(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 February 2004 (19.02.2004)

PCT

(10) International Publication Number
WO 2004/015109 A1

(51) International Patent Classification[7]: C12N 15/12, C07K 14/705, G01N 33/68, A61K 48/00, C07K 16/28, G01N 33/50

(21) International Application Number:
PCT/IB2003/003706

(22) International Filing Date: 12 August 2003 (12.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/402,992 12 August 2002 (12.08.2002) US

(71) Applicant *(for JP only)*: **PFIZER PHARMACEUTICALS INC.** [JP/JP]; Mitsui Building, 1-1, Nishi-shinjuku 2-chome, Shinjuku-ku, Tokyo 163-0461 (JP).

(71) Applicant *(for all designated States except JP, US)*: **PFIZER INC.** [US/US]; 235 East 42nd Street, New York, NY 10017 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **GAJYA, Norikaza** [JP/JP]; Pfizer Global Research and Development, 2, Aza 5-gochi, Taketoyo-cho, Chita-gun, Aichi-ken 479-2393 (JP). **SHINJO, Katsuhiro** [JP/JP]; Pfizer Global Research and Development, 2, Aza 5-gochi, Taketoyo-cho, Chita-gun, Aichi-ken 479-2393 (JP). **TAKI, Kenji** [JP/JP]; Pfizer Global Research and Development, 2, Aza 5-gochi, Taketoyo-cho, Chitagun, Aichi-ken 479-2393 (JP).

(74) Agents: **LUMB, J., Trevor** et al.; Pfizer Inc., 201 Tabor Road, Morris Plains, NJ 07950 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: HUMAN ACID SENSING ION CHANNEL 2B (HASIC2B), PROCESS FOR PRODUCING THE SAME, AND ITS USE

(57) Abstract: A novel polynucleotide sequence, which encodes a novel polypeptide belonging to the proton (H+)-gated cation channel subfamily, human Acid Sensing Ion Channel 2b (hAISC2b), is provided. Since hASIC2b and the other hASICs seem to constitute at least part of the native proton-gated cation channel of nociceptive neurons, cells coexpressing hASIC2b and the other hASICs are useful for a method of screening candidate compounds modulating the perception of acidity with regard to nociception.

WO 2004/015109 A1

# HUMAN ACID SENSING ION CHANNEL 2B (HASIC2B), PROCESS FOR PRODUCING THE SAME, AND ITS USE

## FIELD OF THE INVENTION

The present invention relates to a novel polynucleotide sequence, which encodes a novel polypeptide belonging to the proton ($H^+$)-gated cation channel subfamily, i.e., human Acid Sensing Ion Channel 2b (hAISC2b). The present invention also relates, *inter alia*, to processes of producing the polypeptide and its uses.

## BACKGROUND OF THE INVENTION

$H^+$-gated cation channels are ligand-gated ion channels activated by protons. $H^+$-gated cation channels with different pH sensitivities and kinetics were reported in sensory neurons (1-7), in neurons of the central nerve system (CNS) (7-9), and in oligodendrocytes (10, 38). The extracellular pH in tissue can decrease by more than two pH units during tissue acidosis (11, 38) with inflammation and many ischemic conditions. It is believed that the sensation of pain accompanies a decrease in pH (12, 38). Thus, $H^+$-gated cation channels in sensory nerve endings were proposed to be involved in the perception of pain with tissue acidosis (1, 6, 11, 38).

The ASICs (*A*cid *S*ensing *I*on *C*hannels) are members of $H^+$-gated cation channel subfamily belonging to the ENaC/DEG superfamily (22, 25). The superfamily includes the epithelial $Na^+$ channel (ENaC) (13-17, 38), a family of proteins designated as degenerins (DEG)(18-22, 38), and the FMRFamide-gated $Na^+$ channel (FaNaC) (23, 38).

The four rat $H^+$-gated cation channel subunits (ASIC1-4) were cloned recently and will be briefly discussed below.

Acid Sensing Ion Channel 1 (ASIC1, often referred to as ASIC1a) (24), the first member of the $H^+$-gated $Na^+$ channel subfamily, is expressed in both brain and dorsal root ganglion cells (DRGs). It is activated by pH variations below pH 7. The presence of this channel throughout the brain suggests that $H^+$ might play an essential role as a neurotransmitter or neuromodulator (38). Like other members of the ENaC/DEG superfamily (22, 25), ASIC1 has two transmembrane domains with a large extracellular loop protein component (24). Like the FaNaC channel, it seems to assemble as a tetramer (26). ASIC1 is permeable to not only $Na^+$ and $Li^+$ but also $Ca^{2+}$, and desensitizes rapidly with a single exponential time course (38). ASIC1 is blocked by amiloride and its derivatives, benzamil and ethylisopropylamiloride (38). The transcript encoding ASIC1 is alternatively spliced, which generates an additional derivative of the ASIC1 protein (referred to as ASIC1b) (24, 38). Both of the rat and human ASIC1a proteins and ASIC1b proteins have been cloned. The amino acid sequence of the human ASIC1a (formerly referred to as BNaC2) has been identified in a human cDNA library (32). WO 00/08149 discloses that the rat ASIC1a and human ASIC1a proteins are considered to be functionally equivalent. The amino acid sequences of these two proteins are highly homologous, but are not identical. Substitutions can readily be introduced within the primary sequence of the ASIC1a proteins without influencing their essential functional characteristics.

ASIC2a, mammalian neuronal degenerin homologues was in fact cloned before ASIC1a and previously named MDEG1 (27, 38) (for *m*ammalian *deg*enerin) or BNC1 (28, 38) (for *b*rain *Na*$^+$ channel *1*). ASIC2a shares 67% sequence identity with ASIC1a, and it was demonstrated shortly after the cloning of ASIC1a that MDEG1 is also a $H^+$-gated cation channel (29, 30, 38). That is, cation transport by both ASIC1a and ASIC2a is sensitive to amiloride and regulated by acid. Biophysical properties of these two channels are, however, different in that ASIC2a channel requires more acidic pH values, i.e., pH values below pH 5.5 for activation, desensitizes slower than ASIC1a, and is selective for $Na^+$ over $Ca^{2+}$. The ASIC2a mRNA was detected in neurons of the CNS and sensory neurons. It has been shown that the rASIC2a channel is activated by the same mutations that cause neurodegeneration in *C. elegans*. This suggests that a gain of function of ASIC2a might be involved in human forms of neurodegeneration (27). Both of the rat and human ASIC2a proteins have been cloned (28).

ASIC2b previously named MDEG2 is a splice variant of ASIC2a (29). From mouse and rat brain, ASIC2b has been cloned, which differs in the first 236 amino acids, including the first transmembrane region. This new membrane protein is expressed in both brain and sensory neurons. ASIC2b is activated neither by mutations that bring neurodegeneration once introduced in *C. elegans* degenerins nor by low pH. It can, however, associate both with ASIC2a and another recently cloned $H^+$-gated channel DRASIC (hereinafter also referred to as ASIC3 and will be discussed below) to form heteropolymers that display different kinetics, pH dependencies, and ion

selectivities. Of particular interest is the subunit combination specific for sensory neurons, ASIC2b/ASIC3 (MDEG2/DRASIC). This is because, in response to a drop in pH, the subunit combination gives rise to a biphasic current with a sustained current that discriminates poorly between $Na^+$ and $K^+$, like native $H^+$-gated current recorded in dorsal root ganglion cells. This sustained current is thought to be required for the tonic sensation of pain caused by acids. WO 98/35034 discloses rat ASIC2b protein (29). Human ASIC2b protein, however, had not been cloned until the present invention was made.

ASIC3, that was previously named DRASIC (for *DRG acid sensing ion channel*)(ASIC3), is specifically present in DRGs, is absent in the brain, and displays biphasic kinetics (35) with sustained components. Both ASIC1a (24) and ASIC2a (30) desensitize within a few seconds during prolonged application of extracellular acid (38). Pain associated with tissue acidosis, however, continues until the pH returns to neutral (12, 38). A biphasic H+-gated cation current with a sustained component was described in sensory neurons (1) and was proposed to be responsible for the nonadapting pain with tissue acidosis (1, 11, 38). The specific expression of ASIC3 in sensory neurons and the kinetics of the ASIC3 channel suggest that it is part of the sustained $H^+$-gated cation channel complex in sensory neurons. The sustained ASIC3 current, however, requires a more acidic pH for activation (<pH 4) than the native H+-gated current in sensory neurons (1) (pH=5.8). This suggests that a translational modification or associated subunits are required to form the native $H^+$-gated cation channel. WO 00/08149 discloses the cloning of the rat and human ASIC3 proteins.

Another ASIC channel is ASIC4. ASIC4 is a new protein showing about 45% identity to other ASICs. ASIC4 is 97% identical between rat and human and shows strongest expression in pituitary gland (39). A drop of extracellular pH in Xenopus oocytes cannot activate ASIC4, suggesting association with other subunits or activation by a ligand different from protons.

In brief, ASIC2b (MDEG2) is present in sensory neurons where it modulates the expression of ASIC3 (DRASIC). Coexpression of the two proteins yields a $H^+$-gated current that contains a non-selective sustained component. Thus, it seems very probable that these two units, ASIC2b and ASIC3, constitute at least part of the native proton-gated cation channel of nociceptive neurons (1, 29, 38).

Thus, as the modulation of ASICs can have therapeutic consequences for the human, there is a continued need to provide a new ASIC and its agonists and antagonists. In particular, since hASIC2b and the other hASICs constitute at least part of the native proton-gated cation channel of nociceptive neurons, it is necessary to provide a novel method for screening a candidate substance that can modulate the ASICs by bringing it into contact with transformed cells in which both hASIC2b and the other hASICs have been coexpressed.

## SUMMARY OF THE INVENTION

In a broad aspect, the present invention relates to novel nucleic acid sequences encoding human ASIC2b. In this regard, a specific novel nucleic acid sequence has been isolated and it is to be understood that the invention covers that sequence as well as novel variants,

fragments, derivatives, and homologues thereof.

In another aspect, the present invention relates to novel amino acid sequences. In this regard, a specific novel amino acid sequence has been isolated and it is to be understood that the invention covers that sequence as well as novel variants, fragments, derivatives, and homologues thereof.

Thus, in brief, some aspects of the present invention relate to:

1. Novel nucleotide sequences;
2. Novel amino acids;
3. Assays using said novel sequences;
4. Compounds/compositions identified by use of said assays;
5. Expression systems comprising or expressing said novel sequences optionally together with the other ASICs;
6. Methods of treatment based on said novel sequences;
7. Pharmaceutical compositions based on said novel sequences.

Other aspects concerning the nucleotide sequence of the present invention and/or the amino acid sequence of the present invention include: a construct comprising or capable of expressing the sequences of the present invention; a vector comprising or capable of expressing the sequences of the present invention; a plasmid comprising or capable of expressing the sequences of the present invention; a cell transfected or virally-transduced with a construct/vector/plasmid comprising or capable of expressing the sequences of the present invention; a tissue comprising or capable of expressing

the sequences of the present invention; an organ comprising or capable of expressing the sequences of the present invention; a transformed host comprising or capable of expressing the sequences of the present invention; and a transformed organism comprising or capable of expressing the sequences of the present invention. The present invention also encompasses methods of expressing the same, such as expression in a microorganism; including methods for transferring the same.

## BRIEF DESCRIPTION OF THE FIGURES

Fig. 1A and Fig. 1B show the alignment of deduced protein sequences of hASIC2a (at top) and hASIC2b (at bottom);

Fig. 2A, Fig. 2B, Fig. 2C, Fig. 2D, Fig. 2E, Fig. 2F, Fig. 2G, and Fig. 2H show the nucleotide sequence of hASIC2b;

Fig. 3A, Fig. 3B, Fig. 3C, and Fig. 3D show the amino acid sequence of hASIC2b;

Fig. 4 shows the tissue distribution of human ASIC2b and ASIC2a;

Fig. 5 shows the whole-cell recording from hASIC2b, hASIC2a, and hASIC2a/hASIC2b expressing CHO-K1 cells; and

Fig. 6A shows the electrophysiological properties of acid-sensing current in hASIC2a/hASIC2b co-expressing CHO-K1 cells; The pH dependence of acid-sensing currents in hASIC2a and hASIC2a/hASIC2b co-expression CHO-K1 cells; and Fig. 6B shows comparison of peak current density in hASIC2a and hASIC2a/hASIC2b CHO-K1 transformants.

IDENTIFICATION OF THE SEQUENCE LISTINGS

SEQ ID NO: 1 shows the nucleotide sequence coding for hASIC2b;

SEQ ID NO: 2 shows the corresponding amino acid sequence coding for hASIC2b;

SEQ ID No: 3 shows oligonucleotide probe used in the GENE GENE TRAPPER III experiments;

SEQ ID No: 4 shows oligonucleotide probe used in the GENE GENE TRAPPER III experiments;

SEQ ID No: 5 shows oligonucleotide probe used in the GENE GENE TRAPPER III experiments;

SEQ ID NO: 6 shows the sense primer for hASIC2b;

SEQ ID NO: 7 shows the antisense primer for hASIC2b;

SEQ ID NO: 8 shows the sense primer for hASIC2a;

SEQ ID NO: 9 shows the antisense primer for hASIC2a;

SEQ ID NO: 10 shows the sense primer for GAPDH; and

SEQ ID NO: 11 shows the antisense primer for GAPDH.

