445

Leu Phe Ser Tyr Ile Arg His Trp Asp Gln Asn Glu Arg Phe Leu Val
    450                 455                 460

Val Leu Asn Phe Gly Asp Val Gly Leu Ser Ala Gly Leu Gln Ala Ser
465                 470                 475                 480

Asp Leu Pro Ala Ser Ala Ser Leu Pro Ala Lys Ala Asp Leu Leu Leu
                485                 490                 495

Ser Thr Gln Pro Gly Arg Glu Glu Gly Ser Pro Leu Glu Leu Glu Arg
            500                 505                 510

Leu Lys Leu Glu Pro His Glu Gly Leu Leu Leu Arg Phe Pro Tyr Ala
        515                 520                 525

Ala
```

<210> SEQ ID NO 9
<211> LENGTH: 533
<212> TYPE: PRT
<213> ORGANISM: Rattus sp.

<400> SEQUENCE: 9

```
Met Glu Lys Gly Thr Arg Gln Arg Asn Asn Thr Ala Lys Asn His Pro
  1               5                  10                  15

Asp Arg Gly Ser Asp Thr Ser Pro Glu Ala Glu Ala Ser Ser Gly Gly
             20                  25                  30

Gly Gly Val Ala Leu Lys Lys Glu Ile Gly Leu Val Ser Ala Cys Gly
         35                  40                  45

Ile Ile Val Gly Asn Ile Ile Gly Ser Gly Ile Phe Val Ser Pro Lys
     50                  55                  60

Gly Val Leu Glu Asn Ala Gly Ser Val Gly Leu Ala Leu Ile Val Trp
 65                  70                  75                  80

Ile Val Thr Gly Val Ile Thr Ala Val Gly Ala Leu Cys Tyr Ala Glu
                 85                  90                  95

Leu Gly Val Thr Ile Pro Lys Ser Gly Gly Asp Tyr Ser Tyr Val Lys
            100                 105                 110

Asp Ile Phe Gly Gly Leu Ala Gly Phe Leu Arg Leu Trp Ile Ala Val
        115                 120                 125

Leu Val Ile Tyr Pro Thr Asn Gln Ala Val Ile Ala Leu Thr Phe Ser
    130                 135                 140

Asn Tyr Val Leu Gln Phe Leu Phe Pro Thr Cys Phe Pro Pro Glu Ser
145                 150                 155                 160

Gly Leu Arg Leu Leu Ala Ala Ile Cys Leu Leu Leu Leu Thr Trp Val
                165                 170                 175

Asn Cys Ser Ser Val Arg Trp Ala Thr Arg Val Gln Asp Ile Phe Thr
            180                 185                 190

Ala Gly Lys Leu Leu Ala Leu Ala Leu Ile Ile Ile Met Gly Val Val
        195                 200                 205
```

-continued

```
Gln Ile Cys Lys Gly Glu Phe Phe Trp Leu Glu Pro Lys Asn Ala Phe
    210                 215                 220

Glu Asn Phe Gln Glu Pro Asp Ile Gly Leu Val Ala Leu Ala Phe Leu
225                 230                 235                 240

Gln Gly Ser Phe Ala Tyr Gly Gly Trp Asn Phe Leu Asn Tyr Val Thr
                245                 250                 255

Glu Glu Leu Val Asp Pro Tyr Lys Asn Leu Pro Arg Ala Ile Phe Ile
            260                 265                 270

Ser Ile Pro Leu Val Thr Phe Val Tyr Val Phe Ala Asn Ile Ala Tyr
        275                 280                 285

Val Thr Ala Met Ser Pro Gln Glu Leu Leu Ala Ser Asn Ala Val Ala
    290                 295                 300

Val Thr Phe Gly Glu Lys Leu Leu Gly Val Met Ala Trp Ile Met Pro
305                 310                 315                 320

Ile Ser Val Ala Leu Ser Thr Phe Gly Gly Val Asn Gly Ser Leu Phe
                325                 330                 335

Thr Ser Ser Arg Leu Phe Phe Ala Gly Ala Arg Glu Gly His Leu Pro
            340                 345                 350

Ser Val Leu Ala Met Ile His Val Lys Arg Cys Thr Pro Ile Pro Ala
        355                 360                 365

Leu Leu Phe Thr Cys Leu Ser Thr Leu Leu Met Leu Val Thr Ser Asp
    370                 375                 380

Met Tyr Thr Leu Ile Asn Tyr Val Gly Phe Ile Asn Tyr Leu Phe Tyr
385                 390                 395                 400

Gly Val Thr Val Ala Gly Gln Ile Val Leu Arg Trp Lys Lys Pro Asp
                405                 410                 415

Ile Pro Arg Pro Ile Lys Ile Ser Leu Leu Phe Pro Ile Ile Tyr Leu
            420                 425                 430

Leu Phe Trp Ala Phe Leu Leu Ile Phe Ser Leu Trp Ser Glu Pro Val
        435                 440                 445

Val Cys Gly Ile Gly Leu Ala Ile Met Leu Thr Gly Val Pro Val Tyr
    450                 455                 460

Phe Leu Gly Val Tyr Trp Gln His Lys Pro Lys Cys Phe Asn Asp Phe
465                 470                 475                 480

Ile Glu Ser Leu Thr Leu Val Ser Gln Lys Met Cys Val Val Val Tyr
                485                 490                 495

Pro Gln Glu Gly Asp Ser Gly Thr Glu Glu Thr Ile Asp Asp Val Glu
            500                 505                 510

Glu Gln His Lys Pro Ile Phe Gln Pro Thr Pro Val Lys Asp Pro Asp
        515                 520                 525

Ser Glu Glu Gln Pro
    530


<210> SEQ ID NO 10
<211> LENGTH: 512
<212> TYPE: PRT
<213> ORGANISM: Rattus sp.

<400> SEQUENCE: 10

Met Ala Val Ala Gly Ala Lys Arg Arg Ala Val Ala Ala Pro Ala Thr
  1               5                  10                  15

Thr Ala Ala Glu Glu Glu Arg Gln Ala Arg Glu Lys Met Leu Glu Ala
```

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```
            20                  25                  30

Arg Arg Gly Asp Gly Ala Asp Pro Glu Gly Glu Gly Val Thr Leu Gln
        35                  40                  45

Arg Asn Ile Thr Leu Ile Asn Gly Val Ala Ile Ile Val Gly Thr Ile
    50                  55                  60

Ile Gly Ser Gly Ile Phe Val Thr Pro Thr Gly Val Leu Lys Glu Ala
65                  70                  75                  80

Gly Ser Pro Gly Leu Ser Leu Val Val Trp Ala Val Cys Gly Val Phe
                85                  90                  95

Ser Ile Val Gly Ala Leu Cys Tyr Ala Glu Leu Gly Thr Thr Ile Ser
            100                 105                 110

Lys Ser Gly Gly Asp Tyr Ala Tyr Met Leu Glu Val Tyr Gly Ser Leu
        115                 120                 125

Pro Ala Phe Leu Lys Leu Trp Ile Glu Leu Leu Ile Ile Arg Pro Ser
    130                 135                 140

Ser Gln Tyr Ile Val Ala Leu Val Phe Ala Thr Tyr Leu Leu Lys Pro
145                 150                 155                 160

Val Phe Pro Thr Cys Pro Val Pro Glu Glu Ala Ala Lys Leu Val Ala
                165                 170                 175

Cys Leu Cys Val Leu Leu Leu Thr Ala Val Asn Cys Tyr Ser Val Lys
            180                 185                 190

Ala Ala Thr Arg Val Gln Asp Ala Phe Ala Ala Ala Lys Leu Leu Ala
        195                 200                 205

Leu Ala Leu Ile Ile Leu Leu Gly Phe Ile Gln Met Gly Lys Asp Ile
    210                 215                 220

Gly Gln Gly Asp Ala Ser Asn Leu His Gln Lys Leu Ser Phe Glu Gly
225                 230                 235                 240

Thr Asn Leu Asp Val Gly Asn Ile Val Leu Ala Leu Tyr Ser Gly Leu
                245                 250                 255

Phe Ala Tyr Gly Gly Trp Asn Tyr Leu Asn Phe Val Thr Glu Glu Met
            260                 265                 270

Ile Asn Pro Tyr Arg Asn Leu Pro Leu Ala Ile Ile Ile Ser Leu Pro
        275                 280                 285

Ile Val Thr Leu Val Tyr Val Leu Thr Asn Leu Ala Tyr Phe Thr Thr
    290                 295                 300

Leu Ser Thr Asn Gln Met Leu Thr Ser Glu Ala Val Ala Val Asp Phe
305                 310                 315                 320

Gly Asn Tyr His Leu Gly Val Met Ser Trp Ile Ile Pro Val Phe Val
                325                 330                 335

Gly Leu Ser Cys Phe Gly Ser Val Asn Gly Ser Leu Phe Thr Ser Ser
            340                 345                 350

Arg Leu Phe Phe Val Gly Ser Arg Glu Gly His Leu Pro Ser Ile Leu
        355                 360                 365

Ser Met Ile His Pro Gln Leu Leu Thr Pro Val Pro Ser Leu Val Phe
    370                 375                 380

Thr Cys Val Met Thr Leu Met Tyr Ala Phe Ser Arg Asp Ile Phe Ser
385                 390                 395                 400

Ile Ile Asn Phe Phe Ser Phe Phe Asn Trp Leu Cys Val Ala Leu Ala
                405                 410                 415

Ile Ile Gly Met Met Trp Leu Arg Phe Lys Lys Pro Glu Leu Glu Arg
            420                 425                 430
```