DETAILED DESCRIPTION OF THE INVENTION

According to one aspect of the present invention, there is provided a polynucleotide comprising one or more of:

(a) a polynucleotide encoding the polypeptide as set forth in SEQ ID NO:2;

(b) a polynucleotide comprising a nucleotide sequence of SEQ ID NO:1;

(c) a polynucleotide comprising a nucleotide sequence that has at least 70% identity to the polynucleotide of (a) or (b);

(d) a polynucleotide comprising a nucleotide sequence which is capable of hybridizing to the polynucleotide of any one of (a) to

(c);

(e) a complement to the polynucleotide of any one of (a) to (d); or

(f) a polynucleotide fragment of the polynucleotide of any one of (a) to (e).

Preferably the polynucleotide is isolated and / or purified. Preferably, the polynucleotide comprises a nucleotide sequence that has at least 75% identity to the polynucleotide of (a) or (b). More preferably, the polynucleotide comprises a nucleotide sequence that has at least 80% identity to the polynucleotide of (a) or (b). Even more preferably, the polynucleotide comprises a nucleotide sequence that has at least 85% identity to the polynucleotide of (a) or (b). Yet more preferably, the polynucleotide comprises a nucleotide sequence that has at least 90% identity to the polynucleotide of (a) or (b). More preferably, the polynucleotide comprises a nucleotide sequence that has at least 95% identity to the polynucleotide of (a) or (b).

The polynucleotide described above preferably encodes a human acid sensing ion channel (ASIC) 2b.

The present invention yet further provides a vector comprising the polynucleotide described above.

According to a further aspect of the present invention, there is provided a host cell transformed or transfected with the vector described above. Preferably, the host cell is mammalian, insect, fungal, bacterial or yeast cell.

According to a further aspect of the present invention, there is provided the transcribed RNA product of the polynucleotide described above. There is also provided an RNA molecule or fragment thereof, which is

antisense in relation to the RNA product and is capable of hybridizing to the RNA product.

There is yet further provided a ribozyme or zinc finger protein capable of binding the polynucleotide described above.

According to a yet further aspect of the present invention, there is provided a process of producing a polypeptide or fragment thereof comprising culturing the transformed/transfected host cell under conditions sufficient for the expression of said polypeptide or fragment. Preferably, said polypeptide or fragment is expressed at the surface of said cell. The process preferably further includes recovering the polypeptide of fragment from the culture.

There is also provided by the present invention a process of producing cells capable of expressing a polypeptide or fragment thereof comprising transforming or transfecting cells with the vector described above.

According to a further embodiment of the present invention, there are provided cells produced by the process described above. There is also provided a membrane preparation of said cells.

According to another aspect of the present invention, there is provided a polypeptide or a fragment thereof produced by the process described above.

According to another aspect of the present invention, there is provided a polypeptide comprising:

(a) a polypeptide having the deduced amino acid sequence translated from the polynucleotide sequence in SEQ ID NO:1 and variants, fragments, homologues, analogues and derivatives thereof; or

(b) a polypeptide of SEQ ID NO:2 and variants, fragments, homologues, analogues and derivatives thereof.

There is also provided by the present invention said polypeptide fused with another human acid sensing ion channels (hASICs). Preferably, said another hASICs may be selected from the group consisting of hASIC1a, hASIC1b, hASIC2a, hASIC3, hAISC4, and their derivatives.

There is also provided an antibody against the polypeptides described above.

The present invention yet further provides a compound, which modulates the polypeptide described above. Preferably, the compound antagonizes or selectively antagonized the polypeptide. Alternatively, the compound agonizes the polypeptide.

According to another aspect of the present invention, there is provided a method of screening for substances capable of modulating the polypeptide described above, which comprises:

(a) contacting a substance to be tested with cells expressing at least one molecule of said polypeptide and optionally at least one molecule of an additional human acid sensing ion channel (hASIC) selected from the group consisting of hASIC1a, hASIC1b, hASIC2a, hASIC3, hAISC4, and their derivatives on their surface;

(b) measuring the effects of the substance on the transport functions of said polypeptide and/or at least one of said hASICs and derivatives; and

(c) identifying the substances that have a

positive or negative effect on the transport functions.

Preferably the substance to be tested is in a preselected amount.

According to another aspect of the present invention, there is provided a method of identifying a compound, which binds to and modulates the polypeptide described above, comprising contacting said polypeptide with a candidate compound and determining whether modulation occurs.

Preferably, said method comprises:

(a) contacting a compound with cells expressing at least one molecule of the polypeptide described above and optionally at least one molecule of an additional human acid sensing ion channel (hASIC) selected from the group consisting of hASIC1a, hASIC1b, hASIC2a, hASIC3, hAISC4, and their derivatives on their surface, said polypeptide or at least one of said hASICs or derivatives being associated with a second component capable of providing a detectable signal in response to the binding of a compound to said polypeptide or at least one of said hASICs or derivatives; said contacting being under conditions sufficient to permit binding of compounds to the polypeptide or at least one of said hASICs or derivatives; and

(b) identifying a compound capable of binding the polypeptide or at least one of said

hASICs or derivatives by detecting the signal produced by said second component.

Preferably the compound binds to and (i) antagonizes or selectively antagonizes the polypeptide described above, or (ii) agonizes the polypeptide of described above.

As hASICs are involved in cation transport, modulators (e.g. agonists or antagonists) of the polypeptide of the present invention can find use in interfering with the cation transport process.

Therefore, according to yet another embodiment of the present invention, there is provided the antibody or compound described above for use as a pharmaceutical.

Such antibodies, and compounds, etc., which can modulate the polypeptide of the present invention, can therefore find use in the therapeutic areas which concern aspects of cation transport. Therapeutically useful areas include, but are not limited to, disorders of perception of acidity with regard to nociception and taste transduction, pain, disorders of acid taste, neurodegeneration induced by hyperexpression of ASICs, cerebral neuronal degeneration, Alzheimer's disease, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis, cerebellar ataxia, inflammatory diseases, ischemia, and certain tumors.

Accordingly, there is also provided the use of the compound described above in the manufacture of a medicament for the treatment of a patient having need to modulate the polypeptide described above. Preferably, the treatment is for a patient having a need to antagonize or selectively antagonize the polypeptide. Alternatively, the treatment is for the treatment of a

patient having a need to agonize the polypeptide.

According to a yet further aspect of the invention, there is provided a method for the treatment of a patient having need to modulate the polypeptide comprising administering to the patient a therapeutically effective amount of the compound. Preferably, said method is for the treatment of a patient having a need to antagonize or selectively antagonize the polypeptide. Alternatively, said method is for the treatment of a patient having a need to agonize the polypeptide.

Preferably, said compound is a polypeptide and a therapeutically effective amount of the compound is administered by providing to the patient DNA encoding said compound and expressing said compound *in vivo*.

There is also provided, by the present invention, use of the antibody described above in the manufacture of a medicament for the treatment of a patient having a need to modulate the polypeptide described above. Preferably, said method is for the treatment of a patient having a need to antagonize or selectively antagonize the polypeptide. Alternatively, said method is for the treatment of a patient having a need to agonize the polypeptide.

Yet further provided by the present invention is a method for the treatment of a patient having a need to modulate the polypeptide described above, comprising administering to the patient a therapeutically effective amount of the antibody described above. Preferably, said method is for the treatment of a patient having a need to antagonize or selectively antagonize the polypeptide. Alternatively, said method is for the treatment of a patient having a need to agonize the polypeptide.

According to a yet further aspect of the present invention, there are provided cells genetically engineered *ex vivo* or *in vivo* to express, overexpress, underexpress or to exhibit targeted insertion or deletion of the polypeptide of the present invention. There is also provided by the present invention a transgenic non-human animal comprising such cells.

As discussed above, ASIC2b is considered a modulator subunit of acid sensing ion channels in brain and DRGs (29). RASIC2b is not active by itself, but it can associate with either rASIC2a or rASIC3 to modify their properties. For example, it confers non-selectivity to late $H^+$-induced current. RASIC2b is considered to interact with rASIC2a to form heteromultimers with new properties (29). It has also been shown that the rASIC3 current, like the native proton-gated current in dorsal root sensory neurons, consists of two components: a rapid inactivation current followed by a sustained current (31). It has also been shown that coexpression of rASIC2b and rASIC3 yields a current that looks like a rASIC3-like current (31). RASIC2b is present in sensory neurons where it modulates the expression of rASIC3. Coexpression of the two proteins yields a $H^+$-gated current that contains a non-selective sustained component. Thus, it is very probable that these two units, rASIC2b and rASIC3 are at least part of the native proton-gated cation channel of nociceptive neurons (1, 29, 38).

The amino acid sequence homologies of human ASICs are shown in Table 1 below.

Table 1. Amino acid sequence homologies of human ASICs

| | ASIC3 | ASIC1 | ASIC2a | ASIC2b | ASCI4 |
|---|---|---|---|---|---|
| ASIC3 | 100 | 48.0 | 48.7 | 45.6 | 48.6 |
| ASIC1 | | 100 | 72.0 | 60.3 | 49.7 |
| ASIC2a | | | 100 | 80.6 | 50.4 |
| ASIC2b | | | | 100 | 48.1 |
| ASIC4 | | | | | 100 |

Large changes in extracellular acidity are produced in the brain in the course of ischemia and epileptic seizures. Therefore, this class of ASIC-type channels will certainly be activated in these pathophysiological conditions. This activation would be expected to produce deleterious effects. The effects include cellular depolarization and a significant contribution to the well-known massive $Na^+$ entry, which occurs, especially in ischemia, when the ($Na^+$, $K^+$) ATPase will be less active in pumping $Na^+$ out because of intracellular ATP depletion. Blockers of the $H^+$-gated cation channels that are more specific than amiloride would be important in studying the role of those channels both in pain perception and in physiological and pathophysiological brain functions. Such specific inhibitors have not yet been available, but the search for such blockers will be greatly facilitated with the availability of cDNA clones including hASIC2b cDNA clones of the present invention.

The polypeptide of hASIC2b can be useful for developing a medicament for the treatment or prevention of pathologies entailing the painful perception of acidity found in inflammatory diseases, ischemia, and certain of tumors.

The present invention also provides the transformed cells expressing hASIC2b of the present invention and optionally at least one of other hASICs or their

derivatives. These cells are useful for screening candidate substances that are capable of modulating cation transport by these polypeptides and therefore the perception of acidity with regard to both nociception and taste transduction. This screening can be carried out by bringing a predetermined amount of a substance to be tested into contact with the cells (co)-expressing the hASIC channels and determining the effects of said substance on the currents of said cation channels. These screenings allow for the identification of new drugs that are useful in the treatment or prevention of pain such as analgesics. They also enable the identification of agents that modulate acid taste.

The substances that are isolated and detected by means of the methods described above are also part of the present invention. Thus, the present invention also provides a chemical or biological substance that is capable of modifying the currents of an ionic channel and/or a hybrid channel according to the present invention in the manufacture of a medicament capable of modulating the perception of acidity with regard to nociception as well as taste transduction in a human or animal subject.

The polynucleotide coding for hASIC2b of the present invention or derivative thereof, or a vector comprising the polynucleotide or a cell expressing hASIC2b is also useful for the preparation of non-human transgenic animals used in developing a new drug. These transgenic non-human animals can be those overexpressing or underexpressing said channels, but also "knock-out" animals either deficient in the expression of these channels or in the cation transport activity of these

channels. These non-human transgenic animals are prepared by the methods, *per se*, known in the art, and serve as live animal models in studying pathologies associated with ASIC channels.

The polynucleotide of the present invention or the cells transformed with said polynucleotide can also be used for genetic therapy to compensate for a deficiency in hASIC2b channel at a certain tissue of a patient. Thus, the present invention also provides a drug comprising the polynucleotide of the present invention or the cells transformed by said polynucleotide for the treatment of pathology involving hASIC2b or its derivatives.

In addition to the property of being activated by protons and the resultant applications described above relating to the perception of acidity, hASIC2b having genetic mutations may be involved in some neurodegenerative processes. The death of certain neurons is characteristic of many types of neuronal degenerative disorders such as Alzheimer's disease, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis, and cerebellar ataxia. Only a few deficient genes involved in such neurodegenative processes have been identified. The primitive neural network of the nematode *C. elegans* is a good model of neuronal development and death. The hereditary degeneration in *C. elegans* can be due to mutations of the genes deg-1, mec-4, and mec-10. ASIC2a is activated by the same mutations (27).

Therefore, the present invention provides a use of hASIC2b channel in studying these pathological modifications that may lead to neuronal degenerations.

The screening methods discussed above are useful for identifying substances that can block or inhibit neurodegeneration induced by overexpression or undrexpression of these channels. The ASIC channels have ionic properties in terms of selective permeability by sodium, potassium, lithium, and calcium. The selective permeability may cause excitotoxicity when said ASIC channels are hyperstimulated.

The polypeptide of hASIC2b, an agonist or antagonist of said protein can also be used in the manufacture of a medicament for the treatment of prevention of pathologies involving cerebral neuronal degenerations.

Other characteristics and advantages of the present invention will be seen in the Examples below related to research activities that led to the demonstration and the characterization of hASIC2b channel of the present invention, and in which reference will be made to the annexed sequences and figures.

## EXAMPLES

### EXAMPLE 1

### CLONING OF HUMAN ASIC2b cDNA

Total RNA samples isolated from human dorsal root ganglia (hDRG) was purchased from Analytical Biological Services Inc. (Wilmington, DE), and hDRG cDNA library that contains a total of 1.5 x $10^7$ clones of a size-fractionated (average length: 2.0 kb) oligo (dT)-primed was constructed in pCMVSPORT6 by Life Technologies Inc.

GENE TRAPPER III cDNA Positive Selection System (Life Technologies Inc.) was used to screen novel ASIC clones. Experiments were performed according to the manufacturer's instructions. Degenerate oligonucleotide

probes were designed by the alignment of four published human acid sensing ion channel (ASIC) polypeptide sequences (GenBank accession numbers: AF095897, AF057711, AB010575, and NM_001094). Three oligonucleotide probes (A1: 5'-TTY CCR GCN GTN ACC CCT STG YA-3' (SEQ ID NO: 3); A4: 5'-CTG GAC RTK CAN CAN GAN GAR T-3' (SEQ ID NO: 4); and A9: 5'-GGN YTK TTY ATH GGK GCY AG-3' (SEQ ID NO: 5)) were selected and used in the GENE TRAPPER III experiments and colony hybridization. The degenerate probes were biotinylated by TdT and Biotin-14-dCTP (Life Technlodies Inc.) at 30°C for 1 hr., and the biotinylation of oligonucleotide probes were confirmed by 15% TBE/Urea polyacrylamide gel electrophoresis (Novex). The single-stranded cDNA (ssDNA) was generated from the double-stranded hDRG cDNA library clones with Gene II and Exonuclease III (Life Technologies Inc.) at 30°C for 30 min. The biotinylated ologonucleotide and ssDNA were hybridized at 37°C for 1 hr. Streptavidin paramagnetic beads were added to the hybridization mixture to capture the ssDNA hybridized to the biotinylated probes at room temperature for 30 min. The captured ssDNA were repaired using TP-3000 thermal cycler (TaKaRa) and the Repair Enzymes (Life Technologies Inc.). Repair reaction was carried out with the thermal cycler for one cycle (denaturing step at 90°C for 1 min., annealing step at 55°C for 30 seconds, extension step at 70°C for 15 min. and soaking step at 4°C). *E. coli*. strain DH5α(Life Technologies Inc.) was transformed with repaired cDNAs, and tranferred onto Hybond-N (Amersham) filters prior to hybridization. The cDNA on filters were denatured in the denaturing solution (0.5N NaOH and 1.5M NaCl) at room temperature for 7 min. and neutralized twice in the

neutralizing solution (1.5M NaCl and 0.5M; Tris-HCl, pH adjusted to 7.5) at room temperature for 3 min. The filters were washed with 2x SSC at room temperature for 2 min. The denatured cDNAs on the filters were immobilized by using the CL-1000 ultraviolet cross linker (UVP).

The degenerate oligonucleotide probes were labeled at the 3'-end with fluorescein-dUTP using the Gene Images 3'-oligolabelling kit (Amersham) and hybidization was carried out in the ExpressHyb Hybridization Solution (CLONTECH) at 42°C for 1 hr. The filters were washed twice in 5x SSC with 0.1% SDS at room temperature for 5 min., then in 1x SSC with 0.1% SDS at 42°C for 15 min. Positive clones were selected using the Gene Images CDP-Star detection kit (Amersham) and LAS-1000 imaging system (Fuji Film) according to the manufacturer's instructions. Positive clones were picked up, and their nucleotide sequences were determined in the CEQ2000 DNA analyzer (Beckman). The sequences were analyzed by BLAST search. Among the clones belonging to the ASIC family, a novel splice variant of human (h)ASIC2 with a unique N-terminal 236 amino acids (aa) was discovered, which contained an open reading frame of 1,689 base pairs encoding a protein of 563 aa. This clone was designated as human ASIC2b. The nucleotide sequence and amino acid sequence of hASIC2b are shown in SEQ ID NO: 1.

## EXAMPLE 2

## EXPRESSION PROFILING HUMAN ASIC2b AND ASIC2a

Material and Methods

Expression of human ASIC2a and human ASIC2b transcripts were examined by Reverse Transcription-polymerase chain reaction. Total RNA samples from various human tissues (CLONTECH and ABS) were used in the

reverse transcription reaction. An aliquot of 2µg of total RNA was primed with oligo(dT)$_{12-18}$ and reverse-transcribed with SuperScript II® (Life Technologies Inc.) in a total volume of 20µl. Polymerase chain reaction was performed with 0.5µl of the first strand cDNA in a reaction volume of 20µl.

Primers used were (5'-3', sense/antisense)

hASIC2b: CTG CTC TCC TGC AAG TAC C (SEQ ID NO: 6) / AGC TCT TGG ATG AAA GGT GGC (SEQ ID NO: 7); and

hASIC2a: ACC ACC AAC GAC CTG TAC C (SEQ ID NO: 8) / AGA GGT TTG CCA TCC TCG C (SEQ ID NO: 9).

PCR was performed under the following conditions: PCR conditions were: hASIC2b (94 °C for 1 min; 35 cycles of 94 °C for 20 seconds, 56 °C for 20 seconds, 72 °C for 20 seconds; 72 °C for 5 min), and hASIC2a (94 °C for 1 min; 30 cycles of 94 °C for 20 seconds, 60 °C for 20 seconds, 72 °C for 20 seconds; 72 °C for 5 min). PCR amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was also performed as a control experiment. The sequences of GAPDH-specific primers are as follows:

(5'-3', sense/antisense) GTC TTC ACC ACC ATG GAG AAG GCT (SEQ ID NO: 10) / GTG ATG GCA TGG ACT GTG GTC ATG A (SEQ ID NO: 11).

One-half of the PCR products were electrophoresed on a 2% TAE-agarose gel, stained with ethidium bromide, and photographed under UV light.

Results

As can be seen from Figure 4, transcripts of hASIC2a and hASIC2b were detected in most human tissues examined. Expression of hASIC2a is equally distributed in all tissues examined, however, expression of hAISC2b is

highly expressed in neuronal tissues such as spinal cord, brain, and DRG, and adrenal gland and small intestine. These results suggest an important role of hASIC2b in neuronal functions.

## EXAMPLE 3

## FUNCTIONAL ANALYSIS OF hASIC2b

Materials and Methods

The mammalian expression vectors for hASIC2b and hASIC2a were constructed using appropriate expression vectors such as pcDNA3.1 (Clonetech) according to conventional molecular biological methods. Chinese Hamster Ovary (CHO)-K1 cells were seeded on a 35 mm dish in diameter at a density of 20,000 cells, and then transfected with various combinations of ASIC expression vectors with FuGENE6 transfection reagent (Roche) according to the manufacturer's instructions as follows: the hAISC2b expression vector alone (1μg) for homomeric hASIC2b expression, hASIC2a and green fluorescent protein (GFP) expression vectors (1:2 molar ratio in a total of 1μg) for hASIC2a expression, and hASIC2b/hASIC2a (1:2 molar ratio in a total of 1μg) for heteromeric expression. Cells were used for electrophysiological measurements 2 days after the transfection. Successfully transfected cells were recognized by GFP emission signal. Ion currents were recorded using whole-cell patch clamp technique. Recording was made with an Axopatch 200B amplifier (Axon Instruments). Currents were filtered at 5kHz and digitized by using Digidata 1321A interface. Data were interpolated using Origin6.0 (version 6.0, Microcal). Pipettes were pulled from borosilicate glass and had pipette resistances 1-4MΩ when filled with the

intercellular solution. All recordings were made at room temperature (23±2°C).

The intercellular solution contained 140mM CsCl, 1mM $MgCl_2$, 5mM EDTA and 10mM HEPES, pH 7.2. The extracellular solution contained 140mM NaCl, 5mM KCl, 1mM $MgCl_2$, 2mM $CsCl_2$ and 10mM Glucose and 10mM HEPES, pH 7.0-7.4. The extracellular solutions of pH less than 6.0 were buffered with 10mM MES, but other constituents were identical. The rapid changes in extracellular pH were performed using Rapid Solution Changes (Bio-Logic Co.,).

Results

As can be seen from Figure 5, hASIC2a and hASIC2b were expressed in CHO-K1 cells, and inward currents evoked by 5 sec application of low pH solution were recorded. In ASIC2a expressing cells, acid-induced inward currents were obtained at pH values (2.0-4.0) examined, however, no currents were obtained in ASIC2b expressing cells at any pH values. Thus, it was found that hASIC2b was inactive as an ion channels by itself. Next, hASIC2b was co-expressed with hASIC2a to see the effect on channel properties of hASIC2a. HASIC2a/hASIC2b co-expressing cells showed very small acid-sensing currents compared with hASIC2a expressing cells. These results suggest that hASIC2b exerts inhibitory effect on acid-induced ion currents.

The pH dependence of the acid-sensing currents of hASIC2a and hASIC2a/hASIC2b were examined by decreasing extracellular pH. The $pH_{50}$ value for activation of hASIC2a and hASIC2a/hASIC2b were 3.73±0.09 (n=4) and 3.43±0.17 (n=4), respectively (Figure 6A). There were no significant changes in sensitivity to acidic stimuli

by co-expression of hASIC2a with hASIC2b. Next, as shown in Figure 6B, peak current density was compared between hASIC2a and hASIC2a/hASIC2b transformants at pH2.0, 3.0, and pH4.0.(hASIC2a ; 752.6±140.6 pA/pF at pH2.0, 619.6± 116.8 pA/pF at pH3.0, 284.4±77.7 pA/pF at pH4.0, hASIC2a/hASIC2b ; 112.8±16.3 pA/pF at pH2.0, 88.9±12.0 pA/pF at pH3.0, 21.0±4.83 pA/pF at pH4.0)

A series of studies performed here demonstrated the inhibitory role of hASIC2b in acid-induced currents generated by hASIC2a. That suggests a critical role of hASIC2b in regulating acid-induced currents in the health and disease conditions of human physiological systems.

All documents cited herein, including patents and patent applications, are hereby incorporated by reference.

It will be appreciated that the foregoing is provided by way of example only and modification of details may be made without departing from the scope of the invention.

REFERENCES


1. Bevan, S. & Yeats, J. *J. Physiol.* 433:145-161 (1991)
2. Krishtal, O.A. & Pidoplichko, V.I. *Brain Res.* 214: 150-154 (1981)
3. Akaike, N., Krishtal, O.A. & Maruyama, T. J. Neurophysiol. 63, 805-813 (1990)
4. Kovalchuk, Yu, N., Krishtal, O.A. & Nowycky, M.C. *Neurosci. Lett.* 115: 237-242 (1990)
5. Davies, N.W., Lux, H.D. & Morad, M. *J. Physiol.* 400, 159-187 (1988)
6. Krishtal, O.A. & Pidoplichko, V.I. *Neuroscience* 6: 2599-2601 (1981)
7. Akaike, N. & Ueno, S. *Prog. Neurobiol.* 43: 73-83 (1994)
8. Ueno, S., Nakaye, T., & Akaike, N. *J. Physiol.* 447: 309-327 (1992)
9. Grantyn, R., & Lux, H.D. *Neurosci. Lett.* 89: 198-203 (1988)
10. Sonthebier., H., Perquansky, M, Hoppe, D., Lux, H.D., Gratyn, R. & Kettenmann, H. *J. Neurosci. Res.* 24: 496-500 (1989)
11. Reeh, P.W. & Steen, K.H. *Prog. Brain. Res.* 113: 143-151 (1996)
12. Steen, K.H., Issberner, U. & Reeh, P.W. *Neurosci. Lett* 199: 29-32 (1995)
13. Canessa, C.M., Horisberger, J.D. & Rossier, B.C. *Nature* 361: 467-470 (1993)
14. Lingueglia, E., Voilley , N., Waldmann, H., Lazdunski, M. & Barbry, P. *Febs Lett.* 318: 95-99 (1993)
15. Canessa, C.M., Schild, L., Buell, G., Thorens, B., Gautschi, I., Horisberger, J.D. & Rossier, B.C.