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```
Pro Ile Lys Val Asn Leu Ala Leu Pro Val Phe Phe Ile Leu Ala Cys
        435                 440                 445

Leu Phe Leu Ile Ala Val Ser Phe Trp Lys Thr Pro Leu Glu Cys Gly
    450                 455                 460

Ile Gly Phe Ala Ile Ile Leu Ser Gly Leu Pro Val Tyr Phe Phe Gly
465                 470                 475                 480

Val Trp Trp Lys Asn Lys Pro Lys Trp Ile Leu Gln Val Ile Phe Ser
                485                 490                 495

Val Thr Val Leu Cys Gln Lys Leu Met Cys Val Val Pro Gln Glu Thr
            500                 505                 510


<210> SEQ ID NO 11
<211> LENGTH: 511
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

Met Val Asp Ser Thr Glu Tyr Glu Val Ala Ser Gln Pro Glu Val Glu
  1               5                  10                  15

Thr Ser Pro Leu Gly Asp Gly Ala Ser Pro Gly Pro Glu Gln Val Lys
             20                  25                  30

Leu Lys Lys Glu Ile Ser Leu Leu Asn Gly Val Cys Leu Ile Val Gly
         35                  40                  45

Asn Met Ile Gly Ser Gly Ile Phe Val Ser Pro Lys Gly Val Leu Ile
     50                  55                  60

Tyr Ser Ala Ser Phe Gly Leu Ser Leu Val Ile Trp Ala Val Gly Gly
 65                  70                  75                  80

Leu Phe Ser Val Phe Gly Ala Leu Cys Tyr Ala Glu Leu Gly Thr Thr
                 85                  90                  95

Ile Lys Lys Ser Gly Ala Ser Tyr Ala Tyr Ile Leu Glu Ala Phe Gly
            100                 105                 110

Gly Phe Leu Ala Phe Ile Arg Leu Trp Thr Ser Leu Leu Ile Ile Glu
        115                 120                 125

Pro Thr Ser Gln Ala Ile Ile Ala Ile Thr Phe Ala Asn Tyr Met Val
    130                 135                 140

Gln Phe Leu Phe Pro Ser Cys Phe Ala Pro Tyr Ala Ala Ser Arg Leu
145                 150                 155                 160

Leu Ala Ala Ala Cys Ile Cys Leu Leu Thr Phe Ile Asn Cys Ala Tyr
                165                 170                 175

Val Lys Trp Gly Thr Leu Val Gln Asp Ile Phe Thr Tyr Ala Lys Val
            180                 185                 190

Leu Ala Leu Ile Ala Val Ile Val Ala Gly Ile Val Arg Leu Gly Gln
        195                 200                 205

Gly Ala Ser Thr His Phe Glu Asn Ser Phe Glu Gly Ser Ser Phe Ala
    210                 215                 220