*Nature* 367: 463-467 (1994)

16. Lingueglia, E., Renard, S., Waldmann, R., Voilley, N., Champigny, G., Plass, H., Lazdunski, M. & Barbry, P., *J. Biol. Chem.* 269: 13736-13739 (1994)
17. Waldmann, R., Champigny, G., Bassilana, F., Voilley, N. & Lazdunski, M. *J. Biol. Chem.* 270: 27411-27414 (1995)
18. Chalfie, M. & Woeinsky, E. *Nature* 345:410-416 (1990)
19. Driscoll, M. & Chalfie, M. *Nature* 349: 588-593 (1991)
20. Huang, M. & Chalfie, M. *Nature* 367: 467-470 (1994)
21. Tavernarakis, N., Shreffler, W., Wang, S. & Driscoll, M. *Neuron* 18: 107-119 (1997)
22. Lad, C.C., Hong, K., Kryneli, M, Chalfie, M. & Driscoll, M. *J. Cell. Biol.* 133: 1071-1081 (1996)
23. Lingueglia, E., Champigny, G., Lazdunski, M. & Barbry, P. *Nature* 378: 730-733 (1995)
24. Waldmann, R., Champigny, G., Bassilana, F., Heurteaux, C. & Lazdunski, M. *Nature* 386: 173-177 (1997)
25. Renard, S., Lingueglia, E., Voilley, N., Lazdunski, M. & Barbry, P. *J. Biol. Chem.* 269, 12981-12986 (1994)
26. Coscoy, S., Lingueglia, E., Lazdunski, M. & Barbry, P. *J. Biol. Chem.* 273: 8317-8322 (1998)
27. Waldmann, R., Champigny, G., Voilley, N., Lauritzen, I. & Lazdunski, M. *J. Biol. Chem.* 271, 10433-10436 (1996)
28. Price, M.P., Snyder, P.M. & Welsh, M.J. *J. Biol. Chem.* 271: 7879-7882 (1996)
29. Lingueglia, E., De Weille, J.R., Bassilana, F., Heurteaux, C., Sakai, H., Waldmann, R. & Lazdunski,

M. *J. Biol. Chem.* 272: 29778-29783 (1997)

30. Champigny, G., Voilley, R., Waldmann, R. & Lazdunski, M. *J. Biol. Chem.* 273: 15418-15422 (1998)

31. Waldmann, R., Bassilana, F., De Weille, J., Champigny, G., Heurteaux, C. & Lazdunski, M. *J. Biol. Chem.* 272: 20975-20978 (1997)

32. Garcia-Anoveros, J., Derfler, B., Neville-Golden, J., Hyman, B.T. & Corey, D.P. *Proc. Natl. Acad. Sci.* USA 94: 1459-1464 (1997)

33. Bassilana, F., Champigny, G., Waldmann, R., De Weille, J.R., Heurteaux, C. & Lazdunski, M. *J. Biol. Chem.* 272: 28819-28822 (1997)

34. Chesler, M. & Kaila, K. *Trends. Neurosci.* 15: 396-402 (1992)

35. Krishtal, O.A., Osipchuk, Y.V., Shelest, T.N. & Smirnoff, S.V. *Brain Res.* 436: 352-356 (1987)

36. Nauyen, M.L. & Parsans, S.M. *J. Neurochem.* 64: 1137-1142 (1995)

37. Wolosker, H., De Souza, D.O. & De Meis, L. *J. Biol. Chem.* 271: 11726-11731 (1996)

38. Waldmann, R., Champigny, G., Lingueglia, E., De Weille, J.R., Heurteaux, C. & Lazdunski, M. *Annals New York Academy Of Sciences* 67-76 (April 30, 1999)

39. Stefan Gruender, Hyun-Soon Geissler, Eva-Lotta Baessler, Peter Ruppersberg, *NeuroReport*, Vo.11, No. 85: 1607-16211 (June, 2000)

CLAIMS

1. A polynucleotide comprising one or more of:

(a) a polynucleotide encoding the polypeptide as set forth in SEQ ID NO:2;

(b) a polynucleotide comprising a nucleotide sequence of SEQ ID NO:1;

(c) a polynucleotide comprising a nucleotide sequence that has at least 70% identity to the polynucleotide of (a) or (b);

(d) a polynucleotide comprising a nucleotide sequence which is capable of hybridizing to the polynucleotide of any one of (a) to (c);

(e) a complement to the polynucleotide of any one of (a) to (d); or

(f) a polynucleotide fragment of the polynucleotide of any one of (a) to (e).

2. The polynucleotide according to claim 1, encoding a human acid sensing ion channel (ASIC) 2b.

3. A vector comprising the polynucleotide according to claim 1 or 2.

4. A host cell transformed or transfected with the vector according to claim 3.

5. Transcribed RNA product of the polynucleotide according to claim 1 or 2.

6. An RNA molecule or fragment thereof which is antisense in relation to the RNA product of claim 5 and is capable of hybridizing thereto.

7. A ribozyme or zinc finger protein capable of binding the polynucleotide according to claim 1 or 2.

8. A process of producing a polypeptide or

fragment thereof comprising culturing the transformed/transfected host cell according to claim 4 under conditions sufficient for the expression of said polypeptide or fragment.

9. A process of producing cells capable of expressing a polypeptide or fragment thereof comprising transforming or transfecting cells with the vector according to claim 3.

10. Cells produced by the process according to claim 9.

11. A membrane preparation of the cells according to claim 10.

12. A polypeptide or a fragment thereof produced by the process according to claim 8 or 9.

13. A polypeptide comprising:

(a) a polypeptide having the deduced amino acid sequence translated from the polynucleotide sequence in SEQ ID NO:1 or variants, fragments, homologues, analogues and derivatives thereof; or

(b) a polypeptide of SEQ ID NO:2 and variants, fragments, homologues, analogues or derivatives thereof.

14. The polypeptide according to claim 13 fused with an additional human acid sensing ion channel (hASIC) selected from the group consisting of hASIC1a, hASIC1b, hASIC2a, hASIC3, hAISC4, or their derivatives.

15. An antibody against the polypeptide according to claim 13.

16. A compound, which modulates the polypeptide according to claim 13.

17. A method of screening for substances capable of

modulating the polypeptide according to claim 13, which comprises:

(a) contacting a substance to be tested with cells expressing at least one molecule of the polypeptide according to claim 13 and optionally at least one molecule of an additional human acid sensing ion channel (hASIC) selected from the group consisting of hASIC1a, hASIC1b, hASIC2a, hASIC3, hAISC4, or their derivatives on their surface;

(b) measuring the effects of the substance on the transport functions of said polypeptide or at least one of said hASICs or derivatives; and

(c) identifying the substances that have a positive or negative effect on the transport functions.

18. A method of identifying a compound, which binds to and modulates the polypeptide according to claim 13 comprising contacting said polypeptide with a candidate compound and determining whether modulation occurs.

19. The method according to claim 17, which comprises:

(a) contacting a compound with cells expressing at least one molecule of the polypeptide according to claim 13 and optionally at least one molecule of an additional human acid sensing ion channel (hASIC) selected from the group consisting of hASIC1a, hASIC1b, hASIC2a, hASIC3, hAISC4, or their derivatives on their

surface, said polypeptide or at least one of said hASICs or derivatives being associated with a second component capable of providing a detectable signal in response to the binding of a compound to said polypeptide or at least one of said hASICs or derivatives; said contacting being under conditions sufficient to permit binding of compounds to the polypeptide or at least one of said hASICs or derivatives; and

(b) identifying a compound capable of binding the polypeptide or at least one of said hASICs or derivatives by detecting the signal produced by said second component.

20. Use of the compound according to claim 16 in the manufacture of a medicament for the treatment of a patient having a need to modulate the polypeptide according to claim 13.

21. A method for the treatment of a patient having a need to modulate the polypeptide according to claim 13 comprising administering to the patient a therapeutically effective amount of the compound according to claim 16.

22. The method according to claim 21, wherein said compound is a polypeptide and a therapeutically effective amount of the compound is administered by providing to the patient DNA encoding said compound and expressing said compound *in vivo*.

23. Use of the antibody according to claim 15 in the manufacture of a medicament for the treatment of a patient having a need to modulate the polypeptide according to claim 13.

24. A method for the treatment of a patient having need to modulate the polypeptide according to claim 13, comprising administering to the patient a therapeutically effective amount of the antibody according to claim 15.

25. Cells genetically engineered *ex vivo* or *in vivo* to express, overexpress, underexpress or to exhibit targeted insertion or deletion of the polypeptide according to claim 13.

26. A transgenic non-human animal comprising cells according to claim 25.

# Fig.1A

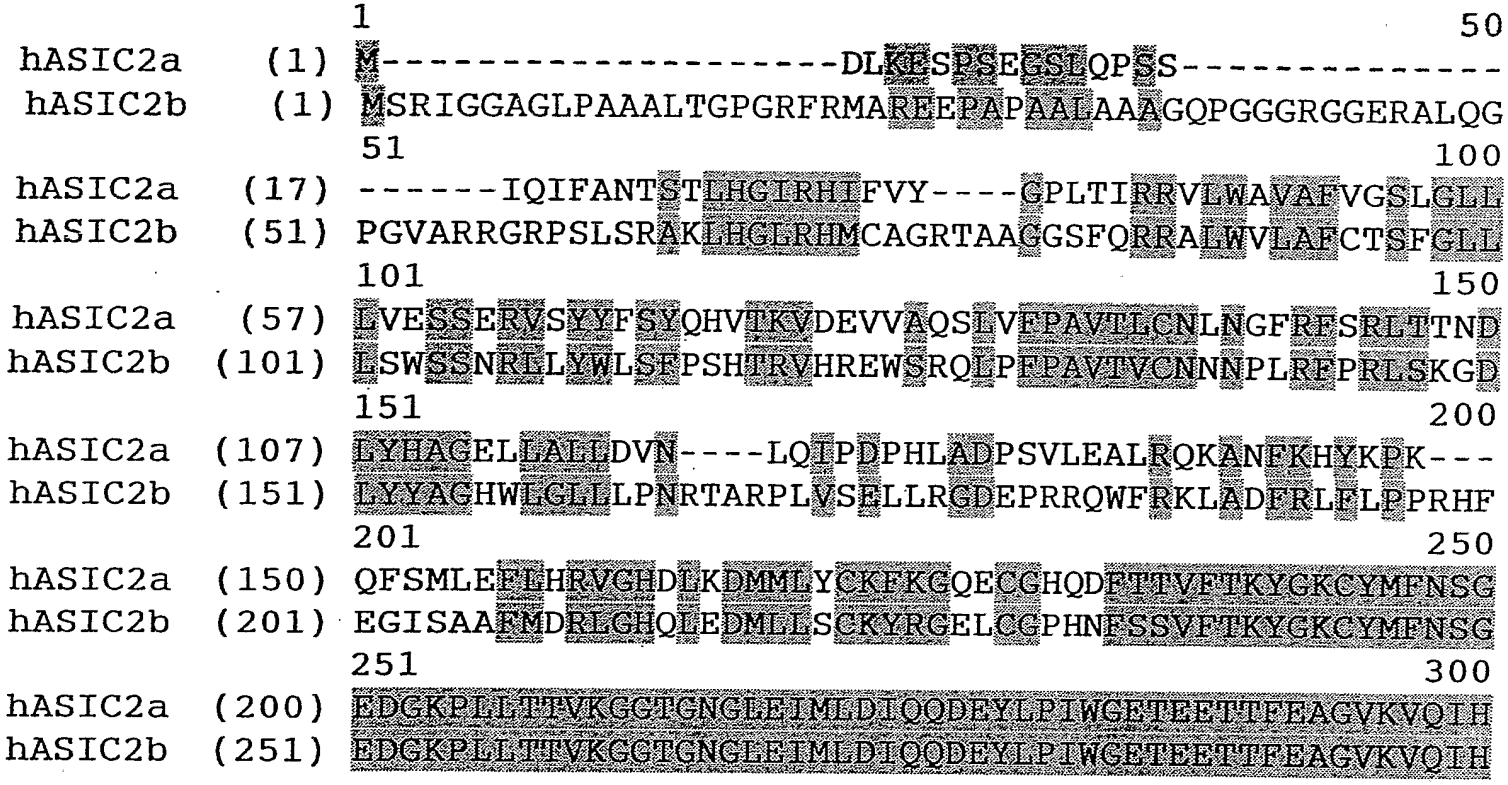
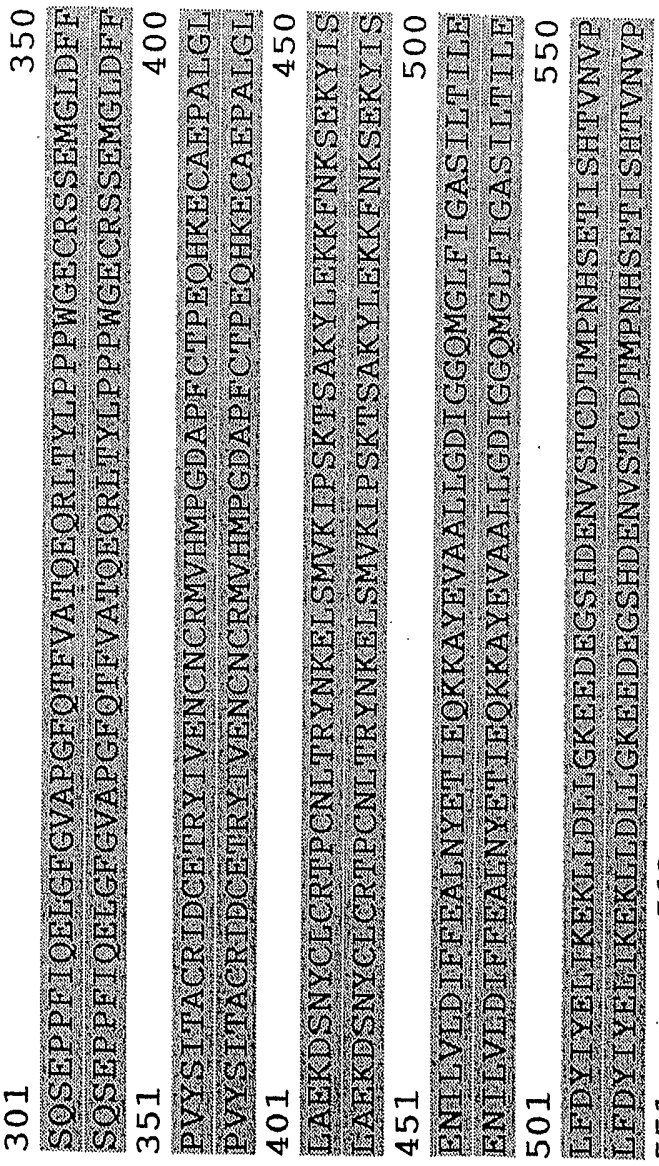