Val Gly Asp Ile Ala Leu Ala Leu Tyr Ser Ala Leu Phe Ser Tyr Ser
225                 230                 235                 240

Gly Trp Asp Thr Leu Asn Tyr Val Thr Glu Glu Ile Lys Asn Pro Glu
                245                 250                 255

Arg Asn Leu Pro Leu Ser Ile Gly Ile Ser Met Pro Ile Val Thr Ile
            260                 265                 270

Ile Tyr Ile Leu Thr Asn Val Ala Tyr Tyr Thr Val Leu Asp Met Arg
```

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        275                 280                 285

Asp Ile Leu Ala Ser Asp Ala Val Ala Val Thr Phe Ala Asp Gln Ile
    290                 295                 300

Phe Gly Ile Phe Asn Trp Ile Ile Pro Leu Ser Val Ala Leu Ser Cys
305                 310                 315                 320

Phe Gly Gly Leu Asn Ala Ser Ile Val Ala Ala Ser Arg Leu Phe Phe
                325                 330                 335

Val Gly Ser Arg Glu Gly His Leu Pro Asp Ala Ile Cys Met Ile His
            340                 345                 350

Val Glu Arg Phe Thr Pro Val Pro Ser Leu Leu Phe Asn Gly Ile Met
        355                 360                 365

Ala Leu Ile Tyr Leu Cys Val Glu Asp Ile Phe Gln Leu Ile Asn Tyr
    370                 375                 380

Tyr Ser Phe Ser Tyr Trp Phe Phe Val Gly Leu Ser Ile Val Gly Gln
385                 390                 395                 400

Leu Tyr Leu Arg Trp Lys Glu Pro Cys Arg Pro Arg Pro Leu Lys Leu
                405                 410                 415

Ser Val Phe Phe Pro Ile Val Phe Cys Leu Cys Thr Ile Phe Leu Val
            420                 425                 430

Ala Val Pro Leu Tyr Ser Asp Thr Ile Asn Ser Leu Ile Gly Ile Ala
        435                 440                 445

Ile Ala Leu Ser Gly Leu Pro Phe Tyr Phe Leu Ile Ile Arg Val Pro
    450                 455                 460

Glu His Lys Arg Pro Leu Tyr Leu Arg Arg Ile Val Gly Ser Ala Thr
465                 470                 475                 480

Arg Tyr Leu Gln Val Leu Cys Met Ser Val Ala Ala Glu Met Asp Leu
                485                 490                 495

Glu Asp Gly Gly Glu Met Pro Lys Gln Arg Asp Pro Lys Ser Asn
            500                 505                 510


<210> SEQ ID NO 12
<211> LENGTH: 515
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Met Glu Ala Arg Glu Pro Gly Arg Pro Thr Pro Thr Tyr His Leu Val
  1               5                  10                  15

Pro Asn Thr Ser Gln Ser Gln Val Glu Glu Asp Val Ser Ser Pro Pro
             20                  25                  30

Gln Arg Ser Ser Glu Thr Met Gln Leu Lys Lys Glu Ile Ser Leu Leu
         35                  40                  45

Asn Gly Val Ser Leu Val Val Gly Asn Met Ile Gly Ser Gly Ile Phe
     50                  55                  60

Val Ser Pro Lys Gly Val Leu Val His Thr Ala Ser Tyr Gly Met Ser
 65                  70                  75                  80

Leu Ile Val Trp Ala Ile Gly Gly Leu Phe Ser Val Val Gly Ala Leu
                 85                  90                  95

Cys Tyr Ala Glu Leu Gly Thr Thr Ile Thr Lys Ser Gly Ala Ser Tyr
            100                 105                 110

Ala Tyr Ile Leu Glu Ala Phe Gly Gly Phe Ile Ala Phe Ile Arg Leu
        115                 120                 125
```

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```
Trp Val Ser Leu Leu Val Val Glu Pro Thr Gly Gln Ala Ile Ile Ala
    130                 135                 140

Ile Thr Phe Ala Asn Tyr Ile Ile Gln Pro Ser Phe Pro Ser Cys Asp
145                 150                 155                 160