1 50
hASIC2a (1) M----------DLKESPSEGSLQPSS----------
hASIC2b (1) MSRIGGAGLPAAALTGPGRFRMAREEPAPAALAAAGQPGGGRGGERALQG
51 100
hASIC2a (17) ------IQIFANTSTLHGIRHIFVY----GPLTIRRVLWAVAFVGSLGLL
hASIC2b (51) PGVARRGRPSLSRAKLHGLRHMCAGRTAAGGSFQRRALWVLAFCTSFGLL
101 150
hASIC2a (57) LVESSERVSYYFSYQHVTKVDEVVAQSLVFPAVTLCNLNGFRESRLTTND
hASIC2b (101) LSWSSNRLLYWLSFPSHTRVHREWSRQLPFPAVTVCNNNPLREPRLSKGD
151 200
hASIC2a (107) LYHAGELLALLDVN----LQIPDPHLADPSVLEALRQKANFKHYKPK---
hASIC2b (151) LYYAGHWLGLLLPNRTARPLVSELLRGDEPRRQWFRKLADFRLFLPPRHF
201 250
hASIC2a (150) QFSMLEFLHRVGHDLKDMMLYCKFKGQECGHQDFTTVFTKYGKCYMFNSG
hASIC2b (201) EGISAAFMDRLGHQLEDMLLSCKYRGELCGPHNFSSVFTKYGKCYMFNSG
251 300
hASIC2a (200) EDGKPLLTTVKGGTGNGLEIMLDIQQDEYLPIWGETEETTFEAGVKVQIH
hASIC2b (251) EDGKPLLTTVKGGTGNGLEIMLDIQQDEYLPIWGETEETTFEAGVKVQIH

Fig.1B


301
350
hASIC2a (250) SQSEPPEIQELGFGVAPGFQTFVATQEQRLTYLPPPWGECRSSEMGLDFF
hASIC2b (301) SQSEPPEIQELGFGVAPGFQTFVATQEQRLTYLPPPWGECRSSEMGLDFF
351
400
hASIC2a (300) PVYSTTACRTDCETRYIVENCNCRMVHMPGDAPFCTPEQHKECAEPALGL
hASIC2b (351) PVYSTTACRTDCETRYIVENCNCRMVHMPGDAPFCTPEQHKECAEPALGL
401
450
hASIC2a (350) LAEKDSNYCLGRTPCNLTRYNKELSMVKIPSKTSAKYLEKKFNKSEKYIS
hASIC2b (401) LAEKDSNYCLGRTPCNLTRYNKELSMVKIPSKTSAKYLEKKFNKSEKYIS
451
500
hASIC2a (400) ENLVLDIFFEALNYETIEQKKAYEVAALLGDIGGQMGLFIGASILTILE
hASIC2b (451) ENLVLDIFFEALNYETIEQKKAYEVAALLGDIGGQMGLFIGASILTILE
501
550
hASIC2a (450) LFDYIYELIKEKLLDLLGKEEDEGSHDENVSTCDTMPNHSETISHTVNVP
hASIC2b (501) LFDYIYELIKEKLLDLLGKEEDEGSHDENVSTCDTMPNHSETISHTVNVP
551
563
hASIC2a (500) LQTTLGTLEEIAC
hASIC2b (551) LQTTLGTLEEIAC

# Fig.2A

```
cccgggatcg ggcgcacgct tccctggcgc gggctgcgcg cggctgccgc tgctgctgct      60
tccactgctg ctgcggactc ccatggcggc tccgcccggc cggggccgcc gccgctgccg     120
ccagccgcgg gctgaatga atg agc cgg att ggc gga gcc ggg ctg ccc gca       172
                    Met Ser Arg Ile Gly Gly Ala Gly Leu Pro Ala
                      1               5                  10
gcc gcg ctc acc ggc ccg gga cgc ttc cgc atg gcc cgc gag gag ccg       220
Ala Ala Leu Thr Gly Pro Gly Arg Phe Arg Met Ala Arg Glu Glu Pro
            15                  20                  25
gcg ccc gcg gcg ttg gcg gct gcc ggg cag ccc ggg ggc ggc aga ggc       268
Ala Pro Ala Ala Leu Ala Ala Ala Gly Gln Pro Gly Gly Gly Arg Gly
        30                  35                  40
ggc gag cgg gcg ctg cag ggg cca ggg gtc gcc cgc agg ggg cgg cca       316
Gly Glu Arg Ala Leu Gln Gly Pro Gly Val Ala Arg Arg Gly Arg Pro
    45                  50                  55
```

# Fig.2B

```
tcg ctg agc cgc gct aaa ctg cac ggg ctg cgg cac atg tgt gcc ggg      364
Ser Leu Ser Arg Ala Lys Leu His Gly Leu Arg His Met Cys Ala Gly
 60                  65                  70                  75
cgc acg gcg gct ggg ggc tcc ttc cag cgg cgg gcg ctg tgg gtg ctg      412
Arg Thr Ala Ala Gly Gly Ser Phe Gln Arg Arg Ala Leu Trp Val Leu
                 80                  85                  90
gcc ttc tgt aca tcc ttc ggc ttg ctg ctg tcc tgg tcc tcg aac cgt      460
Ala Phe Cys Thr Ser Phe Gly Leu Leu Leu Ser Trp Ser Ser Asn Arg
            95                 100                 105
ttg ctc tac tgg ctc agc ttc ccg tca cac acg cgg gtg cac cgc gag      508
Leu Leu Tyr Trp Leu Ser Phe Pro Ser His Thr Arg Val His Arg Glu
        110                 115                 120
tgg agc cgc cag tta ccc ttc ccc gcc gtc act gtg tgc aac aac aac      556
Trp Ser Arg Gln Leu Pro Phe Pro Ala Val Thr Val Cys Asn Asn Asn
    125                 130                 135
ccg ctg cgc ttc ccg cgc ctc tcc aag ggg gac ctc tac tat gcc ggc      604
Pro Leu Arg Phe Pro Arg Leu Ser Lys Gly Asp Leu Tyr Tyr Ala Gly
140                 145                 150                 155
```

# Fig.2C

```
cac tgg ctc ggg ctg ctg ctg ccc aac cgc acc gcg cgc ccg ctt gtc        652
His Trp Leu Gly Leu Leu Leu Pro Asn Arg Thr Ala Arg Pro Leu Val
                160                 165                 170
agc gag ctg ctg cgg ggc gac gag ccg cgc cgc cag tgg ttc cgc aag        700
Ser Glu Leu Leu Arg Gly Asp Glu Pro Arg Arg Gln Trp Phe Arg Lys
            175                 180                 185
ctg gcg gac ttc cgc ctc ttc ctg cct ccg cgc cac ttc gag gga atc        748
Leu Ala Asp Phe Arg Leu Phe Leu Pro Pro Arg His Phe Glu Gly Ile
        190                 195                 200
agc gcc gcc ttc atg gac cgc ctg ggc cac cag ctg gag gac atg ctg        796
Ser Ala Ala Phe Met Asp Arg Leu Gly His Gln Leu Glu Asp Met Leu
    205                 210                 215
ctc tcc tgc aag tac cgc ggc gag ctc tgc ggg ccg cac aac ttc tcc        844
Leu Ser Cys Lys Tyr Arg Gly Glu Leu Cys Gly Pro His Asn Phe Ser
220                 225                 230                 235
```

# Fig.2D

```
tcc gtg ttt aca aaa tat ggg aag tgt tac atg ttt aac tca ggc gag      892
Ser Val Phe Thr Lys Tyr Gly Lys Cys Tyr Met Phe Asn Ser Gly Glu
                240                 245                 250
gat ggc aaa cct ctg ctc acc acg gtc aag ggg ggg aca ggc aac ggg      940
Asp Gly Lys Pro Leu Leu Thr Thr Val Lys Gly Gly Thr Gly Asn Gly
            255                 260                 265
ctg gag atc atg ctg gac att cag cag gat gag tac ctg ccc atc tgg      988
Leu Glu Ile Met Leu Asp Ile Gln Gln Asp Glu Tyr Leu Pro Ile Trp
        270                 275                 280
gga gag aca gag gaa acg aca ttc gaa gca gga gtg aaa gtt cag atc      1036
Gly Glu Thr Glu Glu Thr Thr Phe Glu Ala Gly Val Lys Val Gln Ile
    285                 290                 295
cac agt cag tct gag cca cct ttc atc caa gag ctg ggc ttt ggg gtg      1084
His Ser Gln Ser Glu Pro Pro Phe Ile Gln Glu Leu Gly Phe Gly Val
300                 305                 310                 315
gct cca ggg ttc cag acc ttt gtg gcc aca cag gag cag agg ctc aca      1132
Ala Pro Gly Phe Gln Thr Phe Val Ala Thr Gln Glu Gln Arg Leu Thr
                320                 325                 330
```

# Fig.2E

```
tac ctg ccc cca ccg tgg ggt gag tgc cga tcc tca gag atg ggc ctc      1180
Tyr Leu Pro Pro Pro Trp Gly Glu Cys Arg Ser Ser Glu Met Gly Leu
            335                 340                 345
gac ttt ttt cct gtt tac agc atc acc gcc tgt agg att gac tgt gag      1228
Asp Phe Phe Pro Val Tyr Ser Ile Thr Ala Cys Arg Ile Asp Cys Glu
        350                 355                 360
acc cgc tac att gtg gaa aac tgc aac tgc cgc atg gtt cac atg cca      1276
Thr Arg Tyr Ile Val Glu Asn Cys Asn Cys Arg Met Val His Met Pro
    365                 370                 375
ggg gat gcc cct ttt tgt acc cct gag cag cac aag gag tgt gca gag      1324
Gly Asp Ala Pro Phe Cys Thr Pro Glu Gln His Lys Glu Cys Ala Glu
380                 385                 390                 395
cct gcc cta ggt ctg ttg gcg gaa aag gac agc aat tac tgt ctc tgc      1372
Pro Ala Leu Gly Leu Leu Ala Glu Lys Asp Ser Asn Tyr Cys Leu Cys
                400                 405                 410
agg aca ccc tgc aac cta acc cgc tac aac aaa gag ctc tcc atg gtg      1420
Arg Thr Pro Cys Asn Leu Thr Arg Tyr Asn Lys Glu Leu Ser Met Val
            415                 420                 425
```

# Fig.2F

```
aag atc ccc agc aag aca tca gcc aag tac ctt gag aag aaa ttt aac      1468
Lys Ile Pro Ser Lys Thr Ser Ala Lys Tyr Leu Glu Lys Lys Phe Asn
        430                 435                 440
aaa tca gaa tat ctc atc tca gag aac atc ctt gtt ctg gat ata ttt      1516
Lys Ser Glu Lys Tyr Ile Ser Glu Asn Ile Leu Val Leu Asp Ile Phe
    445                 450                 455
ttt gaa gct ctc aat tat gag aca att gaa cag aag aag gcg tat gaa      1564
Phe Glu Ala Leu Asn Tyr Glu Thr Ile Glu Gln Lys Lys Ala Tyr Glu
460                 465                 470                 475
gtt gct gcc tta ctt ggt gat att ggt ggt cag atg gga ttg ttc att      1612
Val Ala Ala Leu Leu Gly Asp Ile Gly Gly Gln Met Gly Leu Phe Ile
                480                 485                 490
ggt gct agt atc ctt aca ata cta gag ctc ttt gat tat att tat gag      1660
Gly Ala Ser Ile Leu Thr Ile Leu Glu Leu Phe Asp Tyr Ile Tyr Glu
            495                 500                 505
```

Fig.2G

```
ctg atc aaa gag aag cta tta gac ctg ctt ggc aaa gag gag gac gaa   1708
 Leu Ile Lys Glu Lys Leu Leu Asp Leu Leu Gly Lys Glu Glu Asp Glu
     510                 515                 520

ggg agc cac gat gag aat gtg agt act tgt gac aca atg cca aac cac   1756
 Gly Ser His Asp Glu Asn Val Ser Thr Cys Asp Thr Met Pro Asn His
 525                 530                 535

tct gaa acc atc agt cac act gtg aac gtg ccc ctg cag acg acc ctg   1804
 Ser Glu Thr Ile Ser His Thr Val Asn Val Pro Leu Gln Thr Thr Leu
 540                 545                 550                 555

ggg acc ttg gag gag att gcc tgc tga caccctcga gtcacccagc actccct   1858
 Gly Thr Leu Glu Glu Ile Ala Cys Stop
                 560
```

# Fig.2H

```
ccaaacagac cttgaggccc aagacccagg acaaggaaca gcaagctcag gtgggatggc   1918
cccagtgctg gaaagaagca agagcccccc atgcacacat tgcagactag ctgcctagac   1978
ctcgctccgg ccacgtccaa cacgacgcat ccttgggccc cgccgtgcgt ccctcttagg   2038
agagatgagt cacactctgg aactgtccaa gaacgaacct gccatcacat ctcactgcca   2098
gatgtataaa gcacctgcat gctcagactt cttgtggcgc cacctccacg tctgtcttgt   2158
acatgacact cctccacgcg gtttccagtg tccacactgc tgcccgtgca gtgggaccag   2218
attccaggtc caaagtcacc atgaggccac cctggaatca gaactgcaca atcaagaggg   2278
aacccatggg actctctgct acattcagtt cttgtgtcgt ttgtgaaagt tcttaacctg   2338
cccaaaaacc ccctttccc caagctgccc atggggcttc ggcgccaaag gtgacccgcg   2398
ccaacctccc tccccccag tgcctatgac ggcggcacag cagccagcgg gtgggggacg   2458
cctgtgttca cccatggtgc ccatgtcgtt cttctctccc tgtgacacag cttgtacagt   2518
ctgattcttt ttatctgggg taggggggct tttatgtttg tccgatggag atttgttttg   2578
ttttgcttca ttttatgctt ttttatttta gttttgatgt tctgaggttt gctttggttt   2638
ttccattttc tttggcattt atttattcgt gcttcaaatc acagtcatat taaaagctgg   2698
tcttgtggaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaa              2746
```

# Fig.3A

```
Met Ser Arg Ile Gly Gly Ala Gly Leu Pro Ala Ala Ala Leu Thr Gly
 1               5                  10                  15
Pro Gly Arg Phe Arg Met Ala Arg Glu Glu Pro Ala Pro Ala Ala Leu
            20                  25                  30
Ala Ala Ala Gly Gln Pro Gly Gly Gly Arg Gly Gly Glu Arg Ala Leu
        35                  40                  45
Gln Gly Pro Gly Val Ala Arg Arg Gly Arg Pro Ser Leu Ser Arg Ala
    50                  55                  60
Lys Leu His Gly Leu Arg His Met Cys Ala Gly Arg Thr Ala Ala Gly
65                  70                  75                  80
Gly Ser Phe Gln Arg Arg Ala Leu Trp Val Leu Ala Phe Cys Thr Ser
                85                  90                  95
Phe Gly Leu Leu Leu Ser Trp Ser Ser Asn Arg Leu Leu Tyr Trp Leu
            100                 105                 110
Ser Phe Pro Ser His Thr Arg Val His Arg Glu Trp Ser Arg Gln Leu
        115                 120                 125
Pro Phe Pro Ala Val Thr Val Cys Asn Asn Asn Pro Leu Arg Phe Pro
    130                 135                 140
```

# Fig.3B

```
Arg Leu Ser Lys Gly Asp Leu Tyr Tyr Ala Gly His Trp Leu Gly Leu
145                 150                 155                 160
Leu Leu Pro Asn Arg Thr Ala Arg Pro Leu Val Ser Glu Leu Leu Arg
                165                 170                 175
Gly Asp Glu Pro Arg Arg Gln Trp Phe Arg Lys Leu Ala Asp Phe Arg
            180                 185                 190
Leu Phe Leu Pro Pro Arg His Phe Glu Gly Ile Ser Ala Ala Phe Met
        195                 200                 205
Asp Arg Leu Gly His Gln Leu Glu Asp Met Leu Leu Ser Cys Lys Tyr
    210                 215                 220
Arg Gly Glu Leu Cys Gly Pro His Asn Phe Ser Ser Val Phe Thr Lys
225                 230                 235                 240
Tyr Gly Lys Cys Tyr Met Phe Asn Ser Gly Glu Asp Gly Lys Pro Leu
                245                 250                 255
Leu Thr Thr Val Lys Gly Gly Thr Gly Asn Gly Leu Glu Ile Met Leu
            260                 265                 270
Asp Ile Gln Gln Asp Glu Tyr Leu Pro Ile Trp Gly Glu Thr Glu Glu
        275                 280                 285
```

# Fig.3C

```
Thr Thr Phe Glu Ala Gly Val Lys Val Gln Ile His Ser Gln Ser Glu
    290                 295                 300
Pro Pro Phe Ile Gln Glu Leu Gly Phe Gly Val Ala Pro Gly Phe Gln
305                 310                 315                 320
Thr Phe Val Ala Thr Gln Glu Gln Ang Leu Thr Tyr Leu Pro Pro Pro
                325                 330                 335
Trp Gly Glu Cys Arg Ser Ser Glu Met Gly Leu Asp Phe Phe Pro Val
            340                 345                 350
Tyr Ser Ile Thr Ala Cys Arg Ile Asp Cys Glu Thr Arg Tyr Ile Val
        355                 360                 365
Glu Asn Cys Asn Cys Arg Met Val His Met Pro Gly Asp Ala Pro Phe
    370                 375                 380
Cys Thr Pro Glu Gln His Lys Glu Cys Ala Glu Pro Ala Leu Gly Leu
385                 390                 395                 400
Leu Ala Glu Lys Asp Ser Asn Tyr Cys Leu Cys Arg Thr Pro Cys Asn
                405                 410                 415
Leu Thr Arg Tyr Asn Lys Glu Leu Ser Met Val Lys Ile Pro Ser Lys
            420                 425                 430
```

# Fig.3D

```
Thr Ser Ala Lys Tyr Leu Glu Lys Lys Phe Asn Lys Ser Glu Lys Tyr
        435                 440                 445
Ile Ser Glu Asn Ile Leu Val Leu Asp Ile Phe Phe Glu Ala Leu Asn
    450                 455                 460
Tyr Glu Thr Ile Glu Gln Lys Lys Ala Tyr Glu Val Ala Ala Leu Leu
465                 470                 475                 480
Gly Asp Ile Gly Gly Gln Met Gly Leu Phe Ile Gly Ala Ser Ile Leu
                485                 490                 495
Thr Ile Leu Glu Leu Phe Asp Tyr Ile Tyr Glu Leu Ile Lys Glu Lys
            500                 505                 510
Leu Leu Asp Leu Leu Gly Lys Glu Glu Asp Glu Gly Ser His Asp Glu
        515                 520                 525
Asn Val Ser Thr Cys Asp Thr Met Pro Asn His Ser Glu Thr Ile Ser
    530                 535                 540
His Thr Val Asn Val Pro Leu Gln Thr Thr Leu Gly Thr Leu Glu Glu
545                 550                 555                 560
Ile Ala Cys
```

Fig.4

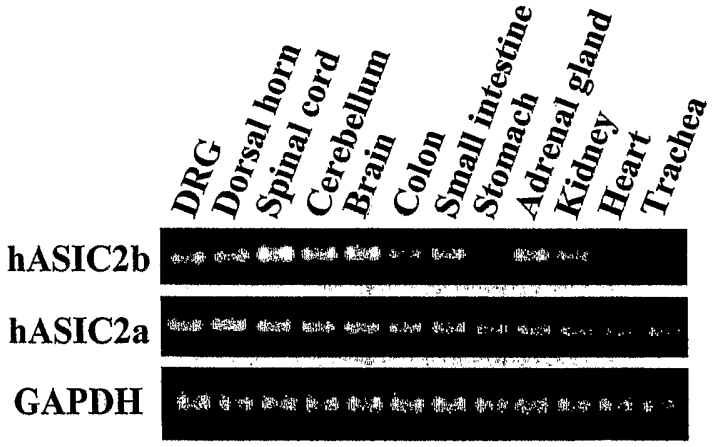

DRG
Dorsal horn
Spinal cord
Cerebellum
Brain
Colon
Small intestine
Stomach
Adrenal gland
Kidney
Heart
Trachea
hASIC2b
hASIC2a
GAPDH