Pro Pro Tyr Leu Ala Cys Arg Leu Leu Ala Ala Ala Cys Ile Cys Leu
                165                 170                 175

Leu Thr Phe Val Asn Cys Ala Tyr Val Lys Trp Gly Thr Arg Val Gln
            180                 185                 190

Asp Thr Phe Thr Tyr Ala Lys Val Val Ala Leu Ile Ala Ile Ile Val
        195                 200                 205

Met Gly Leu Val Lys Leu Cys Gln Gly His Ser Glu His Phe Gln Asp
    210                 215                 220

Ala Phe Glu Gly Ser Ser Trp Asp Met Gly Asn Leu Ser Leu Ala Leu
225                 230                 235                 240

Tyr Ser Ala Leu Phe Ser Tyr Ser Gly Trp Asp Thr Leu Asn Phe Val
                245                 250                 255

Thr Glu Glu Ile Lys Asn Pro Glu Arg Asn Leu Pro Leu Ala Ile Gly
            260                 265                 270

Ile Ser Met Pro Ile Val Thr Leu Ile Tyr Ile Leu Thr Asn Val Ala
        275                 280                 285

Tyr Tyr Thr Val Leu Asn Ile Ser Asp Val Leu Ser Ser Asp Ala Val
    290                 295                 300

Ala Val Thr Phe Ala Asp Gln Thr Phe Gly Met Phe Ser Trp Thr Ile
305                 310                 315                 320

Pro Ile Ala Val Ala Leu Ser Cys Phe Gly Gly Leu Asn Ala Ser Ile
                325                 330                 335

Phe Ala Ser Ser Arg Leu Phe Phe Val Gly Ser Arg Glu Gly His Leu
            340                 345                 350

Pro Asp Leu Leu Ser Met Ile His Ile Glu Arg Phe Thr Pro Ile Pro
        355                 360                 365

Ala Leu Leu Phe Asn Cys Thr Met Ala Leu Ile Tyr Leu Ile Val Glu
    370                 375                 380

Asp Val Phe Gln Leu Ile Asn Tyr Phe Ser Phe Ser Tyr Trp Phe Phe
385                 390                 395                 400

Val Gly Leu Ser Val Val Gly Gln Leu Tyr Leu Arg Trp Lys Glu Pro
                405                 410                 415

Lys Arg Pro Arg Pro Leu Lys Leu Ser Val Phe Phe Pro Ile Val Phe
            420                 425                 430

Cys Ile Cys Ser Val Phe Leu Val Ile Val Pro Leu Phe Thr Asp Thr
        435                 440                 445

Ile Asn Ser Leu Ile Gly Ile Gly Ile Ala Leu Ser Gly Val Pro Phe
    450                 455                 460

Tyr Phe Met Gly Val Tyr Leu Pro Glu Ser Arg Arg Pro Leu Phe Ile
465                 470                 475                 480

Arg Asn Val Leu Ala Ala Ile Thr Arg Gly Thr Gln Gln Leu Cys Phe
                485                 490                 495

Cys Val Leu Thr Glu Leu Asp Val Ala Glu Glu Lys Lys Asp Glu Arg
            500                 505                 510

Lys Thr Asp
        515
```

-continued

<210> SEQ ID NO 13
<211> LENGTH: 502
<212> TYPE: PRT
<213> ORGANISM: Mus sp.

<400> SEQUENCE: 13

```
Met Val Arg Lys Pro Val Val Ala Thr Ile Ser Lys Gly Gly Tyr Leu
  1               5                  10                  15

Gln Gly Asn Met Ser Gly Arg Leu Pro Ser Met Gly Asp Gln Glu Pro
             20                  25                  30

Pro Gly Gln Glu Lys Val Val Leu Lys Lys Lys Ile Thr Leu Leu Arg
         35                  40                  45

Gly Val Ser Ile Ile Ile Gly Thr Val Ile Gly Ser Gly Ile Phe Ile
     50                  55                  60

Ser Pro Lys Gly Ile Leu Gln Asn Thr Gly Ser Val Gly Met Ser Leu
 65                  70                  75                  80

Val Phe Trp Ser Ala Cys Gly Val Leu Ser Leu Phe Gly Ala Leu Ser
                 85                  90                  95

Tyr Ala Glu Leu Gly Thr Ser Ile Lys Lys Ser Gly Gly His Tyr Thr
            100                 105                 110

Tyr Ile Leu Glu Val Phe Gly Pro Leu Leu Ala Phe Val Arg Val Trp
        115                 120                 125

Val Glu Leu Leu Val Ile Arg Pro Gly Ala Thr Ala Val Ile Ser Leu
    130                 135                 140

Ala Phe Gly Arg Tyr Ile Leu Glu Pro Phe Phe Ile Gln Cys Glu Ile
145                 150                 155                 160

Pro Glu Leu Ala Ile Lys Leu Val Thr Ala Val Gly Ile Thr Val Val
                165                 170                 175

Met Val Leu Asn Ser Thr Ser Val Ser Trp Ser Ala Arg Ile Gln Ile
            180                 185                 190

Phe Leu Thr Phe Cys Lys Leu Thr Ala Ile Leu Ile Ile Ile Val Pro
        195                 200                 205

Gly Val Ile Gln Leu Ile Lys Gly Gln Thr His His Phe Lys Asp Ala
    210                 215                 220

Phe Ser Gly Arg Asp Thr Ser Leu Met Gly Leu Pro Leu Ala Phe Tyr
225                 230                 235                 240

Tyr Gly Met Tyr Ala Tyr Ala Gly Trp Phe Tyr Leu Asn Phe Ile Thr
                245                 250                 255

Glu Glu Val Asp Asn Pro Glu Lys Thr Ile Pro Leu Ala Ile Cys Ile
            260                 265                 270

Ser Met Ala Ile Ile Thr Val Gly Tyr Val Leu Thr Asn Val Ala Tyr
        275                 280                 285

Phe Thr Thr Ile Ser Ala Glu Glu Leu Leu Gln Ser Ser Ala Val Ala
    290                 295                 300

Val Thr Phe Ser Glu Arg Leu Leu Gly Lys Phe Ser Leu Ala Val Pro
305                 310                 315                 320

Ile Phe Val Ala Leu Ser Cys Phe Gly Ser Met Asn Gly Gly Val Phe
                325                 330                 335

Ala Val Ser Arg Leu Phe Tyr Val Ala Ser Arg Glu Gly His Leu Pro
            340                 345                 350

Glu Ile Leu Ser Met Ile His Val His Lys His Thr Pro Leu Pro Ala
        355                 360                 365
```

-continued

```
Val Ile Val Leu His Pro Leu Thr Met Val Met Leu Phe Ser Gly Asp
    370                 375                 380

Leu Tyr Ser Leu Leu Asn Phe Leu Ser Phe Ala Arg Trp Leu Phe Met
385                 390                 395                 400

Gly Leu Ala Val Ala Gly Leu Ile Tyr Leu Arg Tyr Lys Arg Pro Asp
                405                 410                 415

Met His Arg Pro Phe Lys Val Pro Leu Phe Ile Pro Ala Leu Phe Ser
            420                 425                 430

Phe Thr Cys Leu Phe Met Val Val Leu Ser Leu Tyr Ser Cys Pro Phe
        435                 440                 445

Ser Thr Gly Val Gly Phe Leu Ile Thr Leu Thr Gly Val Pro Ala Tyr
    450                 455                 460

Tyr Leu Phe Ile Val Trp Asp Lys Lys Pro Lys Trp Phe Arg Arg Leu
465                 470                 475                 480

Ser Asp Arg Ile Thr Arg Thr Leu Gln Ile Ile Leu Glu Val Val Pro
                485                 490                 495

Glu Asp Ser Lys Glu Leu
            500
```

<210> SEQ ID NO 14
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
Synthetic primer

<400> SEQUENCE: 14

ctcttcacat gcatctccac 20

<210> SEQ ID NO 15
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
Synthetic primer

<400> SEQUENCE: 15

ggtacacgac cacacacatc 20

<210> SEQ ID NO 16
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligopeptide

<400> SEQUENCE: 16