Fig. 5

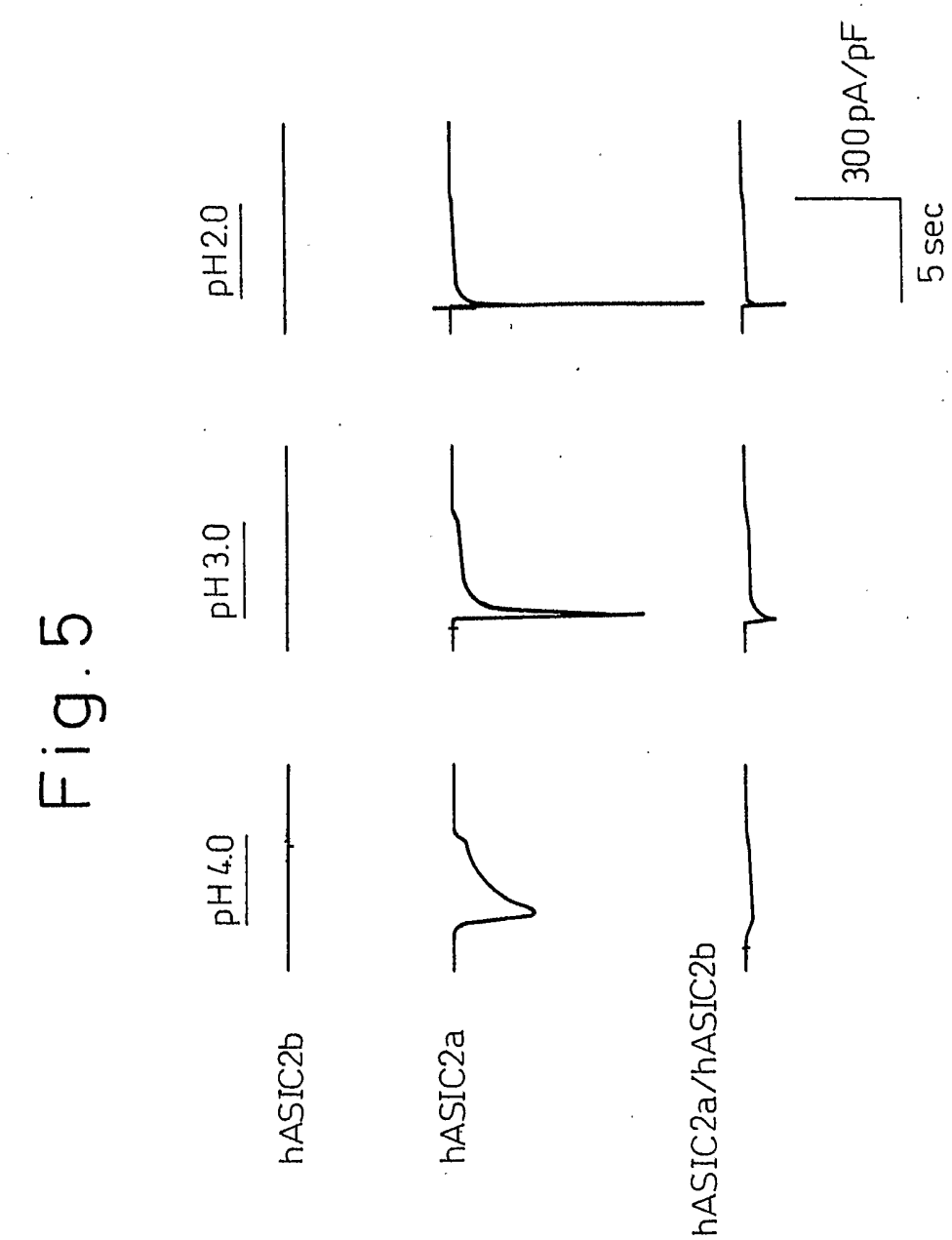

pH 4.0
pH 3.0
pH 2.0
hASIC2b
hASIC2a
hASIC2a/hASIC2b
300 pA/pF
5 sec

Fig.6A

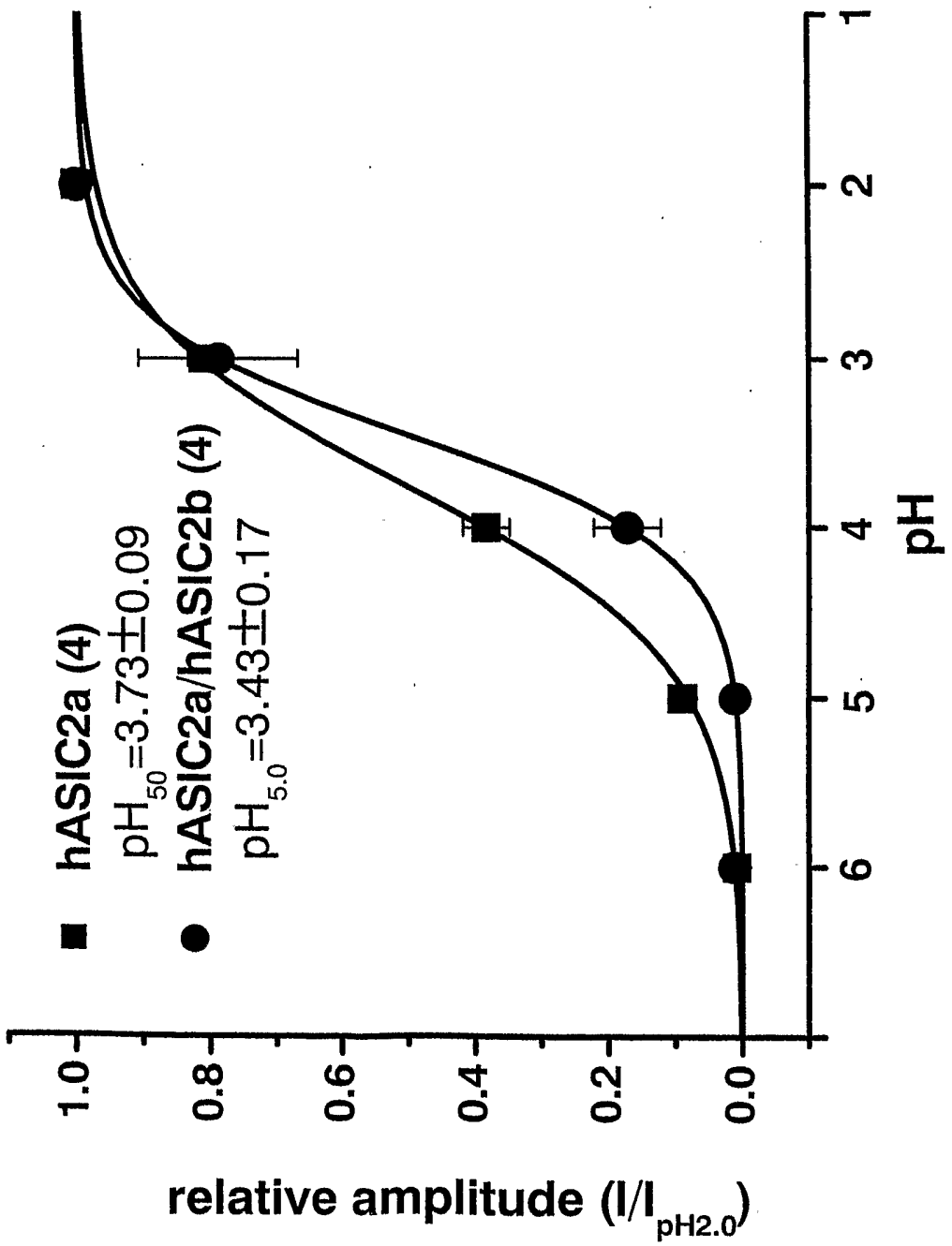
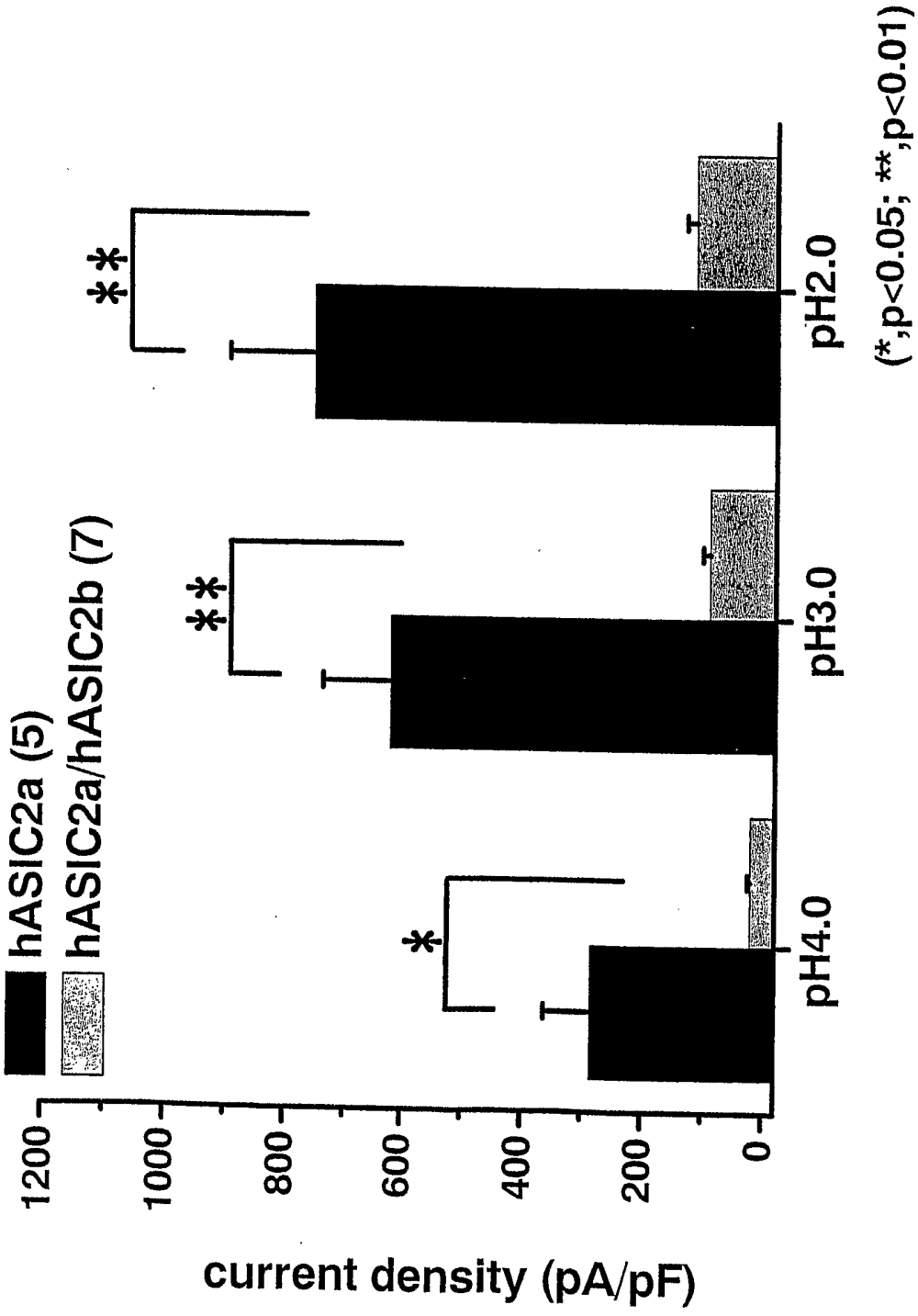

hASIC2a (4)
pH50=3.73±0.09
hASIC2a/hASIC2b (4)
pH5.0=3.43±0.17
relative amplitude (I/IpH2.0)
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
5
6
pH

Fig.6B


hASIC2a (5)
hASIC2a/hASIC2b (7)
current density (pA/pF)
0
200
400
600
800
1000
1200
pH4.0
pH3.0
pH2.0
*
**
**
(*,p<0.05; **,p<0.01)

SEQUENCE LISTINGS

<110> Pfizer Pharmaceuticals Inc. (All Designated States except US);
GAJYA, Norikazu (US only);
SHINJO, Katsuhiro (US only);
TAKI, Kenji (US only)

<120> Human acid sensing ion channel 2b (hASIC2b), process of producing the same, and its use

<130> PC09993A

<150> US 60/402,992
<151> 2002-08-12

<160> 8

<210> 1
<211> 2746
<212> DNA
<213> Human

<220>
<221> CDS
<222> (173) ... (1828)

<400> 1

```
cccgggatcg ggcgcacgct tccctggcgc gggctgcgcg cggctgccgc tgctgctgct      60
tccactgctg ctgcggactc ccatggcggc tccgcccggc cggggccgcc gccgctgccg     120
ccagccgcgg gctgaatga atg agc cgg att ggc gga gcc ggg ctg ccc gca      172
                    Met Ser Arg Ile Gly Gly Ala Gly Leu Pro Ala
                      1               5                  10
gcc gcg ctc acc ggc ccg gga cgc ttc cgc atg gcc cgc gag gag ccg      220
Ala Ala Leu Thr Gly Pro Gly Arg Phe Arg Met Ala Arg Glu Glu Pro
            15                  20                  25
gcg ccc gcg gcg ttg gcg gct gcc ggg cag ccc ggg ggc ggc aga ggc      268
Ala Pro Ala Ala Leu Ala Ala Ala Gly Gln Pro Gly Gly Gly Arg Gly
         30                  35                  40
ggc gag cgg gcg ctg cag ggg cca ggg gtc gcc cgc agg ggg cgg cca      316
Gly Glu Arg Ala Leu Gln Gly Pro Gly Val Ala Arg Arg Gly Arg Pro
     45                  50                  55
tcg ctg agc cgc gct aaa ctg cac ggg ctg cgg cac atg tgt gcc ggg      364
Ser Leu Ser Arg Ala Lys Leu His Gly Leu Arg His Met Cys Ala Gly
 60                  65                  70                  75
cgc acg gcg gct ggg ggc tcc ttc cag cgg cgg gcg ctg tgg gtg ctg      412
Arg Thr Ala Ala Gly Gly Ser Phe Gln Arg Arg Ala Leu Trp Val Leu
                 80                  85                  90
gcc ttc tgt aca tcc ttc ggc ttg ctg ctg tcc tgg tcc tcg aac cgt      460
Ala Phe Cys Thr Ser Phe Gly Leu Leu Leu Ser Trp Ser Ser Asn Arg
            95                  100                 105
ttg ctc tac tgg ctc agc ttc ccg tca cac acg cgg gtg cac cgc gag      508
Leu Leu Tyr Trp Leu Ser Phe Pro Ser His Thr Arg Val His Arg Glu
        110                 115                 120
tgg agc cgc cag tta ccc ttc ccc gcc gtc act gtg tgc aac aac aac      556
Trp Ser Arg Gln Leu Pro Phe Pro Ala Val Thr Val Cys Asn Asn Asn
    125                 130                 135
ccg ctg cgc ttc ccg cgc ctc tcc aag ggg gac ctc tac tat gcc ggc      604
Pro Leu Arg Phe Pro Arg Leu Ser Lys Gly Asp Leu Tyr Tyr Ala Gly
140                 145                 150                 155
cac tgg ctc ggg ctg ctg ctg ccc aac cgc acc gcg cgc ccg ctt gtc      652
His Trp Leu Gly Leu Leu Leu Pro Asn Arg Thr Ala Arg Pro Leu Val
                160                 165                 170
```

```
agc gag ctg ctg cgg ggc gac gag ccg cgc cgc cag tgg ttc cgc aag       700
Ser Glu Leu Leu Arg Gly Asp Glu Pro Arg Arg Gln Trp Phe Arg Lys
            175                 180                 185
ctg gcg gac ttc cgc ctc ttc ctg cct ccg cgc cac ttc gag gga atc       748
Leu Ala Asp Phe Arg Leu Phe Leu Pro Pro Arg His Phe Glu Gly Ile
        190                 195                 200
agc gcc gcc ttc atg gac cgc ctg ggc cac cag ctg gag gac atg ctg       796
Ser Ala Ala Phe Met Asp Arg Leu Gly His Gln Leu Glu Asp Met Leu
    205                 210                 215
ctc tcc tgc aag tac cgc ggc gag ctc tgc ggg ccg cac aac ttc tcc       844
Leu Ser Cys Lys Tyr Arg Gly Glu Leu Cys Gly Pro His Asn Phe Ser
220                 225                 230                 235
tcc gtg ttt aca aaa tat ggg aag tgt tac atg ttt aac tca ggc gag       892
Ser Val Phe Thr Lys Tyr Gly Lys Cys Tyr Met Phe Asn Ser Gly Glu
                240                 245                 250
gat ggc aaa cct ctg ctc acc acg gtc aag ggg ggg aca ggc aac ggg       940
Asp Gly Lys Pro Leu Leu Thr Thr Val Lys Gly Gly Thr Gly Asn Gly
            255                 260                 265
ctg gag atc atg ctg gac att cag cag gat gag tac ctg ccc atc tgg       988
Leu Glu Ile Met Leu Asp Ile Gln Gln Asp Glu Tyr Leu Pro Ile Trp
        270                 275                 280
gga gag aca gag gaa acg aca ttc gaa gca gga gtg aaa gtt cag atc      1036
Gly Glu Thr Glu Glu Thr Thr Phe Glu Ala Gly Val Lys Val Gln Ile
    285                 290                 295
cac agt cag tct gag cca cct ttc atc caa gag ctg ggc ttt ggg gtg      1084
His Ser Gln Ser Glu Pro Pro Phe Ile Gln Glu Leu Gly Phe Gly Val
300                 305                 310                 315
gct cca ggg ttc cag acc ttt gtg gcc aca cag gag cag agg ctc aca      1132
Ala Pro Gly Phe Gln Thr Phe Val Ala Thr Gln Glu Gln Arg Leu Thr
                320                 325                 330
tac ctg ccc cca ccg tgg ggt gag tgc cga tcc tca gag atg ggc ctc      1180
Tyr Leu Pro Pro Pro Trp Gly Glu Cys Arg Ser Ser Glu Met Gly Leu
            335                 340                 345
gac ttt ttt cct gtt tac agc atc acc gcc tgt agg att gac tgt gag      1228
Asp Phe Phe Pro Val Tyr Ser Ile Thr Ala Cys Arg Ile Asp Cys Glu
        350                 355                 360
```

```
acc cgc tac att gtg gaa aac tgc aac tgc cgc atg gtt cac atg cca      1276
Thr Arg Tyr Ile Val Glu Asn Cys Asn Cys Arg Met Val His Met Pro
    365                 370                 375
ggg gat gcc cct ttt tgt acc cct gag cag cac aag gag tgt gca gag      1324
Gly Asp Ala Pro Phe Cys Thr Pro Glu Gln His Lys Glu Cys Ala Glu
380                 385                 390                 395
cct gcc cta ggt ctg ttg gcg gaa aag gac agc aat tac tgt ctc tgc      1372
Pro Ala Leu Gly Leu Leu Ala Glu Lys Asp Ser Asn Tyr Cys Leu Cys
                400                 405                 410
agg aca ccc tgc aac cta acc cgc tac aac aaa gag ctc tcc atg gtg      1420
Arg Thr Pro Cys Asn Leu Thr Arg Tyr Asn Lys Glu Leu Ser Met Val
            415                 420                 425
aag atc ccc agc aag aca tca gcc aag tac ctt gag aag aaa ttt aac      1468
Lys Ile Pro Ser Lys Thr Ser Ala Lys Tyr Leu Glu Lys Lys Phe Asn
        430                 435                 440
aaa tca gaa tat ctc atc tca gag aac atc ctt gtt ctg gat ata ttt      1516
Lys Ser Glu Lys Tyr Ile Ser Glu Asn Ile Leu Val Leu Asp Ile Phe
    445                 450                 455
ttt gaa gct ctc aat tat gag aca att gaa cag aag aag gcg tat gaa      1564
Phe Glu Ala Leu Asn Tyr Glu Thr Ile Glu Gln Lys Lys Ala Tyr Glu
460                 465                 470                 475
gtt gct gcc tta ctt ggt gat att ggt ggt cag atg gga ttg ttc att      1612
Val Ala Ala Leu Leu Gly Asp Ile Gly Gly Gln Gly Leu Phe Ile
                480                 485                 490
ggt gct agt atc ctt aca ata cta gag ctc ttt gat tat att tat gag      1660
Gly Ala Ser Ile Leu Thr Ile Leu Glu Leu Phe Asp Tyr Ile Tyr Glu
            495                 500                 505
ctg atc aaa gag aag cta tta gac ctg ctt ggc aaa gag gag gac gaa      1708
Leu Ile Lys Glu Lys Leu Leu Asp Leu Leu Gly Lys Glu Glu Asp Glu
        510                 515                 520
ggg agc cac gat gag aat gtg agt act tgt gac aca atg cca aac cac      1756
Gly Ser His Asp Glu Asn Val Ser Thr Cys Asp Thr Met Pro Asn His
    525                 530                 535
tct gaa acc atc agt cac act gtg aac gtg ccc ctg cag acg acc ctg      1804
Ser Glu Thr Ile Ser His Thr Val Asn Val Pro Leu Gln Thr Thr Leu
540                 545                 550                 555
```

```
ggg acc ttg gag gag att gcc tgc tga cacccctcga gtcacccagc actccct    1858
Gly Thr Leu Glu Glu Ile Ala Cys Stop
                560
ccaaacagac cttgaggccc aagacccagg acaaggaaca gcaagctcag gtgggatggc    1918
cccagtgctg gaaagaagca agagcccccc atgcacacat tgcagactag ctgcctagac    1978
ctcgctccgg ccacgtccaa cacgacgcat ccttgggccc cgccgtgcgt ccctcttagg    2038
agagatgagt cacactctgg aactgtccaa gaacgaacct gccatcacat ctcactgcca    2098
gatgtataaa gcacctgcat gctcagactt cttgtggcgc cacctccacg tctgtcttgt    2158
acatgacact cctccacgcg gtttccagtg tccacactgc tgcccgtgca gtgggaccag    2218
attccaggtc caaagtcacc atgaggccac cctggaatca gaactgcaca atcaagaggg    2278
aacccatggg actctctgct acattcagtt cttgtgtcgt ttgtgaaagt tcttaacctg    2338
cccaaaaacc ccctttccc caagctgccc atggggcttc ggcgccaaag gtgacccgcg    2398
ccaacctccc tccccccag tgcctatgac ggcggcacag cagccagcgg gtgggggacg    2458
cctgtgttca cccatggtgc ccatgtcgtt cttctctccc tgtgacacag cttgtacagt    2518
ctgattcttt ttatctgggg taggggggct tttatgtttg tccgatggag atttgttttg    2578
ttttgcttca ttttatgctt ttttatttta gttttgatgt tctgaggttt gctttggttt    2638
ttccattttc tttggcattt atttattcgt gcttcaaatc acagtcatat taaaagctgg    2698
tcttgtggaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaa                2746
```