```
Pro Ser Pro Leu Pro Ile Thr Asp Lys Pro Leu Lys Thr Gln Cys
  1               5                  10                  15
```

<210> SEQ ID NO 17
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
oligopeptide

-continued

<400> SEQUENCE: 17

Cys Glu Gly Leu Leu Leu Gln Phe Pro Phe Val Ala
1 5 10

<210> SEQ ID NO 18
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

Glu Ile Gly Leu Leu Ser Ala Cys Thr Ile Ile Ile Gly Asn Ile Ile
1 5 10 15

Gly Ser Gly Ile Phe Ile Ser
20

<210> SEQ ID NO 19
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

Leu Phe Val Trp Val Leu Gly Gly Gly Val Thr Ala Leu Gly Ser Leu
1 5 10 15

Cys Tyr Ala Glu Leu Gly Val
20

<210> SEQ ID NO 20
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

Phe Gly Gly Leu Ala Gly Phe Leu Leu Leu Trp Ser Ala Val Leu Ile
1 5 10 15

Met Tyr Pro Thr Ser Leu Ala
20

<210> SEQ ID NO 21
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

Thr Ala Ser Arg Val Leu Ser Met Ala Cys Leu Met Leu Leu Thr Trp
1 5 10 15

Val Asn Ser Ser Ser Val Arg
20

<210> SEQ ID NO 22
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

Thr Gly Gly Lys Leu Leu Ala Leu Ser Leu Ile Ile Gly Val Gly Leu
1 5 10 15

Leu Gln Ile Phe Gln Gly His
20

-continued

<210> SEQ ID NO 23
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

Leu Ala Leu Ala Phe Leu Gln Gly Ser Phe Ala Phe Ser Gly Trp Asn
1 5 10 15

Phe Leu Asn Tyr Val Thr Glu
20

<210> SEQ ID NO 24
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 24

Asn Leu Pro Arg Ala Ile Phe Ile Ser Ile Pro Leu Val Thr Phe Val
1 5 10 15

Tyr Thr Phe Thr Asn Ile Ala
20

<210> SEQ ID NO 25
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 25

Lys Leu Leu Gly Tyr Phe Ser Trp Val Met Pro Val Ser Val Ala Leu
1 5 10 15

Ser Thr Phe Gly Gly Ile Asn
20

<210> SEQ ID NO 26
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 26

Cys Thr Pro Ile Pro Ala Leu Leu Val Cys Cys Gly Ala Thr Ala Val
1 5 10 15

Ile Met Leu Val Gly Asp Thr
20

<210> SEQ ID NO 27
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 27

Asn Tyr Val Ser Phe Ile Asn Tyr Leu Cys Tyr Gly Val Thr Ile Leu
1 5 10 15

Gly Leu Leu Leu Leu Arg
20

<210> SEQ ID NO 28
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 28

-continued

```
Lys Val Asn Leu Leu Ile Pro Val Ala Tyr Leu Val Phe Trp Ala Phe
  1               5                  10                  15

Leu Leu Val Phe Ser Phe Ile
            20
```

```
<210> SEQ ID NO 29
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 29

Cys Gly Val Gly Val Ile Ile Ile Leu Thr Gly Val Pro Ile Phe Phe
  1               5                  10                  15

Leu Gly Val Phe Trp Arg
            20
```

1.-**14**. (canceled)

15. A method for detection, identification or quantification of action as a substrate of a substance to be tested to the ability of transporting a small neutral amino acid and analogs thereof in a sodium-independent manner using a protein which comprises an amino acid sequence represented by SEQ ID NO: 1 or 4 or an amino acid sequence where one or more amino acid(s) is/are deleted, substituted or added in the said amino acid sequence and which is capable of transporting a small neutral amino acid or analog thereof in a sodium-independent manner.

16.-**22**. (canceled)

23. The method of claim 15, wherein the protein is derived from human being or from mouse.

24. The method of claim 15, wherein the protein is derived from orphan, tissue or cultured cell.

25. A method for determining whether a test substance is a substrate for a protein which comprises an amino acid sequence represented by SEQ ID NO: 1 or 4 or an amino acid sequence where one or more amino acid(s) is/are deleted, substituted or added in the said amino acid sequence and which is capable of transporting a small neutral amino acid or analog thereof in a sodium-independent manner, the method comprising the step of contacting the protein with the test substance, and determining whether the test substance is a substrate for the protein.

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