<210> 2

<211> 563

<212> PRT

<213> Human

<400> 2

```
Met Ser Arg Ile Gly Gly Ala Gly Leu Pro Ala Ala Ala Leu Thr Gly
  1               5                  10                  15
Pro Gly Arg Phe Arg Met Ala Arg Glu Glu Pro Ala Pro Ala Ala Leu
             20                  25                  30
Ala Ala Ala Gly Gln Pro Gly Gly Gly Arg Gly Gly Glu Arg Ala Leu
         35                  40                  45
Gln Gly Pro Gly Val Ala Arg Arg Gly Arg Pro Ser Leu Ser Arg Ala
     50                  55                  60
Lys Leu His Gly Leu Arg His Met Cys Ala Gly Arg Thr Ala Ala Gly
 65                  70                  75                  80
```

```
Gly Ser Phe Gln Arg Arg Ala Leu Trp Val Leu Ala Phe Cys Thr Ser
                 85                  90                  95
Phe Gly Leu Leu Leu Ser Trp Ser Ser Asn Arg Leu Leu Tyr Trp Leu
            100                 105                 110
Ser Phe Pro Ser His Thr Arg Val His Arg Glu Trp Ser Arg Gln Leu
        115                 120                 125
Pro Phe Pro Ala Val Thr Val Cys Asn Asn Asn Pro Leu Arg Phe Pro
    130                 135                 140
Arg Leu Ser Lys Gly Asp Leu Tyr Tyr Ala Gly His Trp Leu Gly Leu
145                 150                 155                 160
Leu Leu Pro Asn Arg Thr Ala Arg Pro Leu Val Ser Glu Leu Leu Arg
                165                 170                 175
Gly Asp Glu Pro Arg Arg Gln Trp Phe Arg Lys Leu Ala Asp Phe Arg
            180                 185                 190
Leu Phe Leu Pro Pro Arg His Phe Glu Gly Ile Ser Ala Ala Phe Met
        195                 200                 205
Asp Arg Leu Gly His Gln Leu Glu Asp Met Leu Leu Ser Cys Lys Tyr
    210                 215                 220
Arg Gly Glu Leu Cys Gly Pro His Asn Phe Ser Ser Val Phe Thr Lys
225                 230                 235                 240
Tyr Gly Lys Cys Tyr Met Phe Asn Ser Gly Glu Asp Gly Lys Pro Leu
                245                 250                 255
Leu Thr Thr Val Lys Gly Gly Thr Gly Asn Gly Leu Glu Ile Met Leu
            260                 265                 270
Asp Ile Gln Gln Asp Glu Tyr Leu Pro Ile Trp Gly Glu Thr Glu Glu
        275                 280                 285
Thr Thr Phe Glu Ala Gly Val Lys Val Gln Ile His Ser Gln Ser Glu
    290                 295                 300
Pro Pro Phe Ile Gln Glu Leu Gly Phe Gly Val Ala Pro Gly Phe Gln
305                 310                 315                 320
Thr Phe Val Ala Thr Gln Glu Gln Ang Leu Thr Tyr Leu Pro Pro Pro
                325                 330                 335
Trp Gly Glu Cys Arg Ser Ser Glu Met Gly Leu Asp Phe Phe Pro Val
            340                 345                 350
Tyr Ser Ile Thr Ala Cys Arg Ile Asp Cys Glu Thr Arg Tyr Ile Val
        355                 360                 365
```

```
Glu Asn Cys Asn Cys Arg Met Val His Met Pro Gly Asp Ala Pro Phe
    370                 375                 380
Cys Thr Pro Glu Gln His Lys Glu Cys Ala Glu Pro Ala Leu Gly Leu
385                 390                 395                 400
Leu Ala Glu Lys Asp Ser Asn Tyr Cys Leu Cys Arg Thr Pro Cys Asn
                405                 410                 415
Leu Thr Arg Tyr Asn Lys Glu Leu Ser Met Val Lys Ile Pro Ser Lys
            420                 425                 430
Thr Ser Ala Lys Tyr Leu Glu Lys Lys Phe Asn Lys Ser Glu Lys Tyr
        435                 440                 445
Ile Ser Glu Asn Ile Leu Val Leu Asp Ile Phe Phe Glu Ala Leu Asn
    450                 455                 460
Tyr Glu Thr Ile Glu Gln Lys Lys Ala Tyr Glu Val Ala Ala Leu Leu
465                 470                 475                 480
Gly Asp Ile Gly Gly Gln Met Gly Leu Phe Ile Gly Ala Ser Ile Leu
                485                 490                 495
Thr Ile Leu Glu Leu Phe Asp Tyr Ile Tyr Glu Leu Ile Lys Glu Lys
            500                 505                 510
Leu Leu Asp Leu Leu Gly Lys Glu Glu Asp Glu Gly Ser His Asp Glu
        515                 520                 525
Asn Val Ser Thr Cys Asp Thr Met Pro Asn His Ser Glu Thr Ile Ser
    530                 535                 540
His Thr Val Asn Val Pro Leu Gln Thr Thr Leu Gly Thr Leu Glu Glu
545                 550                 555                 560
Ile Ala Cys
```

<210> 3
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> oligonucleotide probe A1

<400> 3
ttyccrgcng tnacccctst gya 23

<210> 4
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> oligonucleotide probe A4

<400> 4
ctggacrtkc ancanganga rt 22

<210> 5
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> oligonucleotide probe A9

<400> 5
ggnytkttya thggkgcyag 20

<210> 6
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> sense primer for hASIC2b

<400> 6
ctgctctcct gcaagtacc 19

<210> 7
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> antisense primer for hASIC2b

<400> 7
agctcttgga tgaaaggtgg c 21

<210> 8
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> sense primer for hASIC2a

<400> 8
accaccaacg acctgtacc 19

<210> 9
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> antisense primer for hASIC2a

<400> 9
agaggtttgc catcctcgc 19

<210> 10
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> sense primer for GAPDH

<400> 10
gtcttcacca ccatggagaa ggct 24

<210> 11
<211> 25
<212> DNA
<213> Artificial Sequence

<220>

<221> misc_feature

<223> antisense primer for GAPDH

<400> 11

gtgatggcat ggactgtggt catga 25

# INTERNATIONAL SEARCH REPORT

PCT/IB 03/03706

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/705 G01N33/68 A61K48/00 C07K16/28 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, MEDLINE, WPI Data, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 98 35034 A (HEURTEAUX CATHERINE ;CHAMPIGNY GUY (FR); LINGUEGLIA ERIC (FR); WAL) 13 August 1998 (1998-08-13) The rat ion channel MDEG2 (SEQ ID N°6, claim 9) shows 96.8% identity with SEQ ID N°2 over 563 amino acid overlap, and 90.3% identity with SEQ ID N°1 over 1955 nt overlap. --- | 1-6, 8-15, 17-19 |
| X | WO 00 08149 A (CENTRE NAT RECH SCIENT) 17 February 2000 (2000-02-17) The rat cationic channel 2B (rASIC2B) (cpage 80-82) shows 96.6% identity with SEQ ID N°2 over 563 amino acid overlap, and 90.3% identity with SEQ ID N°1 over 1955 nt overlap. --- -/-- | 1-6, 8-15, 17-19 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 5 December 2003 | 05/01/2004 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31–70) 340–2040, Tx. 31 651 epo nl,<br>Fax: (+31–70) 340–3016 | Vix, O |

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 01 81570 A (UNIV MCGILL ;BABINSKI KAZIMIERZ (CA); SEGUELA PHILIPPE (CA)) 1 November 2001 (2001-11-01) The human ion channel ASIC2A shows 78.9% identity with SEQ ID N°2 over 498 amino acid overlap, and 98.1% identity with SEQ ID N°1 over 1990 nt overlap. --- | 1-6, 8-15, 17-19 |
| X | LINGUEGLIA E ET AL: "A MODULATORY SUBUNIT OF ACID SENSING ION CHANNELS IN BRAIN AND DORSAL ROOT GANGLION CELLS" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 272, no. 47, 21 November 1997 (1997-11-21), pages 29778-29783, XP002068544 ISSN: 0021-9258 The ion channel sequence MDEG shows 97.2% identity with SEQ ID N°2 over 563 amino acid overlap (see Swissprot entry Q61203). --- | 1-6, 8-15, 17-19 |
| X | GARCIA-ANOVEROS J ET AL: "BNAC1 AND BNAC2 CONSTITUTE A NEW FAMILY OF HUMAN NEURONAL SODIUM CHANNELS RELATED TO DEGENERINS AND EPITHELIAL SODIUM CHANNELS" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 94, no. 4, 1 February 1997 (1997-02-01), pages 1459-1464, XP002051359 ISSN: 0027-8424 The ion channel sequence shows 97.2% identity with SEQ ID N°2 over 563 amino acid overlap (see Swissprot entry Q61203). --- | 1-6, 8-15, 17-19 |
| A | GUNTHORPE M J ET AL: "Characterisation of a human acid-sensing ion channel (hASIC1a) endogenously expressed in HEK293 cells." PFLUGERS ARCHIV: EUROPEAN JOURNAL OF PHYSIOLOGY. GERMANY AUG 2001, vol. 442, no. 5, August 2001 (2001-08), pages 668-674, XP002263964 ISSN: 0031-6768 the whole document ----- | 1-6, 8-15, 17-19, 23-26 |

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 7, 16, 20-22

No reach-through compound claims (and use thereof) is allowed:

Present claim 16 relate to a "compound which modulates the polypeptide according to claim 13": such compound is only defined by reference to a its potential interaction/modulation activity with a polypeptide (probably using screening methods such as the one defined in claim 17).

The claim covers all products having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for NONE such products or methods. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT).
A meaningful search cannot be established because it is not possible to determine if any of the presently known substances is falling under the terms of these "modulation substances" product claims. Besides it is noted, that the use of compounds of claim 16 are not rendered novel just because of the fact that the compounds have been identified by the method of claim 17, e.g. such compounds and their specific use can already exist.
The same remark applies for the use (claim 20) of such a compound or method of treatment using such compound as defined in claims 21-22.

Moreover, claim 6 relates to a "ribozyme or zinc finger protein" only defined by its binding characteristic (in absence of structural technical features). Such claim is objected to under Art. 5 and 6 PCT for the same reasons as detailed above.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

PCT/IB 03/03706

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

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

Although claim 24 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 7, 16, 20-22
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

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

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

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

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

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

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

## INTERNATIONAL SEARCH REPORT

PCT/IB 03/03706

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9835034 A | 13-08-1998 | FR 2759372 A1 | 14-08-1998 |
| | | FR 2759373 A1 | 14-08-1998 |
| | | EP 0977844 A1 | 09-02-2000 |
| | | WO 9835034 A1 | 13-08-1998 |
| | | JP 2001514487 T | 11-09-2001 |
| WO 0008149 A | 17-02-2000 | AU 5187999 A | 28-02-2000 |
| | | CA 2336221 A1 | 17-02-2000 |
| | | EP 1102844 A2 | 30-05-2001 |
| | | WO 0008149 A2 | 17-02-2000 |
| | | JP 2002524042 T | 06-08-2002 |
| | | US 6287859 B1 | 11-09-2001 |
| WO 0181570 A | 01-11-2001 | CA 2304494 A1 | 20-10-2001 |
| | | AU 5455301 A | 07-11-2001 |
| | | WO 0181570 A2 | 01-11-2001 |
| | | CA 2406660 A1 | 01-11-2001 |
| | | EP 1290165 A2 | 12-03-2003 |
| | | US 2003219858 A1 | 27-11-2003 |